THESIS ON NATURAL AND EXACT SCIENCES B53

Study on Physico-Chemical Properties and Some Bioactive Compounds of Sea Buckthorn (*Hippophae rhamnoides* L.)

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Declaration: Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for any degree or examination.

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ABSTRACT

Sea buckthorn is a widely spread bush in the world, its natural growing areas being Europe and Asia. In the recent decades, much more attention has been paid to this plant which carries valuable berries ranging from yellow to red in colour.

In Estonia, we already have experience of 10 years in planting sea buckthorn. The first experiments started already 30 years ago in Experimental Station at Rõhu. Nowadays, the researchers in Polli Horticultural Research Centre of the Estonian University of Life Sciences carry out different experiments with sea buckthorn.

Sea buckthorn berries have an abundant chemical composition – they are rich in vitamins, carotenoids, flavonoids, and other biologically active components.

Sea buckthorn berries are arousing interest of nutritional scientists as a potential ingredient for functional food, as well as for pharmacological industry, where different oils and medicines containing sea buckthorn are developed. And, of course, sea buckthorn berries are a valuable addition to various dishes (e.g. salads, sauces etc).

The chemical composition of the berries depends on many factors, such as climate, region, weather, growing year, etc.

In the present thesis, the physical properties of nine different varieties of the berries (size, weight, sphericity, rupture force, moisture content) have been described. Various data on the content of reducing sugars, titratable acidity, pH have been given. Vitamin C is determined by HPLC and the content of vitamin A precursor, β-carotene, is calculated using the UV-spectrophotometric method. In addition, some data on two phenolic compounds – quercetin and catechin – using CE – Capillary Electrophoresis method have been given. Applying TLC method, some carotenoids present in sea buckthorn berries are described.

One subdivision is dedicated to a novel method of encapsulation – entrapment of the biologically active substances of sea buckthorn berries. Preliminary experiments have been made and research has been carried out into the influence of gelling conditions on furcellaran gel bead formation as well as the mechanical properties of encapsulated forms. The entrapment efficiency of furcellaran beads has been characterised by β-carotene content in sea buckthorn juice.

Essential oil of sea buckthorn was obtained by steam distillation and analysed applying GC. Sea buckthorn oil was also analysed with GC and some terpenic compounds were found.

LIST OF PUBLICATIONS

The present dissertation is based on the following papers, which are referred to in the text by the following Roman numbers I-VI:

I. **T. Lõugas**, R. Vokk (2004) Study on sea buckthorn (Hippophae rhamnoides L.) pigments as potential antioxidants – Pigments in Food, more than colours…, (Ed by L. Dufosse), Université de Bretagne Occidentale, Quimper, pp. 238-240

II. **T. Lõugas**, M. Liis, K. Laos, R. Vokk (2005) Physical properties As a Tool For Quality Assessment In Fruit Processing – Annual Transactions The Nordic Rheology Society, Vol 13, 295-299

III. K. Laos, **T. Lõugas**, R. Vokk (2006) Encapsulation of β-carotene of sea buckthorn juice in furcellaran beads – Innovative Food Science and Emerging Technologies, (accepted)

IV. **T. Lõugas**, R. Vokk (2006) Carotenoid analysis in sea buckthorn berries – Pigments in Food, Stuttgart-Hohenheim (accepted)

V. **T. Lõugas**, R. Vokk (2006) Characterization of physical and chemical characteristics of sea buckthorn (Hippophae rhamnoides L.) berries (manuscript)

VI. J. Gorbatsova, **T. Lõugas**, R. Vokk, M. Kaljurand - Comparison of the Various Antioxidants Content of Quick-Frozen and Chilled Stored Sea Buckthorn Berries Using Capillary Electrophoresis (manuscript)

In the appendix of this thesis, copies of papers I-VI have been included. Papers I-VI are reproduced with the permission from the publishers.

THE AUTHOR'S CONTRIBUTION TO PUBLICATIONS

Paper I:

The author performed the experimental work and interpreted the data concerning TLC and UV-spectrophotometry and is the corresponding author. Concerning the CE the author supervised a BSc student.

Paper II:

The author carried out the experimental study together with a BSc student, and interpreted the data obtained. She wrote the paper and is the corresponding author. The author also presented the results at the 14th Nordic Rheology Conference, 1-3 June 2005, Tampere, Finland

Paper III:

The author supervised the experimental study concerning the UV-spectrophotometric analyses and interpreted the results. She is the co-author of the paper.

Paper IV:

The author performed all experimental work and interpreted the results. She wrote the paper and is the corresponding author. The author also will present the results at the 4th International Congress on Pigments in Food, 9-12 October, 2006, Stuttgart-Hohenheim, Germany

Paper V:

The author carried out the experimental study together with MSc and BSc students, and interpreted the data obtained. She wrote the paper and is the corresponding author.

Paper VI:

The author carried out the experimental work concerning the HPLC together with MSc and BSc students, interpreted the results and is the co-author of the paper.

TABLE OF CONTENTS

ABBREVIATIONS

HPLC – high performance liquid chromatography
TLC – thin layer chromatography
CE – capillary electrophoresis
GC – gas chromatography
UV – ultraviolet
LC-MS – liquid chromatography-mass spectrometry
ROS – reactive oxygen species

AVR – Avgustinka
BOL – Botanitcheskaja Ljubitelskaja
BOR – Botanitcheskaja
HPR – Gibrid Pertchika
OTR – Otradnaja
PER – Pertchik
PSR – Podarok Sadu
TRR – Trofimovskaja
VOR – Vorobjevskaja

INTRODUCTION

Sea buckthorn (Hippophae rhamnoides L., Elaeagnaceae) is a temperate bush native to Europe and Asia (Rousi, 1971). It is used as a medicinal plant in Tibet already from 900 A.D. (Lu, 1992). In addition to the medicinal use, the berries of sea buckthorn can be processed into valuable products such as juice and marmalade, and be used for flavouring of dairy products because of their unique taste. Today it is used as a candidate for functional food ingredient - the berries are especially rich in vitamin C and flavonoids.

The flavonoids are potent antioxidants, free radical scavengers and metal chelators; they inhibit lipid peroxidation and exhibit various physiological activities including anti-inflammatory, antiallergic, anticarcinogenic, antihypertensive, antiarthritic and antimicrobial activities. Epidemiological studies have indicated that high flavonoid consumption is associated with reduced risk of chronic diseases like cardiovascular diseases (Puupponen-Pimiä et al., 2001)

The strong association between fruit and vegetable intake and cancer or heart disease prevention has been explained by the content of antioxidant phytonutrients (Halliwell, 1997). Besides the commonly mentioned antioxidants (vitamin C, tocopherols and carotenoids), flavonoids can also act as cancer preventing nutrients (Halliwell, 1997) and contribute significantly to the antioxidative activity of the diet (Rice-Evans et al., 1997).

During the last 10 years the cultivation of sea buckthorn in Estonia has become more popular – there is over 500 ha of sea buckthorn plantations. There are two research institutions in Estonia – the Experimental Station in Rõhu (experiments with sea buckthorn since 1998) and Polli Horticultural Research Centre in Estonian University of Life Sciences – who study the different growing conditions and crop yield. In Estonia there are mostly the sea buckthorn varieties, which are developed under the leadership of professor Trofimov in research institute of the Botanic Garden of the Moscow University.

To select the best varieties of sea buckthorn berries to grow, it is useful to know the different physical and chemical parameters of the berries – the moisture content, dimensions and size distributions of the berries and also the puncture resistance of the berries for packaging and transport. Chemical parameters like the titratable acidity, pH and the content of reducing sugars characterize the different varieties of berries. As the content of bioactive compounds also depends on variety, it is important to evaluate also the content of both vitamin C and β-carotene (precursor of vitamin A) in berries.

It is well known that the content of phytonutrients and also physical and chemical characters depend on both genetic and environmental factors. The purpose of the present study is to characterize the physical and chemical indicators of different sea buckthorn berry varieties grown in Estonia, and some biologically active compounds to characterise.

LITERATURE REVIEW

1.1 Sea buckthorn

Sea buckthorn (*Hippophae rhamnoides* L., *Elaeagnaceae*) is a temperate berry-bearing bush native to Europe and Asia (Rousi, 1971). It was used as a medicinal plant in Tibet already in 900 A.D. (Lu, 1992). Used traditionally as raw material for foods and lately as a candidate for functional food ingredient, the berries are especially rich in vitamin C (up to 20 g/kg fresh berries from some sources) and flavonoids in the juice fraction. Both the soft parts (pulp and peel) and the seeds contain oil and high levels of tocopherols and plant sterols (Kallio et al., 2002).

Sea buckthorn is a hardy, deciduous shrub with yellow or orange berries, which has been used for centuries. In ancient Greece, sea buckthorn leaves added to horse fodder were well reputed to result in weight gain and shiny hair; thus, the Latin name "Hippophae" meaning shining horse. Sea buckthorn occurs as a native plant distributed widely throughout temperate zones between 27° and 69° N latitude and 7° W and 122° E longitude including China, Mongolia, Russia, Great Britain, France, Denmark, Netherlands, Germany, Poland, Finland, Sweden, and Norway (Li and Schroeder, 1996).

