ISA 2007

3rd International Seabuckthorn Association Conference

PROCEEDINGS OF THE 3RD INTERNATIONAL SEABUCKTHORN ASSOCIATION CONFERENCE

Quebec City, Canada • August 12-16, 2007

Editor-in-Chief

David B. McKenzie

Associate Editors

Stefan Cenkowski, Alphonsus Utioh, Tom S.C. Li, Wudeneh Letchamo, Cristina Ratti and Khaled Belkacemi

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PREFACE

This collection of 30 peer reviewed articles represents the published proceedings of a successful international conference – 3rd International Seabuckthorn Association Conference (ISA 2007) – held in Quebec City, Canada, August 12-16, 2007. From nearly 100 abstract title applications received before the conference, just over 50 oral and poster presentations were accepted by the ISA 2007 Scientific Committee. From the pool of scientific presenters, 41 manuscripts were submitted to the ISA 2007 peer review process. Nine manuscripts were withdrawn, two manuscripts were not acceptable, and 30 manuscripts were revised and found acceptable by the scientific committee for publication in these proceedings.

Dr. Tom Li was instrumental in bringing this conference to Canada, so it was appropriate that he authored the introductory paper. Ten papers in the section on seabuckthorn production, cultivation and harvesting practices were authored by a diverse group of scientists from Canada, China, India, Romania and Russia. Thirteen papers in the seabuckthorn processing technology and chemistry section were provided by scientists from Canada, Finland, India, Latvia, Lithuania, Romania and Russia. Six papers in the seabuckthorn products, industry development and marketing section were authored by scientists based in Canada, China, Finland, Germany and Russia. Altogether, 14 papers were included from four regions of Canada. This is an excellent effort by Canadian scientists who have just recently begun work in the exciting area of seabuckthorn research along with 16 papers from scientists in the major seabuckthorn research and production areas of the world.

The many hours of effort by Associate Editors and Reviewers in providing helpful suggestions to manuscript authors is greatly appreciated. The effort required by reviewers and editors to understand a highly technical paper is much greater when an author's first language is not English, and a much greater familiarity with the manuscript is needed before appropriate suggestions can be provided. In particular, I would like to thank Associate Editors Stefan Cenkowski, Alphonsus Utioh, Tom S.C. Li, Wudeneh Letchamo, Cristina Ratti and Khaled Belkacemi.

David B. McKenzie, Editor-in-Chief

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MESSAGE FROM THE CONFERENCE CO-CHAIRS

The 3rd International Seabuckthorn Association (ISA) Conference held from August 12 to 16, 2007 in Quebec City, Canada was a great success. About 140 representatives from 12 countries, including Canada, China, United States of America, Germany, Finland, Russia, Latvia, India, Romania, France, Japan and Bolivia attended the conference. The conference boasted a unique and ambitious theme: "Promoting Seabuckthorn Industry Worldwide – Opportunities and Challenges". Seabuckthorn – a unique plant with many functional therapeutic uses presents both challenges and opportunities. Opportunities – because of its health promoting chemical composition, seabuckthorn can be seen as a key ingredient in functional foods, cosmetic and pharmaceutical formulations. Challenges – in the sense that seabuckthorn is still relatively unknown in North America and many other parts of the world, thus presenting a marketing challenge; not even to mention the harvesting challenge presented by this fruit plant!

The first two conferences of ISA took place in Germany (2003) and China (2005). Very few Canadian growers and researchers were able to attend (six in 2003 and two in 2005). In 2009, the 4th ISA Conference will take place in Russia. The ISA 2007 Conference at Quebec City thus presented a unique opportunity for all seabuckthorn growers, experts and stakeholders in Canada to participate and exchange information about this emerging industry in Canada with world renowned scientists and experts. We thank the Scientific Committee for designing the technical program and presentations to promote sharing and exchange of ideas, and collaborative research discussions. A dedicated day of the conference to address the growers' needs was a very unique aspect of the 2007 conference, and brought about the most participation from growers.

More than 90 papers were presented or posted, and 37 scientists and experts made excellent presentations on the fields of seabuckthorn cultivation and breeding, nutritional and medical applications, processing and technologies, product development and marketing, international co-operation, and seabuckthorn in soil conservation. We thank all those who contributed to the technical papers for presentations in oral and/or poster sessions. Such research efforts help to build sound science for the development of seabuckthorn products.

We thank the Organizing Committee of the ISA 2007 Conference for putting together a strong technical program coupled with networking and social events. We are truly grateful to all sponsors listed in this publication for their support which contributed to the resounding success of the ISA 2007 Conference. Finally, we would like to express our sincere gratitude to Laval University and the Quebec Seabuckthorn Growers' Association for their leadership in the local organization of the conference.

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SUMMARY OF THE EVENT

The 3rd International Seabuckthorn Association meeting was held August 12 to August 16 2007, in Quebec City, Quebec, Canada. The event was proudly organized by the Institute of Nutraceuticals and Functional Foods of Laval University in collaboration with the Quebec Seabuckthorn Growers' Association under the auspices of the International Seabuckthorn Association.

A total of 142 participants from 12 countries attended the meeting:

- From North and South America: Bolivia, United States and Canada, with six provinces represented (Newfoundland and Labrador, Quebec, Ontario, Manitoba, Saskatchewan and British Columbia)
- From Europe: Finland, France, Germany, Latvia, Romania and Russia
- From Asia: China, India and Japan

The objectives of the conference were:

- To share and exchange the updates, findings and progress of seabuckthorn research
- To promote new knowledge about the function of seabuckthorn
- To show new technologies of high quality seabuckthorn products
- To develop long term strategies and partnership for scientific, as well as commercial cooperation on seabuckthorn

The Conference Committee thus developed a very interesting program that included:

- Production: Cultivation and Harvesting Practices; Breeding
- Processing: Technologies; Chemistry - Nutritional, Medicinal and Cosmetic
- Product Development and Marketing: Uses of Seabuckthorn and Its Products

Keynote speakers for each of the themes opened the sessions and provided an overall view of the current and emerging activities in each area. These sessions were designed to promote sharing and exchange of ideas, and collaborative research activities. A poster session was also held to further promote new research activities on seabuckthorn and its derivatives.

This meeting was a genuine occasion to promote the transfer of knowledge on a most promising crop, with the presentation of numerous scientific and technical communications on seabuckthorn production, processing and marketing. Networking and fruitful discussions followed presentations, and the last day's technical farm tour, although challenged by pouring rain, turned out to be a very interesting outing that closed the meeting on a lighter, friendly and very festive note.

PDF files of most of the Powerpoint presentations prepared by the speakers can be viewed on the ISA 2007 website hosted by the Quebec Seabuckthorn Growers' Association: www.isa2007.net.

ACKNOWLEDGEMENTS

The organizers would like to express their gratitude to all their colleagues, teams, collaborators and everyone who has contributed in one way or another to the success of the 3rd International Seabuckthorn Association Conference. This wonderful event would not have been possible without you. Many thanks to the following organizations for their unconditional support:

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PROCEEDINGS

Next Generation of New Botanical Seabuckthorn (*Hippophae rhamnoides* L.)

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Introduction

Seabuckthorn is a hardy, deciduous shrub belonging to the family Elaeagnaceae (Rousi, 1971). It bears yellow through orange to red fruits which have been used for centuries in Europe and Asia for food, therapeutic, and pharmaceutical purpose (Bailey and Bailey, 1978). Seabuckthorn is a dioecious species with male and female flower buds in 3-4 years after seeding. Flowers are formed mostly on 2-year-old wood differentiated during the previous growing season (Bernath and Foldesi, 1992). Neither the male nor the female flowers produce nectar and does not attract insects; thus, pollination of female flowers depends entirely on the wind to spread pollen from male flowers.

Seabuckthorn fruits are among the most nutritious found in the plant kingdom. The importance of the plant is based on the nutritional value of the fruits. The fruits contain an essential oil and high concentration of vitamins A, B1, B2, B6, C, and E. Other important components such as carotene, fatty acid, palmitin, palmitoleic acids, β-sitosterol have been used in therapeutic allocations. Vitamin concentration, especially C and E are as high as 360 mg/100g and 160 mg/100g of fruit weight, respectively. Seabuckthorn is also rich in flavonoids, carotenoid, and water and fat soluble vitamins (Wahlberg and Jeppasson, 1992; Wolf and Wegert, 1993; Solonenko and Shishkina, 1989). The general population has increasing interest in the medicinal use of seabuckthorn and scientific research has shown that claimed therapeutic values of seabuckthorn have increasingly solid support from the scientific community. This trend is reflected in the number of scientific publications in the last 50 years. The number of publications increased from 11 published research papers from 1951-1960 to 37 from 1991-2000, and reached 100 from 2001-mid of 2005 (Li, unpublished data). The quality and specific goals or the research has improved significantly in recent years.

Folklore and ancient tradition to scientifically proven therapeutic values

Seabuckthorn has long been used for relieving coughs, expelling phlegm, improving digestion, promoting blood circulation, relieving abdominal pain, and adjusting irregular menstruation. It also has been used for aiding digestion and alleviating pain (Zheng et al., 1997). Seabuckthorn was officially listed in the Chinese Pharmacopoeia by the Ministry of Public Health and the reputation of seabuckthorn as a medicinal plant was established. More than ten different drugs have been developed from seabuckthorn and are available in different forms, such as liquids, powders, plasters, films, pastes, pills, liniments, suppositories, and aerosols.

Seabuckthorn is regarded as the next generation of new botanical because of its considerable nutritional and therapeutic values for human beings. In a review article, Guliyev et al. (2004) indicated that the extracts of seabuckthorn branches and leaves are used to treat diarrhea, gastrointestinal and dermatologic disorders, colitis and enterocolitis in humans and animals in Mongolia and Russia. Leaves have also been applied as a compress in rheumatoid arthritis in Central Asia. Flowers are used as a skin softener in Tajikistan.

There is evidence that seabuckthorn contains compounds that are anti-cancer in nature and these compounds are present in both the oil and juice fractions (Xu et al., 2001). Seabuckthorn oil can inhibit tumor development of transplanted tumors and both the oil and juice can kill S180 and P388 cancer cells and inhibit strains of human gastric carcinoma (Zhang, 1989). It can inhibit liver cancers induced by aflatoxin B1 and inhibit the formation of N-nitrous compounds preventing the induction of cancers.

Seabuckthorn oil is a good source of α-linolenic acid and other unsaturated fatty acids. It was very difficult to present clear, convincing evidence for the efficacy of these oils in preventing human disease. In the light of repeated observations on the inhibition of platelet aggregation with seabuckthorn oil (Zhao et al., 1990; Johansson et al., 2000), it seems likely that this activity is not associated with these fatty acids. However, the oil has a unique composition of fatty acids, including a spectrum of mono-unsaturated fatty acids and is a rich source of two essential fatty acids, linoleic and α-linolenic and both seed and pulp oils are rich in oleic acid.

There is increasing interest in the physiological role of mono-unsaturated fatty acids relative to the modification of vascular endothelial surfaces and the role this may play in the reduction of thrombosis (Perz-jimmenez et al., 1999; Turpeinen et al., 1998), psoriasis (Hodutu, 1999), and peptic ulcer (Abidov et al., 2002).

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PRODUCTION

The Correlation Between Seabuckthorn Berry Quality and Altitudes of Its Growing Location

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Abstract

Wild Chinese seabuckthorn (*Hippophae rhamnoides* ssp. *sinensis*) stands are broadly distributed in many areas in China. Many ecological factors influence the quality of seabuckthorn berries. Among those factors altitude plays an important role. A study on the correlation between seabuckthorn berry quality and altitude of its growing locations was carried out. The berry samples were collected from low altitudes in the east plain areas to high altitudes in the west mountain areas. A special study was conducted in wild seabuckthorn stands in the Wolong valley, Sichuan, China at altitudes from 1900 m to 3100 m. Results showed that the berry quality (such as weight per 100 berries, soluble substances, organic acids, vitamin C, seed oil and pulp oil contents etc.) rely on altitude of the growing areas. Among quality factors the content of soluble sugar, organic acids and vitamin C have a direct correlation with the growing altitude. At higher altitudes of 1900 to 3100 m, there are greater amounts of organic acids and vitamin C. The results suggest that in the distributed areas of seabuckthorn, establishing seabuckthorn plantations in higher altitude areas can produce better quality berries.

Keywords: seabuckthorn berries, altitude, correlation.

Introduction

Chinese seabuckthorn *(Hippophae rhamnoides* subspecies *sinensis)* is very adaptable to harsh ecological conditions and has large populations. It is the most important species of *Hippophae* and is widely distributed in a large area of north and north-west China. It is estimated that the total area of Chinese seabuckthorn is approximately 2.13 million hectares including wild and cultivated plants which account for 90% of total seabuckthorn resources in the world (Er, 2003).

Chinese seabuckthorn is the main resource for the seabuckthorn industry in China. It is important to know which current seabuckthorn stands produce better quality berries and how to select areas to establish new plantations that could produce the best quality of berries. Many ecological factors influence the quality of seabuckthorn berries, including the altitude at which the berries are grown. Some studies have shown that there are correlations between berry quality and growing altitudes (Yang *et al*., 1988; Yang, 1989; Grazynova *et al*., 1991). This study will report experimental results that detail how the altitude of the growing location influences seabuckthorn berry quality.

Experimental Materials

Comparison of seabuckthorn growing locations: berry samples were collected from 9 locations from the north- east to the south- west of China according to the altitudes of their growing area. Ripe berry samples were collected from low to high altitude in the 2005 season. In order to get representative samples, berries (2 kg samples) were collected from 20 individual seabuckthorn trees in each location.

Comparison of seabuckthorn growing altitude: ripe berry samples were collected from 7 locations at altitudes ranging from 1900 m to 3100 m in the Wolong valley, west Sichuan, China in the 2004-2006 seasons. In order to get representative samples, each berry sample (2 kg) was collected from 20 individual seabuckthorn trees at each altitude.

Methodology

The analysis of chemical components of the berries was made in the laboratory of Chengdu Institute of Biology, Chinese Academy of Sciences. The items of chemical analysis included soluble sugar, organic acids, vitamin C, seed oil and pulp oil contents.

The soluble sugar was determined by Abe Refract Meter; the organic acids by titration; the vitamin C by 2.6-dichlorindopherol sodium; the oil contents of seeds were calculated by dry weight of seeds and the oil contents of pulp were calculated by fresh berries; the oil of seeds and pulp was extracted by petroleum ether (Zhu, 2004). Each analysis of chemical components was replicated 3 times and the final datum comes from the average of 3 analyses.

Results and Discussion

The results of chemical analysis of Chinese seabuckthorn berries (*H. rhamnoides* ssp. *sinensis*) grown in locations at different altitudes in 2005 are shown in Table 1. It can be seen that there is no trend that the weight per 100 berries, the contents of soluble sugar, seed oil and pulp oil are increased along with the increase of altitude, but the contents of organic acids and the vitamin C generally increase with an increase in altitude.

The correlation analysis between altitudes and berry quality of Chinese seabuckthorn (*H. rhamnoides* ssp. *sinensis*) grown in locations at different altitudes in 2005 is shown in Table 2. There are direct correlations between the altitudes and the contents of organic acids and vitamin C. The correlation coefficient is 0.734 (P=0.05) and 0.877(P=0.01) respectively. There is also a high direct correlation between the content of organic acids and content of vitamin C. The correlation coefficient is 0.932 (P=0.01).

Table 1. Results of chemical analysis of Chinese seabuckthorn berries (*H. rhamnoides* ssp. *sinensis*) grown in locations at different altitudes in 2005.

Glowing location	Altitude (m)	Weight per 100 berries (g)	Soluble Sugar (%)	Organic Acids (%)	Vitamin C (mg/100g)	Seed oil content (%)	Pulp oil content (%)
Harbin, Heilongjiang	150	16.80	10.0	2.36	345.5	6.25	0.62
Suilin, Heilongjiang	300	19.20	11.5	2.96	403.0	6.20	0.93
Fuxin, Liaoning	450	18.77	10.5	3.82	460.5	6.78	1.06
Erdos, Inner Mongolia	1200	16.66	13.0	3.08	479.0	8.87	1.05
Fengning, Hebei	1400	15.08	11.5	2.78	581.0	8.14	1.08
Huanglong, Shaanxi	1500	15.52	11.5	4.07	915.0	6.65	0.87
Jiaokou, Shanxi	1600	19.64	11.0	2.73	593.5	6.27	0.64
Wuqi, Shaaxi	1700	14.38	13.0	5.39	1008.0	7.45	1.01
Qingshui, Gansu	1800	16.15	12.0	5.07	1082.0	8.44	1.65
Maoxian, Sichuan	1900	19.10	10.0	5.57	1163.0	7.46	1.03
Wolong, Sichuan	2400	15.18	11.0	6.28	1243.0	6.84	1.02

In order to get a more precise picture about the relationship between the growing altitudes and berry quality, a river valley with a large difference in vertical elevation was selected for an experiment. The experiment was repeated in 3 consecutive years (2004-2006). The results are reported as below:

The results of chemical analysis of Chinese seabuckthorn berries (*H. rhamnoides* ssp. *sinensis*) grown in Wolong valley, Sichuan, China at different altitudes in 2004-2006 are shown in Table 3. The weight per 100 berries did not increase with an increase in altitude, but the content of soluble sugar, organic acids and the vitamin C generally increased along with an increase in altitude.

The correlation analysis between altitudes and berry quality of Chinese seabuckthorn (*H. rhamnoides* ssp. *sinensis*) grown in Wolong valley, Sichuan, China at different altitudes in 2004-2006 is shown in Table 4. There is a direct correlation between the content of soluble sugar and the content of organic acids. The correlation coefficient is 0.962 (P=0.01). There is a direct correlation between the content of soluble sugar and the content of vitamin C. The correlation coefficient is 0.861 (P=0.05). There is also a direct correlation between the content of organic acids and the content of vitamin C. The correlation coefficient is 0.767 (P=0.05).

Table 2. Correlation analysis between altitudes and berry quality of Chinese seabuckthorn (*H. rhamnoides* ssp. *sinensis*) grown in locations at different altitudes in 2005.

Altitude (m)	Weight per 100 berries (g)	Soluble Sugar (%)	Organic Acids (%)	Vitamin C (mg/100g)	Seed oil content (%)	Pulp oil content (%)
Altitude (m)	r 0.400 P 0.222	0.239 0.079	0.734* 0.010	0.877** 0.000	0.367 0.266	0.358 0.280
Weight per 100 berries (g)		-0.510 0.109	-0.327 0.326	-0.382 0.247	-0.409 0.212	-0.282 0.400
Soluble Sugar (%)			0.074 0.829	0.075 0.826	0.586 0.579	0.376 0.255
Organic Acids (%)				0.932** 0.000	0.189 0.579	0.487 0.129
Vitamin C (mg/100g)					0.226 0.505	0.454 0.161
Seed oil content (%)						0.705* 0.015

r- represents for correlation coefficient;
P- represents for significant level;
***** *Correlation is significant at the 0.05 level (2-tailed);*
****** *Correlation is significant at the 0.01 level (2-tailed).*

Table 3. Results of chemical analysis of Chinese seabuckthorn berries (*H. rhamnoides* ssp. *sinensis*) grown in Wolong valley, Sichuan, China at different altitudes in 2004-2006.

Altitude (m)	Harvest year	Weight per 100 berries (g)		Soluble Sugar (%)		Organic Acids (%)		Vitamin C (mg/100g)	
		Data	Average	Data	Average	Data	Average	Data	Average
1900	2004	14.22		7.5		5.55		688.8	
	2005	14.00	**14.46**	6.0	**6.83**	4.56	**5.10**	748.0	**755.1**
	2006	15.15		7.0		5.19		828.6	
2100	2004	14.92		7.0		4.95		795.0	
	2005	14.98	**15.04**	7.5	**7.33**	5.39	**5.16**	973.0	**913.0**
	2006	15.23		7.5		5.15		971.0	
2300	2004	15.88		8.0		4.90		1005.0	
	2005	17.21	**16.55**	7.0	**7.83**	5.62	**5.21**	1163.0	**1059.3**
	2006	16.55		8.5		5.10		1010.0	
2500	2004	15.72		9.0		6.30		1100.0	
	2005	16.04	**16.25**	10.0	**10.00**	6.58	**6.75**	1151.0	**1089.2**
	2006	17.00		11.0		7.36		1016.7	
2700	2004	15.55		10.5		6.70		1164.5	
	2005	15.23	**15.70**	11.5	**11.00**	7.64	**7.28**	1243.0	**1206.0**
	2006	16.30		11.0		7.51		1210.5	
2900	2004	15.20		10.5		7.25		1235.0	
	2005	14.09	**15.13**	10.0	**10.17**	7.17	**7.16**	1381.0	**1304.7**
	2006	16.10		10.0		7.05		1298.0	
3100	2004	15.42		10.5		6.39		1372.5	
	2005	16.50	**15.61**	9.0	**9.83**	6.34	**6.18**	1162.0	**1305.6**
	2006	14.90		10.0		5.81		1381.0	

** The bold number is the average of 3 years.*

Conclusions

The results showed that the berry quality attributes in this study (weight per 100 berries, soluble substances, organic acids, vitamin C, seed oil and pulp oil contents) seemed dependant on the altitudes of its growing location. Among those quality attributes, the contents of soluble sugar, organic acids and vitamin C have direct correlation with the growing altitudes. At elevations of 1900-3100 m the higher the altitude, the higher the contents of soluble sugar, organic acids and vitamin C.

The results suggest that in the distributed areas of seabuckthorn in China, establishing seabuckthorn plantations in higher altitude areas can produce better quality berries.

Table 4. Correlation analysis between altitudes and berry quality of Chinese seabuckthorn (*H. rhamnoides* ssp. *sinensis*) grown in Wolong valley, Sichuan, China at different altitudes in 2004-2006.

	Weight per 100 berries (g)	Soluble Sugar (%)	Organic Acids (%)	Vitamin C (mg/100g)
Altitude (m)	r 0.296 P 0.520	0.845* 0.017	0.742 0.056	0.975** 0.000
Weight per 100 berries (g)		0.366 0.420	0.205 0.659	0.410 0.361
Soluble Sugar (%)			0.962** 0.001	0.861* 0.013
Organic Acids (%)				0.767* 0.044

r- represents for correlation coefficient;
P- represents for significant level;
** Correlation is significant at the 0.05 level (2-tailed);*
*** Correlation is significant at the 0.01 level (2-tailed).*

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Review of Seabuckthorn Research in the Russian Federation and New Independent States (NIS)

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Abstract

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The overview has been conducted on the basis of 250 publications of Russian and NIS scientists on the following topics: natural resources of sbt in the Russian Federation and NIS; sbt ecology, botany and morphology; genetics and breeding; biochemistry; new places of cultivation.

Initially, seabuckthorn was cultivated in Russia about 160 years ago as a decorative plant. It appeared in the collections of most of the botanical gardens at the end of XIX and the beginning XX centuries. The interest in a fruit plant appeared at the same time. Rich biochemical components of all parts of this wonderful plant made it popular and soon it was broadly used in different regions of Russia. Seabuckthorn plant stability to new conditions of cultivation, first of all winter hardiness, is one of the main problems of sbt introduction to the territories of temperate climate.

The yield of wild sbt is low, the biochemical composition of fruit material is not homogeneous and because of high thorns, harvesting of fruits is difficult. Therefore development of new cultivars for orchards is of great importance. The Russian plant breeders are constantly enriching the sbt gene collection and the diversification of methods to make the breeding process more efficient. Some breeding sbt programs are discussed in the paper.

Keywords: seabuckthorn, research, Russian Federation, introduction, genetics, breeding, biochemical composition.

Seabuckthorn range in the Russian Federation and NIS

Seabuckthorn is widely spread over the territory of the former Soviet Union. Many researchers have investigated the sbt natural range. Generally, the sbt range is broad and rag-sporadic which occurs geographically between 2 to 115^0 east and from 27^0 to 68^0 50' north. It grows naturally in Caucasus, Pamir, Middle Asia, in a number of regions in Western Siberia, Altai region, Tuva, Buryatya, Dunube delta, Kalinigrad Region (Eliseev, Fefelov 1977; Eliseev 1982, 1983; Trofimov 1978; Kondrashov 1979; Ermakov, Koikov 1981; Imamaliev, Panteleeva 1980, Imamaliev 1983; Koikov 1980; Korzinnikov *et al*. 1981; Kabulova 1986; Golovatyi 1980; Avdeev 1985; Lebeda, Dzhurenko 1990; Besschetnov 1980; Besschetnov, Kentbaev 2003a,b; Chmyr and Besschetnov 1998).

The seabuckthorn range in Siberia is beginning to be reduced which is why it was included into the Red Books of Irkutsk Region (Zarubin 2001), Buryatiya (The Red Book…, 1988) and included into the list of endangered plants of Tuva (List …, 1989). It was recommended for local conservation in the book "Rear and Threatened plants of Siberia" (Rare and …, 1980).

Hippophae rhamnoides L. systematic

Gatin (1963) described some of the ecological sbt groups. He suggested to divide the sbt range on isolated sbt subspecies according to morphological and chemical composition in fruits**:** *H. rhamnoides* ssp. *altaica*; *H. rhamnoides* ssp. *tianschanica; H. rhamnoides* ssp. *sayanica*).

Trofimov (1967) distinguished four geographical sbt races on the basis of a seed morphological study: Siberian, Central-middle-Asian, Caucasian and West-European.

Eliseev (1982, 1983) subdivided sbt by 5 climatypes related to the territories of former USSR: Siberian, Middle Asian, Caucasian, South-Western and Baltic (North-Western). Each territory refers to different soil and climatic conditions. Siberian sbt climatypes have the shortest period of vegetation and they are the most frost resistant. Eliseev (1982, 1983) has distinguished four ecotypes within Siberian climatypes: Trans-Baikal, Sayanskyi, Altaian, East-Kazakhstan. Within middle Asian sbt climatypes he has distinguish three ecotypes.

Avdeev (1981) noted new subspecies *H. rhamnoides* ssp. *pamiroaltaica* ssp. *nova planta* on the south of Middle Asia.

Sozonova (1985) studied sbt fruits and seeds and she agreed with the Eliseev's sbt subdivision that *H. rhamnoides* L. is polymorphic species, which is presented by ecological groups: climatypes, ecotypes, and populations.

Kondrashov (1996) showed olive and sbt resemblance on morphological and anatomical characteristics of roots, stems, leaves, fruits and seeds, wind pollination, chemical composition of oils, etc. These species have a similar number of chromosomes. Kondrashov (1996) formulated a hypothesis of parallelism in olive and sbt features which is not occasional. He has suggested to conduct a deeper study of remote taxons which could bring us to new constructions in systematic.

Seabuckthorn ecology, botany and morphology

According to many researchers, sbt is adjustable and a non-demanding plant to soil conditions. Soils of natural sbt growth are poor of humus. It can grow on poor sandy soils. Seabuckthorn has a high mortality on solid clay soil. It is more perennial on sandy loam and sandy soils. Black soil and grey podzol soils would be the best according to its genetic origin. Seabuckthorn can be grown in very poor soils including river banks, steep slopes, and acid and alkaline soils. Seabuckthorn is also salt tolerant. It can grow on soils with a pH of 5.5 to 8.3 but does best with soil between 6.0 and 7.0 pH (Gatin 1963; Trofimov 1978, Chmyr and Besschetnov 1998).

Seabuckthorn well stands flooding with cold running well air-enriched water, but does not stand stagnant dissolved air water after plentiful rains or spring snow melting. It is not recommended to have seabuckthorn plantations in areas with close standing of subterranean waters. The level of ground water in spring time should not be more than 50 (60) cm from the surface (Trofimov 1978; Faustov, Ermakov 1978; Koikov 1978).

Seabuckthorn is a light-demanding species. Light is one of the main factors influencing sbt stand development (Rousi 1976).

Ecological preferences of sbt to open spaces and sunshine are connected to a high content of pigment of photosynthetic apparatus. These investigations are carried out in the Central Botanical Garden of Byelorussia. It was found out that there are 2.93~4.36 mg chlorophyll per 1 g of dry mass. Cultivar specification and seasonal changeability of chlorophyll content was established by Garanovich (1995).

The main sbt specifics are: the possibility to develop several layers of horizontal roots and the ability to fix nitrogen by nodules, which form on the lateral roots (Trofimov 1978). This is one of the few plants of a moderate zone having a capacity to fix atmospheric nitrogen with help of the root nodules formations (Khabarov *et al*., 1998).

Morphological sbt features were investigated by many researchers. Leaves were studied by Rousi (1965, 1971) Faustov, Ermakov (1978), Trofimov (1988) Kozhevnikov 2001; buds – by Eliseev (1976a) and Maltseva (1987); flowers and fruit - by Kondorskaya (1967, 1973) Sozonova (1991); seeds – by Trofimov (1988), Mazaeva, Eliseev (1991). Morphological properties of sbt seeds of different origin were investigated in Central Botanical Garden of Byelorussia (Shpital'naya 2003). These investigations permit to distinguish common and individual characteristics as related to climate type as well as between the individual shrubs. The study of measurements of sbt seeds (length, width) confirmed its large diversity.

Similar to other plants, the seed quality is evaluated by the germination energy. Fefelov (2003) found that germination indices do not give objective information about sbt seed quality of different populations. He suggested to introduce changes in the calculation methodology of sbt seed germination energy and introduced new indices for the evaluation of the biological specific characteristics of sbt seeds.

Morphological and biochemical studies on *H. rhamnoides* L. (the forms growing in three different climatic regions of Azerbaijan) were conducted by Novruzov *et al.* (2001). Sbt populations appeared to be characterized by large diversity of morphological signs and biochemical indices. The common features of the studied forms are small fruits, high oil and carotenoid contents.

Sbt from the Great Caucasus and at the border with Azerbaijan is spread by directions of rivers of the mountainous zone (Novruzov *et al*., 2005b). Vegetation begins at the end of February when the average temperature reaches $+6.5^0$C. It is established, that the size of biological productivity of fruits depends on place of growth, density and age structure of the bush and the weather conditions of the previous 2 years. The frost and too much rain during fertilization of flowers and drought during fruit formation negatively influence the quantity of the plant.

Dynamics of physiological conditions (depending on the rhythm of the changes of ecological factors) is an important parameter of adaptation of plant species to the ecological conditions. Besschetnov and Kentbaev (2001) studied the course of dynamics, the accumulation of some compounds, and the rate of hardening of sbt's shoots under different geographical origins.

New places of cultivation

Seabuckthorn can be cultivated in almost all land zones of the Russian Federation. When selecting a place for a seabuckthorn orchard, consideration must be given to a negative reaction of these species, unlike other fruit-berry species, to soils of heavy mechanical structure.

In Russia, seabuckthorn initially was cultivated as a decorative plant more then 160 years ago. It appeared in the collections of most of the botanical gardens at the end of the XIX to the beginning of the XX centuries. The interest in fruit plants appeared at the same time (Volf 1915, Trofimov 1988). Rich biochemical compounds of all parts of this wonderful plant made it popular and it was widely used in the seabuckthorn introduction work in different regions of Russia such as: Nizhnyi Novgorod Region (Eliseev 1972, 1976a, 1980), Vladimir region (Kovalev 1978), Moscow Region (Trofimov 1967, 1976, 1988, Kovalev 1977, Ermakov 1985), and the Northern European part of Russia (Ermakov 1978, Izergina and Gromakovskyi 1980, Nilov 1986, Demidova and Nilov 1988, Demidova 1993, 1995).

Many publications are devoted to sbt cultivation. The most modern monograph was published in Barnaul by Mikhailova (2005) where she presented long-term results on sbt research. She described methods of sbt cultivation, harvesting and suggested principals of sbt selection suitable for mechanical harvesting.

The seabuckthorn introduction in the middle zone of the European part of the country was generally successful, but in some years, seabuckthorn plants in new growing regions were subjected to high mortality. The main reason of seabuckthorn mortality is root systems damping-out in soft winters with much snow (Eliseev, 1976a). So, a "successful introduction of seabuckthorn is not possible without breeding improvement directed to development of forms with high winter resistance, especially resistant against root system damping-out" (Eliseev, 1980).

Seabuckthorn was introduced to the Botanical Garden of Moscow State University by T.Trofimov. He attributed sbt to four geographical races: Western-European, Caucasian, Central Asian and Siberian (Dolgacheva and Aksenova 2001). The experience of growing plants from different populations showed that sbt possesses considerable polymorphism in size, shape, colour of fruits and leaves and degree of thorns. In Moscow climate and soil conditions, the plants from Central Europe and Scandinavia showed higher adaptability and productivity than plants from seeds of Altai and Zabaikalie origin. The plants from Tuva with the high frost resistance were of doubtless interest for this selection.

It is important to take under consideration not only natural range, but specific climatic conditions of place of natural growing and place of its replantation. The duration of vegetation and dormant periods, heat and water supplying regime play an essential role as well (Igoshina *et al*. 1987a, Demidova 2003).

The resistance to new conditions of cultivation, first of all winter hardiness, is one of the main problems of sbt introduction to the territories of temperate climate (Vasilchenko 1970, 1977; Kondrashov 1980a). Seabuckthorn of Fenno-Scandinavian populations have appeared to be the most winter hardy for the conditions of Northern Russia (Demidova 1989b, Demidova and Kolesnichenko 2003).

A study of sbt winter hardiness in laboratory conditions was done by Igoshina *et al*. (1987a). They have found that plants of Baltic sbt, especially Kaliningrad sbt, are more winter hardy in the temperate zone. Yagovtseva and Lobanova (1986) used a method of sbt shoot freezing for selection of male resistant plants. Fefelov *et al*. (2005 a,b) studied winter hardiness of different sbt cultivars and some peculiarities of winter hardiness forming in conditions of temperate climate by the use of artificial freezing. Smertin (2006) investigated resistant sbt genotypes of different geographical origin to the winter injury factors and used these genotypes for further selection and creation of winter hardy sbt cultivars.

The sbt resistance depends on morpho-physiological characteristics of the plant, determined by its genotype (Andreyanov, Korovina 2003). Sbt are affected by lack of water during active growth under the introduction to the Central part of Russia, which accompany by soil compaction on the depth of 20-50 cm. Some adapted sbt forms of Altai and Kaliningrad provenances can compensate lack of water by roots from the depth of 2 m.

The capabilities of sbt acclimatization in two ecotypes with contrasting forest-growing conditions in the clay- semi-desert area of the Pricaspian Plain in Russia were evaluated by Sizemskaya and Sapanov (2005). The authors concluded that planting sbt in such conditions seems to be promising in the depression of mezorelief with available fresh ground water. Planting multifunctional shrubs in these areas makes it possible to optimize the land utilization.

Questions, related to the development of the spontaneously introduced sbt populations were studied by Kozhevnikov (2001) in Ural. Sbt naturalization and development of rare populations of introduced plants were investigated for the first time. The classification of introduced populations was also done. Sbt variability under the development of introduced populations and under secondary introduction was studied. Study of selected forms in natural conditions of Ural was suggested as a way of receiving ecologically diverse breeding material. A term “geoecotop” was suggested as the name of geographically remote sbt ecotypes. That is why spontaneously introduced sbt populations could be considered as cultural geoecotops.

The attempts to introduce Siberian sbt in the southern territories of Russian Federation particularly in Krasnodar Region were done by Igoshina and Korovina (1987b). They have observed mass sbt drying because of climate discrepancy between of the region of introduction and its natural conditions of growing. They made a conclusion that during the introduction process it is very important to take into consideration not only sbt natural range of distribution, but also climatic conditions of regions of its natural inhabitancy and place of introduction. Duration of vegetation and dormancy periods, warmth-and-water regimes play an essential role in it.

The change in population of *Hippophae* in a number of regions in Belarus has been also studied. Different qualities of geographical races and numerous populations have been shown. Their stability and productivity under Belarusian conditions have been measured on the basis of studying the whole ontogenetic cycle (Garanovich 1995, 2003). The main reason for introducing sbt to Belarus is the production of oil and food products from fruits. The complexity of work involving the introduction of sbt to Belarus can be presented as follows: search for and study of the material; study of original forms and kinds in Belarus and selection of the most prospective forms; reproduction of selected forms and mastering the growing technology.

Until the present time industrial plantations of sbt in Ukraine did not exist. Altai cultivars were used for their creation, which appeared to be short-living under Ukraine conditions. The decision was made to breed local, highly productive sbt cultivars that are accustomed to Ukraine’s soil and climate conditions (Lebeda 2001).

Sbt introduction to Donbass (Ukraine) with a continental climate and semiarid conditions started in 1980. Twenty four cultivars and hybrids from Lisavenko Institute of Horticulture, Nizhniy Novgorod Agricultural academy and Don Agricultural Research Institute were tested. The lack of precipitation negatively influenced the sbt yield and berry mass. The berry mass was 10-30% lower than in years with sufficient precipitation. The most tested cultivars were subjected to mycosis. The most resistant in these conditions were cultivars of Baltic origin such as ‘Baltica’, ‘Volna’ and ‘Moryachka’ (Mezhinskyi 2003, 2004).

Genetics and breeding

The yield of wild sbt is low, biochemical composition of fruit material is not homogeneous and because of long thorns, harvesting of fruit is difficult. Therefore, the development of new cultivars for plantations is of great importance (Khabarov, Panteleeva 1988; Panteleeva 2003, 2006).

Goncharov (1995) and Zubarev (2005) indicated that seabuckthorn breeding in the Altai Region started in the 1930's using wild forms. The Siberian plant breeders are constantly enriching the sbt gene collection and the diversification of methods to make the breeding process more efficient.

The first seabuckthorn cultivars were created by scientists of the Lisavenko Research Institute of Horticulture for Siberia (Barnaul, Altai) in 1964 through a very successful breading program. For the period of seventy years more than forty varieties of sea-buckthorn have been bred at the Lisavenko Research Institute of Horticulture for Siberia yielding modern sea-buckthorn varieties that ranged from 7.5 to 18.0 t/ha, mass of 100 berries – 62-120 g (maximum 140 g), length of pedicels – 3-6 mm, content of oil – 4.0-8.0%, carotenoids - 15.0-48.0 mg/100 g, sugars – 5-10%, acidity – 1.0-1.9%. Most of these varieties are thornless. (Zubarev, 2005).

Serious seabuckthorn introduction and selection has been done in the Botanical Garden of Moscow State University by_T.T.Trofimov, V.S. Dolgacheva, N.A. Aksenova; Nizhniy Novgorod Agricultural Academy by I.P. Eliseev, V.A. Fefelov and other; Gus-Hrustalnyi Experimental station by S.N. Kovalev; All-Russian Institute of Medical Plants by B.S. Ermakov, F.F. Potapova; Moscow Agricultural Timiryazev Academy; Michurin All-Russian Horticultural Institute_by V.T. Kondrashov; Cytology and Genetics Institute by G.F. Privalov, N.S. Shapov, L. P. Solonenko; Buryat Fruit-Berry Experimental station by E.G. Sokratova, G.M. Zaharova; Novosibirsk Fruit and berry Growing Experimental Station; Northern Research Institute of Forestry by V.N. Nilov and N.A. Demidova.

The breeding program on sbt in Russia has developed into many directions. The subsequent programs include research work on the trend of developing pest and disease resistance; related to productivity features, such as yield, mass of berries, convenient growth habits (for hand picking) with compact, low crowns, thornless, long fruit stalks; biochemical block, especially high oil, carotene and sugar content; related to complexity of technology aspects mainly different ripening periods (ultra early-ripening and extra late-ripening varieties), and the suitability of sbt for machine harvesting (Kalinina and Panteleeva 1995, Khabarov *et al.* 1997).

The new sbt cultivars developed in the Lisavenko Research Institute of Horticulture for Siberia were based on using a selection of best seedlings from hybridization with small usage of chemical mutation. The annual total volume of the selection makes about 100 combinations of crossings; therefore about 50 000 hybrid seeds are being generated. After a careful selection only 50-60 new hybrids are used in the further work. At present time, the hybrid collection of sbt cultivars in the Institute reached 45 000 plants (Zubarev, 2005). The most productive initial materials appeared to be the cultivars such as 'Chuiskaya', 'Panteleevskaya', 'Prevoskhodnaya', 'Velikan' and 'Inya'. Also, a number of large-fruited elite forms have been developed during the last several years. Cultivars 'Chylishmanka' and 'Tenga' along with other positive characteristics, give an easy separation of fruits from their branches (Panteleeva, Zubarev 2000, 2001; Panteleeva, Oderova 2003).

The successes in sbt breeding work in Siberia have been achieved thanks to: clearly formulated goals; the use of promising parent material; the use of effective methods of obtaining the breeding material; and the selection for the main economic-biological properties (Goncharov, 1995). The Siberian plant breeders enrich constantly the sbt gene pool and developed the donor and gene sources collected on winter hardiness, early ripeness, productivity with high content of oil, vitamin C, sugars, carotenoids and pectin. The material from wild-growing flora is being widely used and varieties and forms from other ecological and geographical locations are being constantly introduced.

In working out new breeding programs, Siberian breeders proceeded from the fact that all varieties must be adapted to the conditions of zones of their expected popularization, that must be superior to all earlier varieties, concerning their basic properties or limiting factors (in the north – early ripening, in the south – drought resistance). Naturally, they must be more resistant, productive and compare favourably with standard varieties in product quality (Goncharov, 1995).

The Siberian plant breeders possess a very rich sbt gene pool and always share it with plant breeders of the other areas of the country and abroad. Broadening the methods used makes the breeding process faster and more effective.

There are not too many varieties suitable for the mechanised harvesting; therefore one of the tasks in the breeding program of the Lisavenko Research Institute of Horticulture for Siberia is to find the cultivars that can be suitable to mechanization. These cultivars need to be: easy in dry separation of fruits, they need to have long pedicels, compact crone, moderate growth and strong branches (Zubarev 2005).

At the same time, mass release of mechanised harvesters is not planned in the near future. Therefore, great attention is paid to varieties suitable for hand picking. Some cultivars, such as 'Chuyskaya', 'Inya', 'Avgustina' and some others, provide productivity on hand picking up to 100-150 kg/8 hours. Moreover, they have unique cultivars with productivity on hand picking up to 200-250 kg/8 hours (Zubarev 2005).

Creation of sbt cultivars with late ripening time could permit a prolonged period of harvesting without change to worse fruit quality and conditions of harvesting. The uses of different ecological forms, which have different duration of vegetative period, are recommended for this purpose (Kondrashov 1999). It is assumed that late ripening cultivars will be suitable for harvesting by cutting of fruited branches, as danger of drying of cut stumps is the lowest during late harvesting (Panteleeva and Gunin, 2003). The late ripening cultivars have a long duration of ripening period which can lasts for 30-35 days for some cultivars. The following conformity was found: the later ripening sbt form, then the period of ripening is longer. It could be explained by lower air temperature and sunlight during the period of developing fruit colour. The most attention in the selection is paid to cultivars 'Shcherbinka-1' and 'Velikan' - the latest ripening forms that were created which differ by the fruit size as well.

Last achievements of the Lisavenko Research Institute of Horticulture for Siberia allow prolonging the period of harvesting to 2.5-3 months (2nd half of July up to the middle of October). They also work on varieties, which can be harvested in winter. The main characteristics of these sbt varieties are stable biochemical compounds of fruit until the time of stable cold temperatures (Zubarev 2005). The further aim of sbt selection is the creation of new cultivars which have complex industrial-valuable features including industrial cultivars with increased amount of carotenoids and dessert cultivars (Zubarev 2000a).

Introducing low sbt trees and shrub-trees to agricultural production will help to increase the harvest and to decrease the work load. But in nature, there are only two types of low forms which inherit the characteristics we need. They are found from the valley of the river Irkut and Kita with an average height of 41 cm and 74 cm, respectively. From their free pollination seedlings were selected series of good types with best harvest and productivity with 90-150 cm height (Ermakov 1995). The possibilities of the selection were enlarged and good results were obtained in the second and third generation by sowing seeds of low forms of sbt.

One of the most rational ways of creating new improved forms with better characteristics and a wide range of ecological adaptation is hybridization of geographically distant forms. This idea was taken as a principle in the work with sbt in the Botanical Garden of Moscow State University (Dolgacheva and Aksenova, 1988, 2001 Dolgacheva *et al*. 1993a,b, Dolgacheva 2005). On the basis of studies on the geographically remote cultivars and forms of sbt, out of the 100 elite samples, 45 of the most promising were selected. They were used in breeding work. Sbt varieties of Altai selection are subject to bacteria and fungus diseases under Moscow conditions and often fall out. Cultivars and forms of the Botanical Garden are notable for high resistance for fungus diseases.

The same method was taken for creation of high winter hardy sbt cultivars in the Russian North (Demidova 1989a, 1989c, 2000; Demidova and Fomina 1998). As it was pointed out, Finnish Sea buckthorn is the most winter hardiness in Arkhangelsk conditions. Finnish seabuckthorn was widely used for the hybridisation. There were selected 12 the most valuable seedlings with high winter hardiness and good berry quality, which could be presented as our new candidates for northern seabuckthorn cultivars.

Having used the methods of the distant ecological-geographic crossings and induced mutagenesis, there were high-yielding technological cultivars with high ecological plasticity created in Nizhniy Novgorod State Agricultural Academy (Fefelov 2005b). The sbt introduction and breeding started there in 1949 by I. Eliseev. The long-term program on the creation of highly productive, technological with high ecological sustainability sbt cultivars for conditions of middle part of Russia started in 1980. Breeding of dessert and industrial cultivars was separated as special direction of the research. Genetically diverse material was obtained to solve further selection problems. The cultivars differ in terms of maturing: from 'Duimovochka' (ultra early in the middle Russia fruit ripen at the end of July) to 'Zarevo' and 'Ryabinka' (late-maturing in the second half of September). Pollen use from male radio-mutant plants was done for the first time in the breeding practice (Fefelov, 2005a). Twenty three cultivars were created by Eliseev and Fefelov for the conditions of the middle part of Russia.

Natural sbt populations are of great interest as a source of valuable initial material for selection. Polymorphism of natural sbt populations was studied by Besschetnov and Kentbaev (2003b). A study was conducted on the territories of Kazahstanian Altai, Dzungarskyi Alatau, Northern Tjan-Shan, Kyrgyzstanian mountain-range where seabuckthorn is growing naturally. They have found high phenotypic diversity in natural sbt populations. Diversity was shown in fruit color, connected with its chemical composition. Clone sbt groups were found with red, orange,

yellow fruits with different shades. Fruit color is stable and is kept during the whole life of the plant, including clone transference on sites with other ecological conditions. Long term experimentation permitted making the conclusion that this is inherited and there is polymorphism in natural sbt populations on fruit color. They have found the domination of orange color in sbt fruits. Fruit shape does not change during the whole plant life and stay the same under cultivation in different ecological conditions. One of the important characteristics of sbt populations, which have adaptation importance, is the leaf blade. It varies substantially: its maximum length (9.9 cm) is 4.5 times longer them minimum (2.2 cm), and the leaf width has the similar ratio. The most stable is the leaf length, variance coefficient of which correspond to middle and low levels (10.8-22.3%) (Besschetnov and Kentbaev, 2003b). The number of thorns on branches varies sufficiently. Variance coefficient (42.2 - 110.0%) is high. The authors concluded that there is a possibility to improve sbt by lowering the number of thorns on sbt brunches.

Male sbt plants on industrial plantations (6-8%) with good pollen productivity provide good fruit yield. Tall plants with a pyramid crown, without thorns, resistant to pests and diseases, winter- and drought hardy and of different flowering time are important characteristics for male sbt plants suitable for industrial plantations. A study of biological specifics of male sbt plants was conducted in orchards and in natural conditions in Buryatiya. The following ecotypes were selected: (i). Tunkiskyi ecotype; (ii). Selenginskyi ecotype; (iii) Hybrid forms (Trifonova 2003). Selected sbt ecotypes could be used for further breeding work for development of new intensive cultivars. Also, research on sbt buds that were frost resistant was conducted. Investigation has shown that buds and tissue of one-year shoots on sbt plants older than 10 years are damaged by frost more than on plants in the age range of 5-8 years old.

Seabuckthorn always produces fruit notwithstanding the climatic conditions during flowering, therefore it is considered insured in horticulture. One of the reasons of that could be its high pollen productivity and high pollen quality or fertility. Questions of pollen fertility have a great practical and theoretical interest. The study of morphological sbt pollen features has been conducted over several years by Nizhniy Novgorod Agricultural Academy (Kuznetsova *et al*., 2003). The effect of climatic conditions on pollen formation, as well the differences between male forms on their potential to develop pollen of high quality was investigated and determined.

Sbt has been grown in severe conditions of the Southern Urals for nearly 30 years. Seven local cultivars were created including one male cultivar ‘Ural’ (Illína and Ilín 2003). The fruits of those cultivars could be recommended for wide use as raw material for further processing. Investigations on pollen fertility were done. ‘Ural’ showed the highest pollen germination ability (46.1%) compared with other male cultivars (like ‘Aley’ and selected forms). ‘Ural’ gave the highest percentage in hybridization (up to 68%) ability.

Research on sbt genetics is conducted by the Institute of Cytology and Genetics in Novosibirsk (Privalov and Solonenko, 2001). The conclusion from that research is that the individual relative variability (IRV) coefficient method may be used in examining genetic structure and mechanism of correlative and cooperative interactions between characters in the process of sbt ontogenesis. The IRV coefficient may be used in studying genetic control of biological variety of characters for various crossing combinations and in plants’ populations.

Sbt is a dioecious species. Nevertheless, some male plants can also develop a few fruits containing viable seeds. “Male” fruits are borne mainly on the base of one-year shoots. The main stages of the fruit development are illustrated by Skuridin and Lobova (2001). The scheme of development and sex polarity of shoots is also explain in that publication.

Skuridin and Baginskaya (2003) reviewed studies of the sbt phenotype correlation and divided it into three groups: morphological correlation, biochemical correlation and combined correlation. Skuridin (2007) published new methods of the sbt selection with the support of genetic correlation signs, which allows for faster creation of new sbt cultivars. He has distinguished conformity in genetical and ecological conjugated sbt quantitative signs.

Polyploidy and chemical mutagenesis can positively influence sbt introduction. Borodina (1976, 1982, 1986) has proved that it is possible to receive high adaptability to new conditions by growing sbt plants by the use of polyploidy. The first studies on experimental mutagenesis in sbt have started at the Institute of Cytology and Genetics in 1959. The research program included: (i) the analysis of the sensitivity of sbt seeds to mutagens; (ii) the development of methods for the identification of mutations in the first mutagen generation (M_1); (iii) the analysis of the frequencies and patterns of induced mutations in the M_1; (iv) screening for the possibilities of using induced mutations in the breeding work. Experimental mutagenesis can greatly facilitate breeding of new varieties thanks to the induction of valuable characters that rarely emerge in natural conditions (Privalov, *et al*. 2003). Privalov and Shchapov (1980) have found that experimentally received sbt triploids can develop dicotyledonous flowers, which

can be used in further selection. Autotetraploids (2n=48) were received by Shchapov and Kreimer (1988a, b). These forms have a higher fruit growth and new sbt cultivars have been selected (Shchapov and Kreimer 1998. The experimental data indicate that the sensitivity of sbt to chemical mutagens is genotype-dependent (Eliseev *et al*. 1982, 1988, Privalov *et al*.2003).

Studies on the effective use of pulse electron and X-ray radiation of nano- and pico-second duration as a variability inductor in the selection of fruit-and –berry cultures was conducted by Russian Federal Nuclear Centre and experts in the sbt selection from Nizhniy Novgorod State Agricultural Academy (Pavlovskaya *et al*. 2003). They investigated irradiated sbt pollen quality, pollen fertilizing capability and its effect on the development of ovaries, berries and seeds.

The seabuckthorn selection studies are conducted in Azerbaijan in the Genetics and Selection Research Institute (Baku) and Institute of Genetic Resources of Azerbaijan Academy of Sciences: 'Zarya-Dabat', 'Zafarani', 'Shafa' and 'Tozlayan' which are suitable for the local conditions and could be suitable for Turkey, Bulgaria, Spain, Portugal and southern part of China. (Imamaliev 1983, 1991).

A unique natural sbt population growing in Ukraine (located in the delta of the Danube) was studied by Lebeda (2001). Some promising forms were selected for breeding purposes. The selected forms are characterised by certain features. The expedition studies to the Danube delta resulted in the selection and description of male forms with economically valuable features (Lebeda *et al*.2005). The selected male sbt forms differ from each other with crown shape, size and colour of a leaf blade, the length of sprout annual increment, and the number of leaves per 10 cm of sprout, period of flowering, and pollen sizes and vitality. These forms have been of interest for use in future selections.

A seabuckthorn breeding program is conducted at the Don Branch of the Horticultural Institute Ukraine Academy of Sciences as well. The cultivar 'Solodka zhinka was created and characterized as a dessert fruit (Mezhenskyi 1999). This fruit contains 15.8-21.9 % of dry matter, 6.4-6.8 % of sugar, 1.5-1.8 % of organic acids, 31.7-35.2 mg/100 g of ascorbic acid, 128.3 mg/100 g of carotenoids. This cultivar is recommended as suitable for the industrial purposes (Mezhenskyi, 2004).

Central Botanical Garden of Belarusian Academy of Sciences provides a sbt breeding work. The breeding pool of cultivars and hybrids has been established (Garanovich, 1995). The existing well known Russian cultivars are used in crossings with different climatypes (Danube, Caucasus, Altai, Siberia, and other). A number of hybrids with large fruits (0.6-0.7 g), with high content of ascorbic acid, carotenoids (18 mg/100g), and low acidity have been obtained. Chemical mutagens can provide a wide diversity of variability.

Biochemistry

Seabuckthorn berries are among the most nutritious and vitamin-rich fruits found in the plant kingdom. Biochemical composition depends both on species and growing conditions. The content of vitamin C in berries varies from 100 to 250 mg/100 g for *Altai* ssp. *Mongolica*. The carotene content ranges from 10 to 50 mg/100 g; content of oil varies from 4.0 to 8.0%; sugar – 5-10%; acidity – 1.0-1.9%. The vitamin E concentration can be up to 160 mg/100g of berries (Eliseev 1989). Seabuckthorn is also rich in flavonoids (vitamin P) and contains appreciable amounts of water soluble and fat soluble vitamins (Solonenko and Shishkina 1989, Shapiro 1989).

Directions of sbt oil use are widely known and are shared by two branches: medicine and cosmetics. Different purposes need various oil compounds. Altai sbt varieties accumulate a high quantity of carotenoids, therefore the oil finds its basic application in medicine and is unsuitable for cosmetics purposes (Zubarev 2005). Sbt is characterised by high variability in oil content in fruit pulp. The variation range for oil content in individual Altai plants is from 2 to 8 in seeds (4 to 15 %) (Obodovskaya 1957, Shishkina *et al*., 1985). In conditions of the West Pamir, fruit pulp oil content was found to be as high as 14.9% (Glazunova *et al*. 1983).

The accumulation of a great deal of oil in the sbt fruit pulp is the main distinctive feature of this plant. It is well known that the reserve oils of most higher plants are localized in seeds. The biological importance of these oils is in their usage while seeds germinate. At the same time there are some plants (including seabuckthorn) which have two fundamentally different types of oil reserves – in seeds and in the pericarp (Berezhnaya 1990). It was shown that pollinators can influence structure and content of oil in sbt fruits. That is why their selection can be successfully used to increase yield and oil quality (Berezhnaya 1990). It was brought to light that sbt habitat conditions strongly

influence fat-acidity structure of hypanthium but practically does not influence the fat-acidity structure of seeds (Berezhnaya 2003).
Sozonova (1991, 1992) explained the physiological causes of oil residing in succulent portions of seed tissues of fruits, particularly of sbt. Special attention was paid to the appropriateness of formation and accumulation of fat inclusions and the fat content. Also, a concept was formed about the role of component parts of sbt fruit in its own development and plant vital activity as a whole.

Mironov *et al*. (1989, 1991) carried out research on the quantity of oil contained in the fruit coat (epidermis and sub-epidermis zone), pulp and seeds. Oil content in fruit is determined on a percent basis of raw mass or dried mass of whole fruits. Sozonova (1988) recommends using diethyl ether for oil determination. She has described various methods of oil determination and its corresponding calculations. Solonenko and Skuridin (1983) used a fast method of oil extraction from raw fruit pulp using chloroform for mass analysis in primary screening of selected material. The authors have recommended regressive equation based on comparative data's of new method of oil determination with traditional method.

The literature provides sufficient evidence that oil content in raw sbt fruits of different ecological-geographical forms and inside populations fluctuates within very wide limits. The highest index of oil content (12.5 – 18.0 %) was ascertained for sbt several forms in West Pamir and Pamir – Altai natural populations. Oil content for these forms accounts for 26 – 34 % in calculation to dry mass (Glazunova *et al*., 1991; Korovina *et al*., 1993; Eliseev, 1976b). But there are also reports on higher oil content for sbt forms from West Pamir populations ranging from 28 to 43 % dry mass (Avdeev, Kreknina, 1987). There are sbt forms with oil content of 10 – 12% and dry mass content of 21 – 37% in the same populations and also in Zakavkazye, in river valleys of Choroh (Adjaria), Bzyb' (Abkhazia), at the basin of river Sevan (Armenia) (Fefelov 1987, Korovina *et al*.,1993). Presence of high-oil content in sbt forms from Armenia confirms research findings of Kondrashov (1980b) regarding oil content of fruits of individual sbt forms 9.0 – 9.7% with dry mass content of 25 – 32%. High oil content sbt were found in Priissykkulye – 7.3 – 9.2% with three sbt forms having as high oil content as 48, 54, and 69% (Malena 1982; Maisuradze, Malena,1988; Malena *et al*.,1984). Shishkina (1967) characterized oil content on a dry basis for the following sbt forms from following populations: Katunskaya in the range of 20-37 %, Chulyshmanskaya, Sayanskaya and Daurskaya in the range of 16-22%.

Research on a sbt Katunskaya natural population carried out by specialists of VILR is shown in work of Malinkovskyi *et al*., (1971a, b) and Potapov *et al*. (1984). The first specimens were selected from sbt of Katunskaya population in NIISS (Barnaul). Fruit oil content was characterized by Kalinina and Panteleeva (1978); Shishkina *et al*. (1985); Panteleeva (1993). Oil content of Altai specimens in Ural is 32–75% lower than during their growth in Altai (Kruchkov *et al*. 1998). Sbt fruits grown in conditions of Chernozemye (Michurinsk) are marked out with its higher oil content (Kondrashov, 1985). Vorobeva (1985) characterized the oil content of the new specimens in conditions of the Novosibirsk region. Data on high oil content in new specimens grown at the botanic garden in Science Academy of Byelorussia, Minsk were given by Garanovich (1991). Oil content in fruits of sbt created at botanic garden of Moscow State University was characterized in work of Trofimov and Dolgacheva (1986); Aksenova, Dolgacheva (1991). Specimens created at Michurin VNII were characterized with rather high oil content (Kondrashov, 1996). Specimens of relatively high oil content were created at Buryatiya Fruit- Berry Station using an analytic selection method such as Atsula – 7.6%, Stepnaya – 6.5%, Sayana – 6.3% (Zakharova, 1986; Zakharova and Baironova, 1993).

A study of plant variabily induced by mutagens was provided in the Institute of Cytology and Genetics (Privalov *et al*. 2003). The experimental results indicated that, under the effect of ionising irradiation, the individual and group variability of plants for fruit oil content widens. After seeds treatment with gamma-rays variability widened from 1.1 to 9.3% (Privalov and Solonenko, 1977).

There are lots of data in the literature indicating that carotenoids content is highest in the red fruit and lowest in the yellow ones. The lowest content of carotenoids is in the milky-white fruit of the Caucasian sbt population (Eliseev 1976b; Korzinnikov *et al*. 1983; Muraviova and Lagazidze, 1985).The data obtained by Privalov *et al*. (2003) show that maximum carotenoids content in red-fruit of sbt half-sibs is twofold in comparison to their yellow counterparts, being 28.3-30.3 mg/100g and 14.0-14.4 mg/100g, respectively. Orange fruits show intermediate values of the carotenoids content between red and yellow ranging from 19.1 to 20.0 mg/100g). Buglova and Shishkina (1978) have studied the correlation between fruit mass and content of carotin and ascorbic acid during 3 years.

They have found negative correlation between mass and carotin (r=–0.71) only in one year. There was no correlation on other 2 years. Different data's were received on correlation between fruit mass – ascorbic acid on the results of 3 years study: (r=–0.50; +0.17; and no correlation). Panteleeva (1993) has studied correlation between fruit mass and carotenoids content during 9 years. She has found out that bigger the fruits then less content of carotenoids in them. Methodology of carotenoids study is described in the book written by Kudritzkaya (1990). She presented carotenoids composition in sbt fruits of different cultivars of Lisavenko Institute (Kudritzkaya et.al. 1991).

A wide variation within individual specimens in ascorbic acid content is a characteristic feature of sbt fruit. It varies from 8.0 to 1400 mg/100g and it has been related to various environmental factors, from the climate to the location of fruit within the tree crown (Daems 1963). Dolgacheva and Aksenova (1993b) have studied influence of weather on the content of vitamin C and acids in sbt fruits. They found out that average summer temperature and sun radiation are the main factors influencing on vitamin C and acid content in sbt fruits. These factors decrease content of vitamin C but don't influence on acid content.

Eliseev *et al.* (1985) made a conclusion that biochemical indexes of sbt fruits are determined first of all by genetical peculiarities of cultivars and in considerably depend on ecological factors. They have found out that content of ascorbic acid correlate with big fruit mass and water content, but content of oil and carotin correlate with content of dry substances and small fruit size.

Byelorussian scientists studied influence of weather conditions of vegetation seasons on oil, phenols and other biological active substances content in sbt fruits during 4 years (Shapiro *et al.* 1986). Optimal weather conditions cause increased accumulation not only oil in sbt fruits but also sugar and organic acids.

Individual variability range for sugar content in wild sbt fruit during the period of their biological ripeness varies from one distribution area to another, being from 0.6-1.2% in Azerbaijan (Imamaliev 1983) to 9.5% in the Altai territories (Kalinina, Panteleeva 1978) and up to 12.4% in the Irkutsk Region (Eliseev, Mishulina 1970). There appears to be a certain relation between the climatic conditions of sbt growth and fruit sugar content.

Sbt fruits are rich in tocopherols (vitamin E) and surpass in its content almost all fruit-bearing plants (Shapiro 1980), but generally, the tocopherols content in sbt fruits varies from 14 up to 50 mg/100g (Bekker, Glushenkova, 2001). Different authors determined tocopherols content (mg/100g) in wild sbt fruits of some regions; Altai – 8 (Obodovskaya, 1957) and 20 (Shugam, 1969); Maly Caucuses (Azerbaijan) – 2.5 – 15. mg/100g (Mamedov, 1984).

It has been determined that sbt seeds contain 30% protein. The content of lysine (the essential amino acid limiting the nutritive value of most proteins of plant origin) is 4%, on the average, which is quite high (Solonenko 2001) Protein content in sbt pulp varies from 0.8 to 1.6%, depending on type of sbt cultivar. Sbt juice contains much non-protein nitrogen (26.5-62.2% of total nitrogen content), depending on the type of cultivar. Most of the non-protein nitrogen is free amino acids of importance in the formation of the organoleptic (taste) proteins of sbt fruit and its processed products. The content of well ingested proteins albumin and globulin containing lysine vary from 3.8 to 6.3%, depending on of sbt cultivar and also of free amino acids, which are high. The entire sbt fruit should be used because it is a source of highly nutritious materials. Electrophoretic patterns of the storage proteins in sbt seeds were analysed for the first time. The patterns were very heterogeneous. Identification of sbt cultivar and breeding of valuable genotypes can be based on electrophoretic protein polymorphism. Sbt seeds contain 30% protein on the average. Seed storage proteins can be used in genetic diversity studies to identify cultivars for selection of valuable genotypes, to clarify phylogenetic relationships, to choose starting sbt forms and control the quality of breeding material.

Literature on flavonoids composition in sbt shows that it has significant variability, depending on the geographical zones of growing and ecological conditions of the habitat. Therefore, in Altai sbt there were found 6 flavonoids substances (Minaeva *et al.*, 1969), 3-5 in northern Caucasian sbt, 5-7 in Buryatiya (Bolotova *et al.* 1983; Potapova *et al.* 1983).

Flavonoids in sbt are presented by catechins, leucoantotsians, flavones, in less degree, by flavones; Antotsians are practically absent. Chlorogen and its isomers, coffee, quinic and gallic phenol-acids were found out in the sbt fruits (Shapiro 1980).

Fruits of selected sbt forms of Buryatiya sbt contain 147-296 mg/100g of poly-phenols (Sokratova *et al.*, 1991). Gachchiladze (1984) measured flavonoids content in sbt fruits of Western Pamir as being in the range from 118 to 854 mg/100g. The common content of poly-phenols in the sbt fruits of the NIISS (Barnaul) selection was

107-269 mg/100g (Shishkina *et al*. 1985); in the fruits of Buryatian selection it was 117-400 mg/100g (Markova 1998). Arbakov *et al*. (1998) showed that Altai cultivars accumulate more poly-phenol substances in Buryatiya conditions then in Altai. The qualitative composition and quantitative content of flavonoids substances in the leaves and fruits of 15 sbt forms growing in the different soil-climatic conditions of Azerbaijan were studied by Novruzov (2001). It was found that the content of flavonoids in the leaves and fruits of these forms varied. It ranged from 110 g/100g in fruits to 1391 mg/100g in the leaves.

Ukrainian researchers (Komissarenko *et al*. 1987) have studied the composition of a phenol fraction of dried sbt fruit pulp. They have found such compounds as catechins, leucoantotsians, coffee acid, flavonols aglucones – quercetin, cempferol, and izoramnetin. Coumarins were found in the fruits of Altai sbt cultivars and in Sayan sbt seedlings (Tribunskaya *et al*. 1970). Their content was determined to be between 1.0-3.6 mg/100g (Vigorov 1976). The content of coumarins in the NIIS cultivars of second generation was between 0.9 and 2.1 mg/100g (Kruchkov *et al*., 1985).

According to the Moscow State University Botanical Garden study of different cultivars the content of vitamin C varies from 21 to 223 mg/100g, oil 2.5-9.6 %, sugar – 2.4-8.1%, acid -1.3-4.3% (Dolgacheva and Aksenova 2001). The main factor, influencing vitamin C and organic acid content in fruits is climatic conditions during vegetative period in the spring-autumn period.

New technical sbt cultivars were created in Nizhniy Novgorod Agricultural. Academy with following content in fruits: vitamin C - more then 100 mg/100g (cv. 'Maria'); carotenoids - more then 20 mg/100g (cv. 'Nizhegorodsky souvenir', 'Nadezhda', 'Zarevo', 'Ryabinka'); oil - more then 4% (cv. 'Nizhegorodsky souvenir', 'Nadezhda', 'Dar Kazakovu') (Fefelov 2005a).

The comparative study of fat-soluble biologically active substances of sbt fruit cake extraction was conducted in Azerbaijan (Novruzov *et al*. 2005a). They have carried out two types of extraction: CO_2 and NR-3. Results of research of chemical composition of extracts from sbt cake indicate that both extracts are appreciably enriched with carotenoids, sterins, tocopherols and others. The content of vitamin C in the extract was 6 times higher than in the standard sbt oil and the carotenoids content was 10 times higher. In composition of both extracts there were substantial amount of wax (up to 25%), which is a natural softener and emulator.

The fatty acid fruit composition of sbt was studied in Ukraine (Dzhurenko and Lebeda 2005). The level of palmitoleic acid in the relative percentage makes up 10.7-12.8% depending on the sbt form and 29.1-37.5% depending on the cultivar. The opposite tendency was noticed in the accumulation of oleic acid: 28.8-37.5% in forms and only 5.5-7.6% in cultivars.

The lignification of annual shoots of sbt was studied by Besschetnov and Kentbaev (2001) in the conditions of Kazakhstan. There are spare substances in sbt shoots such as, starch, sugar and fats which determine high drought and frost resistance of plants. Most of starch is accumulated after 80% of shoot lignification, sugar – at a period from 55 to 85%, fats – from 55 to 100%. Accumulation rates of the substances are sharply slowed down after 100% of shoot lignification.

Smirnova (1998) asserts that taste grades of frozen fruits are lower than of the fresh product. A decrease in quality is a result of cell coat damage caused by ice crystals. The highest freeze stability is established for such cultivars as 'Panteleevskaya', 'Chulyshmanka', 'Zhyvko'. Juice-holding capacity of these cultivars in frozen conditions is more than 98%. Taste change of thawed fruits is towards a more intense sour flavour. It is caused by a sugar content decrease after freezing and storage of 10% and a general acidity increase to 0.1 – 0.7% causing the impartial taste index (sugar–sour index) to decrease. There was no considerable colour change of frozen fruits. Klimina (1988) determined the sugar loss was 5 to 10% over the sbt freeze storage for a year, and at the same time the acidity increased by 0.1 – 0.7% depending on the specimen. In addition, she noted a decrease of ascorbic acid content (mainly at the freezing moment) from 13 up to 54%. Pectin substances amount remain on the level of 73 – 87%.

Researches of Institute Cytology and Genetics and Novosibirsk fruit-berry station working on fruit freezing (Karpova. 1998) also showed cultivar differences regarding the fruit's ability to keep its consistence after thawing.

Cultivars 'Krasnyj Fakel' and 'Ognistaya' appeared to be the best; fruits of cultivars 'Sibirskij Rumyanetc' and 'Ivushka' were slightly deformed and extracted a temperate amount of juice. Great deformation and extraction of large amounts of juice were observed on such cultivars as 'Podruga' and 'Zolotoj Kaskad'. Carotenoid level decreased on 10 – 50% during 3 months storage in a freeze chamber. According to data by Klimina (1988) carotenoid content of frozen fruits of such cultivars as 'Dar Katuni' and 'Chujskaya' did not changed considerably during a year of storage.

Seabuckthorn utilization

Mankind is searching for the panacea from all diseases. Seabuckthorn was used in different medical and prophylactic herb collections in popular medicine from ancient times. Sbt has attracted attention as a prospective technological raw material in Russia since the 40s in the last century. The first sbt oil was produced in Biisk Vitamin Enterprise in 1953 (Koshelev, Ageeva, 2004).

Sbt oil is the main pharmacological product in the Russian Federation. It is used in medicinal preparations for skin therapy (burns, frostbites, eczema, ulcer, and lupus, gastroenterology (stomach and duodenal ulcer) and others. Sbt oil is also used as a vitamin remedy with hypo- and avitaminosis, as a tonic against weakness and mental exhaustion. "Altaivitaminy" processes 3000 tons of sbt berries annually (Koshelev, Ageeva, 2005). Comprehensive utilization of sbt is described in paper written by Koshelev *et al*. (1995) where they introduced 3 types of sbt raw-material and described their industrial manufacturing technology and products.

Sbt has been widely used in Russian traditional medicine (fruit juice, seed oil, plant extract etc.) (Eìdel'nant 1998). Different parts of the plant are quite rich in various biologically active compounds like vitamins, carotenoids, flavonoids, tannins etc.

Pharmacological and clinical research of preparations on the base of sbt were done and presented in the Matafonov monograph (1983). He paid big attention on sbt oil application in medical purposes, its curative and prophylactic action on different diseases.

A problem of the treatment of viral diseases belongs to a most urgent task of modern medicine. The creation of potent, selective and harmless antiviral drugs is one of the most complex tasks of chemotherapy of infections, because RNA and DNA-contacting viruses belong to obligatory intracellular parasites. Among the viral infections influenza occupies a leading place because of a number of people involved in the epidemic. An importance of Herpes viral infection (HVI) as the problem for public health services grows worldwide. Sbt leaf fractions were studied in respect of antimicrobial and antiviral activity (Shipulina *et al*. 1995a,b; Shipulina 2001). "Hiporamin" is a purified tannin fraction from sbt, possessing a wide spectrum of antiviral activity against influenza viruses A and B, herpes simple type 1, adenoviruses type 2, HIP-1, and a mild antimicrobial activity in respect of gram+ and gram- micro-organisms (Shipulina *et al*. 1996, 1997, 1999). "Hiporamin" also exhibited interferon induction activity.

The establishment of the specific chemotherapeutic action of "Hiporamin" on various experimental models of virus infection has been done (Tolkachev, Shipulina 2003). A systematic study has shown that the principle antiviral agents from sbt leaves are hydrolysable tannins. The latter were found in the plant extracts as the complexes with micro and macro elements (Sasov *et al*., 1996). A new spectrophotometric method of tannin determination in sbt leaves and plant extracts was developed by Sheichenko *et al*. (2001a).

A chemical composition of sbt leaves was studied by Sheichenko *et al*. (2001b). They found that the maximum amount of tannins in the leaves and drug "Hiporamin" developed from tannins is observed in the middle of July, while increased amounts of quebrachitol (2-O-methyl-inositol) are accumulated in the autumn period of vegetation (September-October).

Sbt oil is used in cosmetology as it has antioxidant and prooxidant qualities (Tankov 1993). Protective creams for industrial enterprises were created on its basis.

Food value of sbt extract of different degree of concentration was studied by the "Altaivitaminy" Corporation together with the Institute of Cytology and Genetics (Solonenko *et al*., 1993). It was reported that the extract is rich in ascorbic acid (from 450 to 650 mg/100g), carotenoids (8-38 mg/100g) and it displays a sufficiently high acidity. The extract is also rich in mineral composition that includes all macro-and microelements required by the human body.

Bodnar *et al*. (1980) developed a technology for sbt syrup preparation based on the juice concentrate mixed with sugar syrup in a 1:1 ratio and with addition of 10% ethanol as a preservative. Tereschuk and Pavlova (2000) suggested using filtrated and pasteurized sbt juice when preparing tonic drink with milk whey and sugar. Worthwhile enrichment of milk, cream, kefir by sbt juice is shown in research work of Isagulyan *et al.* (1999). These blends are used for medical-prophylactics purposes.

Information about the sbt juice concentration by freezing is presented in the paper of Bagautdinov and Tsapalova (1998) where they showed that the use of a ready product as an additive with light coloured honey could give it golden colour and a unique taste.

Seglina (2007) work in Latvia is devoted to seabuckthorn fruit processing and product development. She has evaluated different seabuckthorn fruits and their processing product quality and investigated the physical and chemical indices of fresh and frozen sbt fruits of widespread cultivars and hybrids in Latvia; assessed the storage possibilities of fresh sbt fruits; assessed the quality of sbt fruit juice; analyzed the physical and chemical indices of the products obtained from sbt fruits and their changes during the processing process and storage time. All these investigations were done for the first time in Latvia.

Research on different sbt specimens and hybrids and their potential usage in the food preserve's industry was carried out by Filimonova (2001a) and Zubarev (1998, 2000b). Filimonova has detected that such cultivars as 'Velikan', 'Zolotistaya Sibiri', 'Obilnaya', 'Prevoshodnaya'and 'Oranjevaya' differ by high content of carbo-hydrates, mainly by glucose and fructose. She has worked out technological demands to sbt fruits for individual preserves groups including stewed fruits, jams, juice with pulp, puree–like products, and also for drying and freezing.

One of the most valuable processed products from sbt berries is freshly pressed juice. The main problem is to prolong its storage time. Studies of shelf-life storage of juices made from different sbt cultivars and enhanced with sweeteners were conducted in Latvia (Seglina *et al*., 2005). As a result of these studies, the most suitable juice for making drinks was found to be juice from cultivar 'Prozrachnaya' by adding fructose as a sweetener. The optimal storage time of freshly pressed juice from sbt berries was 7 days at $+4\pm2^0$C temperature.

Food value of sbt extracts of different degree of concentration was studied by "Altaivitaminy" Corp. together with the Institute of Cytology and Genetics (Solonenko *et al*., 1993). It was reported that the extract is rich in ascorbic acid (from 450 to 650 mg%), carotenoids (8-38mg%) and display a sufficiently high acidity. The extract is also rich in a mineral composition that includes all macro-and microelements required by the human body.

Taste of sbt fruits is very specific. That is why it is limited in use as a dessert product in Russia. The Lisavenko Research Institute of Horticulture for Siberia suggested using biochemical parameters, namely sugar-acid index (SAI), as an estimated level in definition of taste of sbt fruits (Zubarev 2005). The SAI in climatic conditions of Altai territory varies in the high range, both on years and varieties. It was suggested to evaluate the berry taste by groups: Sour – SAI less then 4; Sweet-sour – SAI 4-6; Sour-sweet – SAI 6-8; Sweet – SAI more then 8.
Tests were conducted with the sbt compote quality using 10 new Nizhniy Novgorod cultivars (Ashaeva *et al*. 2003). The best fruits for compote production are large size fruits with a bright yellow colour.

Studies of the sbt fruit suitability for processing juices, syrup and jam started in the Ural State Agricultural Academy in 2002. EHF-technology was used for juice separation and the pulp was made into puree and jam (Evtushenko and Kirsanov 2003). A number of cultivars were selected as suitable for processing and cultivation in Urals.

Information about sbt juice concentration by freezing was presented in the paper of Bagautdinov and Tsapalova (1998) where they showed that the use of a ready product as an additive with a bright honey colour could give it a golden colour and a unique taste.

Sbt pomace could be used for food purposes. Timofeeva (1996) showed how to use it for drinks preparation. She worked out process conditions and recipe of its cooking.

Loskutova (1988) studied a possibility of the sbt juice use in baking bread. The bread was enriched with vitamins P, B_1, B_2, PP, and C by adding sbt juice. Patent (RF 2095986) was received on bread "Tibet" preparation with the use of sbt juice as a biological active additive. The method of bread baking with the addition of sbt extract was worked out and described in the paper by Kichaeva and Sharfunova (1996). The study of the possibility of sbt extract use for toast production was provided.

A technology of fruit wine preparation based on sbt is shown in the paper of Men'shov and Shishkina (1998). They noted that high juice acidity gives spiciness to wine and the use of traditional methods of acidity lowering could cause destruction of ascorbic acid.

Filimonova (2001b) has worked out "The technological demands to sbt fruits" for tinned production including stewed fruits, jams, juice with pulp, puree and also "requirements" for sbt fruits drying and freezing.

Nearly 150 recipes of sbt fruit usage in home cookery as well its application for prophylactic and medical purposes are presented in Eidel'nant's (1998, 2006) books. The ways of a preparation of raw, pasteurized, dry storage, wines, liqueurs, spirits, cold collations and salads, soups, second dishes, sauces, jams, desserts, bakery, cocktails, ice-cream are described in Shishkina book (2000) where she presented 400 recipes of sbt fruits processing.

The review on the sbt research presented in this paper does not claim the full overview of sbt investigations done by Russian and NIS scientists. It is just a small part of substantial amount of research done by them during last 30 years.

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Development of a Branch Shaker to Harvest Seabuckthorn

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Abstract

The objective of this project was to develop a branch shaker to harvest the fruit of the seabuckthorn. Based on our experimental results conducted between 2003 and 2004, we determined that to harvest fruit from the 'Indian Summer' cultivar efficiently by shaking the branches, we had to use a frequency of 40 Hz, amplitude of 15 mm and a vibration time of 10 seconds. Using these parameters, 90% of the fruit was harvested with little damage to the branch and little debris (leaves, buds, wood) being detached from the branches. The prototype used in the 2003 and 2004 tests had several shortcomings. The crankshaft principle used to transform the motor's rotating motion to a back and forth movement was practical for testing various amplitudes but put a great deal of stress on the components. Using an electric motor (1.5 kW) made it possible to conduct trials in the workshop and in the field without using an agricultural tractor, but a generator was needed. The positioning of the prototype was slow and difficult. The experimental prototype developed in 2005 was driven by a hydraulic motor that used a cam to produce the vibration. Although it was in use only a short time, the shaker functioned very well. The quality of the harvested fruit was good if the harvesting was done during the optimal period. Damage to the trees was minimal. Using a branch shaker is an option when harvesting 500 or fewer trees per season.

Keywords: seabuckthorn, berry, harvesting, shrub, shaker, *Hippophae rhamnoides* L.

Introduction

Seabuckthorn (*Hippophae rhamnoides* L.) is a multi-branched thorny shrub that reaches 2-4 m in height at maturity and produces orange berries in August in Quebec, Canada that remains on the shrub all winter. Juice and oils of seabuckthorn berries are used to produce high nutritional and medicinal value products. Unfortunately, it is difficult to harvest the seabuckthorn berries because they are soft at the time of picking and are tightly clustered on two- or three-year old branches (Li and Schroeder 1996). Manual harvesting causes a high percentage of damage as the fruit loses a large amount of biologically valuable substances (Blahovec *et al*. 1995). Manual harvesting is also very slow making mechanical harvesting a key factor for this crop to become profitable in Canada. The dominating principle of mechanically harvesting bush fruits is by shaking. But sea buckthorn generally requires higher frequency and/or amplitude than most other berries (Olander 1995). Large bushes and trees are generally harvested by shaking the trunk or the main branches. Although a trunk shaker would allow the entire bush to be harvested at one time, trunk shakers are typically effective only for bushes that have one central trunk with short branches. Bushes with long, slender branches are more difficult to harvest by shaking the trunk because much of the energy is absorbed by the trunk and branches before it reaches the berries (Olander 1995).

