HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

PHYTOCHEMICAL INVESTIGATIONS ON SEA BUCKTHORN JUICE ENRICHED WITH POMACE

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LIST OF ABBREVIATIONS

%:	Per cent
<:	Less than
>:	Greater than
C:	Control sample of sea buckthorn juice
DHBA:	Dihydroxybenzoic acid
EFA:	Essential fatty acids
FAO:	Food and Agriculture Organization of the United Nations
Fig:	Figure
FRAP:	Ferric Reducing Antioxidant Power Assay
FV:	Fruits and vegetables
H.rhamnoides:	*Hippophae rhamnoides*
ha:	Hectare
HDL:	High-density lipoprotein
HP:	High pressure
HPLC:	High-performance liquid chromatography
P0.5:	Sample of enriched sea buckthorn juice with 0.5% sea buckthorn pomace
P1:	Sample of enriched sea buckthorn juice with 1% sea buckthorn pomace
P2:	Sample of enriched sea buckthorn juice with 2% sea buckthorn pomace
PA:	Phenolic acids
pH:	negative log of hydrogen ion concentration
PME:	pectin methyl esterase
SB:	Sea buckthorn
so., spp:	Species
SSC:	Soluble solid content
THSD:	Tukey's Honest Significant Difference
TPC	Total polyphenolic content
USA:	United States of America
UV:	Ultraviolet
WHO:	World Health Organization
α:	Alpha
THF:	Tetrahydrofuran

CHAPTER 1 – INTRODUCTION

According to the report by the FAO of the United Nations (FAO 2014), 45% of fruit and vegetable wastes and by-products from the fruit and vegetable processing industry are generated around the world.

Depending on fruit and vegetable processing technology (e.g. drying and dehydration, juice technology, fruit jams, canning, jellies, marmalade, paste production, vegetable pickles, and sauerkraut technology), solid (e.g. pomace, pulp, peels, cores, seeds, and stems), as well as liquid (e.g. juices, wash water, chilling water and cleaning chemicals) waste streams are produced (Mirabella et al., 2014). They focused many research efforts on the recovering valuable compounds from these by-products or wastes generated during agricultural and food processing stages. Since these wastes also contain a significant amount of biologically active compounds, they can be used for the recovery of value-added products like polyphenols, glucosinolates, dietary fibers, essential oils, pigments, enzymes, organic acids, etc. (Galanakis et al., 2015; Deng et al., 2015, Heng et al., 2015).

Apart from being a substrate for bioactive compound isolation, fruit and vegetable by-products can contain functional additives in food. Moreover, fruit pomace, a by-product of juice or puree making industry, is a rich source of many nutrients including carbohydrates, minerals, polyphenols and vitamins. Fruit pomace has been utilized as animal feed after ensiling or after drying. Apple pomace, a by-product of the apple juice industry, could a source of dietary fiber and polyphenols in cake production (Sudha et al., 2007), while raspberry (Górecka et al., 2010), white grape (Mildner-Szkudlarz et al., 2013), and blueberry (Mišan et al., 2014) pomace have been used for cookie enrichment. Carrot pomace could be used in bread, cake, dressing, and pickles (Osawa et al., 1995) and onion pomace in snacks (Kee et al., 2000). Citrus by-products (lemon albedo and orange dietary fiber powder) have been added to cooked and dry-cured sausages to increase their dietary fiber content (Fernández-López et al., 2004), while orange juice fibers (peel, pulp, and seeds) have been used as a fat replacer in ice cream (Crizel de Moraes et al., 2013).

Sea buckthorn (SB) (*Hippophae rhamnoides* L.) excels as ingredient of functional foods, being outstandingly suitable because of its biologically active compounds. Primarily it has positive impact on human health because of its high vitamin C, flavonoid, carotenoid, and tocopherol content. In addition, it is rich in unsaturated fatty acids, proteins, and further vitamins (Surykumar and Gupta., 2011; Krejcarova et al., 2015). The wound healing effect of sea buckthorn is confirmed byte most modern studies as well (Upadhyay et al., 2010, Edraki et al., 2014).

Food waste is a critical subject in every industry, in every household; but in many cases, by-

products should not be considered as waste (Perino-Issartier and Abert-Vian, 2011). Fruit and vegetable wastes are produced in large quantities in food industry make up a source of nuisance in landfills because of their high biodegradability (Misi and Forster, 2002). The non-edible portion of fruits and vegetables after processing (waste), such as peels, pods, seeds, skins, etc., accounts for about 10–60% of the total weight of the fresh produce. Because of the significant presence of pectin, minerals, vitamins, and bioactive molecules, this waste offers a huge potential for its conversion into useful products, such as enzymes, ethanol, and bio colours (Sharma et al., 2016). The management of food processing by-products and wastes regarding their reuse and recycling through value addition (Krishna and Chandrasekaran, 2012).

Sea buckthorn pomace is a by-product produced in large quantity during sea buckthorn juice extraction, comprising pulp, seed and skin. It is a good source of phytochemical compounds like phenolic acids and antioxidant. The pomace is usually dried to extend its shelf life for further use, either as a feed supplement or a source of valuable products, e.g. oils extracted from the seeds and the fraction of peel and pulp (Nuernberg, 2015).

Along with reducing the wastes, the purpose behind their utilization is also the extraction of beneficial antioxidants. The present study's hypothesis was: the pomace and juice of sea buckthorn contain phenolic compounds that could add commercial value to these crops. The characterization of these SB pomaces' chemical composition and phenolic profile would contribute to the further development of natural health products and the native fruit industry in SB.

Exploring such material to use them in different ways can help in managing and reducing waste. So, the present study aims to explore the potential of selected fruit waste through phytochemical studies, antioxidant assays. These so-called wastes can also be turned into value-added products. The work will also focus on the various aspects by which these by-products can be utilized into juice industry.

The aim of this doctoral thesis was to investigate the effect of sea buckthorn pomace for several component of sea buckthorn juice during storage:

- Difference of chemical compound compared between samples of SB juice enriched with pomace (0.5%, 1% and 2%) and a control sample of sea buckthorn juice at 18-23°C storage temperature, and monitored for the changes in individual parameters (TPC, FRAP, beta-carotene, flavonoids pH and SSC) for 14 months samples of enriched and control SB juice the sampling took place every. We stored each storage sample in a separate container to avoid oxygen and microbial contamination during time sampling.

- The target of the statistical analysis was to determine whether the storage time or the different quantities of pomace influence changes of biologically valuable compounds during the test period.

Hence, the present investigation entitled “Phytochemical investigations on Sea buckthorn juice enriched with pomace” was undertaken with the following objectives:

1. Collect and prepare pomace and juice from selected SB berry, prepare the samples.
2. Study of storage stability of the juice sample during the time.
3. Analysing soluble solid content potential and pH.
4. Determination of colour parameters in enriched and SB juice.
5. Determination of total polyphenolic content (TPC) and antioxidant capacity (FRAP) in SB juice.
6. Determination of flavonoid values (rutin, quercetin and hydroxy benzoic acid) in enriched and sea buckthorn juice.
7. Determination of beta-carotene in SB juice.
8. Determination of ascorbic acid in SB juice.

CHAPTER 2 – LITERATURE REVIEW

2.1. Sea buckthorn

2.1.1. Brief description and geographical distribution

Sea buckthorn (*Hippophae rhamnoides* L.) is a plant as a shrub or tree, whose size rarely exceeds three to four meters in height, although some varieties can reach up to 20 meters. Its leaves, narrow and lanceolate, are silver-green on the upper surface (Gupta, 2000). The fruits classify as non-climacteric, and in the ripe state can be yellow, orange or red. They come in a slightly rounded oval and are gathered in clusters dense on twigs (Figure 1). Their weight normally varies between 4 and 60g 100^{-1} fruits may exceed 60g in some Russian cultivars. The average weight fruits of the Indian-Summer cultivar, ranges from 20 to 40g 100^{-1} fruits (Li and Beveridge, 2003). Each fruit contains a brown seed, which weighs about 16 mg (Harrison and Beveridge, 2002).

Figure 1. Sea buckthorn fruits (csp_Ursula1964)

All the species of the genus *hippophae* are called Sea buckthorn. Sea buckthorn belongs to the family Elaeagnaceae, which is in the major group *Angiosperms* (flowering plants). Genera in Elaeagnaceae include *Elaeagnus, Hippophae, Lepargyrea* and *Shepherdia*. Number of species under *Hippophae* is still unclear. The classification of genus *Hippophae* has been modified over the years. Six species (*Hippophae rhamnoides, H. salicifolia, H. tibetana, H. neurocarpa, H. gyantsensis* and *H. goniocarpa*) and 12 subspecies of sea buckthorn have been identified(Li and Beveridge 2003), of which two (*Hippophae rhamnoides* L. subsp. sinensis Rousi and *Hippophae rhamnoides* L. *subsp*. *rhamnoides*) are the most used for commercial purposes (Yang and Kallio, 2001).

Many scholars and experts have certified that the genus *Hippophae* originated in the Himalayan mountain regions and then spread to southwest, northwest and northern China and eastern Inner Mongolia. As well as to the northwest regions of Eurasia where one route progressed west to reach the Alps via the Caspian and Black seas before finally arriving at the northwest shore of the Scandinavian peninsula and another route progressed northwest to reach north western Mongolia and southern Siberia in the Russian Federation via India, Nepal, Pakistan, Afghanistan, the Xinjiang Uygur Autonomous Region in China and several Central Asian countries of the former Soviet Union. Sea buckthorn is a typical temperate plant of the Eurasian continent, widely distributed between 27º to 69º N latitude and 7º W to 122º E longitude (Lu, 1990).

Considering the vertical range of elevations, sea buckthorn has a very strong ecological adaptability. It can grow from the seashore of the Baltic Sea in Europe to 5200 m above sea level in the Mount Everest in Asia. In India, sea buckthorn is found in Himalayan regions, the cultivation is mainly concentrated in the cold deserts of Trans-Himalyas (Ladakh, Lahaul and Spiti) at an altitude from 2500 m to 4500 m.

Generally, sea buckthorn grows in temperate regions of the world and it naturally occurs in the arid, semi-arid and high mountainous ecosystems (Figure 2). It grows well in the following climatic conditions: the monthly average temperature of the hottest month is 15 to 25°C and the maximum radiation on clear days in the vigorous growing season is 23500 to 26000 calcm²; annual rainfall ranges from 250 to 500 mm (Lu, 2003).

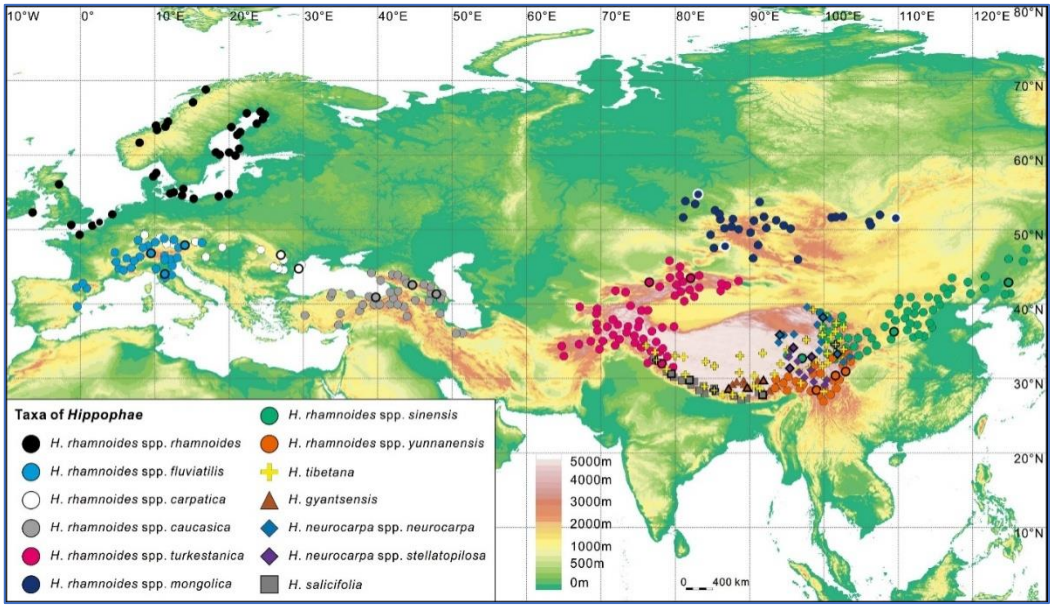


Figure 2. Geographic distribution range of *Hippophae* L. (Jia and Bartish, 2018).

Sea buckthorn (*H. rhamnoides* L.) is a hardy shrub, tolerant of temperatures between -43 and 40°C and drought conditions, yet requires irrigation in regions receiving less than 400 mm of rainfall per year (Li, 2003). Grows best in well-drained soil (e.g. sandy loam) with a pH of 6 to 7, however, can tolerate many soil conditions and pH levels except for extremes. The shrub is known for its extensive root system that develops quickly and is nitrogen fixing. Ripe sea buckthorn berries can be yellow, orange, or red, are spherical to elliptical in shape, and range in size between 3 to 8 mm in diameter (Li, 2003).

Sea buckthorn belongs to the group of thermophilic plant because it needs higher temperature to germinate seeds than those of apple and cherry which can germinate at 1 to 3°C. The word *Hippophae* is derived from the Latin word Hippo means horse and Phaos means shine as it was used in ancient Greece as horse's fodder that increased their weight and made their coat shiny. Sea buckthorn is the general English term given to genus *Hippophae*. It was classified in 1753 in "Species Plantarum" by Karl von Linné at the position 1023(Subedi and Adhikari, 2001).