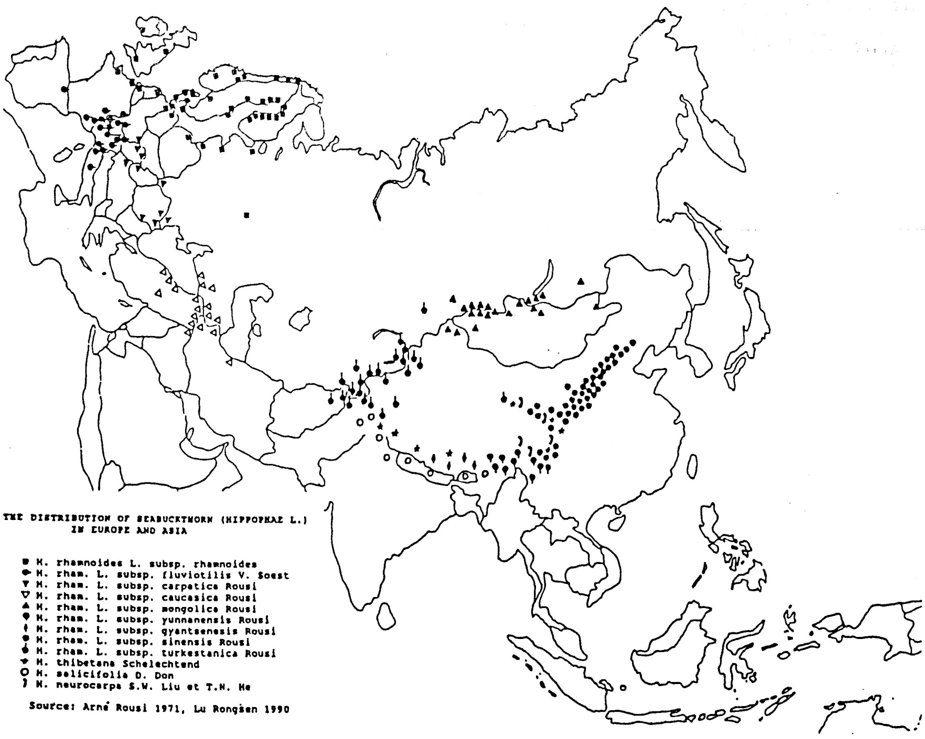

Figure 1 Sea buckthorn distribution in Europe and Asia (Li and Schroeder, 1996)

1.1.1 Taxonomy

Hippophae belongs to the family *Elaeagnaceae.* Arne Rousi classified Hippophae into three species based on morphological variations. *H. rhamnoides* L., *H. salicifolia* D., and *H. tibetana* Schlecht. *Hippophae rhamnoides* was divided further into nine subspecies: *carpatica*, *caucasica*, *gyantensis*, *mongolica*, *sinensis*, *turkestanica*, *yunnanensis*, *rhamnoides*, and *fluviatilis* Rousi (Li and Schroeder, 1996).

1.1.2 Natural distribution

Sea buckthorn's native area is shown in Figure 1. Natural sea buckthorn stands are also widespread in Europe – on river banks and coastal dunes along the Baltic Coast of Finland, Poland, and Germany and on the western coast and along the Gulf of Bothnia in Sweden. In Asia, sea buckthorn is distributed widely throughout the Himalayan regions including India, Nepal, and Bhutan and in the northern parts of Pakistan and Afganistan (Li and Schroeder, 1996).

In Estonia sea buckthorn does not grow naturally, the total area of sea buckthorn plantings in Estonia is about 500-1000 ha (Jalakas et al., 2005).

1.1.3 Description

Sea buckthorn is a deciduous, dioecious shrub, usually spinescent, reaching 2 to 4 m in height. It has brown or black rough bark and a thick grayish-green crown. Leaves are alternate, narrow, and lanceolate with a solver-gray color on the upper side. This calcicolous species tolerates low temperatures, high soil pH of 8.0, and salt spray. The plant's extensive root system is capable of holding the soil on fragile slopes. Sea buckthorn can be planted in marginal soils due to its symbiotic association with nitrogen-fixing actinomycetes. Roots of sea buckthorn also are able to transform insoluble organic and mineral matter in the soil into more soluble states. The plant rapidly spreads by rhizomatous roots, and will quickly colonize adjacent areas (Li and Schroeder, 1996).

1.1.4 Chemical composition, nutritional and medicinal value

Sea buckthorn fruit is rich in carbohydrates, protein, organic acids, amino acids, and vitamins (Li and Schroeder, 1996). Also they are rich in different biologically active compounds.

The chemical composition of sea buckthorn berries varies depending on habitat, species, variety, growing conditions, time of picking etc.

Also the position of a fruit on the tree will determine its nutrient and water status and its exposure to environmental factors such as sunlight or pests and diseases (Aked, 2000).

In Table 1 there is some information about the chemical composition of sea buckthorn berries.

Table 1 Chemical composition of sea buckthorn fruits (Lõugas, 2001).

Component	
Oil	3-15 %
Organic acids	2.6-4 %
Sugars and alcohols	3.5-7 %
Vitamins	
B1	0.02-0.08 mg/100g
B2	0.03-0.056 mg/100g
C	150-1600 mg/100g
P	250-300 mg/100g
K	1-1.5 mg/100g
Carotenoids	6-28 mg/100g
Tocopherols	Up to 145 mg/100g
Coline	110-700 mg/100g
Folic acid	Up to 0.79 mg/100g
Flavonoids	0.2-1.5 %
Quercetine	57 mg/100g
Volatile oil	36 mg/kg
Sterines	Up to 2 %
Pectines	3.7-4.7 %
Mineral elements	
K	159-175 mg/100g
Fe	12 mg/kg
Na	5-10 mg/100g
Mg	20-29 mg/100g
Ca	11-14 mg/100g
Pb	0.045-0.047 mg/kg
Ni	0.28-0.42 mg/kg
Zn	4.5-6.2 mg/kg
Cu	0.12-0.16 mg/kg
Mn	1.8-2.1 mg/kg
Co	0.009-0.01 mg/kg
Cd	0.019-0.023 mg/kg
Cr	0.11-0.13 mg/kg

The nutritional value of the berries is high. Sea buckthorn contains a series of chemical compounds including carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, tannins, etc. They are especially rich in flavonoids and vitamin C. These above-mentioned compounds are of interest not only from the chemical point of view, but also because many of them possess biological and therapeutic activity including antioxidant, antitumoral, hepato-protective and immunomodulatory properties. Vitamin C in sea buckthorn, together with tocopherols and tocotrienols, has a strong antioxidative effect. The berries seem to have preventive effects against cardiovascular diseases, mucosa injuries, and skin problems, evidently through the enhancement of cell membrane regeneration. Because of these effects, sea buckthorn containing bioactive compounds is often used in traditional medicine (Tiitinen et al., 2005; Gulijev et al., 2004).

Some 3000 phytochemicals have been identified to date, although not all have been synthesized and only a few have been studied nutritionally. Those that have been studied have in common an ability to act as antioxidants and they include some well recognized names: the tocopherols (vitamin E), the carotenoids (β-carotene) and quinines (CoQ10). The main classes of phytochemicals with a potential for antioxidant activity are: ascorbic acid, carotenoids, tocopherols, polyphenols, flavonoids, coumarins, terpenoids, anthocyanins, quinines, isoflavones (Shrimpton, 2000).

The strong association between fruit and vegetable intake and cancer or heart disease prevention has been explained by the content of antioxidant phytonutrients (Halliwell, 1997). Besides the commonly mentioned antioxidants (vitamin C, tocopherols and carotenoids), flavonoids can also act as cancer preventing nutrients (Halliwell, 1997) and contribute significantly to the antioxidative activity of the diet (Rice-Evans et al., 1997; Gao et al., 2000).

The physico-chemical properties are the quality indicators of the product. Tang and Tigerstedt (2001) reported that a substantial part of the geographic variation in physico-chemical characteristics of sea buckthorn is associated with the geographic origin of the populations. It follows that it is important to evaluate the quality or physico-chemical properties of the berries in regions where the berries grow.

Each individual fruit or vegetable is unique. Its behaviour is determined by genetic make-up (species, cultivar, clone, etc), its stage of development (maturation, stage of ripening, etc) and the pre- and post-harvest conditions it has experienced (Aked, 2000).

Physical properties of the berries include the appearance, which could be characterised by uniformity of size, shape and colour. To determine the quality of the fruits visually, we have to check the colour and colour uniformity, glossiness, and absence of defects in shape or skin finish and freedom from disease (Aked, 2000).

The firmness of the fruits can easily be measured mechanically. The most common method of assessing firmness is with a penetrometer, which is in common use for evaluating various components of the strength of plant tissues, which change with storage (Aked, 2000). In our work we used a texture analyzer to measure the firmness of the berries, which is one of the characteristics of maturity.

In the present work, the physical properties characterized include size and shape of the berries, as well as the mass and firmness of sea buckthorn berries. The physico-chemical properties subdivision can also include the moisture content.