Khazaei *et al*. (2002) designed and tested a hydraulically-controlled shrub shaker for removing berries. Harvesting trials were conducted during the 2000 and 2001 harvesting seasons using the 'Indian Summer' variety of seabuckthorn. From their 2000 data, amplitude of 25 mm removed a higher percentage of berries and a lower quantity of debris. In 2001, vibration parameters of 25 mm amplitude, and 25 Hz frequency were found to work best for optimum berry harvest, minimum debris removed and caused the least branch, bark, and leaf bud damage.

Tom Li, Research Scientist from Agriculture and Agri-Food Canada at Summerland, collaborated with Mearl's Machine Works from Kelowna, BC to develop a branch shaker from a modified Milwaukee reciprocating saw. According to Li (pers. com.) frequency was about 2800-3200 cycles per minute (46.6 to 53.3 Hz) and amplitude was about 1 or 1.5" (25 to 38 mm). No formal testing was done but machine was able to remove berries from the branches. However, it soon became evident the saw induced too much fatigue and could cause injuries to the operator holding it.

2003 Prototype and Experimental Procedure

This project started in 2003; to prevent operator fatigue, we wanted our shaker to be heavy and hung form a cable so no operator would need to hold it. Research in 2003 was geared at building and testing our first prototype and finding optimal parameters of frequency, amplitude and duration of vibration to harvest the fruit without causing damage to the branches. A branch shaker prototype was built, it was powered by a variable speed 0.75 kW electric motor, powering a crank-shaft assembly which enabled us to change easily the amplitude for experimental needs (Fig. 1 and 2).

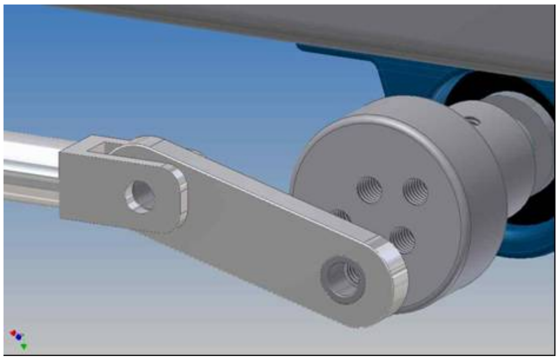

Figure 1. Components of 2003 prototype.

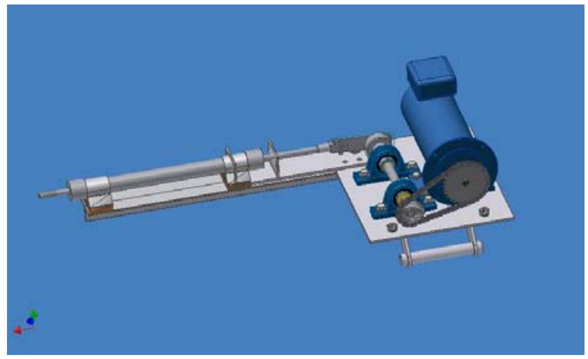

Figure 2. Crank-shaft for amplitude setting.

Two different clamping devices (claws) were tested to hold the branch, a closed (Fig. 3) and an open one (Fig. 4). The branch shaker was suspended from a winch cable attached to an arm mounted on the back of a John Deere Gator on which was also mounted a 3.8 kW, 110V generator.

Figure 3. Closed claw tested in 2003.

Figure 4. Open claw tested in 2003.

Experiments were performed on 'Indian Summer' cultivar at two different orchards: André Nicole at Ste-Anne-de-Beaupré near Québec city and Centre de développement bioalimentaire du Québec (CDBQ) at La Pocatière, Québec between September 29 and October 17 which was late in the season. Shrubs were tested at five frequencies (10, 20, 30, 40 and 50 Hz), five amplitudes (15, 20, 25, 30 and 35 mm) and three shaking durations (5, 15 and 30 s) and every combination was repeated three times. A plastic bag was placed over the branch and attached near the point where the claw was attached to the branch (Fig. 5). The shaker was operated for 5 seconds than the bag was opened and its content was emptied into a plastic sample bag. Then the bag was attached again around the same branch and the shaker was operated for another 10 seconds before its content was emptied into a second sample bag. The bag was attached again around the same branch and the branch shaken for another 15 seconds and content emptied in a third sample bag. Finally, the bag was re-attached and the branch was cut and kept into its bag and placed in a freezer until remaining berries on the branch could be counted and damages to the branch assessed.

Figure 5. 2003 prototype being tested.

Figure 6. Material harvested after 5, 15 and 30 s at 30 Hz and 15 mm.

2003 Results and discussion

The open claw was easy to position but moved a lot during shaking and caused severe injuries to the branches; we quickly realised it could not be used in further experiments. The closed claw was slow to position but caused less injuries; this is the one we used for further experiments. For frequencies of 10 and 20 Hz, 30 seconds were needed to harvest more than 90% of the berries on a branch. At 30 Hz, 15 seconds were enough and for 40 and 50 Hz, 5 seconds were enough. Figure 6 shows typical results after shaking a branch at 30 Hz where most berries were harvested during the first 5 s, some were harvested by shaking for another 10 s, and the last 15 s of shaking harvested only a few berries but many leaves and debris. Shaking longer than needed to harvest the berries increased the amount of leaves and wood debris detached from the branch and also increased the damages done to the branch. Since tests were performed too late in the season, and our electric motor was missing power to perform all the treatments planned, we decided to repeat the experiment in 2004.

2004 Prototype and Experimental Procedure

A new claw was developed and tested in 2004 (Fig. 7). It could be opened and closed by an air powered cylinder with air pressure set at 80 psi (5.5 bars). Other components of the shaker were almost the same except that a 1.5 kW electric motor was used (Fig. 8); twice as powerful as the one used in 2003. Total weight for the shaker was 71 kg; it was suspended from a cable attached to a 12V winch which was used to position the shaker at the right height. The holding arm was mounted at the back of a John Deere Gator. A 3.8 kW 110V electrical generator was mounted on the front and a small electric air compressor was carried in the box to provide compressed air to the air cylinder closing the new claw.

Figure 7. 2004 air operated claw.

Figure 8. Testing 2004 prototype.

Experiments were performed on ‘Indian Summer’ cultivar at two different orchards: from August 9 to 16 tests were done at Pierre Gagnon’s orchard at Dunham, Québec and from August 31 to September 3 at François Dugal’s orchard at Armagh, Québec. Experimental design was to test three frequencies (20, 30 and 40 Hz), three amplitudes

(15, 25, and 35 mm) and three durations (5, 10 and 15 sec.); every combination was repeated five times. Other branches were shaken for 15 s using the same parameters of frequency and amplitudes but without using a plastic bag nor recovering the berries. These branches were identified and left on the tree to enable damage evaluation immediately after shaking and the following year.

2004 Results and Discussion

Figure 9 shows results of harvesting efficacy at both orchards. The standard deviation between the five replications for each test tended to be high for low average harvesting rates but was very low when harvesting rates reached 90%. We consider harvesting 90% of the berries on a branch to be a good harvesting rate. This rate was achieved on Gagnon site when using frequency of 30 Hz and amplitudes of 25 and 35 mm and when using frequency of 40 Hz and amplitudes of 15 and 25 mm. Durations of 10 and 15 s gave best harvesting results. At Dugal site, harvesting 90% and more of the berries was achieved when using frequency of 40 Hz and amplitudes of 15 and 25 mm with durations of 10 and 15 s. Differences in results between the two sites might be due, in part, to an average temperature of 25°C when performing tests at Gagnon site and of only 14°C when performing tests at Dugal site, but they could also be due to other reasons. The new air operated claw performed very well. Damages caused by the claw and to the whole branch were higher at Gagnon site (Fig. 10). There was almost no damage at 20 Hz but increased damages were noted with higher frequencies and longer shaking time. Quantity of leaves and wood debris harvested also increased when using high frequencies and long amplitudes (Fig. 11). After two harvesting seasons with ‘Indian Summer’ cultivar, the best combination for a seabuckthorn branch shaker is a frequency of 40 Hz and amplitude of 15 mm. Shaking duration should be 10 seconds. This combination enabled to harvest over 90% of the berries on the branches while causing low damages to the branches and the trees and detaching few leaves and wood debris. A second choice combination but with a slightly lower harvesting efficacy would be using a 30 Hz frequency, a 25 mm amplitude and a 15 s duration.

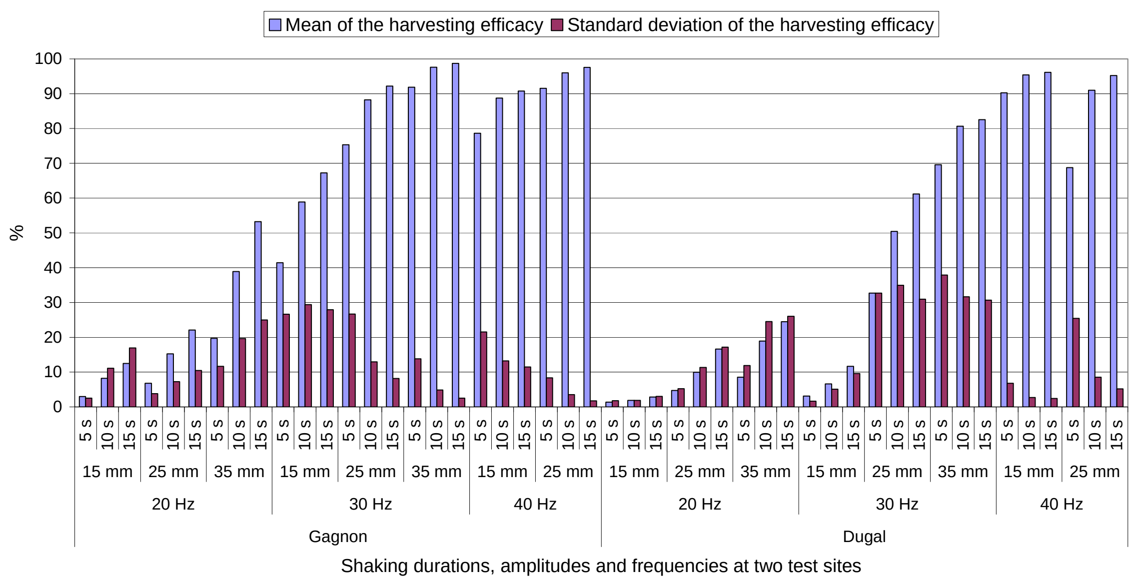

Figure 9. Harvesting efficacy at two sites in 2004.

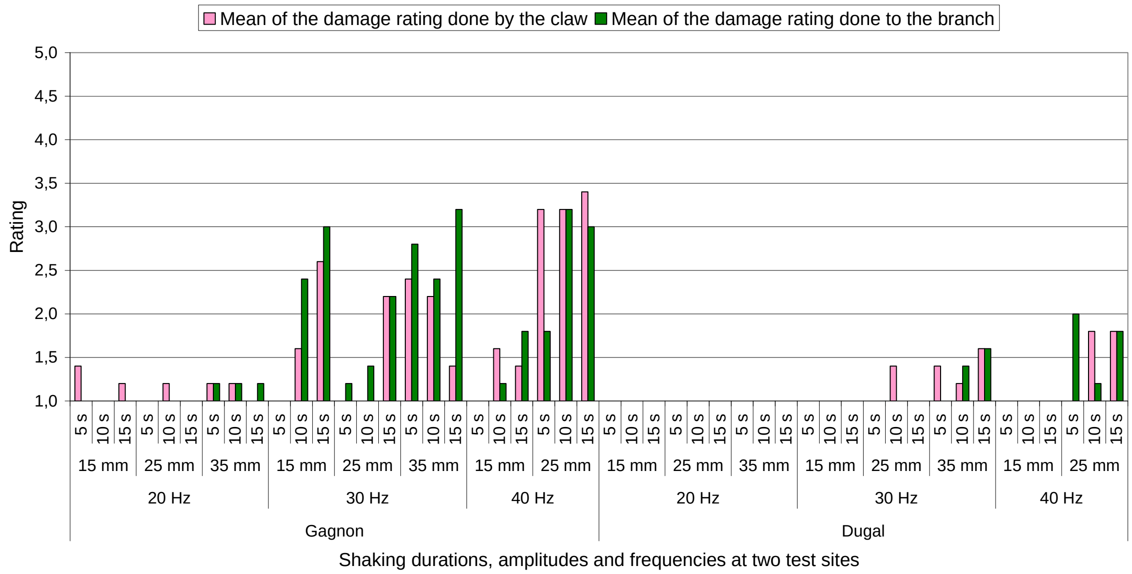

Figure 10. Damages to branches in 2004: claw level and whole branch (1 = none, 5 = high).

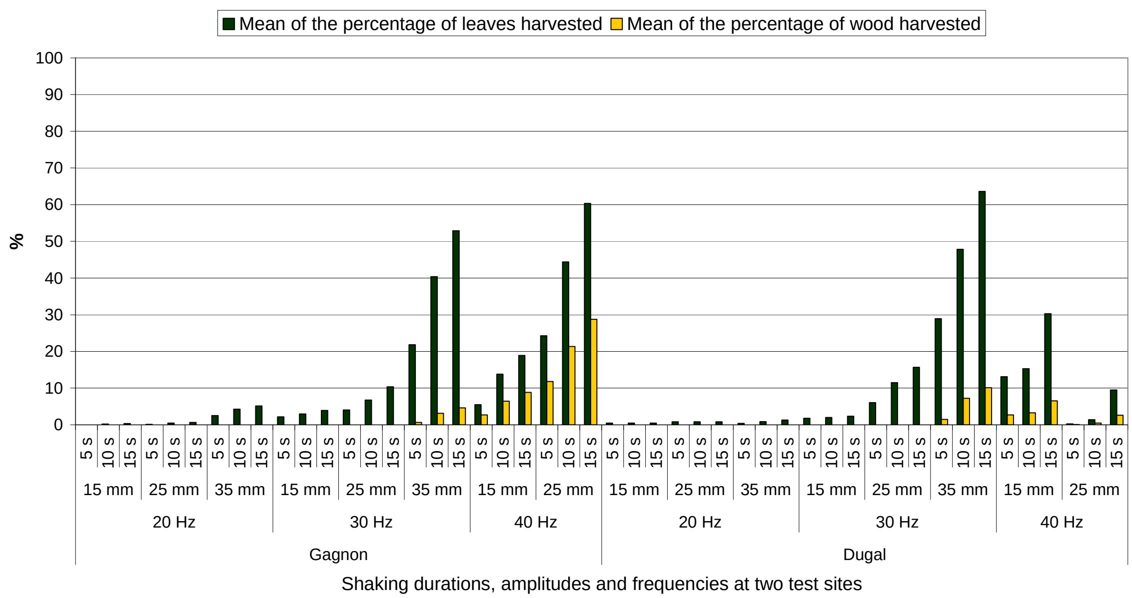

Figure 11. Amount of debris harvested in 2004 (leaves and wood).

2005 Prototype

A new prototype was developed in 2005 to be as close as possible to a commercial unit (Fig. 12). It was powered by a hydraulic motor using the hydraulic power from a farm tractor. This is safer than using an electric motor and cables which could present a risk when used in field conditions. The main difference with our previous prototypes was the use of a cam instead of a crank-shaft to produce the back and forth shaking action. The amplitude was fixed at 15 mm and the cam was balanced by providing counterweight on the same shaft. As the shaft rotated, the cam pushed the arm outwards and a spring brought the arm back (Fig. 13).A ball bearing was mounted at the end of the arm; the spring must be strong enough to keep the bearing rolling on the cam at all times. A simpler and lighter air operated claw was developed (Fig. 14). Total shaker weight was 68 kg. A three point hitch attachment was developed to operate the prototype attached to a farm tractor (Fig. 15).

Figure 12. Prototype developed in 2005.

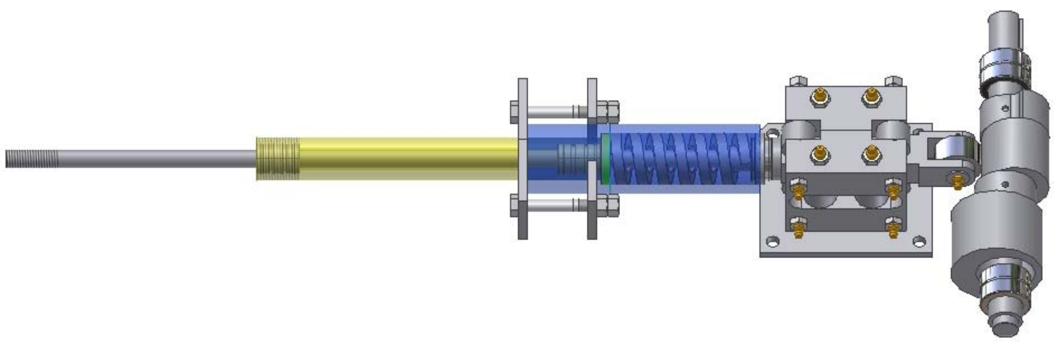

Figure 13. Internal components of 2005 prototype.

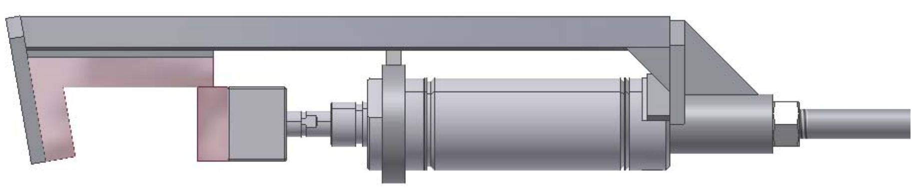

Figure 14. New lighter air operated claw developed in 2005.

2005 Results and Discussion

Preliminary tests performed in a workshop (Fig. 16) showed the system was operating fine but the prototype was not tested in the field. Two growers agreed to preliminary testing in their orchard. The first one was Mr Pierre Gagnon who tried it from August 6 to 18 at his orchard in Dunham. Mr Gagnon used a Mitsubishi MT250D 18.5 kW tractor. Here are some comments from Mr Gagnon: Hydraulic motor overheated after every hour of operation, he had to let it cool for 30 minutes. Between 15 to 20 minutes were needed to harvest one tree. Harvest rate was 8.3 kg of berries per hour. Some trees were harvested at 100% other at 10%, with an average around 40 to 50%; it varied a lot from one tree to another. Harvesting efficacy was better during the morning hours because temperature was lower. Damage varied a lot from one tree to another, vertical branches tended to break more. Quality of berry harvested was good.

Figure 15. Three point hitch attachment. **Figure 16. Tests in workshop in 2005.**

Other tests were performed by Mr André Nicole at Ste-Anne de Beaupré near Québec city from August 20 to September 9, 2005. The branch shaker was installed on a Massey-Ferguson 50 tractor (24 kW). The shaker support system was suspended under the front end loader (Fig. 17). Because his tractor was too wide to get between the rows, he tested the shaker on the surrounding trees. He built a collection basket to collect the berries and harvested about 2.5 kg of berries from each tree. According to Mr Nicole, two people harvested 14 kg of berries per hour with uneven trees and a catching basket difficult to move. He figures 20 kg/h harvest rate would be achievable with uniform trees and better catching system. About 20 to 25% of the branches were injured by the shaker. About 15 minutes were required to harvest one tree and about 50-60% of the berries in the tree were harvested. Two men were able to harvest about 30 to 40 trees per day. Fruit quality was not as good as when cutting and freezing branches. When harvesting with the shaker in lower temperature, quality of harvested berry was better. The branch shaker did not damage the trees severely and part of the damages was due to errors from operators. Mr Nicole does not think he will use this method a lot since it is slower than cutting, transporting, freezing and bashing branches.

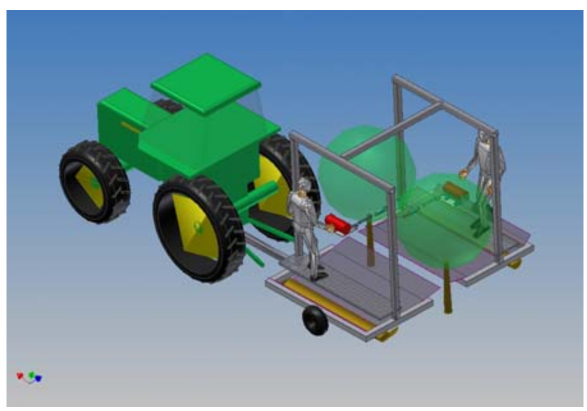

Figure 17. Using prototype with counterweights. **Figure 18. Proposed concept.**

Proposed concept

Our scenario for future was to develop a frame to hold multiple branch shakers each side of the trees and to use a farm tractor to pull the frame and power shakers (Fig. 18). The frame would have mesh or cloth wall on each side and a sloped floor would gather berries falling from trees. A larger farm would use longer frame with more shakers.

Conclusions

Between 2003 and 2005, three prototypes were developed. Harvesting performances and damages to the branches were measured for the first two prototypes. Based on our results, we determined that to harvest fruit from the 'Indian Summer' cultivar efficiently by shaking the branches, we had to use a frequency of 40 Hz, amplitude of 15 mm and a vibration time of 10 s. Using these parameters, 90% of the fruit is harvested with little damage to the branch and little debris (leaves, buds, wood) being detached from the branches. The prototype used in the 2003 and 2004 tests had several shortcomings. The crankshaft principle used to transform the motor's rotating motion to a back and forth

movement was practical for testing various amplitudes but put a great deal of stress on the components. The positioning of the prototype was slow and difficult. The 2005 prototype was designed to be easy and safe to use by farmers. It was driven by a hydraulic motor that used a cam to produce the vibration. It was tried by two growers but not formally tested. Using a branch shaker could be an option when harvesting 500 or fewer trees per season. This prototype is not commercially available. More research and development is needed for this branch shaker before it can be fully used by farmers.

Acknowledgments

We acknowledge great collaboration from the growers and organizations who contributed to this project by accepting and helping our team to perform tests in their seabuckthorn orchards: Mr André Nicole, Pierre Gagnon, François Dugal and Léo Boutin, Centre de développement bioalimentaire du Québec (CDBQ) and Institut de recherche et de développement en agroenvironnement (IRDA) at Deschambault. Thanks to Mr Romain Rioux from CDBQ who helped in assessing damages to trees. This project was funded by Agriculture and Agri-Food Canada.

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Seabuckthorn (*Hippophae rhamnoides* L.) cultivar establishment and survival in a maritime environment

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Abstract

Seabuckthorn trees survived in an ornamental planting at Memorial University of Newfoundland in St. John's for over 30 years, so it was hypothesized that commercial seabuckthorn berry production could be established under the cool, wet coastal conditions of the boreal ecozone in eastern Newfoundland, Canada. The island's climate is strongly influenced by the cold Labrador Current along the northern coastline. July-August temperatures average 15 °C while winter temperatures average (-4 °C). The frost free growing season averages about 130 days. Annual precipitation averages over 1500 mm with about 75 % as rain.

In 2001, ten "thornless" cultivars imported from Russia by a Canadian company were established in a 0.2 hectare field trial on newly cleared land at 47047'N 53012'W 17m elevation above sea level. The pH 4.5 podzolic soil had a shallow hard pan which created a perched water table, so the hard pan was broken up, limed, and tile drainage installed. 'Aley' (male) and the female cultivars 'Rodnitchok', 'Chuiyskaya', 'Karima', 'Vitaminnaya', 'Obilnaya', 'Samarodok', 'Zolotistaya', 'HR-1414', and 'HR-66' were planted at 1850 trees per hectare with 1.5 m within row spacing and 3.6 m between rows in a 1:11 male:female ratio.

There were 16 trees (11 of 325 females, 5 of 33 males) which did not survive their first winter in 2002 due to frost heaving damage followed by diseased roots. All other trees were healthy and had considerable growth over the second growing season. Winter survival was 98% in 2003-2006. Tree vigour was high in all years for the female cultivars, but 'Aley' did not have vigorous growth for the first few years. Tree height increased by about 40 cm per year and tree width increased by about 30 cm per year. Once established, all the seabuckthorn cultivars thrived in the cool, wet climate. In 2007, fruit yields averaged 9.4 tonnes per hectare indicating that the seabuckthorn crop was a commercial success.

Keywords: seabuckthorn, Russian cultivars, Atlantic coastal climate, boreal ecozone.

Introduction

Seabuckthorn (*Hippophae rhamnoides* L.) is a native shrub from Eurasia. Research in western Canada has established production methods for fruit production (Harrison and Beveridge, 2002; Li and Oliver, 1998; Li and Schroeder, 1996, 2000). Manufacture and composition research has shown that the fruit can be made into a nutritious juice, pulp oil may be processed separately, "seabuckthorn yellow" pigment may be extracted from the pulp, or seed oil may be utilized as an ingredient in cosmetics, phytopharmaceuticals, or UV skin protectant preparations (Beveridge, *et al*., 1999).

An ornamental planting of seabuckthorn has been growing in an exposed location near the main administrative building of Memorial University of Newfoundland in St. John's, Canada, for approximately 30 years. The survival of these trees in a high precipitation environment provided support for the plan to establish commercial orchards of seabuckthorn in Newfoundland and Labrador (Hilary Rodrigues, CEO, Natural Newfoundland Nutraceuticals Inc., pers. com. 2001). Cultivars imported from Russia were evaluated in hopes of exploiting this new market.

Materials and Methods

Climate

The climate of the Avalon Peninsula on the east coast of the island of Newfoundland is on or close to major storm tracks throughout the year. Climate is strongly influenced by the Labrador Current, the Gulf Stream and the North Atlantic Ocean which provides cool summers and mild winters. The growing season is about 190 days long at the Salmon Cove field site. Mid-summer air temperatures on the Avalon Peninsula average 15 ^{0}C while winter air temperatures average (-4 ^{0}C). The frost fee growing season averages about 130 days. Annual precipitation averages over 1500 mm with about 75% as rain (Heringa, 1981).

The Salmon Cove site was located approximately 40 km west of St. John's and has a similar overall climate. A climate station was installed at the Salmon Cove site. Climate data collected over the course of the growing seasons for 2003, 2004, 2005, and 2006 was presented graphically in Figure 1. The length of the growing season was described in growing degree days (GDD) above 5 ^{0}C (average of daily maximum and daily minimum air temperatures) where the total GDD from April 1st to October 31st was 1436, 1211 and 1312, for 2003, 2004 and 2005, respectively.

Site Geography and Land Use

The field site was prepared from 1998 to 2000 with the clearing of vegetation and installation of a tile drainage system. This was completed by a labour force supplied by the Salmon Cove Future Development Association Inc. Due to a hardpan causing an interrupted perched water table, additional drainage was added in 2001 in the form of drainage tile placed 60 cm under the seabuckthorn rows using a backhoe.

In fall 2001, seabuckthorn cultivars were planted to determine their performance under coastal Newfoundland conditions. The experiment was divided into 18 rows with different cultivars planted in each row. The entire plot size allocated for the experiment was 36.0 by 54.0 m. There was 3.6 m spacing between rows and 1.5 m spacing within the row (1850 trees per hectare).

In the spring of 2003 the shrubs were pruned for the first time. This and subsequent pruning in late winter each year encouraged proper growth and shape in the trees and maximized light penetration and thus fruit yield. During the experiment composted manure mixed with peat was top dressed each year.

Results and Discussion

In the spring of 2002 it was observed there were 16 trees (11 females, 5 males) which did not survive their first winter in the experimental station. Frost heaving damage to the roots followed by opportunistic root disease appeared to be the cause of loss. All other trees were healthy and had considerable growth over the second growing season. Over the following four years approximately two percent of the trees died with no particular pattern across the cultivars being investigated.

Visual Ratings: 2003-2006

A visual assessment of plant vigour, plant uniformity, and leaf shape and colour was performed each spring. Visual ratings were based on a 1-5 scale where 1 was a negative assessment and 5 indicated a very healthy plant. Plant vigour was compared across all seasons in Figure 2. It was found that vigour was high in all years for all cultivars. Results of plant uniformity and leaf shape and colour ratings were less predictable, as there were only a few minor differences between cultivars or years (Figures 3 and 4).

Growth Data: 2003-2006

In March and April of each season all female seabuckthorn plants were pruned. Height and width measurements were taken at the beginning and end of each growing season to keep track of the growth progress. There was a negative effect of pruning on plant height in the first season including pruning on the male cultivar 'Aley', where the final height in 2003 was lower than that of 2002 (data not shown). However, there was a significant increase in height from 2003 to 2005 and again in 2006 with the exception of the cultivar 'Vitaminnaya' (Figure 5). For 'Vitaminnaya' there was only a slight increase in height. Similarly, tree width had increased significantly from 2003 to 2005 (Figure 6). But in 2006, the results were varied. Some cultivars such as 'Aley', 'HR-1414' and 'Zolotisky'

had experienced significant increases while other cultivars such as 'Chuiyskaya' and 'Samarodok' had "decreased" in width. This "decrease" may be due to vigorous pruning in the spring. Combining all growth observations indicated healthy progress and development of the plants. In 2007, fruit yields averaged 9.4 tons per hectare indicating that the crop was a commercial success.

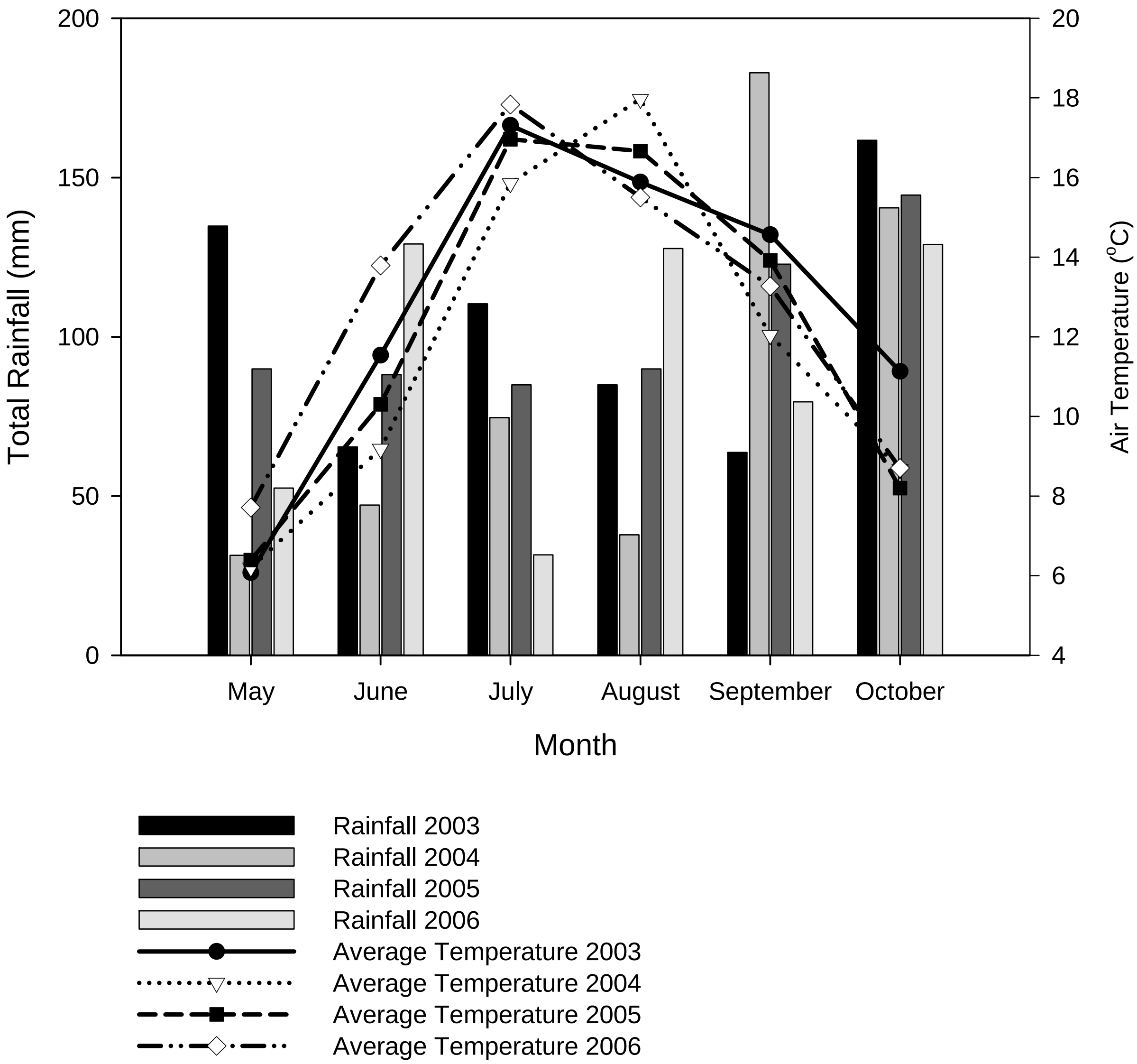

Figure 1. Climate data from the climate station installed in the Salmon Cove Case Site. The total monthly rainfall and average air temperature data is given for the 2003, 2004, 2005 and 2006 growing season. Note that in May 2004 several data points were taken from the St. John's climate station when Salmon Cove data was absent. Also, in July 2006, 15 days of rainfall data are missing.

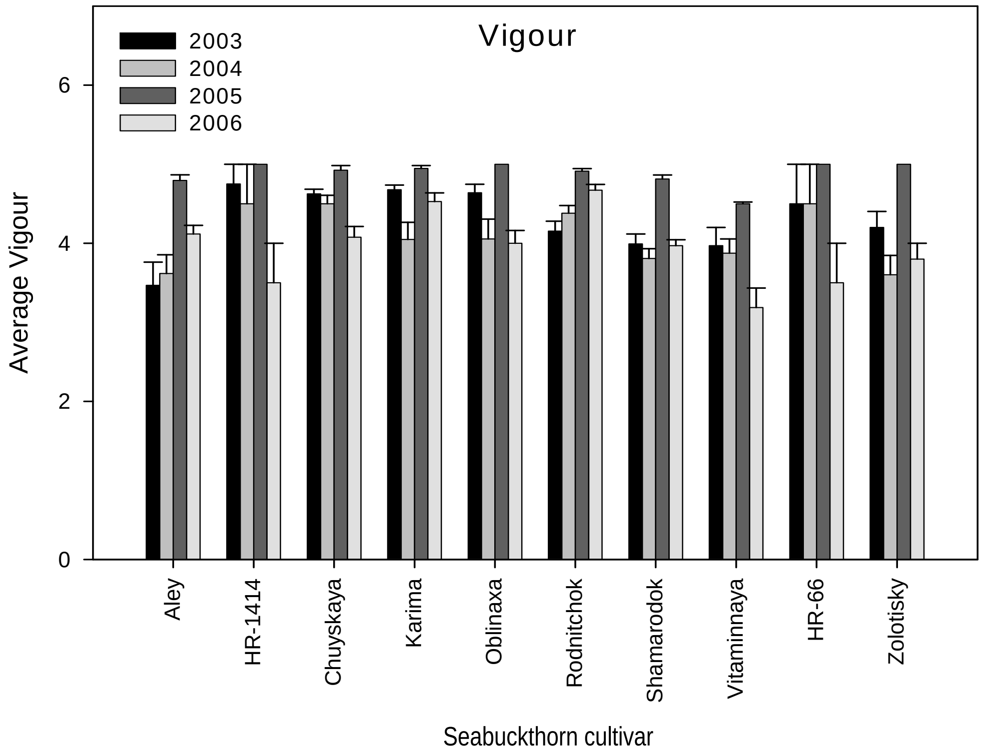

Figure 2. Average visual rating of seabuckthorn vigour from 2003 to 2006.

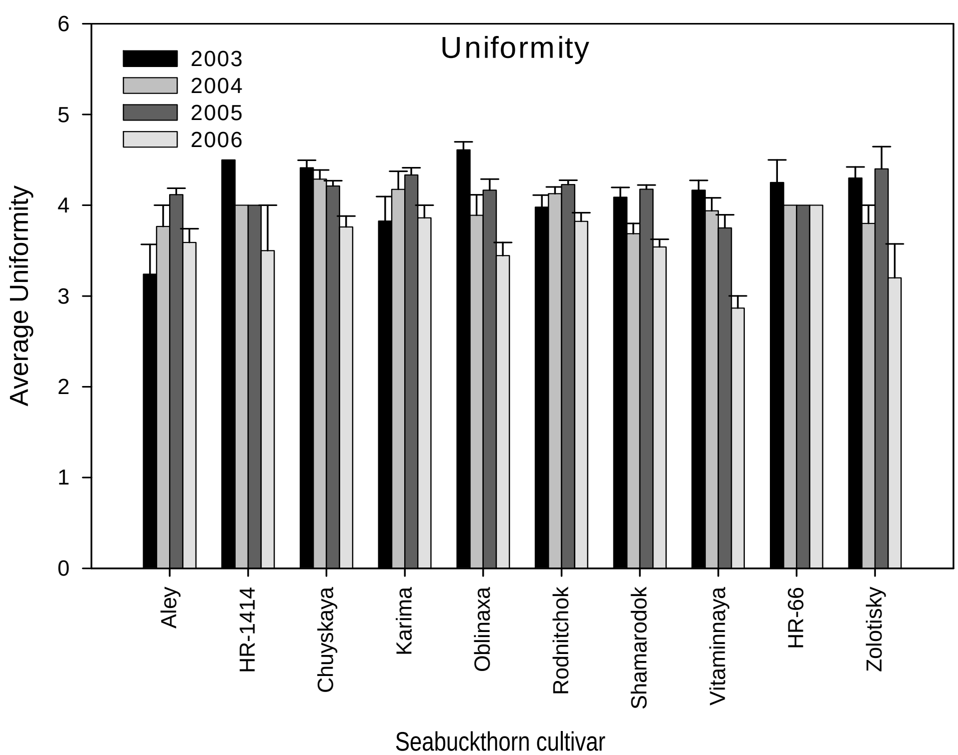

Figure 3. Average visual rating of seabuckthorn cultivar uniformity from 2003 to 2006.

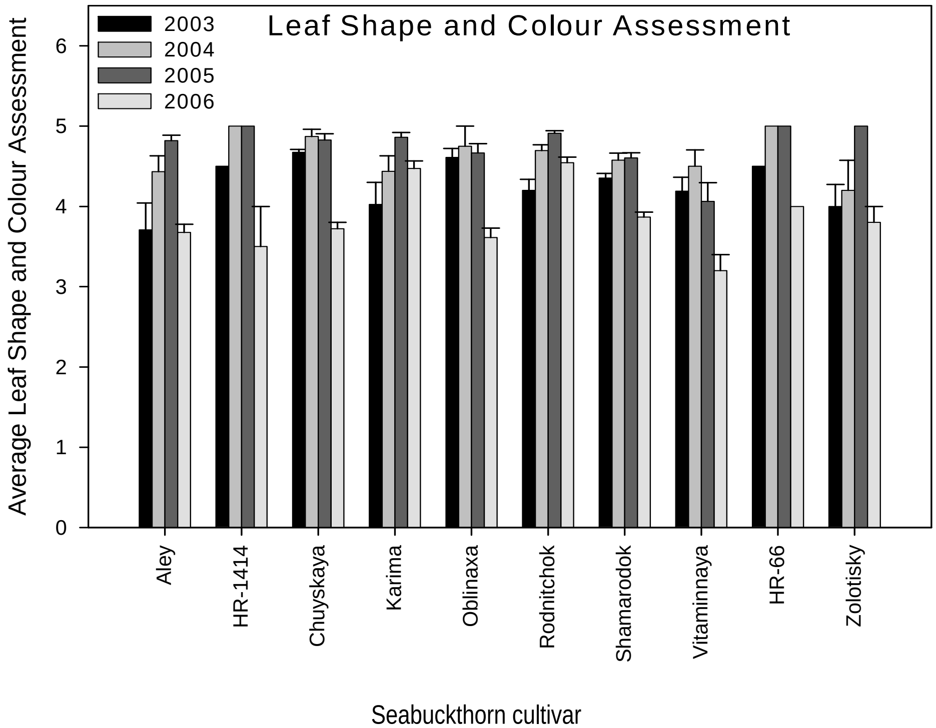

Figure 4. Average seabuckthorn leaf shape and colour assessment. A comparison across four years (2003 to 2006).

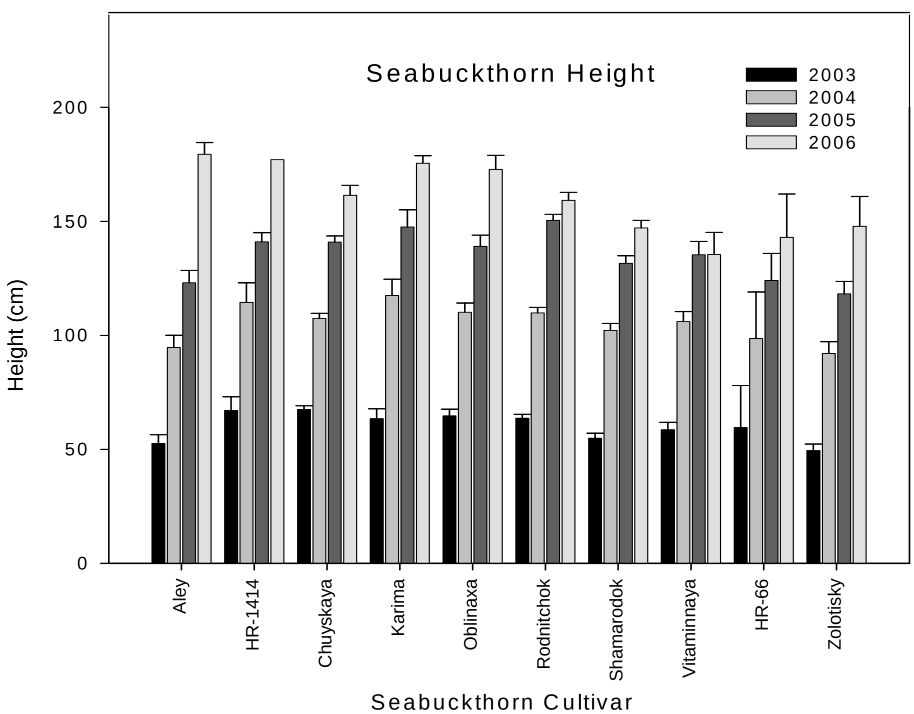

Figure 5. Mean height of seabuckthorn cultivars at the end of each growing season (2003 to 2006).

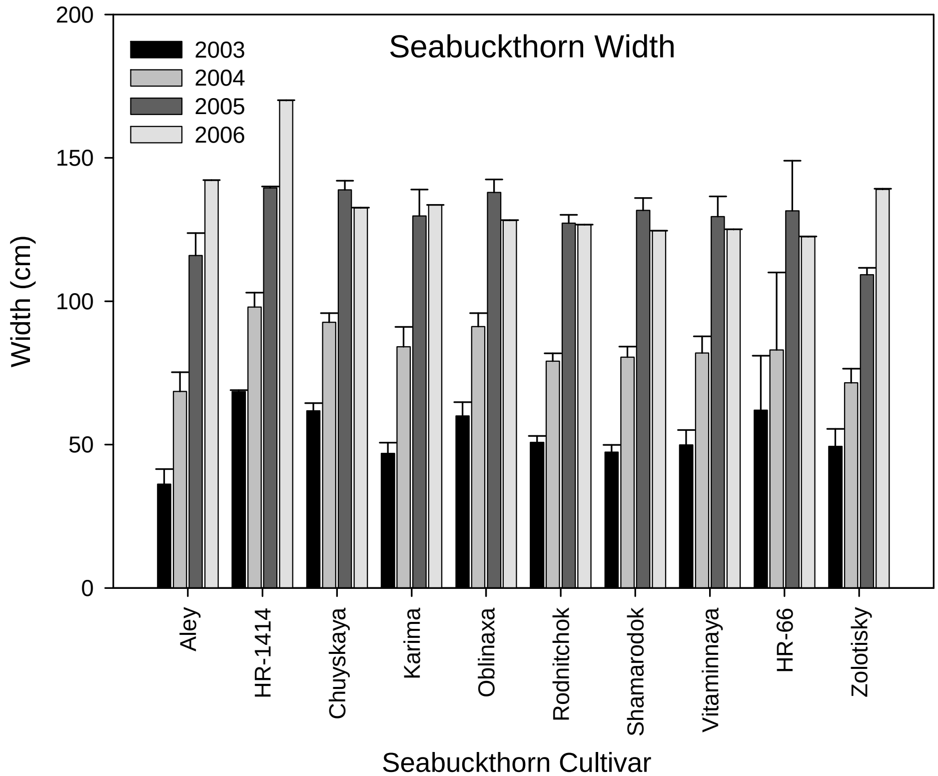

Figure 6. Mean width of seabuckthorn cultivars at the end of each growing season (2003 to 2006).

Conclusions

It was hypothesized that seabuckthorn could be established under the cool, wet coastal conditions of the boreal ecozone in eastern Newfoundland, Canada. In 2001, ten "thornless" cultivars imported from Russia by a Canadian company were established in a 0.2 hectare field trial on newly cleared land at $47^0$47'N $53^0$12'W 17m elevation above sea level. The pH 4.5 podzolic soil had a shallow hard pan which created a perched water table, so the hard pan was broken up, limed, and tile drainage installed. There were 16 trees (11 of 325 females, 5 of 33 males) which did not survive their first winter in 2002 due to frost heaving damage followed by diseased roots. All other trees were healthy and had considerable growth over the second growing season. Winter survival was 98 % in 2003-2006. Tree vigour was high in all years for the female cultivars, but 'Aley' did not have vigorous growth for the first few years. Tree height increased by about 40 cm per year and tree width increased by about 30 cm per year. Once established, all the seabuckthorn cultivars thrived in the cool, wet climate. In 2007, fruit yields averaged 9.4 tonnes per hectare indicating that the seabuckthorn crop was a commercial success.

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Deciphering the low temperature tolerance in seabuckthorn

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Abstract

Seabuckthorn (*Hippophae rhamnoides*) is a low temperature (LT) tolerant plant and can grow up to -40^{0}C. This could be an ideal model system for deciphering LT signaling network and a useful gene resource. Seabuckthorn was developed as an experimental system for genomics and proteomics studies under laboratory conditions. Here we report cloning of a cold induced C-repeat binding factor (CBF) gene which to our knowledge is the first gene cloned from seabuckthorn. The C-repeat binding factor (CBF) is the most well worked out transcriptional activator which is involved in regulating LT tolerance in plants. A reverse transcriptase polymerase chain reaction was carried out with RNA isolated from cold (4^{0}C for 2 hr) treated seabuckthorn seedlings. This yielded an amplicon of 644 bp which was sequenced. BLAST search showed its similarity with *Brassica napus, Brassica juncea*, *Brassica rapa* CBF like protein and dehydration responsive element binding factor2 (DREB2). It was named HrCBF (*Hippophae rhamnoides* CBF). It is a multi copy, LT induced gene. Besides, differential protein expression by cold/freezing was also analyzed and differentially expressed proteins were identified by mass spectrometry. Seedlings were given LT (4^{0}C) and freezing (-15^{0}C) treatment and were resolved on SDS-PAGE gels. Heat Shock Protein (80 kDA) and Actin (41.9 kDa) were significantly down-regulated while a 20 kDa polypeptide was up-regulated by freezing (-15^{0}C). Characterizing the CBF gene and differentially expressed proteins further, would help in understanding the LT signaling in seabuckthorn.

Keywords: seabuckthorn, CBF, antifreeze proteins.

Introduction

Seabuckthorn (*Hippophae rhamnoides turkestanica)* is a hardy, deciduous, nitrogen fixing, medicinal shrub. In has been used as a traditional medicinal plant in the Hindu-Kush region especially in India, Pakistan, China, Bhutan and Nepal. After China, Indian Himalayas are believed to possess the world's second largest seabuckthorn resources. It is found in cold desert and other region of Himalayas comprising the state of Himanchal Pradesh, Ladakh in Jammu and Kashmir, Uttaranchal, Sikkim, and Arunachal Pradesh. Three species of *Hippophae* are found in Indian Himalayas and more are believed to exist (Singh V. 2006). These three are *Hippophae rhamnoides* ssp. *turkestanica*, *H. salicifolia*, *H. tebetana*. Out of these, *H. tebetana* is extremely tolerant to freezing condition and grows at 3000-5300 m above sea level. Other two are distributed in lower region (1500-3800 m) and are relatively lesser tolerant to cold stress. Berries of this plant are the richest source of vitamin C and other vitamins. Various health and medicinal products have been developed from seabuckthorn. Its oils are used to treat cancer, ulcer, skin inflammation and cardiovascular diseases etc (Singh V. 2006). Besides its ecological benefits for soil and water conservation, desertification control, land reclamation has been exploited by China and Russia. Work has been done to study its medicinal properties, growth, regeneration, diversity and sex identification using molecular marker techniques (Singh V. 2006).

There are hardly any studies to understand the molecular mechanism of its cold hardiness. Seabuckthorn survives in very harsh abiotic stress condition and has temperature tolerance up to - 40 ºC. It can be a good system for studying its unique cold hardiness properties. Information generated from this system can be applied to other plants for providing cold tolerance to maximize crop production. Molecular mechanism and the components involved in cold stress tolerance are not yet known in seabuckthorn. Therefore we are trying to understand the stress tolerance mechanism both by genomic and proteomic approach. The genomic approach will give the information about genes while the proteome analysis gives information about the final end product, i.e. the proteins.

Low Temperature (LT) has huge impact on the survival and geographical distribution of plants (Zhu *et al.,* 2007). LT, either chilling or freezing induces a cascade of signaling pathways. Plants differ in their tolerance to chilling (0-15°C) and freezing (<0°C) temperatures (Zhu *et al.,* 2007). Many plants exhibit an increase in freezing tolerance in response to low nonfreezing temperature due to a phenomenon known as cold acclimation (CA) (Thomashow M.F.1999). Among the changes accompanying CA the transcriptional activation and repression of genes are of central importance (Thomashow MF 1999). The products of activated genes are thought to function not only in imparting stress tolerance but also in the regulation of gene expression and signal transduction in stress response (Shinozaki *et al.,* 2003).

Any signal transduction pathway starts with signal perception by sensor followed by the generation of secondary messenger. This perceived signal is transduced and appropriate response is generated. It is believed that stress is perceived by multiple receptors but none have been confirmed for perceiving cold stress (Sharma P. and Deswal R. 2005). Multiple regulatory pathways involved in cold stress response are identified in *Arabidopsis* and other plant systems. (Kazuko Yamaguchi-Shinozaki, 2006). The dehydration-responsive element-binding protein (DREB1)/C-repeat (CRT)-binding factor (CBF) dependent network is most well worked out and probably the most important in transcriptional regulation of cold responsive genes (Thomashow M.F. 1999). This regulon plays a key role in both osmotic and cold stress-responsive gene expression. The CBF's are members of AP2/ Ethylene response element Binding factor (EREBP) family of DNA binding proteins that recognize cold and dehydration responsive DNA regulatory elements known as CRT / DRE (C-rich repeat/Dehydration Responsive Element) (Zhu *et. al.* 2007, Sakuma Y. *et al.* 2002) and in turn influence expression of genes containing CRT/DRE elements. CRT/DRE elements are found in the promoter regions of many cold and dehydration responsive genes of *Arabidopsis*, like COR, (cold-induced), LTI (low-temperature induced) or RD (responsive to dehydration) genes (Thomashaw M.F. 1999). The ability of CBF's to activate the CRT/DRE class of stress responsive genes as upstream transcription factors makes these the key players of transcriptional cascade. In *Arabidopsis* four CBF's are known. The cold stress responsive genes CBF regulon include COR (cold regulated), KIN 1999). Cold stress tolerance is variable amongst plants as some plants are sensitive to even low non-freezing temperature (e.g. Tomato, Rice), some can survive the low non freezing temperature but die in freezing temperature (e.g. *Brassica juncea*) while others can tolerate freezing temperature (e.g. seabuckthorn). The ability of seabuckthorn to grow at freezing temperature makes it a good model system to study LT signaling network vis a vis a good gene source for manipulating other LT sensitive plants. Hence we initiated work on the CBF mediated pathway in seabuckthorn. The cloning of CBF from seabuckthorn, its probable copy no and its expression relation to LT is reported in this manuscript. Our lab is also engaged in dissecting the same pathway from *Brassica juncea*, Tomato, rice and wheat so that a workable comparative model can be built.

Young (15 days old) seabuckthorn seedlings did not shows any drooping at freezing temperature suggesting for existence of antifreeze components. To know if these components could be antifreeze proteins, proteins were extracted and resolved on SDS- PAGE gels from cold/freeze treated young seedlings. The differentially expressed proteins were identified by mass spectrometry. There is no report about any molecular data in seabuckthorn. Our group has initiated some molecular studies to dissect its cold hardiness and here we report the preliminary work including cloning and characterization of "CBF", first gene from seabuckthorn. We have also established a "system" to work under lab conditions. In addition LT induced differential expression of proteins is also analyzed.

Materials and Methods

Plant Material and Stress Treatments

Seabuckthorn (*Hippophae rhamnoides turkestanica)* seeds were washed with 1% teepol to remove any surface infectants and sterilized with 70 % ethanol for two minutes and again washed several time with distilled water. Seeds were soaked in close vessels for five days and were germinated on germination paper. These were kept in dark for two days and then shifted to white fluorescent light at 25^0C for 20 days in 16/8hr light/dark condition. The germination paper was changed twice to avoid growth inhibitors (alkaloids and phenolics) released by seeds. Seedlings were watered regularly and screened for any infection from time to time. For low temperature and freezing stress treatment plants were kept at 4^0C and -15^0C under white fluorescent light for different time points.

RT-PCR Cloning

Total RNA was isolated from fifteen-day-old seedlings by lithium chloride method (Ougham and Davies, 1990). The RNA after two ethanol wash was dissolved in 20 µl of DEPC treated water. It was checked on 1.5% denaturing formaldehyde gel. For cloning of CBF from *Hippophae rhamnoides* the seedlings were given a cold (4^0C, 2hr) treatment. To clone CBF from *Hippophae rhamnoides* PCR was done with following set of primers:

F: CAGTCCGAATTCATGACCTCATTTTCTGCCTTC,
R: GCAGGTCGACATAACTCCAAAGGGACACGTC (fromBNCBF5),

F: ACGCGTCGACATGAAC TCATTTTCAGC,
R: CGAGCTCTTAGTAACTCCAAAGCGACA (fromBNCBF7),

F: TCCGGTTT CCTCAGGCGGTGATTACA,
R: TAAGGACACGTCATCATCTCCCTGAC (AtCBF1),

F: GCGAT GGGTCTTGACGGAAACAATGGTG,
R: TCAGATCATACCAGCATACCCTGCTGTATCG (from At CBF2) (Jaglo *et al*., 2001).

Amplification was observed only with the primer of *Brassica napus* CBF (BNCBF5). Primer sequences were:

F: CAGTCCGAATTCATGACCTCATTTTCTGCCTTC,
R: GCAGGTCGACATAACTCCAAAGGGACACGTC (Gao *et. al.* 2002).

The PCR amplification profile involves 50 sec of denaturation at 95^0C, annealing at 40^0C for 30 sec and extension at 72^0C for 1 min. The amplification was performed for 34 cycles. All PCR reagents were obtained from New England Biolabs USA. The amplified PCR product was cloned at Eco RI site in pGEM-T Easy vector (Promega Corporation, Madison, USA). After screening on Amp/X-gal/ IPTG plate the positive clones were picked and put on liquid culture O/N at 37^0C at 180 rpm. The plasmid isolation was done by alkaline hydrolysis method as described (Sambrook and Russel 2003). The plasmid was digested with Eco RI and the putative positive clones were confirmed by automated DNA sequencing. The gene sequence was submitted to NCBI gene bank under accession no. EF502044.

DNA Isolation and Southern Analysis

Total genomic DNA was isolated using CTAB (Cetyltrethylammonium bromide) method (Sahgai-Maroof *et al.*, 1984). The DNA was dissolved in TE pH 8.0. DNA (10 µg) was restricted with Hind III, Bam HI, Sma I, Eco RI and Xho I for 16-18 hrs, resolved on 1% agarose gel and transferred to Hybon N^+ membrane (Amersham Biosciences Ltd. USA) using alkaline transfer method (Sambrook and Russell, 2003). Pre-hybridization was carried out for 6 hrs in 6X SSC, 0.5% SDS (Amersham Biosciences Ltd. USA), 5X Denhartd's reagent (Amersham Biosciences Ltd. USA) and 2% fish sperm DNA (Sigma) at 65^0C.

For hybridization, full length p32 labeled probe was directly added to the pre-hybridization solution at 65^{0}C for 16 hrs. After hybridization filter was washed with 5X SSC, 0.1% SDS for 10 min at hybridization temperature, 1X SSC, 0.1% SDS for 10 min at hybridization temperature, 1X SSC and 0.1% SDS for 15 min and finally rinsed with 0.1X SSC at RT and autoradiographed. Probe was prepared using 25 ng gel purified DNA (purified using SV WIZARD gel and PCR purification system, Promega) using Prime-a-gene labeling system (Promega Corporation, Madison, USA).

RNA Isolation and Northern Analysis:

RNA isolation was done using lithium chloride method (Ougham and Davis, 1990). For northern analysis 15 µg RNA per lane was loaded, resolved on 1.5% denaturing agarose gel, transferred to Hybond N+ membrane (Amersham Biosciences Ltd. USA) using alkaline transfer method (Sambrook and Russel 2003). Pre-hybridization, hybridization, washing and autoradiography were performed as described for DNA blots.

Protein Extraction and Estimation

LT treated seedlings with their respective control (250mg) were homogenized in 25mM Tris (pH 7.0), 5mM Phenylmethylsulfonilfluoride (PMSF), 20% Glycerol, 0.1% β-mercaptoethenol and 0.5 µl of Protease inhibitor containing 4-(2-aminoethyl)benzenesulfonylfloride (AEBSF), E-64, bestatin, leupeptin, aprotitin in a pestle and mortar on ice. The extract was centrifuge at 12,500 rpm for 30 minutes in Sigma refrigerated centrifuge at 4^{0}C.The supernatant was used for protein estimation (Bradford M.M. 1975) and proteomic studies.

Separation of Protein by SDS-PAGE

Extracted proteins were added to sample buffer (Laemmli 1970) with 1% β-mercaptoethenol and heated at 100^{0}C for 3 minutes before loading. Peptides were separated on different percentage gel (10%, 12%, and 15%) as described by Laemmli 1970. The gels were stained either with coomassie brilliant blue R-250 or by silver staining.

Droop Test

The seedlings of 7 days old *Brassica juncea*, 10 days old *Lycopersicon esculentum*, and 20 days old *Hippophae rhamnoides* were kept in distilled water at -15^{0}C temperature with the respective controls at 25^{0}C for different time points

Bioinformatic Analysis

The open reading frame was determined by ORF finder tool of NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). To know the peptide mass and pI the peptide sequence of each CBF was subjected to compute pI/Mw tool of ExPASy (http://au.expasy.org/tools/pi_tool.html). For multiple alignment and dendrogram CLUSTAL W 1.8 (http://pir.georgetown.edu/pirwww/search/multaln.html) software of protein information resource of Georgetown University was used. Homology analysis was done at basic local alignment search tool protein at NCBI BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and Discussion

RT-PCR Cloning of HrCBF1 from *Hippophae rhamnoides*

For reverse-transcriptase polymerase chain reaction, RNA was extracted using lithium chloride method from LT treated (4^{0}C) seedlings and was used for first strand cDNA synthesis. The cDNA was checked on 1% agarose gel and was used for second strand synthesis. The second strand synthesis was carried out by using primer of BNCBF 5:

Forward: CAGTCCGAATTCATGACCTCATTTTCTGCCTTC, and
Reverse: GCAGGTCGACATAACTCCAAAGGGACACGTC.

The PCR cycle consisted of denaturation at 94^0C for 50 sec, primer annealing at 40^0C for 30 sec and extension at 72^0C for 1 min. The PCR amplification was checked on 1% agarose gel. It showed an amplicon of 643 bp (Figure 1). The amplicon was cloned at Eco RI site of pGEM T Easy vector and was sequenced by automated DNA sequencing. The open reading frame (ORF) finder program of NCBI yielded longest ORF of 636 bp (coding for 211 amino acids).

This translated sequence was used for bioinformatics analysis. It has molecular weight of 23.6 kDa and isoelectric point of 6.6 (http://au.expasy.org/tools/pi_tool.html). The gene was named as *Hippophae rhamnoides* C-repeat binding factor 1 (HrCBF1) and the sequences was submitted to gene bank under the accession number EF502044.

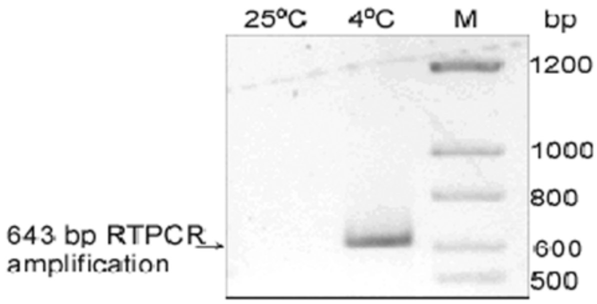

Figure 1. PCR amplification of HrCBF1: agarose gel (1%) showing the RTPCR amplified product of 643bp of HrCBF1. Amplification was done by reverse transcriptase polymerase chain reaction.

HrCBF Structure and Homology Analysis

HrCBF has all typical four domains of CBF namely: N-terminal region, nuclear localization signal, Apetella2 (AP2) and C-terminal activation domain. It also has the CBF signature sequence up-stream (PKKPAGRKKFRETRHP) and downstream (DSAWRL) to AP2 domain. It contains the LWSY signature sequences at the C- tenminal end significance of which is not yet Known. To know the relative position of HrCBF amongst the other CBFs it was subjected to multiple alignment at Georgetown server (http://pir.georgetown.edu/pirwww/ search/multaln.html) and it was found to aligns with *Brassica juncea* CBF (AY887137). The dendrogram shows that HrCBF is probably primitive while monocot CBFs are the advanced ones, while amongst the dicot CBFs the *Capsicum annuum* CBF1a (CaCBF) is the most advanced one (Figure 2). The BLAST-p analysis shows that HrCBF has maximum homology with Dehydration responsive element binding factor (DREB) 2-1 of *Brassica napus* (AAR11858) followed by *B. napus* DREB2-2 (AAR20497), CBF like protein *B. rapa* (AAY 43345), *B. napus* DERB (AAD45625), *B. napus* DREB2-3 (AAR20498) & *B. napus* CBF16.

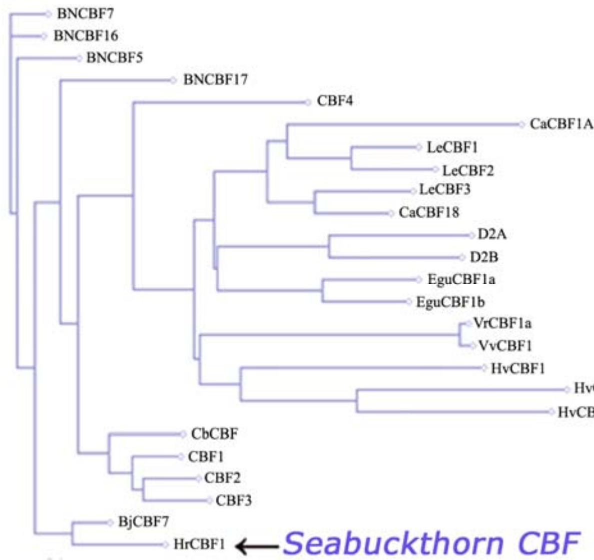

Figure 2. Dendrogram of all CBFs (monocot and dicot): The multiple alignment was done by CLUSTAL W 1.8 program of Protein information resource of Georgetown University. It shows that HrCBF1 aligns with BJCBF.

HrCBF1 is a Multi Copy Gene

Southern blot analysis for seabuckthorn was done after digesting the genomic DNA (10 µg) with Hind III, BamH I, Sma I, Eco R I, and Xho I. Presence of multiple bands in all the lanes suggest that HrCBF is a multi copy gene (Figure 3). CBF can exist as a low to multi copy number gene. In *Arabidopsis thaliana* it is low copy number gene (Stockinger *et al.,* 1997), in *Capsella* a small multigene family (Wang *et al.,* 2004) and in *Eucalyptus* (Kayal *et al.,* 2006), *Vitis* (Xio *et al.,* 2006), *Prunus avium* (Kitashiba *et al.,* 2004), barley and *Brassica napus* (Gao *et al.,* 2002) it is a complex multigene family.

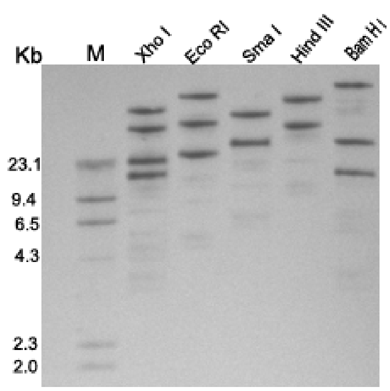

Figure 3. Southern blot analysis of HrCBF1: Total genomic DNA (10µg/lane) of Seabuckthorn was digested with Xho I, Eco R I, Sma I, Hind III and Bam HI. Digested DNA was resolved on 1% agarose gel, transferred to N^+ membrane and probed with αp32[dATP] labeled full length HrCBF1 cDNA.

HrCBF is Induced by Low Temperature

Hippophae rhamnoides 15-20 seedlings were used for treatments as described in materials and methods. The low temperature treatment was given at 4^0C ± 1^0C under constant, white fluorescent light. For transcript analysis total RNA (15 µg) was resolved on denaturing agarose gel (1.5%), transferred to N^+ membrane and was probed with HrCBF1 cDNA. HrCBF1 transcript was induced by 15 min and peaked at 2 hrs (Figure 4). The LT induction pattern of HrCBF1 is similar to *Arabidopsis thaliana* CBF1 / 2 / 3. However, CBFs are also known which deviate from this usual pattern of expression. There are reported CBF which induces as late as 1 day (*Vitis vinifera* CBF3, VvCBF3) and transcript is maintained upto 7 day. *Vitis vinifera* is a LT sensitive plant. Certain CBFs are constitutively expressed like HvCBF2 (Xue 2003), OsDREB1C (Dubozet *et al.,* 2003) and VvCBF1 (Xio *et al.,* 2006). CBF is predominantly, a LT inducible gene, but it is also induced by salt (*Brassica napus,* Gao *et al.,* 2002, *Eucalyptus gunnii,* Kayal *et al.,* 2006, OsDREB1A, Dubozet *et al.,* 2003), ABA (*E. gunnii,* Kayal *et al.,* 2006, VvCBF1 VvCBF2, Xio *et al.,* 2006), drought (VvCBF1, VvCBF2, VvCVF3, Xio *et al.,* 2006) and heat (*Capsicum*, Kim *et al.,* 2004). The transcript analysis of HrCBF1 with respect to other abiotic stresses is underway.

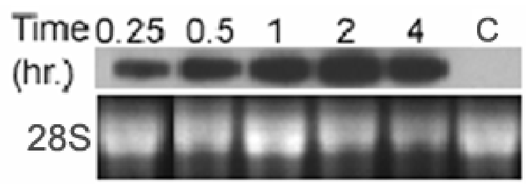

Figure 4. Low temperature (4^0C) induced expression of HrCBF1: HrCBF1 seedlings were give LT treatment for various time points as indicated in figure. Total RNA (15 µg) was extracted, resolved on 1.5% denaturing agarose gel, transferred to N^+ membrane and probed with αP^{32}[dATP] labeled HrCBF1 cDNA probe. Equal loading is shown by 28S RNA in the lower panel.

Droop Test to Analyze Physiological Manifestation of Freezing

LT tolerance being a comparative phenomena, the seedling of seabuckthorn (a very cold hardy plant and tolerant to freezing stress), *Brassica juncea* (low-non freezing stress tolerant) and tomato (cold sensitive plant) were given freezing stress at -15^0C for different time points. *Brassica juncea* and *Lycopersicon esculentum* start drooping by 2:30 hr and were drooped completely by 3:00 hr while seabuckthorn did not droop even in 5 days showing its tolerance to freezing even in young seedlings under lab conditions (Fig 5). This observation is important because this implies that seabuckthorn seedlings express / retain their antifreeze property in young seedlings under laboratory conditions. Therefore this could be used as model system to investigate this extremely important property of this plant.

Figure 5. Young seedlings of *Hippophae rhamnoides* (a) shows freeze tolerance while seedlings of both *Brassica juncea* (b) and *Lycopersicon esculentum* (c) Shows drooping at -15^0C.

Cold/Freeze Regulated Proteins in Seabuckthorn Seedlings.

Seabuckthorn seedlings were given LT (4ºC) and freezing (-15ºC) treatments and the protein was extracted. Extracted protein was resolved on 12 % SDS-PAGE. A 80 kDa heat shock protein and a 41.9 kDa actin were down-regulated while a 20 kDa hypothetical protein was up-regulated by freezing stress at -15ºC as identified by mass spectrometry. Another significant observation is that at 4ºC there is no degradation of RuBisCO while at -15ºC both the small and large sub-units show degradation (figure 6). These results indicated differential regulation of proteins by freezing in seabuckthorn seedlings. To identify more such targets are underway. Antifreeze properties of some of these will be analyzed to understand if any of these are responsible for providing freezing tolerance in seabuckthorn seedlings under laboratory conditions.

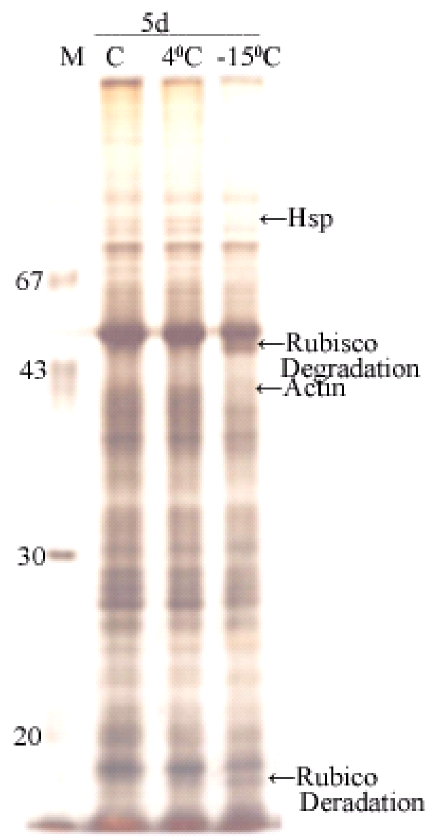

Figure 6: SDS-PAGE silver stained gel showing the profile of protein after LT and freeze treatment to seabuckthorn seedlings: 20 days old seabuckthorn seedlings were given LT and Freeze stress for five days and protein was extracted and resolved on 12% gel. M: molecular weight marker from Amersham Biosciences.

Future Prospect

Presently we are analyzing the freeze induced proteins. The antifreeze properties of these proteins would be investigated to identify if any of these are responsible for providing freezing tolerance to young seedlings. Besides, other isoforms of CBFs would be cloned to get a complete overview of CBF signaling in seabuckthorn. This manuscript describes CBF as first gene cloned from seabuckthorn and the proteomics effort is novel for this plant. In fact, the genomic and proteomic work is lacking with this very important plants. Similar studies can provide a better and clear understanding of the regulatory components and pathways to control metabolism in a precise manner. A precise control could leads to enhanced benefits in form of better medicinal products. Molecular mechanism of cold tolerance of seabuckthorn should be dissected out to understand the low temperature signal transduction pathways of

this commercially important plant both by genomics and proteomics approach. This could provide us with novel cold tolerant genes and promoters for crop improvement.

Acknowledgements

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Characteristics of Mitotic Chromosomes in Some Romanian Seabuckthorn Varieties

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Abstract.

Seabuckthorn is a dioecious species, with marked sexual dimorphism. The mechanism of sex determinism in seabuckthorn is not yet entirely known. Controversies exist even on the diploid chromosome number and on basic number of this species. These are some of reasons for which we studied certain Romanian varieties of *Hippophaë rhamnoides* from cytogenetic point of view, to establish the number and principal morphological characteristics of mitotic chromosomes. The biological material was represented by root tips of plantlets from different Romanian varieties, obtained by seed germination. Squash preparations were used for chromosome analysis. Most of the metaphases, with a good chromosome spreading traits have diploid number 2n=24, although metaphases with other chromosome numbers were observed, as in 'Letea 5' and 'Letea 4' varieties. The average chromosome size was reduced - smaller than 4.0 microns. Detailed cytogenetic measurements were carried out on two Romanian seabuckthorn varieties, 'Coteni-Buhoci' and 'Letea 3'. According to arm ratio, centromeric index, and relative length, we established two chromosome morphotypes: metacentric chromosomes, with median placed centromeres, and submetacentric chromosomes, with submedian placed centromeres. In accordance with the values of analyzed parameters, the karyotype is symmetric, namely relatively less evolved.

Keywords: dioecious species, *Hippophaë rhamnoides*, karyotype, seabuckthorn mitotic chromosomes.

Introduction

Hippophaë rhamnoides L. is a dioecious, anemophylous species, with a marked morpho-physiological polymorphism and with the possibility to fix atmospheric nitrogen. A symbiotic mycorrhizal fungus, *Frankia*, has been found on seabuckthorn roots. The seabuckthorn capacity to fix nitrogen is twice more than that of soybean (Lu, 1992). The average amount of nitrogen fixed in seabuckthorn forest is 30 to 60kg / hectare / year (Zike et al., 1999).

The genus *Hippophaë* L. (*Elaeagnaceae*) includes six species and ten subspecies (Lian, 2000), having great ecological, nutritional, and medicinal values. Regarding the classification, the data are controversial, especially within the genus (Tang, 2002). For example, some authors (Bartish et al., 2002; Sun et al., 2002) sustain the existence of seven species and eight subspecies. The most important species, *Hyppophaë rhamnoides* L., subsp. *rhamnoides* comprising nine subspecies, occupies a large geographical area in Europe and Asia. The last discovered subspecies is *Hyppophaë rhamnoides* subsp. *wolongensis* (Lian et al., 2003). The *rhamnoides, fluviatilis* V. Soest, and *carpatica* Rousi subspecies are represented in Europe, while the *caucasica* Rousi, *turkestanica* Rousi, *mongolica* Rousi, *sinensis* Rousi, *yunnanensis* Rousi, and *wolongensis* subspecies are well represented in Asia. Three of these – *sinensis*, *yunnanensis*, *wolongensis* – are restricted to Chinese habitats.

The domestication can be seen as an evolutive process involving mutation, selection, hybridization, and polyploidization. These processes make possible the obtention of forms with features profitable to human interests. Genetic variation and diversity is the prerequisite tool for plant breeding and improvement. The phenotypic and genotypic studies revealed a great variability in seabuckthorn. The morphological characterization is one of the conventional techniques used to estimate the plant diversity, as tool in selection and breeding. The techniques of molecular biochemistry and genetics are relatively new but more effective in determining the genetic variability in

Hippophaë rhamnoides L.: *RAPD* (Random Amplified Polymorphic DNA) markers – Jeppsson et al. (1999), Bartish et al. (1999; 2000), Chowdhury et al., (2000), Sheng et al., (2006), Sun et al. (2006); *cp DNA* (chloroplast DNA) and *ITS* (internal transcribed spacer) – Bartish et al. (2002), respectively Sun et al. (2002); *AFLP* (Amplified Fragment Length Polymorphism) markers – Ruan and Li (2005), Ruan (2006); *ISSR* (inter-simple sequence repeats) markers – Tian et al. (2004). The data obtained in this way are used to clear the phylogenetic relationships in the genus, to identify the variability in natural populations, to evaluate the wild germplasm resources for their use in improvement of economically important varieties; to realize the genetic maps, and to appreciate the demographic expansion of seabuckthorn varieties.

From economic point of view, the sex determination of seabuckthorn plants is very important. *Hippophaë rhamnoides* L. is a dioecious plant. For commercial production of seabuckthorn berries only 7 – 12 % of male individuals are necessary as producers of fertile pollen and pollinators (http://www.gov.mb.ca/agriculture/crops/fruit/blk01s00.html). For this reason, the breeding efforts are principally orientated towards the development of valuable female phenotypes and to removal of undesired males. Unfortunately, the sex of seabuckthorn plants can not be established before flowering. To improve the effectiveness of breeding programmes it was necessary to find a marker linked to sex determination. For example, Persson and Nybom (1998) investigated the usefulness of RAPD marker in seabuckthorn sex determination. Although in the F1 descendance of one cross, the RAPD marker was present both in male parental and in all male descendants, being absent in all female individuals, this marker can not be considered universal because the determinations effected in F1 progenies of another cross evidenced its presence in only one of the males. Therefore, a sex marker identified in one cross is not necessarily present in another cross (Persson, 2001). The technique of DNA marker assisted sex determination at the seedling stage may be an alternative to the construction of seabuckthorn monoecious plants (Persson and Nybom, 1998; Tang, 2002), advantageous for long-term breeding programmes.

The objective of the present study was to characterize the chromosome number and morphology of some *Hippophaë rhamnoides* varieties grown in Romania

Material and methods

The investigations were carried out using several Romanian genotypes of seabuckthorn, harvested in 2006. To germinate, the seeds were placed on moisten filter paper, at 22 ^{0}C, in dark. At 10-15 mm length, the roots tips were pretreated with a mitotic inhibitor (colchicine, Roundup solution – the pesticides are known to induce C-mitoses), for two or three hours, then in distilled water for two hours. The ethanol-acetic acid (3:l) mixture was used for the fixation of biological material, for 24 hours at room temperature, and 70 % alcohol was used to preserve it. The microscopic preparations were obtained in 45 % glacial acetic acid, by squash method, after 8 minutes hydrolysis, with 50 % hydrochloric acid. For staining, a modified solution of carbol fuxine was used. The microscopic slides were analyzed with Nikon Eclipse 600 light microscope, and the photos were obtained at 100x objective in oil immersion, with a digital camera Cool Pix Nikon, 1600x1200 dpi resolution. The images were processed by Adobe Photoshop programmer. To realize the karyotypes, the metaphases with well-spread chromosomes were kept, and on the basis on the Levan et al. (1964) nomenclature we established the chromosome types.

Results and discussions

The cytogenetic studies establish the chromosome number of a species and help to decipher the morphological particularities of chromosomes and the metric characteristics of these, followed by karyotype construction. The knowledge of chromosome formula, the presence of ploidy level, the existence of some chromosome aberrations can be discussed in relation with respective phenotypes and can direct the activities of selection. All these facts are important to start and sustain the breeding of some plant species of economic interest, such as seabuckthorn. *Hippophaë rhamnoides* is a dioecious species with a pronounced sexual dimorphism and the knowledge of the chromosome characteristics is important. One of the proposed aims is to analyze the chromosome number, their morphological traits, as well as to identify some differences which will permit us to infirm or confirm the existence of heterosomes in this dioecious species.

In dioecious plants, the sex is genetically determined, either by morphologically differentiated sex-chromosomes, either by allele expression at one or several autosomal loci, placed on morphologically non-distinguishable chromosomes. The seabuckthorn sex determination mechanism is not entirely deciphered. There is not much cytogenetic information about this genus, and most data concern the chromosome number. Only one reference (Shchapov, 1979) on the presence of male differentiated heterosomes in seabuckthorn we found, but without details on chromosome type and morphology.

Budashkina, a researcher from Russian Institute for Cytology and Genetics, also specified the existence of a heterosome based sex determinism, namely XY-type for male, and XX-type for female individuals, but without other details or citations from literature (http://us.f543.mail.yahoo.com). She notes an increased bioproductivity (with 25 – 30 %) of polyploids, comparatively to diploid forms, regarding the fruit yield. Based on results obtained in hybridization experiments, Persson (2001) sustained that the sex is genetically determined in seabuckthorn, although nor in this case, evidences on the sex chromosomes existence were not presented. Cytogenetic studies on *Hippophaë rhamnoides* were also accomplished by Darmer (1947, 1952), Cireasa and Dascalu (1983-1984), and Rousi (1965, 1971). In Rousi study (1971), all the 2200 specimens from 33 herbaria, 34 samples from garden populations and 8 nature collections, displayed 2n=24 chromosomes.

In the cited references, the data are quasiunanimous to sustain the existence of a diploid chromosome number 2n=24, for all studied varieties, independently of their Asian or European provenance. Different opinions however exist relative to the *Hippophaë rhamnoides* ploidy level. Rousi (1971) sustains as improbable the situation presented by Darmer (1947) on the existence of 2n=2x=12 diploid forms and 2n=4x=24 tetraploid forms, by emphasizing in this sense the existence, in Eleagnaceae family, of range of variation between 11 - 14 of basic number, that makes more probable existence of x=12 basic number in *Hippophaë* genus. Ruan and Li (2005) note the 2n=24 diploid number, without offer some other details or cite other references. Sobolewska (1926) (see work by. Cooper, 1932) sustained existence of n=6 haploid number in *Elaeagnus angustifolia*, and n=10 haploid number in *Hippophaë rhamnoides* species. Kovacs and Racz (1974) mention the seabuckthorn as having x=6 basic number; they sustain the idea of existence of some 2n=2x=12 diploid varieties, and 2n=4x=24 tetraploid varieties, but these authors did not use (in this case) their results, but older cytotaxonomical studies of Darmer (1947) and Leveque and Gorenflot, 1969.