2.1.2. Global presence of sea buckthorn

The sea buckthorn industry is active primarily in Russia and China with considerable interest growing in Germany and other parts of the world. It has been estimated that with an average production of 200 kg ha^{-1} (berries), the world's annual yield is approximately 280,000 t of berries. The growing demand for health products and cosmetics has lead to extensive international research and commercialization of sea buckthorn. Recently, sea buckthorn oils have been increasing in popularity throughout Japan, Europe, and North America as a result of their nutritional effects being realized in western countries (Yang and Kallio, 2002b).

The sea buckthorn industry has been thriving in Russia since the 1940's, when scientists began investigating the shrub's bioactive compounds(Schroeder and Yao, 1995). The first Russian sea buckthorn factory developed products utilized in the diet of Russian cosmonauts and as a cream for protection from cosmic radiation. There are currently ~6000 hectares of sea buckthorn plantations in Russia (NRCC, 2002).

The sea buckthorn industry in China is more recent, although traditional uses date back many centuries (Schroeder and Yao 1995). Chinese sea buckthorn is very adaptable to harsh ecological conditions and has large populations. It is the most important subspecies of sea buckthorn and is widely distributed in a large area of north and north-west China. It is estimated that the total area of Chinese sea buckthorn is approximately 2.13 million hectares including wild and cultivated plants which account for 90% of total sea buckthorn resources in the world (Er, 2003; Rongsen, 2007). With the establishment of 150 processing factories, over 200 different

products have been developed. The people in the mountainous regions of India are following China's lead (Arimboor et al., 2006).

The sea buckthorn industry in Germany has a long tradition and is based on a total area of - 300 ha (500 ha) (NRCC, 2002). German sea buckthorn products are mainly produced for the valuable supplementary health food market. Significant research has been performed in Finland and Sweden focussing on bioactive compounds, health benefits, and effects of harvest date on quality (Kallio et al., 2002). Many other European countries are growing sea buckthorn; however, little is reported with regards to these markets.

According to the data of the Hungarian Central Statistical Office (HCSO, 2016), sea buckthorn was produced on around 100 hectares in Hungary in 2015. For today, this area is close to 300 hectares. Between 2006 and 2015, the average yield of sea buckthorn in Hungary ranged between 0.8-4.9 t ha^{-1} (HCSO, 2016). Currently, the market is still not saturated, while demand is constantly increasing. Sea buckthorn is usually sold either as berries or pulp. In practice, more sea buckthorn is sold as berries. The most widespread Altai varieties in Hungary include ‘Yantarnaya’, ‘Orangevaya’, ‘Chuiskaya’ and ‘Obilnaya’, while the most popular German varieties in Europe are ‘Hergo’, ‘Leikora’, ‘Oskola’ and ‘Habego’ (Höhne, 2015).

2.2. Nutritional composition

Sea buckthorn, often called "miracle plant", has a chemical composition and nutritional diversity, making it a source of great interest for the food industry functional and nutraceutical products (Oomah and Mazza, 1999).

Sea buckthorn berries are among the most nutritious fruits and rich in vitamins. They contain different vitamins, essential fatty acids, sugars, trace elements, flavonoids, pigments, as well as oil (Yang and Kallio, 2002b). The speciation of this plant is slightly varied. Nevertheless, it has many forms owing to environmental factors and inherited features. Studies with fruits of the subspecies *sinensis* have resulted in vitamins are much higher than those found in other fruits and vegetables (Zeb, 2004a).

2.2.1. Vitamin C

Ascorbic acid in aqueous phase of plasma have significant role in antioxidant mechanism of body. It has many cellular activities that may be directly or indirectly related to its antioxidant properties. It has strong reducing property in solution of pH> 4. In vivo most of the ascorbic acid is maintained in a reduced state by other endogenous reductants. In studies with human plasma lipids, ascorbic acid was found to be far more effective in inhibiting lipid peroxidation initiated by peroxyl radical initiator than other antioxidant components such as protein thiols, urate,

bilirubin and H-tocopherol. Vitamin C is essential for the synthesis of collagen, the most abundant protein in mammals. Vitamin C is sometimes suggested to have anticancer effect by its reaction with and inactivation of free radicals in the body (Sheng et al., 2005).

Sea buckthorn surpasses many other fruit and berry crops in ascorbic acid content and is recognized as one of the most valuable natural sources of vitamin C. The vitamin content of various forms in natural groves varies significantly (Ladaniya, 2008).

The recommended dietary allowance (RDA) for vitamin C is 75 mg day^{-1} for woman and 90 mg day^{-1} for men. Perhaps due to this popularity, the vitamin C content in Sea buckthorn and its antioxidative effect has been widely studied (Zeb, 2004a).

Ascorbic acid – vitamin C is the most important nutrient in sea buckthorn juice. Vitamin C content in the berries were significantly higher than the common vitamin C sources like blackcurrant (177 mg kg^{-1}), lemon (51 mg kg^{-1}), orange (45 mg kg^{-1}), grape (4 mg kg^{-1}), mango (37 mg kg^{-1}) and apple (12 mg kg^{-1}) (Souci et al., 2008). Table 1. shows a comparison of vitamin C concentrations in some fruit. The fruits of sea buckthorn is a good source of vitamin C (500-900 mg 100g^{-1}), which is 4-100 times higher than any vegetable and fruit (Yan-Jun et al. 2011, Bal et al., 2011, Yang and Kallio, 2002b).

Table 1. A comparison of vitamin C concentrations (mg 100g^{-1} of fruit) in some fruit (adapted from Souci et al., 2008)

Fruit	Vitamin C Concentration (mg 100g^{-1}of fruit)
Banana	11
Kiwifruit	44
Orange	45
Black current	177
Sea buckthorn	360-2500 (Li and Schroeder 1996) 450 (Souci et al. 2008)

Being a biologically essential phytochemical for human, vitamin C content in *H. rhamnoides* berries was analysed. It was revealed that soft parts (fresh) of *rhamnoides* berries contained significantly higher other kinds of fruits (banana, orange and black current) amount of vitamin C (2232 mg kg^{-1}). Several authors have reported the high vitamin C content in Chinese berries (3600-25000 mg kg^{-1}) (Beveridge et al., 1999). Sea buckthorn is reputed to be an excellent source of vitamin C, although a wide concentration range has been reported from 2 to 2500 mg 100 g^{-1} juice (Eccleston et al., 2002, Kallio et al.,2002, Sabir et al., 2005). The fruit of the plant has a high vitamin C content – in a range of to 114 to 1550 mg 100g^{-1} (Zeb, 2004) with an average content (695 mg 100g^{-1}) about 15 times greater than oranges (45 mg 100g^{-1} (USDA database) – placing sea buckthorn fruit among the most enriched plant sources of vitamin C.

Storage analysis of vitamin C content and the calculation of its stability parameters are useful instruments to predict the shelf life of food products based on nutrient loss. Kinetic models of thermal degradation are obligate for designing new processes or for the improvement of existing production procedures and, finally, yielding an optimum of product quality (Karhan et al., 2004). Thus, in the past, various studies have determined the effects of processing and of storage on the vitamin C content in a variety of fruit products (Lima et al., 1999, Gil-Izqierdo et al., 2002).

2.2.2. Carotenoids (provitamin A)

Carotenoids are among the most widely distributed pigments and naturally exhibit red, orange and yellow colours. Carotenoids are lipid-soluble pigments, which can be found in many kinds of fruit, vegetables, fungi, flowers and some kinds of animals (Ötles and Çagindi, 2008).

Over 750 different structures of carotenoids have so far been isolated from natural sources; about 500 structures have been fully characterized (Rodriguez-Amaya, 2016). Of the 500 carotenoids known in nature, 39 have been identified in sea buckthorn fruits(Singh, 2005). The carotenoid content can differ depending on the source of the oil (50 and 2139 mg 100 g^{-1}) (Beveridge et al., 1999). The oils of the pulp are richer in carotenoids than seed oils, which usually contain small quantities (20-85 mg 100g^{-1} of oil) (Li and Beveridge, 2003). B-carotene constitutes approximately 15-55% of the total carotenoids, varying its typical contents between 100-500 and 20-100 mg 100g^{-1} in pulp and seed oils respectively (Yang and Kallio, 2002a). The presence of other carotenoids in sea buckthorn fruits, such as α-carotene, γ-carotene, δ-carotene, licopene, β-zeacarotene, cryptoxanthin, sintexanthin, lutein and zeaxanthin have also been reported (Li and Beveridge, 2003).

Table 2. presents the values of the β-carotene content of oils from sea buckthorn fruit pulp and seeds of different species and subspecies reported by Yang and Kallio (2005). Note that the highest values in seed oils and pulp were found in *H. salicifolia* (485.2 mg 100g^{-1} and 97.5 mg 100g^{-1} respectively), while the lowest are listed in species *H. neurocarpa* and *H. rhamnoides* subspecies *mongolica* and *turkestanika* (13.0-18.4 mg 100g^{-1} and 122.7-169.2 mg 100 g^{-1} respectively).

Pop et al. (2014) reported that in total, 27 compounds were identified in berries and 11 compounds in leaves. Among berries, Serbanesti had the highest total carotenoid content, mainly zeaxanthin diester (55 mg 100 g^{-1} DW), and could be the most suitable variety for intensive cultivation and industrial application. In case of leaves the total carotenoid content was lower, on average 4 mg 100 g^{-1} DW. The esterified carotenoids were present only in berries. The results obtained from both berries and leaves indicate that berries are suitable for sample classification

and variety recognition because the leaves were much more variable among samples.

Eccleston et al. (2002) reported a total carotenoid content of 73 mg 100 mL^{-1} for sea buckthorn juice with β-carotene accounting for 45% of this amount.

Table 2. B-carotene content of sea buckthorn fruit oils of different species and subspecies of *Hippophae* L. (mg 100 g^{-1} oil) (from Yang and Kallio, 2005)

Species/ ***Subspecies***	Oil pulp	Seed oil
H. salicifolia	485.2	97.5
H. gyantsensis	271.6	22.1
H. neurocarpa	148.6	13.0
H. tibetana	394.3	17.3
H. rhamnoides* ssp. *sinensis	363.9	33.6
H. rhamnoides* ssp. *yunannesis	364.8	21.2
H. rhamnoides* ssp. *turkestanika	169.2	18.4
H. rhamnoides* ssp. *mongolica	122.7	14.6

2.2.3. Phenolic contents

Phenolic compounds are a large group of secondary metabolites playing diverse and significant roles in plants. Many of them serve as defense compounds against herbivores and pathogens (e.g. lignin and furanocoumarins). Others function in mechanical support (e.g. lignin), in attracting pollinators and fruit dispersers (e.g. anthocyanins), in absorbing harmful ultraviolet radiation (e.g. flavones and flavonols), or in reducing the growth of nearby competing plants (e.g. caffeic acid and ferulic acid). They are biosynthesized by several different routes in plants and constitute a heterogeneous group of compounds (Taiz and Zeiger, 2006). The phenolic compounds of interest in this review include hydroxycinnamic acid conjugates, flavonol glycosides, and anthocyanins.

The total phenolic content of sea buckthorn fruit was reported to range from 114 to 244 mg 100g^{-1} fruit (Gao et al., 2000). These authors reported a strong positive correlation between the antioxidant capacity of the fruit and its total phenolic and ascorbic acid contents. Phenolics, including flavonols, flavones, phenolic acids, PAs and hydrolysable tannins are reported as the major contributors to the biological properties like antioxidant activities of s e a buckthorn berriesandleaves (Eccleston et al., 2002, Zadernowski et al., 2005).

Flavonoids are polyphenolic compounds that have the diphenyl propane (C6-C3-C6) 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. Flavonols represented 87% of all the phenolics analysed in sea buckthorn berry (Hakkinen et al., 1999).

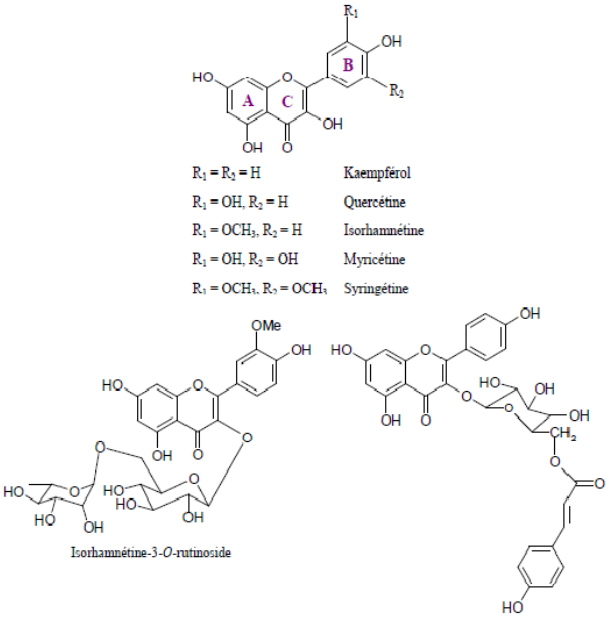
The flavonoid content in the leaves and fruit of sea buckthorn has been reported to range from 310 to 2100 mg $100g^{-1}$ dried leaf and 120 to 1000 mg $100g^{-1}$ fresh fruit, respectively (Tigong et al., 1991). Preparative isolation and purification of flavonoids and protocatechuic acid from sea buckthorn juice concentrate by high-speed counter-current chromatography was also reported (Gutzeit et al., 2007b). HPLC-DAD analysis of flavonoids in sea buckthorn leaves has been described by Zu et al. (2006) and reported the presence of catechin, quercetin, isorhamnetin and rutin. Chen et al. (2007) reported the development of a HPLC fingerprint method for investigating and demonstrating the variance of flavonoids among different origins of sea buckthorn berries. Thirty-four samples were analyzed including 15 *Hippophae rhamnoides* ssp. *sinensis* samples, 7 *Hippophae rhamnoides* ssp. *yunnanensis* samples, 5RW *Hippophae rhamnoides* ssp. *wolongensis* samples, 4NS *Hippophae neurocarpa*ssp. *stellatopilosa* samples and 3 TI *Hippophae tibetana* samples and 12 flavonoids are identified from HPLC chromatograms.