The titratable acidity, pH, reducing sugars, and also different vitamins are the chemical characteristics which are used in the present investigation to describe the sea buckthorn varieties.

1.1.4.1 Sea buckthorn as a source of vitamin C

In *H. rhamnoides*, extensive variations in vitamin C have been revealed among individuals, populations and subspecies. The vitamin C concentration ranges from 28 to 310 mg/100g of berries in the European subspecies *rhamnoides*, from 40 to 300 mg/100g of berries in Russian cultivars belonging to subspecies *mongolica*, from 460 to 1330 mg/100g of berries for subspecies *fluviatilis*, and from 200 to 2500 mg/100g of berries in Chinese subspecies *sinensis*. Temperature, different harvesting time, origin and processing also affect the vitamin C contents of the juice (Zeb, 2004).

Vitamin C (ascorbic acid) is a six carbon weak acid with a pK of 4.2. It is reversibly oxidized with the loss of one electron form the free radical, semidehydroascorbic acid. Compared to other free radical species, semidehydroascorbic acid is relatively stable with a rate constant of decay of approximately 10^{-5} M^{-1} sec^{-1}. Further oxidation of semidehydroascorbic acid results in dehydroascorbic acid which probably exists in vivo in multiple forms. Dehydroascorbic acid can be reduced back to ascorbic acid via the same intermediate radical (Rumsey et al., 1999).

The structure of ascorbic acid was identified as the antiscorbutic factor “vitamin C” about 75 years ago. Since then, an impressive amount of clinical research based on the use of ascorbic acid to cure nearly any disease, from the common cold to cancer, has been conducted. The assumption that ascorbic acid is beneficial to our health is based on a simple syllogism: reactive oxygen species (ROS) are involved in most human diseases; ascorbic acid is an effective antioxidant, therefore ascorbic acid *must* be beneficial in protecting us from diseases (Arrigoni and De Tullio, 2000). Ascorbic acid is an antioxidant which efficiently scavenges toxic free radicals and other reactive oxygen species (ROS) formed in cell metabolism (Arrigoni and De Tullio, 2002).

Vitamin C plays an important role in human physiology as a cofactor or cosubstrate of several enzymes and as an intra- and extracellular antioxidant. Therefore, the content of these components as vitamins and natural antioxidants is an important parameter defining the quality of the berries (Kallio et al., 2002).

1.1.4.2 Sea buckthorn as a source of carotenoids

Carotenoids are among the most common natural pigments, and more than 600 different compounds have been characterized until now, with β-carotene as the most prominent (Stahl and Sies, 2003).

Only plants, bacteria, fungi, and algae can synthesize carotenoids, but many animals incorporate them from their diet. Carotenoids serve as antioxidants in animals, and the so-called provitamin A carotenoids are used as a source for vitamin A. Several reports have demonstrated that plant pigments play important roles in health. The preventive effects have been associated with their antioxidant activity, protecting cells and tissues from oxidative damage (Stahl and Sies, 2003; Schoefs, 2002). Due to their unique structure it can be suggested that they perform specific tasks in the antioxidant network such as protecting lipophilic compartments or scavenging reactive species generated in photooxidative processes. They may further act as light filters and prevent oxidative stress by diminishing light exposure. The possible role of carotenoids as prooxidants and the implication of their prooxidant activity in adverse reactions remain to be elucidated (Stahl and Sies, 2003).

Sea buckthorn fruits (*Hippophae rhamnoides*) are an extremely rich source of carotenoids, a natural, highly conjugated class of pigments. Additional functions for carotenoids, including some non-vitamin A carotenoids, include cancer prevention and treatment, immunoinhancers and in vivo antioxidants (Crapatureanu et al., 1996).

Carotenoids are efficient antioxidants protecting also plants against oxidative damage (Stahl and Sies, 2003).

1.1.4.3 Sea buckthorn as a source of flavonoids

Flavonoids are polyphenolic compounds that have the diphenyl propane (C_6-C_3-C_6) skeleton (Karakaya, 2004).

Flavonoids – a class of secondary plant phenolics, abundant in plant kingdom – are found in many food products of plant origin such as vegetables, fruits, berries, tea and wine. More than 4,000 flavonoids have been identified so far and they have been categorized according to several subclasses, they are widely distributed in the leaves, seeds, bark and flowers of the plants, where these compounds afford protection against ultraviolet radiation, pathogens, and herbivores (Puupponen-Pimiä et al., 2002; Baranowska and Raróg, 2001; Heim et al., 2002).

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Kähkönen et al., 1999).

Fruit and vegetable intake is associated with a reduced risk of cancer and cardiovascular disease (Heim et al., 2002).

The phenolics owe their antioxidant activity mainly to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Kähkönen et al., 1999; Shahidi and Naczk, 1995).

Berries and fruits contain a wide range of flavonoids and phenolic acids that show antioxidant activity. Main flavonoid subgroups in berries and fruits are anthocyanins, proanthocyanins, flavonols, and catechins (Kähkönen et al., 1999).

Among all the flavonoids, which display a significant array of pharmacological activity, quercetin (2-(3,4-dixydroxyphenyl)-3,5,7-trixydroxy-4H-1-benzopyran-4-one) is most commonly presented in food such as onions, tea, apples and red wine. Quercetin is an important dietary constituent because it is most widespread and, consequently, the most studied flavonoid. As all flavonoids, quercetin exhibits potent antioxidant activity, due to free radical scavenging (Pejic et al., 2004).

Flavonoids that exist in foods are usually glycosylated. The linked sugar is often glucose or rhamnose, but can also be galactose, arabinose, xylose, glucuronic acid, or other sugars. The number on sugars is most commonly one, but can be two or three, and there are several possible positions of substitution on the polyphenol. The sugars can be further substituted. The glycosylation influences chemical, physical, and biological properties of the flavonoids (Karakaya, 2004).

In the human body flavonoids act as effective antioxidants mainly due to their phenolic hydroxyl groups. Many of their health-promoting, positive effects are probably based on this property. In addition, flavonoids exhibit various physiological activities including anti-inflammatory, antiallergic, anticarcinogenic, antihypertensive, antiarthritic and antimicrobial activities. Epidemiological studies indicate that they have anticarcinogenic and cardioprotective effects (Puupponen-Pimiä et al., 2002; Baranowska and Raróg, 2001; Careri et al., 2000). Their cardioprotective effects stem from the ability to inhibit lipid peroxidation, chelate redox-active metals, and attenuate other processes involving reactive oxygen species (Heim et al., 2002).

UV/VIS spectroscopy has been used mainly for identification of the flavonoid type and definition of the oxygenation pattern (Baranowska and Raróg, 2001).

Over the past few years, various liquid chromatographic methods with UV-Vis absorption or diode-array ultraviolet, fluorescence and, more recently, with mass spectrometric detection have been developed for the analysis of these substances (Careri et al., 2000).

Figure 2 Possible uses for components in different sea buckthorn plant parts (Li and Schroeder, 1996)

An inverse relationship between the intake of flavonols and flavones and the risk of coronary heart disease, and lung cancer has been shown in epidemiological studies (Häkkinen et al., 1999).

1.2 Application of sea buckthorn

All the sea buckthorn parts - bark, leaves, fruits and seeds - find use owing to their chemical composition. The main fields of use include medicine, food industry and cosmetics.

Sea buckthorn can be used for many purposes shown in Figure 2.

In Figure 3, the various applications of sea buckthorn we used in our study are given in bold.

Figure 3 Possible uses of components of sea buckthorn

1.2.1 Sea buckthorn fruits

Sea buckthorn (*Hippophaë rhamnoides* L.) berries have a unique, strong flavour. They are known to be quite acidic and not very sweet with a mild but characteristic aroma (Tiitinen et al., 2005).

Berries could be added to different canned foods, salads, sauces, marmalades etc.

Sea buckthorn fruits subspecies, particularly *H. sinensis* have been revealed as containing much higher concentrations of vitamins A, B_2, C than other fruits and vegetables such as carrot, tomato, orange etc. Sea buckthorn berries also show appreciable levels of vitamin B_1, P and K. The high vitamin concentrations make sea buckthorn fruit highly suitable for the production of nutritious soft drinks (Zeb, 2004).

1.2.2 Sea buckthorn juice

Sea buckthorn bears a fruit that is high in several nutrients such as vitamin C, carotenoids and tocopherols and which is becoming noticed as a source of nutraceutical materials worldwide. One product which is available from the berry is a cloudy juice that can be obtained either through centrifugation or by using press technologies. Cloudy juices have become a fast-growing sector in the juice industry, especially in Europe, and more recently in North America (Beveridge and Harrison, 2001).

Juice extraction from the berries has not been widely addressed except the casual comment that the berries were pressed. Pressing technologies are standard for the removal of liquid from fruit mash, and there is no reason to suppose that the standard procedures would not work for sea buckthorn berry. The usual press types utilize cloth presses, serpentine belt presses, and screw presses. Rack and cloth and serpentine belt presses are likely to work reasonably well, and apparently give juice yields of ~67% (w/w) (Beveridge, 1999).

Washing the berries with water or addition of a wetting agent or detergent in the wash water would be beneficial. The temperature is normally kept at 40°C. The berries are now ready to extract juice for further processes (Zeb, 2004).