In Romania, investigations performed by Cireasa and Dascalu (1983-1984) on two provenances of *Hippophaë rhamnoides* L. ssp. *carpatica* confirmed presence of 2n=24 chromosomes in root meristems. They established some size and type features for the 12 chromosome pairs. So, the total length ranged between 0.81 - 2.98 microns. The pairs I – IV have the centromere in median position, and the pairs V – XII have submedian placed centromere. More recent studies (Cimpeanu et al., 2004) found too 2n=24, some differences existing in chromosome size (1.85 – 4.68 microns) and chromosome types (one pair with strictly metacentric chromosomes, two chromosome pairs with submedian placed chromosomes, and nine pairs with median centromere).

Our chromosome measurements included length of individual chromosomes (c), long arm length (l), short arm length (s), arm ratio, r (r = l/s), centromeric index, Ic (Ic=100 x s/c), difference between arms, d (d = l - s), and the relative length of each chromosome (expressed as a percentage of the absolute length of each chromosome pair out of the total length of the chromosome complement). These parameters helped us to establish the chromosome morphotypes of analysed seabuckthorn varieties. According to Levan et al. (1964) nomenclature, the chromosome types are established depending on centromere position (median, submedian, subterminal, and terminal), respectively on Ic and r values. Thus, the chromosomes are named metacentric when they have a mean arm ratio of up to 1.7 and Ic=37.5-50.0), submetacentric (r=1.70–2.99 and Ic=37.5–25.0), subtelocentric (r= 3.00–6.99 and Ic=25.0–12.5), and telocentric (r=7.00 and up, and Ic smaller than 12.5).

The small size of seabuckthorn chromosomes and the difficulties in spreading of chromosomes in metaphase plate were serious limitations for our cytogenetic investigations. In a great number of cases, the chromosomes are excessively condensed, even at smaller concentrations of mitotic inhibitor, and do not permit their well spreading and counting. In some situations, the same treatment (exposure time, concentration, type of mitotic inhibitor) induced different degrees of chromosome condensation at the same seabuckthorn variety.

So far, although cytogenetic measurements were carried out on nine Romanian seabuckthorn varieties, satisfactory results were obtained on three varieties, ‘Letea 8’, ‘Coteni-Buhoci’, and ‘Letea 3’, the last two of these being in detail analyzed in this paper. The metaphases with well spread chromosomes presented, in the most cases, a 2n=24 diploid number, although different situations were observed, as in ‘Letea 5’ or ‘Letea 4’ varieties. Together with numerically predominant diploid metaphases (2n=24), cells with other chromosome numbers (2n=32, 36) were present in respective tissues.

The karyomorphological data show that the chromosome complements of the two analyzed varieties, ‘Letea 3’ and ‘Coteni-Buhoci’, have a relative similar pattern (Table 1, Table 2). They have 2n=24 chromosomes of relatively small size, ranging from 1.52 to 3.73 µm in ‘Letea 3’ variety, and from 1.13 to 2.98 µm, in ‘Coteni-Buhoci’ variety. The total length of the haploid complement was 27.87 µm in Letea 3, respectively 22.57 µm in ‘Coteni-Buhoci’. It is possibly that the differences are due to the use of different antimitotic agents in the two cases.

Although both investigated varieties showed identical diploid numbers, their karyotype structure presented some differences. According to Levan et al. (1964) nomenclature, their haploid complements have 8 m + 4 sm chromosome formula, for Letea 3 variety (Table 1, Fig. 1, 2), and 9 m + 4 sm formula, for ‘Coteni-Buhoci’ variety (Table 2, Fig. 3, 4). More precisely, the two varieties have numerically predominant metacentric chromosomes, with median placed centromeres, and submetacentric chromosomes, with submedian placed centromeres. This situation is also valuable for ‘Letea 8’ variety (not presented in detail in this paper), which has the chromosome formula of haploid complement similar to Letea 3 variety, with 8 metacentric and 4 submetacentric chromosomes. The pair III of ‘Letea 3’ variety, and the pair XII of ‘Coteni-Buhoci’ variety have r and Ic values closed to those valuable for subtelocentric chromosome type, but the respective chromosomes can not be included in this category. No secondary constrictions were found. Therefore, based on these data, we sustain that the ‘Letea 3’ and ‘Coteni-Buhoci’ varieties have karyotypes with a great degree of symmetry. Our results are in accordance with the data published by Cireasa and ‘Dascalu (1983-1984), and Cimpeanu et al. (2004). Generally, the karyotypes with the chromosomes smaller than 4 µm, and being preponderantly metacentric (69 %), submetacentric (24 %), or more rarely subtelocentric (7 %) are symmetrical (Acosta et al., 2005). These karyotypes, with small chromosomes, and a higher proportion of metacentric chromosomes, are probably primitive and show relative chromosome stability, being little evolved – they have not been supported significant rearrangements (Stebbins, 1971). Till now, the obtained data do not allow us to presume the existence of sex chromosomes on the basis of their size or morphology. In conclusion, our data on the three Romanian seabuckthorn varieties confirm the existence of some intraspecific variation, but an extensive research is necessary to be developed on a bigger number of varieties.

Table 1. Metric characteristics of mitotic chromosomes in ***Hyppophaë rhamnoides***, **'Letea 3' variety.**

Chromosome pair	Chromosome type	Total length		Long arm		Short arm		Arm ratio	Arm difference	Centromeric index	Relative length
		µm	Limits of variability	µm	Limits of variability	µm	Limits of variability				
I	m	3.73	3.65-3.81	1.96	1.88-2.04	1.77	1.77-1.77	1.11	0.19	47.41	13.38
II	m	3.51	3.48-3.54	1.85	1.82-1.88	1.66	1.66-1.66	1.12	0.19	47.24	12.59
III	sm	2.73	2.71-2.76	1.96	1.93-1.99	0.77	0.77-0.77	2.54	1.19	28.28	9.81
IV	m	2.43	2.38-2.49	1.24	1.22-1.27	1.19	1.16-1.22	1.07	0.06	48.86	8.72
V	m	2.29	2.27-2.32	1.24	1.22-1.27	1.05	1.05-1.05	1.18	0.19	45.78	8.23
VI	sm	2.15	2.10-2.21	1.46	1.38-1.55	0.69	0.66-0.72	2.21	0.77	32.05	7.73
VII	m	2.04	1.99-2.10	1.13	1.10-1.16	0.91	0.88-0.94	1.21	0.22	44.59	7.33
VIII	sm	2.02	1.99-2.04	1.27	1.27-1.27	0.75	0.72-0.77	1.77	0.52	36.99	7.24
IX	m	1.96	1.93-1.99	1.19	1.16-1.22	0.77	0.77-0.77	1.54	0.41	39.44	7.04
X	m	1.80	1.77-1.82	1.08	1.05-1.10	0.72	0.72-0.72	1.50	0.36	40.00	6.44
XI	m	1.69	1.66-1.71	0.99	0.99-0.99	0.69	1.66-0.72	1.50	0.30	40.98	6.05
XII	sm	1.52	1.49-1.55	0.97	0.94-0.99	0.55	0.55-0.55	1.75	0.41	36.36	5.45
Haploid complement length=27.87											

Table 2. Metric characteristics of mitotic chromosomes in ***Hyppophaë rhamnoides***, 'Coteni-Buhoci' variety.

Chromosome pair	Chromosome type	Total length		Long arm		Short arm		Arm ratio	Arm difference	Centromeric index	Relative length
		µm	Limits of variability	µm	Limits of variability	µm	Limits of variability				
I	m	2.98	2.93-3.04	1.52	1.49-1.55	1.46	1.44-1.49	1.04	0.08	49.07	13.22
II	m	2.79	2.76-2.82	1.46	1.44-1.49	1.33	1.33-1.30	1.10	0.17	47.52	12.36
III	m	2.68	2.65-2.71	1.38	1.38-1.38	1.30	1.27-1.33	1.06	0.08	48.45	11.87
IV	m	1.99	1.93-2.04	1.19	1.16-1.22	0.80	0.77-0.83	1.48	0.36	40.28	8.81
V	m	1.77	1.71-1.82	1.02	0.99-1.05	0.75	0.72-0.77	1.37	0.25	42.19	7.83
VI	m	1.69	1.66-1.71	0.97	0.94-0.99	0.72	0.72-0.72	1.35	0.28	42.62	7.47
VII	sm	1.57	1.55-1.60	1.10	0.10-0.10	0.47	0.44-0.50	2.36	0.64	29.82	6.98
VIII	m	1.57	1.55-1.60	0.88	0.88-0.88	0.69	0.66-0.72	1.28	0.19	43.86	6.98
IX	m	1.52	1.49-1.55	0.94	0.94-0.94	0.58	0.55-0.61	1.62	0.36	38.18	6.73
X	sm	1.49	1.44-1.55	0.97	0.94-0.99	0.52	0.50-0.55	1.84	0.47	35.19	6.61
XI	m	1.38	1.38-1.38	0.83	0.83-0.83	0.55	0.55-0.55	1.50	0.28	40.00	6.12
XII	sm	1.13	1.05-1.22	0.83	0.77-0.88	0.30	0.28-0.33	2.73	0.58	26.83	5.02
Haploid complement length = 22.57											

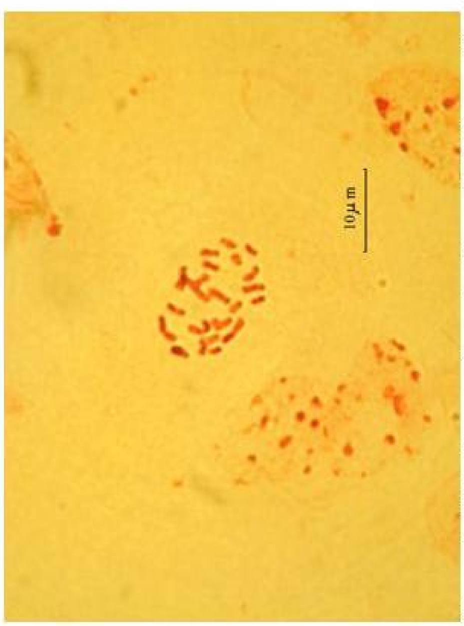

Figure 1. Mitotic metaphase in ***Hippophaë rhamnoides***, 'Letea 3' variety (bar = 10 μm).

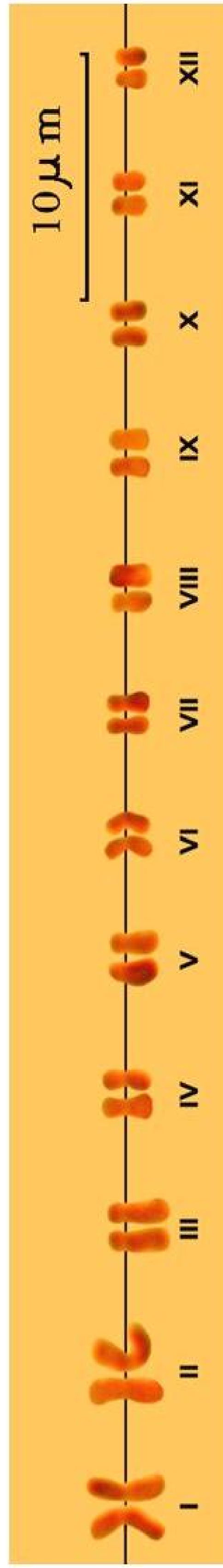

Figure 2. Karyotype in ***Hippophaë rhamnoides***, 'Letea 3' variety (bar=10 μm).

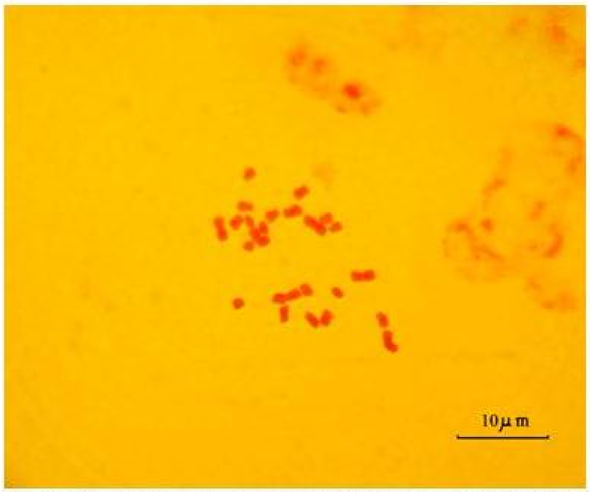

Figure 3. Mitotic metaphase in ***Hippophaë rhamnoides***, 'Coteni-Buhoci' variety (bar = 10 µm).

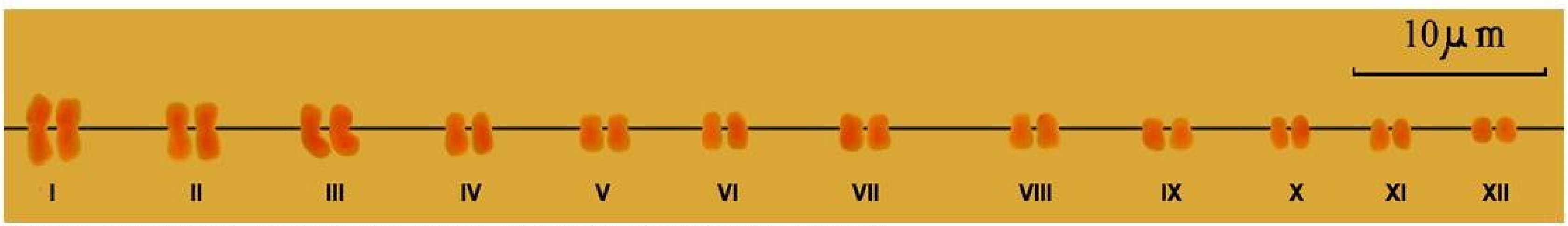

Figure 4. Karyotype in ***Hippophaë rhamnoides,*** 'Coteni-Buhoci' variety (bar = 10 µm).

Conclusion

In 'Letea 3' and 'Coteni-Buhoci' Romanian seabuckthorn varieties, the karyotype is symmetric, with only two groups of chromosomes – metacentric and submetacentric. The average chromosome size is reduced (1.13 - 3.73 microns). The most metaphases presented 2n=24 chromosomes, but we also evidenced cells with other ploidy level.

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Foliar Assimilating Pigments in Different *Hippophaë rhamnoides* L. Varieties in the Romanian flora

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Abstract

Seabuckthorn (*Hippophaë rhamnoides*) is a widely spread plant through the temperate climates of Europe and Asia, including Romania. This species has real medicinal and nutritional values and can also provide a substantial contribution to microclimate regeneration. Relatively few data are published on biochemical composition of seabuckthorn Romanian varieties. For this reason we analysed the variation of foliar assimilating pigments, an important biochemical parameter offering information on one of the basic metabolic processes in plants - photosynthesis. The results indicated this to be the variation dependent on variety and specific environmental conditions

Keywords: *Hippophae rhamnoide*s, seabuckthorn varieties, assimilating foliar pigments, chlorophyll *a*, chlorophyll *b*, carotenoid pigments.

Introduction

Seabuckthorn is a small fruit shrub, pest and disease resistant, spread throughout the spontaneous Romanian flora. This species is very easily adaptable to poor, dry, and even salty soils, without special preferences towards the substrate. Being extremely common in the temperate climates of Europe and Asia, seabuckthorn attracts more and more interest of specialists because of great medicinal, nutritional qualities, and because of its contribution to microclimate regeneration .(Li, 1999; Zeb, 2004b; Cenkowski *et al*., 2006).

The complex chemical composition of seabuckthorn leaves and fruits varies with varieties origin, the cultivation environment, as well as the utilized extraction methods (Tamas and Neamtu, 1986; Li, 1999; Burzo *et al*., 2004; Zeb, 2004a; Burzo *et al*., 2005; Cenkowski *et al*., 2006; Kaminskas 2006). Quantitative and qualitative appreciation of biochemical composition of seabuckthorn foliar apparatus is generally in accordance with geographic area, phenophase, plant genetic characteristics, soil structure, clime, as well as other factors difficult to be foreseen and recorded (Gherghi *et al*., 2001).

In this context, our study was focused on determination of chlorophylls and carotenoids level in some seabuckthorn varieties harvested from Romanian flora and on quantification of a/b chlorophyll ratio. Obtained data were correlated to photosynthesis as a basic metabolism process (Beresiu *et al*., 1983; Parvu, 2002) and as a bioproductive indicator in selection for seabuckthorn intensive cultivation.

Material and methods

The biological material consisted of leaves harvested from one year old offshoots, selected from seabuckthorn varieties originated in different zones of Romania. The analysed varieties were divided in two lots, with the following labelling sequence, according to the origin place: Regarding the names of varieties, we specify that they represent the location of respective varieties in Bacau district (lot A) and in adjacent zones of this (lot B).

Lot A. - The varieties originating from Bacau District:

'B1-Letea 2'; 'B2-Letea 3'; 'B3-Letea 4'; 'B4-Letea 5'; 'B5-Letea 8'; 'B6-Coteni-Buhoci'; 'B7- yellow seabuckthorn'. In this case, the analyses were performed on the averaged samples from homogenized material harvested from entire length of one year old offshoots.

Lot B. - The varieties originating from Romanian regions, other than Bacau Ditrict:

'V1- Serpeni'; 'V2 Serbanesti I'; 'V3- Serbanesti IV', 'V4- thornless seabuckthorn', 'V5- Delta 60 M'; 'V6 - St. George'. In this case, the analyses were conducted on leaves harvested from inferior (basal segment) and superior (top segment of one year old offshoots.

The plants were harvested at full biological maturity. The freshly harvested material (leaves) was processed in order to determine the amount of assimilating pigments, by spectrophotometric method. The assimilating pigments concentration was calculated using specific formulas (Artenie and Tanase, 1981).

Results and discussion

Lot A - The lot of varieties originating from Bacau District (Fig. 1, 2 and 3)

The amount of *a* and *b* chlorophylls (Fig. 1) presented a slight variation in seabuckthorn varieties forming the Letea group, reaching the absolute maxim for the B3 samples (4.78 mg/g fresh matter for chlorophyll *a*, respectively 2.29 mg/g fresh matter for chlorophyll *b*); closed values were registered for B6 and B7. For these two forms,, the amount of chlorophyll pigments had constant increased values (approximately 4.5 mg for chlorophyll *a*, respectively 2 mg for chlorophyll *b*). The lowest levels were noted for B4 (approximately 3.65 mg for chlorophyll *a*, respectively 1.06 mg for chlorophyll *b*) and B1 form (approximately 3.53 mg for chlorophyll *a*, respectively 1.34 mg for chlorophyll *b*).

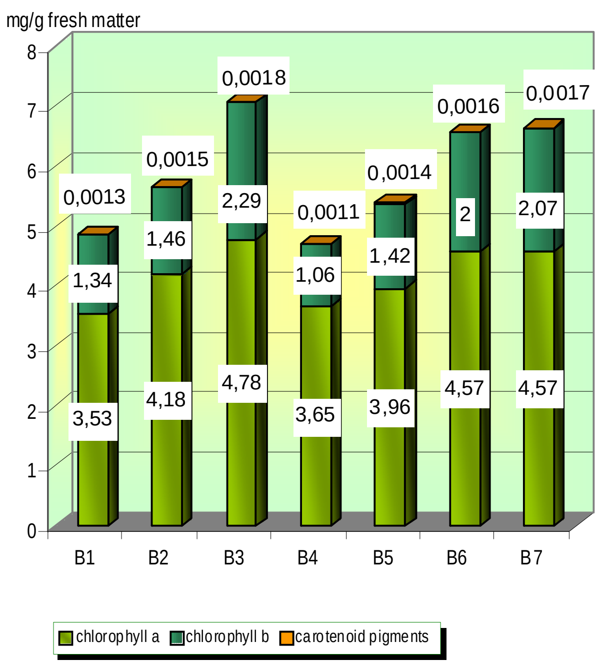

Figure 1. The assimilating pigments amount in *Hippophaë rhamnoides* varieties from Bacau District.

The carotenoid pigments level of varieties from Bacau District are directly correlated to corresponding their chlorophyll level (Fig. 2), and the profiles of the two parameters are similar.

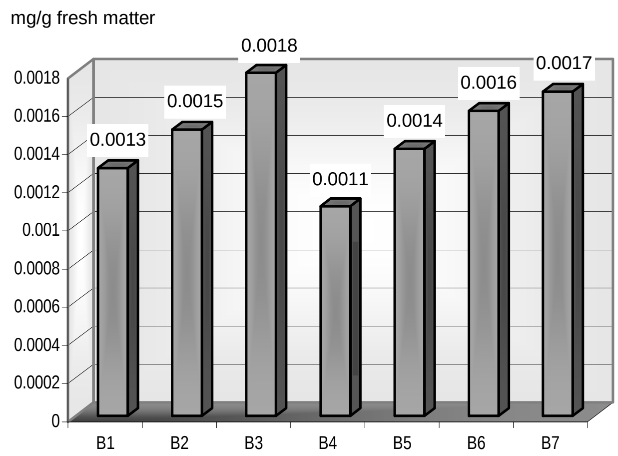

Figure 2. The carotenoid pigments amount in *Hippophaë rhamnoides* varieties from Bacau District.

The a/b chlorophyll ratio, a parameter offering informations on ecological requirements for insolation of analysed plants (Fig. 3), is constantly in favor of the chlorophyll *a*, characterizing from this point of view the seabuckthorn varieties as heliophyll plants.

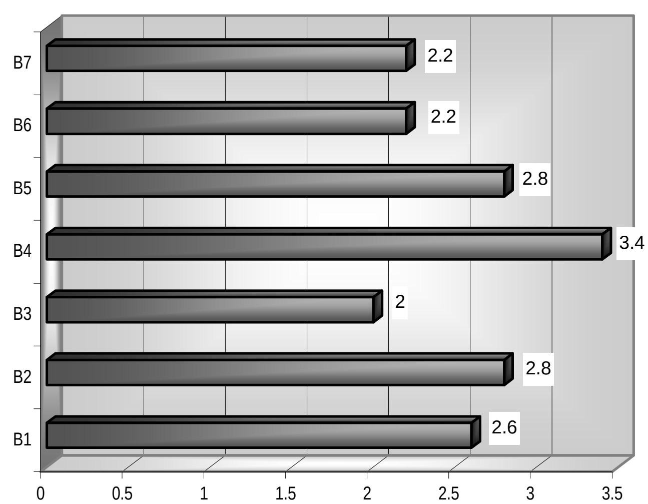

Figure 3. The *a/b* chlorophyll ratio in *Hippophaë rhamnoides* varieties from Bacau District.

Lot B - The lot of varieties originating from Romanian regions, other than Bacau District (Fig. 4, 5 and 6)

The total amount of assimilating pigments (Fig. 4) had increased values in leaves collected from both segments of one year old analysed offshoots, excepting V5, where the amount of assimilating pigments displayed the smallest values, in both analysed segments. We consider that this fact is the expression of a different metabolic reaction to the cultivation conditions offered by the fields from Bacau District. In V4 we noted the maximum biosynthesis in both analysed segments, while V2 presented significant differences of pigments biosynthesis between the two segments.

Therefore, the chlorophyll amount generally registered similar values for leaves harvested from both halves of the one year old offshoots, with slight predominance for the superior fragment for V1, V5 and V6 forms. The V2, V3, and V4 variants had a greater amount of chlorophylls in leaves from the inferior fragment of the analysed offshoots.

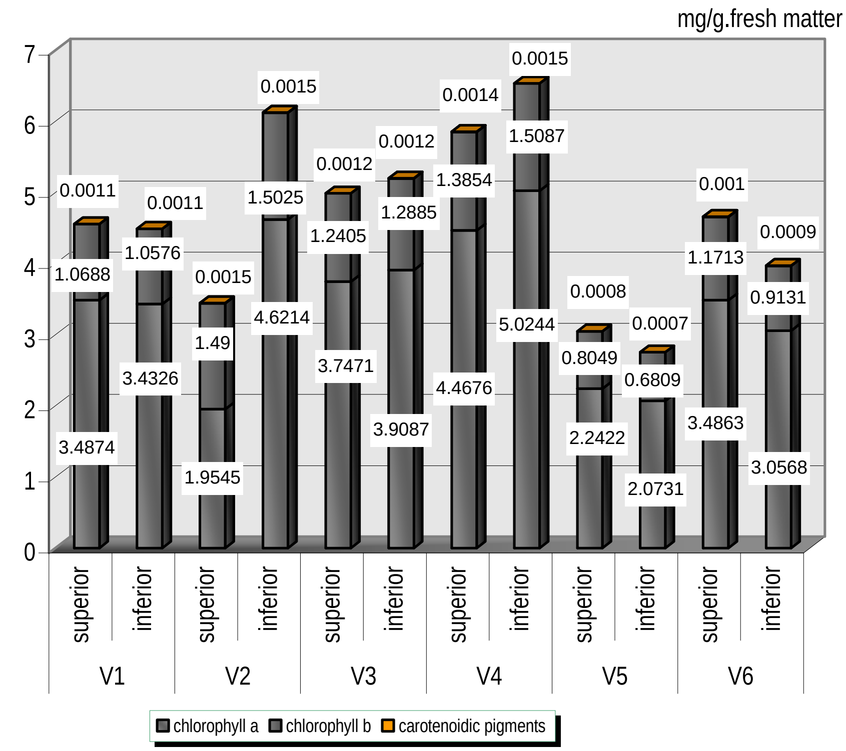

Figure 4. The assimilating pigments amount in leaves of *Hippophaë rhamnoides* plants, originating in Romanian regions, other than Bacau District.

The amount of carotenoid pigments (Fig. 5), as fraction of the assimilating pigments, had a similar way to that of chlorophylls accompanying in plant leaves. V2 and V4 analysed forms exhibited the highest values, followed by V3 and V1, all of these having very closed levels in both segments of offshoots. V5 contained the minimum level of carotenoid pigments, comparatively with the all other varieties originating in Romanian regions, other than Bacau District. Generally, as in the case of varieties harvested from Bacau District, the carotenoid profile resembles that of chlorophylls of respective varieties.

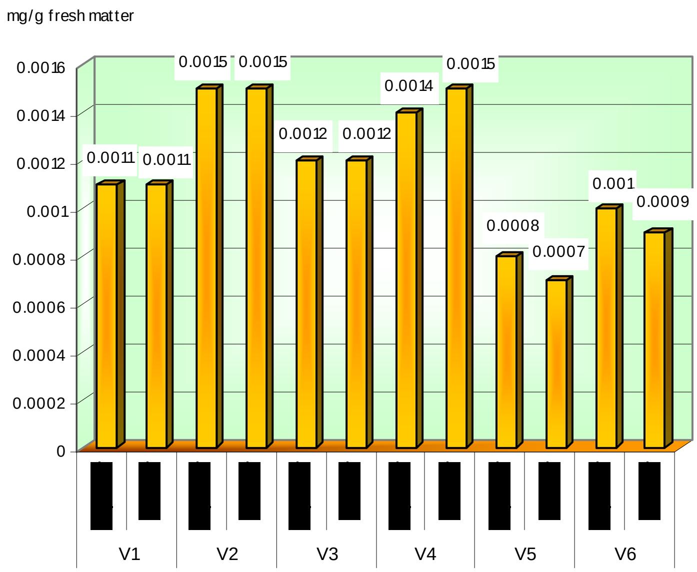

Figure 5. The carotenoidic pigments amount in *Hippophaë rhamnoides* varieties, originating in Romanian regions, other than Bacau District.

The *a*/*b* chlorophyll ratio (Fig. 6) generally is close or slightly higher than 3.0, in favor of the chlorophyll *a*, in leaves from both segments of the analysed one year old offshoots. Only the V2 variant made an exception because *a*/*b* chlorophyll ratio in its inferior segment is reduced to half (1.3). In these conditions we consider that all forms enjoy light and prefer sunny cultivation habitats.

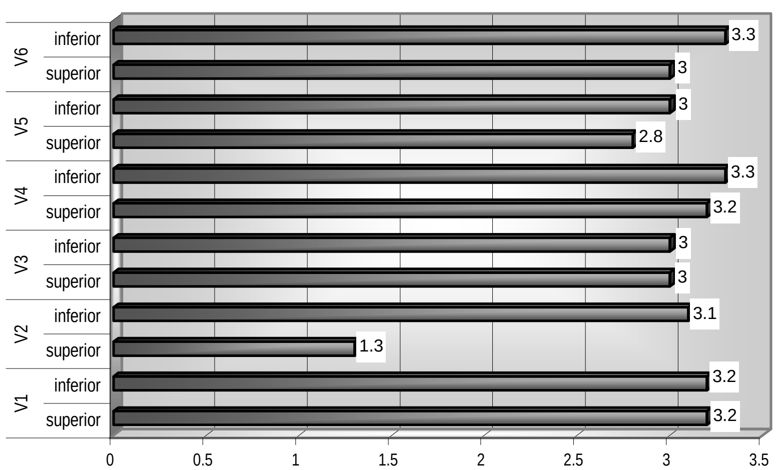

Figure 6. The *a*/*b* chlorophyll ratio in *Hippophaë rhamnoides* varieties, originating in Romanian regions, other than Bacau District.

The content of assimilating pigments constitutes a biochemical parameter that offer us essential information on the manner in which the environmental factors (especially the quantity and the quality of light, in this case) influence the life plant. The knowledge of the assimilating pigments behaviour, of the genetic determination of this quantitative character, and of the manner in which the environmental conditions influence the biosynthesis of these compounds, together other essential biochemical indicators are very important in the selection of high productivity forms.

Conclusions

The amount of chlorophyll pigments represents, for the analysed varieties, well outlined biochemical and physiologic characteristics. For chlorophylls, the amplitude of variability was higher in the lot of varieties originating in Romanian regions, other than Bacau District. For carotenoids, their levels also presented greater differences in the same lot of forms. The predominance of *a*/*b* chlorophyll ratio having values greater than 3.0 suggests that the varieties from Romanian regions, other than Bacau District, are well adapted to the sunny areas where they live.

Acknowledgements

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The Content of Soluble Proteins in *Hyppophae rhamnoides* ssp. *carpathica* Varieties Harvested from Different Regions of Romania

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Abstract

The genus *Hyppophae* is native to the temperate zones of Asia and Europe. Seabuckthorn species are important because of their strong tolerance to soils and air contaminated with toxic pollutants, while it can be used to revegetate the heavily industrialised areas. The quality of the seabuckthorn is often based on the nutritional value of the fruits, which contain most of the valuable health components (carotenoids, lipids, proteins, vitamins, minerals). This paper presents the results of a study regarding the amount of soluble proteins in fruits of some seabuckthorn varieties. The samples were harvested from different regions of Romania, at different periods of time, in autumn. The protein amount varies in fruits (3.99 mg% - 45.35 mg%) and in seeds (1.12 mg% - 171.10 mg%), depending on the geographic region, altitude and soil type, factors which influenced the metabolic processes of plants.

Keywords: *Hyppophae rhamnoide*s ssp. *carpathica*, seabuckthorn varieties, soluble proteins.

Introduction

Research conducted in different parts of the world, including Romania (Socaciu, 2003; Rati and Rati, 2003; Stanescu *et al*., 1977) demonstrate that the seabuckthorn leaves, bark, and especially berries contain numerous bioactive substances with important role in metabolism regulation, immunity and protection of immune system, in therapy of diseases of circulatory and respiratory systems etc. The unusually rich chemical composition of the berries amplified the interest for this plant. Thus, the priority of clinical and laboratory researches is to discover new substances from seabuckthorn and to establish their biological effects.

The concentration of vitamin C in seabuckthorn fruit (100–300 mg/100 g fruit) is higher than that of strawberry, kiwi, orange, tomato, carrot, and hawthorn (Bernath and Foldesi, 1992). Vitamin E content in seabuckthorn (202.9 mg/100 g fruit) is higher than that of wheat embryo, safflower, maize, and soybean (Li, 1999). The most important pharmacological functions attributed to seabuckthorn oil are: anti-inflammatory, antimicrobial, pain relief and promoting regeneration of tissues. Also, the oils of seabuckthorn seeds and berries have factors that protect against some types of cancer (Brad *et al*., 2002). Seabuckthorn oil extracted from seeds is popular in cosmetic preparations, such as facial cream (Li and Wang, 1998, Lu, 1992, Zeb, 2004).

Hippophae rhamnoides is considered, by the content in active substances, a miraculous plant for human health, similar to gingseng (Brad. *et al*., 2002). The oil and fruits of *Hyppophae rhamnoides* are rich in carbohydrates and proteins; the seabuckthorn seeds contain especially globulins and albumins (Li, 1999). The proteins are considered molecular markers in the analysis of plant polymorphisms; they display a great structural diversity in relation with their origin and physiological functions in plant (Brad *et al*., 1986).

The aim of this paper is to establish the amount of soluble proteins in pulp and seeds of the seabuckthorn varieties harvested from different Romanian geographic areas.

Materials and methods

The analyses were performed in the Department of Molecular and Experimental Biology of “Alexandru Ioan Cuza” University Iasi, Romania. The biological material was represented by seabuckthorn - *Hippophae rhamnoides* L. - berries. The berries were collected from seabuckthorn plants placed in different Romanian geographic areas (Buzau district – 10 varieties, Sulina, Danube Delta area – 10 varieties, Bacau district – 6 varieties). The plants from Bacau district are cultivars, while those from Buzau district and Danube Delta are wild, but all have been harvested in the same week of October, 2006.

The quantitative determination of proteins was made both in pulp and seeds. To realize the extraction of soluble proteins, the biological material, was first weighed and well homogenized, and then was subjected to the treatment with 50mM Tris-HCl buffer, pH – 7.0 (5mM dithiothreitol, 10 mM ascorbic acid, 6mM cystein, 1mM EDTA, 0,1% Tritonx100). After centrifugation in Eppendorf tubes, at 3000 x g, for 15 minutes, the supernatant was used to determine the protein level by protein dye binding method based on the Bradford method (Bradford., 1976), using serum albumine (Sigma-Aldrich) as standard. The results are expressed in mg% fresh matter.

Results and discussions

The results obtained after analyses performed in *pulp* of fruits harvested from varieties originated in Bacau district demonstrate that the content of soluble proteins ranged between 22.39 mg% - 45.35 mg% (Figure 1).

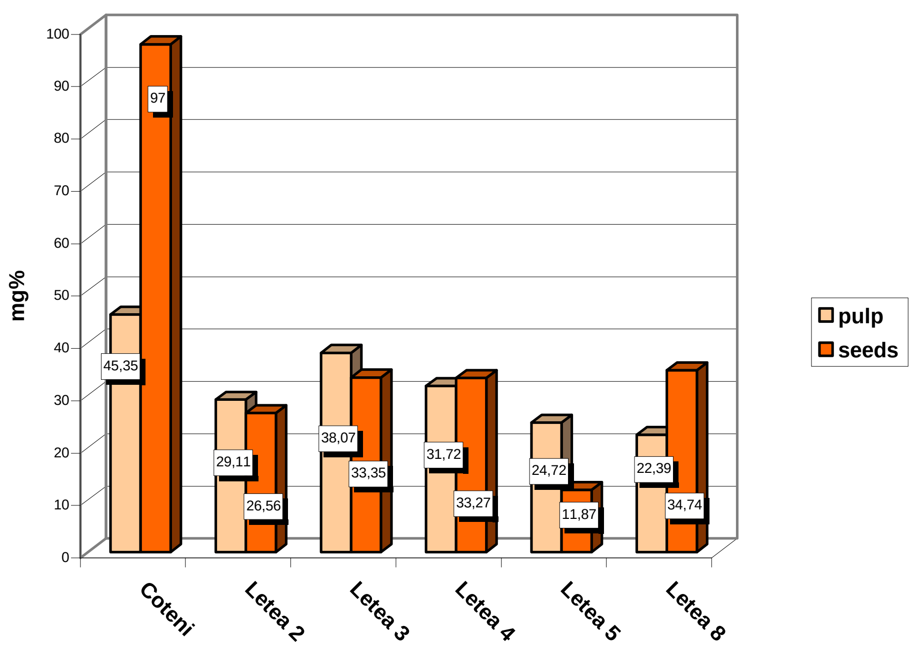

Figure 1. The variation of soluble proteins amount in fruit pulp and seeds of seabuckthorn varieties from Bacau district.

The maximum value of soluble proteins was evidenced at ‘Coteni’ variety – 45.35 mg%, and the minimum at ‘Letea 8’ variety – 22.39 mg%. The other tested varieties displayed intermediary values: ‘Letea 3’ variety – 38.07 mg%, ‘Letea 4’ variety – 31.72 mg%, ‘Letea 2’ variety - 29.11 mg%, ‘Letea 5’ variety – 24.72 mg%. Therefore, between ‘Letea’ varieties, ‘Letea 3’ has the highest content of soluble proteins.

The protein content of *seeds* was also maximum – 97 mg% in ‘Coteni’ variety, followed in this case by the ‘Letea 8’ variety – 34.74 mg%. The amount registered for ‘Letea 5’ variety is considerable reduced by comparison with ‘Coteni’ variety (8 times smaller).

From a perceptual point of view, the comparative analysis evidence that the variation of soluble proteins in pulp and seeds generally depends on variety (Figure 2), both in pulp and seeds. Three of six of analysed Bacau varieties (‘Coteni’, ‘Letea 4’, and ‘Letea 8’) have a content of soluble protein bigger in seeds, comparatively with pulp of seabuckthorn berries, fact in accordance with literature data (Brad, 2002). The other three varieties (‘Letea 2’, ‘Letea 3’, and ‘Letea 5’) contain a quantity of soluble proteins bigger in pulp than seeds, but the differences are relatively small.

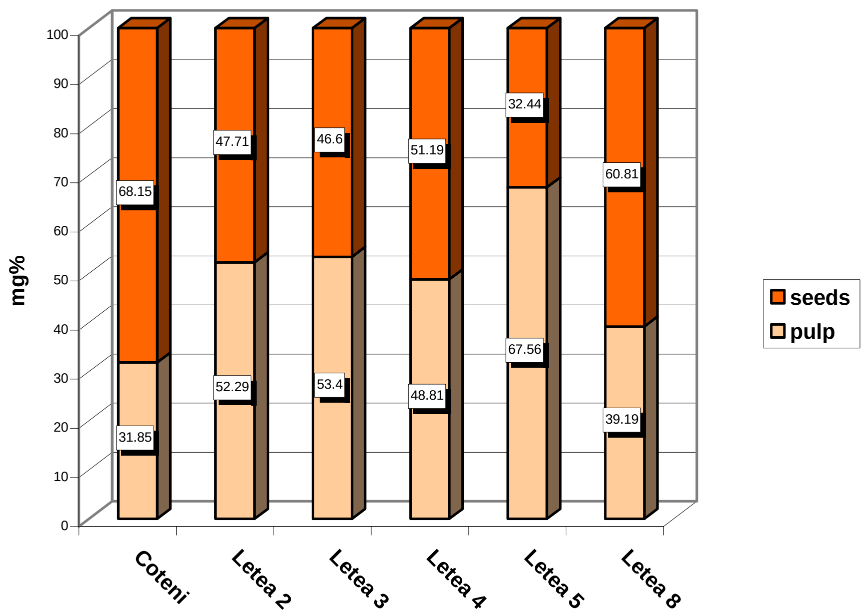

Figure 2. The percentage variation of soluble protein amount in fruit pulp and seeds of seabuckthorn varieties from Bacau district.

The content of soluble proteins in *pulp* of seabuckthorn fruits harvested from another geographic area, the Buzau district (Figure 3), varied between 3.99 mg% - 39.40 mg%. The greatest amount of soluble proteins was observed at ‘Cislau 5’ variety – 39.40 mg%, followed by ‘Cislau 4’ variety – 24.83 mg%, ‘Cislau 1’ variety – 18.44 mg%, ‘Cislau 2’ variety – 18.36 mg%, ‘Cislau 3’ variety – 17.97 mg%, ‘Buzau 1’ variety – 14.84 mg%, ‘Cislau 6’ variety – 11.36 mg%, ‘Chiojdu 2’ variety – 9.38 mg%, ‘Chiojdu 1’ variety– 6.35 mg%, and ‘Buzau 2’ variety – 3.99 mg%. Thus, the minimum amount of soluble protein in pulp was registered for ‘Buzau 2’ variety (10 times smaller than ‘Cislau 5’ variety).

Although these varieties were harvested from the same geographical area, we remark on the large differences at the level of quantity of soluble proteins, probably explained by altitude difference, a fact that obliges the plants to specific adaptations and responses.

It is known that seabuckthorn growth is positively influenced by the sandy loam soils, rich in organic matter. The varieties from Buzau district are originated in three distinct regions. Thus, the ‘Buzau 1’ and ‘Buzau 2’ varieties are from a river bed (at 278 m, respectively 454 m), having an alluvial soil, the Cislau varieties are from a mountain side (at altitudes ranging between 367 m and 398 m), with typical, dark luvic soils (oligobasic and/or holoacids), and the Chiojdu varieties are originated in a terrain fissure (454 m altitude), having an erodisol.

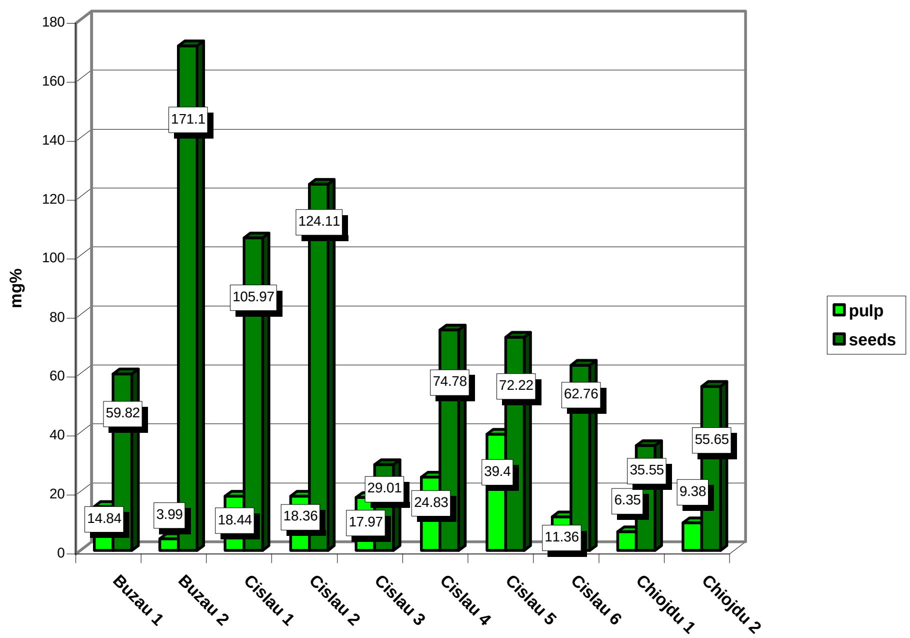

Figure 3. The variation of soluble proteins amount in fruit pulp and seeds of seabuckthorn varieties from Buzau district.

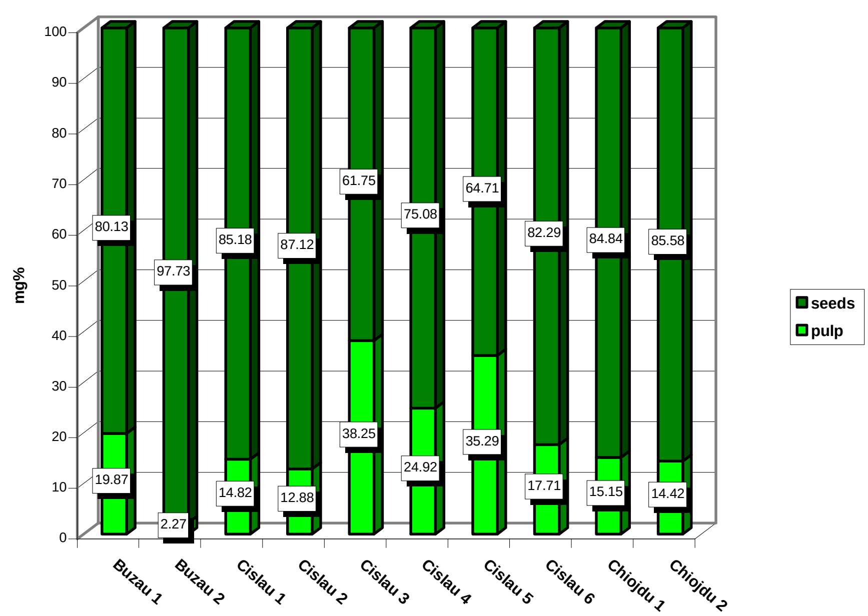

Figure 4. The percentage variation of soluble protein amount in fruit pulp and seeds of the seabuckthorn varieties from Buzau district.

In *seeds* of seabuckthorn varieties from Buzau district, the values of soluble protein are more pronounced at three of these (‘Buzau 2’ – 171.10 mg%, ‘Cislau 2’ – 124.11 mg%, ‘Cislau 1’ – 105.97 mg%) comparatively with the others (Figure 3).

The comparative analysis of percentage variation of soluble proteins from pulp and seeds of the seabuckthorn varieties harvested from Buzau district (Figure 4) evidenced a predominance of soluble proteins in seeds, not in pulp, especially in 'Buzau 2' variety, where the level of pulp soluble protein is extremely low.

In the case of the varieties from Sulina region (an area localized in South-East of Romania – Danube Delta) (Figure 5), the content of *pulp* soluble protein ranged between 18.32 mg%, at Sulina 23, and 53.30 mg%, at Sulina 20. This region is characterized by a very poor soil, more exactly, typical psamosoil, and the altitude is reduced, 6m. In *seeds*, the minimum quantity of soluble proteins was registered for 'Sulina 23' variety – 1.12 mg%, while the maximum value was noted for 'Sulina 9' variety – 46.47 mg%. Unlike Buzau varieties, those originated from Sulina area have, excepting 'Sulina 9', more soluble proteins in pulp, not in seeds. This situation may be caused by factors like: the provenance areas (the altitude of Sulina areas is 6 m, inferior to Buzau area where is 278 – 454 m), the type of soil etc. The most significant differences between the seed and pulp protein amount have been registered for 'Sulina 20', 'Sulina 22', and 'Sulina 23' varieties.

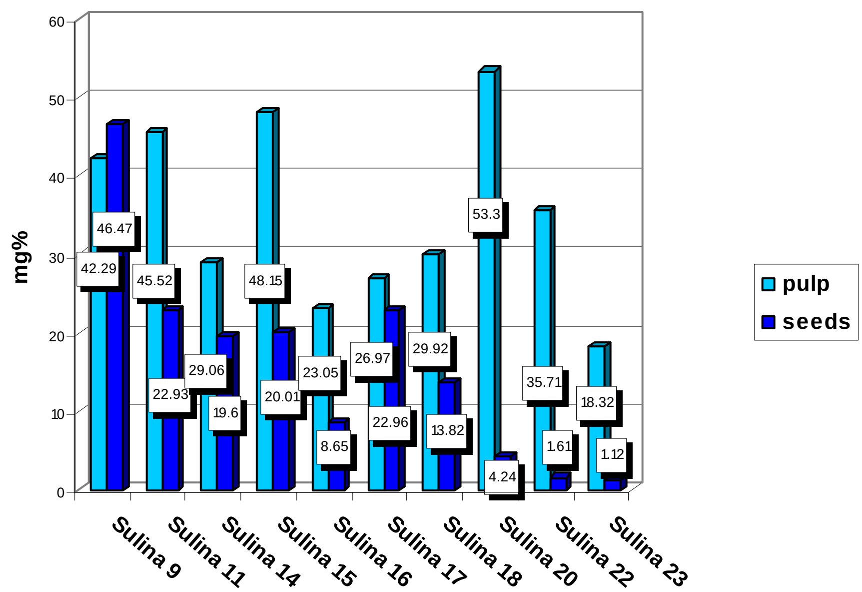

Figure 5. The variation of amount of soluble proteins in fruit pulp and seeds of seabuckthorn varieties from Sulina district.

The percentage variation of soluble proteins amount in fruit pulp and seeds of the seabuckthorn varieties from Sulina district (figure 6) is relatively constant at 'Sulina 23', 'Sulina 22' and 'Sulina 23' varieties but presents fluctuation at the others analysed varieties. We remark that the amount of soluble proteins at 'Sulina 9' variety displayed relatively small differences in pulp and seeds (42.29mg% comparatively with 46.47mg%), while in the other analysed varieties, the pulp content of proteins is higher ('Sulina 11', 'Sulina 14', 'Sulina 15', 'Sulina16', 'Sulina 17', and 'Sulina 18' varieties), while for 'Sulina 20', 'Sulina 22', 'Sulina 23' varieties the values of this parameter were very pronounced.

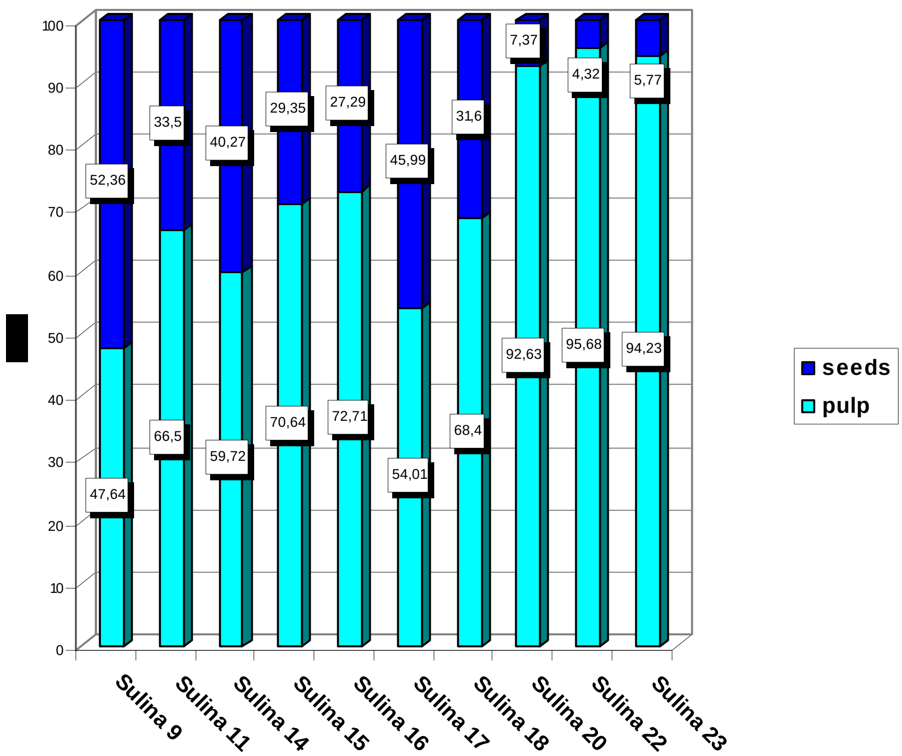

Figure 6. The percentage variation of soluble proteins amount in fruit pulp and seeds of seabuckthorn varieties from Sulina district.

Conclusions

The content of soluble proteins, determined in fruit pulp and seeds of seabuckthorn varieties, depends on the Romanian geographic areas, the type of soil, the altitude, factors influencing the plant metabolic processes. The seabuckthorn varieties originated in Bacau district have relatively constant protein content, both in pulp and seeds. The varieties from Buzau district displayed high protein content in seeds than in pulp, while those from Sulina district have reduced protein content in pulp comparatively with seeds, excepting ‘Sulina 9’ variety.

Acknowledgements

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3rd International Seabuckthorn Association Conference

ISA 2007

PROCESSING

Current and Emerging Processing Technologies for Seabuckthorn (*Hippophae Rhamnoides* L.) and Its Products

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Abstract

Seabuckthorn (*Hippophae rhamnoides* L.) is a hardy deciduous shrub with yellow or orange fruits. It is currently domesticated in many parts of the world like China, Russia, India, Germany, Finland and Canada. Almost all parts of the plant (fruits, leaves and bark) have uses in the food, pharmaceutical, and cosmetic industries due to their content of essential bioactives. Seabuckthorn fruits are usually the main focus for processing because of their high content of vitamins, minerals, carotenoids, flavonoids and amino acids required by the human body. The oils, extracted (by cold press, solvent or supercritical fluid extraction technology) from the seed and pulp (components of the fruit), are often considered the most valuable for their content of fat-soluble vitamins, sterols and essential fatty acids. The high content of palmitoleic acid in the pulp oil makes it attractive to the cosmetic industry as an anti-aging agent. Seabuckthorn fruit juice is high in vitamin C and is often extracted by a combination of pressing and clarification technologies, and sold as single strength juice or blended with other fruit juices and sweeteners to produce nutritional and sport beverages. The juice can also be processed into jellies, syrups, and alcoholic drinks. Seabuckthorn leaves contain important nutrients and bioactives such as flavonoids, carotenoids, and sterols and are often processed by controlled drying for tea blends. The leaves can also be crushed for extraction of essential oil. The bark and seed has also been processed to extract proanthocyanidins. Retaining these nutritional, pharmaceutical and cosmetic attributes of seabuckthorn and its derivatives is dependent on the applicable processing technologies. Current and emerging processing technologies for seabuckthorn will be presented along with their impacts on preserving the important bioactives for their nutritional and health benefits.

Keywords: seabuckthorn, fruits, processing technologies, seed, pulp, bark, juice, oil.

Introduction

Seabuckthorn (*Hippophae rhamnoides* L.) is a hardy deciduous shrub with yellow or orange fruits and has been used for food in both Europe and Asia for centuries (Yang, & Kallio, 2002a). It is currently domesticated in many parts of the world like China, Russia, Mongolia, Kazakstan, India, Nepal, Pakistan, Afghanistan, Hungary, Romania, Switzerland, Germany, France, Britain, Finland, Sweden, Norway and Canada. The wide distribution of seabuckthorn is reflected in its habit-related variation not only in morphology, yield, growth rhythms and cold hardiness, but also in fruit related characters such as fresh weight, chemical and sensory attributes. With the application of modern analytical techniques, almost all parts of the plant (fruits, leaves and bark) are shown to have a unique composition emphasizing its potential as a dietary and medicinal supplement (Li, Beveridge, & Drover, 2007).

The objective of this article is to review the present literature on processing technologies and outline some research, development and commercialization activities at Food Development Centre, Manitoba, Canada on the chemical and medicinal constituents of seabuckthorn of different origin and varieties, so as to get a clear understanding of the compositional importance for future research and development. This article emphasizes on the current and emerging processing technologies for seabuckthorn and their impacts on the important bioactives for their nutritional and health benefits and product development.

Seabuckthorn - A Plant with Many Uses

Seabuckthorn plant is unique for its chemical composition for nutritional, medical and cosmetic uses. Almost all parts of the plant (fruits, leaves, and bark) have uses in the food, pharmaceutical, and cosmetic industries due to their content of essential nutrients and bioactives. The component and uses of the plant parts are shown in Table 1. Research has indicated that extracts isolated from the bark of seabuckthorn may inhibit tumour growth and there are reports that it has successfully treated gingivitis (Xu, Xie, Pan, Yang, Wang, Cenkowski, Hydamaka, & Rao, 2006). The leaves of the seabuckthorn plant also contain many nutrients and bioactive substances (Guan, Cenkowski, and Hydamaka, 2005). Leaves harvested from the male plant can be used to produce tea, tea extracts, tea powder and animal feed (Li, 2007). The extensive root system of seabuckthorn plant offers environmental benefits in soil conservation and land reclamation.

Table 1. The component and uses of the plant parts of seabuckthorn.

Plant Part	Component	Uses
Fruits	Pulp, seed, juice	Food, drinks, pharmaceuticals
Pulp	Oil	Pharmaceuticals, cosmetics, pigment
Seeds	Oil	Pharmaceuticals, cosmetics
Leaves	Sterols, flavonoids, carotenoids	Pharmaceuticals, cosmetics, teas
Bark	Hippophan (5-hydroxytryptamine), proanthocyanidins	Pharmaceuticals
Roots		Soil conservation, land reclamation

Fruits contain many bioactive compounds including vitamin C, vitamin E, flavonoids and carotenoids. The seed and pulp oils are known for their fat-soluble vitamins, plant sterols, and essential fatty acids. The vitamin C content of seabuckthorn ranges from 150 to 2500 mg per 100 gram of fruits. The vitamin C content is dependent on the species, varieties (Yang, & Kallio, 2001), plant location, maturity of the fruits, harvesting time, altitude of growing environment and processing method of seabuckthorn, and is among the highest in the plant kingdom. Health Canada's recommended daily intake (RDI) of vitamin C for adult is 60 mg/day, which means an average Canadian only needs to consume 2.4-40 gram of fruit to meet the vitamin C requirement. Seabuckthorn is known for its high content of α-tocopherol (vitamin E) in its seed oil. An average adult needs to take about 5 g of seabuckthorn seed oil to meet the daily vitamin E requirement. Tocopherol is recognized as the natural antioxidant in the human body. It is believed that high levels of tocopherol minimize skin oxidation, which helps to maintain skin integrity and reduce skin toughening and wrinkling. Seabuckthorn oils are also believed to have a biological protective capacity. The tocopherols and carotenoids can trap and reduce the formation of UV-B induced toxic products in skin cells. Due to these UV-B absorptive properties, seabuckthorn oils may be used by industry as a natural sun screen (Yang, & Kallio, 2006).

Other unique constituents of the seabuckthorn are relatively high amount of unsaturated fatty acids, especially high amount of ω-3 fatty acid in its seed oil. The palmitoleic acid (ω-7) in the pulp oil is known to be the highest in the plant kingdom to date (Yang, & Kallio, 2002b). Table 2 shows the unique constituent in the seabuckthorn fruit or oils. High amounts of carotenoids, flavonoids, and sterols in seabuckthorn contribute to the unique color and antioxidant capacity of the fruits. The palmitoleic acid and carotenoid contents found in seabuckthorn oils are claimed to promote healing of skin burns and the relief of other skin ailments such as eczema and dermatitis. While high organic acid content contributes to the unique tart flavor of the fruits.

Table 2. Unique constituents of seabuckthorn

Constituent	Amount
Lipids: Unsaturated fatty acids: oleic acid (ω-9), palmitoleic acid (ω-7), palmitic acid and linoleic acid (ω-6), and linolenic acid (ω-3), Saturated fatty acids Sterols (mainly β-sitosterol)	6-15% of seed
Carotenoids, including β-carotene, lycopene, zeaxanthin	30-40 mg/100g of fruits
Flavonoids (e.g., mainly isorhamnetin, quercetin glycosides, and kaempferol)	100-1000 mg/100g of fruits
Organic acids (malic and quinic acids)	3.5-4.4 (% malic acid)

Seabuckthorn Processing

Food Development Centre (FDC) has developed an integrated process for seabuckthorn as shown in the flow chart (Figure 1). Seabuckthorn fruits can be air/freeze dried to produce dried fruits or sugar-infused fruits. The fruits can be processed by a pulper/finisher to produce puree, skin and seeds. Puree can be further clarified to clear juice by centrifugation and further processed to single strength juice or blended juice, beverage, jelly and wine. The skin and seeds can be separated by air-drying and sifting through a properly sized screen. The skin (dried pulp) can be incorporated to a nutritional bar or it can be used to extract the pulp oil and yellow pigment. The pigment can be extracted and concentrated by spray drying to yield a yellow powder. The powder contains mainly flavonoids and lower levels of carotene and vitamin E. This product can be used as a natural food colorant and/or as a supplement to boost nutritional values. The seeds are used to extract seed oil by different processing methods, which will be discussed in more details later in this article. The leaves of seabuckthorn can be dried to make tea, and some bioactive components can also be extracted from the leaves (Li, 2007). The bioactive components of bark also can be extracted by different methods (Xu, Xie, Pan, Yang, Wang, Cenkowski, Hydamaka, & Rao, 2006).

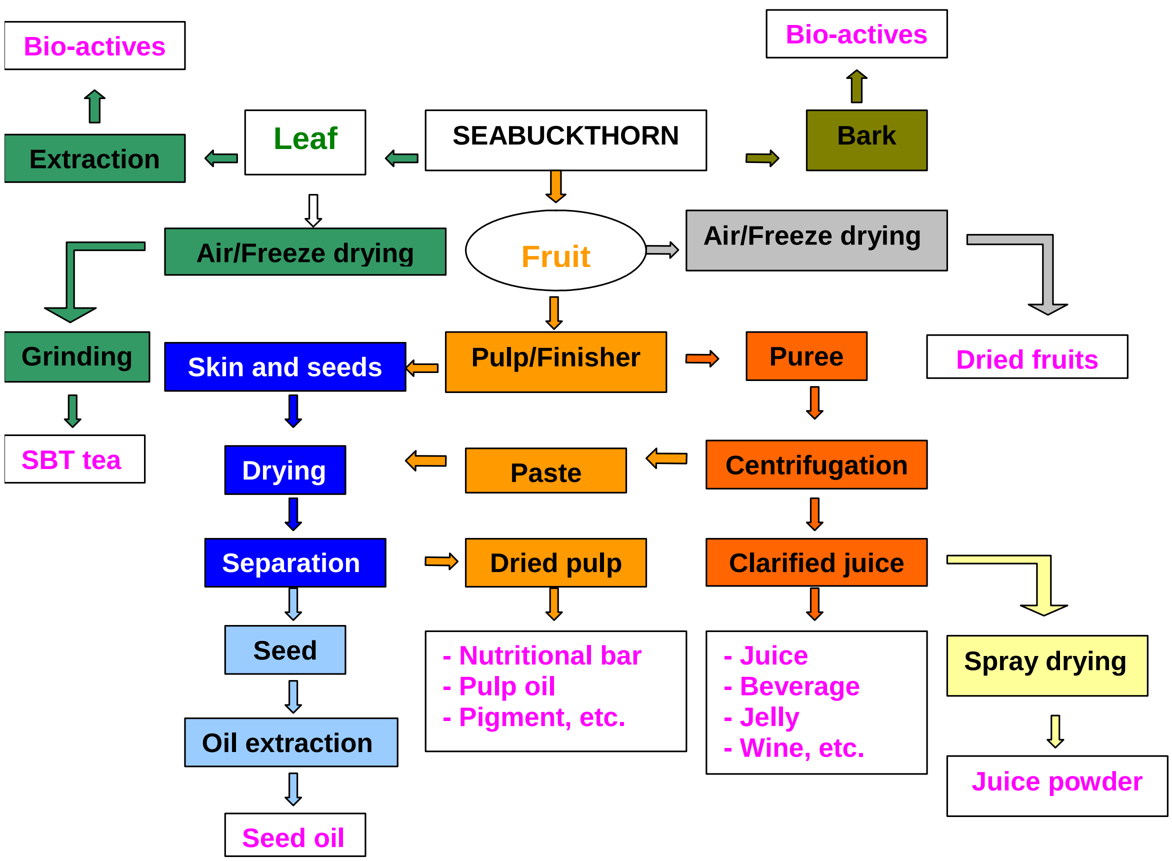

Figure 1. Flowchart of seabuckthorn processing at the Food Development Centre (Manitoba, Canada).

Processing of Seabuckthorn Juice

It is important to remove stems, leaves and other debris from the fruits, and wash the fruits if necessary. Ideally, these procedures should be done during harvest before storage. Juice extraction from seabuckthorn fruit is carried out by pressing technique, using a pulper/finisher. The use of a pulper/finisher ensures that all the fruits are crushed to release the juice without crushing the seeds. The usual press types utilize cloth presses, belt presses and screw presses. The use of both decanter and high-speed centrifuges for seabuckthorn juice has been reported. The decanter centrifuge technology allows for the continuous extraction of juice from fruits or other vegetables (Beveridge, 1997).

Another method for the juice extraction was also reported (Arimboor, Venugopalan, Sarinkumar, & Arumughan, 2006); the frozen seabuckthorn fruits are washed, thawed and crushed in fruit mill. The cold fruits are strained through sieves starting at 2 mm diameter and ending with 0.8-0.1 mm diameter. The last sieve retains the seeds and some peel, and this retenate can be separated into pulp and seed after drying. The remaining mash is heated to 50-60°C and is made up to 5-10% soluble solids with crystalline sugar. The soluble solids are thus increased enhancing easy separation. The mixture is allowed to stand for 1-3 hours and then separated by centrifugation into a turbid juice and pasty sediment. The pasty sediment is then treated with proteolytic, pectinolytic and cellulytic enzyme activities preparation for 2-6 hours at 55°C, and then separated by centrifugation.

Chemical Composition of Juice

The chemical composition of seabuckthorn juice varies with the fruit origin and climate, as well as the method of extraction. The juice yield by a pulper/finisher is about 65% for *sinensis* and 70% for Indian Summer (Wang, & Utioh, 2008). The typical characteristics of Indian Summer seabuckthorn juice are shown in Table 3 (Beveridge *et al*., 2002).

Table 3. Typical characteristics of seabuckthorn juice.

Component	Characteristic/Content
Colour	Yellow
Yield	57 - 75%
Soluble solids	9.3 – 15.5 ^{o}Brix
pH	2.7-3.13
Vitamin C	150-1500 mg/100mL
Organic acid (% malic acid)	3.5 - 4.4
Potassium	100-806 mg/mL
Sodium	17.7-89.8 mg/mL
Zinc	0.43-6.31 mg/mL

Juice Clarification

The juice extracted by pressing method is usually high in solid contents, yielding a very turbid product. The turbidity results from the presence of both insoluble solids and oil droplets suspended in the aqueous juice which produce a very complex juice system (Beveridge, Li, Oomah, & Smith, 1999). Fresh juice extracted from pulper/finisher can separate into three phases: an upper cream phase, juice in the middle, and sediment at the bottom. The upper cream phase can be removed by chilling the juice to below 4°C and skimming off the fat layer (Beveridge & Harrison, 2001). In some countries or area, pulpy juice is accepted by consumers. Clear juice can be produced by using a high speed disk stack type centrifuge or membrane filtration. In centrifugation, the sediment is removed from the bottom of the bowl by the de-sludging mechanism. Homogenization of the juice could remove any oil layer on the juice surface.

Membrane filtration, defined as the separation of components of a liquid or fluid using a thin barrier or film of material (membrane filter) through which fluids and solutes are selectively transported when a driving force is applied, can be used for clarification or concentration of seabuckthorn juice. In the case of seabuckthorn juice clarification, ultrafiltration (membrane filter with 10 to 100 nm pore size) is used. While seabuckthorn juice concentration can be carried out by reverse osmosis process. The advantage of the process is the removal of water without application of heat which allows for retention heat sensitive bioactives in the juice. Cheryan (1998) has reported in detail on the use of membrane filtration technology for clarification, concentration and deacidification of fruit juices. Vincze, Banyai-Stefanovits and Vatai (2007) have recently reported on seabuckthorn juice processing using a combination of microfiltration (MF) and reverse osmosis (RO1), and nanofiltration (NF) and reverse osmosis (RO2) without clarification.

Juice Pasteurization

High-temperature, short-time (HTST) processes, as the name implies, use higher temperatures and shorter times than conventional thermal processes to achieve pasteurization of foods and beverages. Because the products are exposed to high temperatures for short times, there is minimal degradation. In general, temperatures and times for HTST range from 72ºC for 15 sec used for pasteurization of milk to higher temperatures (above 135ºC) for 1-2 seconds. Use of temperatures above 135ºC is generally referred to as ultra-high-temperature (UHT) processing.

HTST processing has been used successfully for many years. HTST pasteurized seabuckthorn juice could turn brown if stored for more than 6 months at 15-20°C. Storage of the processed juice at 2-4°C will prolong the shelf-life for up to 12 months.

Spray Drying Process for Seabuckthorn Juice

Spray drying is a method for drying liquid foods or slurries in which the feed is converted into a fine mist or spray and is in contact with heated air in a drying chamber producing a dry powder. The slurry droplets have diameters in the range 10-300 μm and offering a large surface area to the drying air. Drying time is short, typically within 1-20 seconds. Evaporative cooling of the droplets helps to maintain the surface temperature close to the wet-bulb temperature of the drying air until drying nears completion (Brennan, 1994). The schematic of spray drying process is shown in Figure 2.

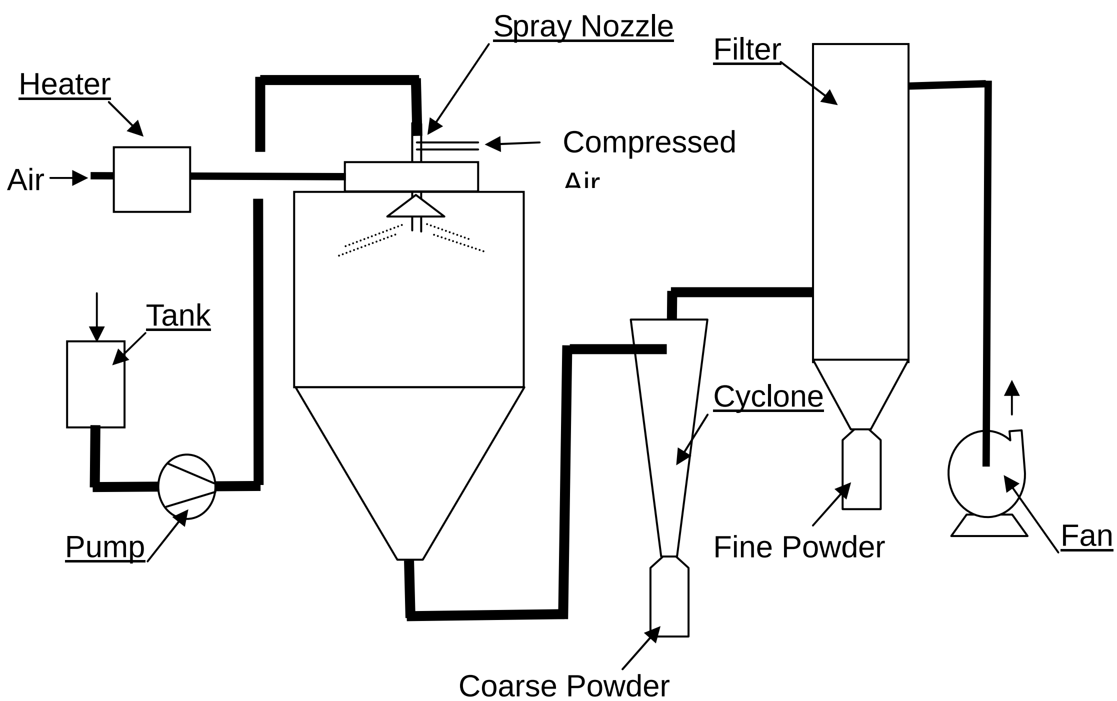

Figure 2. The schematic of spray drying.

The natural liquid state of seabuckthorn juice makes it difficult for incorporation into a wide range of dry food products, cosmetics and nutraceuticals. For this reason free-flowing seabuckthorn juice powder is a desirable product. Seabuckthorn juice powder is also more convenient and stable than the juice itself for prolonged storage; low moisture content greatly reduces microbial activity without the need for freezing or refrigeration. Spray drying is an ideal process for the continuous production of dry powder; a fluid is transformed into a dry powder in a single operation at low product temperatures with short drying times compared to other drying processes, while maintaining the natural nutrients, color and flavour of the hydrated product.

Food Development Centre (Manitoba, Canada) has investigated the possibility of developing seabuckthorn juice powder using spray drying technology and different drying aid materials. The seabuckthorn (SBT) juice was mixed with drying aid materials (corn syrup solids and/or maltodextrin). The mixture was homogenized using the colloid mill (Rheinfeldon CH4310) and this mixture was then spray-dried (Carlise Process System CPS600). The liquid mixture was atomized by a two-fluid nozzle at 4 bars of air pressure. Drying was carried out in a co-current configuration. Inlet air temperatures were 230 ± 1, and 235 ± 1 ˚C, and the outlet air temperature was 85 ± 1 ˚C.

The final product had an intense bright yellow colour and maintained the taste and aroma of seabuckthorn juice. Chemical and microbiological analyses of the spray dried seabuckthorn juice were preformed. Microbiological analysis included Standard Plate Count (SPC), coliforms, yeast and mould counts. All samples where plated in Petrifilm™ plates (3M Canada Inc. London, ON) specific for each of the micro organisms types. The resulting powder had the characteristics summarized in Table 4.

Table 4. Chemical and microbiological characteristics of spray dried seabuckthorn juice.

Characteristic	Value
Moisture Content (%)	4.7%
Starch Content (%)	54.05%
Standard Plate Count (CFU/g)	<10
Coliforms (CFU/g)	<10
Yeast (CFU/g)	5.9×10^3
Mould (CFU/g)	<10

The final product had low moisture content (4.7%) and low microbiological activity. The high amount of starch content (54.04%) in the powder is the result of the drying aid added to facilitate spray drying. The spray drying process and product quality depend greatly on the amount and type of carrier used. Different carriers have different properties of solubility, colour, taste and other important characteristics necessary for spray drying. Corn syrup solids which have a higher dextrose equivalent (DE) value do not possess enough encapsulation power as maltodextrins with lower DE value and produced a very noticeable difference in the quality of the final powder. The ratios of seabuckthorn juice and filler material could be varied depending on the product requirement. There is a strong possibility that using carriers with lower DE could reduce the amount of drying aid material. Further research is required to optimize drying aid to solid content ratio, as well as the drying parameters of the spray dryer for product quality optimization.

Processing of Seabuckthorn Seed Oil

Extraction technologies for seed oil include cold press, solvent extraction, supercritical fluid extraction, Friolex process and enzymatic process. Traditionally, oils are extracted from seed substrates by screw presses (cold press extraction), and more recently organic solvents such as hexane have been used because they are more efficient and provide higher oil yields (Li *et al*, 2007). In 2001, the U.S. Environmental Protection Agency issued regulations on the control of emissions of hexane gas due to its potential carcinogenic properties and environmental concerns. The concerns on the residual amounts of hexane in oils have stimulated research on other technologies for oil extraction. The use of supercritical carbon dioxide for extraction of oils destined for nutraceutical applications has become desirable, because removal of the extracting solvent is complete as residual carbon dioxide volatilizes on exposure to atmosphere.

Cold Press Process

A typical oil press consists of a rotating screw inside a horizontal cylinder. The screw forces the seeds through the cylinder gradually increasing the pressure. Heat is generated by friction or controlled heat is applied to help in the extraction process. It is recommended to heat to no more that 60 to 80 ^oC to maintain good oil quality. The extracted oil escapes from the cylinder through small holes, and the meal (by-product) is collected at the end of the cylinder. Material preparation such as flaking and/or preheating is important in improving oil yields. Figure 3 show a cross-section of oil press.

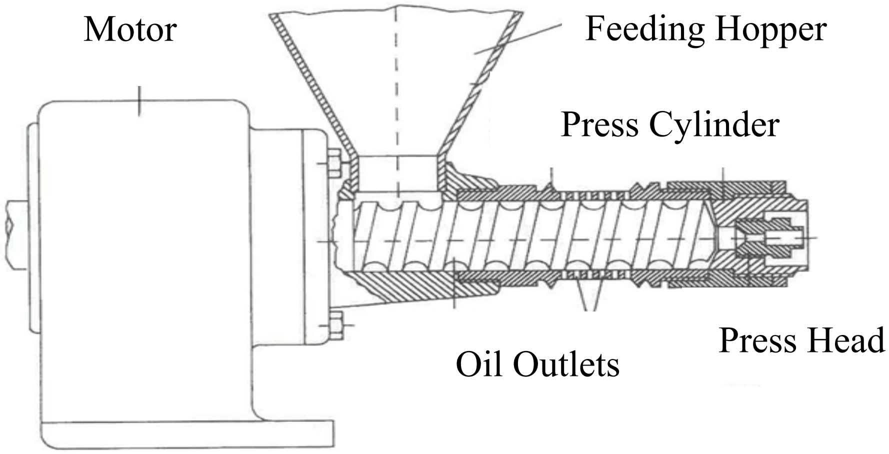

Figure 3: Cross-section of Komet oil press at FDC.

Supercritical Fluid Extraction

Supercritical fluid extraction is a unique separation process that uses properties of gas or liquid above its critical temperature and pressure for extraction. At supercritical condition, the fluid properties are between the gas and liquid. It has high density for good solvent power and low viscosity and high diffusivity for appreciable penetrating power. The most commonly used supercritical fluid for food and nutraceutical is carbon dioxide due to its non-toxic, non-explosive, cost-effective attributes. Carbon dioxide is available in high (food-grade) purity, and has a relatively low supercritical temperature (31 ^{o}C) and pressure (73.8 bar) and can be removed from the extracted products without leaving any residue (King, & Bott, 1993). Oil extraction by supercritical fluid technology is a one step process and allows for fractionation of the extracted oil for targeted components.

Water-based Oil Extraction Process

A water-based oil extraction (Friolex process) is a patented process by Westfalia which uses centrifugal technology for oil recovery. The process uses alcohol as a processing aid to break down the emulsion (water/oil) and a special decanter centrifuge is used for separating the oil from the meal. Oil impurities are removed in a high speed separator. Process is blanketed with an inert gas (nitrogen) to preserve product quality.

Cenkowski, Yakimishen, Przybylski, & Muir (2006) reported the effects of four oil-extraction techniques (solvent extraction using petroleum-ether, supercritical fluid extraction using carbon dioxide (SCFE CO_2), screw cold pressing, and aqueous extraction) on the nutritional quality of seabuckthorn seed and pulp oil for their fatty acids, tocopherols and tocotrienols, total carotenoids, and sterols content. The oil yields were compared against solvent extraction using chloroform/methanol as reference.

The concentration of fatty acids in oil extracted from seeds and pulp was similar in all tested extraction techniques. The predominant fatty acids were linoleic (35.3-36.3%) and linolenic (35.9-38.5%) acids in seed oil, and palmitic (34.4-35.5%) and palmitoleic (34.4-38.5%) acids in pulp oil. α-tocopherol (vitamin E) was the major tocopherol identified in the seed oil (43 to 53% of total tocopherols) and pulp oil (74 to 85% of total tocopherols) and the extracted quantity depended on the extraction technique used. Petroleum-ether extraction gave the highest total carotenoid concentration of 22 mg/100 g in seed oil and 527.8 mg/100 g in pulp oil. The lowest carotenoid concentrations were obtained with 3h-SCFE CO_2 (6.2 and 122.3mg/100g of oil from seed and pulp, respectively). β-sitosterol was the predominant sterol identified in the seed oil (97% range of total sterols for all extraction techniques) and pulp oil (96-98% of total sterols for extraction techniques tested). Petroleum-ether extraction consistently recovered oils having higher amounts of all analyzed nutritional components. Aqueous extraction and cold pressing methods were limited by the oil content of material which could be processed. No oil was recovered from seabuckthorn seeds by aqueous extraction and no oil was recovered from pulp by cold pressing. The SCFE CO_2 method was flexible in extracting both seed and pulp oils having relatively high concentrations of identified nutritional compounds (Yakimishen, Cenkowski, & Muir, 2005).

Processing of Seabuckthorn Leaves

Although the nutritional and medical properties of seabuckthorn fruits are usually the focus of attention, seabuckthorn leaves also contain a variety of nutrients and bioactive substances such as: carotenoids, sterols, flavoniods, fatty acids, minerals, vitamins and other phytochemicals (Mann, Petkau, Crowe, & Schroeder, 2003). Traditionally used in animal feed for rapid weight gain and a shiny coat (Schroeder, & Yao 1995), seabuckthorn leaves also are dried for herbal teas and processed for pharmaceutical and cosmetic applications (Li, 2007).

Drying of Seabuckthorn Leaves

There are two basic technologies employed in drying of seabuckthorn leaves: convection air drying and freeze drying. Convection air drying is the most basic yet effective and commonly used in the industry. Typically, equipment such as a forced-air tray dryer (CPM Wolverine Proctor, Horsham, PA) is used for convection air drying. Freeze drying, or lyophilization, is another drying technology though not commonly used for seabuckthorn leaves. Freeze drying is the process of removing water from a product by sublimation. Freeze drying is an attractive option for drying of heat sensitive high-value products.

The effects of air drying of seabuckthorn leaves on bioactive components have been studied by Guan, Cenkowski, and Hydamaka (2005) and St. George and Cenkowski (2007). The concentrations of total phenolics, carotenoids, and chlorophylls of fresh and dried seabuckthorn leaves (*sinensis*) were determined. Overall, drying of the seabuckthorn leaves resulted in a decrease in the concentrations of these phytochemicals. The degree of reduction depended on the drying time and temperature. For the phenolics, a greater reduction in concentration was observed in the leaves dried at higher temperatures (80 °C or 100 °C) for longer times (to equilibrium moisture contents of 1-3%) compared with those dried at lower temperatures (50 °C or 60 °C).

For the leaves dried to higher final moisture (5-8%), all drying temperatures resulted in a similar final phenolic concentration. The carotenoid and chlorophyll concentrations in the leaves decreased with the increasing temperatures. However, higher temperatures such as 80 °C or 100 °C resulted in similar carotenoid and chlorophyll concentrations in the leaves.

Summary

Processing technologies, both current and emerging, have significant impact on the quality of seabuckthorn products. It is important to evaluate the market potential of the products associated with each technology before implementation. More research is required on processing technologies in order to maximize the quality and yields of seabuckthorn products.