Rosch et al. (2004a) reported the identification of monomeric flavonols and PA from sea buckthorn (*Hippophae rhamnoides*) pomace (Rosch et al., 2004b). Five dimeric PA are identified by HPLC-ESI-MS/MS and by acid catalyzed cleavage in the presence of phloroglucinol. Nine trimeric PA are tentatively identified by HPLC-ESI- MS/MS in the sephadex fractions. The isolated flavan-3-olsand pro-anthocyanidins are potent in scavenging Fermy's salt, a synthetic free radical. They possess antioxidant capacities that are higher or comparable to that of ascorbic acid or trolox. On comparing the antioxidant capacities of monomericflavan-3-olsanddimeric PA, no significant influence from the degree of polymerization (DP) was observed (Figure 3.).

Gorbatsova et al. (2007) report analysis of antioxidant compounds such as trans-resveratrol, catechin, myricetin, quercetin, p-coumaric acid, caffeic acid, L-ascorbic acid, and gallic acid in six different varieties of sea buckthorn berries (sea buckthorn varieties: ‘Trofimovskaja’, ‘Podarok Sadu’, and ‘Avgustinka’) is published. Trans-Resveratrol, catechin, ascorbic acid, myricetin, and quercetin were found in all sea buckthorn extracts. The biggest average antioxidant activity content was found in TR (740 mg $100g^{-1}$ of dried berries). The same varieties gave the highest quercet in content 116 mg $100g^{-1}$ of dried berries).

Rosch et al (2004b) reported the amount of total GA and Pro CA in sea buckthorn berries from Finland by HPLC analysis. Zadernowski et al. (2005) found that the phenolic acid composition in sea buckthorn berries ranged from 3570 to 4439 mg kg^{-1} on dry weight basis.

They tentatively identified 17 phenolic acids in the fruit with salicylic acid accounting for 55 to 74% of the total. The phenolic acids in the fruit were mainly in their esterified and glycosylated forms; where as the maximum free phenolic acids content was 2.3%.



Kaempférol-3-O-(6‘‘-O-coumaroyl) glucoside

Figure 3. Examples of flavonols aglycones and flavonols-O-glycosylates detected in different organs of sea buckthorn, *Hippophae rhamnoides* (Rosch et al., 2004a)

Gao et al. (2000) reported a strong positive correlation between the antioxidant capacity of the fruit and its total phenolic and ascorbic acid contents. Zadernowski et al. (2005) found that the phenolic acid composition in sea buckthorn berries ranged from 3570 ± 282 to 4439 ± 405 mg kg^{-1} on a dry weight basis. They tentatively identified 17 phenolic acids in the fruit with salicylic acid accounting for 55 to 74% of the total. The phenolic acids in the fruit were mainly in their esterified and glycosylated forms, whereas the maximum free phenolic acids content was 2.3%.

The flavonoid content in the leaves and fruit of Sea buckthorn has been reported to range from 310 to 2100 mg 100 g^{-1} dried leaf and 120 to 1000 mg 100 g^{-1} fresh fruit, respectively (Chen, 1991). Eccleston et al. (2002) reported a flavonoid content of 1182 mg L^{-1} Sea buckthorn juice and identified isorhamnetin-rutinoside (355 mg L^{-1}), isorhamnetinglycoside (142 mg L^{-1}), quercetin-rutinoside (35 mg L^{-1}) and quercetin-glycoside (35 mg L^{-1}) as the main flavonoids present. Hakkinen et al. (1999) reported that quercetin was the main flavonoid in European sea buckthorn fruit.

The phenolic compounds of sea buckthorn represent the main group of phytochemicals

which exhibit antibacterial and also antiviral effects. These compounds both suppress gram-negative bacteria (Khan et al., 2010) and reduce gram-positive bacteria (Kumar and Sagar, 2007). A recent study involves a new phytochemical substance called *hipporamin*. It is a phenolic compound from a nature source (Michel et al., 2012). *Hipporamin* positively suppresses a wide spectrum of bacterial as well as viral diseases (Suryakumar and Gupta, 2011).

2.2.4. Sugars

Soluble solid content ranges from 7.0 to 22.7º Brix. The sugar content of sea buckthorn fruits varies according to the origin, the subspecies, the time of the harvest and their state of maturity. It varies between 2.0 and 3.3%, although some fruits originating in Russia can reach up to 7.0% (Singh, 2005). The Indian-cultivar Summer has a content of soluble sugars that varies between 9.3 and 17.3 ºBrix (Li and Beveridge, 2003). Total soluble sugars reported for Chinese origins ranged from 5.6-22.7% in raw juice. Chinese origins show higher concentrations of total sugars than Russian ones which, in turn, are higher in sugars than Finnish origins (Kallio et al., 1999).

Glucose, fructose, xylose are the main sugar components of sea buckthorn berries. Glucose is present in abundance in all the species of sea buckthorn from different origins. Table 3. compares the sugar content among sea buckthorn berries juice from the plants of Chinese and Finnish origin with the three cultivars cultivated in India.

Table 3. Sugar content of sea buckthorn juice of different origin (Stobdan et al., 2011)

Sugar (units)	Chinese origin	Finnish Origin	*Hippophae* spices		
			sinensis	*rhamnoides*	*mongolica*
Glucose (g 100ml^{-1})	5.5	0.9	5.8±1.9	1.4±0.7	5.3±1.6
Fructose (g 100ml^{-1})	3.8	0.2	4.6±1.8	0.3±1.8	2.4±1.2
Mannitol (mg g^{-1})	-	17	-	-	-
Sorbitol (mg g^{-1})	-	314	-	-	-
Xylose (% of total)	0.42	-	-	-	-
Xylitol (mg g^{-1})	-	39.2	-	-	-
Ethyl – beta-D-glucopyranose (g 100ml^{-1})	-		-	0.6±0.6	0.1±0.1
Methyl inositol (g 100ml^{-1})	-	-	0.8±0.3	0.3±0.11	0.2±0.0

Total sugar content of sea buckthorn juice (2.86%) is quite low compared to other fruit crops like mango, apricot, banana, orange and peach having concentrations of 14.8%, 9.24%,

12.23%, 8.4% and 8.39% respectively. Sugar and fruit acids may also effectively influence sensory properties of sea buckthorn juice, playing an important role in market acceptance by the consumers. Low levels of sugar alcohols like mannitol, sorbitol and xylitol are being observed. Relative sugar abundance and absolute sugar content are influenced by factors like type of subspecies, harvesting dates and year. Sugar content pattern can vary during harvesting period depending upon the genetic background of the berries (Stobdan et al., 2011).

2.2.5. Organic acids

The fruits of the sea buckthorn have a high content of organic acids, among which it is possible to mention malic, citric, tartaric, succinic, quinic and oxalic and together constituting around 90% of all the fruit acids in different origins. Large variation in acid content has been observed, which may be due to genotype, origin, harvesting dates and year. Russian origin berries showed relatively lower concentration of total acidity (2.1-3.2 g 100 ml^{-1}), Finnish genotypes have intermediate values (4.2-6.5 g 100 ml^{-1}), while Chinese genotype showed the highest concentration (3.5-9.1 g 100 ml^{-1}) (Stobdan et al., 2010). According to Rongsen (2007), the organic acid content of *Hippophae* berries varies between 1.64-5.95%, being much higher than that of lemon fruit. Table 4. presents the organic acid composition of the sea buckthorn fruit (cv. Summer). It shows the importance of quinic acid, the concentration of which is approximately double the concentration of malic acid. Malic acid was the major organic acid reported in Finnish Sea buckthorn fruit (subspecies *rhamnoides*) with minor quantities of citric and tartaric acid (Kallio et al., 1999). Sea buckthorn grown in Canada, however, has been reported to contain quinic acid as the major organic acid while malic was next, and citric, oxalic and tartaric acids were minor components. Titratable acidity ranges from 3.5 to 7.3% and pH ranges from 2.7 to 3.1 (Beveridge et al., 2002).

Table 4. Organic acid content of sea buckthorn fruit juice cv. Indian-Summer (Adapted from Li and Beveridge, 2003; Beveridge et al., 2002; Stobdan et al., 2010)

Acid (mg / mL)	Values
Malic acid	11.4 - 15.5
Citric acid	1.58 - 2.21
Tartaric acid	0.67 - 3.29
Quinic acid	19.6 - 26.5
Oxalic acid	0.13 - 0.50

2.2.6. Minerals

The fruits of the sea buckthorn contain numerous mineral elements and trace elements. According to Solonenko and Privalov (2005) the mineral composition of the juice is high, and it contains all the 24 essential micro and macro elements. Potassium and Cobalt are the most abundant of all the elements investigated in berries. The Cobalt content is significantly greater than in other fruit juices; Cu, two times greater than in apricots, strawberries, and red currants.

Table 5. compares the mineral elements present in sea buckthorn fruit juices of Chinese and from India, Finland and Pakistan origin.

Table 5. Nutritional attributes of sea buckthorn pulp/juice from different regions

Parameters	India		China[3]	Finland[3]	Pakistan[4]
	Ladakh[1]	Uttarakhand[2]			
Calcium, mg/l	176.6	64-256 125	0.8-1.48 g kg^{-1}	0.27-0.74 g kg^{-1}	70-125
Iron, mg/l	30.9	0.703-1.127	64-282 mg kg^{-1}	22-33 mg kg^{-1}	40-170
Magnesium, mg/l	22.5	0.62-1.92	0.47-0.73 g kg^{-1}	0.56-0.79 g kg^{-1}	139-240
Phosphorous, mg/l	84.2	0.6-0.67 (%)	-	-	110-133
Sodium, mg/l	414.2	0.47-0.63(%)	-	-	20-80
Potassium, mg/l	647.2	10.12-14.87%	6.44-12.2 g kg^{-1}	10.3-14.84 g kg^{-1}	140-360
Zinc, mg/l	1.4	0.817-2.74	8.8-27 mg kg^{-1}	14-27 mg kg^{-1}	-
Copper, mg/l	0.7	0.09-0.133	3.8-12 mg kg^{-1}	6-9.5 mg kg^{-1}	-
Manganese, mg/l	1.06	-	8.7-15 mg kg^{-1}	8.1-17 mg kg^{-1}	-
Selenium, mg/l	0.53	-	7.96-11.3	-	-
Riboflavin, mg/100g	1.45	-	-	-	-
Niacin, mg/100g	68.4	-	-	-	-

([1]Stobdan et al., 2010, [2]Dhyani et al., 2007, [3]Kallio et al., 1999, [4]Sabir et al., 2005)

Specific elements (e.g. calcium, potassium, and trace minerals such as copper, selenium, manganese, and zinc) are recognized for their functionality and are being included as nutraceutical and functional food ingredients (Wildman, 2001).

2.2.7. Oils

Oils can be extracted from the juice, seeds, and pulp and peel of the press cake or (the rind of the cake) decanter waste product. The seed is separated from either the wet (Beveridge, 2003) or dried press cake (Arimboor et al., 2006), ground, and processed by extraction to remove the oil. Cenkowski et al. (2006) investigated several extraction methods for pulp and peel and seed fractions, including solvent extraction using petroleum-ether, SCFE using carbon dioxide (CO_2),

screw pressing, and aqueous extraction.

Besides oil richness, sea buckthorn berry oils are unique in that the compositions of the seed and pulp oils are distinctly different. The seed oil, defined as being highly unsaturated, comprises two essential fatty acids (EFAs), α-linolenic acid or "Omega-3" (18:3π-3) and linoleic acid or "Omega-6" (18:2π-6). The contribution of α-linolenic and linoleic acids of the total fatty acid composition are commonly 20 to 35 and 30 to 40%, respectively. In addition, palmitic (16:0), steric (18:0), oleic (1 B:1 π-9), and vaccenic (18:1 π-7, 11-octadecanoic) acids are also present in seed oil, though low amounts have been reported (Yang and Kallio, 2002b). Palmitoleic (16:1π-7) acid is practically non-existent in seed oil (Yang and Kallio, 2001). Oil from the pulp is characterized as being more saturated and comprises primarily palmitic and palmitoleic acids with lower levels of α-linolenic acid (Kallio et al., 2002b).

Oil from the juice and pulp is rich in palmitic (about 34%), oleic (about 32%), and palmitoleic acids (about 26%), while the oil from the seed contains a higher quantity of unsaturated acids (around 86%), a large part of these being essential fatty acids (linoleic 35% and linolenic acid 26%), and only unimportant concentrations of palmitic acid (about 10%) (Yang and Kallio, 2001).