Sea buckthorn juice can be obtained by either conventional pressing technologies or application of decanter centrifuge technologies. The juice expressed by these techniques is high in suspended solids and provides 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. Natural, freshly-pressed sea buckthorn juice is a cloudy system and juice stored refrigerated overnight separates into three phases; a floating phase, a settled phase and a cloudy aqueous phase between the floating and settled phases (Beveridge and Harrison, 2001).

Sea buckthorn juice is yellow in colour; the high amount of carotene is responsible for this yellow coloration. The presence of some of the other pigments also contributes to the colour (Zeb, 2004).

1.2.2.1 Encapsulation

Encapsulation has found numerous applications in the food and pharmaceutical industries for coating colorants, flavours and other bioactive ingredients in an effort to increase their shelf life (Balassa and Fanger, 1971; Dziezak, 1998; Jackson and Lee, 1991; Kibry, 1991; Shahidi and Han, 1993).

Recently, natural water-soluble polymers have been extensively investigated as a matrix to entrap molecules of biological significance. The loaded beads are then used as immobilised biocatalysts or for controlled release of the entrapped molecules (Ouwerx, 1998).

Furcellaran is an anionic sulphated polysaccharide extracted from the red algae *Furcellaria lumbricalis*. Furcellaran is a copolymer of β- and κ-carrageenan (Hugerth and Sundelöf, 2001) and is usually represented structurally as a repeating unit of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose residues, with a part of the latter existing as a 3,6-anhydro derivative (Glicksman, 1984). Furcellaran forms gels, undergoing a coil (disordered sol state) to helix (ordered state) transition, triggered by a reduction in temperature and/or through ionic interactions. Being non-toxic, biodegradable, biocompatible polysaccharide, furcellaran is a very promising biomaterial for encapsulation.

Carrageenan has often been used as immobilization material. Sipahigil and Dortunc (2001) proved the usefulness of ι- and κ-carrageenan for the controlled release systems of a freely water-soluble drug, verapamil HCl and a slightly soluble drug, ibuprofen. Belyaeva et al. (2004) found that for carrageenan beads containing cross-linked α-chymotrypsin no activity loss upon storage was detected in the course of 2 weeks at least. Cassidy et al. (1997) demonstrated that carrageenan might be a useful long-term carrier for bacteria. Immobilised *Pseudomonas aeruginosa* UG2Lr cells survived well for at least four years.

We did not find any source of information about the application of furcellaran as an immobilizing material.

1.2.3 Residue

A pigment termed "sea buckthorn yellow" can be extracted from sea buckthorn waste material and also from the berries, pressed juice, or pulp. As a by-product it would seem most useful to use the residue remaining after the juice was extracted (Li, 2002; Beveridge, 1999).The waste material could be the press cake remaining after juice extraction or the sediments remaining after centrifugation. In one process the pigment is extracted with low concentrations of alcohol after concentrating the suspension to 11°-13°Brix. The waste material is spray dried to yield a yellow powder. It contains flavones but also, carotene and vitamin E. Supercritical CO_2 has also been used to extract a yellow colouring material from sea buckthorn waste. Pressure had the greatest influence on extraction with yields increasing along with extraction pressure. A yield of 64% total carotenoids was achieved under processing conditions of 60 MPA, 85°C (Li, 2002).

The large volume of "waste" materials from sea buckthorn, such as leaves, fruit, pulp and seed residues from juice and oil extraction, can be developed into a valuable product. Sea buckthorn berry and seed residues contain valuable chemical substances at low concentrations (Li, 2002).

1.2.3.1 Technical solution

Dried sea buckthorn pressing residue can be used for many different purposes such as nutritional supplement as well as a therapeutical item. In Estonia there are some patterns concerning the dried pressing residue. "Granulated dry product made from sea buckthorn pressing residues" (Annist et al., 2004) describes the sea buckthorn "flour" production process. After juice making the pressing residue was dried (not above +75°C) to moisture content 6-8%, and disintegrated. Then the product – sea buckthorn flour – is ready to be used for a number of purposes: to as an additive to food or as a component in food supplements, like in the pattern "Honey mix with pollen (stimopol), rosehip and sea buckthorn addition" (Ehala and Vokk, 2004).

The disintegrated sea buckthorn flour was also used in bread making which had a positive effect on the colour and flavour of bread (Lõugas et al., 2005).

1.3 Essential oil

Essential or volatile oil, which is isolated from natural sources, is a complex mixture consisting of a great variety of volatile, low molecular mass aroma compounds (Margetts, 2005).

Identification of aroma compounds in natural products and foods is still a challenge, despite the sophisticated techniques now available to analysts. Flavour and fragrance compounds are usually present in these matrices at very low concentrations (ppm or even ppb). In addition, they have a wide range of polarities, solubilities, volatilities, and thermal and pH stabilities. The matrices that contain them may be very complex and cause interference with the isolation techniques. Therefore, there is no single, simple method for the identification of aroma compounds (Margetts, 2005).

The most common technique to obtain essential oils is steam distillation, where steam is generated in a separate boiler and then blown through the botanical material which has been placed on racks in a still. Water, and water and steam distillation methods are also commonplace. The steam and the volatile essential oil are condensed, and the oil is then separated from the water. The essential oil may be recovered as a single fraction or as different fractions collected over the duration of the distillation. Essential oils produced using these methods differ from the original oil in the botanical material as a result of changes that take place during the distillation. These changes are due to a number of factors such as the volatility of the components in the steam, hydrolysis due to the mildly acidic distillation conditions, further chemical rearrangements, interaction between the components and losses of components due to their solubility in the distillation water (Margetts, 2005).

MATERIALS AND METHODS

2.1 Materials

In this thesis, varieties of sea buckthorn berries grown in Estonia are described.

Sea buckthorn berries were harvested in September 1998 and 2005 from different cultivars grown in Estonia (marked as BOL – Botanicheskaja Ljubitelskaja, VOR - Vorobjevskaja, AVR - Avgustinka, TRR - Trofimovskaja, PSR – Podarok Sadu, OTR – Otradnaja, BOR – Botanicheskaja, HPR – Gibrid Pertchika, PER – Podarok Sadu). After some experiments the berries were kept frozen for further studies at -40° C. The fruits were cleaned to remove foreign matter.

Some sea buckthorn products like sea buckthorn oil (from the juice), medical oil (sea buckthorn oil produced by "Алтайвитамины", Russia), sea buckthorn seed oil and percolated oil (sea buckthorn dry pressing residue is percolated 5 times with rapeseed oil) were also used. Essential oil of sea buckthorn is prepared by steam distillation. All these oils, except for medical oil from Russia, were prepared on a laboratory scale.

A commercial sample of furcellaran isolated from *Furcellaria lumbricalis* was provided by AS Estagar (Estonia). Sea buckthorn juice for encapsulation was made by separation of crushed berries harvested in Estonia, 2003.

Such carotenoids as β-cryptoxanthine, zeaxanthin, lycopene were obtained from Extrasynthese (Genay, France), whereas β-carotene, quercetin and xanthophyll were obtained from Sigma-Aldrich (Steinheim, Germany). β-sitosterin was obtained from Roth (Karlsruhe, Germany).

All solvents were of chromatographic grade and were purchased from Rathburn Chemicals Ltd. (Scotland) and from Riedel-deHaën (Germany).

2.2 Methods

2.2.1 Moisture content

Paper II and VI

The moisture content was characterized using halogen moisture analyser HR83 (Mettler Toledo, Switzerland).

2.2.2 Dimensions and size distribution of fruit

Paper II and VI

Firmness of berries is one of the most important characteristics for a fresh market cultivar, which is related to both the stage of maturity and the variety itself. Harvested sea buckthorn berries undergo various processing operations both on the estate and in the factory, requiring basic information on the physical properties of the sea buckthorn berries. The information on physical properties, such as size, weight and crushing strength of berries are required for the development of the grading system for berries.

In order to determine the size and shape of the fruit, three principal dimensions, namely length, width and thickness, were measured using a micrometer. The geometric mean diameter (D_g) of the fruit was calculated by using the following relationship (Mohsenin, 1970):

$$D_g = (LWT)^{\frac{1}{3}} \qquad (1)$$

where L is the length, W is the width and T is the thickness.

According to Mohsenin (1970), the degree of sphericity (Φ) can be expressed as follows:

$$\Phi = \left(\frac{(LWT)^{\frac{1}{3}}}{L} \right) * 100 \qquad (2)$$

This equation was used to calculate the sphericity of fruits in the present investigation.

To obtain the mass of the berries, 30 fruits were weighed by a chemical balance reading to an accuracy of 0.0001g before they were frozen.

2.2.3 Puncture resistance

Paper II and VI

The puncture resistance of sea buckthorn berries was characterized using a texture analyzer TA-XT2i (Stable Micro Systems, UK).

The measurements at constant rate 1 mm/s were made with a 5 mm cylindrical probe. The samples were placed centrally on the blank plate, secured on the heavy duty platform, and the probe penetration test is commenced around the mid region of the fruit. The maximum force required to make the puncture on the fruit surface was taken from the force–time curve as shown in Fig. 2. The puncture resistance was measured with 15 single fruits (replications) and the average values are reported.