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Evaluation of Processing and Nutritional Attributes of Seabuckthorn Fractions of 'Indian Summer' and *Sinensis* Varieties

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Abstract

Seabuckthorn (SBT) fruits of both cultivars ('Indian Summer' and *sinensis*) produced in Canada were used to evaluate the processing technologies. Pilot processes were developed for puree extraction, pulp and seed drying and separation. Material balance of each processing procedure from fresh fruits to dried seeds and pulp was also determined. Samples of puree, pulp, seed, and oil from both cultivars were analyzed to establish their nutritional value. SBT seed oil of 'Indian Summer' variety was extracted by supercritical fluid, solvent and cold extraction. The yield and fatty acids profile of SBT seed oil extracted by different extraction methods were compared. It was found that SBT fruits can be successfully processed into major fractions of juice, pulp, seeds, and oil. Each fraction has its unique nutritional composition. Puree from 'Indian Summer' variety was about 5% higher yield and better appearance, flavor and smell compared with that from *sinensis*. The puree, pulp and seed from 'Indian Summer' had much higher fat, vitamin A and ω-7 fatty acids content than that from *sinensis*. The puree and pulp from *sinensis* had much higher sodium and vitamin C content than that from 'Indian Summer'. Pulp from 'Indian Summer' contains 23.5% of oil, compared to 13.7% from *sinensis* pulp. 'Indian Summer' pulp oil had 36.7% of palmitoleic acid (ω-7) in its oil, compared to 33.4% from *sinensis* pulp oil. Pulp oils from both varieties were outstanding for their high content of ω-7 fatty acid.

Keywords: processing, seabuckthorn, seed, pulp, puree, nutritional value, fatty acid profile

Introduction

Seabuckthorn (SBT) is known as *Hippophae rhamnoides* L. and belongs to the family *Elaeagnceae*. It is native to Europe and Asia. SBT is a medium deciduous shrub 2 to 4 meter in height and is very hardy with ability to withstand temperatures of -43 to 40°C (Bailey and Bailey, 1976). Most parts of the SBT plant can be used including the bark, leaves, fruits, and seeds.

A wide range of products can be made from the crop including: pharmaceuticals, cosmetics, teas, animal feed, sport and health drinks, jams and jellies, beverages, dyes, candy, liqueur etc.

SBT fruits are among the most nutritious and vitamin-rich fruits. They are rich in flavonoid, carotenoids, tocopherols, and contain appreciable amounts of water soluble and fat soluble vitamins. Fruits are also rich in proteins and free amino acids. A total of 18 amino acids have been found in seabuckthorn fruit (Beveridge *et al*., 1999). Products containing SBT fruits appear to promote cell tissue regeneration, thus aiding in the healing of wounds, and restoring skin tissue. They also enhance immune activity and disease resistance and destroy harmful free radicals found in human body. In Russia, SBT fruits are often used in home-made cosmetic formulations.

SBT oils extracted from seed and pulp have unique anti-aging properties and as a result, are becoming an important component in many facial creams manufactured in Asia and Europe (Li *et al*., 2007). In addition, the ultra violet (UV)-spectrum of the oil shows a moderate absorption in the UV-B range which makes seabuckthorn derived products attractive for sun care cosmetics. SBT oils contain very high amount of palmitoleic acid, which is a component of skin fat and can support cell tissue and wound healing (Yang and Kallio, 2002). SBT oil is also good for hair care and nail care preparations.

The existing knowledge shows that the composition of seabuckthorn fruits varies greatly according to their varieties and climatic and geological conditions of the growth areas (Yang and Kallio, 2001). Beveridge *et al*. (2002) have reported on the processing effects on the composition of seabuckthorn juice, that research was focused on the juice organic acid. Kallio *et al*. (2002) reported the triacylglycerols, glycerophospholipids and tocopherols in fruits and seeds of seabuckthorn from *sinensis* and mongolica subspecies. Tiitinen *et al*. (2005) reported organic acid and sugar difference among the Finish seabuckthorn varieties. The objective of this research is to evaluate the processing and nutritional attributes of seabuckthorn fractions, including juice, dried seed and dried pulp of ‘Indian Summer’ and *sinensis* varieties. Both ‘Indian Summer’ and *sinensis* are the most popular varieties grown in Canada including Manitoba.

Materials and Methods

Seabuckthorn fruits

Seabuckthorn fruits (‘Indian Summer’ variety) were harvested from mature shrubs at Winkler, Manitoba in 2005 and seabuckthorn *sinensis* fruits were harvested from Branching-Out Orchards, St. Claude, Manitoba in 2005. Immediately after harvesting, the fruits were hand cleaned to remove visible debris, namely dried leaves, branches, and damaged fruits. Cleaned fruits were packed in 12-13 kg portions and were put in cardboard boxes. The fruits remained frozen (at about -15 °C) during transportation from Winkler or St. Claude to Portage la Prairie within 1 hour. The fruits were stored in a walk-in freezer at -20 °C and all samples were used within 8 months.

Pilot processing for juice, seeds and skin separation

Juice extraction

All fruits were initially thawed to about 10 °C at room temperature. Thawed fruits were passed through a stainless steel pulper/finisher (Langsenkamp, Indianapolis, IN), the juice, skin and seeds were collected separately.

Drying

Wet fruit seeds and skin were spread onto perforated drying trays (10 mesh or 1.91 mm perforation size) giving a drying layer of approximately 10 mm. The wet cake was dried at 50 °C for 48 h in a ventilated drying oven (Gas Fired Variable Circulation Laboratory Dryer, Proctor and Schwartz Corporation, Philadelphia, PA).

Separation of seed and skin

The dries seed and skin were carefully removed from the drying trays and emptied into an industrial mixer in approximately 2 kg batches. An industrial mixer (Hobart Cutter Mixer, HCM 300, Hobart Corporation, Troy, OH) equipped with a plastic, two-blade attachment with 1140 rpm served as a threshing unit to gently separate seeds from pulp and remove the white seed skin encapsulating the seeds. The mixer was operated for short time intervals for 1-2 min. This was done to ensure that seeds did not become damaged during threshing.

A vibratory screen separator LS 24S444 (SWECO, Sweco Canada, Toronto, ON) equipped with a stackable arrangement of three screens was assembled for the separation of seeds from pulp. Screens were arranged (top and bottom layer screen openings of 6- mesh (or 3.35 mm) and 10-mesh (or 1.91 mm), respectively to collect three fractions including, debris with pulp, seeds and skin (pulp). When it was necessary, the seeds were further cleaned using compressed air to remove residual pulp that was not removed by mechanical separation.

The seeds were ground using a food processor (Osterizer Blender, Galaxie, ON) for 1 min. Ground seeds were used to extract oil by supercritical fluid extraction (SC-CO_2) and solvent extraction by Soxhlet method. The whole seeds were used to extract seed oil by cold press. A detailed flow chart is shown in Figure 1.

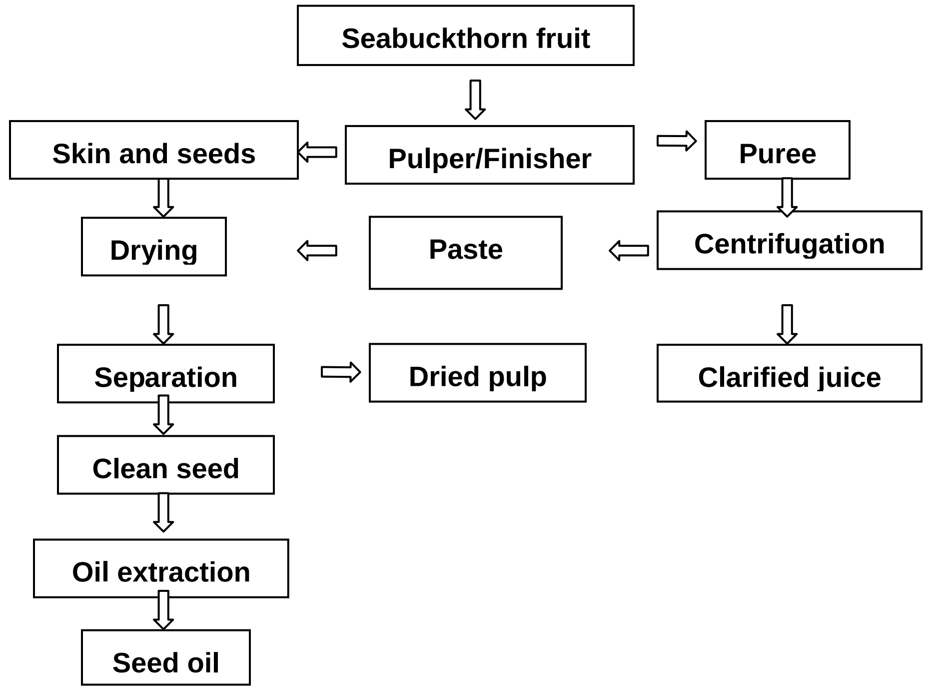

Figure 1: Flow chart for processing of seabuckthorn fruits.

Nutritional analysis

The nutritional analysis of three fractions, seed, skin and juice were conducted at SGS Canada Inc. in Vancouver. All the analytical methods were followed the AOAC (1990) or AOCS procedures (AOCS, 2000).

Statistical analysis

SC-CO_2 extraction of seabuckthorn seed oil at each temperature and pressure condition was carried out in triplicate. All analyses were conducted using Statistical Analysis Program System version 8.2 for Windows (SAS Institute Inc., Cary, NC, USA, 2003).

Results and Discussion

Seabuckthorn processing

Total of 871 kg of 'Indian Summer' and 360 kg of *sinensis* have been processed at Food Development Centre. The yields of seabuckthorn fractions from 'Indian Summer' and *sinensis* varieties are shown in Table 1. The yield of puree from 'Indian Summer' was around 75% which was about 5% higher than that from *sinensis*. The yield of clarified juice from 'Indian Summer' was about 73% which was also about 5% higher than that from *sinensis*. However, the yield of dried (pulp) was about 4.3% higher from *sinensis*. The yields of dried seed from 'Indian Summer' and *sinensis* were similar, about 5%. There is no published comparison on the yield of seabuckthorn fractions from 'Indian Summer' and *sinensis* varieties processed on pilot scale.

Table 1. Yield of seabuckthorn fractions from 'Indian Summer' and *sinensis* varieties.

	'Indian Summer' (%)	*sinensis* (%)
Puree	75.31 ± 0.76	69.64 ± 2.41
Clarified juice	73.00 ± 1.24	67.23 ± 2.09
Dried skin	2.48 ± 0.23	4.36 ± 0.08
Dried seed	5.32 ± 0.52	5.21 ± 0.43
Dried whole fruit	0.06 ± 0.01	0.72 ± 0.56

Nutritional value of seabuckthorn fractions

The nutritional value of seabuckthorn fractions are shown in Table 2. Seabuckthorn juice contains considerably amount of protein in their juice, there was no significant difference between ‘Indian Summer’ and *sinensis*. Dried pulp from ‘Indian Summer’ contains less protein compared with that from *sinensis*, however, dried seed from ‘Indian Summer’ was higher in protein compared with that from *sinensis*. The fat content from ‘Indian Summer’ was significantly higher in those three fractions compared with that from *sinensis*. The higher fat content in ‘Indian Summer’ variety made it more desirable for pulp and /or seed oil extraction.

Seabuckthorn also contained higher amount of fiber, ranging from 31 to 39% in the pulp and from 41 to 46% in the seed. Generally speaking, seabuckthorn from *sinensis* variety contains higher amount of fibre compared to from ‘Indian Summer’.

Another interesting observation is that *sinensis* variety contained almost 6-10 time as much sodium compared with that from ‘Indian Summer’. It was noted that the iron content was significantly higher in seabuckthorn juice from both varieties compared with other fruit or vegetable juice, such as orange juice, apple juice and tomato juice, etc.

Juice and dried pulp from ‘Indian Summer’ had twice as much vitamin A content compared to *sinensis*. However, juice and pulp from *sinensis* had several times higher vitamin C content than that from ‘Indian Summer’.

Table 2. Nutritional value of seabuckthorn juice, dried pulp and dried seed.

	Juice		Pulp		Seed	
Tests	‘Indian Summer’	*sinensis*	‘Indian Summer’	*sinensis*	‘Indian Summer’	*sinensis*
Engery (cal/100g)	53	49	481	429	431	412
Engery (KJ/100g)	222	204	2013	1796	1805	1722
Protein (N x 6.25) (g/100g)	0.6	0.6	6.8	11.9	29.8	24.7
Fat (g/100g)	1.4	0.8	23.53	13.73	12.9	9.1
Saturated Fat (g/100g)	0.6	0.3	8.85	4.87	2.03	1.22
Monosaturated Fat (g/100g)	0.7	0.4	11.12	6.21	2.98	2.13
Polyunsaturated Fat (g/100g)	0.1	0.1	3.56	2.53	7.92	5.75
Trans Fat (g/100g)	< 0.1	< 0.1	< 0.1	0.12	< 0.1	< 0.1
Cholesterol (mg/100g)	< 1	< 1	< 1	< 1	< 1	< 1
Omega 3	0.00	0.00	0.65	0.51	3.78	2.13
Omega 6	0.10	0.10	2.91	2.02	4.14	5.75
Omega 7	0.50	0.20	8.63	4.58	0.70	0.15
Carbohydrates (g/100g)	9.5	9.8	60.6	64.6	49	57.7
Total Sugar (g/100g)	3.4	4.4	7.9	6.9	4.1	5.3
Total Dietary Fibre (g/100g)	<1.0	<1.0	31.0	39.6	41.2	46.5
Sodium (mg/100g)	2.9	27.8	12.0	74.4	6.5	53.7
Calcium (mg/100g)	10.9	8.3	94.6	95.4	53.1	75.5
Iron (mg/100g)	1.1	0.9	5.8	13.9	5.3	6.5
Potassium (mg/100g)	192				677	475
Moisture (g/100g)	88.2	88.5	7.0	8.3	6.3	6.8
Ash (g/100g)	0.3	0.3	2.1	1.5	2	1.7
Vitamin A (IU/100g)	636	308	8212	4397	< 10	< 10
Vitamin A (RE/100g)	191	93	2464	1319	< 10	< 10
Vitamin C (mg/100g)	187	450	442	743	2	2.6
Beta Carotene (IU/100g)					1459	2346

Fatty acids from different fractions

Generally speaking, 'Indian Summer' variety contained more fatty acid as a result of higher fat content compared to that from *sinensis* (Table 3). Table 4 shows the percentage of fatty acids in the three fractions. Juice and dried pulp had similar fatty acid profile which contains higher 16 carbon fatty acids: palmitic acid and palmitoleic acid, account for about 63-79% of total fatty acid. Palmitoleic acid (ω-7) is a unique fatty acid found in the seabuckthorn juice and pulp (Yang and Kallio, 2002). Its content was about 25-36% in juice and 33-37% in dried pulp. The palmitoleic acid (ω-7) content was higher in the juice and dried pulp of 'Indian Summer' variety.

Seabuckthorn dried seed contained more 18 carbon fatty acid than its juice and pulp, accounted for about 60% of total fatty acid. The seed also contained considerable amount of linolenic acid which belongs to ω-3 fatty acid. The ratio of ω-3 to ω-6 fatty acid in seabuckthorn seed was close to 1:1.

Table 3. Fatty acids from different fractions.

	Juice		Pulp		Seed	
Tests	'Indian Summer'	*sinensis*	'Indian Summer'	*sinensis*	'Indian Summer'	*sinensis*
Myristic Acid C14:0					0.03	0.02
Myristoleic Acid C14:1, ω-5				0.04		
Palmitic Acid C16:0	0.6	0.30	8.58	4.62	1.5	0.93
Palmitoleic Acid C16:1, ω-7	0.5	0.20	8.63	4.58	0.7	0.15
Heptadecanoic Acid C17:0					0.03	
Stearic Acid C18:0			0.27	0.21	0.41	0.22
Oleic Acid C18:1, ω-9	0.2	0.20	2.49	1.63	2.25	1.96
Linoleic Acid C18:2, ω-6	0.1	0.10	2.91	2.02	4.14	3.19
Linolenic Acid C18:3, ω-3			0.65	0.51	3.78	2.56
Arachidic Acid C20:0					0.06	0.05
Methyl transmyristelaidate C14:1T				0.12		
Eicosenoic Acid C20:1					0.03	0.02

Table 4. Fatty acid profile presented as percentage.

	Juice		Pulp		Seed	
Tests	'Indian Summer'	*sinensis*	'Indian Summer'	*sinensis*	'Indian Summer'	*sinensis*
Palmitic Acid C16:0	43	38	36	34	12	10
Palmitoleic Acid C16:1, ω-7	36	25	37	33	5	2
Stearic Acid C18:0			1	2	3	2
Oleic Acid C18:1, ω-9	14	25	11	12	17	22
Linoleic Acid C18:2, ω-6	7	12	12	15	32	35
Linolenic Acid C18:3, ω-3			3	3	29	28

Seed oil fatty acid profile by different extraction methods

Seabuckthorn seed oil ('Indian Summer' variety) was extracted by supercritical, Soxhlet and cold press methods. The seed oil recovery by different method is shown in Figure 2. The seabuckthorn seed oil recovery by supercritical and cold press were 90.2% and 40% respectively, based on the assumption that solvent extraction was 100%.

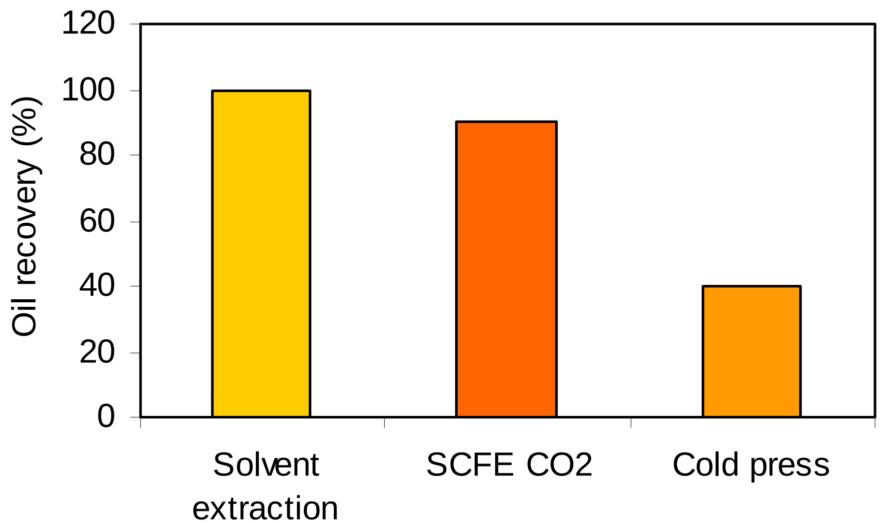

Figure 2. Oil recovery by different extracting methods.

The oil extracted by these methods was analyzed and the results are shown in Table 5. Overall, seabuckthorn seed oil had a similar fatty acid profile regardless of the extracting methods. However, the alpha-tocopherols content was higher when supercritical-CO_2 or solvent extraction was used as compared to cold press.

Table 5. Fatty acid profile of seabuckthorn seed oil extracted by different extraction methods.

Component Name (g/100g)	$SC\text{-}CO_2$ 300 bar/40°C	Soxhlet extraction	Cold press
Myristic Acid C14:0	0.28	0.23	0.15
Myristoleic Acid C14:1, ω-5	0.13	ND	0.11
Palmitic Acid C16:0	11.15	11.60	9.44
Palmitoleic Acid C16:1, ω-7	6.25	5.41	4.46
Heptadecanoic Acid C17:0	0.45	0.23	ND
Heptadecenoic Acid C17:1	0.30	ND	ND
Stearic Acid C18:0	2.56	3.17	2.71
Oleic Acid C18:1, ω-9	15.35	17.40	15.41
Linoleic Acid C18:2, ω-6	31.39	32.02	33.03
Gamma Linolenic Acid C18:3, ω-6	0.10	ND	0.12
Linolenic Acid C18:3, ω-3	31.1	29.23	33.87
Arachidic Acid C20:0	0.40	0.46	0.42
Eicosenoic Acid C20:1, ω-9	0.18	0.23	0.17
Eicosadienoic Acid C20:2	0.06	ND	ND
Behenic Acid C22:0	0.11	ND	0.11
Omega 3 Fatty Acids	31.1	29.23	33.87
Omega 6 Fatty Acids	31.9	32.02	33.15
Trans Fatty Acids	0.11	0.10	ND
Alpha Tocopherols (IU/100g)	260.2	224.0	72.0

ND: not detectable

Seabuckthorn fruits can be commercially processed into puree (or juice), pulp, seeds, and seed oil. The yield of puree form 'Indian Summer' was about 5% higher than that from *sinensis*. 'Indian Summer' juice had better appearance, flavour and aroma compared with that from *sinensis*. Puree, pulp and seed from 'Indian Summer' had higher fat and ω-7 fatty acids content than that from *sinensis*. Juice and pulp from 'Indian Summer' had higher vitamin A content than that from *sinensis*. However, puree and pulp from *sinensis* had higher sodium and vitamin C content than that from 'Indian Summer'. Dried pulp from 'Indian Summer' contains 23.5% of oil, compared to 13.7% from *sinensis* pulp. 'Indian Summer' pulp oil contains 36.7% of palmitoleic acid (ω-7), compared to 33.4% from *sinensis*. Pulp oils from both varieties contain high content of ω-7 fatty acids, which makes seabuckthorn fruit the most important sources of palmitoleic acid in the plant kingdom. Seabuckthorn seed oil can be extracted by different methods and their yield and quality vary depending on the applicable extraction technology.

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Seabuckthorn (*Hippophaë rhamnoides* L.) functional powders: drying methods and quality retention

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Abstract

Convective hot-air drying and freeze-drying were investigated in this study as potential processes to obtain powders of seabuckthorn fruit pulp. Halved seabuckthorn fruits (10 g) were placed in a thin layer in a hot-air drier, and dried at 1 m/s and at 50 or 60°C, or freeze-dried at less than 30 mTorr and at 20 or 50°C shelf plate temperature. An initial characterization of the seabuckthorn pulp (moisture, pH, soluble solids, vitamin C and E, total phenolics and carotenoids) was performed. Water loss was determined at different processing times. The contents of phenolic compounds, total carotenoids, vitamin E and C were determined after drying.

Freeze-drying kinetics was faster than air-drying, probably due to a slower diffusion of moisture in the compact, sugary and oily structure of the air dried tissue. The temperature had an important effect on hot-air drying and freeze-drying kinetics. Drying method and processing times affected the remaining phenolics, carotenoids and vitamin contents of seabuckthorn fruit. Freeze-drying was revealed a superior method–to obtain seabuckthorn powders because of the lower residual moisture content, the ease of grinding as well as the better nutritional retention.

Keywords: seabuckthorn powders, nutraceutical preservation, carotenoids, phenolics compounds, hot-air drying, freeze-drying.

Introduction

Seabuckthorn (*Hippophaë rhamnoides* L.) fruits contain high amounts of natural antioxidants and medicinal compounds including ascorbic acid, carotenoids, flavonoids as well as essential fatty acids [1, 2]. However, seabuckthorn fruits are delicate and, if not properly processed, they have a short shelf life. Transforming these fruits into powders may not only preserve them for longer times but also concentrate their already high nutritional values. One potential problem with dehydration of seabuckthorn fruits is, however, their waxy impermeable skin which impedes the moisture loss. Several pretreatment methods (chemical, mechanical and thermal) have been previously used to overcome this water barrier during drying of different fruits, such as blueberries and cranberries [3]. Thus, the development of an efficient and appropriate drying process is of significant importance to obtain seabuckthorn functional powders.

A functional powder is defined as a dry solid in the form of tiny loose particles designed to provide a specific and beneficial physiological effect on health, performance and/or well-being extending beyond the provision of simple nutrients. The main processes involved in functional powder production are thus drying and grinding. Air-drying is a traditional method of food conservation, which provides an extension of shelf-life, lighter weight for transportation and smaller storage space. This method has been thoroughly applied to dry fruits and pieced foodstuffs [4]. However, it is well known that the quality of an air-dried product is strongly affected by the process particular parameters, such as high temperature, oxygen presence and cell damage. Air-drying can cause changes in the physical properties such as color and structure, as well as the deterioration of aroma compounds or degradation of nutritional substances, thus reducing the product quality. This process is, however, one of the less costly in terms of energy consumption and equipment provision when compared to other dehydration processes (i.e. spray or freeze-drying, etc.). Freeze-drying is based on the dehydration by sublimation of a frozen product. Compared to hot air-drying, freeze-drying can yield high quality products because most of the deterioration reactions are slowed down or practically stopped (i.e. minimization of flavor

and aroma compounds, maximization of nutrient retention, porous structure) due to the absence of liquid water, the absence of oxygen under vacuum and the use of low temperatures. Nevertheless, its production cost is approximately 8 and 4 times higher than conventional air-drying and spray drying, respectively [5].

Oxygen, high temperature and cell damage are usually seen as enemies of nutritional retention during processing. In fact, phenolics compounds could be susceptible to enzymatic degradation during air drying due to the polyphenols oxydases (PPO) activity [6]. On the other hand, carotenoids have a highly unsaturated nature, making them susceptible to degradation by oxidation and thermal processes. Oxidation is the major cause of carotenoids degradation. However, little information on the mechanism involved can be found in the literature. The oxidation of carotene is generally considered to be auto-catalytic, beginning only after an induction period in which radicals are built up and antioxidant depleted [7].

Therefore, the main objective of this study was to investigate the effect of convective hot air-drying and freeze-drying as methods to obtain powders of seabuckthorn pulp. Loss of vitamins C and E, carotenoids and phenolics compounds were measured to test the effect of drying methods on the nutritional characteristics of seabuckthorn fruits. To complete this work, a sorption isotherm at 20oC of air and freeze dried powders was determined.

Material and methods

Material preparation

Seabuckthorn fruits (var. 'Indian Summer') harvested in August 2006 in a farm located in Ste-Anne de Beaupré (Québec, Canada) were used in this project. The fruits were frozen at -18°C immediately after harvesting. The initial moisture, pH, °Brix, titratable acidity, vitamin C, vitamin E, carotenoid and phenolic contents were determined as described later. For the drying experiments, frozen fruits were manually cut into halves and the grains, removed.

Drying experiments

Seabuckthorn samples were dried in a laboratory hot-air tray dryer (Model UOP8-G, Armfield, Hampshire, England) under constant conditions of 50°C and 60°C, and 1 m/s air velocity. Air speed and temperature were measured continuously using an anemometer (LCA 6000, Airflow Development Ltd, Andover, NJ, USA) and T-type thermocouples (Omega Engineering Inc., Laval, Canada), respectively. On the other hand, seabuckthorn samples were freeze-dried in a laboratory freeze-dryer (Freeze-mobile 25L, Virtis Company, NY, USA) at constant heating plate temperatures (20°C and 50°C) and under less than 30 mTorr. Drying curves were obtained by periodic weighing of seabuckthorn samples at different processing times: 2, 4, 6, 8, and 15 hours. Dried seabuckthorn fruits were stored immediately after hot-air or freeze-drying in desiccators in the presence of P_2O_5 for further analysis. The moisture content, vitamin C, carotenoids and phenolics compounds of the dried samples were determined as a function of drying time as described later. Vitamin E was determined after 15 h air and freeze drying.

Sorption isotherms

Freeze-dried and convective-dried samples (approximately 300 mg) were placed over saturated salt solutions (LiCl, NaCl, NaBr, KCl, $MgCl_2$, CH_3COOK) in desiccators at constant temperature (20°C). Final moisture contents were determined when moisture equilibrium was achieved (after 7 days approximately).

Physico-chemical analysis

Moisture content (g water/g dried matter) was calculated using values of dried mass determined by the vacuum oven method [8]. Sea buckthorn samples were placed in a vacuum oven with the presence of P_2O_5 as desiccant. The oven temperature was 50°C with a gauge pressure of 25 in Hg. The samples were kept for 48 h and then taken out of the oven, cooled in a desiccator at room temperature and weighed using a balance (Model Mettler Toledo AB104-S, Greinfesee, Switzerland) with a sensitivity of 0.001g.

Fruits were evaluated for acidity according to the method described by Tang and Tigerstedt [9]. The frozen fruits (40 g) were manually cut in halves and the grains, removed. The fruits were homogenized with 100 ml of water. The homogenate was diluted to 300 ml and boiled for 30 min. After cooling, the homogenate was made up to 300 ml by adding water and then filtered (No.1 Whatman). The filtered aliquot (10 ml) was used for

further analysis. Titratable acidity (TA) was determined by titration with 0.1 N NaOH solution up to the end point of phenolphthalein. The Titratable acidity (TA) was conventionally expressed as malic acid (%).

For solid soluble content (°Brix) and pH determinations, frozen fruits (25 g) were manually cut in halves and the grains were removed. The fruits were crushed and homogenized during 5 minutes (until obtaining a homogeneous juice) using an Ultra-Turrax homogenizer. The juice obtained was then filtered (No.1 Whatman). The filtered aliquots were used for pH and °Brix analysis. The solid soluble content (°Brix) was determined using a digital refractometer Reichert AR 200 (Reichert Inc., Depew, NY, USA) and for pH determination, a pH-meter model SP20 (VWR Symphony, Thermo Orion West Chester, PA, USA).

Carotenoids and phenolics compounds were determined according to the method described by Gao *et al*. [10], with slight modifications. For carotenoids, 1 ml aliquot from lipophilic extract was diluted with 4 ml of hexane and measured at 460 nm (Spectrophotometer Model 8451A, Hewlett-Packard, CA, USA). Quantification was carried out with a calibration curve obtained with a β-carotene standard (Fluka Biochemika, Milwaukee, WI, USA) diluted in hexane. Total carotenoids were expressed as mg/100mg β-carotene equivalent. On the other hand, phenolics extract (100 μL) was mixed with 0.2 ml Folin-Ciocalteau reagent, 2 ml of water and 1 ml of 15% Na_2CO_3, and the absorbance was measured at 765 nm after 2 h incubation at room temperature. Gallic acid (Sigma-Aldrich, Oakville, ON, Canada) was used as standard and the total phenolics were expressed as mg/100g gallic acid equivalent. For the determination of phenolic and carotenoid compounds in dried samples, the fruit was rehydrated to its initial moisture content. Compound retention was determined by dividing the concentration after processing over the initial compound concentration.

Vitamin C was determined according to the method described by Askar and Treptow [11]. Vitamin C was expressed as mg per 100 g fruit. For Vitamin C determination in dried samples, the fruit was rehydrated to its initial moisture.

For the determination of vitamin E, lipid extraction was realized according to the method described by Kallio *et al*. [12]. The extracted lipids were dissolved in 3mL of hexane and analyzed by GC-MS method. Samples were injected into a GC-MS (Model 5890 series 2, Hewlett-Packard, CA, USA) coupled to a MS (Model 5972, Hewlett-Packard, CA, USA) assembled with a ZB-5 0.25x 30m column (Phenomenex, Torrence, CA, USA). The flow rate of the carrier gas (He) was 1mL/min. The split valve with a ratio of 1:20 was opened after 1 minute. The temperature program was initially held for 2 minutes at 150°C, then increased at a rate of 25°C/min to a final temperature of 280°C and held for 10 minutes. The injector temperature was programmed at 280°C and the MS temperature was 300°C. Vitamin E was identified and quantified by comparison with a standard mixture of known composition.

Results and Discussion

The initial composition of seabuckthorn fruits is presented in Table 1. Values obtained in this work are in agreement with literature values for the same cultivar [1, 10, 13].

Table 1. Nutritional and chemical composition of seabuckthorn fresh fruit.

Attribute	Range
pH	2.57 ± 0.03
°Brix	7.91 ± 0.57
Titratable acidity (% malic acid)	1.71 ± 0.04
Moisture content (g/100g) (wb)	87.01 ± 0.12
Vitamin E (mg/100g)	10.85 ± 0.40
Vitamin C (mg/100g)	184.63 ± 23.15
Total phenolics (mg/100g)	175.25 ± 0.24
Total carotenoids (mg/100g)	3.99 ± 0.14

Typical hot-air drying curves for seabuckthorn pulp at 1 m/s air velocity and at different air temperatures is shown in Figure 1. The moisture content decreased exponentially with drying time and thus, hot air drying seemed to take place in the falling rate period. This behavior is in agreement with other results published in the literature on drying of fruits and vegetables [14]. Temperature had a significant effect on the acceleration of seabuckthorn hot-air drying curves.

Freeze-drying curves of seabuckthorn pulp at different heating-plate temperatures (20°C and 50°C) are shown in Figure 2. Moisture content of seabuckthorn samples decreased exponentially with freeze-drying time. A dry layer having high resistance to heat and mass transfer increased within the product during freeze-drying, causing the drying rate to slow down at the end of the process [15].

Both drying methods had an important effect on the remaining moisture content of seabuckthorn samples. However, freeze-drying removed water much faster than hot air-drying (Figures 1 and 2). Freeze-drying kinetics was almost three-fold faster than hot-air drying at comparable temperatures. As an example, the drying time to obtain X/X_0= 0.2 was approximately 6 h for hot-air drying at 50°C while for freeze-drying, 2 h.

An increase in freeze-drying temperature accelerated drying rate and ice sublimation. Moisture content decreased significantly when the heating plate temperature increased from 20°C to 50°C. As an example, after 6 hours of freeze-drying at 50°C, the final moisture content of seabuckthorn samples was 0.064 (g water /g dry mass) and 0.149 (g water /g dry mass) at 20°C (Figure 2). The sublimation duration was already reported to decrease with an increase in temperature during freeze-drying [15, 16].

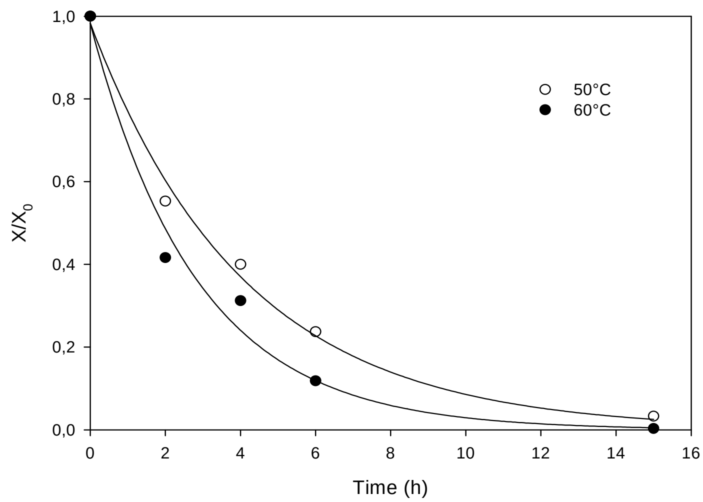

Figure 1. Drying curves of seabuckthorn samples under hot air-drying conditions at 1 m/s air velocity.

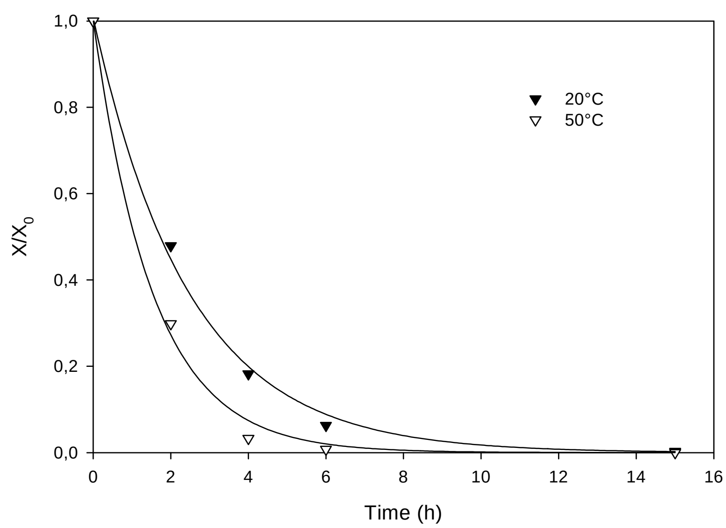

Figure 2. Freeze-drying curves of seabuckthorn samples at 20°C and 50°C.

Table 2 shows the compound retention of the seabuckthorn fruits after 15 h freeze or hot-air drying. The retention of bioactive compounds was more important in freeze-dried samples than hot-dried ones. In fact, freeze-drying keeps double amount of carotenoids, 20% more vitamin C, 10% more phenolics and approximately the same amount of Vitamin E than air-drying. The drying temperature did not show a clear impact on final nutritional retention neither in freeze-dried or hot-dried seabuckthorn samples. Compared to hot air-drying, freeze-drying can yield high quality products because most of the deterioration reactions are slowed down or practically stopped (i.e. minimization of flavor and aroma compounds, maximization of nutrient retention, porous structure) due to the absence of liquid water, the absence of oxygen under vacuum and the use of low temperatures [5, 17].

Table 2. Compound retention (c/c_0) of seabuckthorn fruits after 15 hours drying.

Compound	Air-Drying		Freeze-Drying	
	50°C	60°C	20°C	50°C
Moisture content (dry basis)*	0.25	0.06	0.02	0.01
Vitamin C	0.67	0.61	0.81	0.90
Vitamin E	0.70	0.65	0.66	0.59
Total carotenoids	0.36	0.45	0.78	0.79
Total phenolics	0.89	0.86	0.96	0.99

* (g water/g dry mass)

The sorption equilibrium isotherms of freeze-dried and hot-air dried seabuckthorn samples are shown in Figure 3. Both curves showed a type-II sigmoid adsorption form. Freeze-dried seabuckthorn powders showed a slight higher water sorption than hot-air dried ones, indicating that the former powders will be more hygroscopic during storage. The porous structure generated in the products during freeze-drying can certainly explain their higher water sorption.

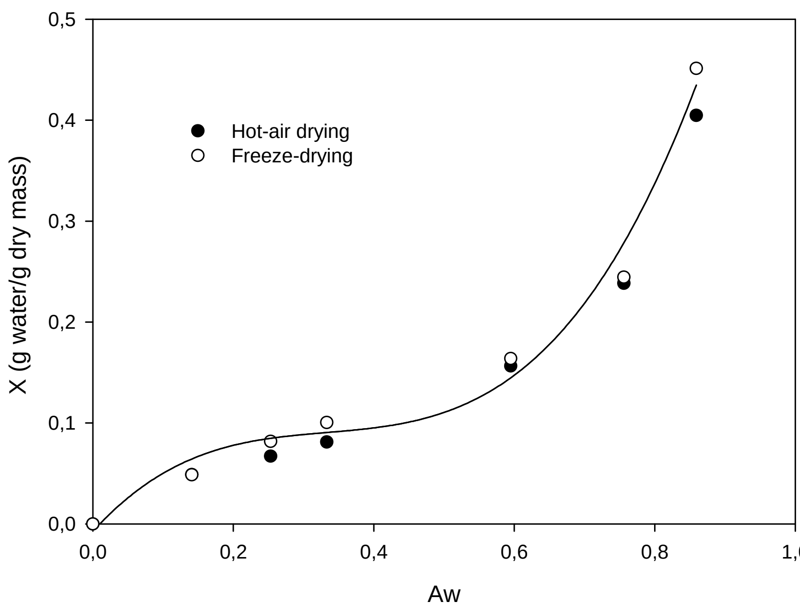

Figure 3. Experimental sorption isotherms of hot-air and freeze-dried seabuckthorn at 20°C.

Conclusion

The influence of drying processes was evaluated to preserve and concentrate bioactive compounds in seabuckthorn fruits. It is possible to make a powder out of seabuckthorn by freeze-drying depending on the mechanical pre-treatment step. Both hot air-drying and freeze-drying had an important effect on the remaining moisture content of seabuckthorn samples. Seabuckthorn freeze-drying kinetics was much faster than air-drying. The bioactive compounds retention was more important in freeze-dried samples than hot air-dried ones. Seabuckthorn powders were hygroscopic.

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Supercritical Fluid Extraction of Seabuckthorn (*Hippophae rhamnoides* L.) Seed Oil

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Abstract

Extraction of seabuckthorn seed oil was performed with supercritical carbon dioxide (SC-CO_2) fluid. In order to investigate the effect of pressure and temperature on the yield of oil and oil yield, five isobaric (200, 250, 300, 350 and 400 bar) and two isothermal (40 and 50 °C) extraction conditions were conducted. The maximum yield of seabuckthorn seed oil, 12.7 mg oil/g CO_2, was obtained at 50 °C/400 bar with 70 g/min CO_2 flow rate, the maximum oil yield after four hour of extraction at that condition was 11.63% (g oil/g seed ×100). Oil composition and α-tocopherol content obtained by both supercritical carbon dioxide (SC-CO_2) and petroleum ether extraction were determined. The seabuckthorn seed oil contains about 5.5-6.5% of palmitoleic acid (ω7), 10.5-12.0% of palmitic acid, 15-17.5% of oleic acid, 31-32.31-32% of linoleic acid (ω6) and 29-32% linolenic acid (ω3). The linolenic acid content of the SC-CO_2-extracted oil was about 2% higher than that obtained by solvent extraction. The total ω 3 fatty acid from SC-CO_2-extracted oil was higher than that from solvent extraction. Three different fractions collected from SC-CO_2-extraction had similar fatty acid profiles. However, the α-tocopherol content was much higher in the first fraction (F_1) than that from second (F_2) and third (F_3) fractions. While vitamin A content was higher in F_1 and F_3, β-carotene was higher in F_2 and F_1.

Keywords: Supercritical CO_2, extraction, seabuckthorn, seed oil, fatty acid, linolenic acid.

Introduction

Seabuckthorn (*Hippophae rhamnoides* L.) has become a crop of interest for the Canadian farmer, consumer, health food market, and food processing industry (Harrision and Beveridge, 2002). Seabuckthorn is native to Europe and Asia and the species *rhamnoides* has been cultivated in Russia, China, Finland, Mongolia, Germany, and other areas of Europe (Li and Schroeder, 1999). Seabuckthorn is a desirable crop because the fruit juice is high in vitamins A, C, iron and antioxidants, the seed is high in vitamin E and oil content, especially high in ω-3 fatty acid.

Seabuckthorn seed and pulp oils are considered the most valuable components of the fruit, which contain a unique fatty acid composition, fat soluble vitamins and plant sterols (Yang and Kallio, 2002). The seed oil, contains two essential fatty acids, α-linolenic acid (C18:3, ω-3) and linoleic acid (C18:2 ω-6) and very high vitamin E. The contribution of α-linolenic acid and linoleic acid are commonly at 20-35% and 30-40% range, respectively (Yang and Kallio, 2002). Seed oil contains α-, β-, γ-, and δ-tocopherol isomers which comprise 93-98% (84-318 mg/kg of fruit) of the total tocopherols. Alpha-tocopherol (vitamin E) constitutes 76-89% of tocopherols in whole fruit (Kallio *et al*., 2002).

The process used to separate oil from oil-bearing materials has a direct effect on the extractability and quality of oil. Supercritical fluids have been used to extract a wide range of components from crops. These extracts range from essential oils to phytochemicals. Carbon dioxide (CO_2) is one of the most extensively used supercritical fluids because of its relatively low critical temperature and pressure (Tc=31.1 °C, Pc=73.8 bar). The low critical temperature of CO_2 makes it attractive for processing heat-sensitive flavors, pharmaceuticals and labile lipids. In addition, CO_2 is an inert, easily available, odorless, tasteless, non-corrosive, environment-friendly, and GRAS (generally regarded as safe), nontoxic and nonflammable, prevents oxidative degradation, is inexpensive and has a relatively high dissolving power compared to other supercritical fluids (Hierro and Santa-Maria, 1992). High-pressure extraction and fractionation technology employing supercritical CO_2 is an alternative technique for oil extraction and refining (Dunford and King, 2000). A few research groups reported extracting seabuckthorn seed oil by supercritical CO_2 extraction (Cenkowski, Yakimishen, Przbylski and Muir, 2006; Yakimishen, Cenkowski and Muir, 2005; Manninen, Haivala, Sarimo and Kallio, 1997; Stastova, Jez, Bartlova and Sovova, 1996). However, there is no report for the fractionation of the final products by the supercritical CO_2 extraction. The effects of

temperature and pressure on the oil yield also remain unclear. The objectives of this study were to determine the optimal conditions for extracting the seabuckthorn seed oil and to fractionate the final products by using three collecting cyclones. The quality of the extracted oil was also measured in terms of its fatty acid composition and some oil soluble components.

Material and Methods

Seabuckthorn fruits

Seabuckthorn fruits ('Indian summer') were harvested from mature shrubs in Winkler, Manitoba in 2005. Immediately after harvesting, the fruits were hand cleaned to remove visible debris, namely dried leaves, branches, and damaged fruits. Cleaned fruits were packed in 12-13 kg portions and were put in cardboard boxes. The fruits remained frozen (at about -18 °C) during transportation from Winkler to Portage la Prairie for about an hour. The fruits were stored in a walk-in freezer at -20 °C and used within 8 months for this research.

Juice extraction

All fruits were initially thawed to about 10 °C at room temperature. Thawed fruits were passed through a stainless steel pulper/finisher (Langsenkamp, Indianapolis IN, USA), the juice was collected separately from skin and seeds.

Drying

Wet fruit seeds and skin were spread onto perforated drying trays (10 mesh or 1.91 mm perforation size) giving a drying layer of approximately 10 mm. The wet cake was dried at 50 °C for 48 h in a ventilated drying oven (Gas Fired Variable Circulation Laboratory Dryer, Proctor and Schwartz Corporation, Philadelphia, PA) to remove moisture.

Separation of seed and skin

The dried seed and skin were carefully removed from the drying trays and empties into an industrial mixer in approximately 2 kg batches. An industrial mixer (Hobart Cutter Mixer, HCM 300, Hobart Corporation, Troy, OH) equipped with a plastic, two-blade attachment with 1140 rpm served as a threshing unit to gently separate seeds from pulp and remove the white seed skins encapsulating the seeds. The mixer was operated for short time intervals for 1-2 min. This was done to ensure that seeds did not become damaged during threshing.

A vibratory screen separator LS 24S444 (SWECO, Sweco Canada, Toronto, ON) equipped with a stackable arrangement of three screens was assembled for the separation of seeds from pulp. Screens were arranged (top and bottom layer screen openings of 6- mesh (or 3.35 mm) and 10-mesh (or 1.91 mm), respectively to collect three fractions including, debris with pulp, seeds and skin (pulp). When it is necessary, the seeds were further cleaned using compressed air to remove residual pulp that was not removed by mechanical separation.

The seeds were ground by using a food processor (Osterizer Blender, Galaxie, ON) for 1 min. Moisture content was determined according to AOCS method 2-54 (AOCS, 1993). Oil content was measured by Soxhlet extraction using petroleum ether (40-60 °C) for 5 h followed by solvent removal under vacuum at 40 °C for 2 h.

Supercritical fluid extraction

Supercritical fluid extraction unit (Thar Technologies, Inc. Pittsburgh, PA) was used for this study. Carbon dioxide (99.5% purity, Praxair, Brandon, MB) was compressed to the desired pressure by using a high pressure pump. Pressure was controlled by an automatic back-pressure regulator.

Three hundred and fifty (350) grams of ground seed sample was loaded into an extraction vessel (1000 mL) which was heated with a heating ring. The extracts were collected in three cyclones at 120 bar at 45 °C, 90 bar at 40 °C and 50 bar at 35 °C, respectively and three fractions were collected from three cyclones and fractions were named as F1, F2 and F3. The picture of the supercritical fluid extraction unit is shown in Figure 1.

SC-CO_2 extraction of oil was performed at temperatures of 40 and 50 °C, pressures of 200, 250, 300, 350, 400 bars, and CO_2 flow rates of 70 and 50 g/min for 4-7 hours. The extracted oil fractions were collected at time intervals of 1, 2, 3, 4 and 5 hour for CO_2 flow rates of 70 g/min, and 1.4, 2.8, 4.2, 5.6 and 7.0 hour for CO_2 flow rates of 50 g/min. The amount of each fraction was determined by weight. The collected fractions were combined or stored separately at 4 °C with sealed containers.

Figure 1. Supercritical units used for this project.

Chemical analysis

Fatty acid (FA) compositions of oil samples were determined as described by Yang and Kallio (2001). α-tocopherol was analyzed by HPLC equipped with HP 1050 series auto injector and Shimadzu-RF 535 fluorescence detector with wavelengths at 330 nm for emission and 298 nm for extinction. α-tocopherol was separated on a normal-phase column (Supelcosil-LC-Diol, 25 cm × 4.6 mm i.d., 5 µm particle size, Supelco) with the mobile phase flow rate at 1 ml/min. The mobile phase was a mixture of n-hexane/isopropanol (99.4:0.6). The data were integrated and analyzed using Shimazdzu Class-VP Chromatography Laboratory Automated Software system (Shimadzu Corporation). Standards of α-tocopherol (Sigma Chemical Co., St. Louis, MO) was dissolved in hexane and used for identification and quantification of peaks. The amount of tocopherol in the extract samples was calculated as International Unit (IU) tocopherol per 100 g oil sample using external calibration curves.

Statistical analysis

SC-CO_2 extraction of seabuckthorn seed oil at each temperature and pressure condition was carried out in triplicate. All analyses were conducted using Statistical Analysis Program System version 8.2 for Windows (SAS Institute Inc., Cary, NC, USA, 2003).

Results and Discussion

The particle size distribution (%, w/w) of the ground seeds was as follows: 1%, 1.4-1.7 mm; 68%, 1.0-1.4 mm; 26%, 0.5-1.0 mm; 4%, 0.25-0.5 mm. The seeds contained 6.3% (w/w) moisture. Oil content was 12.9% (w/w) as determined by Soxhlet extraction using petroleum ether (40-60 °C) for 5 hours. The proximate analysis results of seabuckthorn seed are shown in Table 1.

Table 1. Proximate analysis results of seabuckthorn seed.

Item	Content (%) (wet base)	Content (%) (dry base)
Moisture	6.3	
Dry matter	93.7	
Crude protein (×6.25)	29.8	31.8
Crude fiber	41.2	44.0
Fat	12.9	13.8
Ash	2.0	2.1

Figure 2 presents extraction curves for oil extracted by SC-CO_2 at 40 °C/300 bar at the CO_2 flow rates 50 and 70 g/min, respectively. The slope of the initial linear portion corresponded to the solubility of the oil in SC-CO_2. The maximum yield of seabuckthorn seed oil, 12.7 mg oil/g CO_2, was obtained at 50 °C/400 bar with 70 g/min CO_2 flow rate, the yield is similar with flaxseed (Bozan and Temelli, 2002) at 11.34 mg/g CO_2, corn and soybean oil at 12 mg/g CO_2 (Christianson, Friedrich, List, Warner, Bagley, Stringfellow, and Inglett, 1984; Friedrich and List, 1982). The maximum oil yield after four hours of extraction at that condition was 11.63% (g oil/100g seed) which extracted abut 90% of the seed oil. The extraction is faster at the CO_2 flow rate at 70 g/mL compared to that at 50g/mL (Figure 2a). However, there are no difference in terms of CO_2 usage and the oil yield at both flow rates. Obviously, it is recommended for industry to use the higher CO_2 flow rate to reduce the operating time (Figure 2b).

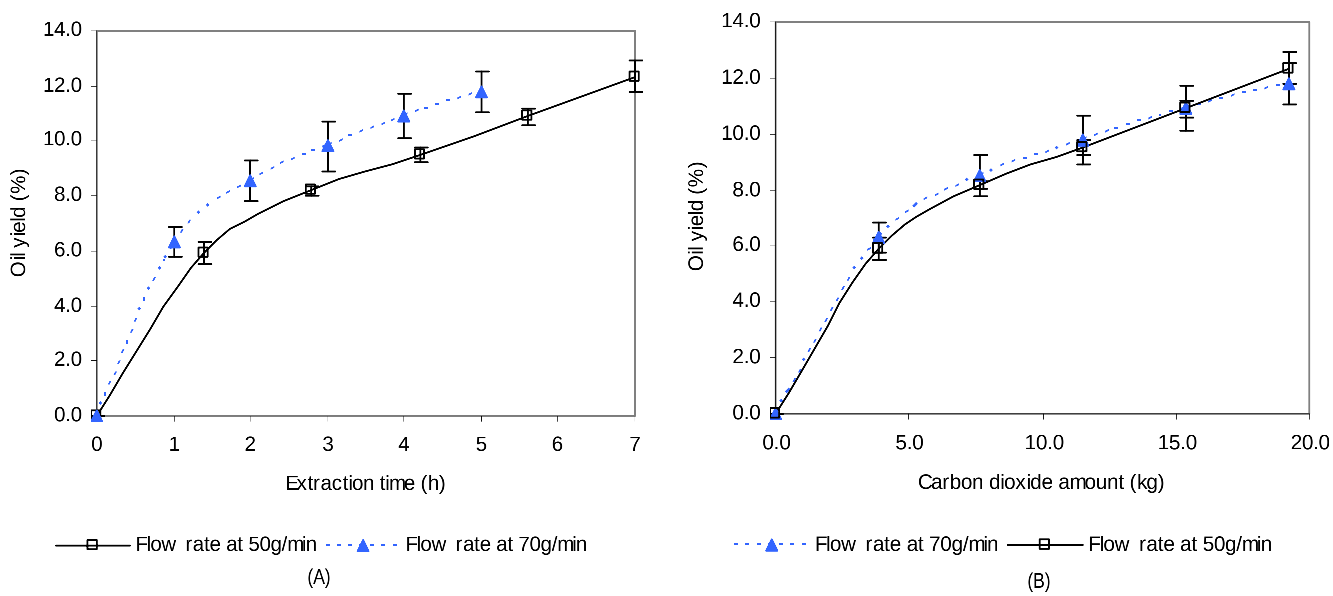

Figure 2. Seabuckthorn seed oil yield (%) as a function of the CO_2 flow rate and extraction time (A) and the amount of SC-CO_2 used (B) at CO_2 flow rates 50g/min and 70g/min at 40 °C/300 bar.

As expected, the yield of seabuckthorn seed oil increased with pressure as a result of the increase in the density of CO_2 at 50 °C, however, the yield increased with pressure from 200 to 350 bar but slightly decreased at the pressure at 400 bar at 40 °C extraction as a result of the well-established crossover of the solubility isotherms (Friedrich and Pryde, 1984; Stahl, Schutz and Mangold, 1980). Yield was higher with extraction temperature at 50 °C compared with extraction at 40 °C (Figure 3).

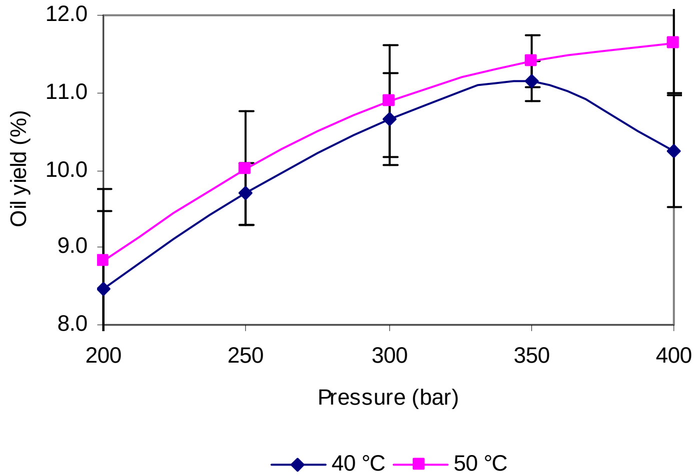

Figure 3. Yield of seabuckthorn seed oil as a function of temperature and pressure.

Based on the fatty acid compositions, the seabuckthorn seed oil obtained by SC-CO_2 under different temperatures and pressures contained similar fatty acid profile. Generally speaking, seabuckthorn seed oil contained similar fatty acid profile by SC-CO_2 and solvent extraction methods, which contained about 5.5-6.5% of palmitoleic acid (ω7), 10.5-12.0% of palmitic acid, 15-17.5% of oleic acid, 31-32.31-32% of linoleic acid (ω6) and 29-32% linolenic acid (ω3) as shown in Table 2. Our results are in agreement with Yang and Kallio (2001) who reported the fatty acid profile of seabuckthorn seed oil by solvent method. However, the linolenic acid content of the SC-CO_2-extracted oil was about 2% higher than that obtained by solvent extraction. The total ω 3 fatty acid from SC-CO_2-extracted oil was higher than that from solvent extraction.

Table 2. The fatty acids (%) and alpha tocopherols composition of seabuckthon seed oil extracted by different conditions (200 bar 40 ºC, 300 bar 40 ºC, 400 bar 40 ºC, 300 bar 50 ºC) of supercritical and Soxhlet extraction.

Component Name	200/40	300/40	400/40	300/50	Soxhlet extraction
Myristic Acid C14:0	0.24	0.28	0.3	0.33	0.23
Myristoleic Acid C14:1, ω-5	0.13	0.13	0.13	0.14	
Palmitic Acid C16:0	10.62	11.15	10.96	10.98	11.60
Palmitoleic Acid C16:1, ω-7	6.15	6.25	6.40	6.62	5.41
Heptadecanoic Acid C17:0	0.29	0.45	0.38	0.40	0.23
Heptadecenoic Acid C17:1	0.29	0.30	0.30	0.31	
Stearic Acid C18:0	2.51	2.56	2.48	2.49	3.17
Oleic Acid C18:1, ω-9	15.2	15.35	14.94	15.01	17.40
Linoleic Acid C18:2, ω-6	31.86	31.39	31.37	31.41	32.02
Gamma Linolenic Acid C18:3	0.13	0.10	0.13	0.13	
Linolenic Acid C18:3, ω-3	31.76	31.1	31.82	31.41	29.23
Arachidic Acid C20:0	0.4	0.40	0.40	0.40	0.46
Eicosenoic Acid C20:1, ω-9	0.18	0.18	0.18	0.18	0.23
Eicosadienoic Acid C20:2	0.06	0.06			
Behenic Acid C22:0	0.1	0.11	0.10	0.1	
Omega 3 Fatty Acids	31.76	31.1	31.82	31.41	29.23
Omega 6 Fatty Acids	32.05	31.9	31.5	31.54	32.02
Trans Fatty Acids	0.08	0.11	0.11	0.09	0.10
Alpha Tocopherols	180.4	260.2	237.5	201.3	

The fatty acids composition of three fractions of final oil products are shown in Table 3. The seabuckthorn seed oil was extracted at 300 bar at 40 ºC, collected at 120 bar at 45 °C for fraction 1 (F1), 90 bar at 40 °C for fraction 2 (F2) and 50 bar at 35 °C for fraction 3 (F3). Fraction 3 had slight higher myristic acid (C14:0), myristoleic acid (C14:1, ω-5) and eicosadienoic acid (C20:2) contents compared with that of fraction 1 and fraction 2. The alpha tocopherol was much higher in fraction 1 than that of fraction 2 and 3. Beta carotene content was higher in fraction 1 and 2.

Samples in three collecting cyclones with different chemical composition might result from the different collecting pressure and temperature and their solubility properties in different phases of CO_2. Up to date, there is no information about the seabuckthorn seed oil collected by different fractions. Based on these results, it is possible to concentrate certain nutrients in seabuckthorn oil by supercritical fluid extraction.

Table 3. The fatty acids (g/100g), alpha-tocopherol, vitamin A and beta-carotene contents of three fractions of seabuckthorn seed oil extracted by 300 bar at 40 ºC.

Component Name	F1	F2	F3	Mixture of F1, F2 and F3
Myristic Acid C14:0	0.28	0.24	0.58	0.28
Myristoleic Acid C14:1, ω-5	0.04	0.03	0.12	0.13
Pentadecanoic Acid C15:0	0.13	0.12	0.18	
Palmitic Acid C16:0	10.93	10.39	11.84	11.15
Palmitoleic Acid C16:1, ω-7	6.39	5.38	6.37	6.25
Heptadecanoic Acid C17:0	0.47	0.39	1.11	0.45
Heptadecenoic Acid C17:1	0.31	0.25	0.33	0.30
Stearic Acid C18:0	2.54	2.66	2.59	2.56
Oleic Acid C18:1, ω-9	15.33	15.78	15.12	15.35
Linoleic Acid C18:2, ω-6	31.52	32.33	31.32	31.39
Gamma Linolenic Acid C18:3, ω-6	0.12	0.13		0.10
Linolenic Acid C18:3, ω-3	31.11	31.40	29.15	31.1
Arachidic Acid C20:0	0.39	0.43	0.38	0.40
Eicosenoic Acid C20:1, ω-9	0.18	0.20	0.16	0.18
Eicosadienoic Acid C20:2	0.06	0.06	0.28	0.06
Behenic Acid C22:0	0.10	0.11	0.13	0.11
Omega 3 Fatty Acids	31.11	31.4	29.15	31.1
Omega 6 Fatty Acids	31.7	32.5	31.6	31.9
Trans Fatty Acids	0.10	0.10	0.10	0.11
Alpha Tocopherols (IU/100g)	259.7	79.8	87.1	
Vitamin A (IU/100g)	823	555	806	
Beta Carotene (IU/100g)	14276	16224	7864	

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Identification and Quantification of an Unknown Sugar Derivative in Seabuckthorn (*Hippophaë rhamnoides*) Berries

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Abstract

Sugars are important components influencing both the sensory and nutritional properties of seabuckthorn berries. Both the content and composition of sugars vary considerably among different subspecies of seabuckthorn. In addition to the commonly known fructose, glucose and sucrose, sugars of varying identities have been reported in seabuckthorn berries. In the present study, a major unknown sugar derivative was isolated from seabuckthorn juice by a solid phase extraction procedure followed by a preparative high performance liquid chromatography (HPLC) method. The isolated compound was identified as ethyl β-D-glucopyranoside by gas chromatography – mass spectrometry (GC-MS) and ^{1}H and ^{13}C nuclear magnetic resonance (NMR). The identification was confirmed by ^{1}H and ^{13}C NMR analysis of the synthesized reference compound ethyl β-D-glucopyranoside and co-injection of the reference compound with seabuckthorn sample in gas chromatography. In berries of *Hippophaë rhamnoides* ssp. *rhamnoides* the level of ethyl β-D-glucopyranoside increased during the harvesting period accompanied by a decrease in glucose content. This indicates the active role of a biochemical pathway converting glucose into its derivatives in this subspecies. In contrast, the level of this compound remained negligible in berries of *H. rhamnoides* ssp. *sinensis* throughout the harvesting period. The presence and relative abundance of ethyl β-D-glucopyranoside may be an important chemotaxonomic feature characterizing different subspecies of seabuckthorn.

Keywords: Seabuckthorn, *Hippophaë rhamnoides*, Sugars, Ethyl β-D-glucopyranoside, Chemotaxonomic characteristics.

Introduction

Content and composition of sugars and acids as well as sugar:acid ratio are important compositional features strongly influencing the sensory and nutritional properties of seabuckthorn berries (Tang et al., 2001; Tiitinen et al., 2005). The total contents of sugars and acids have been widely reported for seabuckthorn berries of different origins. The major sugars in seabuckthorn berries are generally known to be fructose and glucose with very small amounts of sucrose present in some berries. In addition, sugars of varying identities have been reported every now and then in seabuckthorn samples without clear information about the methods used for the identification of these compounds. This fact indicates the presence of additional sugars or sugar derivatives after fructose, glucose, and sucrose in the berries. In the present study, an unknown compound was isolated from the sugar fraction of seabuckthorn berries. The identification of this compound was carried out by gas chromatography – mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). The quantification of this compound in the juice of different seabuckthorn samples was carried out with gas chromatography - flame ionization detection (GC-FID).

Materials and Methods

Berry samples

Berries of cultivated *Hippophaë rhamnoides* ssp. *rhamnoides* were picked from Finland at different harvesting dates in 1998. During the same harvesting period wild berries of *H. rhamnoides* ssp. *sinensis* were collected from two natural growth sites in Shanxi Province, China. The berries were loosely frozen right after picking and kept at -20˚C until analyzed.

Fractionation of seabuckthorn juice and isolation of unknown compound

After thawing the frozen berries in a microwave oven, juice was extracted manually by pressing. Right after pressing, juice was filtered and fractionated into sugar and acid fractions using a solid phase extraction (SPE) procedure (Kallio et al., 2000). The juice sample was diluted by a factor of 1:20 by addition of 0.1 N NaOH water solution and Mili Q water. An aliquot of the dilution mixture was applied on a preconditioned Isolute strong anion exchange chromatography (SAX) column (International Sorbent Technology, Hengoed, UK). From the SAX column the sugars and sugar derivatives were eluted with Mili Q water. The unknown compound was isolated from the sugar and sugar derivative fraction with high performance liquid chromatography (HPLC) on a Luna NH_2 phase column (Phenomenex, Torrance, CA) using a mobile phase of acetonitrile:water (80:20) with a flow rate of 1.4 ml / min.

Identification of unknown sugar derivative

^{1}H and ^{13}C nuclear magnetic resonance spectra of the isolated and synthesized sugar derivative were recorded on a Bruker Avance 400 spectrometer in deuteriated water as a solvent at 400.12 and 100.61 MHz, respectively. Chemical shifts (δ) are quoted in parts per million (ppm) and the coupling constants (J) in Hertz (Hz). The following abbreviations are used to describe the multiplicity: d, doublet; t, triplet; q, quartet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dt, doublet of triplets; dq, doublet of quartets. Two dimensional spectra were run as a HHCOSY90 standard 2D experiment for HH homonuclear correlation and a HCCOW standard 2D experiment for CH correlation.

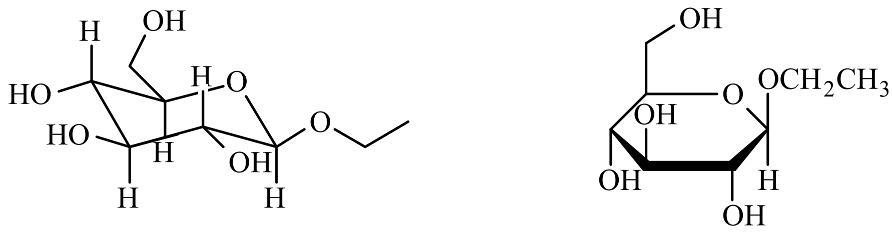
^{1}H NMR (D_2O): δ 4.49 (d, $J_{1,2}$ = 8.0 Hz, 1H, H_1), 3.99 (dq, ^2J = 10.0 Hz, ^3J = 7.1 Hz, 1H, -CH_2-CH_3), 3.93 (dd, $^2J_{6'6}$ = 12.3 Hz, $^3J_{6'5}$ = 2.2 Hz, 1H, $H_{6'}$), 3.75 (dq, ^2J = 10.0 Hz, ^3J = 7.1 Hz, 1H, -CH_2-CH_3), 3.73 (dd, $^2J_{66'}$ = 12.3 Hz, $^3J_{65}$ = 6.0 Hz, 1H, H_6), 3.50 (t, J_{32} = J_{34} = 9.1 Hz, 1H, H_3), 3.47 (ddd, J_{54} = 9.6 Hz, J_{56} = 6.0 Hz, $J_{56'}$ = 2.2 Hz, 1H, H_5), 3.39 (dd, J_{43} = 9.1 Hz, J_{45} = 9.6 Hz, 1H, H_4), 3.27 (dd, J_{23} = 9.1 Hz, J_{21} = 8.0 Hz, 1H, H_2) and 1.25 ppm (t, J = 7.1 Hz, 3H, -CH_3). ^{13}C NMR (D_2O): δ 101.8 (C_1), 75.9 (C_3), 75.8 (C_5), 73.1 (C_2), 69.6 (C_4), 66.2 (-CH$_2$-CH_3), 60.8 (C_6) and 14.2 ppm (-CH_3).

Quantification of sugars in seabuckthorn juice

A water solution of sorbitol was added into the filtered juice as an internal standard before SPE. After the SPE process, the sugar fraction was evaporated under nitrogen to dryness followed by a further drying process over night in a desiccator. Trimethyl silyl (TMS) derivatives of the sugars were prepared by incubation in the reagent Tri-Sil (trimethyl chlorosilane and hexamethyl disilazane in pyridine, Pierce, Rockford, IL) in a screw-cap glass vial at 60 ˚C for 30 min. The TMS derivatives of the sugars and sugar derivatives were analyzed with a Varian 3300 gas chromatograph equipped with a flame ionization detector (Varian, Limerick, Ireland) using a methyl silicone Supelco Simplicity-1 fused silica column (30 m, id 0.25 mm, film thickness 0.25 μm) (Bellefonte, PA) (Kallio et al., 2000). Glucose was quantified with the international standard sorbitol corrected with corresponding response factor obtained with reference compounds; ethyl glucose was quantified as sorbitol without application of any correction factor.

Results and Discussion

The structure of the unknown sugar derivative was unambiguously determined as ethyl *β*-D-glucopyranoside (Figure 1) by high resolution ^{1}H and ^{13}C NMR spectroscopy and confirmed by mass spectrometry. All peaks of the NMR spectra were explicitly assigned by the aid of 2D HHCOSY90 and HCCOW standard 2D experiments for the HH homonuclear and CH correlations, respectively. The spectra were identical to those obtained for purified ethyl glucose synthesized by the method reported by Goncalves et al. (2004). The identification was further confirmed by co-injection of the synthesized reference compound with seabuckthorn samples in GC analysis.



Molecular formula: $C_8H_{16}O_6$

M.wt. 208

Figure 1. Structure of ethyl β-D-glucopyranoside.

At the end of August 1998, the content of ethyl glucose was 0.2-0.4 g in 100 ml juice of *H. rhamnoides* ssp. *rhamnoides* berries. The content of this sugar derivative increased considerably during the harvesting period from the end of August to the end of November 1998, accompanied by a decrease in the level of glucose (Figure 2-A). This indicates the presence of an active pathway converting glucose to its derivative in this subspecies during the harvesting period. In the Chinese berries of *H. rhamnoides* ssp. *sinensis,* the content of ethyl glucose remained at trace levels throughout the harvesting period, whereas the level of glucose increased dramatically from 2.5 g / 100 ml juice at the end of August to 7-8 g / 100 ml juice in late November 1998 (Figure 2-B). The clear difference in the profile and changing dynamics of glucose and the glucose derivative during the harvesting period suggests that different biochemical pathways are active in seabuckthorn probably due to the genetic difference between subspecies or induced by different local climates. This compositional characteristic may be a potential chemotaxonomic factor differentiating seabuckthorn subspecies from one another.

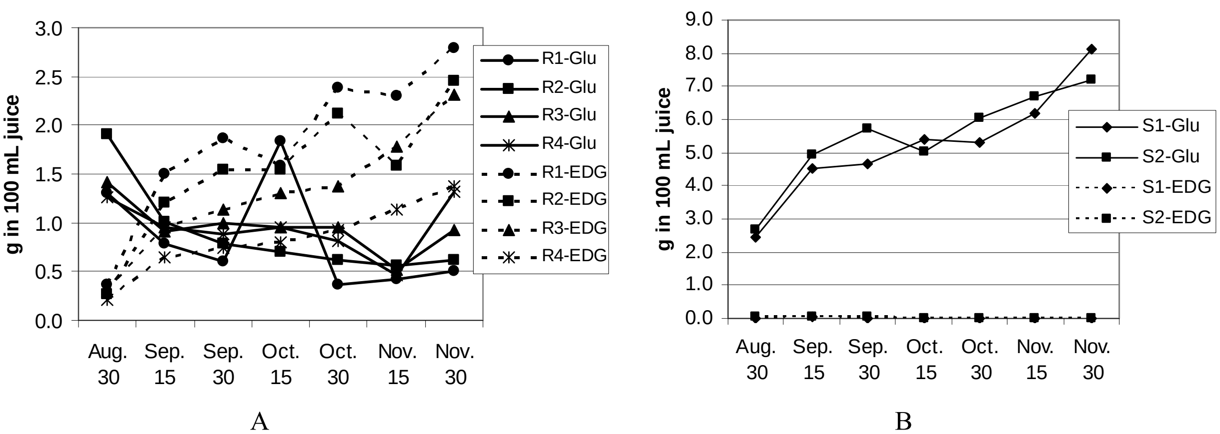


Figure 2. Changes in content of glucose and ethyl glucose in cultivated berries of *H. rhamnoides* ssp. *rhamnoides* (A) and wild berries of *H. rhamnoides* ssp. *sinensis* (B) during the harvesting period in 1998. R1, R2, R3 and R4 are cultivars selected from wild ssp. *rhamnoides;* S1, wild berries of ssp. *sinensis* from Wenshui, Shanxi, China; S2, wild berries of ssp. *sinensis* from Xixian, Shanxi, China; Glu, glucose, and EDG, ethyl β-D-glucopyranoside.

Acknowledgement

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Polar Constituents of Himalayan (Ladakh) Seabuckthorn Leaves

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Abstract

Investigation on Indian seabuckthorn leaves (*Hippophae rhamnoides* from Ladakh, Himalayas) was carried with special reference to flavonoids. The methanolic fraction of seabuckthorn leaves showed prominent reactions for phenolics but contrary to other reports, only weak response for flavonoids. Adsorption and gel-permeation chromatography, complexation with metal ions, selective precipitation *etc.* was used to separate the individual constituents. Since flavonoids and phenolics usually occur in the bound forms, the polar fractions of seabuckthorn leaves were subjected to acid hydrolysis. Isorhamnetin and quercetin were identified (UV, NMR, MS, HPLC, TLC) as the main flavonoids. Substantial amount of gallic acid was also isolated. Glucose was identified as the main carbohydrate from the hydrolysate. Prior to hydrolysis the amounts of free flavonoids and phenolic acids were very small. Thus, it is inferred that flavonoids and phenolics are present in the bound form such as gallotannins and glycosides. For tannins, extraction was carried out with 70% aqueous acetone by percolation. The solvents were removed below 45^0C. Column chromatography using Silica gel, Sephadex, and centrifugal chromatography (chromatotron) were used for fractionation. Phytosteroids (*e.g.* sitosterol, stigmasterol), triterpenoids (*e.g.* ursolic acid), β-carotene (only in small amount), querbrachitol were some of the other compounds isolated from seabuckthorn leaves fractions. A detailed study on the antioxidant activity of compounds and fractions from seabuckthorn leaves was carried out and compared with those of other phenolics and flavonoids. Dyeing potential (for food and textiles) were also evaluated. Isolation, characterization, antioxidant and dyeing activities of seabuckthorn constituents or fractions are discussed.

Keywords: seabuckthorn, flavonoids, phenolics, isorhamnetin, quercetin, gallo-tannins, ursolic acid, water-solubles, natural dyes, antioxidants.

Introduction

Seabuckthorn berries have been in use for centuries in Mongolia, Tibet, Russia, and China in folk medicine and as food supplements. From 1950's onwards, seabuckthorn attracted global attention and systematic investigations on its berries were initiated. More recently, the discovery of bioactivities of constituents of seabuckthorn leaves (SBL) triggered chemical and biological investigations on the leaves. Seabuckthorn (*Hippophae* species) occurs in India abundantly in the Ladakh region of Jammu – Kashmir, cold deserts of Himachal Pradesh, and in the dry temperate areas of Uttaranchal and Sikkim (1). Among the different species, *Hippophae rhamnoides* L., is the most dominant and abundant plant. *H. salicifolia* and *H. tibetana* are the other species which occur in India.

The yields of the phytochemicals from seabuckthorn show enormous variations depending on the geographical location, genetic make-up, ecology, stage of maturation *etc.*(2). In addition to these intrinsic reasons, the apparent variability is also due to different modes of extraction and analytical techniques to estimate phytochemical content (3). Biological and phytochemical investigations on Indian seabuckthorn have been initiated only recently (4). A brief account of phytochemical investigations on seabuckthorn from the Ladakh region is presented below.

Materials and Methods

The plant material was collected in the month of July 2004 from the Ladakh region of Jammu & Kashmir, by the Field Research Laboratory (FRL), Defense Research and Development Organization (DRDO), Leh. This was duly identified by Dr. O. P. Chaurasia, Scientist, FRL as *Hippophae rhamnoides,* ssp *Turkestanica* (5). Different parts of the plant were dried in shade and studied individually. Realizing the chemical diversity of constituents of seabuckthorn, different methods of extraction were employed. Sequential extraction was carried out in a Soxhlet extractor using solvents of varied polarities. In an alternative approach, the plant material was refluxed with appropriate solvents such as methanol and the concentrate from the extract was subsequently fractionated. Extraction was also carried out using 70% aqueous acetone or aqueous methanol at room-temperature to avoid possible loss of

heat - labile constituents such as tannins. The solvents from the extracts were removed on a rotary evaporator (Heidolph, Laborota 4001, Germany) under reduced pressure and the concentrates thus obtained were fractionated by partitioning with a variety of solvents such as petroleum ether (PE), diethyl ether, chloroform ($CHCl_3$), ethyl acetate (EtOAc), methyl ethyl ketone (MEK), *n*-butanol, etc. The separation of individual components was carried out by column chromatography, preparative thin layer chromatography (TLC), centrifugal chromatography (Chromatotron MODEL 79247) and gel-permeation chromatography. The progress of separation of compounds/extracts was monitored by TLC, circular paper chromatography and high performance liquid chromatography (HPLC, Shimadzu, CTO-104SUP). For the molecular characterization, chemical tests, optical spectroscopy (IR, UV), ^1H NMR, ^{13}C NMR (Bruker ADVANCE DPX_{300} series) and mass spectroscopy (JEOL JMS 600 and Shimazdu GC -MS 5050 series) were used.

Results and discussion

Carotenoids

A red oil, obtained on removal of the solvent from the PE extract of seabuckthorn fruit (SBF), was fractionated by chromatography on silica gel column using mixture of PE with increasing proportions of EtOAc. The fractions were analyzed (TLC) using appropriate solvent systems. Four prominent coloured spots (2 red and 2 yellow) could be seen on TLC analysis (PE- EtOAc; 7: 3) of the fractions. They turned blue when sprayed and heated with an ethanolic solution of antimony trichloride indicating their carotenoid nature. Chromatotron (centrifugal chromatography) was very convenient for the preparative scale separation of carotenoids. Preparative TLC (silica gel, PE: EtOAC; 7:3) was also used successfully for the separation of the individual compounds. The identities of carotenoids were established spectroscopicaly as β-carotene and minor amounts of lycopene, β-cryptoxanthin, zeaxanthin, rubiaxanthin and gazaniaxanthin. Carotenoids were also present in the seed oil. The amount of β-carotene in the pulp (1007 mg/100g of pulp) was higher than that of seed (949 mg/100g). The non-polar fractions of seabuckthorn leaves (SBL) contain only very small amount of carotenoids. PE extract of SBL was dark green in colour. The chlorophyllous materials were removed by partitioning with aqueous methanol.(MeOH) and hexane. The extract (70% MeOH) was filtered and the filtrate partitioned with hexane. The yellow hexane extract was subjected to column chromatography (silica column) using PE with increasing amount of EtOAc. The amount of carotenoids in SBL was very low.

Steroids, Triterpenoids, Fatty substances

In addition to residual β-carotene, the oil from the $CHCl_3$ extract of SBF shows the presence of fatty acids/esters, tocopherols, steroids and triterpenoids. Column chromatography of the $CHCl_3$ extract yielded sitosterol, β-amyrin, uvaol, ursolic acid, oleanolic acid, and betulinic acid. The compounds were characterized by ^1H and ^{13}C NMR spectroscopy. Ursolic acid (M^+ 456) was further purified by chromatotron. It is important to note that ursolic acid is known for many medicinal properties such as anti-inflammatory, anti- cancer (induction of apoptosis), anti-microbial, anti-viral and anti-hyperlipidemic activities (6). It is under clinical trial for possible use in skin melanoma (7). Sitosterol and related phytosterols are reported to have hypocholesterolemic and anti-ulcerogenic activities (8). Sitosterol and ursolic acid are the major component in the chloroform extract of SBF. In addition to steroids and triterpenoids, unsaturated fatty acids were also obtained by column chromatography. The presence of tocopherols was also inferred.

For the fatty acid analysis of the oil, the methyl esters of the fatty acids (prepared using MeOH-H_2SO_4) were analyzed by GC-MS. The following fatty acids were identified: palmitic (23%); palmitoleic (42%), stearic (0.57%); oleic (29%); linoleic (4%) and linolenic (0.5%) acids. The presence of palmitoleic and linolenic acids is noteworthy due to their possible actions on many physiological processes such as cholesterol lowering, stroke prevention, *etc*. (9). For the study of non-fatty constituents, the oil was saponified using ethanolic sodium hydroxide (NaOH). The unsaponifiable portion was fractionated by elaborate column chromatography.

The presence of steroids and triterpenes referred above were also isolated. The $CHCl_3$ extract of seeds and leaves were also studied by column chromatography. Seed extract yielded mainly sitosterol and tocopherols. The main constituent of the $CHCl_3$ extract of SBL was ursolic acid (TLC; PE/EtOAc, 7:3) and its amount was higher than in the fruit or seed.

Ascorbic acid

Ascorbic acid plays important role in imparting nutritional quality to seabuckthorn products. The content of ascorbic acid varies considerably with season, location and age. Ascorbic acid content of SBF and SBL were estimated spectrophotometrically. The estimation is based on the reduction of 2, 6-dichloro phenol to indophenol by ascorbic acid (10). Ascorbic acid contents of SBF were compared with that of SBL, lemon and gooseberry (amla, *Phyllanthus emblica*). From experiments it could be concluded that SBL contains more ascorbic acid (126 mg/100 g) when compared to SBF (66 mg/100 g) or lemon (15 mg/100).