2.2.8. Other compounds

Tocopherol and tocotrienol are collectively known as vitamin E (Rafalowski et al., 2008). Some studies reported that the total content of tocopherols and tocotrienols in sea buckthorn berries (ssp. *sinensis* and *mongolica*) ranged from 56 to 140 mg kg^{-1} of whole berries, and the total content of tocotrienols varied from 1.5 to 8.1 mg kg^{-1} of whole berries. α-tocopherol was the predominant tocopherol found in sea buckthorn berries, where it constituted 49% of total tocopherols in seed buckthorn seed oil (Beveridge et al., 1999) All groups of tocopherols are rich in sea buckthorn seed oil as compared to pulp oil except in β-tocopherols. Whereas, tocotrienols were observed more concentrated in pulp oil. The antioxidant vitamin E content of sea buckthorn seed oil makes it a valuable contributor in helping the body fight and eliminates free radicals (Kallio et al., 2002, Cenkowski et al., 2006).

Vitamin K is a group of structurally similar, fat-soluble vitamins found in foods and in dietary supplements. Vitamin K is found in nature in two forms – K1, also called phylloquinone, and is found in plants and vitamin K2, also called menaquinone, which can be synthesized by many bacteria. Vitamin K1 (phylloquinone) in sea buckthorn amounts to about 1.2 mg %, i.e. more than in ash berries (Rishavy et al., 2013; Tie et al., 2011).

Content of vitamin K1 in sea buckthorn berries ranges from 21 % up to 186 % (wet weight) depending on the storage time and temperature. During the industrial juice production,

the technological processing of the berries caused a loss of about 36–54 % phylloquinone in the manufactured juice. The following processing steps, leading to the concentrated juice, result in the complete depletion of phylloquinone (Gutzeit et al., 2007a, b).

Leaves of female and male plants are reported to contain an average of 21.1 and 20.6 g protein 100 g^{-1} dried leaf, respectively (Stobdan et al., 2010). The protein content of the fruit varies with the variety and geographical location and is reported to vary from 0.79 to 3.11% on a fresh weight basis (Bekker and Glushenkova, 2001).

The volatile compound composition of sea buckthorn fruit essential oil was analyzed by Cakir (2014). Thirty compounds could be identified representing 94.6% of the oil. The main compounds of this oil are ethyl dodecanoate, ethyl octanoate, and decanol and ethyl decanoate. Most volatile compounds in sea buckthorn leaves are tetracosane (10–40%), hexadecanoic acid (<0.1–11%), octadecatrienoic (5–27%), tetracosene (3–11%), and eicosanol (<0.1–13%).

The sterol content of sea buckthorn fruit oil varies between 2.2 and 8.8%, and varies according to origin, subspecies, oil extraction method and time of fruit picking (Li and Beveridge, 2003). Yang et al. (2001) found varying sterol contents between 12 and 23 g kg^{-1}, 10 and 29 g kg^{-1} and 13 and 33 g kg^{-1} in seed oils, parts soft tissues (pulp and skin) and whole fruits respectively, in the subspecies *sinensis* and *rhamnoides*. Sitosterol constituted between 57 and 76% of the total sterols of the seeds, and between 61 and 83% of the total sterols of the soft tissues. Cenkowski et al. (2006) reported β-sitosterol as the main sterol identified in seed oils (about 97% of total sterols) and pulp (96-98% of sterols totals), in the Indian-Summer cultivar.

2.2.9. Sensory properties of berries

Sensory properties constitute a very important aspect of berry quality for a successful fresh market. The term sensory is defined as relating to the senses. Sensory assessment or evaluation is accordingly based on appearance, taste, aroma, sound and texture of food as perceived though the senses (ASTM Committee E-18 1978).

Chemical constituents of the berries have strong impacts on the sensory quality, thus affecting the consumer liking and acceptance of berries and berry products. The ratio between sweetness (sugars) and sourness (acids) has been regarded as a critical factor affecting the sensory quality of berries (Tiitinen et al., 2005). In sea buckthorn berry, intense sourness induced mainly by abundance of malic acid would have a negative influence on the pleasantness (Laaksonen et al., 2016, Ma et al., 2017a). The astringent and bitter of sea buckthorn berries have been reported to have a correlation with the contents of flavonols, proanthocyanidins (PAs) and ethyl b-D-glucopyranoside (EG) (Ma et al., 2017a; Ma et al. 2017b). Besides these non-volatile compounds, odour-active volatiles have crucial influence on the sensory quality of sea

buckthorn berries (Lunden et al., 2010). The amount of ethanol correlated with the intensity of pungent odour, and the concentration of propyl 2- methylbutanoate is related to the fermented odour (Tiitinen et al., 2007; Lunden et al., 2010). Previous researches have revealed that olfactory stimuli accompanying with sweet or sour-tasting foods may induce the enhancement of the associated taste quality in fruit (Sung et al. 2019). Moreover, aroma is also a good indicator of freshness, quality and authenticity of sea buckthorn products (Caprioli et al., 2016).

Nowadays, sea buckthorn berries are processed into a wide range of products including juice, jam and food additives. For the majority of fruits, sweetness is the most important tastant. A sugar: acid ratio of 15-16 apple-juices produces the optimal balance of sweetness-sourness in this product. In citrus fruits, a sugar: acid ratio over 12 gives the most acceptable product. Sweetness also correlates closely with overall liking of soft fruit juice. However, in sea buckthorn berries, the highest value of sugar: acid ratio that has been analysed in a Chinese genotype should be not over 5 (Kallio et al., 1999).

2.3. General information on sea buckthorn plant

2.3.1. The pomace

The commercial production of sea buckthorn (*Hippophae rhamnoides*) juice results in a large amount of pomace, which is utilized rather efficiently or discarded as a waste, so considerable amounts of nutrient are lost (Galanakis, 2012). The pomace, one by-product of sea buckthorn berries. Sea buckthorn pomace is a precious product which contains not only important nutrients but also high-quality oils (Figure 4.). The pomace of sea buckthorn contains mainly α-, ß- and γ-carotens, lycopene, and zeaxantin. Vitamin B group is mainly represented by B_1 (thiamine), B_2 (riboflavin), B_6 (pyridoxine), vitamin PP (nicotinamine, niacin, vitamin B_3), and folic acid necessary for nucleic acid synthesis. The vitamin C content depends on the variety and natural conditions. Plants growing in Central Asia contain 150–200 mg 100 g^{-1}, and Alpine plants contain around 800 mg 100 g^{-1} (Eccleston et al., 2002; Fatima, 2012).

Sea buckthorn berry pomace contained a total of 1.068 mg kg^{-1} phenolic acids, of which 58.8% was derived from phenolic glycosides. Free phenolic acids and phenolic acid esters constituted 20.0 % and 21.2 %, respectively, of total phenolic acids in sea buckthorn berry pomace. The total phenolic acid content in seed kernel (5,741mg kg^{-1}) was higher than that in berry pomace and seed coat. Gallic acid was the predominant phenolic acid both in free and bound forms in sea buckthorn berry parts and leaves (Arimboor et al., 2008).

After pressing, the fruit consists of juice, 74.5%; seed, 6.54%; and residue/pomace, 19.45%.

Figure 4. Sea buckthorn pomace (source from Seabuckwonders ®)

2.3.2. The Seed

Sea buckthorn is a single seeded fruit. The seed is ovate-oblong with a length of 4 to 7 mm, a breadth of 2.5 to 3.5 mm and a thickness of 1.6 to 2.2 mm. The skin of the seed is greyish-brown or dark brown, leathery and lustrous. A parchment-like ovarian wall surrounds the seed. *Hippophae salicifolia* seed is globose and seem fissured on one side with length 3 to 4.5 mm long, a breadth of 2.5 to 3 mm wide and 1.5 to 2 mm thick. It tastes sour (Figure5.). Sea buckthorn belongs to the group of thermophilic plants. Ideal temperature for germination of seed is 24º to 26ºC (Ansari, 2003).

Figure 5. Seed of *Hippophae rhamnoides* (source from Seabuckwonders ®)

The seed represents only 10% of the whole fruit. Major chemical composition of sea buckthorn seed is carbohydrate, lipid (fat) and protein. The seed contains 10 to 20% of oil depending upon the species of the plant (Singh, 2001). *Hippophae tibetana* contains 19.51 % oil which is highest among all the species of *Hippophae*. The seed oil contains 12 % to 20 % saturated fatty acids and 88.3% to 89.1 % unsaturated fatty acids, particularly Linolenic acid

(32.3%), linoleic acid (40.8%) and oleic acid (15%) (Schroeder and Yao, 1995). Other constituents of the seed oil included gamma and alpha tocopherols (Li and Beveridge, 2003).

2.3.3. Leaves

Sea buckthorn leaves (Figure 6.) and branches presently create waste-/by-products of harvesting after pruning the plants. The leaves of sea buckthorn (*Hippophae rhamnoides* L.) can be a rich source of nutrients and biologically active substances. Their levels depend on growing conditions, agricultural technology and climate.

The leaves are small (usually 3 to 8 cm long and 0.4 to 1 cm wide), alternate, linear, lanceolate and covered on the backside with silvery stellate scales that reflect sunshine and reduce moisture loss (Lu, 2003). Leaves of sea buckthorn are used to manufacture various products due to the fact that the leaves contain many nutrients and bioactive substances.

Figure 6. Leaves of *H. rhamnoides* L. (picture by *Maxsol7*)

Leaves are equally rich in important antioxidants, including ß-carotene, vitamin E, catechins, ellagic acid, ferulic acid, folic acid. They also contain significant quantities of calcium, magnesium and potassium (Upadhyay et al., 2010). The leaves of sea buckthorn have been reported to contain higher contents of phenolic compounds and antioxidant activities than the berries, due to the high content of nutrients and bioactive compounds such as minerals, vitamins, fatty acids, carotenoids and phenolic compounds. Total phenolics were 12.7 %, out of which 92 % were in the form of hydrolysable tannins. The content of hydrolysable tannins was quite high, when compared to other locally available fodder species. It was reported that the flavonoid content in leaves ranges from 319 to 2100 mg 100 g^{-1} of air-dried leaves (Hellström et al., 2013; Pop et al., 2014; Tian et al., 2017).

The minerals in sea buckthorn leaves, such as iron, phosphorus and magnesium, have a positive effect on the processes of absorption of vitamins and other nutrients, and therefore

enhance tissue building and prevent anemia. The fresh sea buckthorn leaves are rich in carotenoids (26.3 mg 100 g^{-1}) and chlorophyll (98.8 mg 100 g^{-1}), which are considered quality indicators for green vegetables. Sea buckthorn leaves also contain high quantities of protein (21%), of which 0.73% is lysine and 0.13% is methionine and cystine (Biswas et al., 2010; Patial et al., 2013).

2.4. Physiological and microbiological effects of sea buckthorn berries

A wide spectrum of physiological effects of sea buckthorn berries and berry products has been reported, including antioxidant (Eccleston et al., 2002; Chawla et al.,2007), radio-protective and anti-tumor (Padmavathi et al., 2005; Teng et al., 2006) inhibition of LDL cholesterol oxidation and platelet aggregation (Johansson et al., 2000), anti-hypertensive (Pang et al., 2008), immunomodulation and cytoprotective effects (Geetha et al., 2002), protection from gastric ulcer (Xing et al., 2002), reduction of atopic dermatitis (Yang et al., 2000),and wound healing (Gupta and Flora, 2006).

Investigated the antioxidant activity of sea buckthorn fruits and its relationship with maturity (Gao et al., 2000). The study demonstrates that capacity of phenolic and ascorbate extracts to scavenge radicals decreased significantly with increased maturation and the changes were strongly correlated with the content of total phenolics and ascorbic acid. Antioxidant capacity of the lipophilic extract increases significantly with maturation and corresponds to the increase in total carotenoids (Figure 7.). Eccleston et al. (2002) reported that sea buckthorn juice was rich in antioxidant and moderately decreased the susceptibility of LDL to oxidation. Alcohol and water extracts of various sea buckthorn seeds are found to possess high levels of antioxidant and antibacterial activities and these activities are attributed to the high phenolic content in Sea buckthorn seeds (Chauhan et al., 2007).

Johansson and coworkers showed that sea buckthorn oil inhibited platelet aggregation in humans (Pang et al., 2008). A similar inhibitory effect to aspirin on platelet aggregation induced by collagen in mouse femoral artery was reported for a total flavones extract from sea buckthorn (Cheng et al., 2003). This ability to prevent in vivo thrombogenesis suggested that sea buckthorn fruit consumption may help prevent cardiac and cerebral thrombosis in humans.

In contrast to the in vitro studies Suomela et al. reported (2006) that sea buckthorn flavonols,ingestedwithoatmealporridge,donothaveasignificanteffect on the levels of oxidized LDL, C-reactive protein, and homocysteine, on the plasma antioxidant potential, or on the paraoxonaseactivityin human (Suomela et al., 2006). They also showed that flavonols in oatmeal porridge were rapidly absorbed, and a relatively small amount of sea buckthorn oil added to the porridge seemed to increase the bioavailability of flavonols considerably.

Michel et al. (2012) report that the active agent contained in sea buckthorn manly inhibit *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Yersinia enterocolitica* and *Enterococus faecalis* bacteria. These effects are mainly shown by extracts from sea buckthorn leaves. Oil obtained by pressing is a very effective inhibitor of bacterial growth, especially of *Escherichia coli* (Christaki et al., 2012). Also, Kaushal and Sharma (2011) confirmed that sea buckthorn seed oil showed good antimicrobial properties (growth inhibition zone diam. 4.0 mm) against *Escherichia coli*.

SB has also shown unique biological properties against viral diseases, antiviral activity against the influenza virus and herpes virus. The suppressing effect on the influenza virus is provided by inhibition of virus neuraminidase present in the virus (Michel et al., 2012).

Figure 7. Sea buckthorn berries (picture by Silkrute)

In another study Nersesyan and Muradyan (2004) showed that sea buckthorn juice protects mice against genotoxic action of the anticancer drug cisplatin (Nersesyan and Muradyan, 2004).