2.2.4 Titratable acidity, pH and reducing sugars

Paper II and VI

The titratable acidity is determined by titration with 0.1N NaOH solution to the end point of phenolphthalein. Titratable acidity is expressed in g of acid per 100 g of product by using the factor for citric acid (0.064).

The pH is measured using digital pH-meter Sentron 2001 (Intergrated Sensor Technology, the Netherlands).

The reducing sugars were determined according to the Lane-Eynon volumetric method (AOAC, 1995). Sugars were expressed as the percentage of reducing sugars.

2.2.5 HPLC

Paper II and VI

Vitamin C content was determined using the HPLC method. Samples (5 g) were homogenized with mortar and pestle with extraction solution (0.0095M KH_2PO_4 buffer (pH 3.0) with 5% methanol) and transferred into 50-ml volumetric flask. The sample was filtered through a Whatman no. 41. 10 ml of extracts were centrifuged at 27,000 *g* for 5 min at 20°C. Sample aliquots were filtered through a 0.45 μm poly(tetrafluoroethylene) syringe filter prior to injection. Analysis was performed using Gilson 321 HPLC pumps equipped with a Gilson UV/VIS-151 detector. Reverse-phase separation was attained using a C-18 column Inertsil ODS-2, 5µ (250 mm, I.D. 4.6 mm) and pre-column Inertsil ODS-2, 5µ (20 mm, I.D. 4.6 mm). The mobile phase was 0.0475M KH_2PO_4 buffer (pH 3.0) with 3% methanol and 2% acetonitrile. The flow rate was 1.0 mL/min, and the detection wavelength was 245 nm. The device was calibrated with standard solution before injection.

2.2.6 β-carotene content

Papers I, II, IV, VI

To determine the content of β-carotene the UV-spectrophotometric method was used (USP XXII, 1990).

2.2.7 TLC

Papers I, IV

Plates of silica gel Polygram ® SIL G (Macherey-Nagel, Germany) (20 x 20 cm, thickness 0.25 mm) were used. Standard solutions were prepared in chloroform. In our study the optimal developing solvent system consisting of hexane – ether – ethyl acetate (7:2:1), found in a preliminary study (Lõugas, 2001), was selected for further investigations and 5% phosphorus-molybdenic-acid solution in ethanol was used as a developer.

Extracts of sea buckthorn berries were prepared using chloroform as a solvent. The standards and berry samples were carried to the TLC plates with pipettes, microcapillary calibrated, 20µl (Drummond Scientific Company, U.S.A.). TLC plates were loaded with 20µl of samples (both standards (see p. 20) and extract samples were put on the same plate).

2.2.8 CE

Papers I, VII

Several methods have been developed to analyse the polyphenols in plant matrices. Thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC) and HPLC-mass spectrometry (LC-MS) are the most powerful analytical separation methods (Vaher and Koel, 2003).

Capillary electrophoresis has gained widespread interest as a favourable technique for the determination of pharmacologically interesting compounds in biological matrices such as plants. The most attractive advantages of CE are rapidity of the method, small sample amounts required and a strictly limited solvent waste (Vaher and Koel, 2003).

Different methods to determine the flavonoids, eg rutin, quercetin, have been used – UV-spectrophotometrically using Kalman filter approach (Hassan et al., 1999).

Capillary electrophoresis analyses were performed using an in-house built CE system described in *Paper VII.*

In the present work CE separation was performed with 20mM sodium tetraborate (pH 9.3), using an applied voltage of 16 kV at 25°C. The detection was performed at 210 nm. The standards and samples were injected into capillary gravitationally by elevation of the inlet buffer vessel for 12 cm at a fixed time of 20. The capillary was conditioned prior to use with 1M NaOH for 20 min and with H_20 for 30 min. After each run, the capillary surface was regenerated by sequential washing with 0.1M NaOH, H_20, and the separation buffer for 3 min each.

2.2.9 Encapsulation

Papers III, V

2.2.9.1 Preparation of the gel beads

Furcellaran gel beads were prepared by using ionotropic gelation process. The furcellaran solution with or without sea-buckthorn juice was dropped through a syringe (2 mm in diameter) into the magnetically stirred aqueous salt solutions. The solidified white beads were formed immediately and allowed to stand for 15 minutes in the cation solution with mild agitation at room temperature. The gel beads were filtered and washed with distilled water before measurement. The diameter of beads formed in different conditions varied from 4.3 to 5 mm, while the diameter of the beads realised in a particular condition varied within ±0.2 mm. Furcellaran concentration, type and concentration of cations and the outer phase volume were varied in different experiments and their influence on the bead shape was studied by visual observation.

2.2.9.2 Gel strength of the gel beads

The measurements were performed on a TA-XT2i Texture Analyser (Stable Micro Systems, Surrey, UK), using the data analysis software package Texture Expert Exceed. The bead was placed in the centre of the compression plate after gentle drying on filter paper. The 12 mm Cylinder Probe was used with a crosshead speed of 0.2 mm/s. Measurement was repeated 10 times on different beads formed under identical conditions.

2.2.9.3 Encapsulation efficiency of the beads

Beads were crushed using a mortar and a pestle and β-carotene was extracted from a sample applying USP XXII method. The sample was assayed spectrophotometrically at 455 nm for β-carotene. Furcellaran did not interfere with the absorbances at these wavelengths.

2.2.10 Steam distillation

There is a variety of different methods of obtaining volatile oils ranging from cold or hot expression, aqueous infusion, organic solvent extraction, steam distillation and supercritical carbon dioxide (CO_2). Of these, steam distillation and organic solvent extraction are the most commonly used (Deans, 1991).

The basic principle involved in steam distillation is to boil the fresh or dried plant material in distilled water, a process that results in the disruption of the gland cells and release of the volatile substances contained therein. The steam plus volatile oil passes through a cold-water condenser allowing the volatile oil fraction to float on top of the water. Collection can be made by drawing out the water followed by the volatile oil. Typically, steam distillation for 3 to 5 hours required, while the oil yield is around 1-2% (v/w) (Deans, 1991).

To obtain essential oil from fresh sea buckthorn berries, steam distillation of 4 hours was used (on Clevenger's apparatus).

2.2.11 GC

The chemical composition of the sea buckthorn *H. rhamnoides* L. oil and essential oil was determined using gas chromatography. Analyses were carried out on a Chrom-5 gas chromatograph, with a flame ionization detector, fitted with a fused silica capillary column coated with Polymetylsiloksane NB 30 as a stationary phase (50 m × 0.2 mm I.D., 0.25 μm). The carrier gas was He at a flow rate of 0.4 ml/min. The oven temperature was programmed to 320°C, the column temperature ranged from 50°C to 260°C. The sample injected consisted of 2 μl of extract diluted in hexane. To obtain the results the integrator SP 4100 (Spectra-Physics) was used.

PRESENT INVESTIGATION

3.1 Objectives

The objectives of the current study were as follows:

- To study the physical and chemical characteristics of sea buckthorn berries
- To study the content of some bioactive compounds in sea buckthorn berries
- To study the sea buckthorn oil and essential oil (obtained by steam distillation) by GC
- To carry out comparative evaluation of different investigated sea buckthorn varieties

3.2 Results and discussion

3.2.1 Moisture content of raw material

The moisture content was measured in all nine species of sea buckthorn berries. The average values are reported and are presented in *Paper V, Table 1 and* in *Paper II, Table 1*. The values are in the range of 84.12-86.87 % and 80.33-85.82 % in 1998 and 2005, respectively.

In *Paper II, Table 1*, also the water activity of the berries is given.

3.2.2 Dimensions and size distribution of the fruit

Three principal dimensions, namely length, width and thickness, were measured using a micrometer. These data are given in *Paper V, Table 2* and in *Paper II, Table 2*. The length of the berries is between 12.19-15.24 mm in 1998 and 10.64-13.71 mm in 2005, the longest berries in 1998 are those of Pertchika variety and the shortest are of Podarok Sadu variety, but in 2005, those of Botanitcheskaja Ljubitelskaja and Podarok Sadu, respectively. The width of the berries is in the range of 9.23-11.69 mm in 1998 and 7.79-9.03 mm in 2005, the widest berries in 1998 belong to the variety Avgustinka and the narrowest to the variety Pertchika, in 2005, to Trofimovskaja and Podarok Sadu, respectively.

According to formula 1 in *Paper V* there is calculated geometric mean diameter, which is between 10.28-12.57 mm in 1998 and 8.64-10.36 mm in 2005, largest value belongs to Avgustinka variety in 1998, the smallest value to

Podarok Sadu, in 2005, however, to Trofimovskaja and Podarok Sadu, respectively.

Sphericity is calculated on the assumption of formula 2 in *Paper V*. The certain values are given in *Paper V, Table 2* and in *Paper II, Table 2*. This formula takes into account the length, width and thickness of the berries and as a result it is possible to evaluate the shape of the berries. Most spherical berries in 1998 are in Avgustinka variety; simultaneously most oval berries are in variety Pertchika, in 2005 also Avgustinka and Botanitcheskaja Ljubitelskaja, respectively.