Phenolics, Flavonoids, Tannins

Seabuckthorn from different parts of the world is reported to contain flavonoids in substantial quantities (11). However, no details on the isolation and characterization of flavonoids from Indian seabuckthorn are available. The polar fractions of SBL (collected from Ladakh, India) give prominent reaction for phenolics and tannins but only weak reaction for flavonoids. The fresh leaves (SBL), collected from the campus of FRL were extracted with $CHCl_3$ and ethanol. The concentrates were obtained after removal of solvents at reduced pressure. The ethanolic concentrate was a dark brown mass, which was refluxed with PE followed by $CHCl_3$ to remove the unextracted less polar constituents. Carotenoids, phytosteroids, triterpenoids were isolated from these extracts but no substantial amount of free phenolics or flavonoids were present in these extracts. The resulting extract gave positive ferric reaction (blue black) reminiscent of gallic acid derivatives e.g. tannins. The Shinoda's test (12) (pink or red colour with Mg + HCl) was only weakly positive suggesting the presence of small quantity of flavonoids. A TLC analysis of the crude extract was carried out in a variety of solvent systems such as toluene: ethyl acetate: acetic acid; (5:4:1) or EtOAc: MeOH: H_2O (50:17:13) or EtOAc: MEK: MeOH: H_2O (5:3:1:1). Usually free flavonoids/phenolics appear as distinct spots in theses solvent systems.

No significant spots were noticed in these solvent systems in the crude MeOH extracts showing the absence of significant amounts of free flavonoids or phenolics (12).Therefore, it was inferred that the flavonoids/phenolics are present mainly in the bound state such as glycosides or esters and the extract is an intricate mixture of glycosides/esters. The presence of glycosides/carbohydrates was inferred from a positive Molisch's (α-naphthol and H_2SO_4) test. The presence of tannins could be inferred from the ready formation of a precipitate on addition of bovine serum albumin (BSA) solution and strong ferric reaction.

Attempts to isolate individual compounds from the crude extract by gel permeation chromatography (Sephadex) or adsorption chromatography (SiO_2) were partially successful. Some of the heavy metals are known to complex with the compounds containing appropriately placed phenolic hydroxls. A broad separation of flavonoids/phenolics from other constituents could be carried out by complexation with heavy metal salts such as lead acetate. The compounds were regenerated from the lead complex by careful acidification with dilute sulfuric acid (1N) to pH 2.0. Lead sulfate was removed by centrifugation; the supernant contained the phenolics, flavonoids and tannins. It was positive to ferric reaction, Shinoda's test and gave a precipitate with BSA solution. To resolve the complexity of the polar extracts, hydrolysis was attempted under different conditions. No significant change was observed by using various enzymes such as effective microbe preparation (commercial EM, a consortium of microbes), emulsin, pectinase, and diastase. Therefore chemical hydrolytic procedures were then employed.

To optimize the conditions, hydrolysis was carried out under diverse experimental conditions. The crude extract was treated with sulfuric (7% aqueous) or hydrochloric (10% aqueous) acid or oxalic acid or acetic acid for different time intervals and at different temperatures. Use of aqueous sulfuric acid for 1 hour at 70^0C was found to be optimum. Appearance of characteristic spots in TLC with the solvent system, toluene: ethyl acetate: acetic acid; (5:4:1) was indicative of hydrolysis. After completion of hydrolysis (monitored by TLC), the reaction mixture was exhaustively extracted with EtOAc. Initial extracts were yellow in colour (green ferric reaction) while the later fractions were less coloured (blue black ferric reaction). The concentrate obtained from the extract was subject to extensive adsorption chromatography followed by gel permeation chromatography. Three compounds, A, B, C were isolated. Compounds A and B gave yellow spots in TLC (toluene: ethyl acetate: acetic acid; 5:4:1) while C appeared as a cream coloured spot which gave a blue black colour with $FeCl_3$ spray. The flavonoids could also be isolated by centrifugal chromatography (chromatron). Contrary to earlier reports on occurrence of large quantities of flavonoids in several species of *Hippophae*, only a small amount could be isolated from the plant collected from Ladakh. The products were analyzed by NMR spectroscopy and mass spectrometry. The use of UV spectroscopy in conjunction with shift reagents was very useful in the identification of the constituents.

Compound A: It gave a green colour with alcoholic $FeCl_3$ and dissolved in aqueous sodium hydroxide (1%) giving a yellow solution. The yellow spot in TLC intensified on exposure to ammonia vapors. The Shinoda test gave pink colouration. A yellow precipitate was obtained with ethanolic lead acetate solution. The UV spectrum showed prominent absorptions at 225, 278, and 370 nm. The compound showed molecular ion at 316 in the mass spectrum. In the ^1H NMR spectrum signals for one methoxyl and five aromatic protons were observed. A tetraacetate was obtained on acetylation using acetic anhydride and pyridine. A methyl ether was obtained by refluxing with dimethyl sulfate in acetone medium in the presence of anhydrous potassium carbonate. The methylation product was identified as quercetin pentamethyl ether. The analysis of data, along with UV spectral studies using shift reagents (like $AlCl_3$, $AlCl_3$ + HCl, sodium methoxide, sodium acetate and sodium acetate + boric acid) showed that the major compound was isorhamnetin (3'- methyl quercetin). This was confirmed by direct comparison with an authentic sample (obtained from the library of compounds of the senior author). It is the major flavonoid in SBL.

Compound B: It answered ferric reaction (green) and pink colour was obtained in Shinoda's test. It dissolved in aqueous alkali giving a yellow coloured solution. A yellow precipitate was obtained with lead acetate solution. This compound showed a tendency towards oxidation when exposed to air or ammonia vapors. Its UV spectrum (256, 268sh, 371 nm) was similar to those of quercetin and related compounds. A molecular ion at 302 was obtained in the mass spectrum. The compound was then acetylated (acetic anhydride/pyridine). The acetate was identified as quercetin pentacetate. A methyl ether (quercetin methylether) was obtained when an acetone solution of B was refluxed with dimethyl sulfate in the presence of anhydrous potassium carbonate. On the basis of data collected, compound B was identified as quercetin which was confirmed by direct comparison of its properties with authentic quercetin.

Compound C: It is a white amorphous powder, soluble in ethanol/methanol and water. It gave prominent colour with $FeCl_3$ (bluish black colour). It dissolved in alkali (1% aqueous sodium hydroxide) solution giving a pink colour. It did not respond to the Shinoda Test. From the proton and carbon NMR spectra, compound C appeared to be a small molecule. It produced an acetate suggesting that C has many hydroxyls. It gave a prominent UV absorption at 274 nm in methanol. These properties suggest that compound C could be gallic acid. This contention was supported by directly comparing UV, NMR, TLC and paper chromatography data with an authentic sample of gallic acid.

Glucose and rhamnose and an additional quantity of gallic acid were identified from the aqueous part of the hydrolysate. The presence of sugars and gallic acid suggest that flavonoids are present as glycosides and esters. Indications of presence of minor amounts of kaempferol, myrcetin and polar acids such as ellagic acid were also obtained.

With a view to extract intact glycosides, tannins and related compounds, extraction of SBL was also carried out using 70% aqueous methanol and 70% aqueous acetone at ambient temperature (13). Heavy metals such as lead are known to form water insoluble complexes with a variety of phenolics. This method was used in the present investigation. Addition of lead acetate solution to the concentrate in methanol gave a yellow precipitate which was separated by centrifugation. The lead complex was decomposed by careful addition of dilute sulfuric acid (1 N) to pH 3 to recover the compounds. The product could be isolated by gel-permeation chromatography (Sephadex LH-25). In order to identify the products, the resulting product was hydrolyzed with acid. Isorhamnetin and quercetin were isolated and characterized. In addition, presence of other flavonoids, possibly kaempferol, and myrcetin was indicated. Two sugars namely, glucose and rhamnose were identified from the aqueous part. Gallic acid was isolated suggesting the presence of hydrolysable tannins. The flavones may be present as glycosides. However flavonoids, sugars and tannins may be present as a complex which is yet to be characterized.

Percolation of the SBT leaves (pre-extracted with 70% acetone) with water gave an extract which contained flavonoids and gallic acid derivatives. Column chromatography of the extract on Sephadex G25 resulted in the isolation of two fractions: I) -eluted with 100% water and II) -eluted with 50% water-MeOH. On paper chromatography 'I' showed the presence of at least two compounds (Ferric positive). Acid hydrolysis of 'I' gave quercetin and rhamnose. The fraction 'II' contained gallic acid and other unidentified compounds. This experiment showed that some of the flavonoids/phenolics are highly water soluble and escape extraction even with 70% aq. acetone.

The concentrate from the methanolic extract of SBF (fruits) gave a brown mass. It answered tests for flavonoids but separations of individual components in pure states were not successful. The SBF- MeOH concentrate therefore was partitioned with MEK and water. The concentrate from the MEK extract gave a dark-green colour with $FeCl_3$ indicating the presence of phenolics. Red colour was obtained in Shinoda's test suggesting the presence of flavonoids. The presence of glycosides/carbohydrates was inferred from a positive Molisch's (α-naphthol and H_2SO_4) test. The UV spectra using shift reagents indicated the presence of flavonoids. To further resolve the fraction, acid

hydrolysis was carried out. UV spectral studies using shift reagents like $AlCl_3$, $AlCl_3$ + HCl, sodium methoxide, sodium acetate and sodium acetate + boric acid showed that the major compound was isorhamnetin. Spectral studies of minor fractions showed the presence of quercetin, myrcetin and kaempferol. The presence of gallic acid in the hydrolysate suggests the possible presence of hydrolysable gallo-tannins in the mixture before hydrolysis. In addition to phenolics and flavonoids, a colourless crystalline carbohydrate derivative, quebrachitol (inositol -2 -methyl ether), was also isolated. Quebrachitol is a useful template for asymmetric synthesis. It is also known for its bioactivity. The complex formed by quebrachitol with phenolics show anti viral activity. It may be interesting to note that many of thenaturally occurring phenolics/flavonoids, may be present as complexes with scaffolds such as carbohydrates, ascorbic acid, gallic acid, and ellagic acid. The well known ayurvedic medicinal plant, *Phyllanthus emblica,* was reported to be is one of the richest sources of ascorbic acid. However a careful reinvestigation shows that ascorbic acid is not present in the native state in the berries of this plant but is present as a complex with other phytochemicals such as gallic acid (14).

Antioxidant activity of seabuckthorn extracts.

The antioxygenic, antiatherosclerotic, anti-aging properties of seabuckthorn products have been reported. In the present study the antioxidant activity of seabuckthorn extracts were studied for their radical scavenging activity using a DPPH (1, 1-diphenyl-2-picrylhadrazyl) model system.

The free radical scavenging activity of PE, $CHCl_3$, MeOH and MEK extracts of SBF were studied. The radical scavenging power of these extracts was only moderate. Very significant activity was observed for the extracts from the leaves of seabuckthorn (SBL). The polar fractions such as MeOH and MEK extracts showed very significant free radical scavenging activity. This may be due to the presence of phenolic acids, flavonoids and tannins. SBL-MeOH and SBL-MEK extracts showed significant activity when compared to standard antioxidants such as tocopherols or BHA. EC_{50} value of quercetin was found to be much higher compared to that of isorhamnetin. Thus the introduction of a methyl group at 3’ position greatly reduces antioxidant activity. Expectedly, polyhydroxy flavonoids showed high radical scavenging activity. Some representative data on anti-oxygenic activity of extracts and isolated compounds are presented below in Table 1.

Table 1. EC_{50} (DPPH) values for compounds/Extracts

	Sample	EC_{50}
1	SB tea extract (FRL)*	1200
2	SB Flavone (commercial)**	5700
3	Rhodiola methanolic extract	5900
4	Nilgiri tea leaves**	100
5	Quercetin	35
6	Isorhamnetin	620
7	BHA	115
8	Ginseng powder**	18000
9	Gossypin	200
10	Gossypetin	<20
11	Quercetagetin	<20
12	Hebifolin	60
13	DARL tea***	6400

**A product of the Field Research Laboratory, (DRDO), Leh, Ladakh,*
***Commercial sample*
**** A product of the Defense Agricultural Research Laboratory, (DRDO)*

Comparative antioxidant activity of other related natural compounds was also evaluated. Antioxidant activities of α-tocopherol, ellagic acid, ursolic acid etc were also studied by the above method. At the concentration level of 200 ppm, ellagic acid showed high radical scavenging activity (88%) compared to BHA (70%), α-tocopherol (51%) and ursolic acid (8%). Antioxidant activity of some other naturally occurring flavonoids was also determined. Gossypetin and quercetagetin (both hexahydroxyflavones) and gossypin, and hebifolin (both glycosides) showed good anti-oxygenic activities.

Seabuckthorn Extracts as Dyes

The great chemodiversity of seabuckthorn constituents (for example carotenoids, tocopherols, sterols, fatty acids, polyphenolics, flavonoids, tannins, *etc.*) suggests their possible use as a food as well as textile dyes. A detailed investigation was carried out to evaluate the dyeing properties of different extracts. The non-polar extracts, which are rich in carotenoids, impart yellow colour to food materials such as vegetable oil, butter, milk and curd. The colours are stable and have high tinctorial properties. The use of seabuckthorn extracts for food materials has an added advantage in that they enrich them with important nutrition supplements such as antioxidants and vitamins. The polar extracts of SBF also gave similar colours but with low tinctorial values and less stability. SBL is rich in polyphenolics and tannins. Dyeing properties of the polar extracts were investigated on fibers such as cotton, silk, nylon and wool and detailed dyeing characteristics were determined. A camel brown colour was obtained in all fabrics. The range of colour and tinctorial values could be modified or enhanced substantially by the judicious use of mordants. Thus, the use of seabuckthorn as a source of natural dyes will contribute to its value-addition and help in providing additional economic benefit to the farmers.

Conclusions

There is an increasing realization of the possible multifarious uses of seabuckthorn products. Though considerable work on bioactivities of seabuckthorn has been carried out, not much on phytochemical studies on Indian seabuckthorn has been reported. Seabuckthorn products have been found to be useful for skin care, as a radiation protectant, as a sunscreen, and for cardiovascular diseases, wound healing, nutritive health drink, *etc*. It contains a cocktail of nutraceuticals like vitamins (carotenoids, tocopherols), essential fatty acids, and phenolics (flavonoids, tannins *etc*). The non-polar fraction of seabuckthorn includes β-carotene, sitosterol, ursolic acid, essential fatty acids, *etc*. The polar fraction mainly contains organic and phenolic acids, flavonoids, glycosides and hydrolysable and condensed tannins. The present investigation revealed that many of the components of seabuckthorn are highly polar and are extracted with water. As in some other plants, the phytochemicals of SBT may not be present in the free state but in form of macro molecular complexes with other constituents. The glycosides and tannins were best isolated from the aqueous acetone or aqueous. methanolic extracts o by gel-permeation chromatography or by precipitating with lead acetate. Glucose and rhamnose were identified (by paper chromatography) as the sugar components of hydrolysis. The radical scavenging power of the polar extracts of leaves of seabuckthorn are found to be high in a DPPH model antioxidant system compared to that of non-polar extracts. The non-polar extracts, which are rich in carotenoids, impart yellow colour to food materials such as vegetable oil, butter, milk and curd The polar extracts were found to be useful for dyeing of textile fabrics. The yield of the phytochemicals shows considerable variations due to geographical, climatic, and ecological reasons. In addition, the reported variations could also be due to errors incurred in the methods of extraction and estimation.

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Biologically Active Substances in Seabuckthorn and Production of Functional Foods

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Abstract

The biochemical compounds of 25 seabuckthorn cultivars (cvs) were studied. The nutrient content in the investigated cvs varied: ascorbic acid- 44.8 (in 'Mosaika') to 168.4 mg/100 g ('350 let Michurinsky'); vitamin P – 33.0 ('Ocharovanie') to 141.0 mg/100 g ('Krasnoplodnaya'), carotene – 1.1 ('Ulybka') to 4.95 mg/100 g ('Pamyat Indiry'). The cvs with soluble solids contents greater than 12.0% were isolated. The formulations and production practices for natural and functional foods from seabuckthorn were developed. All of the products developed have elevated contents of vitamins and biologically-active substances and the antioxidant activity was found to be more than 600.0 mkg/ml. The product, "stewed fruit from seabuckthorn, dietary and of low caloric value", has a higher carbohydrate content (1.24%), "jelly from seabuckthorn with Lactulose" had a higher vitamin C content (90.0 mg/100 g), whereas the "pears in nectar from seabuckthorn" product had an arbutin content of 6.5% (per dry weight) and a chlorogenic acid content of 18.0 mg/100 g. These products are tonic and improve digestion. In general they are classified as prophylactic, acting against overweight, diabetes and metabolic disturbances.

Keywords: seabuckthorn cultivars, biologically active substances, vitamins, processing.

Introduction

Over the past several years, in connection with the decline in the condition of the environment and increases in the incidence of chronic human diseases, one of the most important trends in the canning industry is the development and production of new natural, functional foods.

Seabuckthorn is a polyvitaminous crop. Seabuckthorn fruit is an important raw material for technological processing. The State Register has registered more than 70 Seabuckthorn cvs, these cultivars are admitted for usage in different regions of Russia, and 22 of the cvs have been patented. A great contribution into development of new seabuckthorn cultivars was made by research breeders in Siberia, especially at the M.A. Lisavenko Research Institute of Siberian Horticulture. For the last 50 years, the breeders at this Institute have released more than 40 seabuckthorn cultivars, 19 of them have been inserted into the State Register in nine regions (Kalinina, 2005).

The best cultivars are characterized by the following traits: winter-resistance, complex resistance to diseases, dwarfing, shoots which are not closely thorned, and showing a high, annual yield (16-24 t/ha), large-sized fruit (up to 1.4g) with a long stalk and dry-scar. In addition they possess higher contents of vitamins and biologically active substances, for example, vitamin C – up to 200 mg/100 g, carotenoids – more than 50 mg/100 g, and oil – up to 10%).

The biochemical evaluation of new seabuckthorn cvs, as well as the need to determine the technologically-relevant properties which are necessary for the production of natural functional foods, and the development of new foods with a designed compound and functional properties is the focus of this paper.

Materials and methods

The biochemical compounds of 25 seabuckthorn cultivars have been studied using traditional biochemical methods (i.e. soluble solids contents, sugars, vitamins C and P, carotene, etc.). The formulation and processing technology for obtaining natural foods, which are protective, dietetic, colourant and preservative-free, from seabuckthorn is developed (Makarov, Vlazneva, 2007).

Results and discussion

It was found that the seabuckthorn fruit cvs investigated differed in biochemical composition. The ascorbic acid content in the investigated cvs varied between 44.8 (‘Mosaika’) and 168.4 mg/100 g (‘350 let Michurinsky’). In some years the fruits of the latter cultivar accumulates more than 260 mg/100 g of vitamin C. Several cvs from Siberia, for example, ‘Naran’, ‘Baikalsky Rubin’, ‘Yatsula’, ‘Tengá’, and ‘Oranagevaya’, are characterized by high contents of ascorbic acid (Kalinina, 2005).

The seabuckthorn fruits are also rich in P-active catechines, with the content being cultivar dependent, and varying between 39 (‘Ocharovanie’) and 141 mg/100 g (‘Krasnoplodnaya’). In some favourable years, the fruit of the ‘Krasnoplodnaya’ cv possesses a vitamin P content above 300 mg/ 100 g. Cultivars with higher carotene contents are of great interest for the development of natural functional foods.

In the Medium Zone of Russia, the investigated cvs show carotenoid contents ranging from 1.1 (‘Ulybka’) up to 5.0 mg/100 g (‘Pamyat Indiry’); however, their content may, at times, be above 10 mg/100 g.

The breeders of Siberia released new cultivars of seabuckthorn (‘Tengá’, ‘Zarnitsa’, ‘Pamyati Zakharovoi’, ‘Zhivko’, etc.) in which the carotenoid contents are above 30 mg/100 g (Kalinina, 2005, 2005a). Releases such as these, which exhibit higher soluble solids in the fruit, help to improve food quality after processing, and also to reduce the input of raw material / per unit of the food produced. Thus the following seabuckthorn cvs with higher soluble solids and sugars (9-12%) were identified: ‘Prelest’, ‘Uspekh’, ‘Lyubimaya’, ‘Altaiskaya’, ‘Augustina’, and ‘Samorodok’.

On the basis of research and technological investigations, the following new types of natural foods, which are both dietetic and prophylactic, are assayed and introduced into practice at the Experimental (M-Kons 1) Centre in Michurinsk (Russia):

“Pear in nectar from seabuckthorn” – consists of pear halves submerged in seabuckthorn nectar, and enriched with inulin and β-carotene. The technical process involves several steps in the preparation of raw material, the preparation of syrup, packing, filling in (syrup), capping and sterilization. The product contains up to 19 mg/100 g of vitamin C, 4.3 mg% of β-carotene, 20 mg/100 g P-active catechines, 65% arbutin, 18.0 mg/100 g chlorogenic acid and 2.5 % of nutrient fibre. The antioxidant activity of product is not less than 600 mkg/ml. The consumption of this product will strengthen immunity and remove heavy metal salts from the human body.

“Jelly from seabuckthorn with lactulose” – is made from fresh seabuckthorn with the addition of pectin, fructose and lactulose. The technical process includes the preparation of raw material, rubbing, mixing of the components, short-term cooking loss after boiling, packing, and pasteurization. The product contains 90 mg/100 g of vitamin C, 1.4 mg/100 g of β-carotene, 196 mg/100 g of p-active catechines, 1.0% protopectin and 2.2 mg/100 g anthocyanins. The antioxidant activity of product is 700 mkg/ml. The jelly is sold for dietetic purposes, does not contain sucrose, and is a good source of vitamins, anthocyanins and pectin substances.

“Stewed fruit from seabuckthorn”- is a dietary and low energy value product from cooked, fresh seabuckthorn fruit with the addition of the natural sweetener “Swyta” (fermentatively-treated steviozid), enriched with dihydroquercetine and ascorbic acid. The production process includes: the preparation of raw material, preparation of syrup, packing, filling in syrup, capping, and sterilization. The product contains 40 mg/100 g of vitamin C, 30 mg/100 g of p-active catechines, 1.2% pectin and a lesser amount of carbohydrates (1.2%). The antioxidant activity of product is not less than 650 mkg/ml. The stewed fruit is characterized by a low energy value (about 16 kcal/100 g) and has tonic properties, promotes better digestion, reduces the need for sugar, and can be regarded as prophylactic against overweight, diabetes, and metabolic disturbances.

The desirable attributes of the three above seabuckthorn-based products are three-fold: high nutrient value, good organoleptic quality and a positive, directed physiological effect. More traditional products are characterized only by the two first attributes. The products which have been developed are not medicines and are not able to cure a person from any particular disease, but they are extremely important as prophylactic agents against diabetes, cardiovascular diseases and normalization of digestion with their unique properties. In addition, these foods help humans resist stress.

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Seabuckthorn (*Hippophaë rhamnoides* L.) Pulp Oil Fractionation by Solvent Crystallization Process

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Abstract

Seabuckthorn pulp oil was fractionated at different temperatures using a solvent crystallization process with acetone. Crystallization experiments were carried out under a controlled cooling rate of 0.25ºC/min without agitation. The obtained liquid (LF) and solid (SF) fractions were analyzed for their fatty acid and triacylglycerol compositions. Their melting profiles were characterized by differential scanning calorimetry. Fractionation at -15ºC yielded about 20%w/w of LF comprised of 80.9% unsaturated fatty acids, with palmitoleic acid accounting for around 53%. On the other hand, SF was richest in palmitic acid, which reached a maximal concentration of 58% at a crystallization temperature of -1ºC. LF and SF fractions exhibited different triacylglycerol compositions. LF were richer in triacylglycerol molecules with acyl carbon numbers of 50 and 52 (C50 and C52) than SF, which contained a higher amount of C48. The melting curves of LF and SF showed multiple endothermic transitions, as commonly observed in vegetable oils.

Keywords: seabuckthorn, *Hippophaë rhamnoides*, solvent fractionation, palmitoleic acid, melting curves, fatty acids, triacylglycerols, crystallization.

Introduction

Seabuckthorn berries (*Hippophaë rhamnoides* L.) have been recognized for many years for their high nutrient and phytochemical content (Beveridge, Li, Oomah & Smith, 1999). The berry pulp has a high oil content and fluctuates commonly between 30-35% w/w (Yang & Kallio, 2002; 2002a). Seabuckthorn pulp oil (SBPO) contains high levels of palmitic (C16:0, 17-47%) and palmitoleic (C16:1n-7, 16-54%) acids, and minor amounts of oleic (C18:1 n-9, 2-35%) acid (Yang & Kallio, 2002).

A process allowing for increased palmitoleic acid levels, and lower palmitic acid concentrations could make the SBPO more interesting. Klaas and Meurer (2004) reported a palmoleic acid content increase of about 50% using a procedure comprising oil extraction from sea buckthorn pomace, followed by oil transesterification, fractional distillation and urea crystallization. In spite of the high concentration of palmitoleic acid obtained in the final ester concentrate (81.9% w/w), the low yield of the process (about 4%) and the possible formation of carcinogenic ethyl or methyl carbamates when using urea (Canas & Yurawecz, 1999), could limit its application in the food and pharmaceutical industries.

SBPO exhibits a wide melting range, from approximately -30ºC to 20ºC (Gutiérrez, Ratti & Belkacemi, 2008). This may allow the fractionation by crystallization of a series of triacylglycerols at temperatures below their melting points by rigorous control of the oil cooling rate (O'Shea, Devery, Lawless, Keogh & Stanton, 2000). Fractional crystallization is a well-established fat modification process based on the difference of melting points of the triacylglycerols. There are two main types of fractionation processes: dry and solvent fractionation. Both processes use simple procedures consisting of complete fat melting, slow and controlled cooling of oil, gentle or no agitation of the oily mother liquor to promote the development of crystals, and finally, separation into liquid and solid fractions (Timms, 2005). Although dry fractionation is the simplest and most economical technology, the separation of the fat crystals from the liquid matrix is often difficult and incomplete (Mohamed, 1999). Solvent fractionation,

using mainly acetone or hexane, is commercially applied to produce specialty oils from a wide selection of edible oils (Yokochi, Usita, Kiamisaka, Nakahara & Suzuki, 1990; Vanputte & Bakker, 1987). According to Timms (2005), the main advantages of using a solvent are the short crystallization times and the high separation efficiency. Preliminary dry fractionation experiments of SBPO showed that the phase separation is very difficult, because of the high viscosity of the SBPO at temperatures below 0ºC. The aim of this work was investigate the enrichment of SBPO in palmitoleic acid by means of a solvent crystallization process using acetone at a crystallization temperature ranging between -15ºC to 15ºC.

Materials and Methods

Berries

Seabuckthorn berries (*Hippophaë rhamnoides* L. cv. 'Indian-Summer') from Sainte-Anne-de-Beaupré (Québec, Canada) were hand cleaned and stored at -30ºC in sealed plastic bags until the beginning of the experiments.

Oil extraction

Air-dried seabuckthorn pulp was extracted with hexane at 50ºC in sealed flasks with continuous agitation for about 24 h. After extraction, the flask contents were filtered under vacuum, and the solvent was separated from the miscella using a rotary film evaporator (Büchi R-205, Cole-Parmer, Vernon Hills, IL, USA). The oil was collected, evaporated under nitrogen, and stored in sealed amber glass vials at -20ºC until the crystallization processes and analysis were performed.

Solvent crystallization processes

Samples of SBPO (~1.5 g) were placed into a 15-mL polypropylene centrifuge tube and mixed with acetone, using a 1/5 m/v oil to solvent ratio. Crystallization processes were carried out at different temperatures (15, 5, -1, -5, -10 and -15ºC) without agitation. The centrifuge tubes were placed into a thermoregulated bath (HAAKE C, HAAKE, Germany) containing a glycol/water mixture. The bath cooling rate was controlled by means of a digital temperature programmer (HAAKE PG 41, HAAKE, Germany), as follows: from 30ºC (isothermal for 30 min) to the crystallization temperature (TC) at 0.25ºC/min, and held at the crystallization temperature for about 24 h. The bath temperature was registered over time using a temperature data logger (HOBO H8 4-Channel Outdoor/Industrial External Input Logger, Onset Computer Corporation, USA). At the end of crystallization process, liquid (LF) and solid (SF) fractions were separated by filtration, evaporated under nitrogen, weighed, and stored at -20ºC until subsequent fatty acids, triacylglycerol and DSC analysis. The yields (%w/w) of LF and SF were calculated for each TC using Eq. (1) and (2), respectively.

$$LF\ yield\,(\%) = \frac{Mass\ of\ LF\,(g)}{Mass\ of\ SBPO\,(g)} \times 100 \qquad \text{(Eq. 1)}$$

$$SF\ yield\,(\%) = \frac{Mass\ of\ SF\,(g)}{Mass\ of\ SBPO\,(g)} \times 100 \qquad \text{(Eq. 2)}$$

Analytical methods

Fatty acid composition

The fatty acid compositions of the obtained LF and SF were determined by gas chromatography (GC). Samples were converted into their methyl esters (FAME) and analyzed on a 5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, CA). The oven temperature was programmed as follows: from 60ºC (isothermal for 1 min) to 190ºC at 20ºC/min, and isothermal period of 30 min at 190ºC. The injector and detector temperatures were set at 250°C. Hydrogen was used as carrier gas. Component separation was performed on a BPX-70 capillary column (60 m×0.25 mm i.d.×0.25 μm film thickness; SGE, Melbourne, Australia). Fatty acids were identified by comparing their retention times with those of the FAME standards purchased from Nu Chek Prep (Elysian, MN, USA) under the same conditions. Peaks were integrated using a Hewlett-Packard ChemStation software.

Triacylglycerol composition

A gas chromatography (GC) method was used for the determination of the triacylglycerol (TAG) composition of the obtained LF and SF. Samples were dissolved in octane and analyzed on a 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA). The oven temperature was programmed as follows: from 250ºC to 360ºC at 3.0ºC/min, and isothermal period of 15 min at 360ºC. Injector and detector temperatures were set at 400°C. Hydrogen was used as carrier gas. The GC separation method was performed on a RTX-65TG capillary column (30 m×0.25 mm i.d.×0.1 μm film thickness; Resteck, Bellefonte, PA, USA). The identification of the peaks was achieved by comparing their retention times with those of the standards purchased from Nu Chek Prep (Elysian, MN, USA) and Sigma-Aldrich (Sigma, St. Louis, MO, USA) under the same conditions. Peaks were integrated using a Hewlett-Packard ChemStation software.

Melting profiles

The melting profiles of the crude SBPO and the obtained LF and SF were determined by differential scanning calorimetry (DSC) using a differential scanning calorimeter (Pyris 1 DSC, Perkin Elmer, Norwalk, CT, USA) equipped with an intracooler II (Perkin Elmer, Norwalk, CT, USA). During analysis the system was purged with nitrogen at 30 mL/min. The melting curves were obtained within the temperature range of -50ºC to 50ºC. Samples (~10 mg) were cooled to -50ºC and held at this temperature for 5 min, and then heated at 5ºC/min. An empty pan was used as an inert reference to balance the heat capacity of the sample pan. Calibration of DSC was carried out using indium (mp = 156.6ºC, ΔHf = 28.71 J/g). Data were analyzed using a thermal analysis software (Pyris 1 Version 3.5, Perkin Elmer).

Statistical analysis

All assays were carried out in duplicate. Analysis of variance by the general linear models (GLM) procedure and mean comparisons by the least significant difference (LSD) test were performed using the Statistical Analysis System (SAS Institute, 2000).

Results and Discussion

Fractions yields

Figure 1 shows the yields of the LF and SF obtained after solvent crystallization of crude SBPO as a function of crystallization temperature (T_C). As depicted, the SF yields increased as T_C decreased, whereas LF yields decreased. When T_C decreased from -5ºC to -10ºC, the SF yields doubled (31.3±1.0%w/w *vs*. 62.0±1.3%w/w, $p<0.05$), whereas the LF diminished about of 50% (67.7±0.9%w/w *vs*. 35.8±0.3%w/w, $p<0.05$). As observed in Figure 1, the solvent crystallization process at -15ºC yielded approximately 20%w/w of LF. Because of the low LF yields that can be obtained below this temperature, no lower crystallization temperatures were tested.

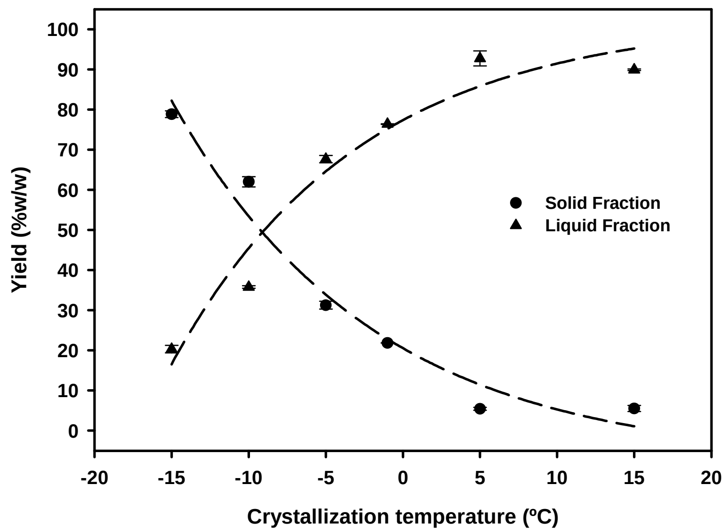


Figure 1. Yields of the LF and SF obtained after solvent crystallization of SBPO with acetone at different temperatures during 24 h, using a 1/5 m/v oil to solvent ratio.

Fatty acid compositions of the crude SBPO and its LF and SF

Table 1 presents the fatty acid profiles of the crude SBPO and its LF and SF obtained after solvent crystallization processes. As can be seen, the crude oil was mainly rich in palmitoleic (C16:1, 41.4%), palmitic (C16:0, 34.7%) and linoleic (C18:2n-6, 10.2%) acids. Similar values were recently reported for seabuckthorn (cv. Indian-summer) pulp oil (Gutiérrez, Ratti & Belkacemi, 2008).

In LF, it can be seen that except for palmitic, oleic and arachidic acids, the concentrations of the other fatty acids increased as crystallization temperature (TC) decreased. When TC decreased from 15 to -15ºC, liquid fractions were lower in saturated fatty acids (SFA, 37.9% to 19.1%), and richer in monounsaturated (MUFA, 51.0% to 65.5%) and polyunsaturated (PUFA, 11.1% to 13.8%) fatty acids. The crystallization process at TC of -15ºC diminished the proportion of palmitic acid by 60%, and increased the concentrations of palmitoleic and linoleic acids by around 27 and 37%, respectively, when comparing to the initial values of these fatty acids in crude SBPO. At this temperature, the concentrations of palmitoleic and palmitic acids in LF found in this study, were slightly higher (52.9% vs. 52.1%) and significantly lower (13.9% vs. 40%), respectively, than those reported by Klaas and Meurer (2004) in the distillates of the fatty acids methyl esters obtained after seabuckthorn pulp oil transesterification.

In SF, it can be noticed that except for palmitic acid, the concentrations of the other fatty acids decreased to their lowest values at TC=-1ºC, and then they increased as TC further decreased. The crystallization process at -1ºC yields 21.9%w/w of a solid fraction with the highest concentration of SFA (60.1%), and with the lowest proportions of MUFA and PUFA (31.6% and 8.3%, respectively). At this temperature, the concentration of palmitic acid increased by around 67%, while the proportions of palmitoleic and linoleic acids diminished around 35% and 26% respectively, in comparison to their initial values in crude SBPO. Palmitic acid reached its highest concentration (58.1%) at TC=-1ºC, whereas palmitoleic and linoleic acids were 26.4% and 7.5%, respectively.

Table 1. Fatty acid composition of the liquid and solid fractions obtained after solvent crystallization of sea buckthorn pulp oil with acetone at different temperatures during 24 h, using a 1/5 m/v oil to solvent ratio[a].

T_C	Fatty acid composition in liquid fractions (LF) (%)									
(ºC)	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1*n*-9	C18:1*n*-7	C18:2*n*-6	C18:3*n*-3	C20:0
-15	0.8^{a}	13.9^{a}	52.9^{a}	3.0^{a}	1.2^{a}	2.7^{a}	9.9^{a}	14.0^{a}	1.4^{a}	0.2^{a}
-10	0.6^{b}	22.2^{b}	48.3^{b}	2.5^{b}	1.0^{b}	3.0^{b}	8.5^{b}	12.5^{b}	1.3^{b}	0.2^{a}
-5	0.5^{c}	26.8^{c}	46.5^{c}	2.1^{c}	0.9^{b}	3.2^{c}	7.9^{c}	10.9^{c}	1.0^{c}	0.2^{a}
-1	0.5^{c}	28.8^{d}	45.1^{d}	2.1^{c}	1.0^{b}	3.1^{c}	7.5^{d}	10.7^{c}	1.0^{c}	0.2^{a}
5	0.4^{d}	34.6^{e}	41.4^{e}	2.0^{d}	0.9^{c}	3.1^{c}	6.5^{e}	10.1^{d}	1.0^{c}	0.2^{a}
15	0.4^{d}	34.6^{e}	41.4^{e}	1.9^{de}	0.8^{c}	3.1^{c}	6.5^{e}	10.1^{d}	1.0^{c}	0.2^{a}
T_C	Fatty acid composition in solid fractions (SF) (%)									
(ºC)	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1*n*-9	C18:1*n*-7	C18:2*n*-6	C18:3*n*-3	C20:0
-15	0.3^{a}	39.6^{a}	39.1^{a}	1.7^{a}	0.8^{a}	3.1^{ab}	5.6^{a}	9.0^{a}	0.8^{a}	0.2^{a}
-10	0.3^{a}	40.3^{a}	38.1^{b}	1.7^{a}	0.8^{a}	3.1^{a}	5.6^{a}	9.1^{a}	0.9^{b}	0.2^{a}
-5	0.2^{b}	53.0^{b}	29.3^{c}	1.5^{b}	0.3^{b}	2.8^{c}	3.3^{b}	8.3^{b}	0.9^{b}	0.1^{b}
-1	0.1^{c}	58.1^{c}	26.4^{d}	1.4^{c}	0.4^{c}	2.9^{cd}	2.4^{c}	7.5^{c}	0.8^{a}	0.1^{b}
5	0.4^{d}	33.9^{de}	40.4^{e}	2.0^{d}	0.8^{a}	3.0^{e}	6.6^{d}	11.0^{d}	1.6^{c}	0.2^{a}
15	0.3^{d}	33.0^{d}	39.1^{a}	2.1^{e}	0.8^{a}	2.9^{d}	7.0^{e}	13.0^{e}	1.4^{d}	0.3^{c}
	Fatty acid composition in crude oil (%)									
	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1*n*-9	C18:1*n*-7	C18:2*n*-6	C18:3*n*-3	C20:0
	0.4	34.7	41.4	1.9	0.9	3.0	6.5	10.2	0.9	0.2

[a] For each fraction, means in a column followed by the same letter are not significantly different by LSD test at the 5% level.

Triacylglycerol composition of the crude SBPO and its LF and SF

The triacylglycerol (TAG) compositions of the crude SBPO and its LF and SF obtained after the solvent crystallization processes, are presented in Table 2. The crude oil contained mainly TAG with acyl carbon number (ACN) of 48 and 50, both representing approximately 95% of the total TAGs (57.1% and 38.2%, respectively). TAG of 48 acyl carbons with 2 double bonds (DB) (48:2) constituted the major ACN:DB of these species of TAG, followed by 48:1 and 48:3 (29.0%, 19.6% and 8.4%, respectively). In the case of TAG of 50 acyl carbons, the most abundant ACN:DB were 50:2 and 50:3 (15.5% and 13.8%, respectively). The patterns of the molecular weight distribution of TAG of the crude SBPO obtained in this study were in accordance of those reported recently by Yang and Kallio (2006) for berries of sea buckthorn of different origins.

As it can be noticed in Table 2 for LF, when the crystallization temperature (TC) decreased from 15ºC to -15ºC, overall TAG species with 48 acyl carbons decreased (57.1% to 38.2%), while those with 50 acyl carbons increased (38.2% to 48.4%). The crystallization process at -15ºC diminished the monounsaturated TAG species from 23.8% to 1.4%, and augmented the proportions of polyunsaturated TAG species from 75.4% to 96.3%, when comparing to the initial values of these TAG species in crude SBPO.

In agreement with their fatty acid compositions, the concentrations of TAG species in SF showed a plateau at TC= - 1ºC as can be observed in Table 2. At this temperature, TAG species with 48 acyl carbons were 66.1%, while those of 50 acyl carbons decreased to 31.9%. After solvent crystallization process at TC= -1ºC, a solid fraction with the highest concentration of monounsaturated TAG species and the lowest proportions of and polyunsaturated TAG species (64.8% and 34.5%, respectively) can be recovered.

Table 2. Triacylglycerol (TAG) composition of the liquid and solid fractions obtained after solvent crystallization of sea buckthorn pulp oil with acetone at different temperatures during 24 h, using a 1/5 m/v oil to solvent ratio[a].

T_C (ºC)	TAG in liquid fractions (%)													
	C46:1	C46:2	C48:1	C48:2	C48:3	C50:0	C50:1	C50:2	C50:3	C50:4	C50:5	C52:2	C52:3	C52:4
-15	0.1^{d}	1.0^{d}	0.9^{f}	15.0^{e}	22.3^{d}	2.2^{d}	0.4^{f}	6.5^{d}	24.5^{f}	13.0^{e}	1.8^{b}	0.5^{a}	4.8^{c}	6.9^{d}
-10	0.2^{cd}	0.7^{c}	3.2^{e}	26.7^{b}	14.4^{d}	1.3^{c}	1.3^{e}	11.9^{c}	21.2^{e}	7.9^{d}	1.0^{a}	1.4^{bc}	4.6^{c}	4.2^{c}
-5	0.4^{ab}	0.5^{b}	4.8^{d}	33.0^{d}	10.5^{c}	1.0^{a}	1.7^{e}	14.8^{b}	18.7^{d}	5.7^{c}	0.7^{a}	1.7^{cd}	4.0^{bc}	2.6^{b}
-1	0.4^{ab}	0.5^{b}	8.1^{c}	30.7^{c}	9.6^{b}	0.9^{ab}	2.4^{b}	15.4^{b}	17.4^{c}	5.6^{c}	0.7^{a}	1.9^{d}	3.7^{bc}	2.7^{b}
5	0.3^{bc}	0.3^{a}	17.9^{b}	26.8^{b}	8.2^{a}	1.0^{a}	3.7^{a}	15.8^{a}	14.9^{b}	4.4^{b}	0.6^{a}	1.3^{bc}	2.8^{ab}	1.8^{ab}
15	0.4^{ab}	0.4^{ad}	17.7^{b}	26.6^{b}	8.2^{a}	0.8^{a}	3.8^{a}	15.9^{a}	14.7^{b}	4.4^{b}	0.7^{a}	1.8^{cd}	2.9^{ab}	1.9^{ab}
T_C (ºC)	TAG in solid fractions (%)													
	C46:1	C46:2	C48:1	C48:2	C48:3	C50:0	C50:1	C50:2	C50:3	C50:4	C50:5	C52:2	C52:3	C52:4
-15	0.4^{bc}	0.2^{acd}	21.6^{f}	29.8^{ab}	5.3^{d}	0.5^{b}	4.4^{b}	17.5^{e}	12.5^{be}	2.3^{e}	0.4^{c}	1.4^{a}	2.3^{c}	1.4^{bd}
-10	0.4^{bc}	0.3^{ac}	24.5^{e}	26.9^{c}	5.6^{d}	0.5^{b}	4.9^{b}	17.4^{de}	11.9^{e}	2.5^{e}	0.4^{c}	1.2^{a}	2.2^{ac}	1.2^{b}
-5	0.3^{c}	0.1^{d}	47.8^{d}	13.1^{e}	3.3^{c}	0.3^{b}	8.4^{d}	17.1^{d}	5.9^{d}	1.3^{d}	0.2^{b}	0.5^{bc}	0.8^{bd}	0.5^{ce}
-1	0.3^{c}	0.2^{cd}	54.3^{c}	9.5^{d}	2.3^{b}	0.4^{b}	10.2^{c}	16.7^{c}	3.9^{c}	0.6^{c}	0.2^{b}	0.3^{c}	0.4^{d}	0.3^{e}
5	0.3^{bc}	0.3^{ac}	18.3^{b}	27.0^{c}	8.3^{a}	0.8^{a}	3.7^{a}	15.7^{a}	14.7^{a}	4.0^{a}	0.7^{a}	1.6^{a}	2.5^{c}	1.8^{d}
15	0.6^{a}	0.7^{b}	20.1^{a}	30.1^{b}	8.5^{a}	0.9^{a}	4.6^{b}	15.0^{b}	12.8^{b}	3.1^{b}	0.6^{a}	1.0^{ab}	1.1^{b}	0.7^{ac}
	TAG in crude oil (%)													
	C46:1	C46:2	C48:1	C48:2	C48:3	C50:0	C50:1	C50:2	C50:3	C50:4	C50:5	C52:2	C52:3	C52:4
	0.5	0.4	19.6	29.0	8.4	0.8	3.7	15.5	13.8	3.7	0.7	1.0	1.9	1.1

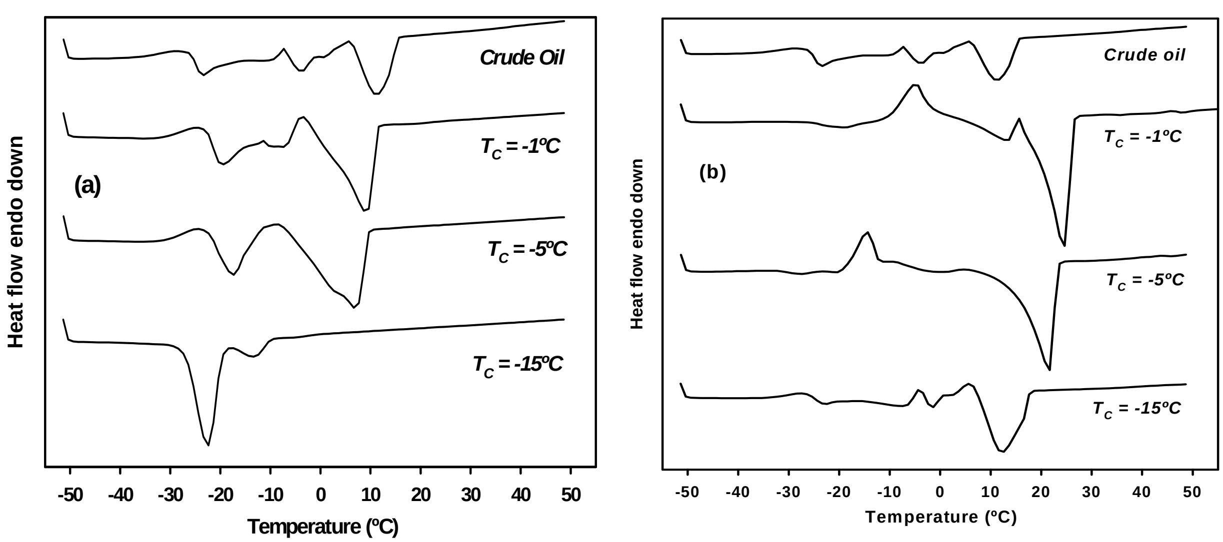
[a] For each fraction, means in a column followed by the same letter are not significantly different by LSD test at the 5% level.

Melting profiles of the crude SBPO and its LF and SF

Figures 2a and 2b show the melting profile curves recorded using DSC, when heating crude SBPO and its liquid and solid fractions at 5ºC/min from -50 to 50ºC. The melting behavior of crude SBPO is a complex phenomenon showing at least three well separated endotherms, which indicate the presence of three major groups of triacylglycerols melting independently between -30ºC and 20ºC. Melting of crude SBPO started at approximately - 26ºC with a broad low-temperature endotherm (LTE) which spanned until -7ºC, whose peak maximal temperature (T_m) and melting enthalpy (H_m) were about -23.5ºC and 24 J/g, respectively. The middle-temperature endotherm (MTE) extends from -7ºC to about 7ºC, with a T_m of -3.7ºC, and a H_m of about 16 J/g. The high-melting triacylglycerols (HMT) group exhibited a prominent endothermic peak between about 7ºC and 17ºC, with a T_m of approximately 11ºC, and a H_f of about 30 J/g. The average total melting enthalpy (ΔH) of the crude SBPO was about 75 J/g.

As shown in Figures 2a and 2b, the solvent crystallization processes caused marked changes in the thermal characteristics of the liquid and solid fractions obtained after crystallization processes at -1ºC, -5ºC and -15ºC, compared with the crude SBPO. The melting curve of the LF obtained at T_C=-1ºC (Figure 2a) shows a broad endothermic peak between -23ºC and -3ºC (T_m=-18ºC), followed by a second prominent endothermic peak spanned between -3ºC and 13ºC (T_m=9ºC). For this fraction, the average total melting enthalpy (ΔH) was nearly 74 J/g. On the other hand, the melting behavior of the LF obtained at T_C=-5ºC showed two well-defined and separated endothermic peaks. The first occurred between -23ºC and -10ºC had a T_m=-17.6ºC, whereas the second occurred between about -8ºC and 10ºC had a T_m=6.9ºC. The ΔH for this fraction was approximately 67 J/g. When melting, the LF obtained at T_C=-15ºC exhibited two overlapping peaks between -30ºC and -18ºC (T_m=-22.5ºC), and between -18ºC and -10ºC (T_m=-13.1ºC), with an average total melting enthalpy (ΔH) of about 62 J/g. Thus, as shown in Figure 2a, the liquid fractions obtained after solvent crystallization processes at -1ºC and -5ºC were lower in HMT, in contrast to the liquid fraction obtained at T_C=-15ºC, which was higher in LMT.

Figure 2b presents the melting profiles of the solid fractions obtained after crystallization processes at -1ºC, -5ºC and -15ºC. The melting curves of the SF obtained at -1ºC and -5ºC were characterized by the presence of one broad exothermic peak between -19ºC and 3ºC (T_m=-3.5ºC) and between -20ºC and 0ºC (T_m=-12.8ºC) respectively, prior to the melting of their HMT as indicated by the prominent endothermic peaks exhibited between about 15ºC and 27ºC (T_m=25.7ºC), and between 6ºC and 25ºC (T_m=22ºC), respectively. Moreover, the melting curve of the SF obtained at -1ºC showed a small endothermic transition with T_m=14.1ºC, before the melting of the HMT. On the other hand, the melting curve of the SF obtained at T_C=-15ºC, was similar to the crude SBPO melting curve, because of the high weight yield found at this crystallization temperature (~80%w/w). The average total melting enthalpies (ΔH) of the SF obtained after crystallization processes at -1ºC, -5ºC and -15ºC were 78.1, 75.1 and 75.7 J/g, respectively.



a) b)

Figure 2. Melting curves of crude SBPO and their LF (a) and SF (b) obtained after solvent crystallization with acetone at different temperatures during 24 h, using a 1/5m/v oil to solvent ratio (Heating rate = 5ºC/min).

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Osmotic dehydration of seabuckthorn (*Hippophaë rhamnoides* L.) fruits

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Abstract

Seabuckthorn presents interesting economic potential due to its well-known nutraceutical properties. In spite of its high nutritional content, seabuckthorn fruits are fragile and rapidly perishable, limiting its production at large scale. The development of an efficient conservation process is thus of significant importance in terms of commercial strategy.

The effect of osmotic dehydration in combination with hot-air or vacuum drying was investigated regarding the physicochemical characteristics of seabuckthorn fruits. First, a comparison of different pre-treatments was done in order to optimize the seabuckthorn skin permeability during osmotic dehydration. For this purpose, fruits were treated by different techniques: immersion in liquid nitrogen, steam blanching, freeze/thaw cycles, and no treatment (control). Based on the results, immersion in liquid nitrogen was found to be the best treatment to maximize skin permeability and to increase sugar gain during osmotic dehydration. The second part of this study was the evaluation of drying kinetics during osmotic dehydration of seabuckthorn fruits pre-treated with liquid nitrogen, followed by vacuum or hot-air drying. Loss of vitamin C, carotenoids and phenolics compounds was also measured to test the effect of drying methods on the nutritional characteristics of seabuckthorn.

Sugar intake and partial dehydration of seabuckthorn samples increased with osmosis time and reached a maximum value after 5 h treatment. The post drying method (vacuum or hot-air) showed a significant impact on the remaining moisture content of seabuckthorn samples. The final moisture content was significantly lower in air-dried samples than vacuum-dried ones.

Keywords: seabuckthorn, osmotic, dehydration, carotenoids, phenolics**.**

Introduction

In last few years, seabuckthorn (*Hippophaë rhamnoides* L.) gained great interest around the world, including Canada. This plant is primarily valued for its golden-orange fruits, which provide vitamin C, vitamin E, flavonoids, oils rich in essential fatty acids, and other healthful components [1]. However, these fruits are fragile and delicate and, if not properly stored or processed, they have a short shelf life. The development of an efficient conservation process is thus of significant importance in terms of commercial strategy.

Dehydration is an important processing operation in the food industry and it is an interesting option for the seabuckthorn conservation. Hot-air drying is a commonly used method for fruit dehydration but can cause, changes in the physical properties such as color and structure, as well as the deterioration of aroma compounds or degradation of nutritional substances, reducing product quality [2, 3]. An alternative to conventional drying is osmotic dehydration, which is a gentle and low-energy process that can be used to preserve high nutritional products due to low temperatures and lack of oxygen during the process. Osmotic dehydration achieves a twofold transformation of the food item, by both a decrease in water content and an increase in solute incorporation [4]. Osmotic dehydration can only remove moisture from the fruit up to a certain level, which is still high for long term preservation, so it should be followed by another process to further lower the water content of the fruit. Hot-air or vacuum drying methods can be used to remove the remaining water from the fruit after osmotic dehydration.

In spite of its high nutritional content, seabuckthorn fruits also present a unique characteristic, a waxy outer skin. This waxy layer is an important barrier for sugar impregnation and water loss during osmotic dehydration. Physical or chemical pre-treatments are necessary to overcome the waxy barrier and thus to maximize skin permeability and sugar gain during osmotic dehydration. The main goal of this work was thus to evaluate osmotic dehydration in

combination to hot-air or vacuum drying for seabuckthorn fruit preservation, with the following specific objectives: a) study of pre-treatments to optimize the seabuckthorn water and sugar skin permeability; b) determination of osmotic and drying kinetics; c) analysis of vitamin C, carotenoids and phenolics compounds losses after processing.

Material and Methods

Seabuckthorn (var. 'Indian Summer') were manually harvested in August 2006 in a farm located in Ste-Anne de Beaupré (Québec, Canada). After harvesting, the fruits were frozen at -18°C. The initial moisture, pH, water activity (A_w), solid soluble content (°Brix), titratable acidity, vitamin C, carotenoid and phenolic contents, color and fruit weight were determined as described later. The osmotic solution was prepared by mixing food grade sucrose with distilled water.

This study was done in two set of experiments. The first set was conducted to evaluate the effect of different pre-treatments in order to optimize the seabuckthorn skin permeability during osmotic dehydration. For this purpose, the fruits were treated by different techniques: immersion in liquid nitrogen, steam blanching, freeze/thaw cycles, and no treatment (control samples). After each pre-treatment, the osmotic dehydration was carried out for 6h at 40°C. Moisture content, pH, sugar content (based on °Brix determination) and water activity (Aw) were then evaluated.

From these results, the pre-treatment allowing both a better water loss and sugar intake was chosen for the second part of this study. The goal of the second set of experiments was to investigate the effect of combined osmotic and hot-air or vacuum drying. To test the effect of drying methods on the nutritional characteristics of seabuckthorn fruits, the determination of carotenoids and phenolics compounds and vitamin C content was carried out after the osmotic treatment and after hot-air and vacuum drying.

Pretreatments

Steam blanching was carried out using a deep container with boiling water. The frozen fruits (20g) were suspended in a sieve above the boiling water and heated only by the steam. The fruits were sufficiently spaced from each other to maximise the contact surface between the fruit and the steam. This treatment was carried out for two minutes.

Other frozen fruits were subjected to two freeze/thaw cycles. The first one was at temperatures of -10°C/ -2°C and the second, at -30°C /-10°C /-2°C (3 hours at each temperature). Each cycle was repeated three times. A temperature-controlled incubator (Sanyo Incubator MIR 153, Sanyo Scientific. Hayward, CA, USA) was used for these experiments.

Liquid nitrogen was used to pre-treat another part of the fruits by immersion until the total evaporation of liquid. Then the fruits were left at ambient temperature for 2 minutes. The immersion in liquid nitrogen was repeated three times.

Untreated seabuckthorn samples were used as control.

Osmotic Dehydration

In the experiments made to evaluate the effect of pre-treatments, the fruits were kept in the osmotic solution (60°Brix) for 6 h at 40°C. On the other hand, in the experiments made to study the process of osmotic dehydration followed by hot-air or vacuum drying, the samples were kept in the osmotic solution for 5 h at the same temperature. A basket was used to keep the fruits immersed in the osmotic solution. After osmotic dehydration, the basket with seabuckthorn samples was removed, and the fruits were drained and blotted with absorbent paper to remove the excess of solution. The temperature and soluble solid content of the solution were monitored throughout each experiment using a thermometer and a hand-held refractometer (Model Shilac, Fisher Scientific Ltd., Nepean, ON, Canada). Samples were used to measure quality parameters such as water activity, water loss, sugar gain and pH.

Drying

After osmotic dehydration, the seabuckthorn samples were dried by hot-air or vacuum drying. For hot-air-drying experiments, a laboratory hot-air tray dryer (Model UOP8-G, Armfield, Hampshire, England) was used. Air speed and temperature were 1 m/s and 50°C respectively. Air speed and temperature were measured continuously using an anemometer (LCA 6000, Airflow Development Ltd, Andover, NJ, USA) and T-type thermocouples (Omega Engineering Inc., Laval, QC, Canada), respectively. Seabuckthorn samples were also dried in a vacuum oven (Lab-Line instruments, Inc, Melrose Park, IL, USA) at 50°C with a gauge pressure of 25 in Hg. Drying curves were obtained by periodic weighing of seabuckthorn samples at different processing times (2, 4, 7, and 15 hours).

Seabuckthorn samples were stored immediately after drying in desiccators in the presence of P_2O_5 for further analysis. The moisture content, vitamin C, carotenoids and phenolics compounds of dried samples were determined after 15 hours of hot air and vacuum drying.

Physico-Chemical Analysis

Moisture content (g water/g dried matter) was calculated using values of dried mass determined by the vacuum oven method [5]. Seabuckthorn samples (5g) were placed in aluminium dishes in a vacuum oven with the presence of P_2O_5 as a desiccant. The oven temperature was 50°C with a gauge pressure of 25 inHg. The samples were kept in the oven until moisture equilibrium was achieved and then taken out of the oven, cooled in a desiccator and weighed using a balance (Model Mettler Toledo AB104-S, Greinfesee, Switzerland) with a sensitivity of 0.001g.

Fruits were evaluated for acidity according to the method described by Tang and Tigerstedt [6]. The frozen fruits (40 g) were manually cut in halves and the seeds were removed. The fruits were homogenized with 100 ml of water. The homogenate was diluted to 300 ml and boiled for 30 min. After cooling, the homogenate was made up to 300 ml by adding water and then filtered (No.1 Whatman). The filtered aliquot (10 mL) was used for further analysis. Titratable acidity (TA) was determined by titration with 0.1 N NaOH solution up to the end point of phenolphthalein. TA was conventionally expressed as malic acid (%).

For soluble solid content (°Brix) and pH determination, frozen fruits (25 g) were manually cut in halves and the seeds were removed. The fruits were crushed and homogenized for 5 minutes (until obtaining a homogeneous juice) using an Ultra-Turrax homogenizer. The juice obtained was then filtered (No.1 Whatman). The filtered aliquots were used for pH and °Brix analysis. The soluble solid content (°Brix) was determined using a digital refractometer Reichert AR 200 (Reichert Inc., Depew, NY,USA) and for pH determination, a pH-meter model SP20 (VWR Symphony, Thermo Orion West Chester, PA, USA).

The water activity was measured by using an A_w-meter (Aqualab, Meyer Service & Supply Ltd., ON, Canada), calibrated with saturated salts solutions providing constant relative humidity. The color was measured by using a Chromameter (Model Minolta meter CR-300, Mississauga, ON, Canada).

Carotenoids and phenolics compounds were determined according to the method described by Gao *et al.* [7] with slight modifications. For carotenoids, 1 ml aliquot from lipophilic extract was diluted with 4 ml of hexane and measured at 460 nm (Spectrophotometer Model 8451A, Hewlett-Packard, CA, USA). Quantification was achieved by a calibration curve obtained with β-carotene standard (Fluka Biochemika, Milwaukee, WI, USA) diluted with hexane. Total carotenoids were expressed as mg/100mg β-carotene equivalent. On the other hand, phenolics extract (100 μL) was mixed with 0.2 ml Folin-Ciocalteau reagent, 2 ml of water and 1 ml of 15% Na_2CO_3, and the absorbance was measured at 765 nm after 2 h incubation at room temperature. Gallic acid (Sigma-Aldrich, Oakville, ON, Canada) was used as standard and the total phenolics were expressed as mg/100g gallic acid equivalent. For the determination of phenolic and carotenoid compounds in dried samples, the fruit was rehydrated to its initial moisture content.

Vitamin C was determined according to the method described by Askar and Treptow [8]. Vitamin C was expressed as mg per 100 g. For Vitamin C determination in dried samples, the fruit was rehydrated to its initial moisture.

Results and Discussions

The composition of seabuckthorn fruits is presented in Table 1. An average fruit weighs 0.41 g ± 0.01 and contains 87.01 % ± 0.12 moisture. These values are closer to the average reported in the literature for the 'Indian Summer' cultivar [1]. Vitamin C (ascorbic acid) represents a nutrient of major importance in seabuckthorn fruits because it is present in large quantity [1]. In fact, ascorbic acid level was 184.63 ± 23.15 (mg/100g). The result of Vitamin C compares favourably with the average result of 174.70 ± 30.70 (mg/100g) published in the literature [9]. Considering that fresh orange fruit contains 45 mg/100g [10], the amount of Vitamin C in seabuckthorn is significant. The content of soluble sugars in fresh seabuckthorn was 7.91 ± 0.57 °Brix. This value appears to be lower than the values for 'Indian Summer', which ranges in the literature from 9.3 to 17.3°Brix [1].

Seabuckthorn fruits var. 'Indian Summer' had an average pH of 2.57 ± 0.03 and an average titratable acidity of 1.71% ± 0.04 calculated as malic acid (Table 1). The titratable acidity values are similar to those reported by Beveridge *et al*. [9], while the pH is slightly lower than the one published in the same study. Various investigations showed high variability in seabuckthorn fruit composition depending on variety, maturity and growing location. However, considering the present results and the ones reported in previous studies, it can be concluded that this type of variability could be present even in fruits of the same cultivar.

Seabuckthorn fruits are particularly abundant in bioactive molecules such as phenolics and carotenoids compounds. The carotenoids and phenolics compounds in the fresh fruit are present at levels of 3.99 ± 0.14 and 175.25 ± 0.24 (mg/ 100g), respectively. Beveridge *et al*., [1] reported values of carotenoids ranging from 9.4 to 34.5 (mg/100g) for 'Indian Summer' cultivar, which are higher than the present results. On the other hand, Gao *et al*. [7] reported carotenoids contents from 1 to 6.5 mg/100g for the Botanitjetskaja variety, 8.2 to 13.3 (mg/100g) for Aromatnaja, and 5.1 to 8.0 (mg/100g) for Trofimovskaja. In contrast to the carotenoids, data for total phenolic content in seabuckthorn fruits were not found in the literature for 'Indian Summer' variety. However, the total phenolics content reported in the present study (175.25 ± 0.24 mg/100g) is similar to that found for other cultivars such as Botanitjetskaja (156.6 to 177.4 mg/100g), Trofimovskaja (114 to 209.8 mg/100g) and Arotmanaja (187.9 to 244.1 mg/100g) [9]. Phenolics content observed in seabuckthorn fruits is lower to that of other fruits such as cranberries (709 mg/100g), blueberry (503 mg/100g) and strawberry (366 mg/100g) [11].

Table 1 Composition of seabuckthorn fresh fruit (obtained in this work).

Property	Value
Weight (g/fruit)	0.41 ± 0.01
pH	2.57 ± 0.03
Color	L : 54.73 a :18.16 b : 47.11
°Brix	7.91 ± 0.57
Titratable acidity (% malic acid)	1.71 ± 0.04
A_w	0.97 ± 0.01
Moisture content (g/100g) (wb)	87.01 ± 0.12
Vitamin C (mg/100g)	184.63 ± 23.15
Total phenolics (mg/100g)	175.25 ± 0.24
Total Carotenoids (mg/100g)	3.99 ± 0.14

Table 2 shows a comparison of the different pre-treatments made in order to optimize the seabuckthorn skin permeability during osmotic dehydration. Water loss and solid gain are mainly controlled by the raw material characteristics, but certainly influenced by the possible pre-treatments [4]. The freezing/thawing cycles had no effect on the osmotic dehydration of seabuckthorn fruits because water loss and sugar gain were similar to the control treatment (Table 2). From these results, immersion in liquid nitrogen was found to be the best skin pre-treatment allowing 36.27% water loss and 19.96% sugar intake after 6 hours of osmotic dehydration. The percentage of water loss and sugar gain is similar to steam blanching, but immersion in liquid nitrogen would be a better option because, contrary to the steam blanching, the fruit does not burst during treatment. Immersion in liquid nitrogen could be beneficial from two standpoints: a) the low temperature of liquid nitrogen (-195°C) causing changes and considerable breakdown of the waxy skin layer allowing both a quick water loss and an increase solute incorporation, and thus b) the sugar/acid ratio is improved giving a better taste to final product.

Table 2. Effect of different pre-treatments on osmotic dehydration of seabuckthorn fruit.

Treatment	Water loss (%)	Sugar content (g/100g)*	A_w	pH
Control	29.87	11.31	0.91	3.16
Freeze/thaw cycles (-30°C/-10°C/-2°C)	28.30	14.33	0.91	3.24
Freeze/thaw cycles (-10°C/-2°C)	23.85	12.38	0.90	3,13
Steam blanching	39.14	18.76	0.88	3.33
Immersion in liquid nitrogen	36.27	19.96	0.84	3.34

* based on °Brix determination.

Figure 1 shows the hot-air and vacuum drying kinetics curves of osmotically dehydrated seabuckthorn fruit. The moisture content decreased exponentially with drying time and thus, both hot air and vacuum drying seemed to take place in the falling rate period. The same behaviour was reported in the literature on fruits dehydrated by combined

osmotic and drying processes [12, 13]. Both post drying methods had an important effect on the remaining moisture content of seabuckthorn samples. Hot-air removed water much faster than vacuum drying. In fact, air-drying kinetics was almost two-fold faster than vacuum drying. As an example, the drying time to obtain X/X_0= 0.1 was 7 h for vacuum drying while it was 4 h for hot-air drying.

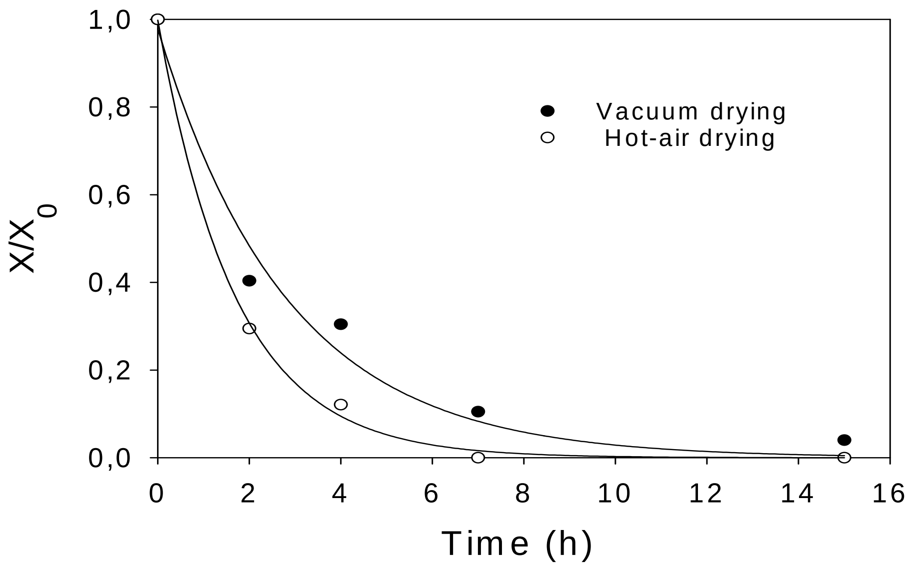

Figure 1. Drying curves of osmotically dehydrated seabuckthorn fruit (pre-treated in liquid nitrogen and after 5 h of osmotic treatment).

Table 3 shows the final concentration of vitamin C, total carotenoids and total phenolics after osmotic dehydration, hot-air drying or vacuum drying. Osmotic dehydration causes a reduction of some bioactive compounds (Table 3). Carotenoids and phenolics had a slight loss after osmotic dehydration while vitamin C decrease was significant (22%, 12% and 78%, respectively). Vitamin C (ascorbic acid) is a very soluble compound leaving the fruit together with moisture as osmotic dehydration proceeds.

On the other hand, vacuum drying keeps better the phenolics compounds than hot-air drying (25% loss compared to 39%). Phenolics compounds could be susceptible to enzymatic degradation during air drying. In fact, the oxygen catalyzes the enzymatic oxidation of phenols by the polyphenols oxydases (PPO) [14]. In vacuum drying, the activity of PPO would be partly reduced by the lack of oxygen. For the other compounds, however, the total losses were significant after vacuum drying or air-drying (approximately 85% vitamin C and 50% carotenoids). Vitamin C is a heat-sensible vitamin and carotenoids have a highly unsaturated nature which makes them susceptible to degradation by oxidation and thermal processing [15].

Drying after osmotic dehydration caused color deterioration of the seabuckthorn fruit (browning) and 'collapse' of its structure giving a sticky and compact final product. This change was more pronounced in air-dried samples than vacuum-dried ones.

Table 3. Nutritional composition of seabuckthorn fruit after osmotic dehydration and hot-air or vacuum drying.

Treatment	Concentration (mg/100g)		
	Total carotenoids	Total phenolics	Vitamin C
Fresh fruit	3.99 ± 0.14	174.25 ± 0.24	184.63 ± 23.15
After osmotic dehydration*	3.11 ± 1.31	153.33 ± 0.21	39.16 ± 8.15
After air drying*	2.13 ± 0.42	106.28 ± 0.81	26.69 ± 4.54
After vacuum drying*	1.89 ± 0.35	130.68 ± 0.07	28.93 ± 15.1

* concentration (g/100g of rehydrated fruit).

Conclusion

This study focused on the preservation of seabuckthorn fruits by combined osmotic and drying processes. Pre-treatments such as immersion liquid nitrogen allowed a quick water loss and sugar intake through the waxy skinned fruit. Moisture content was greatly reduced in air-dried samples compared to vacuum dried ones. Sugar intake gives a better taste to the final product. An improvement of organoleptic properties through sugar intake is important since seabuckthorn fruit is not generally appreciated by consumers due to its high acidity. However, concentration of some nutritional compounds was dramatically reduced after the two drying processes.

Acknowledgments

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Enzymatic Oil Extraction of Seabuckthorn (*Hippophaë rhamnoides* L.) Pulp

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Abstract

Seabuckthorn pulp contains interesting health-beneficial oil. This oil is currently obtained by pressing, centrifugation and solvent extraction with hexane or supercritical carbon dioxide of the berries. Enzymatic oil extraction was thus investigated in this work due to its technological advantages in comparison to current methods. It eliminates solvent consumption, reduces energy requirements, and enables high yields of good quality oils. Three commercial enzymes (Pectinex Ultra SP-L, Celluclast 1.5L and Viscozyme L) were evaluated for oil extraction from the seabuckthorn pulp obtained after juice separation. Extractions were carried out for 24 h at pH 5.0 and 50ºC, using different enzyme concentrations. The obtained crude oils were analyzed for their fatty acid (FA) and triacylglycerol (TAG) compositions, and fractionated using solid-phase extraction. The melting profiles of different oil samples were characterized by differential scanning calorimetry (DSC). The average oil yields obtained with Viscozyme L (18.6%w/w) and Pectinex Ultra SP-L (17.0%w/w) were significantly higher ($p<0.01$) than those attained with Celluclast 1.5L (13.9%w/w). For all enzymes, the FA profiles of oils were characterized by high concentrations of palmitoleic (~40%) and palmitic (~35%) acids, with minor proportions of linoleic (~10%) acid. These oils were mainly rich in TAG with acyl carbon number of 48 (~56%) and 50 (~39%), with trace amounts of 46 and 52. Lipid fractionation of crude oils yielded about 97% of neutral lipids. The melting curves of the oils showed the presence of three major endotherms melting separately between -30ºC and 20ºC, for which melting temperatures were -24ºC, -4ºC and 10ºC.

Keywords: seabuckthorn, *Hippophaë rhamnoides*, enzymatic oil extraction, melting curves, fatty acids, triacylglycerols, solid phase extraction (SPE).

Introduction

Seabuckthorn berries (*Hippophaë rhamnoides* L.) have a high lipid content, including tocopherols, tocotrienols, carotenoids, as well as omega-3 and omega-6 fatty acids families (Yang and Kallio, 2002a). The oil contents of seeds and pulp range commonly between 10-15% and 30-35%, respectively (Yang and Kallio, 2001; 2002a). The pulp oil contains high amounts of palmitoleic (C16:1*n*-7, 16-54%) and palmitic acids (C16:0, 17-47%), whereas the seed oil is highly unsaturated, containing mainly linoleic (C18:2*n*-6, 30-40%) and α-linolenic (C18:3*n*-3, 20-35%) acids (Yang and Kallio, 2002a). Both seed and pulp oils have shown beneficial effects on human health (Yang and Kallio, 2002a).

The most used techniques to obtain commercial seed and pulp seabuckthorn oils are solvent extraction and supercritical fluid extraction (Yang and Kallio, 2002a). Aqueous extraction and pressing have been also reported recently (Yakimishen et al., 2005). Other methods comprise the use of additional vegetable oils to extract oils from seeds and pulp, to obtain oils of mixed composition (Li and Beveridge, 2003).

Enzymes have been used in oil extraction of various fruits and seeds, and the results in both laboratory and industrial scale have been excellent (Domínguez et al., 1994). There has been little study in the field of enzymatic oil extraction of seabuckthorn berries. Only one study (Nikolov and Heilscher, 2002) indicates the use of enzymes to obtain seabuckthorn oils and food-grade seabuckthorn juice.

Given the increasing interest and applications of seabuckthorn pulp oil, and with the aim to avoid the use of toxic organic solvents, the objective of this work was to investigate the extraction yields and composition of oils from seabuckthorn pulp, using three commercial enzymes (Pectinex Ultra SP-L, Celluclast 1.5L and Viscozyme L).

Materials and Methods

Berries

Seabuckthorn berries (*Hippophaë rhamnoides* L. cv. 'Indian Summer') from Sainte-Anne-de-Beaupré (Québec, Canada) were hand cleaned and stored at -30ºC in sealed plastic bags until the beginning of the experiments.

Enzymatic oil extraction

Seabuckthorn berries were washed with boiling water for short periods of time, to inactivate deteriorative enzymes, and then rinsed with cold water. The juice, obtained using a commercial juice extractor, was allowed to stand 48 h in the refrigerator at 4ºC. The upper pulp phase, whose dried mass was measured by the vacuum oven method, was treated enzymatically for 24 h at 50ºC, after adjusting the pH to 5.0 with NaOH, with three commercial enzyme preparations (Celluclast 1.5 L from *Trichoderma reesei*, Pectinex Ultra SP-L from *Aspergillus aculeatus* and Viscozyme L from a group of *Aspergillus*) purchased from Sigma-Aldrich (St. Louis, MO). Five different enzyme/substrate concentrations (0.25, 0.5, 1.0, 2.0 and 4.0% v/v) were tested and compared with a control sample (without enzymes). Extractions were carried out in Erlenmeyer flasks under permanent agitation (400 rpm) using a thermoregulated bath. After centrifugation at 10000 rpm during 60 min at 25ºC (Refrigerated Multipurpose Centrifuge 5804 R Eppendorf, Germany), the supernatant oils were collected, weighed, and stored under nitrogen in sealed amber glass vials at -20ºC until lipid analysis. Oil extraction yields (%w/w) were calculated as the mass ratio percentage of extracted oil (g) to dried pulp material (g).

Analytical methods

Fatty acid composition

The fatty acid compositions of the obtained oils were determined by gas chromatography (GC). Samples were converted into their methyl esters (FAME) and analyzed on a 5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, CA). The oven temperature was programmed as follows: from 60ºC (isothermal for 1 min) to 190ºC at 20ºC/min, and isothermal period of 30 min at 190ºC. The injector and detector temperatures were set at 250°C. Hydrogen was used as the carrier gas. Gas chromatography separation peaks were performed on a BPX-70 capillary column (60 m×0.25 mm i.d.×0.25 μm film thickness; SGE, Melbourne, Australia). Fatty acids were identified by comparing their retention times with those of the FAME standards purchased from Nu Chek Prep (Elysian, MN, USA) under the same conditions. Peaks were integrated using Hewlett-Packard ChemStation software.

Triacylglycerol composition

A GC method was used for the determination of the triacylglycerol (TAG) composition of the obtained oils. Samples were dissolved in octane and analyzed on a 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA). The oven temperature was programmed as follows: from 250ºC to 360ºC at 3.0ºC/min, and isothermal period of 15 min at 360ºC. Injector and detector temperatures were set at 400°C. Hydrogen was used as the carrier gas. The GC separation method was performed on a RTX-65TG capillary column (30 m×0.25 mm i.d.×0.1 μm film thickness; Resteck, Bellefonte, PA, USA). The identification of the peaks was achieved by comparing their retention times with those of the standards purchased from Nu Chek Prep (Elysian, MN, USA) and Sigma-Aldrich (Sigma, St. Louis, MO, USA) under the same conditions. Peaks were integrated using Hewlett-Packard ChemStation software.

Melting profiles

Melting profiles of the oils were determined using a differential scanning calorimeter (DSC) (Pyris 1 DSC, Perkin-Elmer, Norwalk, CT, USA) equipped with an intracooler II (Perkin-Elmer, Norwalk, CT, USA). The system was purged during analysis with nitrogen at 30 mL/min. The melting curves were performed within the temperature ranges of -50 to 50ºC. Samples (~10 mg) were cooled at -50ºC and held at this temperature for 5 min, and then

heated at 5ºC/min. An empty pan was used as inert reference to balance the heat capacity of the sample pan. Calibration of the DSC was carried out using indium (*mp* = 156.6ºC, ΔH_f = 28.71 J/g). Data were analyzed using a thermal analysis software (Pyris 1 Version 3.5, Perkin Elmer).

Lipid fractionation

Separation of individual lipid fractions was achieved using solid-phase extraction (SPE) cartridges (Bakerbond SPE amino [NH2] disposable extraction columns, J.T. Baker Inc., Phillipsburg, NJ, USA) as described by Oomah et al. (2000). The cartridges were preconditioned with 2-mL methanol, 2-mL chloroform, and 4-mL hexane before use. Lipid fractions were recovered by sequential elution under vacuum with 4-mL each of chloroform/isopropanol (2/1, v/v), diethyl ether/acetic acid (95/5, v/v), and methanol, to separate neutral lipids, free fatty acids and phospholipids, respectively. The collected eluted fractions were evaporated under nitrogen, weighed, and stored at -20ºC for subsequent fatty acid analysis.

Experimental design and statistical analysis

The experimental units were grouped in three blocs, and the treatment structure adopted was a 3×5 factorial design (three enzymes×five concentrations). Results were analyzed using the analysis of variance by the general linear models (GLM) procedure. To analyze the effects of the independent variables on the oil extraction yields, qualitative contrasts and means comparisons by the least significant difference (LSD) test were performed according to the Statistical Analysis System (SAS Institute, 2000). Probability of p<0.05 was considered statistically significant.

Results and Discussion

Extraction yields

After settling, seabuckthorn juice separates into an emulsion pulp layer on the top, an aqueous phase in the middle, and some solids on the bottom. After removing the solids and the aqueous phase, the moisture content of the cream pulp phase was 84.8±0.6%. The effects of the enzymatic treatments on oil extraction yields from this material are shown in Figure 1. When comparing with the control sample (oil yield 11.6±0.7%), it is clear that enzyme concentrations ≥ 0.5% v/v improve the oil extraction yields of the cream pulp phase. It was also observed that the higher oil yields were obtained with Viscozyme L and Pectinex Ultra SP-L. In contrast, Celluclast 1.5L produced the lowest oil yields, reaching its maximum performance at a concentration of 1.0 % v/v.

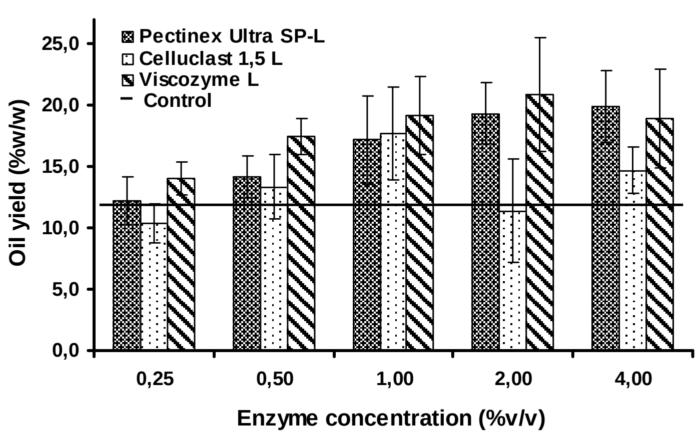

Figure 1. Oil extraction yields from seabuckthorn pulp using three commercial enzymes at different concentrations.