Geetha et al. (2002) found that concentrated (500µgmL^{-1}) alcoholic extracts of fruit and leaves of sea buckthorn could inhibit chromium-induced free radical apoptosis and DNA fragmentation and restored antioxidant status to that of control cells in a lymphocyte in vitro model system. The leaf extracts have a cytoprotective effect against chromium induced cytotoxicityas well as immunomodulating activity (Pang et al., 2008).

The preventive effect of Sea buckthorn extracts on liver fibrosis was also demonstrated through a clinical study (Gao et al., 2003). Sea buckthorn proanthocyanidins reported to play an important role in healing of acetic acid-induced gastric lesions in mice, possibly by the acceleration of the mucosal repair (Xu et al., 2007).

2.5. Medicinal applications

Throughout history, sea buckthorn berries have been used in Tibetan and Mongolian medicines. The berries, being recognized as a medicinal ingredient were listed in the Chinese Pharmacopeia in 1977. Although sea buckthorn berries contain numerous nutritional components, many of the health benefits are attributed to the berry oils, which have demonstrated many pharmacological functions (Oomah and Mazza, 1999).

Clinical investigations on the medicinal uses of sea buckthorn were first conducted in Russia during the 1950s. However, many publications are only case reports rather than scientific investigations and have been written in Russian and Chinese. For these reasons, validation research on health claims associated with the oils is needed. Currently, some health claims are being evaluated in Europe (Yang and Kallio, 2002b).

The various kinds of sea buckthorn extracts was proved to be efficient against several other common diseases such as the diabetes (Kim, 2013), the gastric ulcer (Huff et al., 2012), in tumor therapy (Ali and Ahmad, 2015; Chakraborty et al., 2015), cardiovascular disease prevention, immune system restoration, and anticancer applications (Yang and Kallio, 2002b; Geetha et al., 2002).

2.5.1. Cancer therapy

The literature describing the role of *Hippophae* in prevention and control of cancer is limited, however certain analysis of the known experimental research information on anticancer by *Hippophae* available at present. The inhibition of *Hippophae* oil on the cancer cells was not as effective as the positive medicine, for example, the cancer inhibition rate of phosphamide was twice as much as *Hippophae*, the possible mechanisms of antimutagenic action of the sea buckthorn oil, have been discussed (Nersesian et al., 1990). Most of the work done in this area has been with laboratory animals. Reports on the potential of a *Hippophae* extract (an alcohol extract, which would mainly contain the flavonoids) to protect the bone marrow from damage due to radiation; this study also showed that the extract might help faster recovery of bone marrow cells (Agrawala and Goel, 2002). In China, a study was done to demonstrate faster recovery of the hemopoietic system after high dose chemotherapy in mice fed the sea buckthorn oil (Chen, 2003). The seed oil has been found to enhance non-specific immunity and to provide antitumor effects in preliminary laboratory studies. The effects of vitamins from other diet sources on cancer therapy in amphibian and other animals has established, however well-designed clinical studies with sea buckthorn are needed to validate its effects and exact mechanism on cancer patients in humans (Chandra et al., 2018).

2.5.2. Cardiovascular therapy

Hippophae is used as anti-cardiovascular medicine. The sea buckthorn is hypothesized to lower the risk of cardiovascular diseases in several studies, which was review by Sayegh et al. (2014).

Some simple formulas based on sea buckthorn have been developed recently which in intended for use in treatment of coronary heart disease and sequelae of heart attack and stroke, through improving blood circulation and restoring cardiac function. (Xiao et al., 2003).

Salahat et al., (2002) show that the major factors leading to the atherosclerosis are the lipid oxidation damage and antioxidation treatment could significantly inhibit the atherosclerosis formation and the incidence of coronary heart disease have a close relation with HDL cholesterol.

There is increasing evidence to support the hypothesis that free radical-mediated oxidative processes contribute to atherogenesis (Eccleston et al., 2002). More recently the ability of antioxidant nutrients to affect cell response and gene expression has been reported *in vitro*, providing a novel mechanistic perspective for the biological activity of antioxidants. Sea buckthorn (*Hippophae rhamnoides* L.) is rich source of antioxidants both aqueous and lipophilic, as well as polyunsaturated fatty acids. It was found that antioxidants rich sea buckthorn juice affects the risk factors (plasma lipids, platelet aggregation and plasma soluble cell adhesion protein concentration) for coronary hearts disease in humans (Eccleston et al., 2002).

2.5.3. Gastrointestinal ulcers

Gastric ulcers are growing fast in human being, especially in the developing countries like Pakistan, due to unfavourable and non-assessed diet, ignorance, carelessness. Sea buckthorn berry is traditionally used in the treatment of gastric ulcers and laboratory studies confirm the efficacy of the seed oil for this application (Xing et al., 2002). Its functions may be to normalize output of gastric acid and reduce inflammation by controlling pro-inflammatory mediators. The antiulcerogenic effect of a hexane extract from *Hippophae rhamnoides* was tested on indomethacin and stress induced ulcer models. As a result, hexane extract from *Hippophae* was found to be active in preventing gastric injury (Suleyman et al., 2001).

2.5.4. Liver diseases

A clinical trial demonstrated that sea buckthorn extracts helped normalize liver enzymes, serum bile acids and immune system markers involved in liver inflammation and degeneration (Gao et al., 2003). In addition, sea buckthorn oil protects the liver from damaging effects of toxic chemicals, as revealed in laboratory studies.

Some studies have shown that sea buckthorn contains lots of vitamin A precursors including β-carotene and unsaturated fatty acids. The sea buckthorn could protect the liver from damage induced by CCl_4. A combination of an antiviral drug and sea buckthorn in treating patients with chronic hepatitis-B could shorten the duration for the normalization of serum ALT (Zeb, 2004, Zeb and Mehmood, 2004).

2.5.5. Skin diseases

An ingredient of the oil, palmitoleic acid, is a component of skin. It is considered a valuable topical agent in treating burns and healing wounds. This fatty acid can also nourish the skin when taken orally if adequate quantities of sea buckthorn or its oil are consumed; this is a useful method for treating systemic skin diseases, such as atopic dermatitis. Sea buckthorn oil is already widely used alone or in various preparations topically applied for burns, scalds, ulcerations and infections. It is an ingredient in sun block. *Hippophae* oil has UV-blocking activity as well as emollient properties and it is an aid in promoting regeneration of tissues (Goel et al., 2002).

It was established that *Hippophae* seed oil had functions like improve the human immunity, remove blood stasis and promote blood circulation, anti-inflammation and pain reliever, increase the tissue regeneration etc. and had magic effects in treating burns and scalds. This medicine is easy to use and reliable in the effectiveness without any side effect and could widely use in clinical treatment of burns and scalds (Yang and Kallio, 2005).

2.5.6. Other properties

Sea buckthorn products are used for curing varieties of other diseases. Sea buckthorn oil act as strong antioxidant and used as for balancing the immune system. Sea buckthorn flavonoid could significantly increase the anti-hyperlipemia condition. The medicinal applications of sea buckthorn products are increasing regularly, with recent worldwide health applications and increasing popularity in the developing countries (Geetha et al., 2002; Yang and Kallio, 2002b).

2.6. Processing methods

Hundreds of commercial products (e.g. including pharmaceuticals, nutraceuticals, beverages and foods, cosmetics and skin preparations, sun blocks, fermented products, animal feeds, and pigment) containing sea buckthorn derivatives have been developed in Europe and Asia (Schroeder and Yao, 1995) with product development now extending to North America. Once harvested, berries are perishable and must be cooled to 4 – 6°C if they are to be used within a few days (Li and Beveridge, 2003). If usage or processing is to be delayed beyond a few days,

the berries must be frozen (e.g. individual quick frozen). The berries can be thawed and used or processed when required. Alternatively, berries may be processed immediately and stored as pasteurized or sterilized, final products.

The favoured processing path of sea buckthorn berries is depicted Figure 8. The preliminary steps prior to juice separation include fruit selection, inspection and washing. Fruit selection and inspection is necessary to remove diseased, damaged, and pest-infested berries (Beveridge et al., 1999) followed by washing, recommended for the removal of microorganisms, dust, and dirt. The inclusion of berry washing is controversial as it may result in the dilution of soluble solids and the introduction of foreign chemicals or microorganisms (Beveridge et al., 2002).

The investigation into juice extraction has included decanter centrifugation, rack and cloth and serpentine belt pressing, and screw pressing (Arimboor et al., 2006). At this point the processing branches off in two directions: 1) juice and 2) press cake processing. The three main resulting products include juice, oils juice, seed, and pulp and peel, and yellow-orange pigment (pulp and peel) (Beveridge et al., 1999).

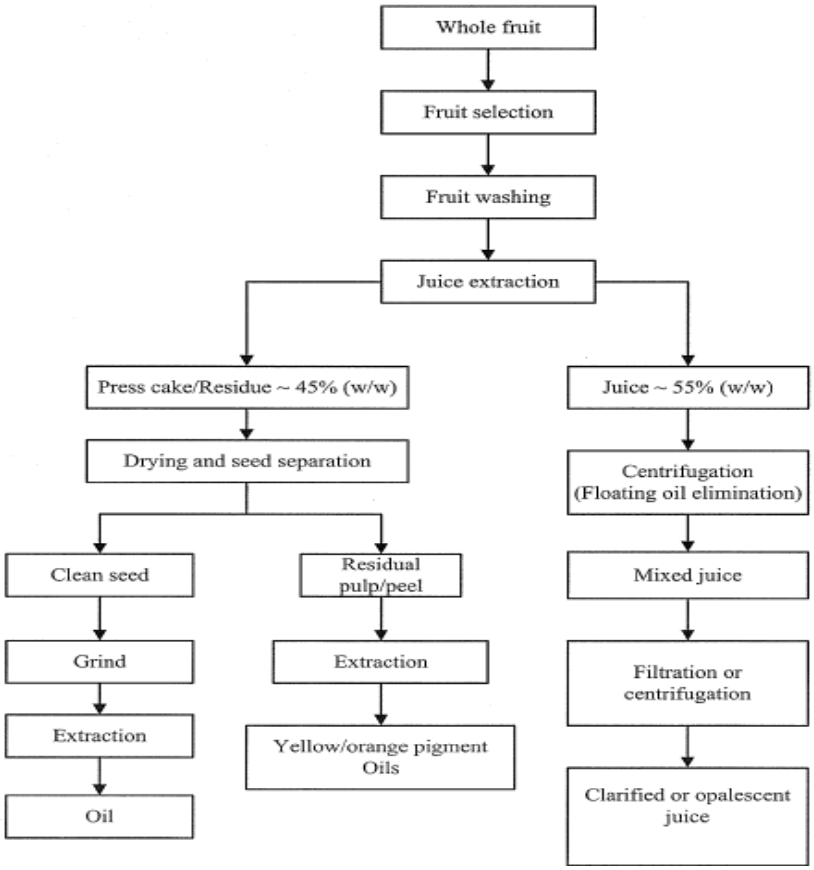


Figure 8. Processing plan for sea buckthorn berries. (Adapted from Beveridge et al., 1999; Zielenska and Nowak, 2017)

Storage and juice processing

Storage conditions may affect the content of bioactive compounds depending on product, packaging, duration of storage, light, temperature, humidity and other environmental factors in the storage room. Both tocopherols and carotenoids are relatively stable compared with the water-soluble ascorbic acid, which decreases more quickly (Kalt, 2005).

Temperature is crucial for maintaining the bioactive compounds in a product. Lower temperature decreases metabolic activity, although if too low the product may be damaged. If light is used during storage, it may affect compounds involved in photosynthetic activity, such as carotenoids, tocopherols and chlorophyll, also non photosynthetic activities involving enzymes may affect the content of bioactive compounds (Wills et al., 2007).

Manea and Buruleanu (2009) reported that thermal pasteurization of the sea buckthorn juice at 80°C for 10 min led to significant losses of vitamin C (approximately 86%) within a period of 3 months of storage at refrigeration conditions. Other researchers observed that storage of thermal treated (HTST 90°C, 45 s) sea buckthorn juice for 7 days at 6°C resulted in a degradation of vitamin C of about 11%–12% (Gutzeit et al., 2008).

Sea buckthorn berries juice is a nutritious beverage, rich in vitamin C and carotenoids with high antioxidant activity.

The main requirements for a freshly squeezed sea buckthorn juice production are the cloud stability and antioxidant activity retention after processing. Appropriate process technologies and conditions have to be applied in order to inactivate PME, responsible for cloud loss, while maintaining the nutritional characteristics and antioxidant activity of the juice. Alexandrakis et al. (2014) studied and modelled the effect of thermal treatment and HP processing on the inactivation kinetics of endogenous PME and on total antioxidant activity alteration. Thermal treatment significantly affected PME inactivation and residual antioxidant activity. Processing even at mild process conditions (60°C for 1 min) resulted in 2.5-fold antioxidant activity reduction and 50% PME inactivation compared to untreated sample. Pressure and temperature acted synergistically for PME inactivation that followed first-order kinetics with a residual PME activity at all pressure–temperature combinations used (200–600 MPa and 25°C–35°C). The effect of temperature and pressure on the inactivation rate constants was expressed through the activation energy and activation volume, respectively. Values of 163 $kJmol^{-1}$ and −17 $mL\ mol^{-1}$ at reference pressure of 600 MPa and reference temperature of 35°C were estimated, respectively. Antioxidant activity of the samples was expressed through the determination of the effective concentration (EC50). A slight increase in sea buckthorn antioxidant activity when applying pressures (200–600 MPa) at ambient temperature (25°C) was observed compared to the corresponding value of untreated juice. Processing at higher temperatures did not significantly

alter the total antioxidant activity of sea buckthorn juice. For sample treated at 600 MPa–35°C for 5 min, a 5% reduction of total antioxidant activity was observed.