Also there is given the mass of the berries in *Paper V, Table 1* and in *Paper II, Table 2*. The lightest berries in 1998 are in Podarok Sadu variety and they weight 0.51 g, in 2005 the same variety has the lightest berries (0.49 g). Variety Gibrid Pertchika in 1998 and Trofimovskaja in 2005 have the heaviest berries, 0.71 and 0.85 g, respectively.

3.2.3 Puncture resistance of the fruits

A typical force-time curve for puncture resistance of sea buckthorn fruit is shown in *Paper V, Figure 1* and in *Paper II, Figure 2*. When the probe moves down onto the fruit, a rapid rise in force is observed. During this stage the sample is deforming under the applied force but there is no puncturing of the tissues. This stage ends abruptly when the probe punctures through the skin and begins to penetrate into the internal tissue of the sample, often called the bioyield point. The bioyield point occurs when the probe begins to penetrate into the fruit, causing irreversible damage. The first peak is the force required to puncture the surface of the sample. The second peak is obtained as a result of the prongs penetrating through the lower surfaces. This peak is due to the movement of the prongs towards the base plate.

It is usually desired that, whilst being ripe, the fruit still maintain a high degree of mechanical strength to protect the fruit from damage, such as bruising, during transport and handling. The rupture force of sea buckthorn berry varieties before and after freezing in two different years is shown in *Paper V, Figure 6*. As it can be seen, the strongest fruits were from the Podarok Sadu variety (303.87 g) and the weakest were from Botanitcheskaja Ljubitelskaja variety (199.43 g). The puncture resistance of the berries is influenced on freezing and defrosting, as it can be seen on *Figure 6* in *Paper V*. The fresh berries are stronger than the berries which are frozen and melted. The puncture resistance decreases after defrosting differently, variety Otradnaja has the biggest decrease (65 g) in the puncture resistance. As all the characteristics of the berries are influenced by climatic and other conditions, we can see, that the puncture resistance is not the exception – some varieties of berries collected in 1998 have after freezing higher values of puncture resistance than the berries collected in 2005 (*Figure 6* in *Paper V*, variety Avgustinka).

3.2.4 Determination of pH, titratable acidity and reducing sugars content in sea buckthorn berries

Applying the Lane and Eynon method (AOAC, 1997) we obtained data on the content of reducing sugars in sea buckthorn berries. The latter are given in *Paper V, Table 3*. The results concerning the pH, titratable acidity of sea buckthorn berries and sugar/acid ratio are also given in *Paper V, Table 3.*

The pH is in the range of 2.9 to3.1, which is quite normal for sea buckthorn berries. The AVR, BOL and VOR varieties have the lowest value, whilst the highest value belongs to the varieties PSR and TRR.

The titratable acidity is in the range of 2.33 g/100g (variety OTR) to 3.00 g/100g (variety AVR).

The content of reducing sugars is 1.88-2.65%. It is quite a common range for sea buckthorn berries. One variety (AVR) has the lower content of reducing sugars.

The sugar/acid ratio is from 0.3 to 1.14. As the reducing sugars content of variety AVR is low, the sugar/acid ratio for that variety is low, too.

3.2.5 HPLC and vitamin C

Using the HPLC we found a not very high vitamin C content in sea buckthorn berries grown in different regions of Estonia as shown in *Paper V, Figure 3*. The berries collected in 1998 have higher vitamin C levels (69-154 mg/100g) than the berries collected in 2005 (48-96 mg/100g). Climatic conditions, region of plantation, harvesting time are among the many factors influencing the content of vitamin C in sea buckthorn berries.

In Figure 3 the HPLC chromatogram for sea buckthorn variety Pertchik is presented.

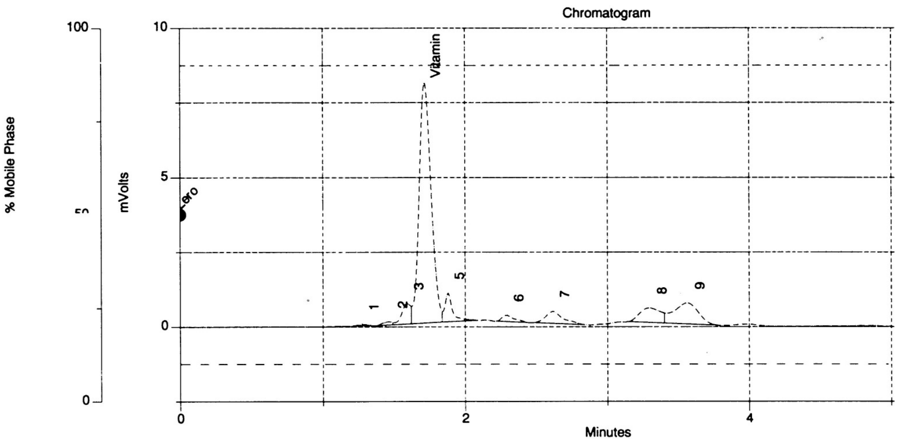

Figure 4 HPLC Chromatogram for sea buckthorn berry variety PER

3.2.6 β-carotene content

Applying the method in USP XXII, the content of β-carotene in different sea buckthorn berry varieties was calculated. In Figure 4, the spectrum of β-carotene is presented.

The β-carotene content in sea buckthorn berries varies according to the species and in 1998 is in the range of 9.1-25.3 mg/100g, whereas in 2005 it is in the range of 11 to 26.6 mg/100g. Results are shown in *Paper I, Paper IV, Table 2* and in *Paper V, Figure 4*. There is a dependence between the content of β-carotene and the colour of the berries (β-carotene is one of the main colour components). β-carotene content is also influenced by the growing year and other conditions, but it is not so sensitive as compared to vitamin C.

In addition, β-carotene content in different sea buckthorn preparations has been evaluated and compared with some commercial oil products purchased from the local drug stores. In *Paper V, Figure 4,* the obtained spectrophotometric data are presented.

Figure 5 Spectrum of β-carotene in sea buckthorn juice

3.2.7 TLC - carotenoids

For the experiments previously worked out different solvent systems (Lõugas, 2001) were used. Two solvent systems: hexane – ethyl acetate – methylene chloride (8.1:1) for β-carotene, lycopene, xanthophyll, β-sitosterin and β-cryptoxanthine; and hexane – ethyl acetate (6:4) for zeaxanthin were used.

Respective R_f values are reported in *Paper I* and *Paper IV*.

3.2.8 CE – quercetin, catechin and vitamin C

The CE – capillary electrophoresis method (Vaher and Koel, 2003) with some modifications was used and the first results to get optimal separation for quercetin in sea buckthorn berries are reported in *Paper I*.

Our previous work has shown that CE is an appropriate method for separating quercetin in complex mixtures (e.g. sea buckthorn berries). With the help of this method, it is also possible to determine other phenolic compounds, like catechin etc. The present study has given the results which are presented in *Paper VI, Table 1*. The results of determining the content of catechin in sea buckthorn berries are also given in *Paper VI, Table 1*.

Vitamin C content was also determined using improved CE method. We investigated different sea buckthorn extracts (CH_3OH/KH_2PO_4 and CH_3OH/H_20) to compare the results (CE and HPLC) and now we can assert that both methods are appropriate to determine both vitamin C and quercetin. All these results are reported in *Paper VI, Table 1*.

3.2.9 Encapsulation

The furcellaran concentration varied between 4% and 8% where the beads of typical spherical form were formed. When the furcellaran concentration was under 4% flakes formed and when it was over 8% a very viscous solution was obtained whereas no spherical beads formed. The gel strength increased along with increasing concentration of furcellaran. These results are presented in *Paper III, Figure 1*.

Dependence of gel strength on salt (KCl and $CaCl_2$) concentration in gel beads formed by dropping an 8% w/w furcellaran solution into various salt solutions is shown in *Paper III, Figure 2*. Both KCl and $CaCl_2$ were suitable for bead formation. Gel strength increased as KCl and $CaCl_2$ concentration was growing, and was more marked in case of KCl solution. The critical concentration necessary to form beads was 4% for KCl solution and 10% for $CaCl_2$ solution. Under these concentrations flake like particles was formed. The hardest and best spheres in shape were obtained by dropping 8% furcellaran dispersion into a 10% KCl solution.

The outer phase volume was investigated to optimise this formulation (*Paper III, Figure 3*). It can be seen that if the outer phase volume is small (furcellaran:KCl solution 1:1 or 1:2), the gel strength of the beads is small as well. When the outer phase volume increased, the gel strength as well as the spehiricity of the gel beads improved. The maximum gel strength was obtained in a furcellaran:KCl ratio of 1:4. Further increase of the outer phase volume decreases the gel strength only slightly.

The influence of sea buckthorn juice addition to the furcellaran gel beads properties has been studied in the following conditions: 8% furcellaran solution with different sea buckthorn juice concentrations was dropped into a 10% KCl solution in the ratio 1:3. The dependence of gel strength on the sea buckthorn juice concentration is shown in *Paper III, Figure 4*. It can be seen that sea buckthorn juice addition decreases the strength of the furcellaran gel beads. This may be caused by the acid degradation of furcellaran.