No significant differences were found between the oil yields obtained with Viscozyme L and Pectinex Ultra SP-L, and their average values (18.6% and 17.0%, respectively) were significantly higher (p<0.01) than those obtained with Celluclast 1.5L (13.9%). On the other hand, as shown in Figure 1, the oil yield increases as the enzyme concentration increases. However, from concentrations higher than 1.0%v/v, the oil extraction yields were not significantly different.

The results found in this study are in accordance with those reported by Nikolov and Heilscher (2002) who obtained a seabuckthorn pulp oil yield between 1-3%, using enzymes containing pectolytic and acid proteinase activity (Novozyme AP and Rohapect D5L) for about 24 h at 20ºC and 50°C.

Fatty acid composition

The fatty acid composition of the extracted oils is summarized in Table 1. As shown, the fatty acid profiles were similar, and independent of the type of enzymes. The major fatty acids were palmitoleic (39.5%), palmitic (36.1%) and linoleic (10.8%) acids. Similar values were recently reported for pulp oil from the same cultivar (Gutiérrez et al., 2008), while Yang and Kallio (2001) reported lower values of palmitic (26.7% and 27.8%) and palmitoleic (27.2% and 32.8%) acids for the subspecies *sinensis* and *rhamnoides* respectively, and higher values of oleic acid (~17%) for both subspecies.

Table 1. Fatty acid composition (%) of seabuckthorn pulp oils extracted with three commercial enzymes

Fatty acid	Pectinex Ultra SP-L	Celluclast 1.5 L	Viscosyme L
C14:0 (Myristic)	0.4±0.0	0.4±0.0	0.4±0.0
C16:0 (Palmitic)	36.1±0.3	36.1±0.4	36.2±0.3
C16:1 (Palmitoleic)	39.4±0.3	39.5±0.3	39.5±0.3
C17:0 (Margaric)	2.1±0.1	2.1±0.1	2.1±0.1
C18:0 (Stearic)	0.8±0.0	0.8±0.0	0.8±0.0
C18:1*n*-9 (Oleic)	2.8±0.0	2.8±0.1	2.8±0.0
C18:1*n*-7 (Vaccenic)	6.2±0.2	6.2±0.2	6.2±0.2
C18:2 *n*-6 (Linoleic)	10.8±0.3	10.8±0.3	10.8±0.3
C:18:3 *n*-3 (α-Linolenic)	0.9±0.0	0.8±0.0	0.9±0.1

Triacylglycerol composition

The TAG compositions of the oils obtained after enzymatic extraction are presented in Figure 2. As it can be observed, enzyme extracted seabuckthorn pulp oils contained mainly TAG with acyl carbon number (ACN) of 48 and 50 with 1–3 double bonds (C48:2, C48:1, C50:3, C50:2 and C48:3), representing between 90-95% of the total TAGs. These results are in accordance with those reported recently by Yang and Kallio (2006) for seabuckthorn berries of different origins. Analogous to the fatty acid compositions, the TAGs profiles were similar and independent of the type of enzymes.

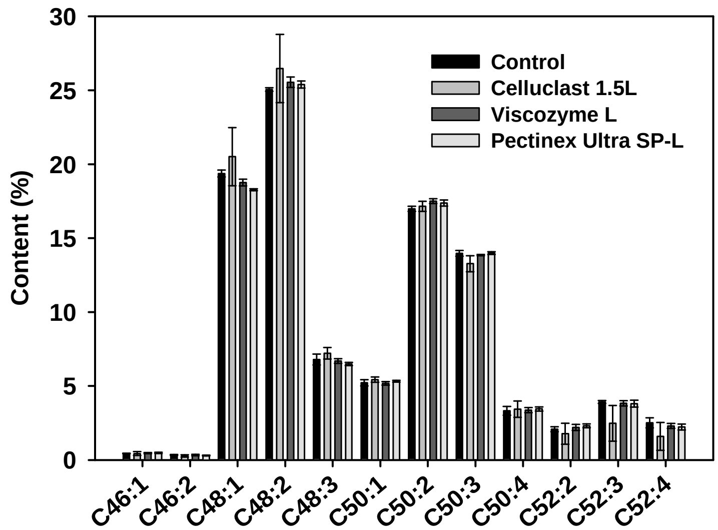

Figure 2. Triacylglycerol composition of seabuckthorn pulp oils extracted with three commercial enzymes.

Lipid fractions obtained by SPE

Enzyme extracted seabuckthorn pulp oils consisted mainly of neutral lipids (NL) (~97%), with minor amounts of free fatty acids (FFA) (~2%) and phospholipids (PL) (1%) (Table 2).

Table 2. Fatty acid composition (%) in individual lipid fractions of oils from seabuckthorn pulp, extracted with three commercial enzymes.

	Pectinex Ultra SP-L			Celluclast 1.5 L			Viscozyme L		
	NL	FFA	PL	NL	FFA	PL	NL	FFA	PL
Weight (%)	96.8	2.2	0.9	97.8	1.5	0.9	97.9	1.5	0.9
C14:0	0.4	2.2	2.1	0.4	1.9	2.1	0.4	2.6	2.0
C16:0	36.4	38.3	27.5	36.5	37.9	29.7	36.4	38.6	31.9
C16:1	39.6	36.0	32.6	39.6	36.2	34.4	39.8	34.9	33.3
C17:0	2.1	1.8	2.1	2.1	2.0	2.2	2.1	1.9	2.3
C18:0	0.8	1.7	3.6	0.8	1.6	2.6	0.8	1.8	3.3
C18:1n-9	2.8	3.0	2.5	2.8	2.8	2.4	2.7	2.8	2.1
C18:1n-7	6.0	5.6	9.0	6.0	5.6	7.9	6.0	5.5	7.5
C18:2	10.5	9.9	12.5	10.5	10.3	12.3	10.5	9.9	11.5
C:18:3	0.9	1.6	8.1	0.9	1.6	6.3	0.9	2.0	6.8

NL: Neutral lipids. FFA: Free fatty acids. PL: Phospholipids

The fatty acids profiles of the NL fractions were very close to the corresponding crude oils, because of the quantitative primacy of this fraction in the oils. In the FFA fractions, palmitoleic acid was about ~35%, whereas the proportions of myristic, palmitic, stearic and α-linolenic acids were slightly higher than in the neutral lipid fractions. On the other hand, the phospholipid fractions of the oils were poorer in palmitic and palmitoleic acids, but richer in stearic, vaccenic, linoleic and α-linolenic acids, than in the neutral lipid fraction. The proportions of linoleic and α-linolenic acids reached 12% and 8%, whereas palmitoleic was about 33%. Kallio et al. (2002) and Yang and Kallio (2001) reported contents of linoleic (32%, 22% and 24%), palmitoleic (22%, 15% and 19%), palmitic (21%, 16% and 17%) and α-linolenic (10%, 15% and 13%) acids in the PL fractions of the sea buckthorn pulp oils from subspecies *mongolica*, *sinensis* and *rhamnoides* respectively.

Genetic differences among subspecies, as well as climate, soil conditions, cultivating activities and lipid extraction processes could explain the differences (Yang and Kallio, 2002b).

Melting profiles

The DSC melting curves of the extracted oils are shown in Figure 3. As it can be observed, no significant differences were found between the melting profiles of the oils extracted with different enzymes, confirming the similarities in their triacylglycerol compositions. The oil melting curves indicate the presence of three major groups of triglycerides melting independently between -30ºC and 20ºC. The heating profiles showed that there is one major peak at 10ºC, and two minor peaks at -4ºC and -24ºC. The low melting endotherm (LME), representing the melting transition of the more unstable crystals of the low melting triacylglycerols, had a melting enthalpy of about 20 J/g. The melting enthalpies of both medium (MME) and high melting endotherms (HME), corresponding to medium and high melting triacylglycerols, are given in Table 3. There were no significant differences between the melting temperatures and enthalpies of individual endotherms. According to Timms (1980), the large area of the highest melting peak would partly due to the higher heat of melting of the TAG groups corresponding to this endotherm. Another reason which could explain this situation is connected to the presence of high melting triacylglycerols crystallizing together with lower melting triacylglycerols in a quasi-stable mixture.

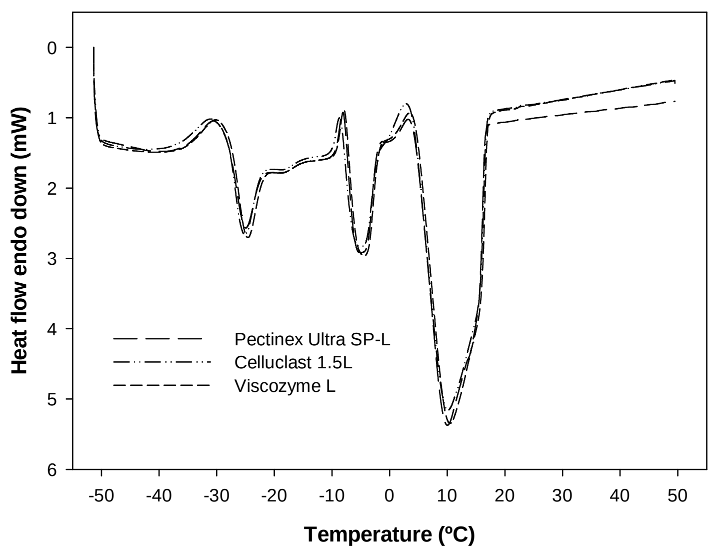

Figure 3. Melting profiles of oils from seabuckthorn pulp extracted with three commercial enzymes (Scan rate: 4ºC/min).

Table 3. Thermal characteristics of enzyme extracted sea buckthorn pulp oils[a].

Enzyme	LME		MME		HME	
	ΔH_m (J/g)	T_m (ºC)	ΔH_m (J/g)	T_m (ºC)	ΔH_m (J/g)	T_m (ºC)
Pectinex Ultra SP-L	20.6±0.4	-24.4±0.4	11.3±0.5	-4.6±0.6	43.6±0.4	10.4±0.3
Celluclast 1.5 L	20.5±3.2	-24.3±1.0	12.0±1.3	-3.9±0.9	40.6±4.0	10.7±0.5
Viscozyme L	21.6±1.5	-24.1±0.5	12.1±0.4	-4.1±0.3	43.3±1.2	10.7±0.3

a n=3. LME: Low melting endotherm. MME: Middle melting endotherm. HME: High melting endotherm.
T_m: Melting temperature. ΔH_m : Melting enthalpy.

Conclusions

Enzymatic extraction is a technique that could be applied to obtain seabuckthorn pulp oil. Among enzymes used in this study, Viscozyme L and Pectinex Ultra SP-L gave an oil extraction yield significantly higher (p<0.01) than that attained with Celluclast 1.5L (18.6%w/w, 17.0%w/w *vs.* 13.9%w/w, respectively). Fatty acid analysis revealed that palmitoleic (~40%), palmitic (~35%) and linoleic (~10%) acids were the main fatty acids in pulp oil extracts. No significant differences were found between the fatty acid compositions when comparing the type of enzymes.

Seabuckthorn pulp oils contained mainly TAG with acyl carbon number (ACN) of 48 and 50 with 1–3 double bonds (C48:2, C48:1, C50:3, C50:2 and C48:3), representing between 90-95% of the total TAGs. Lipid fractionation of pulp oils, obtained by SPE, yielded mainly neutral lipids (~97%). The melting profiles of pulp oils showed multiple endothermic transitions, as observed normally in DSC analysis of vegetable oils.

Acknowledgments

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Total Lipids and Carotenoids Content in Different Biotypes of *Hippophaë rhamnoides* L., Harvested in Romania

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Abstract

Seabuckthorn berries are well known in Eastern Europe for their healthful components but they are also a unique source of rare and valuable lipids. The antioxidant activity of the oil is stronger because of high carotenoids levels. In this paper, the results of total lipids and carotenoids content in seabuckthorn fruits and seeds harvested in two Romanian areas (Sulina, different places located at altitudes between 0.8 m and 12 m; and Buzau, different places located at altitudes between 278 m and 454 m) are presented. Seabuckthorn berries were harvested at full biological maturity. Lipids were extracted with ethyl ether and evaluated gravimetrically after the solvent was removed. Carotenoids, after the elimination of all the carotenoid denaturizing substances, were extracted in diluted acetone and estimated spectrophotometrically. The total lipids content of whole berries varied from 15.28 g/100 g dry matter to 38.42 g/100 g dry matter and, from 15.04 g/100 g dry matter to 32.41 g/100 g dry matter for the seeds. Carotenoid content of whole berries varied from 15.03 mg/100 g dry matter to 141.36 mg/100 g dry matter. Total lipids and carotenoids content show a meaningfully variation due to biotypes and pedoclimatic conditions of the harvested plants ecosystem.

Keywords: *Hippophaë rhamnoide*s, seabuckthorn biotypes, dry matter, water content, lipids, carotenoids.

Introduction

Seabuckthorn (*Hippohaë rhamnoides* L), a plant found in the temperate zones of Europe and Asia, represents a valuable biologic material for the medical and nutritional industries. It can be very different depending on the microclimate point of view. The plant easily accommodates to poor, dry and even salt-affected soils, without manifesting any special preferences regarding the lithologycal substratum (Bereşiu *et al*., 1983).

The seabuckthorn's complex chemical structure varies depending on the environmental conditions and the extraction methods which are used. Generally, *Hippophae rhamnoides* contains vitamin C, mineral elements, monosaccharides, organic acids, free amino acids, large quantities of carotenoid pigments and vitamin E, volatile compounds and different flavonoids, fat acids, triacyglycerol, glycerophospholipids, phytosterols, esters of zeaxanthin, α-tocopherol, phenolic compounds, etc. For the qualitative and quantitative estimation of seabuckthorn composition, the following factors have to be taken into account: the geographical zone of the habitat, a certain period of developing, the plant's genetic characteristics, soil structure, climate conditions, as well as other factors which are hard to foresee and register (Kaminskas *et al*., 2006). The exact time of harvesting is important for the seabuckthorn analysis, because it was noted that after becoming physiologically mature, there are some environmental factors which determine changes of the chemical composition (Gherghi *et al*., 2001).

Material and methods

The biological material that has been used consists in seabuckthorn pulp and seeds, harvested in the moment of complete biological maturity of the plant, from different ecosystems in Romania.

The seabuckthorn were named with Arabic numbers: **1**-Buzău; **2**-Valea Pătârlagelor; **3**-Cislău 1; **4**- Cislău 2; **5**-Cislău 3; **6**-Cislău 4; **7**-Cislău 5; **8**-Cislău 6; **9**-Chiojdu 1; **10**-Chiojdu 2; **11**-Sulina 1; **12**-Sulina 2; **13**-Sulina 3; **14**-Sulina 4; **15**-Sulina 5; **16**-Sulina 6; **17**-Sulina 7; **18**-Sulina 8; **19**-Sulina 9; **20**-Sulina 10. The biotypes noted from 1 to 10 have been harvested from different zones, situated at altitudes between 278 and 454 meters and the ones noted from 11 to 20 have been harvested from zones situated at altitudes between 0.8 and 12 meters.

The dried substance and water quantities were determined using the gravimetric method, maintaining the biological material at a temperature of 105°C until constant weight. The results were expressed in grams of dried biological material/100g fresh biological material.

The quantity of total lipids was determined using the Soxhlet gravimetric method, which consists in the extraction of the total lipids from the analyzed materials, in a warm environment, using specific organic solvents. The extraction using petroleum ether was also performed, which is considered as the most efficient (Cenkowski and al., 2006). The total lipids are calculated depending on the degreased sample's weight and the results are expressed in grams/100g dried biological material (Artenie and Tanase, 1981).

The carotene content is determined using a spectrophotometric method**.** The vegetal material was triturated with a reactive mixture (anhydrous sodium sulfate, calcium oxide, anhydrous sodium carbonate) that retains the water, the colored substances, except for carotenes, and that prevents the carotene decomposition. The carotenes are extracted with acetone, petroleum ether or gasoline from the homogenous mixture. In our experiments we have used acetone. The acetone extract of pigments was transferred into a separatory funnel and mixed with petroleum ether. The acetone was removed by washing two or three times with distilled water. Like a result the carotenes entirely pass in petroleum ether layer. The carotene etheric solution was dried with anhydrous sodium sulfate and filtered. To isolate the chlorophylls and the xanthophylls we have added in the resulted solution dicalcium phosphate. After another filtration the obtained solution was diluted in a volumetric flask. The analyzed pigments from the obtained extract are determined at 450nm using a Shimadzu UV-Visible spectrophotometer (UV-1700 series) spectrophotometer . The amount of carotenes was calculated in terms of β-carotene using a standard solution. The standard solution was prepared with 72mg potassium dichromate diluted up to 100ml with distilled water. The absorbance of these solutions was determined at 450nm and corresponded to 0.00416mg carotenes/ml (Artenie and Tanase, 1981).

Results and discutions

The content of dried substance in the pulp, in samples 1-10, showed a variation from 15 g% for sample 5 (Cislau 3) to 19.43 g% for sample 3 (Cislau 1) (Figure 1). The average content of dried substance in these samples is 16.9 g%. The water content presented logically a reversed variation, being 83.1 g% the average value. For the seeds, the dried matter had a small variation, from 86.63 g% to 89.1 g%, and water content ranged from 10.9 g% to 13.37 g% (Figure 2).

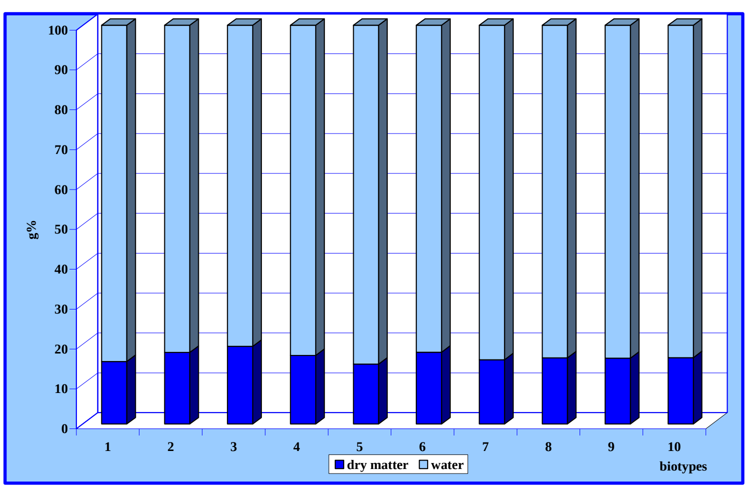

Figure 1. Dried matter and water content in seabuckthorn pulp for biotypes 1-10.

In the case of seabuckthorn biotypes 11-20 harvested from the Sulina zone, the maximum value of the dried matter content determined in the pulp was found for biotypes 14 (Sulina 4) and 15 (Sulina 5), and the minimum value, for biotype 19 (Sulina 9) (Figure 3). For the water content, the situation is the reverse. The determinations on fruit seeds resulted generally in more uniform percentages, the average value being 86.21 g%, for the dried matter and 33.79 g% for water (Figure 4).

A particular situation is the one of biotype 1 (Buzau), which registers the lowest value of dry matter content (82.78 g%) and, respectively, the highest value of water content from all the samples in this set.

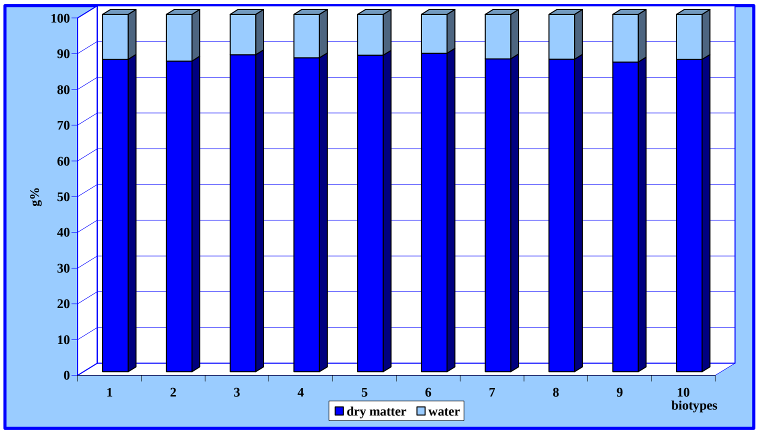

Figure 2. Dried matter and water content in the seabuckthorn seeds for biotypes 1-10.

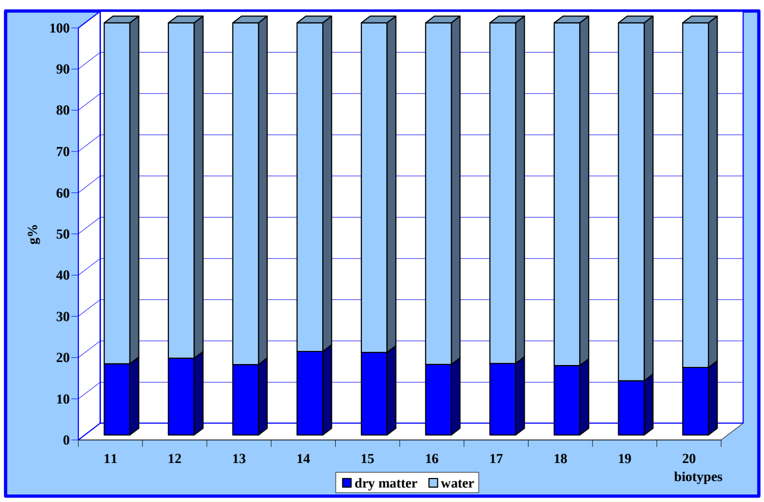

Figure 3. Dried matter and water content in the seabuckthorn pulp for biotypes 11-20.

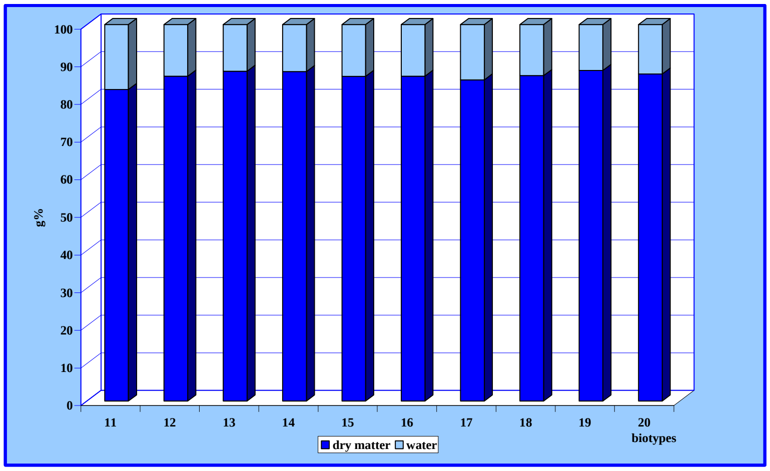

Figure 4. Dried matter and water content in the seabuckthorn seeds for biotypes 11-20.

Analyzing the results in consideration to the altitude where the biological material was harvested, slight differences concerning the dried matter and water quantity were noticed. The average value of dried matter quantity (16.9 g%) was found for the seabuckthorn samples harvested from the Buzau zone, which is situated at approximately 360 meters altitude, which is lower than the one registered at the samples taken from the Sulina zone (17.42g %), situated at approximately 6 m altitude. This behavior is justified by environmental particularities of different locations.

The seabuckthorn contains important quantities of lipids in both the pulp and the seeds. The reference literature indicates oil quantities varying from 29% to 48% in the pulp and from 10% to 15% in the seeds (v/v).

The data analysis revealed a high fluctuation of the total content of lipids. This behavior is obvious if is noted that in the pulp samples the values have a minimum of 16.38 g%, for biotype 9, and a maximum of 38.08 g%, for biotype 3 from Buzau (Cislau 1). In the case of the seeds, the fluctuation is smaller, the minimum content of total lipids being 21.40 g% for biotype 8 (Cislau 6) and the maximum content, 32.41 g% for biotype 4 (Cislau 2) (Figure 5).

The total content of lipids in the seeds is higher or equal than the one determined in the pulp for the majority of biotypes in this series of analyzes.

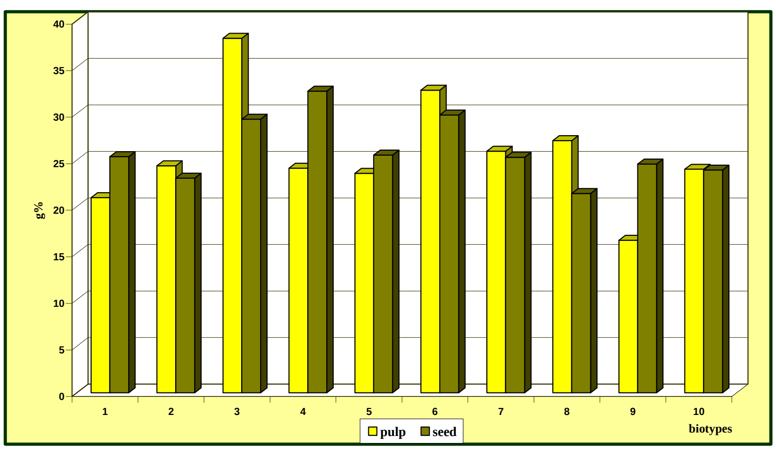

Figure 5. Total lipid content in seabuckthorn pulp and seeds for biotypes 1-10.

The results of the determinations done on biotypes 11-20 were also characterized by the ample fluctuations of lipid content in the pulp. A maximum value of 38.42 g% was found for biotype 1 (Buzau) and a minimum value of 15.28 g%, for biotype 8 (Cislau 6) (Figure 6).

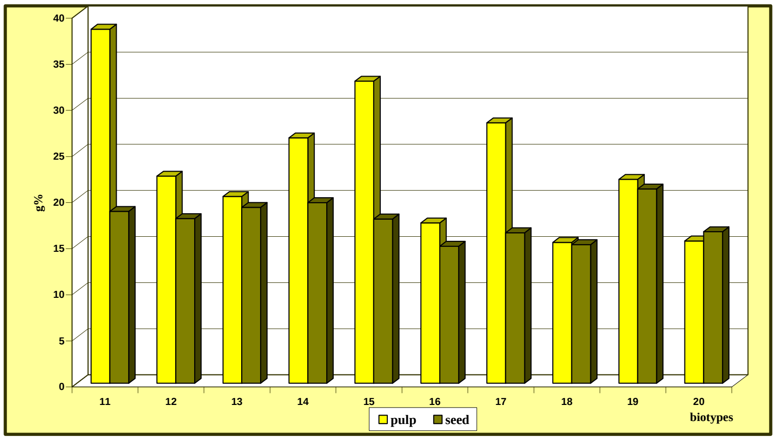

Figure 6. Total lipid content in seabuckthorn pulp and seeds for biotypes 11-20.

As previously found, the fluctuation of total lipids content in the seeds was low. In most cases, the values were close to the average one, 17.67 g%. A maximum of 21.08 g% for biotype 19 (Sulina 9) and a minimum of 14.87 g% for biotype 16 (Sulina 6) was determined (Figure 6). In most of the cases, the total lipid quantity in the pulp was higher or almost equal than that of the seeds. The total content of lipids was different depending on the nature of the analyzed material and the biotic conditions of the ecosystem where the plants were harvested from.

This research showed a specific variation of carotenoid content in the seabuckthorn pulp, depending on the plant material, in accordance to the literature (Anderson *et al*., 2007). Thus, in the case of the first ten biotypes, the pigment content registered maximum values for biotype 4 (Cislau 2), followed by 8 Cislau 6) and 3 (Cislau 1) . For the rest of the cases, the investigated parameter remained below the average value (Figure 7).

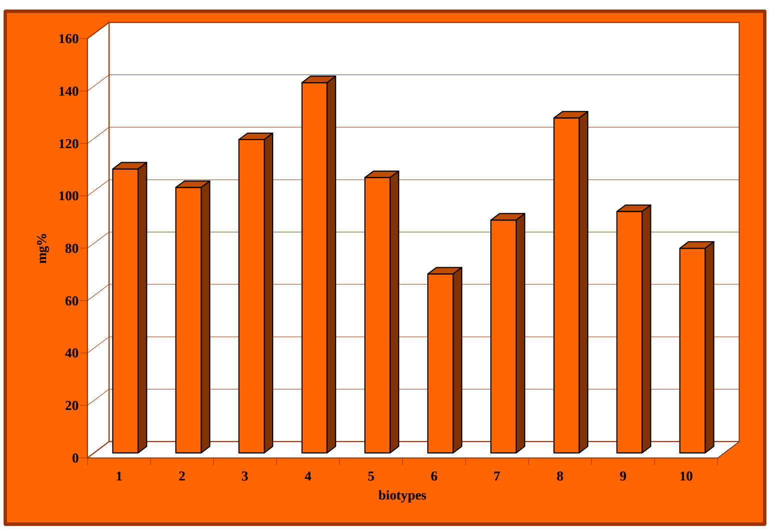

Figure 7. Carotenoid pigment content in the seabuckthorn pulp for biotypes 1-10.

In the case of biotypes 11 to 20, the carotenoid content had a marked variation, with minimum values for biotype 17 (Sulina 7) and approximately 7 times bigger values for biotype 12 (Sulina 2) (Figure 8).

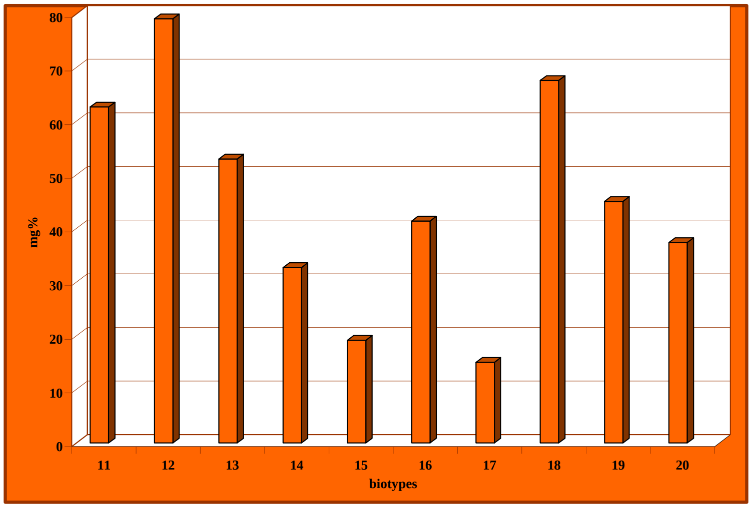

Figure 8. Carotenoid pigment content in the seabuckthorn pulp for biotypes 11-20.

The rest of the biotypes showed an average content of carotenoid pigments. Biotypes 11 (Sulina 1), 12 (Sulina 2) and 18 (Sulina 8) presented a high potential for carotenoid pigments biosynthesis.

Conclusions

The content of dry matter in the seabuckthorn pulp and seeds harvested in low altitude areas are superior to those in high altitude areas.

The content of total lipids is different depending on the altitude of the harvesting areas. Superior values of the indicator were found for higher altitudes. The distribution of the lipids in the pulp or seed presented different variation at the two altitudes.

The content of carotenoid pigments depended also on the altitude, their biosynthesis being clearly stimulated by the conditions from the higher altitudes.

Acknowledgment

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Biochemical Characterization of Seabuckthorn (*Hippophae rhamnoides* L.) Grown in Latvia

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Seabuckthorn fruits are among the richest sources of vitamins, minerals, organic acids, polyphenols and the other biologically active substances useful for human nutrition. The plantations of seabuckthorn have been increasing during the latest 10 years in Latvia. Therefore the objective of this work is actually to test the content of biologically active compounds of seabuckthorn cultivars grown in Latvia. Our country is located near the Baltic Sea, and the place of the trials is in the Dobele district. The annual sunshine is 4522 hours; the sum of temperatures above 5 ^{o}C – 2423 ^{o}C; length of vegetation period – 200 days; the absolute minimal temperature is -36 ^{o}C, which occurs only each 5-10 years. The aim of the study was to determine the content of soluble solids, total acids, vitamin C and E, total carotenoides and phenolic compounds in cultivars adapted to the Latvian climate: 'Avgustinka', 'Prozrachnaya', 'Botanicheskaya Lubitelskaya', 'Luchistaya', 'Trofimovskaya' and 'Podarok Sadu'. Seabuckthorn cultivars grown in Latvia are of Russian origin. The venue of the research was the Fruit and Berry Processing Center of Latvia State Institute of Fruit-Growing. Biochemical analyses were made during 2004 – 2005. The average biochemical content of all cultivars was: soluble solids - 8.3 ^{o}Brix, total acids – 3 %, vitamin C - 84.9 mg 100 g^{-1}, vitamin E - 23.2 mg 100 g^{-1}, total carotenoids - 14.5 mg 100 g^{-1}, total phenolic compounds - 126.1 mg 100 g^{-1}. As a result, the highest content of biologically active compounds was found for cultivars 'Trofimovskaya' and 'Podarok Sadu'.

Keywords: seabuckthorn, soluble solids, acids, carotenoids, phenols, vitamin C, E.

Introduction

Seabuckthorn was used in medicine already since 618-907 A.D., however, as a cultivated plant it is known since the 19th century. Biochemical content of seabuckthorn fruits can be characterized as diverse, they contain many different biologically active compounds: vitamins (especially C, E, K1, as well as of P and b group), carotenoides, sterols, tocoferols, unsaturated fatty acids (linolic, linoleinic, oleinic acids), phenolic compounds (leicoanthocyans, catehin, flavonols, flavonoides, triterpenoides), mineral substances (copper, zinc, cobalt, molybdenum, manganese, iron, calcium, magnum, lead, phosphorus) (Singh, 2003; Singh, 2005). The experiments made on animals, as well as clinical investigations on humans carried out in China and Russia allow to consider the processing products of seabuckthorn fruits as functional food (Varshney, 2003; Singh, 2005). The fruits of seabuckthorn have high antioxidative capacity, as it was confirmed by the experiments of many researchers (Geetha *et al*., 2001; Gao, 2005; Allman *et al*., 2005; Singh, 2005b). Biochemical content of seabuckthorn fruits is dependent on the origin of plants, climatic conditions, growing place (Yao, 1994; Rongsen, 2003), season of vegetation and stage of maturity (Korovina and Fefelov, 2005). The fruits contain in average 79% of water, 3% of carbohydrates, 3% of acids, 4% of oil, as well as vitamins, mineral substances and other compounds – in total 11% (Heilscher, 2003). The content of soluble solids can vary from 2.85 to 22.74 ^{o}Brix depending on climate, origin and stage of maturity (Piir and Kelt, 1998; Antonelli *et al*., 2005; Dwivedi *et al*., 2005).

The fruits of seabuckthorn contain a diversity complex of vitamins, of which vitamin C is the most important. Scientific investigations had proved that fruits of seabuckthorn contain 4 – 100 times more vitamin C than in many other fruits or vegetables (Singh, 2005). As it was found, seabuckthorn fruits of European origin can contain vitamin C till 373 mg 100 g^{-1} (Yao *et al*., 1992; Tang, 2002; Antonelli *et al*., 2005). The content of vitamin C of each cultivar differs depending on the growing place (Bieniek and Kawecki, 2001; Aksenova and Dolgacheva, 2003; Jalakas *et al*., 2003; Kawecki *et al*., 2004; Univer *et al*., 2004).

Carotenoides are pigments of plant origin, which determine the color of fruit skin (from yellow to red). Different carotenoides occur in the fruits of sea buckthorn: α-, β-, γ- carotenes, licopen, xanthol, zeaxanthin, etc. (Jamyansan and Badgaa, 2005; Novruzov, 2005a). The lowest content of carotenoides depending on the origin and cultivar has been mentioned as 0.5 – 10.4 mg 100 g^{-1} (Novruzov, 2005), but some data show very high content: even 120 mg 100 g^{-1} (Antonelli *et al*., 2005). Researchers from Moscow reported 11.2 – 65.5 mg 100 g^{-1} (Aksenova and Dolgacheva, 2003), however scientists from Novgorod indicated 9.5 – 18.7 mg 100 g^{-1} (Korovina and Fefelov, 2005).

Vitamin E (tocopherol) is an important vitamin soluble in fats, occurring in seabuckthorn fruits. The content of it varies 1 to 12 mg 100 g^{-1} (Kallio *et al*., 2002). The data of vitamin E mentioned are very different depending on species and subspecies. For example, the content of vitamin E in the region of Caucasus was 2.5 – 15.0 mg 100 g^{-1} (Mamedov, 1984; Singh, 2005). Higher contents (to 36 mg 100 g^{-1}) were also mentioned. If compared to other fruits, a high content is found in the fruits of cloudberries and black currants (31 and 23 mg 100 g^{-1} respectively) (Heinonen *et al*., 2005).

Flavonoids are the largest group of phenolic compounds, having an antioxidative capacity, occurring in plants, also in seabuckthorn fruits. High content of flavonoids and other biologically active compounds is found not only in the fruits, but also in the seabuckthorn leaves (Tsybikova *et al*., 2005; Gonchova and Glushenkova, 2005). The content of flavonoids in the fresh fruit is estimated as 552.0 mg 100 g^{-1} (Novruzov, 2005).

Seabuckthorn is becoming popular in Latvia during the last 10 years, however it is considered as a nontraditional crop. Unfortunately, the industrial processing of seabuckthorn fruits is not yet developed, and large quantities are exported unprocessed. This situation can be explained by the lack of interest in processing seabuckthorn fruits from the existing companies by the fact that seabuckthorn products are not well known in the market (except the oil). Only some local products are available for consumers, produced using the practical experience of producers.

Until now data on the biochemical content of seabuckthorn cultivars grown in Latvia were not available in publications. Therefore, the objective of this research was to determine the biochemical content of seabuckthorn cultivars which are grown in Latvia, to evaluate their amount of biologically active compounds: vitamin C and E, total acids, carotenoides and phenolic compounds.

Material and methods

The research material – fruits of seabuckthorn (*Hippophae rhamnoides* L.) cultivars, widely grown in Latvia, was harvested at “Baltplant” Ltd, Dobele region in 2004 and 2005. These cultivars are:

1. ‘Avgustinka’ –dark yellow fruits with a little marked spot at the stalk, cylindrical-oval shape, the average mass of the fruit – 0.58 g;
2. ‘Prozrachnaya’ –light orange fruits with darker spots at both fruit ends, barrel shaped, the average mass of the fruit – 0.72 g;
3. ‘Botanicheskaya Lubitelskaya’ –yellow fruits, oval shaped, longish, the average mass of the fruit – 0.55 g;
4. ‘Podarok Sadu’ –yellow-orange fruits, oval shaped, big, the average mass of the fruit – 0.76 g;
5. ‘Trofimovskaya’ – orange-red fruits, oval shaped, longish, the average mass of the fruit – 0.46 g;
6. ‘Luchistaya’ –light yellow fruits, oval shaped, the average mass of the fruit – 0.60 g.

Seabuckthorn fruits were frozen in a “PORKKA BF 710” freezer at the temperature -25 ± 1 ^{o}C. Frozen fruits were packed in polypropylene bags (1–1.5 kg in each) and stored in a “VTK 201 V” low-temperature chamber at a temperature of –18 ± 1 ^{o}C for three months.

The following biochemical contents were determined for seabuckthorn cultivars:

- The content of soluble solids (^{o}Brix), determined at a temperature of 20 °C with an ATAGO N20 digital refractometer (deviation of measuring instrument face value ±0.1%) (ISO 2173:2003);
- The content of total acids (%) was determined by titrating with 0.1N NaOH (ISO 750 – 1998);
- The content of ascorbic acid (vitamin C) (mg 100 g^{-1}) was determined with the iodine method (T-138-15-01:2002);
- The total content of phenols (mg 100 g^{-1}) was determined with the method of spectrometry, by using a spectrometer UV-1650-PC at wave length 765 nm (Singleton *et al*., 1999);
- The content of vitamin E in samples was established by a modified method. The method is based on the extraction of carotenoides with petroleum benzene. The same solvent was used for the determination both of carotenoides and vitamin E.

Five (5) g of seabuckthorn berries were crushed and put into a 100 ml retort. Ten (10) ml of 96% ethanol were added and the solution was mixed for 5 minutes. After adding of twenty five (25) ml of petroleum benzene mixing was continued 4 hours. Then thirty (30) ml of H_2O was added and mixed for 15 minutes. The sample was filtered. The sediment was rinsed with ten (10) ml of 96% ethanol and two times with H_2O. After the separation of both layers the upper one was used for analyses. Yellow petroleum benzene layer was put in twenty five (25) ml retort and refilled till the mark.

Analysis

Two (2) ml of petroleum benzene were taken and put into twenty five (25) ml retort. Ten (10) ml of ethanol were added and mixed. Then one (1) ml of 0.5% 2.2'-dipiridyl solvent was added in ethanol and mixed; one (1) ml of 0.2% $FeCl_3$ solvent added in ethanol and the retort was placed in a dark place. After 15 minutes ethanol was added to the retort till the mark. The absorbance of light was measured with a spectrophotometer at 500 nm. At the same time a control substance was prepared in the same way as the analyzed substance, only instead of the analysis two (2) ml of petroleum benzene were taken. The content of vitamin E was found by using a graduation curve and calculated by using the formula:

$$X = \frac{c \cdot 12.5 \cdot 100}{a}, \quad \text{mg } 100 \text{ g}^{-1}$$

where:

c = The content of vitamin E by using a graduation curve, mg/25ml;
12.5 = factor of sample dilution;
a = weight of sample, g.

Analysis for determination of carotenoides

Two (2) ml of petroleum benzene solvent were taken and put in twenty five (25) ml retort, refilled till the mark. The absorbance was read at 450 nm. Petroleum benzene was used as control.

By using hierarchy analysis and multicriteria (integrated assessment) basic principles (Martinov, 1987), and calculating the sum of several qualitative indicators and the coefficient of qualitative indicators, it is possible to assess seabuckthorn cultivars and determine the best. The coefficient value of the maximum qualitative indicator was taken as 1 ($0 < K < 1$). The following qualitative indicators were included in the assessment: the content of soluble solids, total acids, carotenoides, phenols, vitamins C and E in seabuckthorn fruits.

Estimation was carried out by using the formula (1):

$$K_i = \frac{I}{I_{\max}}, \qquad (1.)$$

where:

K_i = coefficient of qualitative indicators;
I = value of qualitative indicators;
I_{max} = maximal value of qualitative indicators.

Meteorological data of the growing seasons are shown in Table 1.

Table 1. Meteorological data in years of 2004 – 2005.

Month	Average daily temperature, °C		Precipitation, mm		Length of sunshine, hours	
	2004	2005	2004	2005	2004	2005
April	6.93	6.23	1.00	8.57	8.72	7.03
May	10.70	11.20	15.33	20.00	7.71	8.40
June	13.87	14.40	32.67	16.10	7.96	9.60
July	20.13	18.47	16.33	20.53	10.89	9.90
August	17.73	16.53	24.33	40.33	6.71	7.23
September	13.00	13.63	20.67	14.77	4.80	5.57
October	5.00	7.60	18.00	11.77	3.08	4.10

Data were statistically elaborated by using SPSS for Windows and MS Excel. Two-factor variance analysis (Scheffe test) was applied for estimating the effect of the cultivar and the year, significance level at p_{crit}=0.05. The closeness of the coherence between the stated indicators was determined according to Pearson's correlation analysis.

Results and discussion

One qualitative indicator for the seabuckthorn is the soluble solids content. They characterize the content of acids, sugars, pectin compounds, tannins, soluble amino-acids and some other substances occurring in fruits. The content of soluble solids in fruits was affected by the year (p=0.03) and cultivar (p=0.00). The lowest content of soluble solids was found for the cultivar 'Podarok Sadu' (7.75 ^{o}Brix in average), but the highest –for the cultivar 'Trophymovskaya'- (9.00 ^{0}Brix in average) (Figure 1).

If comparing the content of soluble solids in the years 2004 and 2005, the greatest difference was found for the fruits of 'Botanicheskaya Lubitelskaya': it was larger in 2005 (9.0 ^{o}Brix) than in 2004 (7.1 ^{o}Brix). Similar results were obtained in the fruits of the cultivar 'Luchistaya' and 'Podarok Sadu': the content of soluble solids was larger in 2005 (9.0 and 8.1 ^{o}Brix respectively) than in 2004 (8.1 and 7.4 ^{o}Brix respectively).

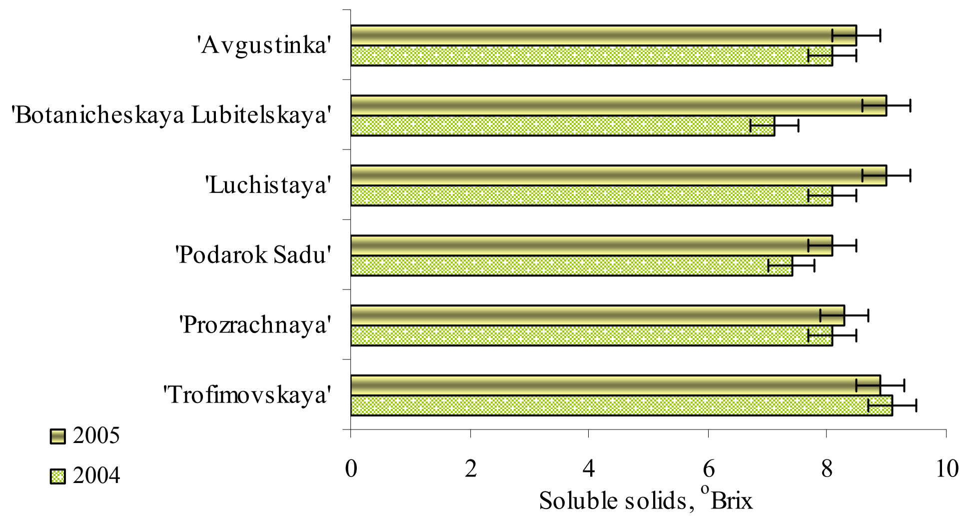

Figure 1. The content of soluble solids in seabuckthorn fruits.

Seabuckthorn fruits have a pleasant sour or sweet-and-sour flavor without a bitter taste. The total acidity of the fruits is formed by ascorbic acid, malic acid, oxalic acid and succinic acid. The total content of the acids in the fruits is dependent on the origin of seabuckthorn and their growing place (climatic conditions). Significant difference in the content of total acids was determined among the investigated samples (p<0.05). The content of the total acids varied from 2.55 to 3.10 % in the fruits of cultivars 'Podarok Sadu', 'Luchistaya', 'Botanicheskaya Lubitelskaya', 'Avgustinka'; however, the content was higher in the fruits of 'Prozrachnaya' and 'Trofimovskaya' to 3.55 % (Figure 2). The content of the total acids had a tendency to decrease in 2005 for almost all cultivars if compared to the content in 2004. Similar data have been obtained in Estonia, where the content of total acids of the cultivars 'Botanicheskaya Lubitelskaya' and 'Avgustinka' was found as 3.0; 2.9 and 2.5 % respectively (Jalakas *et al*., 2003).

The biological activity of fruits and berries is directly linked with their content of vitamins. High content of vitamin C is a very characteristic trait of seabuckthorn fruits. The differences in the content of vitamin C of frozen seabuckthorn fruits were significant (p=0.00). The content of vitamin C in the fruits was in average 84.92 mg 100 g^{-1}. The lowest content was found for the cultivar 'Prozrachnaya': 72.55 mg 100 g^{-1} in average, and the highest one – for the cultivar 'Podarok Sadu' 112.36 mg 100 g^{-1} in average (Figure 3). Increasing of vitamin C was observed for all cultivars in 2005, especially for 'Avgustinka', 'Botanicheskaya Lubitelskaya', 'Podarok Sadu' and 'Luchistaya'. It can be explained by the daily temperature in July and August of 2005 (during formation and maturing of berries), which was lower than in 2004. These conditions promoted the formation of vitamin C in fruits.

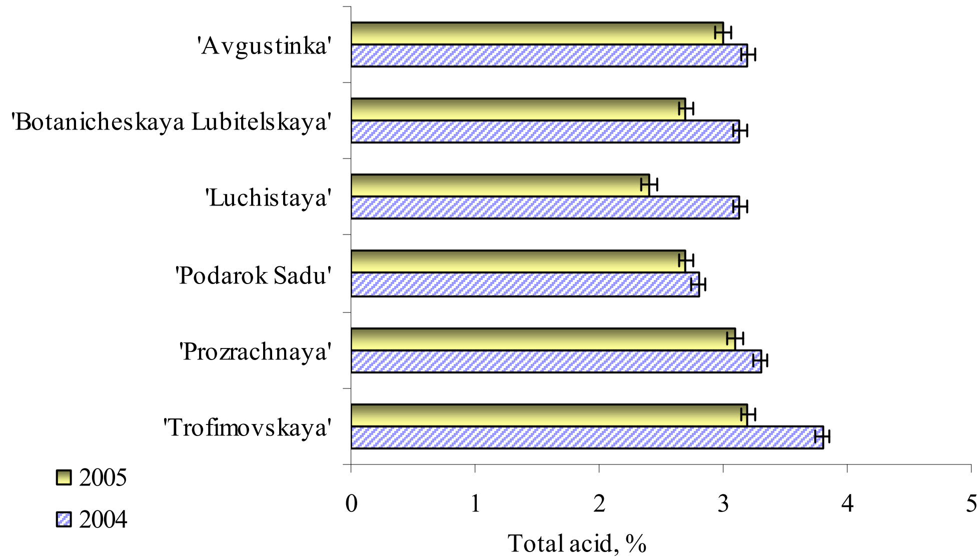

Figure 2. The total content of acids of seabuckthorn fruits.

The content of vitamin C was higher in the cultivars 'Avgustinka', 'Botanicheskaya Lubitelskaya' and 'Trofimovskaya' grown in Latvia as compared to those grown in Estonia (Jalakas *et al*., 2003; Univer *et al*., 2004), but lower than those of Germany (Fischer and Albrecht, 2003; Singh and Mörsel, 2005).

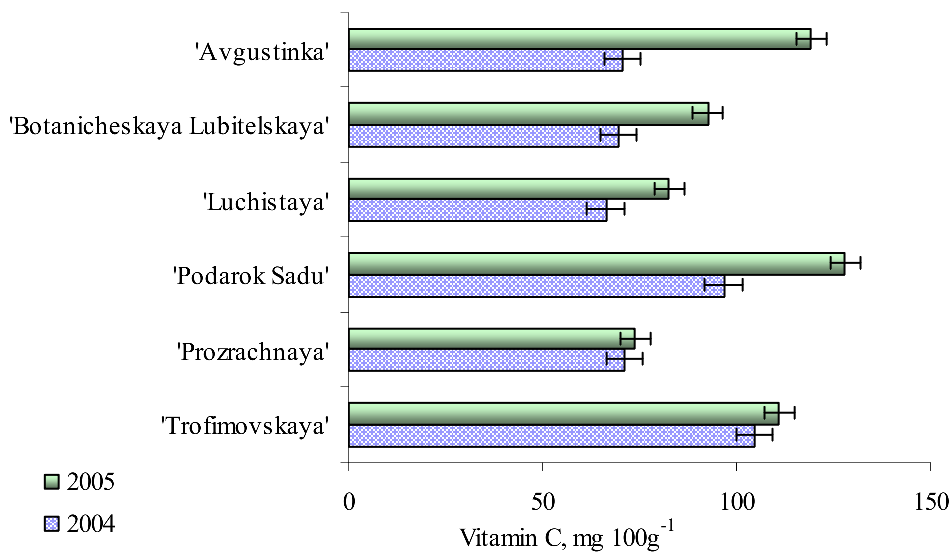

Figure 3. The content of vitamin C of seabuckthorn fruits.

The content of vitamin E is an important parameter of seabuckthorn fruits. Moreover, their content of vitamin E can be higher than in the popular oily plants. The highest amount of vitamin E contained the cultivar 'Podarok Sadu': 32 mg100 g^{-1} in average, but the lowest one – 'Luchistaya': 16.76 mg 100 g^{-1} in average (Figure 4). The content of vitamin E differed significantly depending on cultivar (p=0.00). The lowest content of vitamin E observed in the fruits of cultivars 'Luchistaya', 'Avgustinka' and 'Botanicheskaya Lubitelskaya' from 16.76 to 17.88 mg 100 g^{-1} (p = 0.61), but the highest one – in cultivars 'Trofimovskaya' and 'Prozrachnaya': from 27.48 to 28.64 mg 100 g^{-1}) (p = 0.26). It was observed that the content of vitamin E was higher for some cultivars in 2005 than in 2004 ('Prozrachnaya', 'Podarok Sadu', 'Avgustinka'), but for other cultivars ('Trofimovskaya', 'Luchistaya', 'Botanicheskaya Lubitelskaya'), it was lower. The changes in vitamin E may be influenced by the cultivar specific response to temperature and precipitation during growing season.

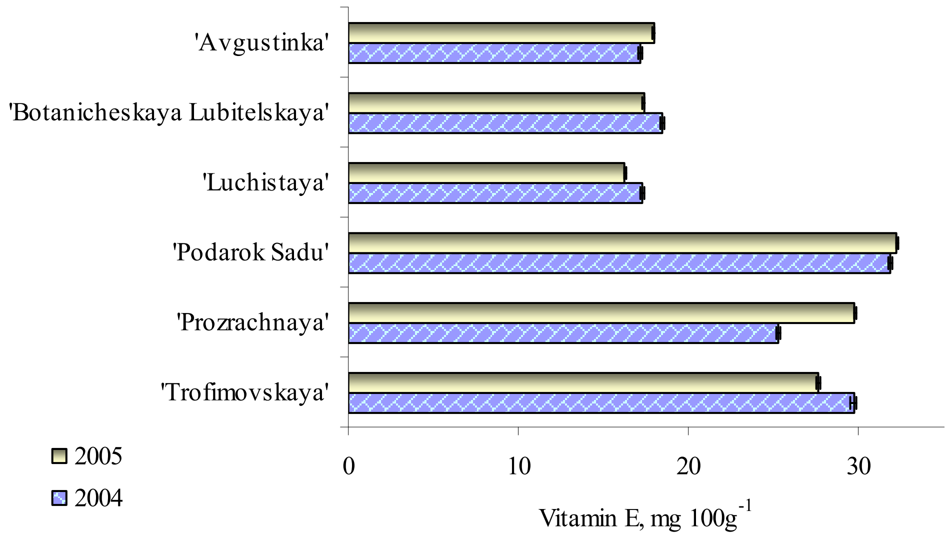

Figure 4. The content of vitamin E in seabuckthorn fruits.

The content of carotenoides was influenced by meteorological conditions. It has been shown that an important role during formation of carotenoides is played by the air temperature, the amount of sunny hours, as well as the amount of cloudy and sunny days during the growing season (Novruzov, 2005). Large amount of sunny days, high air temperature, relatively low sum of precipitation and decreased level of air moisture, often observed in May, June, at the beginning of July, provide for a high content of carotenoides in the fruits. Unfavorable meteorological conditions (spring, frosts, rain and low air temperature) in spring and summer influence negatively the formation of carotenoides.

The highest content of total carotenoides was determined in the fruits of ‘Podarok Sadu’ (characteristic red overcolor, 21.35 mg 100 g^{-1}), but the lowest – in the fruits of ‘Luchistaya’ (characteristic yellow overcolor, 8.75 mg 100 g^{-1} in average) (Figure 5).

The total content of carotenoides in some cultivars, if comparing the yield of year 2004 to year 2005, increased (‘Botanicheskaja Lubitelskaya’ and ‘Prozrachnaya’), but decreased in the fruits of cultivar ‘Prozrachnaja’.

The data on the content of total carotenoides obtained in the investigations of several researchers are different. They vary from 0.5 to 30.3 mg 100 g^{-1} (Aksenova and Dolgacheva, 2003; Privalov *et al*., 2003; Korovina and Fefelov, 2005; Novruzov, 2005; Antonelli *et al*., 2005). The data obtained in our research on the content of total carotenoides of the cultivars grown in Latvia are similar to those obtained in Germany (Fischer and Albrecht, 2003; Singh and Mörsel, 2005).

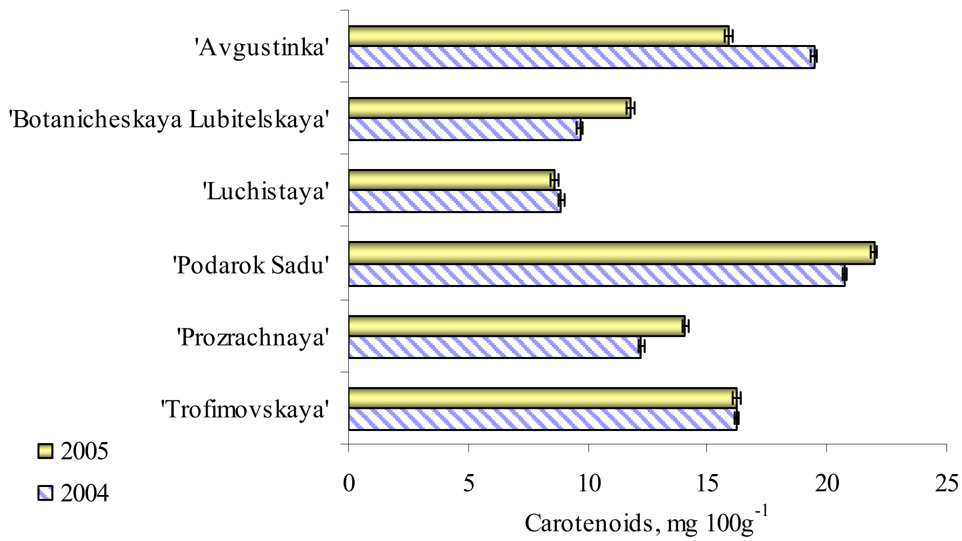

Figure 5. The total content of carotenoides of seabuckthorn fruits.

Phenolic compounds are a large group of organic substances and they have an important role in photosynthetic processes. Phenolic compounds are divided into several groups, of which the larger is flavonoides. They are found in many parts of seabuckthorn – in the fruits, leaves and shoots (Tsybikova *et al*., 2005; Gonchova and Glushenkova, 2005). The content of total phenolic compounds of the cultivars included in the research was different. The highest content of total phenolic compounds was found in the fruits of the cultivars 'Botanicheskaya Lubitelskaya' and 'Podarok Sadu' (141.7 mg 100 g^{-1} in average), and the lowest one – in 'Prozrachnaya' (105.36 mg 100 g^{-1} in average) (Fig. 6). It was observed that content of total phenolics was higher in 2005 ('Prozrachnaya', 'Luchistaya' and 'Podarok Sadu') than in 2004. However, the content of total phenolic compounds in the fruits of the other cultivars in 2005 was smaller, which may be influenced by their response to the different meteorological conditions during growing season.

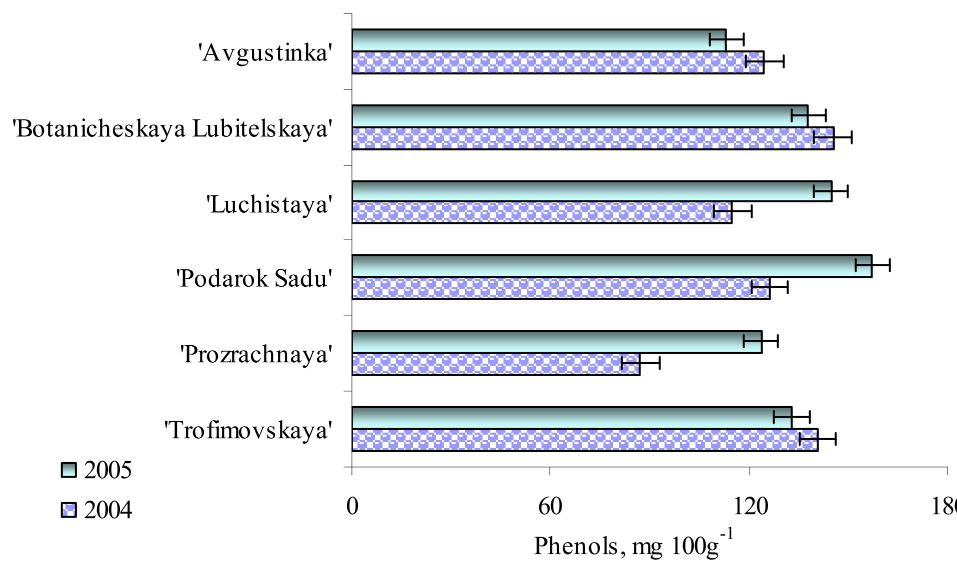

Figure 6. The total content of phenolic compounds of seabuckthorn fruits.

Several correlations between different compounds in seabuckthorn fruits were found (Table 2).

Table 2. Correlation between different compounds in seabuckthorn fruits.

	Soluble solids, °Brix	Content of total acids, %	Vitamin C, mg 100 g^{-1}	Vitamin E, mg 100 g^{-1}	Carotenoids, mg 100 g^{-1}	Phenolic compounds, mg 100 g^{-1}
Soluble solids, °Brix	1					
Content of total acids, %	0.30*	1				
Vitamin C, mg 100 g^{-1}	0.38*	**0.70****	1			
Vitamin E, mg 100 g^{-1}	0.16*	0.37*	**0.65****	1		
Carotenoides, mg 100 g^{-1}	0.16*	0.31*	0.53**	**0.74****	1	
Phenolic compounds, mg 100 g^{-1}	0.28*	**0.77****	**0.87****	0.52**	0.47**	1

* correlation significant (significance level 95%);
** correlation significant (significance level 99%)

The total amount of acids in seabuckthorn fruits was composed mainly by ascorbic acid. One of the first and widely spread theories explaining the biological efficiency of polyphenols, is based on a close connection of polyphenol with ascorbic acid. Phenolic compounds stabilize the ascorbic acid in plants and in products of plant origin. Ascorbic acid does not work as polyphenol oxidizing inhibitor but restores the oxidized phenols (Baraboi, 1976). This accounts for the positive correlation of the existing phenolic compounds and the ascorbic acid present in seabuckthorn.
Seabuckthorn fruits are rich in pigments and lipids; they are located in the cell membranes and fruit mesocarp. A great deal of carotenoid-lipid compounds are accommodated in the fruit cell membranes where polar lipids work as a connectors between polar (lipid) and non-polar (carotenoid) parts. The stage of seabuckthorn fruit ripeness affects the closeness of mutual correlation (Zeb, 2004).

The highest coefficient of qualitative indicators among the seabuckthorn cultivars grown in Latvia more widely was found for the cultivars 'Podarok Sadu' and 'Trofimovskaya' (0.72), but the lowest one – for the cultivar 'Luchistaya' (0.56). The results of the present research demonstrated that these cultivars may be recommended for processing.

Conclusions

1. The study carried out with 6 cultivars widely grown in Latvia showed that the content of vitamin C, E, carotenoides and phenols was depending on the year and the cultivar significantly;

2. The average biochemical content of all cultivars was the following: soluble solids - 8.3 °Brix, total acids - 3 %, vitamin C - 84.9 mg 100 g^{-1}, vitamin E - 23.2 mg 100 g^{-1}, total carotenoides - 14.5 mg 100 g^{-}1, total phenolic compounds - 126.1 mg 100 g^{-1}.

3. Investigations on the correlation between different compounds indicate on a strong correlation (correlation coefficient $|r| > 0.8$) between the content of total phenols and the content of vitamin C, and a moderate correlation between the content of total acids and the content of total phenols ($0.5 < |r| > 0.8$).

4. It is recommended to use the seabuckthorn cultivars 'Podarok Sadu' and 'Trofimovskaya' for processing, as they have high content of biologically active compounds.

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PRODUCTS, INDUSTRY DEVELOPMENT AND MARKETING

Product Development and Marketing of Seabuckthorn

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Abstract

Seabuckthorn has a long history of application in traditional Tibetan and Mongolian medicines. For decades seabuckthorn has been listed in the Chinese Pharmacopea as a medicinal plant. Medicines based on seabuckthorn extracts have been developed and marketed in China and Russia. The unique composition and health effects of seabuckthorn are increasingly recognized in western countries. Seabuckthorn berries and berry fractions are used as bioactive ingredients for foods, food supplements and personal care products. Opportunities and challenges coexist in the product development and marketing of seabuckthorn. Increasing health consciousness of the consumers presents great potential market for seabuckthorn. The quality of raw materials is of crucial importance to guarantee the efficacy and safety of the products. In addition to the great compositional variation among different subspecies, cultivars and varieties of seabuckthorn, environmental factors at the growth sites strongly influence the quality of the berries. There is a considerable risk of damaging sensitive components and introducing harmful contaminants during the harvesting and pre-handling of the raw materials. Careful screening and optimization of processing technologies are important measure to ensure that bioactive components are maintained and enriched in the natural forms. Standardized and constant compositions, together with well substantiated health claims are essential for the success of seabuckthorn products. Attention shall be paid to the regulatory aspects when developing and marketing seabuckthorn products for specific markets.

Keywords: seabuckthorn, *Hippophaë rhamnoides*, product development, processing technology, marketing, health products, health food regulations.

Introduction

Seabuckthorn (*Hippophaë rhamnoides*) is becoming an increasingly popular source of active ingredients for functional food, food supplements, personal care products and pharmaceutical preparations. For the success of new products, multiple aspects shall be considered during the product development and marketing. This paper will discuss some of these aspects, where some characteristics of seabuckthorn shall be taken into account.

Compositional characteristics of Seabuckthorn

Seabuckthorn refers to several species of *Hippophaë*. *Hippophaë rhamnoides* is the most important and common species, which is further classified into different subspecies naturally distributed in different regions of Europe and Asia (Rousi 1971). Many commercial cultivars/varieties with varying characteristics are available originally from countries such as Russia, China, Germany, Finland and other Baltic countries.

Seabuckthorn berries contain a wide range of bioactive components. The content of vitamin C is often very high, up to 2% in the juice of some wild berries from China. The berries are also rich in polyphenols such as flavonoids. Lignans, secoisolariciresinol and matairesinol, are also an important group of water-soluble bioactive components in seabuckthorn berries. The major sugars in the berries are fructose and glucose with small amount of sucrose. Some sugar derivatives are also present in the berries. The major fruit acids are malic acid and quinic acid. Typically wild seabuckthorn berries have a high acid content. For most subspecies of *H. rhamnoides*, a high content malic acid combined with a low sugar:acid ratio gives the berries a characteristic sour and slightly bitter taste.

Seabuckthorn has a single seed in each berry. The seeds of seabuckthorn contains typically 7-12% oil, which mainly consists of triacylglycerols of the two essential fatty acids, linoleic (18:2n-6) and alpha linolenic (18:3n-3) acids. The n-6/n-3 fatty acid ratio is close to 1:1 making seabuckthorn seed oil an ideal ingredient for correcting the imbalance of n-6 and n-3 fatty acids in the western diet. The seed oil is rich in tocopherols, tocotrienols, carotenoids and plant sterols in the unsaponifiable fraction. Like some other species (*e.g.* olive) of the family *Elaeagnaceae*, seabuckthorn contains oil in both seeds and fruit pulp/peel. The oil from the fruit pulp/peel differs from the seed oil by high contents of palmitoleic acid (16:1n-7) and higher levels of carotenoids (Yang and Kallio, 2002).

Considerable variation has been found in the content and composition of different bioactive components among different subspecies and cultivars of seabuckthorn. In addition to the origins, harvesting date significantly influences the composition of the berries (Kallio *et al*. 2002a, 2002b; Raffo *et al*. 2004, Yang *et al*. 2006). The relatively polar compounds such as vitamin C, sugars and acids in the juice fraction are strongly influenced by subspecies/cultivars, harvesting time and growth conditions. The vitamin C content in wild berries of ssp. *sinensis* is 10-20 times higher than the levels in the berries of ssp. *rhamnoides* and *mongolica* (Kallio *et al*., 2002). Berries of some commercial cultivars originally from the Altai region have high sugar content and relatively low acid content, especially low malic acid content. These lead to a higher sugar:acid ratio and a very pleasant fruity taste of the berries. As an example of the influence of harvesting time, Figure 1 presents changes in vitamin C content of seabuckthorn berries during the harvesting period from the end of August to the end of November, 1998 (Kallio *et al*., 2002b). The oil content in seeds, fruit pulp/peel, the fatty acid composition of fruit pulp/peel as well as the content of carotenoids and tocopherols in different fractions all vary considerably with subspecies, cultivars and varieties (Yang and Kallio, 2005). Figure 2 shows the fatty acid composition of the pulp/peel fraction of berries of three subspecies of seabuckthorn (Yang and Kallio 2002).

It is difficult to define in general which subspecies, variety or cultivar is the best for human health since each subspecies/cultivar/variety has its own advantages. That is why it is important to define the purpose of application and compositional target of the product first. Based on the application, products with optimized and standardized composition shall be manufactured to best achieve the health effects expected.

In addition to the berries, the leaves of seabuckthorn are also a potential source of functional ingredients. Seabuckthorn leaves are rich in different types of bioactive components such as polyphenols, proteins, amino acids, lipids and minerals.

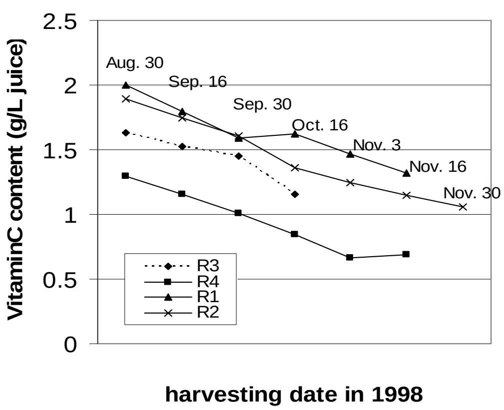

Figure 1. Changes in vitamin C content in seabuckthorn berries during harvesting period in 1998. R1, R2, R3, and R4 are four cultivars of *H. rhamnoides* ssp. *rhamnoides* cultivated in Finland.

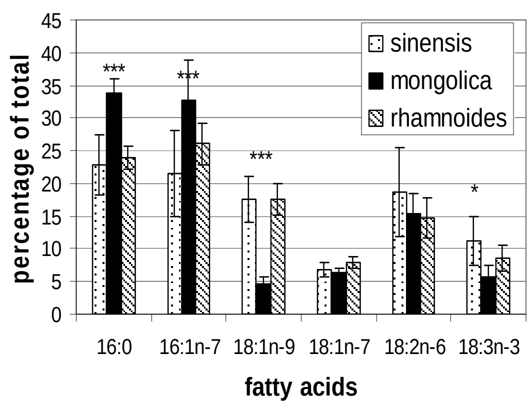

Figure 2. Fatty acid composition of oil of fruit pulp/peel fraction of different subspecies of sea buckthorn.
*** P < 0.05, ** P < 0.01, *** P < 0.001, showing the differences among the subspecies.**

Processing technology

Seabuckthorn berries, leaves, and fractions of berries and leaves concentrated with targeted bioactive components are potential raw materials of food, food supplements, and pharmaceutical and personal care products. After harvesting, different technologies may be used for processing yielding extracts and products of different compositional characteristics (Xin *et al*. 1997). The pre-handling and processing procedures shall be designed to minimize the damage of the most important bioactive components in the raw materials. Often specific processing

technologies are chosen to isolate some targeted components or to produce extracts and fractions concentrated with these components. Due to the great variation in the composition of seabuckthorn raw materials, it is important that the processing technologies are optimized to standardize the final composition of the products. The environmental impact of the processing chain shall be evaluated and minimized.

Figure 3 shows an overall scheme of berry processing for extracts to be used in different types of products. Often juice is pressed from fresh and freshly melted frozen berries. The press cake fraction is usually dried for further separation of seeds from the fruit flesh and peel before the extraction of seed oil and pulp/peel oil. Oil can be manufactured from seeds and dry pulp/peel by cold-pressing and extracting with organic solvents, vegetable oils or supercritical CO_2. Centrifugation alone and in combination with enzymatic treatment can be used for isolating oil from juice. The residues after oil extraction can be used for further isolation of active components of higher polarity such as proteins, amino acids and phenolic compounds. Berries, berry juice and powders of berry and juice are raw materials for food and functional food. Oils and phenolic extracts from the berries, juice, fruit pulp/peel and seeds are active ingredients of health and personal care products.

Product hygiene

Pesticides are not a major problem for seabuckthorn raw material since a majority of the global seabuckthorn resources are wild bushes growing in the nature. Even for the small amount of cultivated seabuckthorn, pesticides are seldom used. Environmental pollution at the growth sites may increase the content of heavy metals and a range of harmful chemicals in seabuckthorn berries and leaves. The harvesting and pre-handling process shall be carefully monitored to avoid contamination of heavy metals, chemicals, and pathogens. The growth of moulds and other microbes shall be strictly controlled. Depending on the physical-chemical properties of the contaminants, it is possible to remove these substances during the processing. On the other hand, some contaminants may be enriched by some specific processing technologies. Heavy metals are transferred efficiently from berries to juice during juice pressing. Often heavy metal contaminants present in the raw materials in the oxidized forms or as salts. During oil extraction process the heavy metals remain in the extraction residues. On the other hand, heavy metals are often coextracted with more polar compounds such as phenolic compounds and glycosides. When organic solvents are used during the processing, solvent removal shall be carefully monitored to restrict the solvent residues in the extracts to a safe level.

Supercritical CO_2 extraction (CO_2-SFE) is a technology used for extracting oils from seabuckthorn raw materials. The advantages of CO_2-SFE are mild temperature, absence of oxygen and free from conventional organic solvents, avoiding thermal and oxidative damage to the active components and organic solvent residues in the extracts. CO_2-SFE does not transferred heavy metals and microbes from raw materials to the extracts.

Substantiation of product efficacy

In vitro studies and animal experiments are often the first steps for investigating the biological activities of active ingredients and products. However results of these studies shall not be extrapolated to humans. Well designed, independent human intervention/clinical studies are essential for demonstrating the physiological effects and for evaluating the proper dosage for the targeted health benefits. There is a vast body of literature published indicating a wide range of health effects of seabuckthorn berries, leaves and fractions processed with different technologies from the berries and the leaves. A majority of seabuckthorn literature is published in Chinese and Russian. During the recent years, active seabuckthorn research has been carried out in Western countries as well. The existing literature is of great reference value for developing seabuckthorn health products and for studying the physiological effects of these products (Guliyev *et al*. 2004, Singh *et al*. 2005). Often literature alone is not enough. In many countries, health claims made for functional foods and nutraceuticals need to be based on product specific studies published in peer-reviewed international scientific journals. Large scale clinical studies are often costing and maybe too expensive for small and medium-sized industries. Thus cooperation between companies as well as joint research among industrial partners and research institutes may provide ways of investigating the health effects of seabuckthorn products with reasonable input of resources.

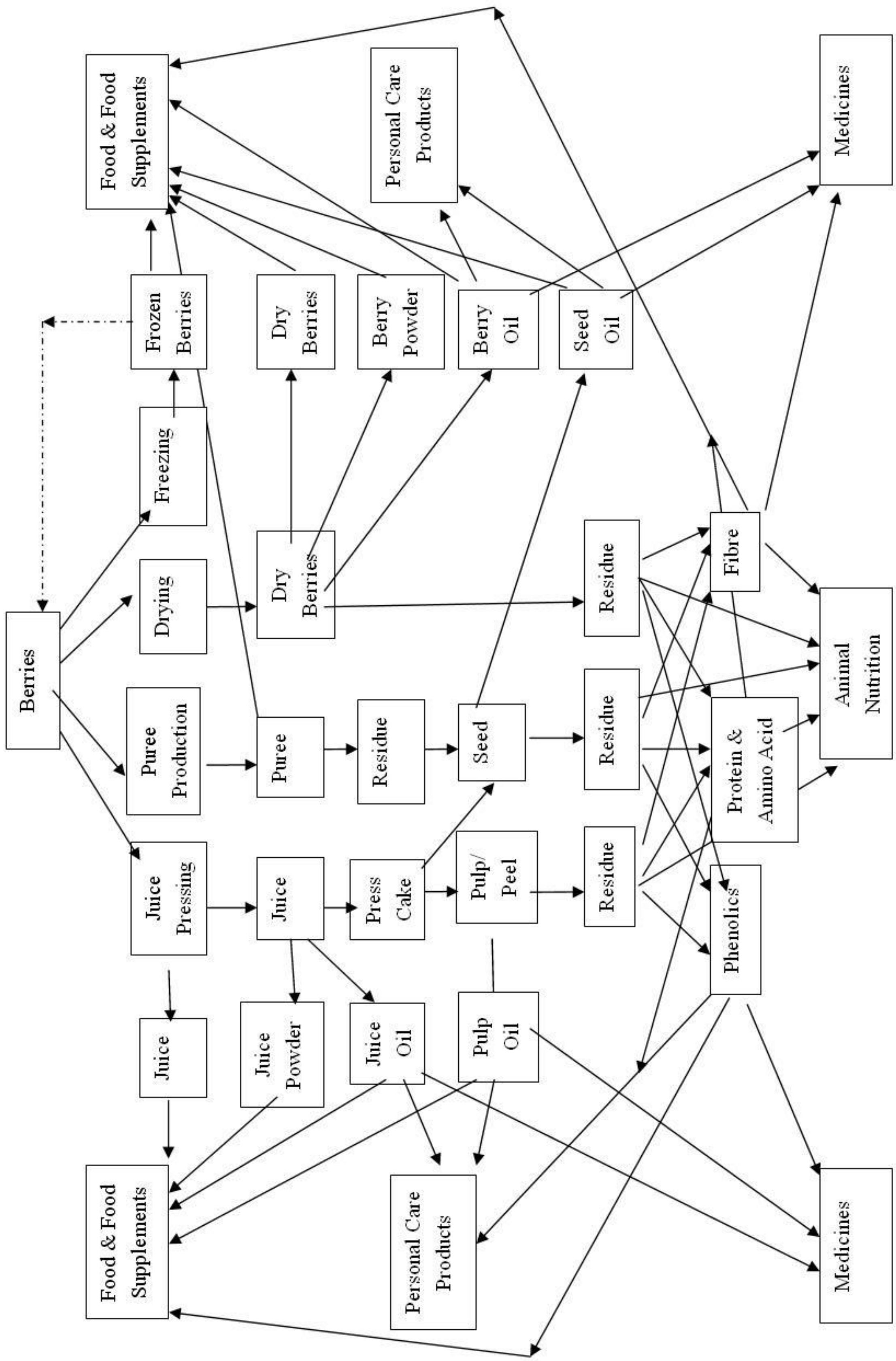

Figure 3. Processing of seabuckthorn berries for different applications.

Regulatory aspects

Legal and regulatory aspects need to be taken into account already at the early stage of product development. Despite the common principle of consumer protection, there are considerable variations among regional and country based laws and regulations for manufacturing and marketing food, medicines, and health and personal care products. Furthermore, new regulations and updates are coming into force all the time to supervise the operation of specific product categories.

In the European Union (EU), the food category includes conventional food, functional foods, dietetic products and food supplements. In addition to the general food law and regulations, manufacturing and marketing of functional foods, special dietetic products and food supplements shall comply with a range of special directives set for regulating these activities in EU. Among these, the regulation on novel food (The European Parliament and Council, 1997), food supplement directive (The European Parliament and Council, 2002) and the health claim regulation (The European Parliament and Council, 2006) have attracted much focus of the field. At the moment, it is important to notice the lack of harmony among EU member states in specific measures taken at national levels due to cultural and historical reasons as well as different interpretation of the EU regulations by the national authorities.

Seabuckthorn is native to Europe and Asia and lately introduced to America. With increasing amount of research activities on seabuckthorn, the plant is getting better known in Western countries. However, the utilization and commercialization of seabuckthorn products at industrial scale are still new practice in many countries. Varying numbers of regulatory issues need to be cleared depending on products and the targeted markets of these products. At the moment, most of the seabuckthorn resource is in China, other Asian countries and Russia. The manufacturers of seabuckthorn products in these countries have a clear advantage of a better access to raw materials compared with their counterparts in other parts of the world. In order to gain success in the international markets, it is of crucial importance that these manufacturers work together with local authorities, experts and partners in the targeted countries.

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Seabuckthorn (*Hippophae rhamnoides* L.) Products Overview

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Abstract

Seabuckthorn (*Hippophae rhamnoides* L.) (SBT) has become a crop of interest in many parts of the world. Almost all parts of the seabuckthorn shrub can be used in the food, nutraceutical, pharmaceutical and cosmetic industries. SBT fruits have high amounts of vitamins, minerals, flavonoids, carotenoids and fatty acids and can be processed into dried fruits, infused fruits or fractionated into puree, seed and pulp. SBT juice contains high amounts of vitamins A, C, iron and antioxidants; it can be produced to single strength juice or blended with other fruit juices and sweeteners to produce nutritional and sport beverages. It can be further processed into concentrated SBT juice, jellies, syrups, dried to SBT juice powder, fermented into SBT wine and other alcoholic drinks, SBT soy sauce and SBT vinegar. SBT pulp contains high amounts of palmitoleic acid (ω-7), which offers a unique characteristic to the pulp. Dried pulp can be incorporated into nutritional bars and bakery products to enhance their nutritional value and provide an attractive aroma and colour. SBT pulp and seed oils are the most valuable products due to their fat-soluble vitamins, sterols and fatty acids; they can be used directly in food, pharmaceutical and cosmetic industries. SBT pulp and seed oils can also be produced for encapsulated products as a functional food or processed into micro-encapsulated oil as a functional ingredient. SBT leaves and extracts are used for tea blends and body care products. The leaves can also be crushed for extraction of essential oil and flavonoids. The bark and seed have also been used to extract proanthocyanidins. SBT products and some of their nutritional value and potential health benefits will be presented in this paper.