These conditions are proposed as effective process conditions for sea buckthorn juice cold pasteurization, based on the higher antioxidant activity retention and simultaneous PME inactivation (Alexandrakis et al., 2014).

2.7. Current trends in sea buckthorn applications

In Europe and Asia, 10 different drugs manufactured from sea buckthorn components have been reported and are available in liquid, powder, paste, pill, and spray form. For example, popular pill products include vitamin C tablets because of the high vitamin C concentration in sea buckthorn berries, there are several other value-added products being manufactured including teas (from leaves) and animal feed (leaves, pulp, and seed residues).

Despite its highly acidic nature and exotic flavour, sea buckthorn berries have a good potential for producing various processed products like ready-to-serve beverage, squash, syrup, jam and jellies (Bal et al., 2011). Judicious blending of sea buckthorn juice/pulp with other fruits such as papaya, apple and orange in different ratios could be a promising way for processing of sea buckthorn and for minimizing astringency. Products on the market from sea buckthorn range from oil, juice, and food additives to candies, jellies, cosmetics, and shampoos (Schroeder and Yao, 1995).

Sea buckthorn fruit can be used to make pies, jams, lotions and liquors. The juice or pulp has other potential applications in foods or beverages (Bal et al., 2011). For example, in Finland, it is used as a nutritional ingredient in baby food. Fruit drinks were among the earliest sea buckthorn products developed in China. Sea buckthorn-based juice is even popular in Germany, Scandinavian and Nordic countries. It provides a nutritious beverage, rich in vitamin C and carotenoids.

For its troops confronting extremely low temperatures, India's Defence Research Development Organization established a factory in Leh to manufacture a multi-vitamin herbal beverage based on sea buckthorn juice (Cenkowski et al., 2006). The seed and pulp oils have nutritional properties that vary under different processing methods (Cenkowski et al., 2006; Bal et al., 2011). Sea buckthorn oils are used as a source for ingredients in several commercially available cosmetic products and nutritional supplements. Jams from the berries are fermented products from the pulp. Juice, pulp oil, seed oil, cream and pigments are the main commercial products from sea buckthorn berries.

Based on 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) (Savelyev et al.,2007):

Pear in nectar from sea buckthorn — consists of pear halves submerged in sea buckthorn 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^{-1} of vitamin C, 4.3 mg 100$g^{-1}$of β-carotene, 20 mg 100 g^{-1} P-active catechins, 65% arbutin, 18.0 mg 100 g^{-1} chlorogenic acid and 2.5% of nutrient fibre. The antioxidant activity of product is not less than 600 mg mL^{-1}. The consumption of this product will strengthen immunity and remove heavy metal salts from the human body.

Jelly from sea buckthorn with lactulose —is made from fresh sea buckthorn 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^{-1} of vitamin C, 1.4 mg 100 g^{-1} of β-carotene, 196 mg 100 g^{-1} of p-active catechins, 1.0% protopectin and 2.2 mg 100 g^{-1} anthocyanins. The antioxidant activity of product is 700 mkg mL^{-1}. 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 sea buckthorn —is a dietary and low energy value product from cooked, fresh sea buckthorn fruit with the addition of the natural sweetener 'Swyta' (fermentatively-treated steviozid), enriched with dihydroquercetin 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^{-1} of vitamin C, 30 mg 100 g^{-1} of p-active catechins, 1.2% pectin and a lesser amount of carbohydrates (1.2%). The antioxidant activity of product is not less than 650 mg mL^{-1}. A low energy value (about16 kcal 100 g^{-1} characterized the stewed fruit) 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 sea buckthorn-based products are threefold: high nutrient value, good organoleptic quality and a positive, directed physiological effect. The two first attributes characterized more traditional products only. 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 (Savelyev et al., 2007).

In a study by Li and Schroeder (1996) and Li (2003) possible uses for components in different sea buckthorn plant parts were given as listed in Table 6.

Over 250 products of food (jam, compote, juice, fruit wine, vodka, liqueur, soft drinks,

etc.), medicinal and cosmetic importance are prepared from sea buckthorn fruits. Sea buckthorn is a high valuable medicinal plant. Existence of vitamins in the content of fruits resulted in it becoming named as a poly-vitaminic fruit (Musayev, 2013).

As the plant's leaves also have got vitamins and other valuable substances, they are used as useful forage for agricultural animals and pets. Price of sea buckthorn is not measured only for its use in medicinal and food productions (Musayev, 2013). Sea buckthorn is valuable forestry species. It has strong capacity to produce root suckers, which give opportunity for its use for mitigation of soil erosion and this is a good way of reclamation of lands, marginalized in agriculture. Because sea buckthorn usually grows on the upper layer of soil, strongly branches and it has got multi-storied root system as well root tubers are formatted there and by their help free nitrogen of the air is absorbed as well it enriches the soil with the nitrogen as legume plants (Musayev, 2013).

Table 6. Sea buckthorn components and product categories (Li and Schroeder, 1996; Li, 2003)

Components		Product categories		
Bark	Pharmaceuticals Cosmetics			
Leaves	Pharmaceuticals Cosmetics Tea Animal feed			
Fruit	Oil	Pharmaceuticals Drinks Food products Cosmetics		
	Juice	Sports drinks Health drinks		
		Pulp	Food Beverages Brewery	
			Oil	Pharmaceuticals Cosmetics
			Residues	Animal feed
Seeds	Oil	Pharmaceuticals Cosmetics		
	Residues	Animal feed		

That is why it is possible to cultivate sea buckthorn on poor soils without fertilisation, including soils that have been structurally disturbed including those areas which structures are destroyed by open mining activities. Use of the sea buckthorn as a fitomeliorant increases biological productivity of soil and returns them to agricultural circle. Moreover, sea buckthorn is used for afforestation in steppes and fixing riverbanks (Musayev, 2013). Since sea buckthorn has been

used since ancient times in common medicines for curing many diseases affecting humans and other animals, the commercialization of sea buckthorn based nutritious products would be a great achievement in alternative nutritional diet sources (Kumar et al., 2011).

CHAPTER 3 - MATERIAL AND METHODS

3.1. Collection of sea buckthorn berries

The berries of the SB (*Hippophae rhamnoides* L.) cultivar 'Leikora' was collected from the orchard of Superberry Ltd. (North latitude 47° 10′ 29″, East longitude 20° 11′ 47″) near Szolnok in middle Hungary (Figure 10.). Berries were collected during October of 2017 at the stage of commercial maturity, as judged by juiciness and appearance. The berries were cleaned to remove damaged, diseased, or pest-infected fruits. Approximately twenty-four kilograms of fruit was collected at harvest. Following collection, the samples were placed in freezer bags and were transferred to a cooler containing ice within two hours after harvesting (Figure 9). Samples were transferred to a -20ºC freezer within five hours of harvesting and stored under these conditions until analyzed.

Figure 9. Maps of Szolnok in Hungary

3.2. Preparation of samples for experiment

Work on Sea buckthorn (*Hippophae rhamnoides* L.) was initiated in the laboratory of Department of Fruit and Vegetable processing Technology, at the Hungarian University of Agriculture and Life Science. During the separation of juice, seeds, and shells of the berries, we followed the technological process used in industrial practice. First, freeze berries were thawed at room temperature for 30 min. and then berries were heated to 80-85°C next to continuous mixing to inactivate the enzymes and to facilitate the extraction of juice and oil in subsequent steps. The berries were squeezed using fruit pulping and squeezer equipment, during which the juice and the pomace were, separated (Figure 10.). The Sea buckthorn juice was of orange colour. 9600 mL of juice was extracted from 24 kg of berries. The Figure 11. represents the juice extraction process from sea buckthorn berry.

Based on the experiments of Furulyás et al. (2018) the pomace was dried at 80ºC until moisture content became lesser than 10% by atmospheric dryer (LMIM, Esztergom, Hungary). Pomace with unbroken seed was obtained as a residue and was grinded by grinder (Retsch GM 200). After separating juice was added dried pomace from sea buckthorn. The treatment was made as control sample of sea buckhorn juice (C), without pomace, sea buckthorn juice 99.5% + pomace from sea buckthorn 0.5% (P0.5), sea buckthorn juice 99% + pomace of sea buckthorn 1% (P1) and sea buckthorn juice 98% + pomace of sea buckthorn 2% (P2).

Figure 10. Juice extraction process from Sea buckthorn berries

The drinks were filled into 100 ml transparent glass bottles and sealed. Extracted juice was heated at 90°C, for 10 min. Finally, all samples were cooled in a cold water bath and stored at room temperature for physicochemical analysis at an interval of 2 months for 14 months. Three parallel measurements were conducted for each sample.

After thawing the physicochemical parameters and antioxidant properties were measured. An overview of key activities undertaken during Sea buckthorn juice samples processing and analyses are provided in Figure 12.

Extraction pre-treatment was used to the total polyphenolic content and antioxidant activity measurement. During the extraction, 1 g of each sample was dissolved in 30 ml of the solution consisting of 60% distilled water, 39% methanol and 1% formic acid. The mixture was put in ultrasonic bath for 15 minutes and the homogenate was centrifuged at 5000 rpm for 10 min. This extraction is needed to perform some further measurements (Figure 13.).