To examine the entrapment of β-carotene in sea buckthorn juice, the content of β-carotene in dried furcellaran beads was measured using the UV-spectrophotometric method (USP XXII). β-carotene content in sea buckthorn juice was 11.5 mg%. As it is shown in *Paper III, Figure 5* the β-carotene content increased with increasing the juice concentration in gel beads. While using 100% juice for making the furcellaran beads, the β-carotene content in the beads was 11.12 mg%, showing that the loading efficiency of β-carotene from sea buckthorn juice in furcellaran capsules was 97%. This factor suggests that furcellaran beads may be applied for β-carotene immobilisation.

3.2.10 Steam distillation to obtain essential oil

Steam distillation, using Clevenger's apparatus, was used to obtain essential oil from fresh sea buckthorn berries. Mixture of berries (moisture content 86%) was used. The yield of essential oil was 72 mg/kg, which is double compared with data given in literature (Li, Schroeder, 1996). We used the essential oil for further studies to characterise the oil composition.

3.2.11 GC analysis of oils

For GC analyses two different oils from sea buckthorn berries were used; one of them being sea buckthorn oil (separated from the juice surface) and the other essential oil obtained in steam distillation.

The essential oil consists of 52 components, not all of which components have been defined. The main components are alcohols, alkanes and esters. Sea buckthorn oil consists of 28 components and not all of them have been identified either. The respective results are given in Table 2. Sea buckthorn oil contains 3 terpenic compounds – α-Thyjone, (E)-β-Caryophyllene and (Z)-Nerolidol – we have not found any information about that in literature. To obtain more detailed information, GC-MS analyses have been done and further investigation is needed.

These results are not comparable with the data in other papers (Cakir, 2004; and others). The composition of oils depends on the growing region as well as some other factors as mentioned before.

Table 2 The composition of essential oil and sea buckthorn oil

	essential oil		**oil**	
1	2-Hexen-1-ol	0.2	α-Thyjone	0.5
2	(E)-2-Heptenal	0.2	n-Undecane	0.3
3	Ethyl hexanoate	3.8		0.3
4	Hexyl acetate	0.1		1.2
5		0.3	Decanal	0.8

6	n-Nonanal	0.7	n-Tridecane	0.2
7	Aliphatic ester	6.5		0.9
8		0.3	n-Tetradecane	1.8
9	1-Decen-3-ol	4.7	(E)-β-Caryophyllene	2.6
10	n-1-Nonanol	0.3	n-Pentadecane	3.6
11	Ethyl octenoate	0.8	(Z)-Nerolidol	0.4
12	Ethyl octenoate	5.6	n-Hexadecane	0.8
13	Octyl acetate	0.1	n-Heptadecane	3.0
14	Aliphatic ester	11.2	n-Octadecane	3.9
15		0.5	n-Nonadecane	3.6
16	n-1-Decanol	0.3		1.1
17	n-Tridecane	0.1		10.3
18	Ethyl decenoate	0.7	Palmitic acid?	21.0
19	n-1-Tetradecene	0.6		1.4
20	Ethyl decanoate	1.1	n-Eicosane	3.2
21	n-Tetradecane	0.3		1.4
22	Aliphatic alcohol	37.8	n-Heneicosane	5.2
23		3.0		3.6
24	n-1-Dodecanol	0.2	Stearinic acid?	18.4
25	n-Pentadecane	0.7		2.0
26	Aliphatic aldehyde	4.7	n-Docosane	1.3
27	n-1-Tridecanol	0.1		1.9
28	Ethyl dodecanoate	0.4		4.8
29	Dodecyl acetate	0.3		
30	Aliphatic alcohol	0.3		
31	n-1-Tetradecanol	0.1		
32	n-Heptadecane	0.4		
33	Myristic acid	0.2		
34	Ethyl myristate	0.1		
35	n-Octadecane	0.3		
36	n-Nonadecane	0.3		
37	Palmitic acid	0.2		
38	n-Eicosane	0.2		
39		0.3		
40		0.1		
41	n-Heneicosane	0.2		
42	n-Docosane	0.1		
43	Tricosene?	1.0		
44	Tricosene?	0.8		
45	n-Tricosane	1.6		
46		0.4		
47	n-Pentacosane	0.3		
48		0.3		
49		0.3		
50	n-Heptacosane	0.3		
51		0.4		
52	n-Nonacosane	0.4		

Comparative analysis of different varieties of sea buckthorn berries showed that even grown in the same area, the varieties of berries differ from each other in their physical and chemical characteristics. In addition, the physical and chemical characteristics depend on climatic conditions, including the growing year.

In the present thesis sea buckthorn berries grown in two different years have been compared. The size and sphericity of the berries varies from variety to variety also depending on the growing year. And all the other characteristics, eg moisture content, vitamins content etc vary in the same way. It can be said that two identical berries do not exist.

Some trends in varieties of berries can be observed, for example, the variety PSR has the highest β-carotene content every year.

Vitamin C content in sea buckthorn berries is more sensitive to climatic conditions – it is not possible to point out certain variety with the highest content.

CONCLUSIONS

Based on the current study, the following conclusions can be made.

Sea buckthorn is a very valuable source of bioactive compounds for food industry, and some physical properties of the berries and chemical characteristics are given.

Moisture content is in the range of 84,12-86,87% and 80,33-85,82% in1998 and 2005, respectively.

Calculated geometric mean diameter was between 10,28-12,57 mm in 1998 and 8,64-10,36 mm in 2005. The largest value belongs in 1998 to Avgustinka variety, the smallest value to Podarok Sadu, in 2005 Trofimovskaja and Podarok Sadu, respectively.

Most spherical berries in 1998 are of Avgustinka variety; whereas the most oval berries are of the variety Pertchika, in 2005 also of Avgustinka and Botanitcheskaja Ljubitelskaja, respectively.

The average mass of berries varied from 0.5 g to 0.69 g and from 0.49 g to 0.85 g, in 1998 and 2005, respectively. The lightest berries in 1998 and 2005 are in Podarok Sadu variety. The varieties Gibrid Pertchika in 1998 and Trofimovskaja in 2005 had the heaviest berries.

The rupture force depends on berries – if they are fresh or frozen. While the strongest fruits were of Podarok Sadu variety and the weakest those of Botanitcheskaja Ljubitelskaja variety, the puncture resistance decreases differently after defrosting. The variety Otradnaja has the biggest fall in puncture resistance. As all the characteristics of the berries are influenced by climatic and other conditions, we can see that puncture resistance is not an exception – after freezing, some varieties of berries collected in 1998 have higher values than the berries collected in 2005.

The pH of the berries is in the range of 2.9-3.1; the titratable acidity is between 2.33-3.00 g/100g; the content of reducing sugars is 1.88-2.65%.

The vitamin C content in sea buckthorn berries varies with varieties and growing years, in 1998 it is in the range of 69-154 mg/100 g. In 2005 the results are between 48-96 mg/100 g. The results obtained by different methods, namely HPLC and CE are similar.

The β-carotene content also varies being determined by various factors. As the β-carotene is the main carotenoid in sea buckthorn berries, we can evaluate this by visual observation and get confirmation by experiments: the β-carotene content is 9.1-25.3 mg/100 g in 1998 and in 2005 is in the range of 11-26.6 mg/100g. In addition, we have also evaluated the β-carotene content in some sea buckthorn preparations and commercial products.

Using the TLC method, β-carotene, lycopene, xanthophyll, β-sitosterin, β-cryptoxanthine and zeaxanthin have been identified in sea buckthorn berries.

Phenolics – quercetin and catechin – have been determined by CE. Two different extraction methods were used.

We used successfully furcellaran as a beads forming agent to entrap β-carotene from sea buckthorn juice. It follows that furcellaran beads may serve as an immobilisation agent of β-carotene and other bioactive compounds in sea buckthorn.

Using the steam distillation method we obtained the essential oil from sea buckthorn berries. The yield was 72 mg/kg.

The results of GC analyses are as follows: the essential oil consists of 52 different components, not all have been identified. Sea buckthorn oil has 28 different components and among them 3 terpenic compounds – α-Thyjone, (E)-β-Caryophyllene and (Z)-Nerolidol – have been found.

In addition, it could be pointed out that the nutritional value in terms of the content of vitamins and other biologically active compounds is better in varieties TRR and PSR for use in food products, for example, for preparing juice.

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KOKKUVÕTE

Astelpaju on maailmas laialt levinud põõsas, tema looduslikud levialad on Aasias ja Euroopas. Viimastel aastakümnetel on hakatud sellele väärtuslike marjadega põõsale pöörama järjest suuremat tähelepanu.

Eestis on juba kümneaastane kogemus astelpaju istandikega. Esimeste astelpaju katsetega alustati Rõhu katsepunktis juba 30 aastat tagasi. Täna viivad läbi erinevaid katseid astelpajuga ka Eesti Maaülikooli juures tegutseva Polli Aiandusuuringute Keskuse teadlased.

Astelpaju keemiline koostis on väga rikkalik sisaldades rohkesti vitamiine, karotenoide, flavonoide ja teisi bioloogiliselt aktiivseid toimeaineid.