Keywords: seabuckthorn fruit, product, seed, pulp, bark, juice, oil.

Introduction

Seabuckthorn is a shrub or small tree of the genus *Hippophae*. It is a deciduous shrub native to China, Mongolia, Russia and Eurasia. The genus belongs to the family *Elaeagnaceae*, which consists of six species and, among them is the most economically important one, *Hippophae rhamnoides*, commonly known as seabuckthorn. *Hippophae rhamnoides* is further divided into ten subspecies (Yang & Kallio, 2002). 'Indian Summer' and *Sinensis* are the most popular varieties grown in Canada. Even though the nutritional values vary from species to species and from varieties to varieties, seabuckthorn fruit contains a higher content of vitamin A, C, E, iron, carotenoids and flavonoids than most fruits. Seabuckthorn also contains higher omega 7 fatty acid. Almost all parts of the plant can be processed into a wide spectrum of products that fit the rapidly growing nutraceutical and health food markets. Products are made from the fruits, as well as the oil, leaves and bark. The objective of this work is to review seabuckthorn products from the research and development project conducted at the Food Development Centre (Portage la Prairie, Manitoba) as well as products in the market.

Seabuckthorn Processing

The seabuckthorn fruit can be processed into many stable consumer products. The Russian's began to use seabuckthorn fruit for making good quality wine, jam and jelly in the 19th century for domestic use (Lu, 1992). An integrated process developed at the Food Development Centre for the preparation of various seabuckthorn products is shown in Figure 1.

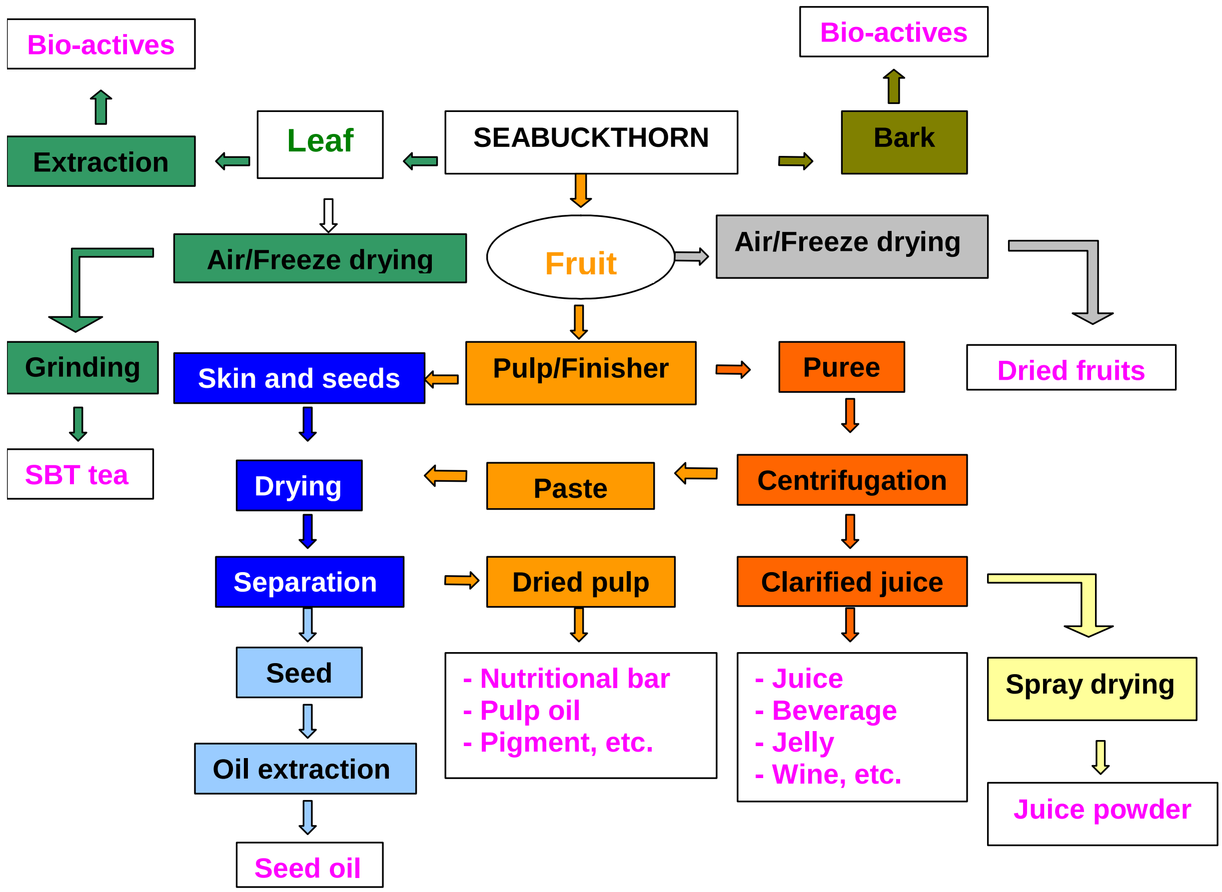

Figure 1. Seabuckthorn processing flow chart.

Juice extraction is often the first step in processing of seabuckthorn fruit. Fruits were thawed to about 10°C at room temperature. Thawed fruits were passed through a stainless steel pulper/finisher (Langsenkamp, Indianapolis IN, USA); the juice, skin and seeds were collected separately. The extracted juice was clarified using a centrifuge; a small amount of sludge (paste) was removed. The clarified juice can be used to supplement health drinks and foods. Seabuckthorn fruit yields 60% to 75% juice. The juice is high in organic acids and has a low pH (around 2.7). High vitamin C (0.6 %), vitamin E (0.16%) and iron (1.1 mg %) contents have been reported in the juice (Li, 2002; Meseyton, 2007).

Seabuckthorn Infused Fruit

Figure 2 shows a picture of infused seabuckthorn fruit developed at the Food Development Centre. Seabuckthorn fruit was infused with apple juice concentrate and dried; the final infused fruit product contains whole fruit, which is rich in seed oil and fibre. Infused fruit is a potential ingredient for nutritional bar enrichment. The infused fruit had strong aroma, tart fruity flavour, appealing orange/red colour and chewy dried fruit texture. Infused seabuckthorn fruits can also be coated with chocolate and marketed as candies.

Figure 2. Infused seabuckthorn fruits ('Indian Summer') with apple juice concentrate.

Seabuckthorn Juice Products

Seabuckthorn juice is an excellent material for developing beverages, blended beverages, jam, juice (Beveridge, 1999), fruit leather and fruit jelly (Meseyton, 2007). Meseyton (2007) reported that seabuckthorn juice mixed with apple juice concentrate produced a pleasant beverage with high nutritional value. However, the jam product contained almost no vitamin C, due to vitamin C degradation during heat treatment. The pictures of some seabuckthorn products developed at the Food Development Centre are shown in Figure 3.

Beverage Jam Wine Puree

Figure 3. Pictures of seabuckthorn beverage, jam, wine and puree.

Selvamuthukumaran et. al. (2007) reported that seabuckthorn mixed fruit jelly was prepared by blending seabuckthorn juice with papaya, watermelon or grapes in varying proportions to obtain an acceptable level of total soluble solids (TSS) and acidity in the final product. Among the above blends, the seabuckthorn grape jelly exhibited good organoleptic characteristics with a high sensory score. The shelf stability of the jelly samples was evaluated at ambient temperature and at 37°C for a period of six months. The physiochemical properties of the seabuckthorn grape jelly, such as TSS, reducing sugar, acidity and browning significantly increased during storage at 37°C. Total sugars, vitamin C, vitamin E, total phenols, total carotenoids and total anthocyanins decreased significantly during storage at 37°C. The sensory attributes of the seabuckthorn grape jelly remained acceptable up to six months of storage under ambient temperature conditions when stored in polyethylene terephthalate (PET) bottles. The microbial load of the jelly under the above conditions was also found to be within acceptable limits.

Seabuckthorn Juice Powder

The natural liquid state of seabuckthorn juice makes it difficult for incorporation into a wide range of dry food products, cosmetics and nutraceuticals. For this reason, free-flowing seabuckthorn juice powder is a desirable product. Seabuckthorn juice powder is also more convenient and stable than the juice itself for prolonged storage; low moisture content greatly reduces microbial activity without the need for freezing or refrigeration. Spray drying is an ideal process for the continuous production of dry powder; a liquid is transformed into a dry powder in a single operation at low product temperatures with short drying times compared to other drying processes, while maintaining the natural nutrients, colour and flavour of the hydrated product. A picture of seabuckthorn powder produced at the Food Development Centre is shown in Figure 4.

Figure 4. Picture of spray dried seabuckthorn powder.

Seabuckthorn Dry Mix Smoothie

A seabuckthorn dry mix smoothie prototype was developed at the Food Development Centre using the spray dried seabuckthorn juice powder. The smoothie base is a yellow powder with a seabuckthorn aroma and flavour. Soluble fibre was incorporated to increase the fibre content in the product in order to achieve a fibre content claim on the label. Vitamin C and antioxidant activity were measured as present in significant levels in the dry mix. The following sensory attributes were taken into account in the formulation of the seabuckthorn smoothie:

- Sweetness: The sweeteners sucralose, erythitol, aspartame and acesulfame-K were tested in the formulation to achieve a balanced flavour with no aftertaste in a low sugar product such as the smoothie. Sucralose left a slight aftertaste according to the trained panelists used in the sensory evaluation and was excluded from further tests. The combination of aspartame and acesulfame-K were chosen for this product and tested at different ratios. Erythitol was used to give a refreshing flavour.
- Tartness: A tart flavour should be slightly noticeable and acceptable in the smoothie flavour, which is a characteristic of seabuckthorn fruit flavour. The challenge was to balance the tartness flavour of the fruit with the sweetener. Acidulants were tested in different ratios to achieve a balanced and acceptable flavour in the final product. Sensory evaluation was used as a key tool to reaching a balanced flavour.
- Flavours: Blending different flavours in the smoothie product improves its acceptability. The addition of natural cream flavour offered a more rounded flavour to the product. Flavoured fruit crystals such as strawberry were also used to minimize the seabuckthorn flavour, thus offering choices for different consumer taste preferences.
- Texture: To reach the desired thickness and creaminess, ingredients such as gums and starches were tested. It was observed that some of the starch ingredients (corn starch) interfered with the product's flavour. A mixture of gums (cellulose gum, xanthan gum, carrageenan) produced the best results for texture without interfering with the flavour. Different concentrations were tested and sensory evaluation was conducted to determine the most acceptable level of gum.
- Fibre: Inulin was chosen as an ingredient for the smoothie product. As a soluble fibre, 8% was added to the formulation in order to obtain a dietary fibre claim based on the reference amounts as per Health Canada regulations.

Pulp and Seed Separation

Wet fruit seeds and skin were spread onto perforated drying trays (10 mesh or 1.91 mm perforation size) giving a drying layer of approximately 10 mm. The wet cake was dried at 50°C for 24 h in a forced-air drying oven (Gas Fired Variable Circulation Laboratory Dryer, Proctor and Schwartz Corporation, Philadelphia, PA). The dried seed and skin were carefully removed from the drying trays and emptied into an industrial mixer in approximately 2 kg batches. An industrial mixer (Hobart Cutter Mixer, HCM 300, Hobart Corporation, Troy, OH) equipped with a plastic, two-blade attachment with 1140 rpm served as a threshing unit to gently separate seeds from pulp and remove the white seed skins covering the seeds. The mixer was operated for short time intervals for one to two minutes. This was done to ensure that seeds did not become damaged during threshing.

A vibratory screen separator LS 24S444 (SWECO, Sweco Canada, Toronto, ON) equipped with a stackable arrangement of three screens was assembled for the separation of seeds from pulp. Screens were arranged (top and bottom layer screen openings of 6- mesh (or 3.35 mm) and 10-mesh (or 1.91 mm), respectively to collect three fractions including debris with pulp, seeds and skin (pulp). When it was necessary, the seeds were further cleaned using compressed air to remove residual pulp that was not removed by mechanical separation. The dried pulp from two seabuckthorn varieties is shown in Figure 5.

Figure 5. Dried pulp from *Sinensis* (left) and 'Indian Summer' (right).

Seabuckthorn Pulp Products

Dried seabuckthorn pulp contains high amount of fibre, vitamin C and iron, and can be used in nutritional bar formulation. Other ingredients in the nutritional bar formulation can be used to balance strong tart seabuckthorn flavour; the amount of dried 'Indian Summer' pulp in the formulation can be up to 10% of the bar mass. (Meseyton, 2007). A picture of a nutritional bar is shown in Figure 6.

Figure 6. Nutritional bar containing seabuckthorn pulp.

Seabuckthorn Seed Oil

Important pharmacological functions of the seed oil include anti-inflammatory, anti-microbial, relieving of pain and promoting regeneration of tissues (Li, 2002). Seabuckthorn seed oil was used to treat radiation damage, burns, scalds, duodenal ulcers, gastric ulcers, skin ulcers and other skin damage. Three extraction methods for seabuckthorn seed oil were compared at the Food Development Centre, namely solvent extraction, cold press extraction and supercritical fluid extraction (SFE CO_2). A schematic flow diagram of SFE is shown in Figure 7. The details of the extraction methods have been reported (Wang & Utioh, 2007). Seabuckthorn seed oil is widely used as a dietary supplement, as well as in the cosmetic industry.

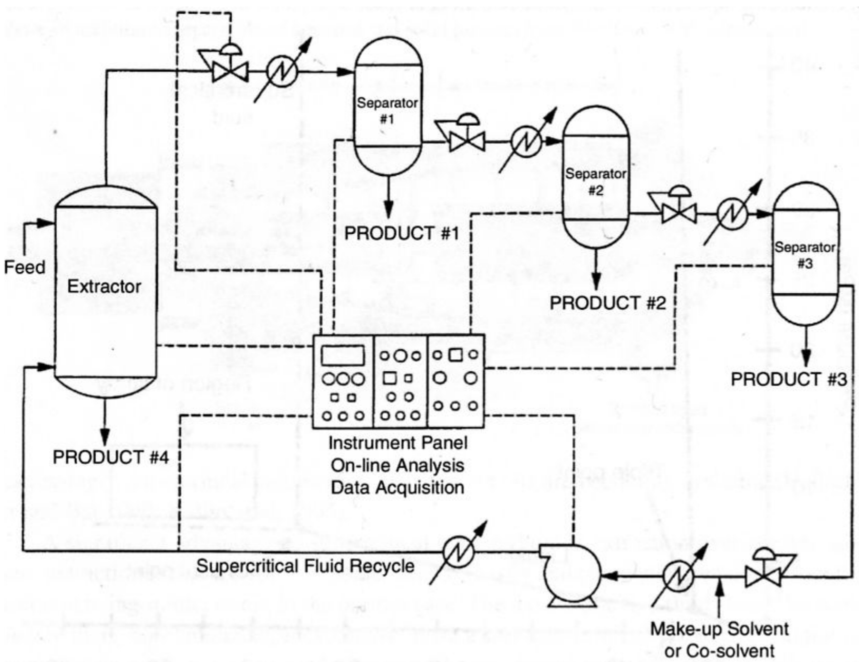

Figure 7. Schematic flow diagram of SFE.

Seabuckthorn Pulp Oil

Pulp oil is another valuable co-product of seabuckthorn processing. Pulp oil has an extremely high content of carotenoids, tocopherols and sterols with its characteristic fatty acid, palmitoleic acid, (Arimboor, Venugopalan, Sarinkumar, Arumughan & Sawhney, 2006). The pulp oil obtained by process shown in Figure 1 retained its characteristic seabuckthorn flavour. The presence of palmitoleic acid (ω-7) as the predominant fatty acid is unique among oils of plant origin (Yang & Kallio, 2002). Palmitoleic acid is reported to facilitate the fluidity of cell membranes in a similar manner to polyunsaturated fatty acids, but with low susceptibility to peroxidation, and improves the metabolism of vascular smooth muscle cells (Yamori, Nara, Tsubouchi, Sogawa, Ikeda & Horie, 1986). Pulp oil is an ingredient for cosmetic industry for skin care products.

Pigment

A "seabuckthorn yellow" pigment can be extracted from seabuckthorn pulp or skin. The pigment can be extracted with alcohol or using supercritical CO2 methods. Chen et al. (1995) and Liu et al. (1989) reported on the seabuckthorn pigment extracted with low concentrations of alcohol. The pigment contains flavones, but also carotene and vitamin E. The pigment is an orange-yellow powder that dissolves in water and alcohol. The colour is stable at pH < 6.5, but unstable at pH > 7. β-carotene is dominant among the pigments in seabuckthorn, the concentration of which varies depending upon the place of origin (Zhang, 1987). Supercritical CO2 has also been used to extract a yellow colouring material from seabuckthorn waste. Operating pressure had the greatest influence on extraction yields increasing with extraction pressure. A yield of 64% total carotenoids was achieved under processing conditions of 60 MPa and 85°C (Messerschmidt et al., 1993).

Seabuckthorn Tea Product

Sea buckthorn leaves contain nutrients and bioactive substances. These include flavonoids, carotenoids, free and esterified sterols, triterpenols and isoprenols (Li, 2002). Various products can be made from the air dried leaves, including teas and tea powders.

Animal Feed

One potentially large market for seabuckthorn is nutraceutical products for animals. The large volume of "waste" material from seabuckthorn, such as leaves, fruit, pulp and seed residues from juice and oil extraction, and soft branches could be developed into value-added animal feed products. Seabuckthorn leaves contain approximately 15% protein, and fruit and seed residues also contain minor, but high value components, such as polyphenols.

Conclusions

Seabuckthorn could be called a super fruit because of its commercial value and its many health promoting properties. The fruit can be processed for juice rich in vitamin C and oils rich in fat soluble vitamins A and E, sterols and essential fatty acids. Seabuckthorn leaves can be dried for tea formulations or as raw materials for bioactive components. The "waste" material of seabuckthorn processing can be further processed into high value animal feed.

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Utilization of Seabuckthorn Fruit Pulp and Juice as Functional Food Ingredients

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Abstract

Seabuckthorn (SBT) berries are considered a functional food due to their high content of unsaturated fatty acids, vitamins, carotenoids, flavonoids and dietary fibre. Processing SBT berries for seed oil results in nutrient rich co-products (juice and pulp). SBT fruit pulp is high in dietary fibre while the raw juice is rich in vitamins A and C; positioning them as functional ingredients suitable for the food industry. Prototype food products were developed using the co-products of SBT fruit processing. Dried fruit pulp was used in an unbaked energy bar and clarified juice was used for beverage development. The fruit pulp and juice are very tart, strongly flavoured and bright orange/red; thus they present unique product formulation challenges. Pulp and juice from two SBT varieties ('Indian Summer' and *sinensis*) were evaluated. Pulp and juice from 'Indian Summer' berries were more tart and more orange/red than those from *sinensis*; nutritional differences also existed between the two varieties. 'Indian Summer' dried pulp and juice were used in the final bar and beverage formulations. The fruit pulp was incorporated into the bar at 2, 4, 6 and 10%. Increasing the level of pulp affected bar appearance, flavour, texture and nutritional profile. Initial results indicate SBT fruit pulp may contribute humectant properties to the bar. Beverages containing 30, 35 and 45% SBT fruit juice (10.9° brix) were formulated. Adding 30% juice produced a pleasantly tart and fruity beverage with an attractive opaque orange colour. It is important to minimize heat processing to preserve colour and vitamins of the product.

Keywords: *Hippophae*, 'Indian Summer', *sinensis*, dietary fibre, pulp, juice, beverage, bar, functional food.

Introduction

Seabuckthorn (*Hippophae rhamnoides* L.) is a hardy bush with nutritious fruit, indigenous in Central Asia and Europe and now also grown in North American. The species is classified into nine subspecies (Kallio, Yang, Peippo, Tahvonen, & Pan, 2002). 'Indian Summer' and *sinensis* are the most abundant and commercially viable varieties in Canada (Li, 2003). Seabuckthorn fruit has a unique nutritional profile and a strong flavour. It is known to be quite acidic and not very sweet with a mild but characteristic aroma (Tiitinen, Hakala, & Kallio, 2005). Lange, Klein, Gerber, Bauer, Fetkenhauer & Sievert (1991) characterized the flavour of seabuckthorn as "whey-like" and indicated that consumers might find the flavour unpleasant. Therefore, combining seabuckthorn (SBT) juice or pulp with other ingredients to balance its strong flavour could result in unique, nutritious products with appealing sensory qualities. The objectives of this study were to incorporate the by-products of seabuckthorn seed oil extraction (juice and dried pulp) into a beverage and an energy bar and to determine the sensory and nutritional properties of the final products containing SBT pulp or juice. Utilizing SBT by-products as functional food ingredients could result in the creation of unique products with health and wellness benefits.

Material and Methods

Seabuckthorn fruit

Seabuckthorn fruit ('Indian Summer' variety) was harvested from mature shrubs at Winkler, Manitoba in 2005 and seabuckthorn *sinensis* fruit was harvested from Branching-Out Orchards, St. Claude, Manitoba in 2005. Immediately after harvesting, the fruit was hand cleaned to remove visible debris, namely dried leaves, branches, and damaged fruit. Cleaned fruit was portioned into 12-13 kg lots and packed in cardboard boxes. The fruit remained frozen (at about -15 °C) during transportation from Winkler or St. Claude to Portage la Prairie, Manitoba. The fruit was stored at -20 °C and all samples were used within 8 months.

Pilot processing for juice, seeds and skin separation

Juice extraction

All fruits were thawed at room temperature to about 10 °C. Thawed fruits were passed through a stainless steel pulper/finisher (Langsenkamp, Indianapolis, IN) to separate puree from pulp and seeds. Extracted puree was clarified using a centrifuge (SB7-02, Westfalia, Burlington, ON) then placed in plastic pails (20 L capacity) and stored at -20 °C.

Drying

Wet fruit seeds and pulp were crumbled onto perforated drying trays (10 mesh or 1.91 mm perforation size) resulting in a drying layer of approximately 10 mm. The wet cake was dried at 50 °C for 48 h in a ventilated drying oven (Gas Fired Variable Circulation Laboratory Dryer, Proctor and Schwartz Corporation, Philadelphia, PA) to remove moisture. Final moisture content of the 'Indian Summer' pulp was 7.0% and 8.3% for the *sinensis* pulp.

Separation of seed and skin

The dried seed and pulp were carefully removed from the drying trays and transferred to an industrial mixer in approximately 2 kg batches. An industrial mixer (Hobart cutter mixer, HCM 300, Hobart Corporation, Troy, OH) equipped with a plastic, two-blade attachment with 1140 rpm served as a threshing unit to gently separate seeds from pulp and remove the white seed skins encapsulating the seeds. The mixer was operated for short time intervals for 1-2 min. This was done to ensure that seeds did not become damaged during threshing.

A vibratory screen separator LS 24S444 (SWECO, Sweco Canada, Toronto, ON) equipped with stackable screens was used to separate seeds from pulp. The top screen had 6-mesh (3.25mm) openings and the bottom screen had 10-mesh (1.91 mm) openings. Three fractions were collected: debris with pulp, seeds and pulp. When necessary, the seeds were further cleaned using compressed air to remove residual pulp not removed by mechanical separation. A detailed flow chart is shown in Figure 1.

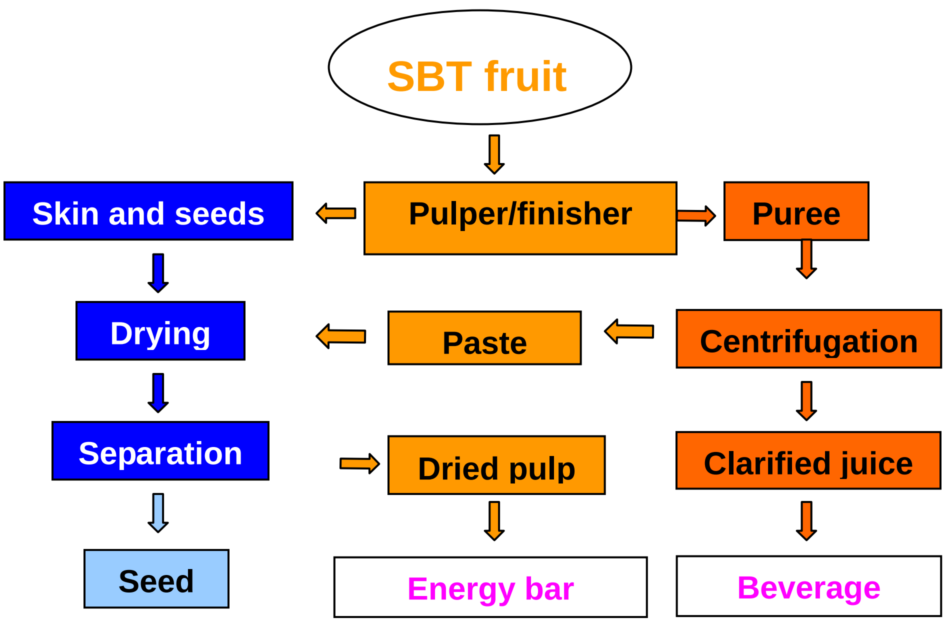

Figure 1. Flow chart for processing of seabuckthorn fruit at Food Development Centre, Portage la Prairie, MB.

Seabuckthorn beverage preparation

During prototype development, the beverage ingredients were manually combined in a pot, heated on the stove to boiling, hot filled into glass bottles and allowed to cool at room temperature. The pulp and oil naturally present in the SBT juice caused the beverage to separate, thus a shearing step was added when the beverage formulation was

scaled-up. The formulation was scaled-up to a 50-kg batch using a 40-gallon steam kettle (MKDL, Garland, Cleveland, OH), in-line shear mixer (19289-275LS, Silverson, East Longmeadow, MA) piston filler (PPF1-10-1, Hauser, Greenwood, IN) and positive displacement pump (UR 30, Waukesha, Pine Bush, NY). The beverage was heated to boiling, pumped through the shear mixer to the filler, hot filled, capped and air cooled at room temperature.

Seabuckthorn energy bar preparation

The lab method involved blending the dry ingredients using a large bowl and whisk. The binder ingredients were manually combined in a pot and heated to 110 °C. The cooked binder was poured over the dry ingredients and manually combined until the dry ingredients were moistened. The mixture was distributed in a saran lined baking pan, covered with saran and then evenly compressed by passing a rolling pin across the surface several times. The top layer of saran was removed and the mixture was cooled at room temperature before cutting it into bars. The bar formulation was scaled-up to a 15 kg batch using an industrial mixer and dough hook (L-800, Hobart Corporation, Troy, OH) electric kettle (KET-6-T, Cleveland Range, Cleveland, OH) and custom built bar extruder (Sperling Industries, Sperling, MB). The dry ingredients were blended on speed 1 for one minute using the mixer and dough hook. The binder ingredients were heated on high to 110°C in the electric kettle then transferred to the mixer. The ingredients were mixed using a dough hook on speed 1 for 1.5 minutes. The mixture was cooled to about 45 °C then placed in the hopper of the bar extruder and extruded into bars.

Nutritional analysis

Nutritional analysis of the SBT pulp and juice were conducted by SGS Canada Inc. in Vancouver. All methods followed the AOAC (1990) or AOCS procedures (AOCS, 1993). Canadian nutrition facts panels for the SBT beverage and bar were generated by the Genesis™ R&D SQL nutritional Labelling software program (Esha Research, Inc. Lemon Cove, CA, USA).

Sensory evaluation

During the product development process, the most acceptable beverage and energy bar formulations were determined using in-house sensory panels.

Results and Discussion

Seabuckthorn juice nutritional composition

The nutritional composition of raw SBT juice is shown in Table 1. SBT juice from the ‘Indian Summer’ variety was about 75% higher in total fat than that from the *sinensis* variety. More specifically, juice from the ‘Indian Summer’ variety had about twice the levels of saturates and omega 7 (palmitoleic acid) fatty acids. In addition, juice from the ‘Indian Summer’ variety contained about twice the vitamin A of *sinensis*. Juice from the *sinensis* variety had over twice the vitamin C content of that from ‘Indian Summer’.

Table 1. Composition of raw seabuckthorn juice *.

NUTRIENT	SEABUCKTHORN VARIETY	
	‘INDIAN SUMMER’	*SINENSIS*
Protein (g/100g)	0.6	0.6
Fat, Total (g/100g)	1.4	0.8
Saturates	0.6	0.3
Omega 6	0.1	0.1
Omega 7	0.5	0.2
Omega 9	0.2	0.2
Vitamin A (IU/100g)	636	308
Vitamin C (mg/100g)	187	450

* Based on a single sample analyzed by SGS Canada Inc.

A comparison of the nutritional composition of SBT juice and other common fruit juices is shown in Table 2. The nutritional composition of SBT juice is unique compared to other commonly consumed fruit juices as SBT juice contains substantially more fat, vitamin A and vitamin C and a significant amount of iron.

Table 2. Comparison of nutritional composition of seabuckthorn juice with other common fruit juices *.

	Seabuckthorn ('Indian Summer') raw	Orange raw	Grapefruit raw	Cranberry canned unsweetened	Prune canned	Tomato canned no salt
Energy (kcal/100g)	53	45	39	46	71	17
Protein (g/100g)	0.60	0.70	0.50	0.39	0.61	0.76
Fat (g/100g)	1.40	0.20	0.10	0.13	0.03	0.05
Carbohydrates (g/100g)	9.50	10.40	9.20	12.20	17.45	4.24
Calcium (mg/100g)	11	11	9	8	12	10
Iron (mg/100g)	1.10	0.20	0.20	0.25	1.18	0.43
Vitamin A (IU/100g)	636	200	440	45	3	450
Vitamin C (mg/100g)	187	50	38	9.3	4.1	18.3

* Seabuckthorn data is from the Food Development Centre and is based on a single sample. Data for the other fruit juices is from USDA Nutrient Database for Standard Reference and the sample number is unknown to the author.

Seabuckthorn juice sensory attributes

The SBT juice was a bright orange colour, opaque with some surface oil, pulpy, with a very tart fruity flavour and a distinctive aroma. Sensory differences existed between 'Indian Summer' and *sinensis* varieties. The 'Indian Summer' juice was a darker red orange while *sinensis* juice was orange. Juice from the 'Indian Summer' variety was more tart and less sweet than that from *sinensis*. These varietal sensory differences could provide flexibility when formulating products with SBT juice.

Sea buckthorn beverage formulation considerations

Beverages containing 30, 35 and 45% SBT juice were prepared and evaluated. The SBT juice was very tart ('Indian Summer' pH 2.7), not very sweet (10.9 °Brix) and strongly flavoured, thus a sweetener was required and 30% SBT juice was considered the maximum level for beverage palatability. The SBT juice contained a substantial amount of pulp and a small amount of oil which rapidly separated from the juice and the beverage even after high shear mixing. The SBT juice and the SBT beverage exhibited browning with heat exposure. Minimal heat treatment is recommended to preserve product colour and limit the loss of heat sensitive nutrients such as vitamin C. 'Indian Summer' juice produced a brighter coloured slightly more appealing beverage than *sinensis* juice.

Seabuckthorn beverage nutritional profile

Figure 2 shows the Canadian nutrition facts panel for the SBT beverage. A 250 mL beverage serving provides an adult with 240% of the daily value of vitamin C and 15% of the daily value of vitamin A. According to the Guide to Food Labelling and Advertising (Health Canada, 2003), the following nutrient content claims can be made for the SBT beverage: "excellent source" of vitamin C and "good source" of vitamin A. Although SBT juice is rich in health promoting antioxidants, health claims for antioxidants are currently not allowed in Canada. However, the nutritional profile of the SBT beverage would still favorably position it in the functional beverage category.

Dried seabuckthorn pulp nutritional composition

The nutritional composition of dried SBT pulp from the 'Indian Summer' and *sinensis* varieties is shown in Table 3. SBT pulp from the 'Indian Summer' variety had about twice the vitamin A, saturated fatty acids, mono unsaturated fatty acids and total fat compared to *sinensis*. Polyunsaturated fatty acids were also higher in the 'Indian Summer' pulp. *Sinensis* pulp contained about twice the vitamin C and protein of 'Indian Summer' and more fibre. These differences in nutritional composition between the varieties could be an important consideration when developing functional foods containing dried pulp.

Nutrition Facts Valeur nutritive Serving Size 250 mL / Portion Servings Per Container Portions par contenant	
Amount Teneur	% Daily Value % valeur quotidienne
Calories / Calories 240	
Fat / Lipides 1 g	**2** %
Saturated / saturés 0.4 g + Trans / trans 0 g	**2** %
Cholesterol / Cholestérol 0 mg	**0** %
Sodium / Sodium 15 mg	**1** %
Carbohydrate / Glucides 58 g	**19** %
Fibre / Fibres 0 g	**0** %
Sugars / Sucres 51 g	
Protein / Protéines 0.4 g	
Vitamin A / Vitamine A	15 %
Vitamin C / Vitamine C	240 %
Calcium / Calcium	4 %
Iron / Fer	4 %

Figure 2. Seabuckthorn beverage nutrition facts panel according to the Guide to Food Labelling and Advertising (Health Canada, 2003)

Table 3. Comparison of nutritional composition of dried seabuckthorn pulp from 2 varieties*

NUTRIENT	'INDIAN SUMMER'	*SINENSIS*
Protein (g/100g)	6.80	11.90
Fat (g/100g)	23.53	13.73
Saturated (g/100g)	8.85	4.87
Monounsaturated (g/100g)	11.12	6.21
Polyunsaturated (g/100g)	3.56	2.53
Omega 3 (g/100g)	0.65	0.51
Omega 6 (g/100g)	2.91	2.02
Omega 7 (g/100g)	8.63	4.58
Fibre (g/100g)	31.00	39.60
Vitamin C (mg/100g)	442	743
Vitamin A (IU/100g)	8212	4397

***** Based on a single sample analyzed by SGS Canada Inc.

Dried seabuckthorn pulp sensory attributes

Dried seabuckthorn pulp had an orange colour, distinctive aroma, very tart fruity flavour, fibrous texture and a slightly gritty/powdery mouth feel if ground. 'Indian Summer' pulp is more tart and more red than *sinensis* pulp. The strong tart flavour of the dried SBT pulp could limit its use but the attractive colour could create formulation opportunities.

Seabuckthorn energy bar formulation considerations

When formulating the bar, sensory evaluations by 3-5 FDC staff guided the development process. The strong tart flavour of the SBT pulp was balanced by a sweetener and other intensely flavoured ingredients. The attractive colour of the dried pulp provided visual appeal to the bar and its fibrous composition created interesting chewy textures. SBT pulp was incorporated at 2, 4, 6 and 10% levels. Increasing the level of dried SBT pulp in the formulation resulted in bars which were more orange/red (based on visual observations), fruitier, tangier and denser. Up to 10% dried pulp was successfully used in the bar formulation, although SBT flavor and color mask the appearance and

flavor of the other bar ingredients at the 10% level. Based on FDC staff perception, bars with 6% pulp may be more acceptable as the flavour of seabuckthorn is unfamiliar to North Americans and unexpected in bars. Periodic verbal feedback solicited from 20-30 FDC staff and the general public suggested the sensory quality of the bar containing 6% SBT pulp was well received. It was also observed that bars containing SBT pulp resisted drying out and hardening suggesting SBT pulp could play an important role in extending the shelf life of bars.

Seabuckthorn energy bar nutritional profile

The Canadian nutrition facts panel for the SBT energy bar is shown in Figure 3. A 30-g serving of the energy bar provides 2g fibre, 1g omega 3 fatty acids, 2g omega 6 fatty acids and 15% of an adult's daily value for vitamin C. According to Health Canada's Guide to Food Labelling and Advertising, the following nutrient content claims can be made for the SBT energy bar: "source" of fibre, "source" of omega 3 and 6 fatty acids, "source" of vitamin C, "source" of iron, low sodium, low cholesterol, low in saturated fat and trans fat free. The SBT energy bar addresses many current nutrition concerns thus creating opportunities for it as a health and wellness product.

Nutrition Facts
Valeur nutritive
Serving Size 1 Piece (30 g)
Portion (30 g)

Amount / **Teneur**	**% Daily Value** / **% valeur quotidienne**
Calories / Calories 120	
Fat / Lipides 6 g	**9** %
Saturated / saturés 0.5 g + Trans / trans 0 g	**3** %
Polyunsaturated / polyinsaturés 3 g	
Omega-6 / oméga-6 2 g	
Omega-3 / oméga-3 1 g	
Monounsaturated / monoinsaturés 1.5	
Cholesterol / Cholestérol 0 mg	**0** %
Sodium / Sodium 20 mg	**1** %
Carbohydrate / Glucides 17 g	**6** %
Fibre / Fibres 2 g	**8** %
Sugars / Sucres 10 g	
Protein / Protéines 4 g	
Vitamin A / Vitamine A	4 %
Vitamin C / Vitamine C	15 %
Calcium / Calcium	2 %
Iron / Fer	8 %

Figure 3. Nutrition facts panel for the seabuckthorn energy bar according to the Guide to Food Labeling and Advertising (Health Canada, 2003)

Conclusions

A pleasing beverage was prepared using 30% SBT juice and a sweetener. Canadian nutrient content claims for vitamin A and C can be made for the beverage. Minimal heat exposure was necessary to prevent beverage browning and loss of heat sensitive nutrients. An attractive tasty energy bar was prepared by incorporating 6% SBT pulp. Tasty bars containing 10% SBT pulp were also prepared but SBT flavor and color masks the flavor and appearance of the other bar ingredients. Increasing SBT pulp also created a denser bar. Bars containing 6% SBT skin can be considered a functional food as Canadian nutrient content claims can be made for fibre, omega 3, omega 6 and vitamin C. Sensory and nutritional differences existed between 'Indian Summer' and *sinensis* varieties. 'Indian Summer' pulp and juice were darker orange/red and more tart compared to *sinensis*. 'Indian Summer' pulp contained about twice the fat and vitamin A, about half the vitamin C and significantly less fibre and protein compared to *sinensis* pulp. Similar results were obtained for the juice, where 'Indian Summer' contained about twice the vitamin A and about half the vitamin C of *sinensis*. These varietal differences provide formulators the ability to

manipulate the sensory or nutritional profile of products containing SBT ingredients.

Acknowledgement

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Assessment report on the seabuckthorn market in Europe, Russia, NIS-Countries and China Results of a market investigation in 2005

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Abstract

The commercial cultivation and exploitation of seabuckthorn berries using their secondary compounds such as flavonoids, vitamins and carotenes for high quality food products or even to produce basic products such as juices is differently developed in Europe, Asia and Russia/New Independent States. The aim of the presented market study was to collect the important figures of the seabuckthorn market giving an overview of market strategies. For the investigation of the seabuckthorn industry Nahrungs- Ingenieurtechnik GmbH (NIG) developed a detailed questionnaire in English language, which was further translated into German, Chinese and Russian. The questionnaire was based on theoretical aspects of data mining for market studies. The questionnaire has been widely distributed among key player and core partners identified in the seabuckthorn business in Europe and Asia, together with the Forest Institute Archangelsk and the International Center for Research and Training of Seabuckthorn (ICRTS) Beijing. The information requested in the questionnaire, ranged from cultivation area, products sold (fresh, frozen berries, leaves), price, and market, to processing of berries and leaves, types of half-products and their market, as well as final products, their price and market. The information derived from the questionnaire provides a wide overview of the market situation and the development of the seabuckthorn industry as well as different marketing strategies for seabuckthorn products in the investigated countries. This report provides an original comprehensive overview of the current situation in the seabuckthorn business and its trends in Europe, China, Russia and the New Independent States (NIS).

Keywords: seabuckthorn market, berries, half and final products, prices, production amount, Europe, Russia, China.

Introduction

Seabuckthorn (*Hippophae rhamnoides*) is native to Europe and Asia and has been known and used by humans for centuries. The natural distribution of seabuckthorn extends in Eurasia from China, Altai-Region (Russia, Kazakhstan), Hindukush- Himalaya Region (India, Pakistan), Carpathians to the Alps and Pyrenees. A second band of distribution reaches the riparian states of the North and Baltic Sea from north-western France and south-eastern England to Lithuania, Estonia, Finland, and Norway.

During the last decade seabuckthorn has attracted special attention and became an important subject for domestication in many countries as it is one of the most interesting plants for human use. However, the commercial cultivation and exploitation of seabuckthorn berries using its secondary compounds such as flavonoids, vitamins and carotenes for high quality food products or even to produce basic products such as juices is differently developed in Europe, Asia and Russia/NIS. The presented study compared the seabuckthorn market situation in Europe, Asia and Russia/NIS countries in terms of the raw material market (berries and leaves), the half product market and the final product market with special focus on food applications. The collection of the figures for Russia and the NIS countries as far as China were done by the European-Asia Network (EAN) Project Partners, the Forest Institute Archangelsk and the ICRTS in Beijing.

Material and Methods

Market research is a systematic process of the collection and analysis of information about the market as a base for marketing decisions.
There are two methods for data surveys; the secondary research based on old internal or external data bases and the primary research based on interviews or observations. Both methods of data survey were used for the presented market study.

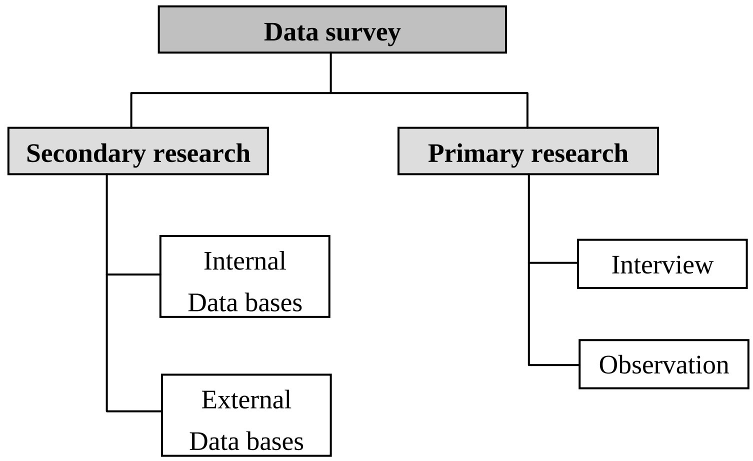

Figure 1. Schema of data surveys.

For the investigation of the seabuckthorn industry as primary research NIG developed a questionnaire in the English language, which was further translated into German, Chinese, and Russian.

The questionnaire was divided into 3 parts, each one corresponding to the following categories:

1. raw materials such as berries and leaves
2. half products such as juice, puree, etc.
3. final products, mainly focusing on food products (seabuckthorn is also applied in the cosmetic and pharmaceutical industry)

The questionnaires were widely distributed among key player and core partners identified in the seabuckthorn business in Europe and Asia. It was sent for instance to National Seabuckthorn Associations and farmers associations (i.e. Europe) with the aim of distributing it among their members. Conferences and other events were also employed to distribute questionnaires to the participants, which sometimes permitted a fast feed-back (e.g. in Beijing and Moscow).

Results and Discussion

The project partners found some difficulties getting valid responses to the questionnaires by some companies. Some of the questionnaires sent back contained no figures regarding produced product amounts and the actual selling prices, since many of the companies were not willing to provide this data. For that reason, it has only been possible to build a qualitative statement of product range.

Table 1: Investigated countries and response of the questionnaires.

Country	Sent Questionnaires	Response	Response factor [%]
China	53	26	49,06
Russia	57	5	8,77
NIS-Countries	18	3	16,67
Baltic countries	15	4	26,67
Germany	17	11	64,71
Romania	2	2	100,00
Italy	1	0	0,00
Sweden	1	1	100,00
Finland	1	1	100,00
Great Britain	1	0	0,00
Czech Republic	2	0	0,00
Summary	**168**	**53**	**31,55**

For assessing the situation in the Scandinavian countries, the project partners turned to government institutions, since no feed-back has been obtained from other sources, (i.e. seabuckthorn associations). A joint effort with these institutions has permitted an assessment of realistic figures of the seabuckthorn industry in those countries. That is the reason for a "1" entered for Finland and Sweden in Table 1, since the questionnaire was filled out according to the general situation of the whole country. The assessment of the situation in Russia and the NIS countries has borne many difficulties as well.

The feedback from those countries was insufficient, however, the partners intensively tried to get an overview by means of contacting and interviewing government institutions and stakeholders of the countries. A response of 31.5 % for market studies is in general an expectable result.

Seabuckthorn raw material market

For the description of the raw material market, only the figures of the researched cultivation area have been considered. It is known that a huge area of wild seabuckthorn can be found in most of the investigated countries, especially in China. Due to a lack of accurate data, an investigation of these resources for raw material has not been possible. It is well known that a lot of berries are harvested yearly from wild seabuckthorn resources.

As expected, China represents the world's biggest seabuckthorn cultivated area. Most of these cultivated areas in China have been very successfully established for erosion control, and an area of 130000 hectares per year is still in demand according to the plan of the Chinese government. The use of these areas for harvesting berries is not very efficient due to topographical conditions.

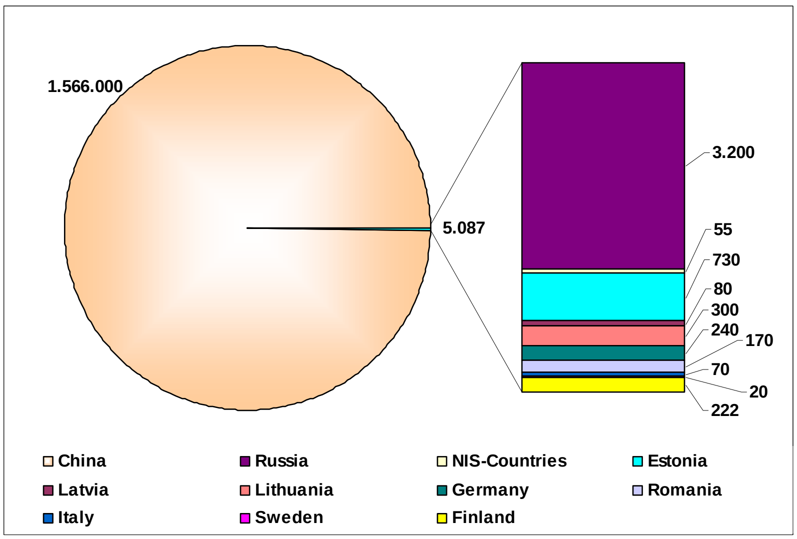

Figure 2: Cultivation area of Seabuckthorn in the investigated countries (in hectares).

The information corresponding to the seabuckthorn cultivated area in the NIS countries indicates 55 hectares. This figure seems unrealistic compared with the figures provided by Rongsen, 1992. However, no further information for plantations could be gathered. The situation in the Baltic countries has dynamically developed with more than 1000 hectares in plantations erected in the last decade.

The investigated countries harvested approximately 40000 tons of seabuckthorn berries from cultivated areas in 2005 (Figure 3). Figures 2 and 3 do not differ in harvesting strategies for

- fresh berries (fresh sold),
- fresh berries (frozen stored and sold) and
- frozen harvested (winter harvested).

Fresh berries represented more than 70 % of the world harvest; the fresh harvested berries were sold directly to the processing companies or to the end consumer without intermediate storage. Approximately 20 % of the berries were harvested as frozen berries in winter time. Only 10 % of the berries were harvested as fresh berries and will be marketed as frozen berries until the new harvesting season.

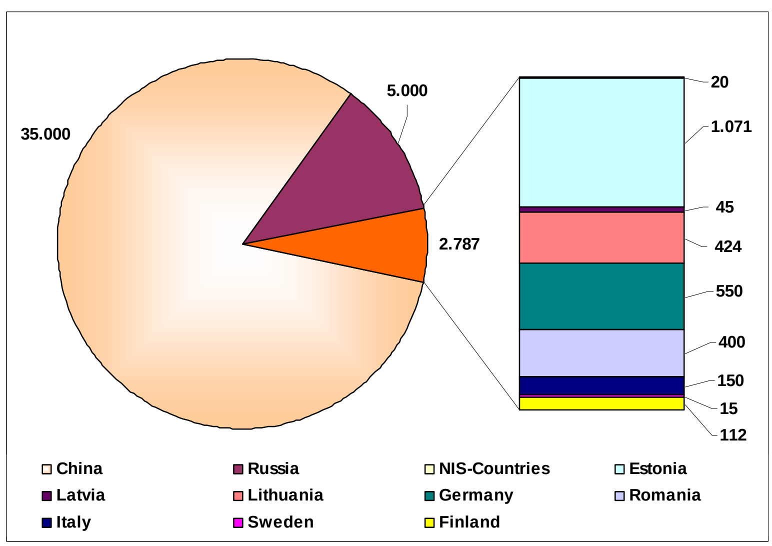

Figure 3. Berries currently harvested in the investigated countries (tons).

The given prices in USD have been calculated in EUROs with a rate of 0.8575. It has not been possible to assess how prices differ for fresh and frozen material in some countries, namely Russia, the NIS countries, and Romania. For some countries the market prices were given without a range. The price range depends on the kind of material (i.e. conventional or organic berries).

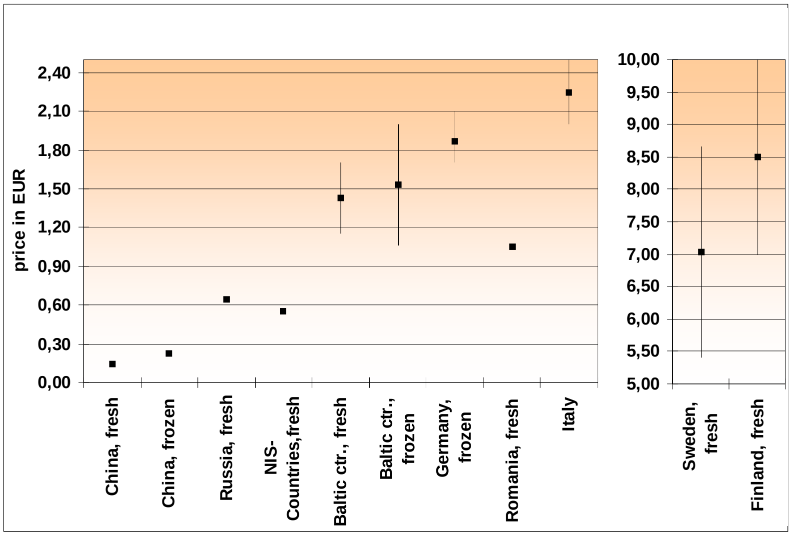

Figure 4. Selling price range for frozen and fresh Seabuckthorn berries (given in EUR/kg) in the investigated countries.

In Western Europe most of the berries will be marketed as certified organic raw material, which is one of the reasons for the higher prices there. Other reasons for the general price range are the quality of the berries as well as the labour costs in the countries.

The berry market volume is app. **14.4 Mill EURO** in the investigated countries, based on the price average and the harvested amount in the year 2005; China represents about 53 %.

Seabuckthorn half product market

The response of processing companies to the questionnaires in comparison with the response of seabuckthorn farmers was very sluggish. There is no statistics collection program for the associations or governmental institutions for production amount of different half products. The data source for the authors was constituted only on the answers from companies. The most complete figures were given by the German and Chinese processing companies, followed by the Baltic countries, and Russia together with NIS countries. The authors tried to estimate the figures of the other countries in a separate category (others).

The market volume of half products produced from seabuckthorn berries and seabuckthorn leaves represents a volume of approximately **42 Mill. EURO**. Very interesting is the range of different half products. It can be seen that China and Germany have the widest range of seabuckthorn half products while Russia and the NIS countries concentrate mainly on pulp oil. A comprehensive base of different half products is important for the development of different final products as shown in the section on final products.

Table 2. Average of yearly (2005) produced seabuckthorn half products depend on origin.

	Germany	Baltic Countries	China	Russia (incl. NIS)	Others
Sea Buckthorn juice	120 t	420 t	12000 t	162 t	115 t
Sea Buckthorn puree (pulp)	480 t	300 t	3000 t		35 t
Sea Buckthorn juice concentrate	13 t	10 t	300 t	50 t	8 t
Sea Buckthorn juice powder	6 t		300 t		
Sea Buckthorn puree concentrate	0,3 t				
Sea Buckthorn seeds	35 t	20 t	350 t	33 t	13 t
Sea Buckthorn dried skins	11 t	10 t		5 t	2 t
Sea Buckthorn pulp oil					
CO_2- extracted	1 t		20 t		5 t
centrifuged	3,5 t	10 t	34 t	70 t	2,8 t
solvent extraction				2,5 t	
Sea Buckthorn seed oil					
CO_2- extracted	1 t		30 t		5 t
cold pressed	0,8 t		25 t		1,7 t
solvent extraction			50 t		
Sea Buckthorn dried leaves	0,5 t	3 t	600 t		
Flavone powder			5 t		
Sea Buckthorn pomace dried	60 t	10 t	600 t		15 t

The market segments were calculated by given production amount and prices per country.

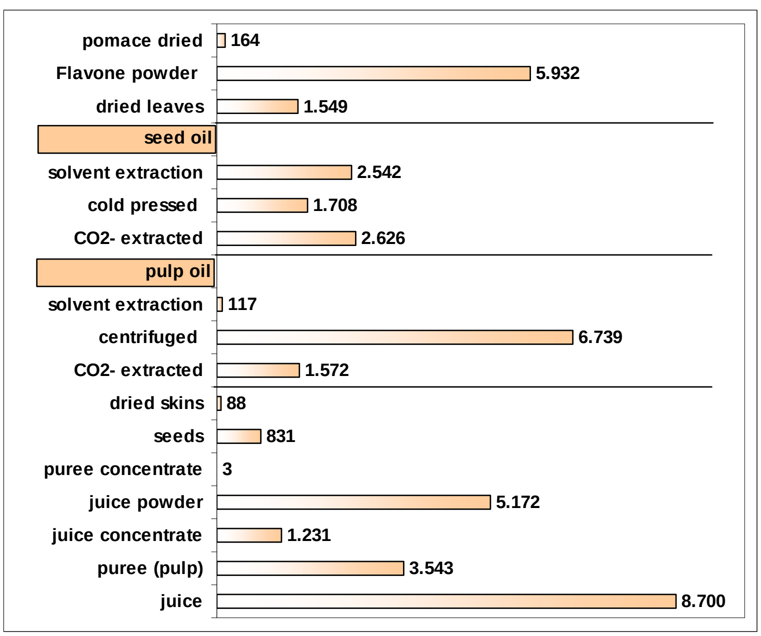

Figure 5. Market for Seabuckthorn half products, 2005, value in thousand Euros.

The highest turnover was realised with pulp oil, yearly app. **8.5 Mill EURO** per annum and more than 80 % produced by centrifugation – figure 5. The pulp oil content of the berries depends on the varieties and ranges from 1 – 5 %.

The reason for the low market level of the main products, seabuckthorn juice and puree, were the low prices in China and the small market segment in Russia and NIS countries.

A very interesting product is flavone powder. It is expected that the product will grow above average in the next years following the market segments of health products or nutraceuticals.

Approximately 70% of the global half-products were produced in China. The marketing of the products takes place mainly in the national market. Internationally, the products were sold in South Asia, Middle East, Japan, Korea, North America, and partly in Belgium and Finland.

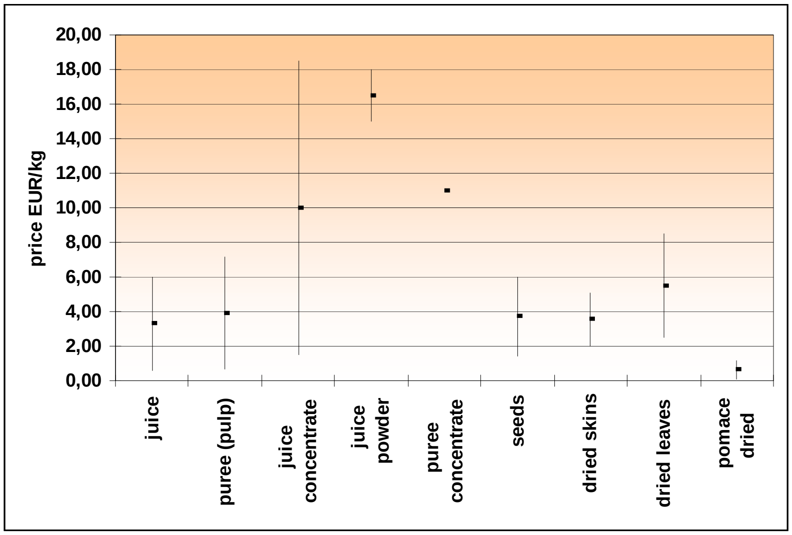

Figure 6a. Selling price range for Seabuckthorn half fabricates (non oil) in the investigated countries.

The German suppliers market their products nationally and internationally mainly in Western Europe and North America. The Russian producers sell their products in the national market and also in international markets such as Mongolia, Korea and North America. Products from Baltic countries are exported in the Scandinavian countries, Germany, and North America.

Figure 6 shows the market prices of the different seabuckthorn half-products depending on the type of product. When no price range is found in the figure, the product is only produced in one country.

It is remarkable that the selling prices of the manufactures vary approximately by ten times for some non-oil products such as juice, puree, juice concentrate and dried pomace. The reasons for these differences are the prices of the berry, the different processing technologies and costs, the different quality standards for the products, and the general situation in the markets.

On the other side, the selling price of seabuckthorn powder produced in Western Europe, Baltic countries, or China differ only by about 17%, but there is variation in the powder quality from 50% to 20% of fruit content.
The generally accepted opinion that Western European manufacturers will get the highest prices is not right for all half-products in the market. The manufacturers of the Baltic countries could meet the highest prices for puree, juice, and dried leaves for instance.

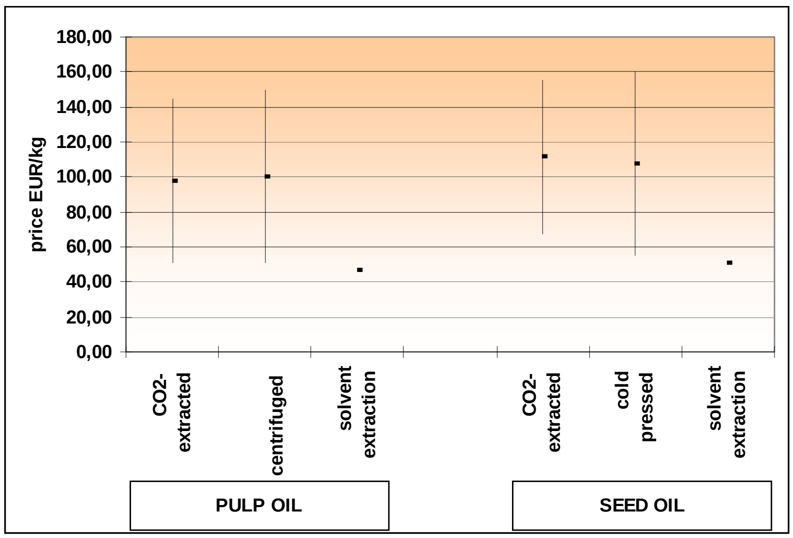

Figure 6b. Selling price range for Seabuckthorn oil in the investigated countries.

Oils produced by CO_2- extraction are an average 10 – 15% more expensive than other oils. The extraction of oils by organic solvents is only used in China and partly in Russia. The main producer of pulp oil is Russia, while the main producer of seed oil is China. Most of the oils are sold in the national market. In Western Europe we find high prices for sea buckhorn oils. However, the market volume today represents only about 6% of the production amount and 11.4% of the volume of sale. Growth opportunities are seen in the Southwestern European market because seabuckthorn is almost unknown there.

Seabuckthorn final product market

There are three main market segments for seabuckthorn half-products: the food market, the cosmetic market and the pharmaceutical market (Table 3). This study shows the investigation results mainly for the food market. For the German cosmetic market some figures could only be estimated based on the replies.

In the Russia and especially in China, the oils are also used in pharmaceutical products. Figures for the cosmetic and pharmaceutical market in Russia could not be summarised since no sufficient feed back from producers was obtained.

Table 3. Calculated production amount by final products.

	Germany	Baltic Countries	China	Others
	production amount in t/a			
Seabuckthorn raw juice (100%)	71,5	215,0	900,0	50,0
Seabuckthorn nectar	535,0	200,0	700,0	800,0
Seabuckthorn wine	74,0	100,0	3,0	
Seabuckthorn vinegar	-	-	1.300,0	
Seabuckthorn blended juice	510,0	50,0	1.000,0	700,0
Seabuckthorn jam	27,8	640,0	1.300,0	25,0
Seabuckthorn jelly	10,5	-	-	
Seabuckthorn sweeties, bars	9,0	-	-	2,0
Seabuckthorn tea				
leaves tea	-	3,0	45,0	
with dried berries	2,8	-	-	
Seabuckthorn cosmetics				
cremes	344,4	-	100,0	150,0
lotions	230,0	-	-	170,0
others	279,0	-	-	180,0
Seabuckthorn Nutraceuticals				
Flavone powder capsules	-	-	5,0	
Pulp oil capsules	0,3	2,0	6,0	2,0
Seed oil capsules	-	-	18,0	1,0
Fruit powder capsules	-	-	0,4	
Seabuckthorn syrup	64,0	-	-	

The categories “others” refers to Russia, the NIS countries, and the other European countries estimated on the given figures or experiences.

Table 3 shows significant differences in the marketing of final seabuckthorn products. In Western Europe and especially in Germany the production of cosmetic products is well developed, while in the other investigated countries an enormous market potential still exists. On the other hand the marketing of nutraceuticals is an important market segment, clearly shown by the turnover in China. The North American and Japanese markets especially offer opportunities for nutraceuticals producers. Table 3 generally shows the opportunities for manufacturers to develop different market segments in the investigated countries.

Table 4 . Share of final products' yearly turnover sorted out by products, value in thousand EURO.

	turnover per annum in thous. EUR
Seabuckthorn raw juice (100%)	100.451,20
Seabuckthorn nectar	3.290,20
Seabuckthorn wine	381,51
Seabuckthorn vinegar	273,00
Seabuckthorn blended juice	3.096,00
Seabuckthorn jam	2.852,58
Seabuckthorn jelly	116,03
Seabuckthorn sweeties, bars	320,00
Seabuckthorn tea	
leaves tea	788,16
black with dried berries	96,30
Seabuckthorn cosmetics	
cremes	41.659,00
lotions	21.535,00
others	32.762,50
Seabuckthorn Nutraceuticals	
Flavone powder capsules	3.390,00
Pulp oil capsules	4.061,26
Seed oil capsules	11.677,96
Fruit powder capsules	33,90
Seabuckthorn syrup	2.420,10
Summary	**229.204,69**

It is notable that the marketing of raw seabuckthorn juice represents approximately 43% of the whole turnover with app. **229 Mill EURO** in the estimated countries (Table 4). The reason for this is the marketing success of Chinese manufactures in Asia. They were able to reach a price of approximately 100 EUR/kg. The product is sold as a healthy drink in 30 ml bottles for daily use. It is expected that the market share for cosmetics will also strongly rise outside Germany and some Western European countries.

The 35% market share of the German manufacturers strongly depends on the cosmetic market. More than 80% of the German turnover comes from the cosmetic market, while approximately. 80% of the turnover of the Chinese manufacturers (market share 52%) is reached by selling raw juice for health drinks.

The price range for raw seabuckthorn juice could not be represented in Figure 8, because it ranges from 1,14 EUR/kg to 106 EUR/kg. This variation shows the possibility of different marketing strategies.

There are different food products summarized under one head line in Figure 8. For example we find in China nectars with 10 – 20 % juice content, whereas we usually find in Germany 25 % of juice content. Another example is the jam: in Estonia jam produced from the whole berries is found, while in Germany, jams do not contain any seed parts. It was impossible to differentiate all the products depending on the quality, so the prices have to interpreted with this knowledge.

The development of new products finds limitations because of existing standards and laws applied in different countries. However producers can still find a place for development and marketing.

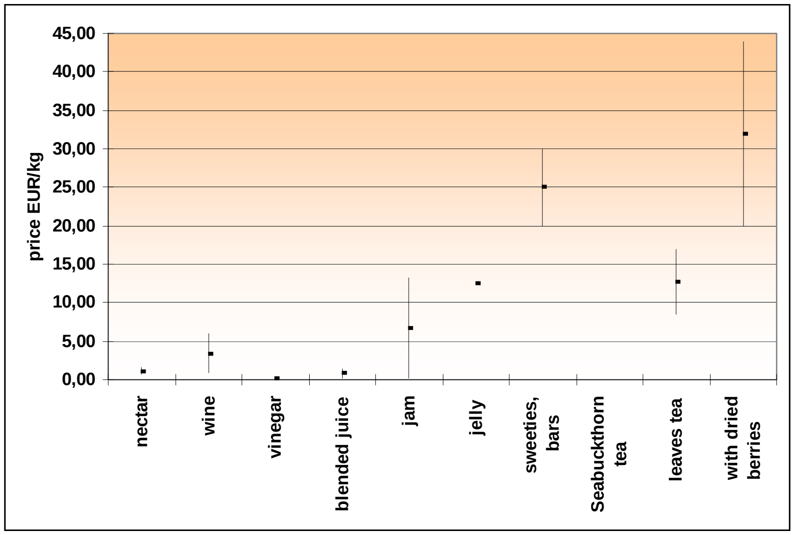

Figure 7. Price range for Sea Buckthorn final products.

Conclusions

The presented figures of the investigated seabuckthorn market segments illustrate the general situation of the seabuckthorn business in the different countries. It is known that some administrative problems and technical gaps exist in some states, which hinder successful marketing of seabuckthorn products.

The collection of berries from wild seabuckthorn resources could not be investigated. However, the amount of such berries plays a role in Romania, Baltic countries, and NIS-countries.

Romania, China and the Baltic countries invest in new seabuckthorn plantations, partly with new varieties. In China the new cultivations will be mostly used for erosion control, of course also with views for the use of the berries and leaves.

The differences found in the berries selling prices depend on

- different expected or demanded qualities of the processors, (certificates, standards...),
- costs for harvesting (different harvesting strategies and technologies) and storage,
- labour costs level.

In the segment of seabuckthorn half-products, interesting different marketing strategies could be found. While China and Germany market the seabuckthorn with the greatest variability of half-products, Russia concentrates on the pulp oil and the Baltic countries sell only a small range of half-products. The diversification of different seabuckthorn half-products opens the market for different types of final products. In Germany, the use of seabuckthorn oils in cosmetics is well developed. However, in China the cosmetic market for seabuckthorn products for people is not developed yet. The main interest in Russia is the separation of the seabuckthorn pulp oil, but a great opportunity exists for the manufacturers to develop the market for high quality non-oil products, because a big part of the berries is not being used at the present moment for the production of food products.

For the marketing of final products sufficient figures could be only retrieved from Germany, China, and some of the Baltic countries. It can be seen that only in Germany the market for seabuckthorn cosmetics is well developed, while in the other countries the manufacturers have a great potential for product development and marketing.

Despite a strong growth in some emerging markets, the growth in the worlds food consumption has been suppressed by static performances in the established markets like Germany and Russia, where impending population declines are leading to reduced demand. However much activity in market sub-sectors (health food, nutraceuticals, cosmeceuticals) is detected. To achieve success in this frame, it is necessary to displace a competitor or competitive product (Rice 2006).

The seabuckthorn industry has to become more active in helping its customers with a greater level of support in order to be more successful in the marketing of seabuckthorn products. The potential of seabuckthorn products on a high quality level is given and can be used if the manufacturers develop successful marketing strategies besides the current marketing as a natural ecological food product, like mainly in Germany.

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The Effect of Irrigation, Cultivars and Mulch Types on Nutrient Availability and Vegetative Growth of Seabuckthorn (*Hippophae rhamnoides* L.)

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Abstract

Soil nutrient availability, leaf nutrient status, and vegetative growth of six *Hippophae rhamnoides* L. cultivars ('Askola', 'Hergo', 'Golden Rain', 'Mary', 'Sunny' and 'Tatjana') were measured in 2004 and 2005, under combinations of two irrigation regimes and two mulch types, on Ile d'Orléans, Québec, Canada. Irrigation was triggered by tensiometers when soil moisture tension reached either 25 kPa or 60 kPa. Black plastic and chipped tree residues were compared as mulching materials. Experimental design was a split-strip-split-plot, with irrigation in main plot, cultivar in subplot, and mulch in sub-subplot. Factorial combinations were replicated three times in plots bearing ten plants each, for a total of 720 plants. Irrigation regime and cultivars had no significant effect on soil N, P, K, Mg and Ca. In 2004, plastic mulch significantly increased N mineralization, whereas wood mulch led to significant increase in soil K content. In 2004, foliar Mg levels were significantly greater on plastic than on wood mulch, whereas both foliar K and Ca were enhanced by wood mulch. In 2005, wood mulch led to a significantly greater foliar K level only. In 2004, all cultivars' increment in trunk diameter was greatest under a combination of wood mulch and soil moisture tension of 25 kPa, whereas in 2005 this irrigation regime improved this parameter only for 'Hergo' and 'Sunny cultivars. In 2004, height growth of 'Hergo', 'Sunny' and 'Tatjana' cultivars, was more important on plastic than on wood mulch, whereas 'Golden Rain' grew best on wood mulch. In 2005, all cultivars grew better on wood mulch.

Keywords: seabuckthorn, *Hippophae rhamnoides*, irrigation, mulch, soil nutrient availability, leaf nutrient status, tensiometer.

Introduction

Seabuckthorn, *Hippophae rhamnoides* L., a plant introduced to Canada and first used a wind-break or ornamental, is now grown commercially in orchards. In Quebec the first such orchards were set up in the late 1990s and are mainly home to seedlings of cv. 'Indian Summer,' developed in western Canada.

With few seabuckthorn orchards currently operating in North America, an expansion of its production in Quebec requires the selection of cultivars adapted to conditions prevailing in Quebec. In addition, further knowledge must be developed with respect to managing the crop. As a result, this industry's sustainability and market competitiveness requires, in a first phase, the carrying out of cultivar trials. Such trials provide an excellent means of developing our expertise in the management of the seabuckthorn crop and can lead to a greater understanding of conditions favourable to its growth.

When planted, the seabuckthorn is subject to strong competition from surrounding vegetation (Singh, 2003), such that weeds constitute the main factor limiting its growth and survival (Li, 1999). However, as this shrub is shallow-rooted (Levandovsky, 2003), mechanical weed control can be damaging. Consequently, it is recommended that the soil not be disturbed any deeper than 8 cm (Gonchar and Sadan, 1986, cited in Singh, 2003). As no herbicide is registered in Canada for use in seabuckthorn orchards, one is expected to use mulch for weed control, a practice which has been well documented (Carter and Johnson, 1988; Skroch *et al*., 1992; Hembry and Davies, 1994).

Water availability is another important factor in establishing an orchard. Under our climatic conditions total annual precipitation is generally sufficient to meet a seabuckthorn crop's needs; however, its temporal distribution remains variable and unpredictable. Even if the plant's small size limits transpirational water losses, depending on climatic conditions, evaporative losses from the soil surface may become significant. As the soil volume tapped by the roots is rather limited in the first years after planting, the plant quickly suffers from drying of the topsoil. A number of cultural practices, such as mulching the soil surface (Larsson et Bath, 1996), favour an effective maintenance of soil moisture and limit water losses. As long periods of drought can endanger young seedlings, it may become necessary, when water conservation practices and rainfall fail, to provide additional water through irrigation

This research project's primary aim was to determine, in support of stakeholders in the development of the production of this crop in Quebec, the factorially combined effects of two irrigation regimes, six cultivars and two types of mulch on soil nutrient availability, mineral nutrition and vegetative growth of young seabuckthorn shrubs.

Materials and methods

Plant material

The current trials involved six female seabuckthorn cultivars: two German and four Latvian. These were chosen on the basis of their high fruit yield, potential to adapt to winter conditions in Quebec, and commercial availability.

The German female cultivars 'Askola' and 'Hergo' and pollinators 'Pollmix 1', '3' et '4' were developed at the Friesdorf Floriculture and Orchard Management Experiment Station, near Bonn, Germany. Two year old seedlings, purchased for the study in the spring of 2003, were propagated by cuttings by the Späth'sche Baumschulen Co. (Berlin, Germany). At planting, seedlings of cultivars 'Hergo' et 'Askola' were 30 and 50 cm tall, respectively, excluding their root systems.

Originally developed by Dr. T. Trifimov at Moscow State University by crossing subspecies *rhamnoides*, *mongolica* and *fluvialis*, cultivars 'Sunny', 'Golden Rain', 'Mary', 'Tatjana' and the pollinitor 'Lord' were selected by Andrejs Bruvelis and Karlis Blums after a series of backcrosses with subspecies *rhamnoides* (Bruvelis, 2003). One to five year old seedlings of these five cultivars were obtained in Newfoundland, Canada, where they had been propagated by cuttings. At planting, the seedlings were between 10 and 15 cm tall, excluding their root systems.

Irrigation regime

The two irrigation regimes were managed on the basis of soil moisture tension, as monitored by an array of Irrometer© tensiometers. A total of 24 tensiometers (2 × 2 × 3 × 2 - irrigation regime × mulch × block × no. per plot) were installed in orchard plots housing the Latvian cultivar 'Sunny.' Tensiometers were grouped in pairs, so as to obtain adjacent measurements at depths of 15 cm and 30 cm.

Under the first irrigation regime, good water availability was maintained throughout, with irrigation being triggered when root zone soil moisture tension (15 cm depth) exceeded 25 kPa (*in situ* field capacity (θ_{fc}) + 15 kPa). Under the second irrigation regime, more limited water availability conditions prevailed, with irrigation only being triggered when soil moisture tension exceeded 60 kPa. When readings from four of six tensiometers equaled or exceeded the threshold soil moisture tension, irrigation was applied for a period of 90 minutes, allowing the soil moisture tension to return to near zero (~0 kPa). Irrigation water was applied through a drip irrigation system installed along the base of the stems. This system was installed beneath the mulch prior to the seabuckthorn's planting. Spaced 20.3 cm apart, the emitters could provide irrigation at a rate of 1L hr^{-1} at an operating pressure of 103 kPa.

Mulches

Two types of mulch were investigated: a forestry-grade black plastic mulch, and a 0,15 m thick wood chip mulch. Both mulches extended 0,45 m to either side of the base of the plants. The wood chip mulch originated from branches and twigs of several deciduous tree species (*Acer* spp., *Sorbus* spp. and *Fraxinus* spp.)

Experimental set up and monitoring

The experimental orchard was set up near Saint-Laurent, Ile d'Orléans, Quebec (46º 52' N lat., 70º 54' W long.), in plant hardiness zone 4b. Mean annual precipitation is 1100 mm, with that falling between November and April being largely in the form of snow. May to September mean monthly precipitations are 118, 108, 129, 114 and 127 mm, respectively. The Monthly mean of daily maximum and minimum air temperatures are shown in Figure 1 (30-years, 1971-2000).

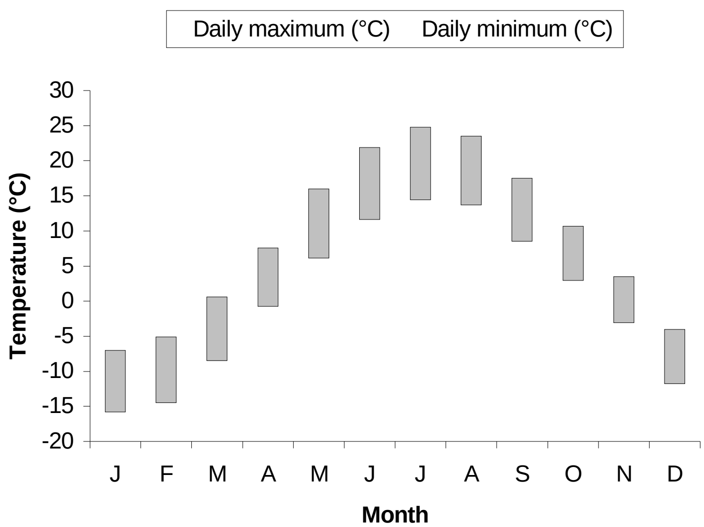

Figure 1. Monthly mean of daily maximum and minimum air temperatures.

Planting density was 2 184 plants ha^{-1} and row orientation north-south. Throughout the period of study, vegetative cover was maintained between the mulched rows and this was mown on a regular basis during the growing season. Both mulches provided adequate weed suppression throughout the period of study. No fertilization was applied. No pesticide applications were made, as, in Quebec, no such products are registered for use on this crop.

In order to raise its soil pH to near neutrality, in October 2002 and 2003 the site's sandy clay loam was amended with 2.2 Mg ha^{-1} and 6.7 Mg ha^{-1} of lime, respectively. Preliminary soil sampling was done in May 2004 to establish initial field fertility levels (Table 1). Nutrient analysis showed the soil to be low in P, moderately rich in K and Mg, and adequate in Ca content (CRAAQ, 2003).

Table 1. Content of major soil nutrients (mg/kg) at the Saint-Laurent, Quebec orchard, prior to seabuckthorn planting, spring 2004.

NH_4^+	NO_3^-	P (mg/kg)	K	Mg	Ca
1.26	8.69	53	209	133	4223

No pruning was done at planting. In the spring of 2004, prior to bud-break, all female plants were pruned for shape, so as to favor a dominant apex. In the spring of 2005 a light pruning was applied to eliminate smaller branches at the base of the trunk. For plants of the Latvian cultivars, which were younger than those of the German cultivars, an apex was chosen and two lateral branches were maintained. For the German cultivars, a dominant apex was selected. The plants showed no winter freeze damage, even though the German cultivars aouting was rather late.

Three factors were investigated in this study. Main plots were split into two sub-plots, according to their irrigation regime, and further into four sub-sub-plots according to the type of mulch applied. The randomization of the six cultivars was constrained by the fact that these were planted in single rows across the breadth of the main plots. Such a design was justified, given the orchard's commercial avocation. Indeed, since the fruit of different cultivars ripen at different times, harvesting is much simplified if there is only one cultivar per row. The randomization of mulch types in the sub-subplots suffered from no such constraints. The experimental design was thus a *split-strip-split-plot* with repeated measures, the strip representing the cultivar rows within the main plots. The layout included 72 experimental units, each housing 10 seabuckthorn, for a total of 720 plants. To these were added one guard row each between blocks A and B and between blocks B and C. These guard rows were made up of 160 pollinator plants, including cultivars ‘Pollmix 1’, ‘3’ and ‘4’ as well as ‘Lord’ (Figure 2).

Block 1

Main plot

Sub-plot (strip)

I1 C2 P1	I1 C2 P2	I2 C2 P1	I2 C2 P2
I1 C3 P1	I1 C3 P2	I2 C3 P2	I2 C3 P1
I1 C4 P1	I1 C4 P2	I2 C4 P1	I2 C4 P2
I1 C1 P1	I1 C1 P2	I2 C1 P1	I2 C1 P2
I1 C5 P2	I1 C5 P1	I2 C5 P1	I2 C5 P2
I1 C6 P1	I1 C6 P2	I2 C6 P2	I2 C6 P1

Sub-sub-plot

Figure 2. Experimental Design (*Split-strip-split-plot*).
Main plots: two irrigation regimes, one triggered at 25 kPa of soil-moisture tension (I1) and one triggered at 60 kPa (I2). Sub-plots: six cultivars, namely ‘Askola’ (C1) ‘Golden Rain’ (C2), ‘Hergo’ (C3), ‘Mary’ (C4), ‘Sunny’ (C5) and ‘Tatjana’ (C6). Sub-sub-plots: plastic mulch (P1) or wood chips mulch (P2).

Plant growth and nutrition variables measured

In both years of the experiment, each shrub’s height, defined as the tallest point on the stem, and stem diameter 10 cm above the soil surface were measured in the spring, prior to budbreak and in the fall after growth cessation. The increment in diameter or height was calculated as the difference between the spring and fall measurements. In both years, levels of soil minerals ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$, P, K, Ca and Mg) were measured in composite samples drawn, at two week intervals, from the top 15 cm of soil of each of the 72 experimental units. Six such samplings were completed in 2004 and four in 2005.

Similarly, leaf mineral (N, P, K, Ca and Mg) levels were measured in composite samples drawn from all 72 experimental units. Two foliar samplings were completed in each year: July 19 and September 4 in 2004, and July 18 and August 29 in 2005. These dates correspond, respectively; to a period of active plant growth, and to one when growth has ended, but nutrients have yet to be translocated to the roots.

Statistical analyses employed the PROC MIXED procedure in SAS (Littell *et al*., 1996).

Results and discussion

Annual differences in weather conditions over the study's 2-year period resulted in the proportion of soil water contributed by precipitation differing significant from year to year. In 2004, water inputs from rainfall were regular and sufficient to maintain soil moisture levels above irrigation thresholds until July 27, when the season's first 25 kPa threshold-triggered irrigation occurred. The 60 kPa threshold was never reached in 2004, so this treatment received no irrigation over the entire season. Early in 2005, and all through the season, irrigation was necessary to maintain soil moisture levels above irrigation thresholds. For the period of June through August 2005, 60 days with no or less than 2 mm of rain were recorded.