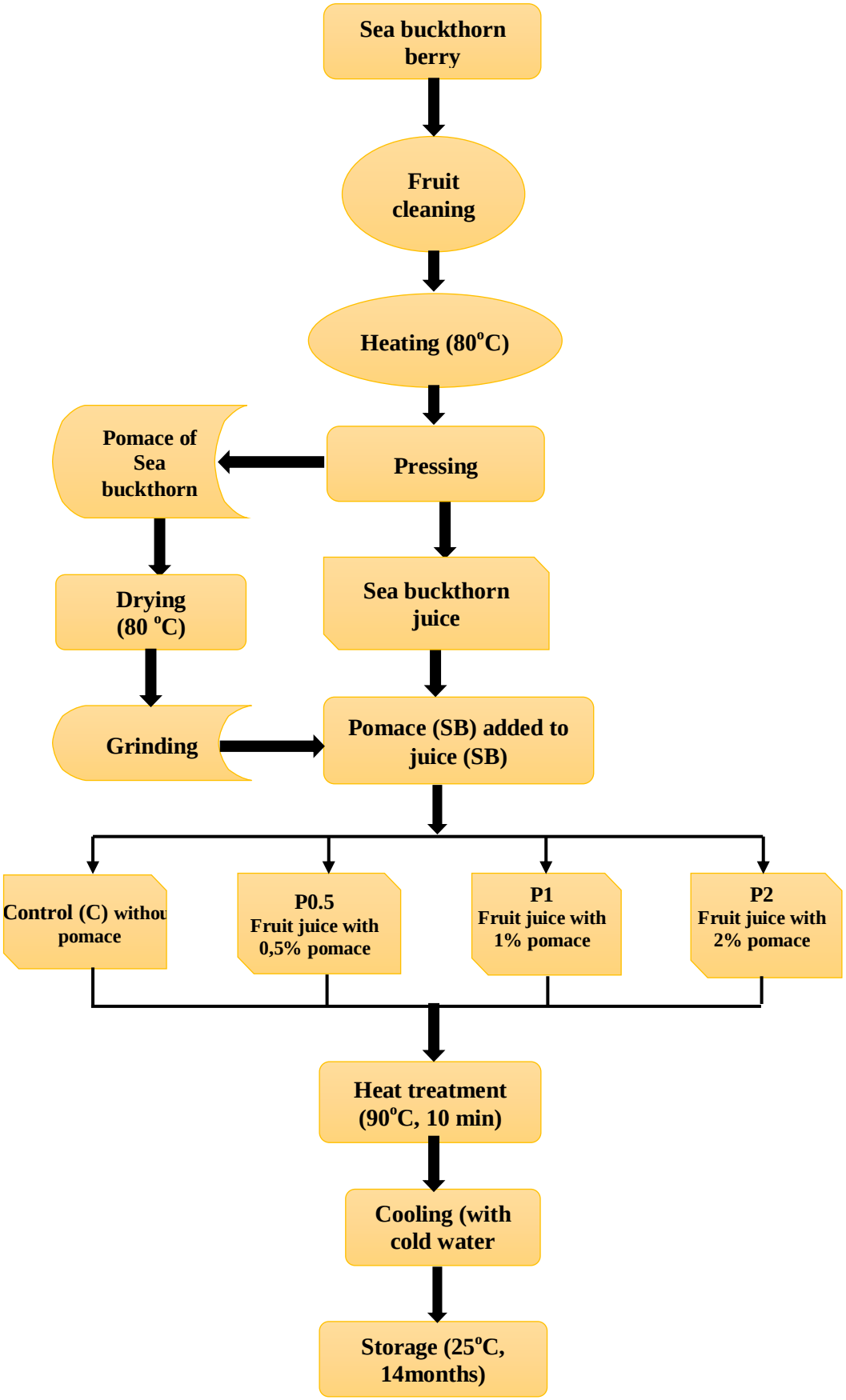


Figure 11. Process flowchart of Sea buckthorn juice extraction

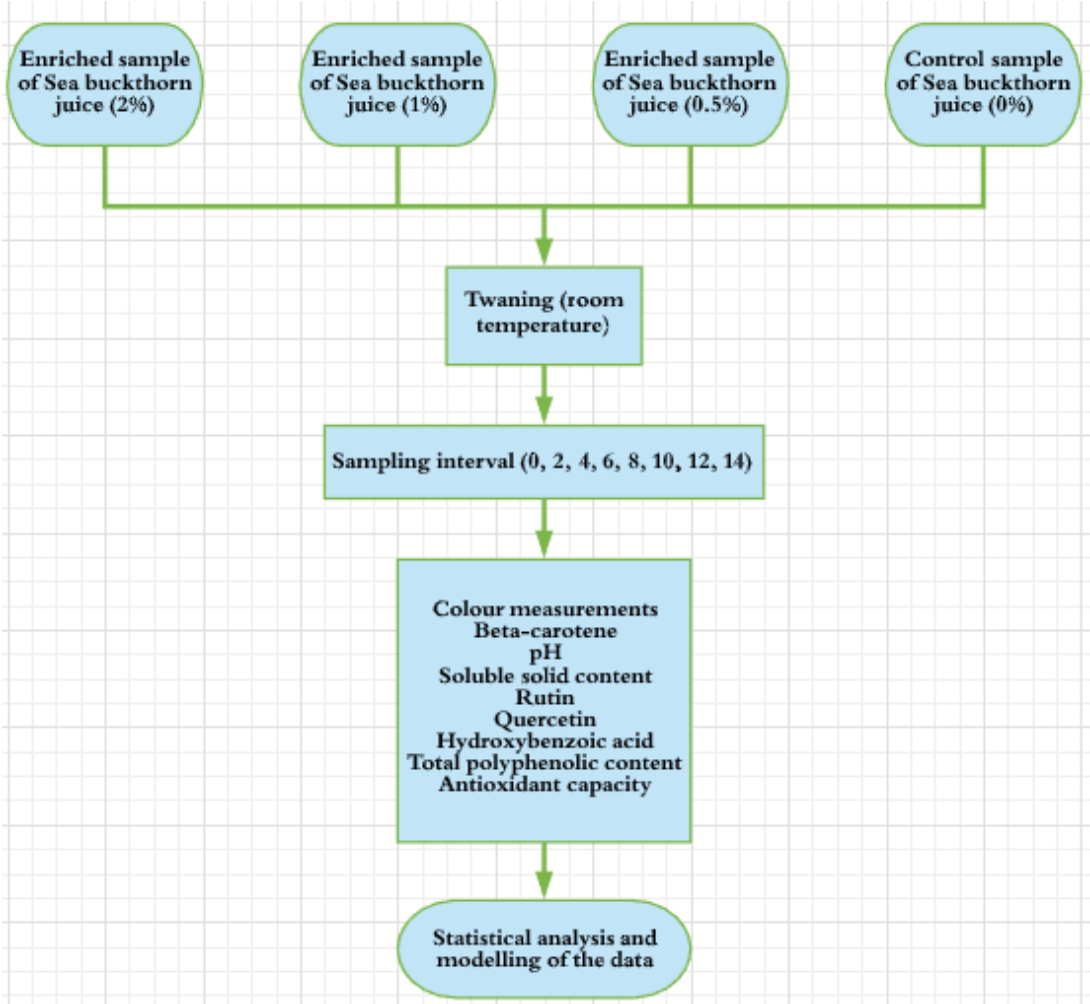


Figure 12. Overview of key steps undertaken during processing and analysis of sea buckthorn juice samples

All the reagents were analytical grade purchased from Sigma-Aldrich Chemical Co. (3050 Spruce Street, St. Louis, MO 63103, USA).

Figure 13. Control and enriched samples of Sea buckthorn before experiment

3.3. Methods

3.3.1. Soluble solids content

Soluble solids were expressed as ºBrix and determined by refractometer. The fruit was thawed to 15 ± 5°C and juice was expressed by placing 3 to 5 fruit between two layers of cheesecloth and manually squeezing the sample. The ºBrix was determined on the juice using an ATAGO DBX-55 digital refractometer with temperature compensation according to Codex Alimentarius 558/93/EEC. Fruit samples were analysed in triplicate.

3.3.2. pH value

Sample pH was determined in triplicate at room temperature using a Testo 206 pH measuring instrument. Three-point calibration was accomplished employing pH 7.0, 4.0 and 2.0 buffers.

3.3.3. Colour parameters

CIELAB tristimulus colour coordinates were measured with a Konica Minolta CR 410 manual digital colour meter. The colour meter was standardized using a white tile (standard no. LS-13903) with colour coordinates L* = 94.65, a* = -0.82 and b* = 0.83. Frozen fruit was thawed to 15 ± 5°C at room temperature. The thawed fruit (25 ± 1 g) was transferred to a glass sample cup (Hunter Associates) and placed over the analysis port on the colour meter (Pauli, 1976).

The results were expressed in the CIE LAB system with L* (the lightness coordinate), a* (the red/green coordinate, with +a* indicating red, and -a* indicating green) and b* (the yellow/blue coordinate, with +b* indicating yellow, and -b* indicating blue) colourimetric coordinates. Calculation of colour difference between two samples using the following formula:

$$\Delta E^{*} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Evaluation of ΔE * using in Table 7.

Table 7. Summary of colour difference

ΔE*	Sensable difference
0-0.5	Not noticed
0.5-1.5	Hardly noticeable
1.5-3.0	Noticeable
3.0-6.0	Clearly visionable

The hue angle (θ*) of the samples was determined by calculating tan-1 b*/a*. Samples were analyzed in triplicate.

Chroma = (a*+b*)½

Hue = Tan-1 (b/a)

3.3.4. Total polyphenolic content

Total phenolic content (TPC) was determined according to the Singleton and Rossi (1965) method. Samples were prepared with Folin-Ciocalteu's reagent and sodium-sulphate solution. One mL of appropriately diluted sample was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of 15% (w/v) sodium carbonate in a 20 mL test tube. Samples were held for 2 hours at 22 ± 2°C before measurement. The colour change taking place during the reaction can be detected on 765 nm by Hitachi U-2900 spectrophotometer (Hitachi High Technologies Europe GmbH, Krefeld, Germany).

The total phenolic content was determined from a gallic acid calibration curve prepared and analyzed concurrently with the fruit samples. Standard solutions of gallic acid were prepared in ddH_2O at concentrations ranging from 10 to 50 mg L^{-1}. The calibration curve was constructed by plotting the absorbance at 765 nm (A765) versus standard concentration. Calibration curves (y=0,1988*x-0,0847) had correlation coefficients R^2=0,9802. The results can be expressed as gallic acid equivalent (µg gallic acid equivalent (GAE) mL^{-1}). All samples were analyzed in triplicate.

3.3.5. Evaluation of antioxidant capacity (FRAP)

Antioxidant capacity (FRAP) was determined by Ferric Reducing Ability of Plasma (FRAP) assay according to the method of Benzie and Strain (1996). The stock solutions included 300 mM acetate buffer (3.1g sodium acetate tripyridyl-s-triazine) solution in 40mM hydrochloric acid, and 20 mM ferric chloride solution and then warmed at 37°C before using. Fruit extracts (150µL) were allowed to react with 2.85 mL of the FRAP solution for 30 min in the dark conditions. The iron ion ($Fe3^+$) reducing ability of the antioxidants in the samples is shown by blue colour change (λ=593 nm). The antioxidant capacity can be expressed as ascorbic acid equivalent after calibration (µg ascorbic acid (AA) mL^{-1}). Calibration curves (y=0,346*x-0,146) had correlation coefficients R^2=0,9974.

3.3.6. Identification and quantification of β-carotene

β-carotene (7235-40-7; Sigma-Aldrich Chemical Co.) was determined by Waters Co. (USA) HPLC instrument according to Ficzek et al. (2019). The extraction of carotenoids from 5 g sea buckthorn fruits was carried out under subdued light with 15 mL tetrahydrofuran (THF) for 12 h at +4°C, using Edmund Büchler SM 30-control shaker (Hechingen, Germany) at 150 rpm. The supernatant of extraction was decanted to 1.5 ml Eppendorf-tubes and centrifuged at 15,000 rpm (Hettich Mikro 22R centrifuge, Tuttlingen, Germany) for 5 min at -5°C.

The supernatant was filtered through 0.45 µm Millex HN syringe filter unit (SLHV 013 NL,

PVDF Durapore, Millipore Co., Billerica, MA, USA), and finally injected onto the HPLC. Each extraction was performed in triplicate.

HPLC instrument from Waters Co. (USA) includes the 2487 dual absorbance detector (analytical wavelength 450 nm), 1525 binary HPLC pump (temperature of the sample compartment set to +5°C), column thermostat (set to 30°C), in-line degasser AF and 717 plus autosampler (temperature of the sample compartment set to 5°C). Empower TM2 software was used to control the analysis. The column type was Symmetry C18, 5 µm, 4.6 × 150 mm. The pressure on the column was 11.55±0.07 MPa and the volume injected into the column was 20 µL. The conditions regarding mobile phase were modified after Bushway (Bushway, 1986). The mixture ACN:MeOH:THF (50:45:5, V/V/V) flowing at 1 mL min^{-}1 was used as eluent.

3.3.7. Identification and quantification of flavonoids

Sample preparation for phenolic components. 1 g samples were extracted in 10 mL methanol (containing 1% HCL and 1% BHT) for 12 h in the dark at 4°C, using an Edmund Bühler SM 30 control shaker (200 rpm min^{-1}). The supernatant was decanted and centrifuged in Eppendorf tubes in a Hettich Mikro 22R centrifuge (15,000 rpm min-1 for 5 min). The supernatant was filtered on a 0.45µm Millex® HV Syringe Driven Filter Unit (SLHV 013 NL, PVDF Durapore), purchased from Millipore Co. (Bedford, MA, USA), and injected into the HPLC system. The quantities of the individual phenolic compounds are given in µg g^{-1}.

Analytical conditions. The Waters High-Performance Liquid Chromatography (purchased from Waters Co., 34 Maple Street, Milford, MA, USA) was equipped with 2487 Dual λ Absorbance Detector, a 1525 binary HPLC pump, and in-line degasser, a column thermostat (set at 40°C) and an 717 plus autosampler (set at 5°C), and was controlled using Empower TM2 software. A Kinetex C18 2.6µm 150 × 4.6 mm column (Phenomenex, 411 Madrid Avenue Torrance, CA 90501-1430, USA) was installed. The gradient mobile phase was A: H2O:MeOH:H_3PO_4=940:50:1, B: MeOH (0–30 min: A 100%–10%, 30–30.1 min: 10%–100%, 30.1–31: A 100%) with a flow rate 1 mL min-1, the pressure in the column was 4200±10 psi at a column temperature of 30°C. The running time was 25 min. Each injected volume was 20 µL. The sampling rate was 10 pt sec^{-1}, and the phenolic components were monitored at a wavelength of 280 nm. The retention times of the standards were rutin (28.8 min), quercitrin (24.4 min), dihydroxybenzoic acid (22.83 min).

3.3.8. Identification and quantification of ascorbic acid

1 g of sample was homogenized repeatedly with 0.01 mol L^{-1}metaphosphoric acid (6 X 20 mL) and centrifuged at 2200 × g for 5 min (Cooling Centrifuge C-30, REMI). The supernatant

was filtered through a0.45 μm filter and analyzed immediately. Juice samples were also treated with met phosphoric acid, centrifuged and filtered. A 20 μL sample was injected into a Shimadzu LC-8A HPLC equipped with a Phenomenex C-18 ODS-2column (5 μm, 250 mm × 4.60 mm; Luna) and a PDA detector (SPD-M 10AVP) set at 260 nm, and 3.7 mmol L^{-1} phosphate buffer (pH 4) at a flow rate of1mL/min was used as mobile phase. Authentic standards of ascorbic acid(0.5–5 μg mL^{-1}) were used for optimizing the HPLC conditions. Results are reported as the mean ± standard deviation of three independently extracted samples.

3.3.9. Statistical analysis

All experiments were conducted in more than triplicates. There was two factors pomace treatment and storage time. Treatment factor had four levels 0%, 0.5%, 1% and 2% of pomace concentration represented as control C, enriched P0.5, P1 and P2 respectively. Time factor had 8 levels 0, 2, 4, 6, 8, 10, 12, and 14 months. Assumptions for Normal distribution of data and homogeneity of variance were checked.

Normality was proved by Kolmogorov-Smirnov test. Homogeneity of variance was checked by Levene's test for each factor. Two factor complete randomized design ANOVA was selected for analysis since two factors were involved with one dependent variable. Turkey post hoc test was run for significant variables. Statistical evaluation was done by using IBM SPSS V25 in 95% confidence interval.

CHAPTER 4 - RESULTS AND DISCUSSION

4.1 Soluble solid content

The soluble solid content of the fruit is usually obtained from assessing the degrees ^{o}Brix of the fruit. The soluble solid content measures and includes the soluble carbohydrates, organic acids, proteins and minerals of the fruit. It represents from 10 to 20% of the fruit's fresh weight and increases as fruit mature to produce a less acidic, sweeter fruit. The grower must aim to produce an acceptable balance of soluble solid content.

The observations for soluble solid content of the storage enriched and control samples of sea buckthorn juice were taken on every two months. The soluble solids content changes of the control sample and enriched of sea buckthorn juice (C, P0.5, P1 and P2) samples illustrate the Table 8.