Astelpaju marjad on nii toitumisteadlaste huviorbiidis, kui võimalik funktsionaalse toidu komponent, kui ka farmaatsiatööstusele huvipakkuv lähteaine, kus on välja töötatud mitmeid astelpaju sisaldavaid õlisid ja ravimeid. Loomulikult on astelpaju marjad ka väärtuslik lisand erinevatele toitudele (näiteks salatid, kastmed jne).

Astelpaju keemiline koostis sõltub paljudest erinevatest faktoritest, mõne näitena võiks tuua kliima, kasvatuspiirkond, ilm, kasvuaasta jne.

Antud töös on uuritud mõningaid astelpaju füüsikalis-keemilisi näitajaid ja bioaktiivseid komponente. Füüsikalistest näitajatest on määratud kahel erineval aastal kasvanud marjade suurust, kaalu, sfäärilisust, tugevust ja niiskusesisaldust. Uuritud on ka redutseerivate suhkrute ja tiitritavate hapete sisaldust ning pH-d. Vedelik-kromatograafia meetodiga on määratud vitamiin C sisaldus astelpaju marjades ning kasutades UV-spektrofotomeetria meetodit on leitud vitamiin A prekursori β-karoteeni sisaldus nii astelpaju marjades kui ka astelpaju toodetes ja saadustes. Kapillaar-elektroforeesi meetodiga on uuritud kahe fenoolse ühendi – kvertsetiini ja katehhiini – sisaldust. Ka on võrdlevalt uuritud vitamiin C sisaldust, mis lubab väita, et mõlemad meetodid, nii HPLC kui ka CE on sobivad meetodid erinevate bioloogiliselt aktiivsete ainete määramiseks. Õhukese kihi kromatograafia abil on tuvastatud erinevate karotenoidide sisaldus astelpaju õlis ja saadustes/marjades.

Üks alajaotus on pühendatud uudsele meetodile – kasutades furtsellaraani kapseldatakse bioloogiliselt aktiivsed toimeained. Tehtud on eelkatsed, kus uuriti geelistumise tingimusi furtsellaraani kapslite moodustumisele ning kapslite mehhaanilisi omadusi. Furtsellaraani kapslite kapseldamise võimet on kirjeldatud β-karoteeni sidumisvõimega astelpaju mahlast.

Veeaurudestillatsiooniga eraldati astelpaju marjadest eeterlik õli, mida analüüsiti gaas-kromatograafil. Uuriti ka astelpaju õli ja tehti kindlaks kolme terpeense ühendi – α-tujoon, (E)-β-karüofülleen and (Z)-nerolidool – olemasolu selles.

APPENDIX 1

ARTICLE I

T. Lõugas, R. Vokk (2004) Study on sea buckthorn (*Hippophae rhamnoides* L.) pigments as potential antioxidants in *Pigments in Food, more than colours… Ed. by L. Dufosse, ISBN 2-9521516-0-1, 238-240*

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APPENDIX 1

ARTICLE II

T. Lõugas, M. Liis, K. Laos, R. Vokk (2005) Physical Properties as a Tool for Quality Assessment in Fruit Processing – *Annual Transactions of the Nordic Rheology Society, Vol. 13, 295-299*

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APPENDIX 1

ARTICLE III

K. Laos, T. Lõugas, R. Vokk (2006) Encapsulation of β-carotene of sea buckthorn (Hippophae rhamnoides L.) juice in furcellaran beads – ***Innovative Food Science and Emerging Technologies (accepted)***

APPENDIX 1

ARTICLE IV

T. Lõugas, R. Vokk (2006) Carotenoid analysis in sea buckthorn berries – *Pigments in Food, Stuttgart-Hohenheim (accepted)*

APPENDIX 1

ARTICLE V

T. Lõugas, R. Vokk (2006) Characterization of physical and chemical characteristics of sea buckthorn (*Hippophae rhamnoides* L.) berries (*manuscript*)

APPENDIX 1

ARTICLE VI

J. Gorbatšova, T. Lõugas, R. Vokk, M. Kaljurand (2006) Comparison of the Various Antioxidants Content of Quick-Frozen and Chilled Stored Sea Buckthorn Berries Using Capillary Electrophoresis (*manuscript*)

APPENDIX 2

ELULOOKIRJELDUS

1. Isikuandmed

Ees- ja perekonnanimi	Tiina Lõugas
Sünniaeg ja –koht	22.01.1975, Kostivere
Kodakondsus	Eesti

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3. Hariduskäik

Tallinna Tehnikaülikool	2001-2006	Bio- ja toiduainetehnoloogia/doktorant
Tallinna Tehnikaülikool	1998-2001	Bio- ja toiduainetehnoloogia/magister
Tallinna Tehnikaülikool	1993-1998	Toiduainete töötlemine/insener
Kehra Keskkool	1990-1993	

4. Keelteoskus

Eesti keel	emakeel
Inglise keel	hea
Vene keel	rahuldav

5. Teenistuskäik

Sept 2004 - …	TTÜ, teadur

6. Teadustegevus

2002 – 2003	ETF grant nr. 5189 täitja
1998 – 2000	ETF grant nr. 3426 täitja
1998 – …	teadus- ja uurimistöö seoses õpingutega

7. Kaitstud lõputööd

Magistritöö	Astelpaju bioaktiivsete komponentide uurimine
Diplomitöö	Astelpajuõli kromatograafiline lahutamine

8. Teadustöö põhisuunad

bioaktiivsed komponendid,
marjade füüsikalis-keemilised omadused

APPENDIX 2

CURRICULUM VITAE

1. Personal particulars

First name and surname	Tiina Lõugas
Date and Place of birth	22.01.1975, Kostivere
Citizenship	Estonian

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3. Education

Tallinn Univ. of Technology	2001-2006	Bio-and Food Technology/PhD
Tallinn Technical University	1998-2001	Bio-and Food Technology/MSc
Tallinn Technical University	1993-1998	Food Technology/graduate engineer
Kehra Secondary School	1990-1993	

4. Languages

Estonian	native language
English	good
Russian	satisfactory

5. Professional experience

Sept 2004 - …	Tallinn Univ. of Technology, research scientist

6. Scientific research

2002 – 2003	ESF grant nr. 5189 executor
1998 – 2000	ETF grant nr. 3426 executor
1998 – …	research and study

7. Defended thesises

MSc thesis	Study on bioactive compounds of sea buckthorn
Diploma work	Chromatographic analysis of sea buckthorn oil

8. Current research program

Bioactive compounds

Physico-chemical properties of berries

APPENDIX 3

PUBLICATIONS RELATED TO THE TOPIC

1. A. Menert, **T. Lõugas**, R. Vokk and A. Orav – Identification of biologically active compounds of plant origin // 6th Wartburg Aroma Symposium, Eisenach, 10-13 April, 2000
2. R. Vokk, A. Menert, **T. Lõugas**, T. Sillamaa – Sea Buckthorn *Hippophae rhamnoides* L. II. Vitamins C and content in sea-buckthorn preparations. A preliminary study on antimicrobial activity // Food and Nutrition, TTU, Tallinn 2000, Vol. 8, 41-48
3. R. Vokk, **T. Lõugas** – Sea buckthorn – a valuable natural source for food enrichment // Conference "Nutritionists meet Food Scientists and Technologists", Porto, 11-14 April, 2000
4. R. Vokk, **T. Lõugas**, T. Sillamaa, M. Kravets – Toidu funktsionaalsed komponendid taimsest toormest // Eesti XXVII Keemiapäevad, 15.-16.November 2001, Tallinn
5. Kravets, M., Vokk, R., **Lõugas, T.** - "Nutrient and non-nutrient dietary bioactive plant compounds." // New Functional Ingredients and Foods, 9-11 April 2003, Copenhagen, Denmark
6. **Lõugas, T.**, Vokk, R., Keskpaik, L., Koel, M. - "Sea buckthorn and its bioactive compounds" // NJF's 22nd Congress "Nordic Agriculture in Global Perspective", 1-4 July Turku, Finland
7. **Lõugas, T.** – "Sea buckthorn and its bioactive components" // Food and Nutrition, TTU, Tallinn 2003, Vol. 11, 34-38
8. **T. Lõugas**, K. Laos, R. Vokk, M. Friedenthal – "Encapsulation of bioactive compounds of sea buckthorn in furcellaran beads" // "Marine Biopolymers – Structure, Functionality and Applications", A satellite to the XVIII International Seaweed Symposium, 27-29 June 2004, Trondheim, Norway
9. **T. Lõugas**, K. Laos, A. Mändmets, R. Vokk, M. Friedenthal – "Gel strength and encapsulation efficiency of furcellaran beads" // 4th International Congress on Food Technology: 18-19 February 2005, Piraeus, Greece "Accessing Useful Technologies…. Optimizing Food Safety and Nutrition"
10. **T. Lõugas,** T. Veskus, K. Martverk, R. Täht, K. Rada, R. Vokk – „Sea buckthorn and its new fields of use" // INTRADFOOD2005 25-28 October 2005 Valencia, Spain, Innovations in traditional foods Proceedings Ed by Pedro Fito and Fidel Toldra Vol 1, 719-722