Soil nutrients

In the 2004 season, NO_3^--N and K levels differed significantly between the two mulch types, but those of P, Mg and Ca did not. At all 6 sampling dates in 2004, significantly greater ($P \leq 0.001$) soil NO_3^--N levels were found under black plastic mulch than under wood chips mulch (Figure 3).

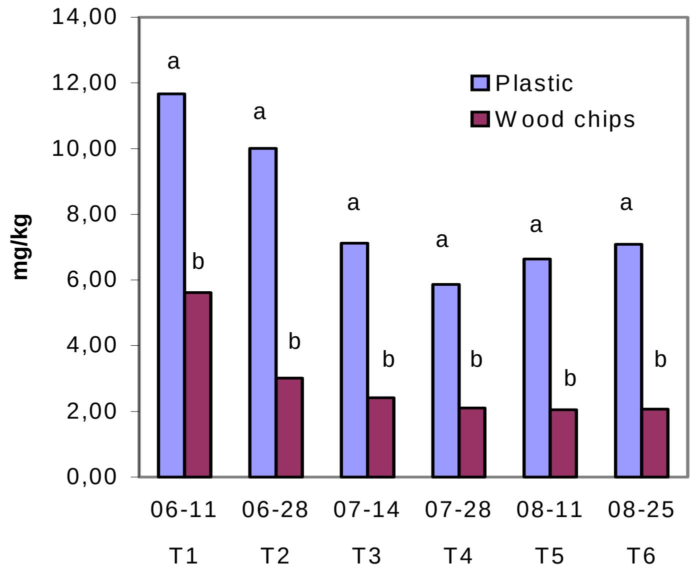

Figure 3. Seabuckthorn orchard topsoil (0-15 cm) NO_3^--N levels (mg kg^{-1}) as a function of the interaction between mulch type and sampling date (P × T), for the 2004 season.

The lower soil NO_3^--N levels observed under the wood chips (*vs*. plastic) mulch suggest less effective nitrification, as reported by Halvorson (2002). Such a drop in the nitrification rate could explain the significantly higher levels of soil NH_4^+-N measured under wood chips (*vs*. plastic) mulch (Figure 4) in late June (T2; $P \leq 0.001$), mid-July (T3; $P \leq 0.006$), mid-August (T5; $P \leq 0.005$) and late August (T6; $P \leq 0.002$).

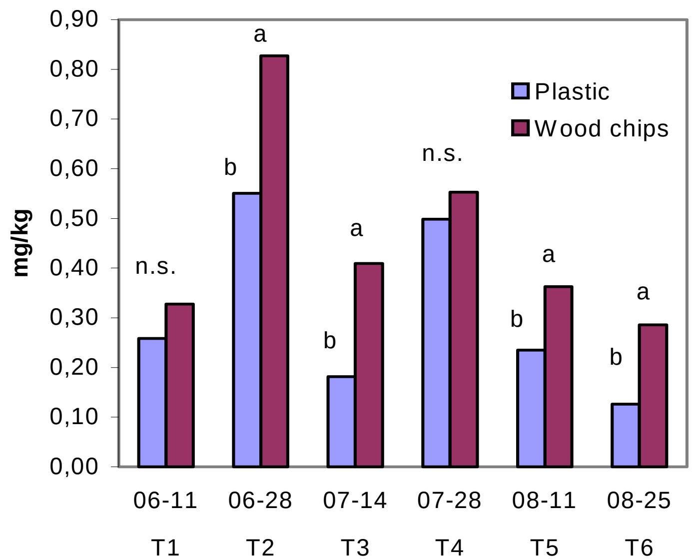

Figure 4. Seabuckthorn orchard topsoil (0-15 cm) NH_4^+-N levels (mg kg^{-1}) as a function of the interaction between mulch type and sampling date (P × T), for the 2004 season.

For all 2004 sampling dates (T1—T6), soil K levels were significantly greater ($P \leq 0.001$) under wood chips (*vs*. plastic) mulch (figure 4). The rise in soil K over the season can be attributed to the leaching of this element from the wood chips (Fraedrich and Ham, 1982; Merwin *et al.*, 1995).

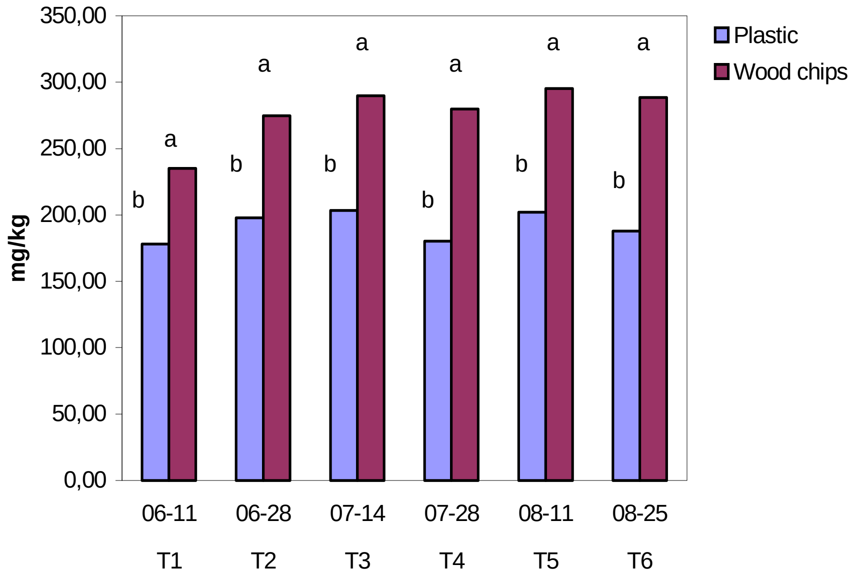

Figure 5. Seabuckthorn orchard topsoil (0-15 cm) K levels (mg kg^{-1}) as a function of the interaction between mulch type and sampling date (P × T), for the 2004 season.

Mineral nutrition

Mulch type also clearly influenced leaf mineral uptake; indeed, in 2004, leaves from wood chips mulched seabuckthorn bore more K than their plastic mulched counterparts (Figure 6), confirming the results of Merwin *et al*. (1995). Leaf K and Ca levels were significantly ($P \leq 0.001$) enhanced by wood chips (*vs.* plastic) mulch, while the converse was the case for leaf Mg ($P \leq 0.04$).

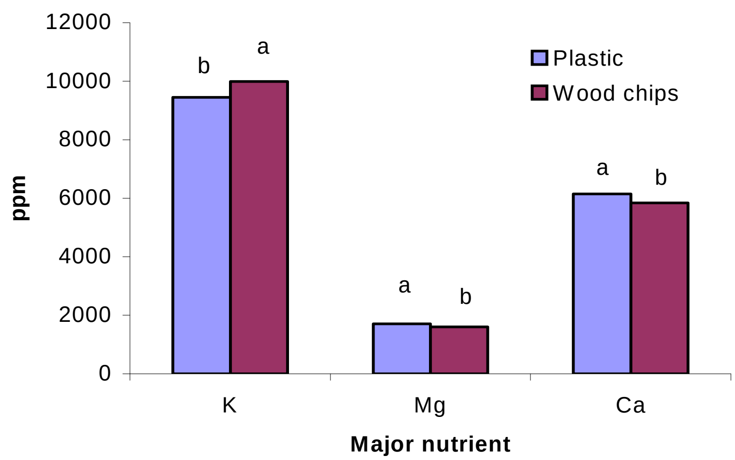

Figure 6. K, Mg, and Ca levels (ppm dry weight basis) in seabuckthorn leaves as a function of mulch type for the 2004 season.

In 2005, notwithstanding mulch type not significantly influencing topsoil K levels, K levels in the leaves of seabuckthorn plants grown on wood chips mulch were once again greater than those grown on plastic mulch (Figure 7). In this regard, soil moisture tension measurements showed a quicker drying out of soil under plastic (*vs.* wood chips) mulch. Given that transport of K through the soil occurs by mass flow (Marshner, 1986), greater soil moisture content would tend to enhance plant K uptake.

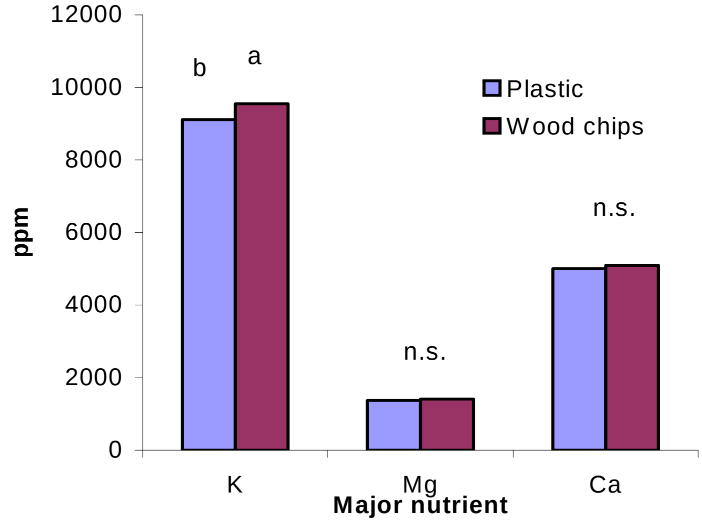

Figure 7. K, Mg, and Ca levels (ppm dry weight basis) in seabuckthorn leaves as a function of mulch type for the 2005 season.

Stem diameter

Significantly greater stem diameter increments were measured under the 25 kPa (*vs.* 60 kPa) soil moisture tension irrigation threshold, but mulch type only showed a significant effect within the 25 kPa irrigation regime, where diameter increased more with wood chips than plastic mulch ($P \leq 0.001$) (Figure 8). By allowing better nutrient uptake (Marschner, 1986; Patterson *et al.*, 1990; Bignami *et al*., 2000), the elevated moisture conditions under the wood chips (*vs.* plastic) mulch may have enhanced seabuckthorn plant stems' radial growth.

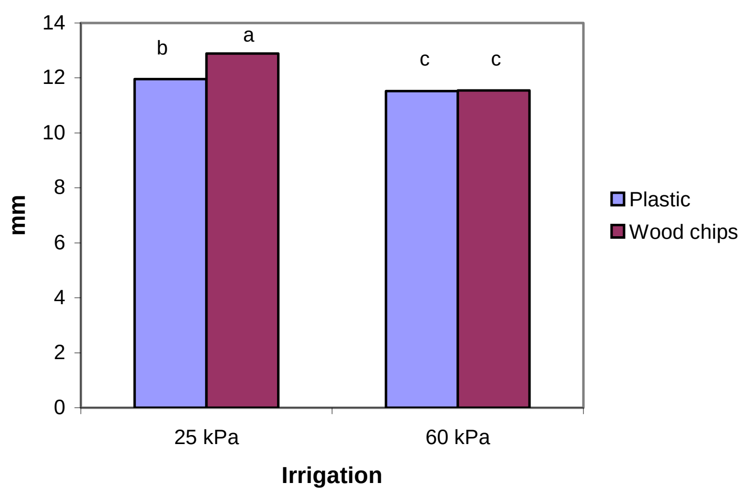

Figure 8. Increase in seabuckthorn stem diameter (mm) as a function of interacting irrigation × mulching treatments (I × P), for 2004 season.

In 2005, cultivars were differentially affected by the irrigation regime; indeed, the increment in stem diameter of cultivars 'Hergo' and 'Sunny' was significantly greater under the 25 kPa (*vs.* 60 kPa) soil moisture tension threshold irrigation regime ($P \leq 0.04$, $P \leq 0.02$, respectively), whereas the other cultivars showed no such difference (Figure 9).

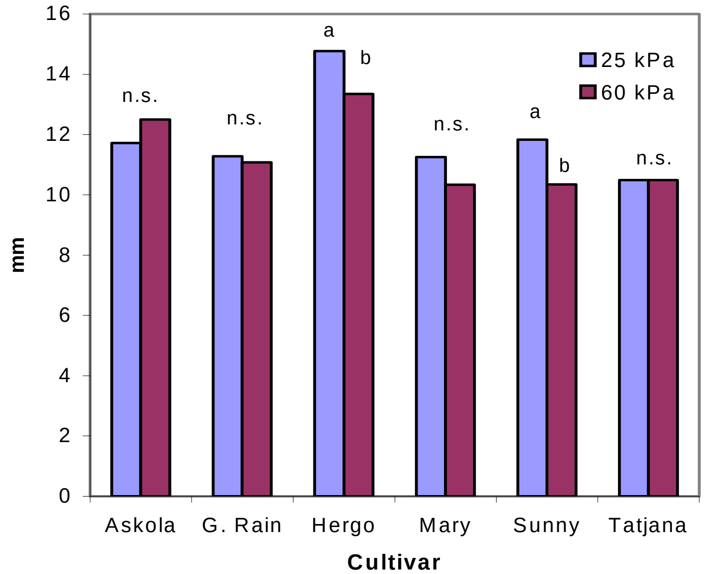

Figure 9. Increase in seabuckthorn stem diameter (mm) 10 cm from the soil surface as a function of interacting irrigation × cultivar treatments (I × C), for the 2005 season.

Plant height

In 2004, the increment in growth in height of seabuckthorn plants of cultivars 'Hergo', 'Sunny' and 'Tatjana' was significantly greater ($P \leq 0.03$, $P \leq 0.002$, and $P \leq 0.02$, respectively) on plastic (*vs.* wood chips) mulch. These growth differences were of 6.9 cm, 8.9 cm and 6.2 cm, respectively. The growth of seabuckthorn, a symbiotic nitrogen-fixing plant, may have been enhanced by the warmer soil temperatures under the plastic mulch (Gupta and Singh, 2003). However, cultivar 'Golden Rain' grew better on the wood chips (*vs.* plastic) mulch ($P \leq 0.03$) (Figure 10).

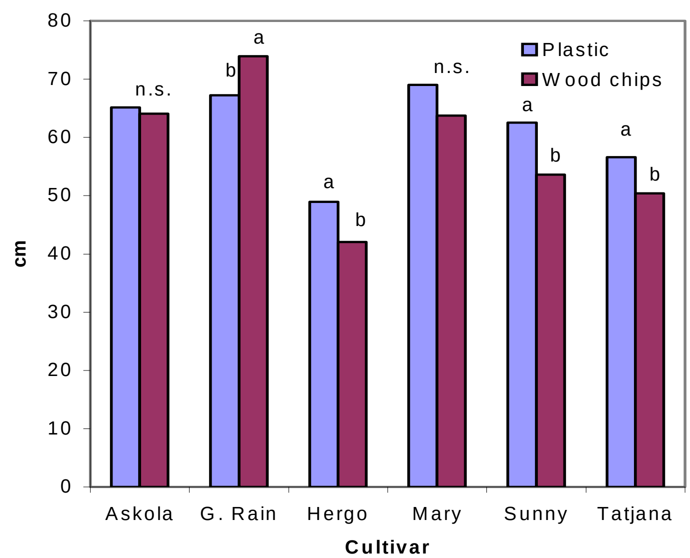

Figure 10. Increase in seabuckthorn plant height (cm) as a function of interacting cultivar × mulch treatments (I × C), for 2004 season.

In the drier 2005, the mean increments in height across cultivars were 57.5 and 61.9 cm on plastic and wood chips mulches, respectively (Table 2). The improved soil moisture conditions under the wood chips mulch, and the resulting enhancement in plant nutrient uptake, may account for the greater increment in height with this type of mulch. However, it is difficult to attribute these results to a single specific factor. Results obtained in other studies vary and the explanations postulated by their authors remain similarly unspecific. Larsson *et al*. (1997) showed lesser currant bush growth with wood chips (*vs*. plastic) mulch, whereas Merwin *et al* (1995) and Foshee *et al*. (1999) showed the contrary.

Table 2. Increment in height (cm) of seabuckthorn plants mulched with wood chips *vs.* black plastic, for the 2004 and 2005 growing seasons.

Mulch (P)	2004	2005
Plastic	61.6	57.5
Wood chips	58.0	61.9
F value	**	***

, * differences between mulches in a given year, significant at 0.01 or 0.001, respectively

Conclusions

The use of irrigation to supplement natural rainfall and of mulch to control weeds is methods effective in the successful establishment of seabuckthorn orchards. While such interventions clearly modify the plants' immediate environment, plant-soil interactions are complex, and the present study has only addressed a limited number of the issues at hand, namely the impact of two irrigation regimes and two types of mulching material on the availability of soil nutrients, their uptake by seabuckthorn plants, and the latter's vegetative growth.

The two irrigation regimes differentially affected increments in stem diameter and height of young seabuckthorn plants, whereas the different mulch types differentially affected mineral nutrition and growth of these plants. However, both the seabuckthorn cultivar and the meteorological conditions under which it grew influenced treatment effects.

Acknowledgements

This project was undertaken in collaboration with la Ferme Maurice et Philippe Vaillancourt. The authors thank Drs Adrien N'Dayegamiye and Mohammed Lamhamedi, as well as Mrs. Marie-Pierre Lamy and Mr. Marcel Giroux for their collaboration. Mrs. Michèle Grenier is thanked for her help in the statistical analysis of the results. The project was funded by the Institut de recherche et de développement en agroenvironnement (IRDA), Economic Development Canada (EDC), and l'Association des producteurs d'argousiers du Québec (APAQ).

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Design of a Mechanical Harvester for Sea Buckthorn Berries

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Abstract

Recent interest in commercial production of seabuckthorn in the Canadian Prairies requires an economical harvesting solution. A hydraulically-controlled shrub shaker has been designed and tested for removal of these berries. Harvesting trials were conducted over four consecutive harvesting seasons beginning in 2000 using the 'Indian Summer' variety of seabuckthorn. For each harvest season, changes were made to the prototype harvester to address issues such as berry removal efficiency, minimization of shrub damage, berry collection efficiency, and maneuverability. With the Mark I prototype, vibration parameters of 25 mm amplitude and 26 Hz frequency were found to optimize the ratio of berries to debris removed. With the Mark II prototype, special attention was paid to the damage inflicted by the harvester to the shrubs. Shaking at 25 Hz and 25 mm caused less damage to the shrubs than shaking at 20 Hz and 36 mm. The goal of the Mark III prototype was to increase maneuverability of the harvester and to add the capability to collect the berries as they fell from the shrub. A rigid platform was connected to the front of a John Deere industrial compact excavator. Collection efficiency was less than 70 % and many of these berries were damaged by impact with the rigid platform. Maneuverability was poor because the harvester had to approach each individual shrub perpendicular to the row of shrubs. The Mark IV prototype consisted of a flexible collector mounted to the side of the harvester that enabled the harvester to drive parallel to the row of shrubs. Collector efficiency was 56 % because the shrubs had been allowed to sucker and the collector could not be extended past the centerline of the row.

Keywords*:* Fruit, harvesting, shrub, shaker, *Hippophae rhamnoides* L.

Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a multi-branched thorny shrub that reaches 2-4 m in height at maturity (Li and Schroeder, 1996). The fruit, produced in late August, is a yellow-orange berry that remains on the shrub well into the winter. It is difficult to harvest sea buckthorn berries because they are soft at the time of picking and are tightly clustered on two- or three-year old branches (Li and Schroeder, 1996; Blahovec *et al*., 1995). Recent interest in the establishment of a sea buckthorn industry in Canada has identified the need for mechanical harvesting equipment for sea buckthorn berries.

Mechanical fruit harvesters can be classified as either direct harvesters or indirect harvesters (Olander 1995). A direct harvester relies on direct contact with the fruit, while an indirect harvester causes the fruit to be removed without physically touching it. Indirect harvesters have typically been based on shaking, but some other principles have been used like vacuum suction units (Varlamov and Gabuniya, 1990), quick freezing units (Wegert and Wolf 1990; Gaetke and Triquart 1993), and "whole branch harvesters" (Gaetke and Triquart, 1992; Olander, 1995). Quick freezing units were used to freeze the berries so that they would be easier to remove by shaking (Wolf and Wegert, 1993). Following this treatment, the berries were removed by beating the branches. "Whole branch harvesters" have been developed based on the hypothesis that it is possible to expose every part of the branch to the same vibration if only a small portion of the branch is being shaken (Olander, 1995). Thus, "whole branch harvesters" rely on the removal of an individual branch to subject it to a consistent shaking motion. The most obvious disadvantage of

"whole branch harvesters" is that a fruit crop can only be harvested on a biennial basis. There is incentive, therefore, to develop a harvester that will permit annual fruit harvest. Although a trunk shaker would allow the entire bush to be harvested at one time, trunk shakers are only effective for bushes that have one central trunk with short branches. Bushes with long, slender branches are more difficult to harvest by shaking the trunk because much of the energy is lost before it reaches the berries (Olander, 1995). Bantle *et al*. (1996) showed that a foliage shaker is appropriate for some fruit crops native to Saskatchewan (i.e., saskatoon, chokecherry, and pin cherry) using vibration frequencies above 10.8 Hz and vibration amplitudes above 19 mm. The sea buckthorn bush has somewhat similar structure to saskatoon, chokecherry, and pin cherry.

Experiments with harvesting of sea buckthorn by shaking have been conducted at several places. Stan *et al*. (1985) used a black currant harvester to test seven cultivars of seabuckthorn. Only one cultivar could be successfully harvested with a vibration of 18.5 Hz and an amplitude of 25 mm. Gaetke *et al*. (1991) tested the seabuckthorn cultivar Hergo at three different frequencies (20, 25, and 30 Hz) and three different amplitudes (13, 18, and 25 mm). At amplitude of 25 mm, a frequency of 25 Hz was required for adequate removal. A Swedish prototype was tested with amplitudes of 40 and 55 mm at frequencies up to 25 Hz (Olander, 1995). For some cultivars, the harvesting results were acceptable.

Mann *et al*. (2001) used a branch shaker to determine the best combination of shaking frequency and shaking amplitude to remove seabuckthorn berries. Experiments were conducted at four harvest dates (September 28, 1999; October 13-14, 1999; November 2-3, 1999; and January 13-14, 2000). They found frequencies < 14 Hz were ineffective at removing > 50% of the berries, regardless of amplitude. At frequencies of both 20 and 25 Hz, the percentage of berries removed by shaking increased linearly with increasing amplitude. The combination of 25 Hz and 32 mm produced the best result during the November harvest date when 98% of the berries were removed within 15 s of shaking. During the January test, the branches were brittle and many broke when shaken at 25 Hz.

Attempts at harvesting seabuckthorn berries have typically experienced problems of fruit and bark damage and low efficiency. Therefore, these factors must be considered in the design of a sea buckthorn berry harvester. It should be noted that we did not attempt to assess root damage caused by shaking. Although Abdel-Fattah *et al*. (2003) speculated that vertical displacement might be associated with root damage in almond trees, Li *et al*. (2005) concluded that damage to the roots of orange trees was only an issue when soil water was low. Any drought-stress symptoms quickly disappeared when soil water was replenished. For this research, it has been assumed that root damage to sea buckthorn shrubs is not a concern.

This paper describes the design and evaluation of a mechanical harvester designed to remove sea buckthorn berries by shaking. The harvester was developed over four years. During the first year (Mark I prototype), the primary objective was to confirm the optimum combination of amplitude and frequency for removal of the berries. Based on observed, but not rigorously documented, damage to the shrubs, modifications were made to the clamping device (Mark II prototype). During the second year of evaluation, the primary focus was to assess the nature and impact of the damage to the shrubs. For the third year, maneuverability of the harvester was improved by mounting the shaking arm onto a tractor (Mark III prototype). A berry collector was also added to the harvester. For the final year, the collector was re-designed to minimize berry damage (Mark IV prototype).

MARK I PROTOTYPE

Features of the Mark I Prototype

A full-size shrub shaker was designed and fabricated for field-testing in the autumn of 2000. The shaker consisted of a hydraulically-operated arm mounted on a trailer (Fig. 1 and 2) with a clamping device used to attach the shaker to the shrub (Fig. 3). Oscillatory motion was created by a cam mechanism. The entire harvester was powered by a hydraulic motor, and was controlled from an operator station, both of which were situated on the trailer.

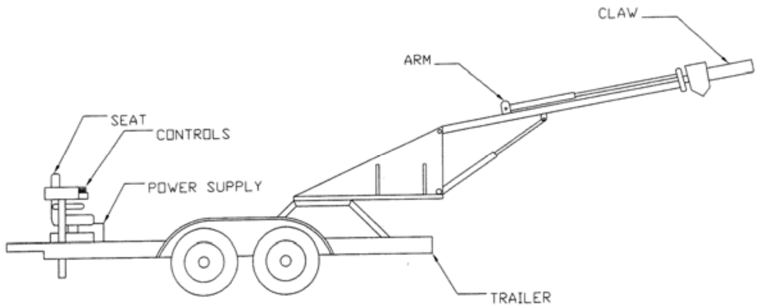

Figure 1. Schematic of the Mark I prototype. A claw for grasping a shrub was located at the end of a rotatable arm. Movement of the arm and claw was controlled from an operator seat near the front of the flat-deck trailer.

Figure 2. The Mark I prototype, with shaking arm extended, sitting in a seabuckthorn orchard.

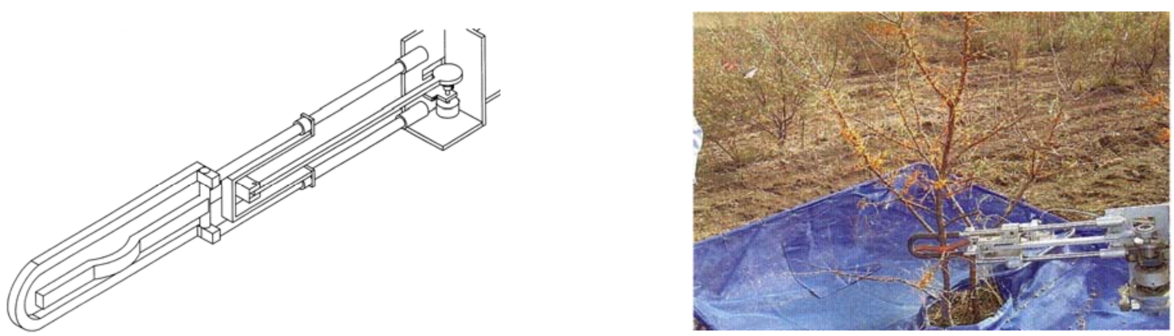

Figure 3. Schematic of the clamping mechanism used on the Mark I prototype (left). View of the clamping mechanism attached to a seabuckthorn shrub (right).

Evaluation of the Mark I Prototype

Five years old, unpruned seabuckthorn shrubs of the cultivar 'Indian Summer' located at the Prairie Farm Rehabilitation Administration (PFRA) Shelterbelt Center, Indian Head, SK were selected for testing. The effect of shaking amplitude (19, 25, and 32 mm) and shaking frequency (8, 13, 18, 23, and 26 Hz) on mass of berries and debris removed was studied. Each trial was replicated three times. Testing occurred on October 18 and 19, 2000. The conclusion from testing with the Mark I prototype is that sea buckthorn shrubs should be shaken at 23 Hz using an amplitude of 25 mm. Based solely on visual observation, the Mark I prototype caused extensive damage at the point where the clamp was attached to the shrub. This damage to the tree bark could provide an entry point for pests or disease, and must be avoided to sustain a healthy orchard.

MARK II PROTOTYPE

Features of the Mark II Prototype

As mentioned above, the clamp of the Mark I prototype caused excessive damage to the bark of the shrub. To minimize damage caused by the clamping mechanism, the angle at which the shaking arm extended from the trailer was reduced so that the clamping device intercepted the shrub at a right angle. The clamping device was improved by increasing the contact area and coating it with a rubber material.

The new clamping device consisted of a steel tube machined to a 50.8-mm inside diameter, and cut in half along its length (Fig. 4). Aluminum bushings were fit into the steel shells. Sets of these bushings were bored out to 19, 25.4, and 31.7 mm. They were cut in half along their length similar to the steel tubing and were lined with rubber to grip the shrub (Fig. 5). The outside diameters of these bushings were machined so that they could rotate and slide inside the steel tube to relieve stress on the shrub bark. The bushings were easily removed to allow quick changeover for shrubs with trunks of different sizes.

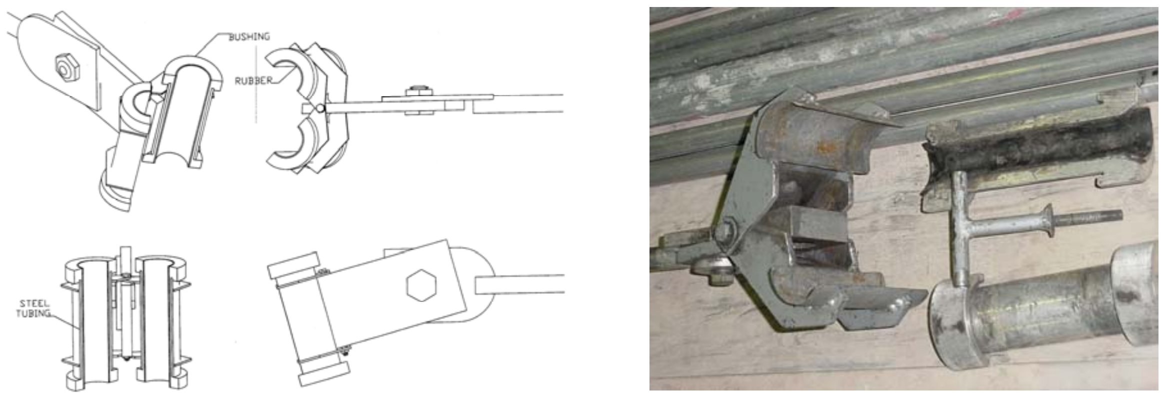

Figure 4: Schematic of the clamping mechanism used on the Mark II prototype (left); photograph the actual mechanism (right). The mechanism was lined with rubber to minimize bark damage on the shrubs.

Evaluation of the Mark II Prototype

Trials were conducted in the fall of 2001.The 5-year-old orchard shrubs selected for testing were on average 2 m tall, unpruned, and of the cultivar 'Indian Summer' located at the PFRA Shelterbelt Center, Indian Head, SK. Two combinations of amplitude and frequency were selected for testing: 25 mm amplitude at 25 Hz and 36 mm amplitude at 20 Hz. Between three and ten replicates were completed for each treatment.

For each trial, a shrub with a main branch or trunk that had at least 20 cm of trunk with no branches was selected to attach the claw. Collection baskets, designed to fit around the base of the shrub, were used to collect the fruit and debris removed by shaking. The claw was positioned and fastened manually, as close to the bottom of the shrub as possible.

The actuator was started and run for 5 s. After this 5-s trial was completed, the berries and debris from the hoppers were collected in bags. The actuator was then run for 15 and 60 s, respectively. Again, all berries and debris were collected and bagged. The samples were manually sorted into two classifications; *harvest* and *debris*. The *harvest* consisted of the berries and *debris* consisted of bark, stems, and branches. The harvest and debris samples were weighed separately.

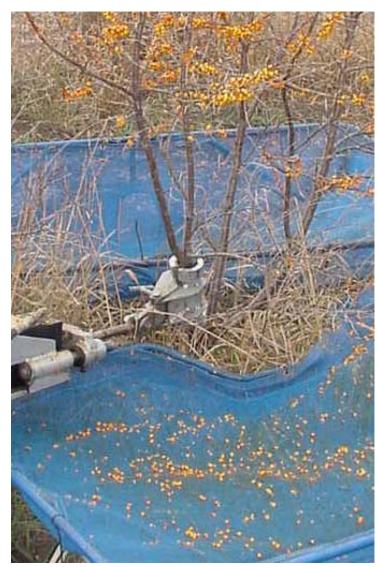

Figure 5. Clamping mechanism used with the Mark II prototype attached to a sea buckthorn shrub.

A "damage key" was proposed by researchers of the PFRA Shelterbelt Center, Indian Head, SK (Table 1). Two damage assessments were completed; one following the harvesting trials in the fall of 2001 and one during the spring of 2002. The initial assessment was used to establish the damage inflicted by the harvester. The second assessment was used to determine whether the shrubs were able to recover from the damage inflicted by the harvester.

Table 1. Key used to assess the damage inflicted upon the sea buckthorn shrubs by the berry harvester prototype.

Descriptor	Key	Level	Meaning
Branch damage	Light	1	0-24% of branches damaged
	Medium	2	25-74% of branches damaged
	Heavy	3	75-100% branches damaged
Bark damage	Light	1	top layer of bark rubbed off
	Medium	2	outer layer removed
	Heavy	3	complete disruption of vascular tissue
Leaf bud damaged	Light	1	0-24% of leaf buds damaged
	Medium	2	25-74% of leaf buds damaged
	Heavy	3	75-100% leaf buds damaged
Overall condition of shrub	Good	1	shrub visibly vigorous, no appreciable change in condition compared to non-tested shrub
	Fair	2	shrub noticeably less vigorous than non-tested shrub but no serious damage visible
	Poor	3	shrub significantly less vigorous than non-tested shrub; serious damage present

The combination of 25 Hz and 25 mm caused the least damage to shrubs (as assessed immediately following the harvesting trials). For this combination, the overall condition of shrubs was good for all tested shrubs. When the damage assessment was completed in the following spring, it was observed that shrubs shaken with the combination of 25 Hz and 25 mm made a better recovery.

MARK III PROTOTYPE

Features of the Mark III Prototype

The trailer-mounted harvester unit was replaced by a tractor-mounted unit to improve maneuverability of the harvester (Fig. 6). A John Deere industrial compact excavator (Fig. 7) was selected for its high hydraulic flow rate of 33.6 L per minute compared to its power rating of 9.2 kW at 2300 revolutions per minute. A hydraulically controlled A-frame or scissor style arm was designed and manufactured. A stock hydraulic cylinder having a 25-mm stroke was used to provide the shaking action. A solenoid valve and PLC controller that allowed the operator to select different shaking frequencies activated the cylinder.

The berry collector was simply a rigid platform (steel frame in-filled with plywood) attached to the blade mounts of the tractor unit (Fig. 8). A slot in the platform allowed the collector to be maneuvered into place surrounding a shrub. The platform covered an area of 4 m^2. With the collector attached to the front of the tractor unit, it was necessary to approach the row of shrubs with the tractor unit perpendicular to the row.

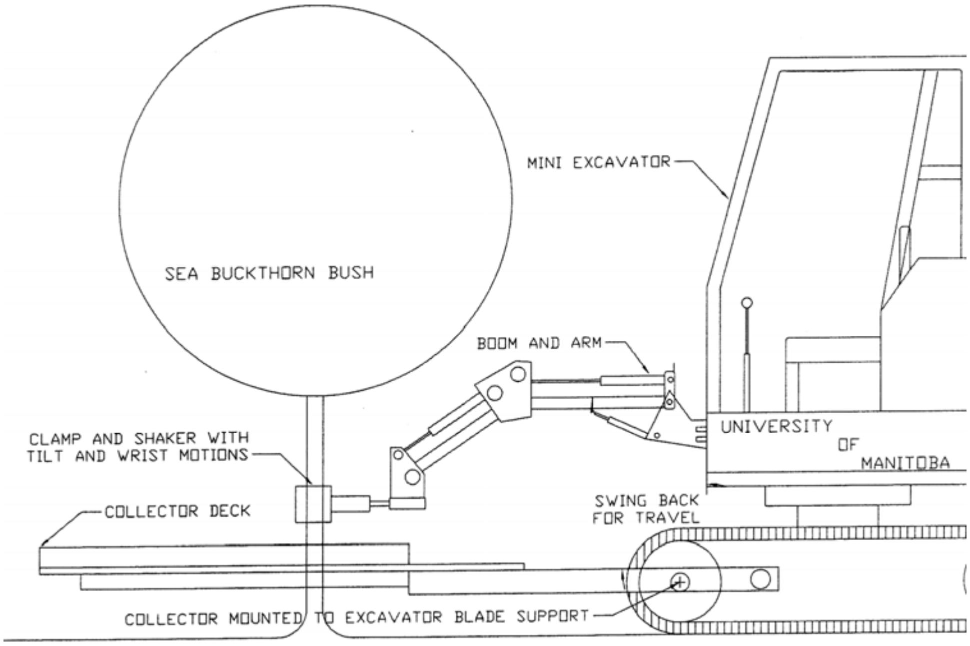

Figure 6. Schematic of the Mark III prototype illustrating the shaking arm and collector deck in relation to a tractor unit.

Figure 7. John Deere industrial compact excavator with shaker arm and berry collector mounted from the front of the tractor unit (Mark III prototype).

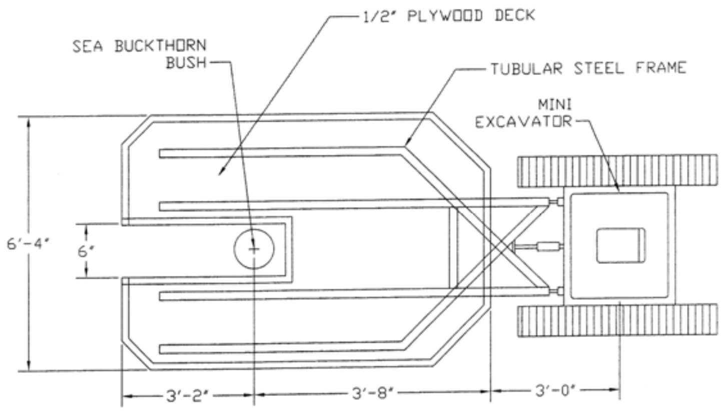

Figure 8. Schematic of the rigid berry collector attached to the front of the tractor unit (Mark III prototype).

After considering the different physiological characteristics of the target trees, a claw was developed to handle branches from 13 to 38 mm in diameter. Two cantilevered hands with interlocking fingers were mounted to a hydraulic cylinder (Fig. 9). A block in the back of the cylinder was screwed onto the shaft of the cylinder and connected to the hands with a pin. Retracting the shaft pulled the block back and closed the claw. This entire assembly was threaded onto the shaker cylinder. Mounted on the back of the shaker cylinder was a rack and pinion to provide a rotational movement, or wrist action, to grab branches. A hydraulic cylinder activated from the operator's platform operated the rack.

Evaluation of the Mark III Prototype

The testing conducted with the 'Indian Summer' seabuckthorn cultivar was with approximately 25 year-old shrubs located at the PFRA Shelterbelt Centre in Indian Head, SK. Harvesting occurred on September 25 and 26, 2002. Once the frequency and amplitude were set, the harvester was maneuvered into position with the collector plate around the base of the tree and with tarps placed on the ground outside the area covered by the collector plate. The claw of the harvester was positioned around the test branch and clamped with enough pressure to hold the branch without damage. Each branch was shaken until all the berries were harvested to a maximum of 90 s. After the harvest interval, the berries were collected from the tarp and collector. The unharvested berries were picked from the branch.

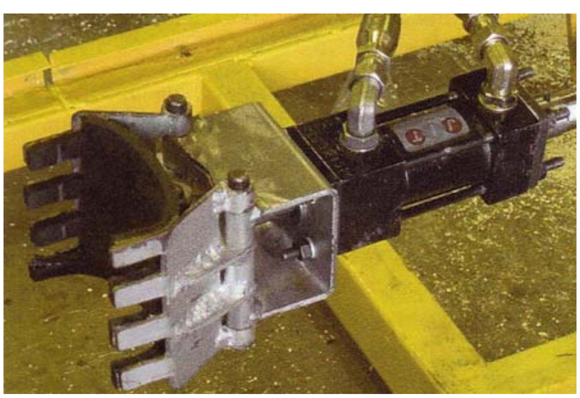

Figure 9. The clamping mechanism for the Mark III prototype consisted of two cantilevered hands with interlocking fingers controlled by a hydraulic cylinder.

Although the Mark III prototype had been designed to produce a shaking frequency of 25 Hz at 25 mm amplitude, the harvester was unable to achieve these conditions. When adjusted to generate a 25 mm amplitude, a frequency of only 14 Hz was obtained. If shaking frequency of 25 Hz was set, an amplitude of only 13 mm was obtained. In total, 17 branches were tested with diameters ranging from 13 to 50 mm. Harvest efficiency ranged from 3 to 40 %, with a mean of 17 %. Of the berries removed by shaking, less than 70% remained on the collector. The rigid collector, mounted to the front of the tractor unit, contributed to berry damage (impact as the berries hit the rigid platform).

Tree damage was observed and was attributed to operator error. Considerable time was spent aligning the claw to the target branch. The height difference between the operator and the claw, and the heavy foliage that obscured sightlines, made alignment of the claw difficult. Most damage occurred while trying to align the claw to the branch. The outside of the claw did considerable damage to nearby branches. The claw did not cause significant damage to the shrub during actual shaking.

Overall, the Mark III prototype was a disappointment. Despite improved maneuverability compared with the previous prototypes, the inability to generate the necessary shaking conditions yielded poor harvesting results. The rigid collector contributed to excessive berry damage.

MARK IV PROTOTYPE

Features of the Mark IV Prototype

Modifications were made to the piston in the cylinder to improve the capabilities of the shaking arm. Subsequent testing verified that the modification produced a consistent displacement of 25 mm throughout the frequency range.

A collector was mounted to the side of the tractor unit (Fig. 10). This allowed the tractor unit to drive parallel to the row of shrubs in the orchard without turning. The collector consisted of screen mesh suspended between frames constructed of square tubing. The collector was designed to extend underneath the canopy when the tractor unit was positioned adjacent to the shrub to be harvested. A slot allowed the collector to be positioned so that it surrounded the shrub. At this point, the collector was manually extended. In its extended position, the mesh covered an area of 4.0 m^2. A future improvement would be to mechanize the positioning of the collector. After harvesting a shrub, the collector was retracted for movement to the next shrub. No modifications were made to the claw because it worked well and caused minimal damage during shaking.

Evaluation of the Mark IV Prototype

Evaluation of the Mark IV prototype followed the same procedures as was done for the Mark III prototype. The orchard that had been harvested in the previous year did not produce fruit in 2003 because it was an extremely dry year. Evaluation of the prototype occurred in an alternate orchard with several different varieties of sea buckthorn. Harvesting occurred between September 8 and 11, 2003. Harvest efficiency varied from 20 to 100 % (with a mean of 76 %) for 22 branches harvested in September 2003. Because the shrubs had been allowed to sucker in this orchard, the collector could not be extended past the centerline of the row. Thus, the collector covered approximately one-half of its design area. Collection efficiency was calculated as 56 %.

Figure 10. Mesh berry collector attached to the side of the tractor unit (Mark IV prototype).

Conclusions

A hydraulically–controlled shrub shaker has been designed and tested for removing berries. Harvesting trials were conducted in four consecutive seasons beginning in 2000 using the 'Indian Summer' variety of sea buckthorn. The shaking arm was initially mounted on a trailer, but was subsequently mounted on a tractor unit to improve maneuverability. A collector was added to catch the berries as they fell from the shrub. Proper grooming to prevent suckering of the shrubs is necessary for the collector to be effective. A rubber-lined claw was used to grasp individual branches for shaking. An amplitude of 25 mm at a frequency of 25 Hz was found to produce the best harvest efficiency without causing excessive debris in the harvested sample. The combination of 25 Hz and 25 mm also caused the least branch, bark, and leaf bud damage.

Acknowledgements

The authors thank the Manitoba Rural Adaptation Council (MRAC) and the Agriculture Development Fund - Saskatchewan Agriculture and Food for funding of this study and Merle Kroeker, Kris Frederickson, Gillian Roos, Erron Leafloor, Javad Khazaei, Jim Aitken, Jarrett Wylde, Art Smith, Ken Carmichael, and Dwayne McGhee for their technical assistance.

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EAN-SEABUCK: a successful story of international seabuckthorn cooperation

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Abstract

Hippophae rhamnoides (seabuckthorn) is native to Europe and Asia. It has been used for centuries in traditional Russian and Chinese medicine and it is rich in bioactive compounds, such as vitamins, minerals, and essential oils. The plant offers a huge potential as raw material for premium food, cosmetic, and pharmaceutical products. However, commercial cultivation and exploitation is poorly developed in some areas due to a lack of know-how in harvesting and processing technologies, and marketing. In other areas (Europe), there is a shortage of raw material.

During the last two years, the European project EAN-SEABUCK, financed by the European Commission under the 6th Framework Program, has been dedicated to the successful establishment of an integrated cooperation network between Europe and Asia, including Russia and the Newly Independent States. The purpose of this network was to create a joint sustainable utilization of seabuckthorn, to improve technical knowledge and know-how on seabuckthorn production, from cultivation and harvesting to processing and product development, leading in the long-term to the establishment of a solid seabuckthorn industry. Current research has been assessed, as well as the production and marketing situation, and Best Available Technologies (BAT) have been identified. Training modules on the different topics has been designed, collecting feedback to optimise training effectiveness. Training has been successfully carried out in areas of China, Russia and Uzbekistan addressed to seabuckthorn farmers and processors. A win-win situation has been achieved by mobilising scientific and technological capacities to the benefit of the seabuckthorn international community.

Keywords: *Hippophae rhamnoides*, seabuckthorn, network, sustainability, international cooperation.

Introduction

Seabuckthorn (*Hippophae rhamnoides*) is a plant belonging to the *elaeagnaceae* genus and is native to Europe and Asia. For centuries, traditional medicine in Russia and China has recognised the value of this plant for its richness in bioactive substances, especially vitamins, minerals, and essential oils. Seabuckthorn is a pioneer plant which grows in poor, sandy soils and fixes nitrogen with the aid of bacteria. It can tolerate an alkaline environment just as well as extreme fluctuations in temperature and high saline concentrations. Over 95% of the seabuckthorn worldwide (total sum of all plantation and wild stock) is of Asian origin, of which 85% stem from China alone, where it is mainly used for the protection against wind and water and recovery of degraded soils (EAN-SEABUCK project partners 2007). Seabuckthorn berries are amongst the most vitamin-rich fruits found in flora. They also offer a very interesting spectrum of oils and flavonoids. Their high content of essential fatty acids (in particular unsaturated fatty acids such as Omega 3 fatty acids) and phytosterols make the plant interesting for the pharmaceuticals and cosmetics industries. Several studies have proven that seabuckthorn contains over 190 bioactive compounds (Code 2001). The cultivation of seabuckthorn and the processing of the berries offer less-developed, remote rural areas with extreme climatic conditions a chance for additional income for the population. Improving the economic situation there helps to stop/slow down the migration into cities – a major social problem today in countries like China, Russia or Newly Independent States (NIS) countries. Besides the berries, the remaining parts of the seabuckthorn plant (leaves and branches) could be useful as a source of animal feed and firewood.

TTZ Bremerhaven, together with the NIG-Nahrungsingenieurtechnik GmbH (both in Germany), the International Center for Research and Training on Seabuckthorn (ICRTS) in China and the Northern Research Institute of Forestry in Arkhangelsk (Russia), launched the EAN-SEABUCK (European Asian Network - Sea Buckthorn) project (EAN-SEABUCK project 2005-2007). The objective of the project, which was awarded funding from the European Commission for two years, was to identify the most important players in the various areas of the seabuckthorn industry in Europe, China, Russia, and in the NIS and to encourage the formation of a network for a transnational exchange of experience in order to guarantee the sustained use of seabuckthorn fruit.

Background

Development of the seabuckthorn industry is quite varied in different countries. A study completed in 2001 by NIG (NIG 2001) and a more recent study finished in 2006 carried out under the frame of the EAN-SEABUCK project (EAN-SEABUCK project partners 2007) reveal the potential of seabuckthorn cultivation and processing in Asia and Europe. The limiting factor is still a lack of know-how and access to harvesting and processing technologies, and in product development and marketing. It has been identified that the harvesting (e.g. time of harvesting, harvesting technologies applied) and processing technologies (e.g. oil separation, juices, and puree) commonly applied in Asia are the crucial quality determining factor for seabuckthorn half-products (e.g. juice, puree) and oils which limits their commercialisation. Limitations due to harvesting methods applied, topographical features, and transport conditions are the reason for very low output in cultivated areas in Asia. The commercial cultivation and exploitation of seabuckthorn berries is thus not sufficiently developed in those areas.

In Europe, Germany and Romania (Carpathians) are the leaders in seabuckthorn cultivation and exploitation. Seabuckthorn is mainly used as ingredients for the production of a wide range of food, cosmetic, and pharmaceutical products. However, the incorporation of seabuckthorn in products in Europe is yet limited due to the restricted availability of high quality raw material and half products in sufficient quantities. The four EAN-SEABUCK project partners aware of the positive environmental, nutritional, economic, and social effects encountered by the increase of the sustainable cultivation of seabuckthorn established the European Asian Network for the development of strategies to enhance the sustainable use of seabuckthorn (EAN-SEABUCK). This would provide benefits for Europe (as major equipment supplier, customer (food industry) and consumer market), Russia/NIS (developing producer and consumer market) and China (as major producer and consumer market).

Objectives

The objectives of the EAN-SEABUCK project are:

- to establish a European-Asian network for the trans-national technology and know-how transfer, exchange of best practices for joint sustainable utilisation of the immense Asian seabuckthorn crop and development of the Russian/NIS market on Sea Buckthorn,
- to improve and exchange knowledge between Europe and Asia in the field of seabuckthorn harvesting and processing – in order to achieve better product quality and safety to reach international quality standards and thus make the resulting products available for the European market.
- in the long-term to establish a seabuckthorn industry in the targeted countries and thus provide employment alternatives for less developed and climatically disadvantaged remote rural areas mitigate against migration into cities,
- to increase the competitiveness of the European food industry in the use of bio-active compounds derived from seabuckthorn of Asian and Russian/NIS origin,
- to increase the use of bio-active compounds in the European diet – a matter which is limited only due to the availability of seabuckthorn on the European market,
- to support vitamin supply and healthy nutrition of the relevant Asian and Russian/NIS population by increased valorisation of the endemic seabuckthorn fruit,
- to support anti-erosion measures and to solidify the cooperation between European and Asian agriculture, food and rural development sectors

Implementation

In order to achieve the objectives of the network, the following activities, besides others, have been implemented:

- Investigation of the actual requirements and lack of know-how in Asian countries (main seabuckthorn producing countries) and Russia/NIS (developing seabuckthorn production); exploration of the potential in the respective as well as European markets; identification of main players, decision-makers and multipliers (state organizations or non-governmental organizations, able to multiply knowledge).

- Market review of food and non-food applications for seabuckthorn and intermediate seabuckthorn products in Europe and Asia. Actual information about the extent of commercial seabuckthorn cultivation, the degree of utilisation, and current technologies for seabuckthorn processing was collected. A questionnaire in four different languages (English, Chinese, Russian, and German) was developed for this purpose and extensively distributed among the identified key players. On the basis of the results, a comparative analysis was undertaken with regard to market and price developments in the entire net product chain, i.e. from the raw material (berries, leaves) to semi-finished products (juice, puree, oil) to end products (foodstuffs, cosmetics, remedies). The study, which was finished in 2006, demonstrated that the commercial processing of seabuckthorn berries into qualitatively high-value food products, cosmetics, or pharmaceuticals has developed very differently between the regions of Europe, Russia, NIS, and China.
- Definition of international quality and safety standards for seabuckthorn to address standards, practices, legislation, and policies on fruit processing and quality assurance in the fruit processing sector. Quality and safety requirements for seabuckthorn products (raw materials, half- and final products) in the involved countries were furthermore investigated, with consideration of legal as well as industrial requirements. The gaps and needs detected during the investigations were used to identify BAT for cultivation, harvesting, and processing/preservation with consideration of economic and technical aspects.
- Assessment of the state of the art in technology in order to select the BAT for seabuckthorn harvesting and processing best suited for the targeted countries; China, Russia and NIS.
- Organisation of workshops and training on seabuckthorn harvesting and processing as well as quality assurance, product development, marketing, and commercialisation. Special trans-national work groups (German/Russian/Chinese) including representatives of each project partner and external experts were established to develop five different training modules on the mentioned topics covering the whole seabuckthorn production chain. The training program was structured as follows (Figure 1).
- Broad dissemination of the EAN-SEABUCK results via multiple media including: an electronic EAN-SEABUCK newsletter, a seabuckthorn manual, and a multilingual project web-site as the main dissemination and communication tool for the public with restricted areas for partner exchange and concerted involvement of stakeholders. The EAN-SEABUCK project has been, and will be presented at many conferences and fairs.
- Networking between Europe and Asia in joint expert workgroups.
- Review of current research activities on seabuckthorn in Asia and Europe for a sustainable implementation that is supported by recommendations for future research policies, and by the creation of further international co-operation projects.

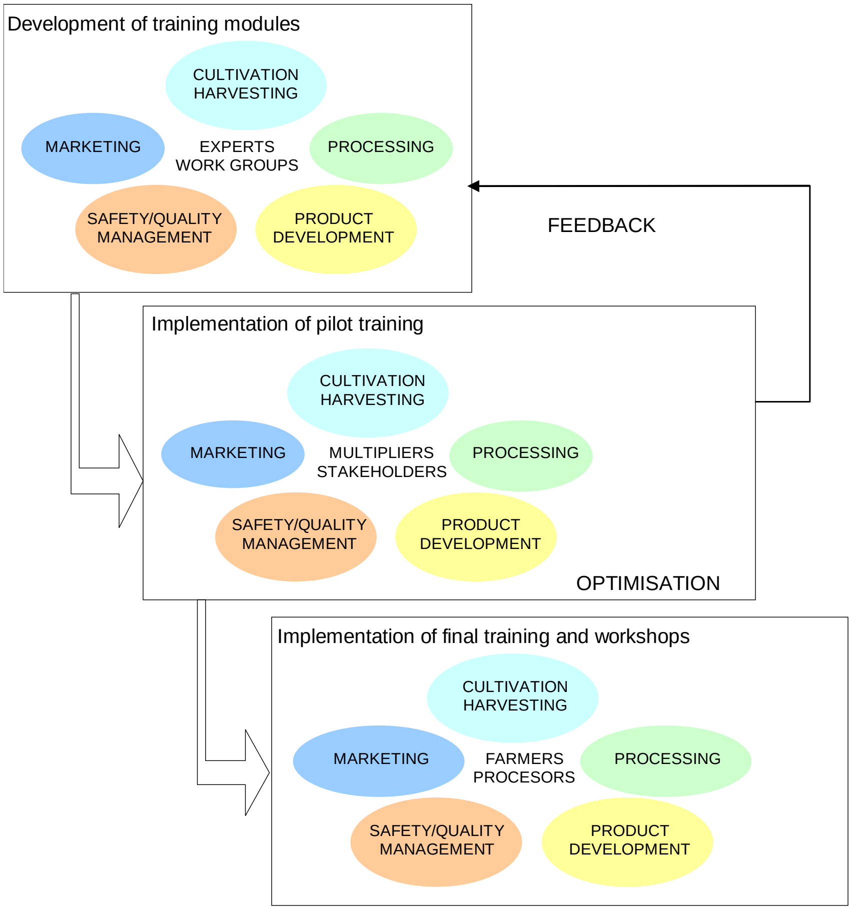

Figure 1. EAN-SEABUCK training program.

Dissemination

The EAN-SEABUCK website (*www.eanseabuck.com*) is the main project dissemination tool, providing the following services:

- General information about the project in the English, Russian and Chinese languages
- A key-players database with search facilities
- Report on current situation and trends of the seabuckthorn business
- Report on quality and safety requirements in Europe, China, and Russia
- Catalogue of Best Available Technologies (cultivation, harvesting, and processing)
- Training materials in English, Chinese, and Russian
- Seabuckthorn manual in English, Chinese, and Russian
- Multilingual project leaflet

All public documents produced during the project can be downloaded from the website upon free registration.

Apart from its website version, the seabuckthorn manual is available in its printed version in Chinese, Russian, and English. The seabuckthorn manual consists in a collection of five booklets on the following main topics: seabuckthorn cultivation and harvesting, processing of seabuckthorn, development of seabuckthorn products, quality and safety management of seabuckthorn and its products, and marketing and commercialisation of seabuckthorn products.

Conclusions

The purpose of EAN-SEABUCK is the improvement and exchange of knowledge and know-how on seabuckthorn production for the achievement of better product quality and safety, and the establishment of a long-term seabuckthorn industry that involves the international area of activity of the project (Eurasia).

In addition to the enormous economic potential of seabuckthorn, the achievement of economic alternatives in rural areas for combating the migration into cities, the prevention of desertification and saving of soil fertilisation by plantations of seabuckthorn, as well as the use of by-products such as plant leaves and branches for animal feed and fire wood are foreseen as a long-term socio-economic impact of the network. The project partners expect four main outcomes from the established Eurasian network: the facilitation of a sustainable production of seabuckthorn, with low-input farming and good yields; the production of safer products of a higher quality in larger quantities; the access of industry to larger resources of raw material; and the development of a market for industry for harvesting and processing equipment.

Acknowledgements

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Investigations Regarding the Cicatrizing Action of the Gels with *T. Gallica* compared with *H. rhamnoides*

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Abstract

This project investigated the cicatrizing action of gels with gemmoderivatives from *Tamarix gallica* (french tamarisk) in a concentration of 5% total extract from the fresh shoots in gel, compared with gels from *Hippophae rhamnoides* (seabuckthorn), in the same concentration. The gemmoderivatives are extracts of the buds and other very young parts of plants.

Taking into account that the oil extracted from seabuckthorn has cicatrizing properties, the present research, studied if *T. gallica*, in abundance in Romania, has the same properties.

The assessment of the cicatrizing action was done with the gemmoderivatives from french tamarisk - a 5% concentration of fresh shoot extracts in gel preparation compared with the 5% seabuckthorn fresh shoot total extracts in gel. The gels were prepared with carbopol 990 and triethanolamine 0.5%. The experimental study was done on white Wistar rats, aged 5-8 months, weighing 200±10 g, epilated on the dorsal side. The burns were provoked after ethylic ether anesthesia of the animals with a metallic device with the diameter of 1 cm, heated at 105˚C (221˚F). The treatment was applied for 14 days.

For the untreated controls the complete healing of the wounds provoked by burning, was produced in 15-16 days. For the healing of the wounds of the animals treated with the seabuckthorn gel, the complete healing of the wound was after 8-10 treatment days and for the animals treated with french tamarisk gel, after 9-10 days. The gels obtained from the oil of seabuckthorn and from the gemmoderivatives of french tamarisk have a similar cicatrizing action.

Keywords: *H. rhamnoides, T. gallica,* gemoderivative, cicatrizing.

Introduction

The flora from the Danube Delta in Romania is very particular and consists of various species of trees including french tamarisk and seabuckthorn. Due to the fact that they grow in the same environment and also due to the fact that in Romania they have the same popular name "cătina", red for tamarisk and white for seabuckthorn, and also due to the fact that both species have in their composition among other constituents, polyphenolic compounds, we intended to study if these have similar actions. The oil extracted from seabuckthorn contains increased quantities of carotene, explaining its cicatrizing action. In the present paper we studied if the tamarisk extract, incorporated in a gel formulation, has a cicatrizing action comparable to seabuckthorn.

Materials and methods

The evaluation of the cicatrizing action was made with the gel of french tamarisk in a concentration of 5% compared with the gel of seabuckthorn 5%. The gel was obtained with Carbopol 990, triethanolamine 0.5%, and purified water.

The experimental study was made on Whistar white rats, weighing 200±10 g. The animals were hosted in mobile cages in a special shelter according to the European Convention regarding animal protection.

The clinically healthy animals were distributed in batches of 8-10 animals. The first batch consisting of 8 animals was the control batch. All the animals were epilated in the dorsal area. The wounds were burns produced by using a round metallic device 1 cm in diameter, heated with saline water NaCl 5%, at 105ºC (221˚F). The heated disk was applied on the epilated dorsal area of the animals, which were previously anaesthetised with ethylic ether, and maintained for 10 seconds. The treatment with the studied gels was made daily, during 14 days. The evolution of the wounds was followed from every two days by the measurement in millimeters of the treated surfaces compared with the untreated controls and with those treated with the gel base.

During the experimental period we followed the clinical state of the animals. The statistical evaluation of the results was made with the Student's t-test (4).

Results and discussion

The control animals had an initial wound surface of 2.316 cm^2; after 12 days the healing percentage was 58.24%. The total healing was produced after 15-16 days.

The treatment of the animals with 5% seabuckthorn gel was produced after 8-10 treatment days. Similar results were obtained after treating of the animals with gel of tamarisk. Total healing was produced after 10 treatment days; the healing of the skin being completely without scars (Figures 1 and 2).

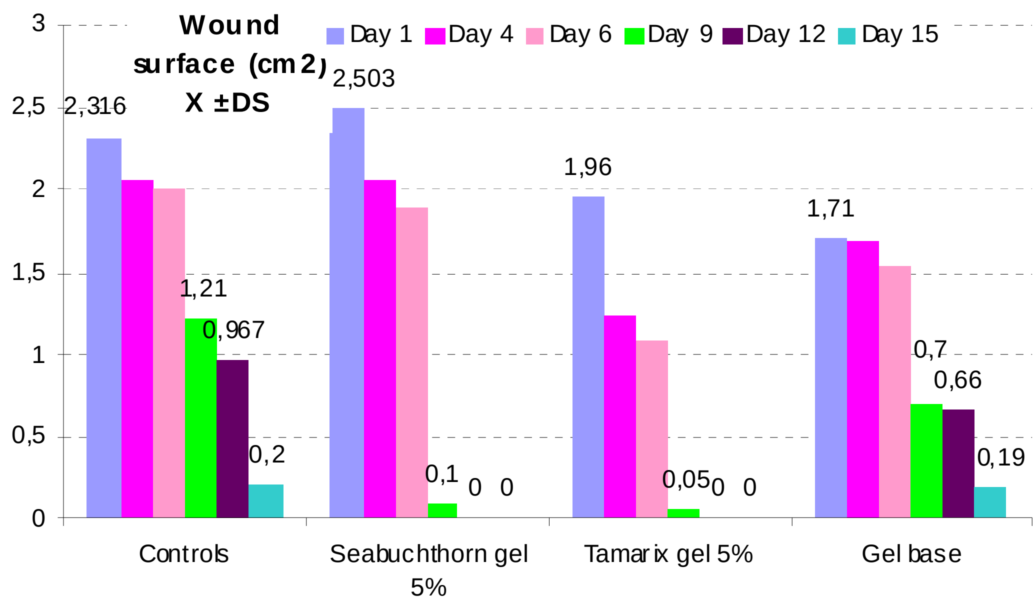

Figure 1. The cicatrising effect of the gels of seabuckthorn and french tamarisk on the experimental wounds in rats (wound surface - cm^2).

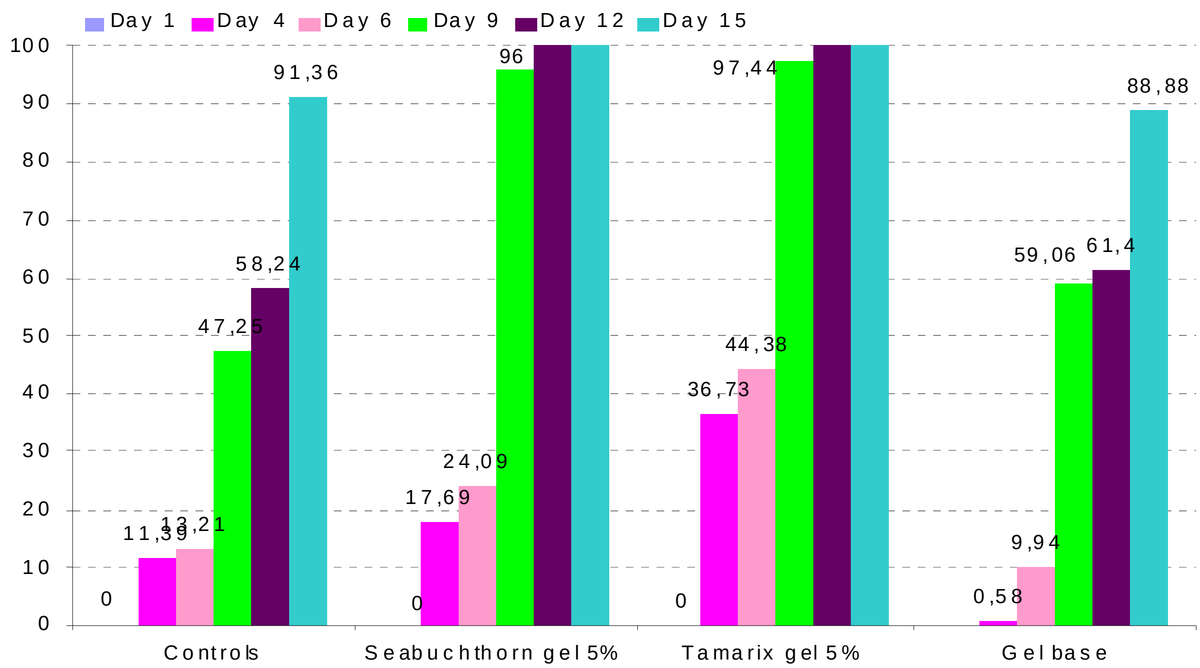

Figure 2. The cicatrising effect of the gels of seabuckthorn and french tamarisk on the experimental wounds in rats (effect %).

Figure 3. Photos representing the cicatrisation of the provoked wounds in controls at different intervals A - Day 3, B – Day 9, C- Day 15.

Figure 4. Photos representing the cicatrisation of the provoked wounds in treated subjects – 5% seabuckthorn gel at different intervals A- Day 3, B – Day 9, C- Day 15.

Figure 5. Photos representing the cicatrisation of the provoked wounds in treated subjects – 5% tamarisk gel at different intervals A- Day 3, B – Day 9, C- Day 15.

Figure 6. Photos representing the cicatrisation of the provoked wounds in treated subjects with gel base at different intervals A- Day 3, B – Day 9, C- Day 15.

Despite the fact that the two plants with the same popular name are part of different species, both presented cicatrizing properties that are explained by their complex chemical composition. Among the constituents responsible for the cicatrizing action are the carotenoids, polyphenolic compounds, proanthocyans, and vitamins.

Conclusions

The treatment with the 5% seabuckthorn (Figure 4) and 5% tamarisk (Figure 5) gels, presented a similar cicatrising effect. The healing of the wounds took place after the treatment with the seabuckthorn and tamarisk gels after 9-10 days of therapy compared with the untreated controls (Figures 3 and 6) that healed after 15-16 days.

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Research Regarding the Gemmoderivatives of *Tamarix gallica* compared with *Hippophae rhamnoides* on the gastric secretory activity and motility on rats

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Abstract

The present study investigated the influence of the gemmoderivative from fresh shoots of *Tamarix gallica* (french tamarisk) compared with the gemmoderivative from fresh shoots of *Hippophae rhamnoides* (seabuckthorn) on the gastric secretory action and intestinal motility in rats. The gemmoderivatives are extracts of the buds and other very young parts of plants (fresh shoots).

The experimental study was done on white Wistar rats, weighing 190±10 g, kept under food restriction during 24 hours. The assesment was made for the single extracts (or mother-macerates) derivatives of *T. gallica* and seabuckthorn after the elimination of the glycerin-alcoholic solution. From the dry extract we obtained aqueous solutions of the macerate in a concentration of 5%, given in dosage of 1 ml solution/100 g body weight animal.

The extraction solution of *T. gallica* lowered the volume of the gastric secretion by 29.73%, the seabuckthorn extract solution decreased by 37.41% and the gastric acidity with 30.50% and 35.40% for seabuckthorn respectively. The gastric motility of the animals treated with *T. gallica* was lowered by 31.87% in unstimulated transit and by 17.68% for the $MgSO_4$ stimulated intestinal transit animals.

For the seabuckthorn treated animals, the gastric motility was lowered by 21.62% in unstimulated transit and by 15.38% for the stimulated transit animals. The decrease of the gastric acidity in the animals treated with tamarisk and seabuckthorn is due to the presence of the flavones and anthocyanosides in the investigated extracts, the decrease of intestinal motility is due to the presence of the tannins.

The extracts from the buds and shoots of seabuckthorn and french tamarisk produced the decrease of the gastric secretion volume, the gastric acidity, the decrease of the unstimulated and $MgSO_4$ stimulated intestinal motility. The tamarisk and the seabuckthorn presented a pronounced gastro-protective effect in experimental ulcers provoked by indometacin.

Keywords: seabuckthorn, french tamarisk, gastric acidity, intestinal motility, gemmotherapy.

Introduction

The gemmoderivatives are obtained by extraction from fresh embryonic vegetal tissues - buds and young shoots - in a balanced mixture of alcohol, water, and glycerin, known as glycerin-hydro-alcoholic extracts. By this extraction procedure a complete extraction of the vegetal compounds is accomplished without denaturation of the active principles, keeping intact the synergic action. The bioactivity of the gemmoderivatives is the result of the interaction of the constituents that constitute the genetic program of the plant development.

The first step in this research is the chemical analysis of *Tamarix gallica* and *Hippophae rhamnoides*. In the tamarisk extracts were identified flavones, anthocyanosides, tannins, saponosides, carotenoids, sterols; in the seabuckthorn extract were evidenced flavones, anthocyans, triterpenes, vitamin C, B_1, B_2, B_3, D, E, and carotenoids.

In the specialty literature it is confirmed the presence of these constituents in the composition of the french tamarisk and seabuckthorn extracts. (Istudor, V.2001, Parvu C. 1997, Joerg Gruenwald, 2000).

The present study investigated the influence of the gemmoderivative from fresh shoots of tamarisk on the gastric secretor action and intestinal motility in rats, compared with the gemmoderivative from fresh shoots of seabuckthorn. It also investigated the action of these extracts on the experimental ulcers provoked in rats.

Materials and methods

The measurements were made on the gemmoderivatives of *T. gallica* and *H. rhamnoides* after the elimination of the glycerin-hydro alcoholic solution. From the extracts we obtained aqueous solutions in concentration of 5%.

The measurement of the action on the gastric secretion was made by the Shay method of ligature of pylori. The study included batches of 8 white male, Wistar race rats, weighing 190±10 g, kept under food restriction during 24 hours. The treatment was made with the aqueous extraction solutions of tamarisk and seabuckthorn in concentration of 5% 1 ml/100 g animal. As a reference substance for the gastric secretion inhibition it was used famotidine in a dose of 10 mg/kg body weight, orally. The control batch received only a solution of 0.9% NaCl, 25 ml/kg body weight. After 4 hours the animals were sacrificed under chloroform anesthesia with, the abdominal area was opened and the stomach excised. We collected the gastric content, we measured the volume and after centrifugation we measured the gastric acidity by the Töpfer Linossier method (Mihele D, 2000).

For the assessment of the action on the normal intestinal transit we took into study batches of 8 white mice, both sexes, weighing 22±2 g, total food restricted for 18-20 hours before the beginning of the tests. Tha animal batches were treated with the analysed solutions in a dose of 1 ml/100 g body weight, orally. The control batch received a solution of NaCl 0.9%, 25 ml/kg body weight orally.

After 30 minutes from the administration of the tested solutions, the animals received by gavage 0.2 ml carbon suspension 5%.

For the determination of action on stimulated intestinal motility after 30 minutes from the administration of the tested solutions, the animals received by gavage, a 0.2 ml suspension of carbon 5% with $MgSO_4$ solution 15%. After 15 minutes the animals were sacrificed with chloroform and then the digestive tract was excised from the cardia to the *caeccum*. We measured the distance from the pylory to the carbon black coloured limit of the intestinal bolus.

For each animal we expressed the distance of the carbon suspension as a percentage of the total length of the intestine. We calculated the media for each batch and we compared with the control batch.

The statistical evaluation of the results was made with the Student's t-test (Simionovici M., Carstea Al., Vladescu C., 1983).

Results and discussion

The action on the intestinal motility

The normal intestinal transit in rats was lowered by 21.62% and the stimulated intestinal transit was lowered by 15.38% by the extraction 5% seabuckthorn solution. The tamarisk solution decreased the normal intestinal transit by 31.87% and the $MgSO_4$ stimulated transit by 17.68% (Table 1, Figure 1).

Table 1. The action of the extracts used in the study on the intestinal transit in rats.

Tested product	Distance of the carbon transit (cm)			
	Normal transit		Stimulated transit with $MgSO_4$	
	X±e.s. [a]	Effect % [b]	X±e.s.	Effect %
CONTROLS	51.80	-	67.60	-
Tamarisk 1 ml sol. 5%/100 g animal	35.30	-31.87	55.70	-17.68
Seabuckthorn 1 ml sol. 5%/100 g animal	40.60	-21.62	55.70	-15.38
Papaverine HCl 50 mg/kg body weight, orally	31.10	-41.89	42.30	-37.42

[a] x ± e.s. = media ± standard error, p<0.05 = Statistical significance
[b] Effect % = [transit length Test (cm) - transit length Control (cm) /transit length Control (cm)]x 100

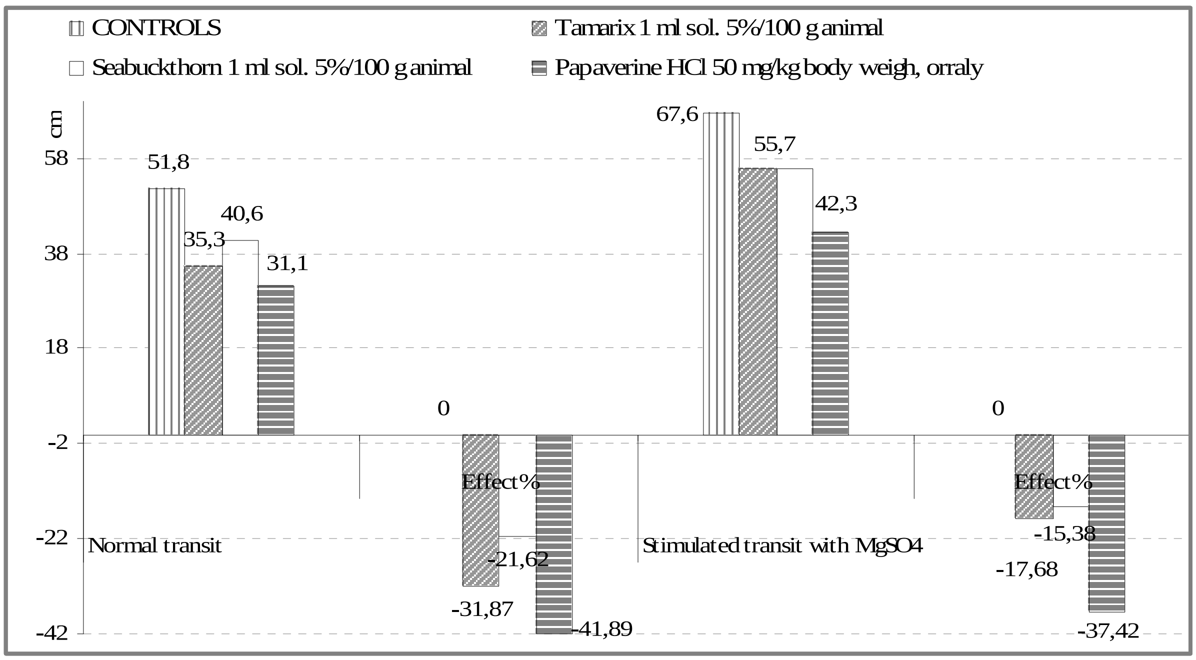

Figure 1. The action on the intestinal transit in rats of the extracts used in the study.

The action on the gastric secretory activity

The extraction solution of the seabuckthorn solution produced a decrease of the gastric secretion in rats of 37.41% and of the gastric acidity of 35.40% (Table 2, Figure 2). The tamarisk extraction solution reduced the gastric secretion volume in rats by 29.73% and the gastric acidity was reduced by 30.50%.

The extraction solution of *T. gallica* reduced the gastric acidity by 13.55% compared with untreated controls; the seabuckthorn reduced the gastric acidity by 15.62% (Table 2, Figure 2)

Table 2. The action of the extracts took used in the study on the gastric secretory activity in rats.

Tested product	Dose /route	Gastric content volume (ml)		Total acidity (mEq/l)	
		X±e.s.[a]	Effect %.[b]	X±e.s.	Effect %
CONTROLS	-	4.25	-	17.34	-
Famotidine	10 mg/kg body weight orally	2.40	-43.52	7.37	-57.49
Tamarisk	1 ml sol. 5%/100 g animal orally	2.98	-29.73	12.05	-30.50
Seabuckthorn	1 ml sol. 5%/100 g animal orally	2.66	-37.41	11.20	-35.40
Indometacin	20 mg/kg body weight i.p [c]	5.50	+29.41	25.00	+44.17
Indometacin + Tamarisk	20 mg/kg body weight i.p. + 1 ml sol. 5%/100 g orally	4.80	+12.94	14.99	-13.55
Indometacin + Seabuckthorn	20 mg/kg body weight i.p + 1 ml sol. 5%/100 g orally	4.65	+9.41	14.63	-15.62

[a] x ± e.s. = media ± standard error, $p<0.05$ = Statistical significance
[b] Effect % = [Test - Control / Control] x 100, [c] i.p.= intraperitoneally

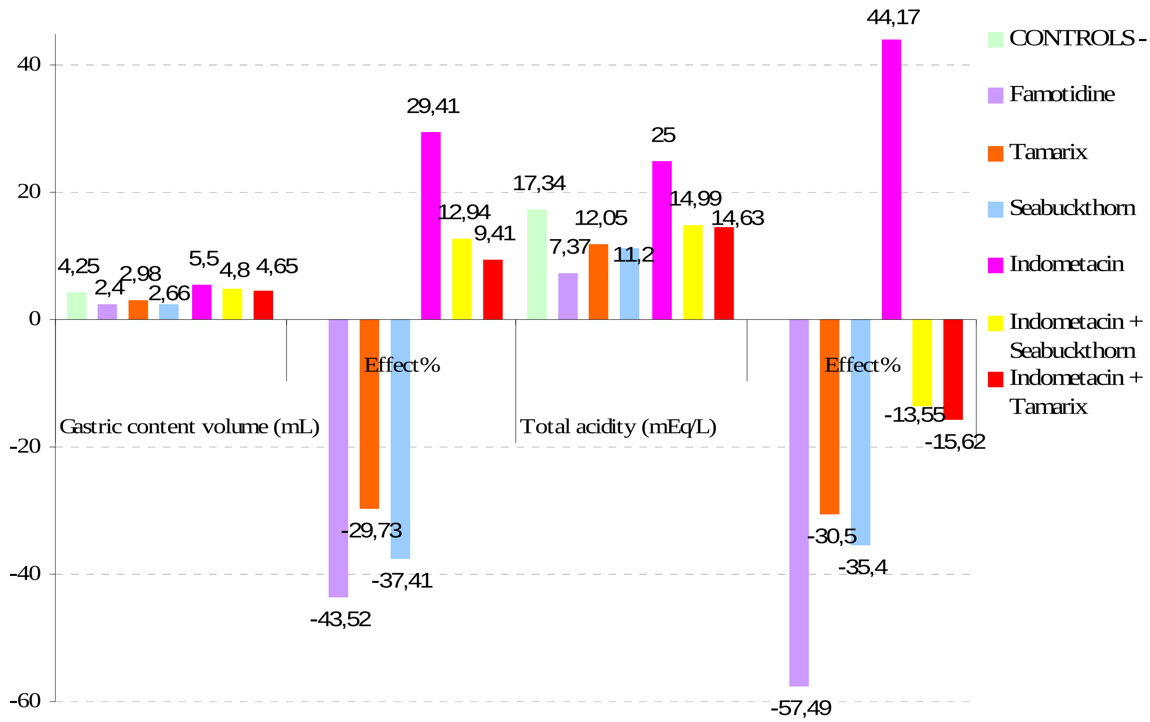

Figure 2. The action of the extracts took used in the study on the gastric secretory activity in rats.

The action on the experimental ulcers provoked by indomethacin

The experimental ulcers showed that the extraction solutions of tamarisk and seabuckthorn have protective action on the experimental ulcers provoked by indometacin and decrease the gastric acidity.

The protective effect on the experimental ulcers provoked by indometacin is also observed by the reduction of the number of ulcers after the treatment of the animals with seabuckthorn extract and tamarisk extract (Figure 3).

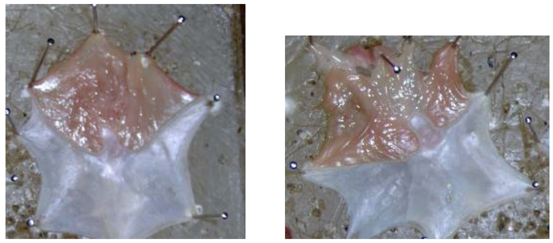

Figure 3a. Microphotos representing the protective effect of seabuckthorn on the indomethacin experimentally induced ulcers.

Figure 3b. Microphotos representing the protective effect of tamarisk on the indomethacin experimentally induced ulcers.

Figure 3c. Microphotos representing the controls in indomethacin experimentally induced ulcers.

The decrease of the extraction solutions of *H. rhamnoides* and *T. gallica* is due to the presence of flavones and anthocianosides and the intestinal motility is reduced due to the presence of tannins.

Conclusions

The extract of the gemoderivate of the fresh shoots of *H. rhamnoides and T. gallica* produced a reduction of the volume of gastric secretion, a reduction of gastric acidity and a reduction of the normal and $MgSO_4$ stimulated transit.

The extraction solutions of *H. rhamnoides* and *T. gallica* presented a pronounced parenteral protective effect in the experimental ulcers provoked by indomethacin.

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