Table 8. Effect of pomace treatment and storage time (months) on soluble solids of the sea buckthorn juice (^{o}Brix)

Month	Control	P0.5	P1	P2
Initial	9.50 ± 0.17^{aA}	9.82 ± 0.02^{abcAB}	10.02 ± 0.12^{abB}	10.32 ± 0.33^{aB}
2	8.95 ± 0.18^{aA}	9.64 ± 0.05^{aB}	9.93 ± 0.12^{abBC}	10.19 ± 0.20^{aC}
4	9.00 ± 0.17^{aA}	9.90 ± 0.17^{abcBC}	9.65 ± 0.28^{aB}	10.33 ± 0.06^{aC}
6	9.18 ± 0.13^{aA}	9.88 ± 0.11^{abcB}	9.98 ± 0.37^{abBC}	10.50 ± 0.17^{aC}
8	9.42 ± 0.34^{aA}	10.14 ± 0.09^{cB}	10.29 ± 0.17^{bB}	10.60 ± 0.10^{aB}
10	9.10 ± 0.17^{aA}	9.80 ± 0.17^{abB}	10.20 ± 0.10^{abBC}	10.60 ± 0.17^{aC}
12	9.07 ± 0.21^{aA}	10.00 ± 0.17^{bcB}	10.10 ± 0.10^{abBC}	10.43 ± 0.15^{aC}
14	9.23 ± 0.15^{aA}	10.07 ± 0.06^{bcB}	10.23 ± 0.21^{bB}	10.47 ± 0.50^{aB}

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

In our experiment enriched P0.5, P1 and P2 samples retained 3.32%, 5.47%, and 8.63% more soluble solids content, respectively, compared to control (C) sample. Thus, the lowest soluble solids content values were observed for the control (C) sample (9.5^{o}Brix) and the highest soluble solids content value was observed for enriched P2 sample (10.32^{o}Brix) in the initial day. All samples of sea buckthorn juice are slightly higher values than Raffo et al., (2004), who studied 'Leikora' (7.5^{o}Brix) and Ficzek et al. (2019), who measured 5.6 ^{o}Brix in case of 'Leikora' variety . However similar and higher values were measured Tiitinen et al. (2005) (7.4-12.6^{o}Brix), in Turkish (10.1-14.8^{o}Brix) (Ercisli et al., 2007), and Chinese (10.2-22.7^{o}Brix) genotypes (Zhang et

al., 1989; Tong et al., 1989).

Effect of storage

The soluble solids content value of the enriched samples of sea buckthorn juice (P0.5, P1 and P2) were gradually increased to 10.07, 10.23 and 10.47°Brix respectively during 14 months of storage, whereas control (C) sample was decrease from 9.5to 9.23 °Brix during storage time (Figure 14.). The soluble solid content showed a rapid decrease in all sea buckthorn juice samples in the first two months. Thus, the lowest soluble solids content values were observed for the two months control (C) sample (8.95 °Brix) followed by enriched P0.5 sample (9.64 °Brix). The decline in soluble solid content (°Brix) in some treatments might be due to the degradation of total soluble substances due to the prolonged storage period.

In most of the sample of sea buckthorn juice, the maximum increase in soluble solids content was recorded at 8th months of storage and after that, it started declining except for enriched P2 sample, where the increasing trend was recorded till the end of the investigation. We observed the highest soluble solids content values were observed in the enriched P2 sample (10.6 °Brix) followed by enriched P1 sample (10.29 °Brix) in 8 months. The soluble solid content values of control (C) sample were decreased to 2.84% at the end of storage.

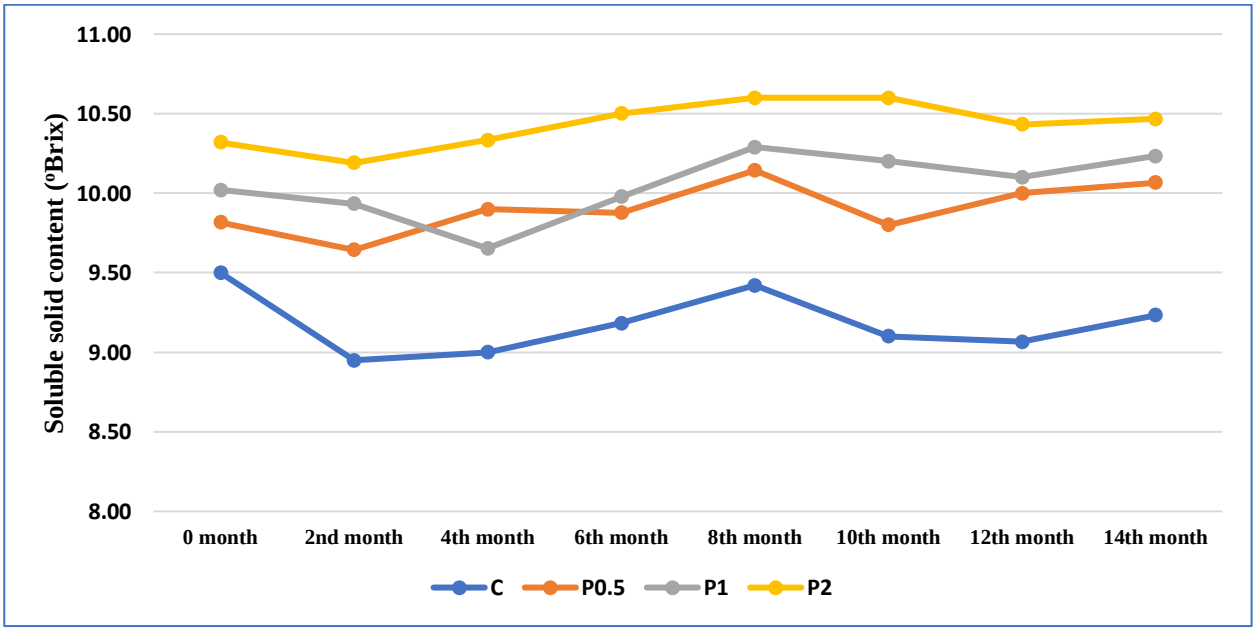


Figure 14. Soluble solid content of sea buckthorn juice samples during 14 months storage at room temperature

Enriched P0.5, P1 and P2 samples retained 9.10%, 10.83% and 13.43% more soluble solid content than the control (C) sample after 14 months. This gradual increase might be associated

with the transformation of pectic substances, starch or other polysaccharides into soluble sugar, monosaccharides and oligosaccharides (Deka, 2000).

During storage time, enriched P2 sample was more stable than other sea buckthorn juice (C, P0.5 and P1) samples. All enriched sea buckthorn juice samples exhibited higher soluble solid content (^{o}Brix) than the control (C) sample. In addition, soluble solid content in sea buckthorn juice increased in case of different pomace content during storage time.

Results showed that storage time and pomace content significantly affected the level of soluble solid content (Table 8. and Annex.2.) of sea buckthorn juice. Both pomace treatment and storage time had significant effect on soluble solids of buckthorn juice. Pomace treatment, $F (3, 64) = 165.777$, $P<0.001$ and storage time, $F (7, 64) = 5.921$, $P<0.001$. Moreover, there was no significant interaction between pomace treatment and storage on the soluble solids content of the juice, $F (21, 64) = 1.218$, $P = 0.268$

Soluble solid content (°Brix) of sea buckthorn berries is 10.19–22.74°Brix (highest) for Chinese varieties as reported by Zhang et al. (1989) and 9.3–17.3°Brix (lowest) for cv. Indian-Summer, as reported by Li et al. (2003). But the soluble solid content (°Brix) of pulp from berries is 26.2–27.9 (Arimboor et al., 2006) and 8.86–9.72°Brix (Dhyani et al., 2007) for Indian varieties.

4.2. pH value

Foods with a pH below 4.6 are called high-acid food. This limit was set, because in foods below pH 4.6, *Clostridium botulinum* spores cannot sporulate and produce toxin. The optimum pH for growth of microorganisms is close to neutral (pH 7), and most bacteria do not grow below pH 4.6.

In our results, control (C) sample was high-acid than other enriched samples of sea buckthorn juice (P0.5, P1 and P2). Our experiment enriched P1 and P2 samples retained 3.40% and 5.53% less acidity (pH higher), respectively, compared to control (C) sample on the initial day. The pH value of enriched and control samples of sea buckthorn juice presented the Table 9.

The sea buckthorn juice samples (C, P0.5, P1 and P2) on the initial day were 2.33 to 2.48. The pH was slightly different between enriched P0.5, control (C) samples and enriched samples (P1, P2). Sea buckthorn pomace slightly improved from pH value of sea buckthorn juice. Other authors have approximate result, which agrees with previously reported results for sea buckthorn in Finland (2.7–2.9 pH, Tiitinen et al. 2005). Escisli et al. (2007) was recorded that sea buckthorn juice pH values varied from 2.63 to 2.98. Sea buckthorn juice had pH values around 2.5 units (Chirila et al., 2014) and the measured pH value of 2.8 of the juice products (Gutzeit et al., 2008).

Table 9. Effect of pomace treatment and storage time (months) on pH of sea buckthorn juice

month	Control	P0.5	P1	P2
Initial	2.35 ±0.00^{aA}	2.33 ±0.01^{aA}	2.43 ±0.00^{abB}	2.48 ±0.00^{bcC}
2	2.46 ±0.01^{cAB}	2.47 ±0.01^{bAB}	2.48 ±0.01^{bcdA}	2.50 ±0.00^{bcdB}
4	2.40 ±0.01^{bA}	2.43 ±0.00^{bAB}	2.45 ±0.01^{abcdBC}	2.49 ±0.01^{bcC}
6	2.42 ±0.01^{bcA}	2.45 ±0.01^{bAB}	2.51 ±0.03^{deB}	2.45 ±0.02^{abAB}
8	2.31 ±0.01^{aA}	2.34 ±0.01^{aAB}	2.38 ±0.02^{aB}	2.45 ±0.01^{abC}
10	2.32 ±0.01^{aA}	2.43 ±0.02 ^{bB}	2.43 ±0.00^{abcB}	2.42 ±0.01^{aB}
12	2.43 ±0.00^{bcA}	2.45 ±0.01 ^{bA}	2.50 ±0.01^{cdeB}	2.53 ±0.02^{cdB}
14	2.43 ±0.01^{bcA}	2.47 ±0.01 ^{bA}	2.55 ±0.01^{eB}	2.55 ±0.00^{dB}

Superscript with mall case letters indicates significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

Effect of storage

The pH of control and enriched samples of sea buckthorn juice during storage was estimated every 2 months (Figure 15.). The pH values were gradually increased to 2.43, 2.47, and 2.55 during 14 months of storage. An increase in pH values was recorded for all samples of sea buckthorn juice.

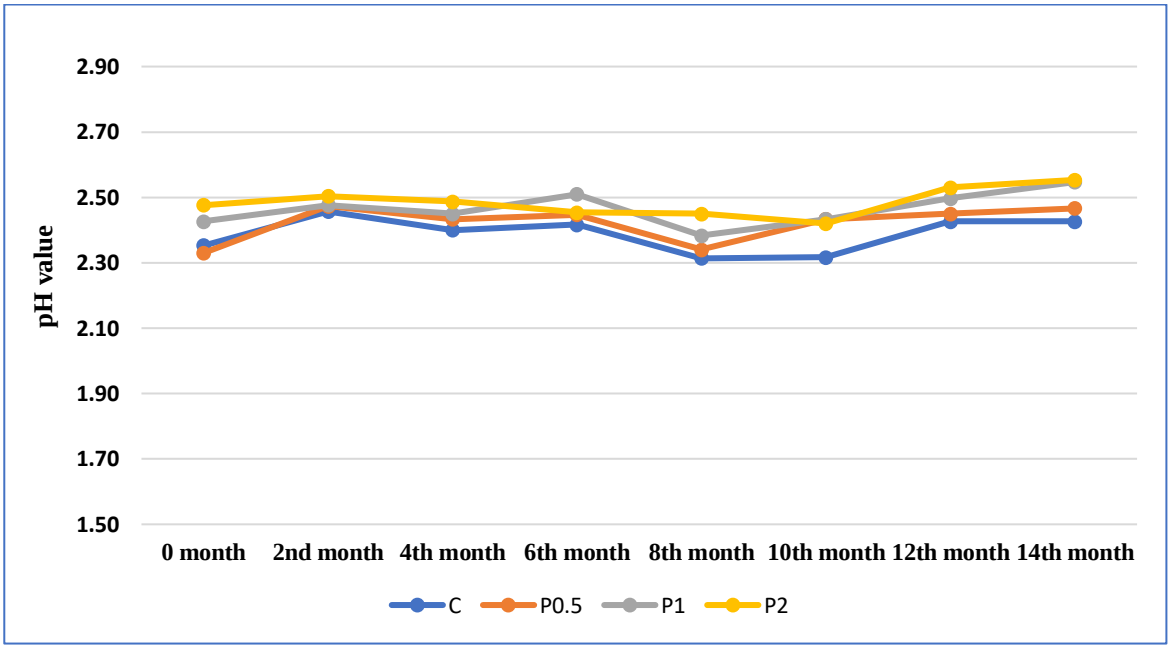


Figure 15. pH value of sea buckthorn juice samples during 14 months storage at room temperature

The minimum pH value was observed for the control (C) sample at 8 months. At 12 months, it comes up to 2.43. In enriched P2 samples a maximum pH 2.55 was recorded at 12

months. In first 2 months pH value of each sample increased (between 0.81% and 4.68%) and after 4 months all samples of sea buckthorn juice pH value decreased (between 0.4% and 2.44%).

During storage time, the maximum increase was observed enriched P0.5 sample (6.00%) followed by P1 sample (4.93%), while minimum increase was observed in control (C) sample (3.40%) followed by enriched P2 sample (2.82%).

After 14 months, enriched P0.5, P1 and P2 samples had higher pH value (1.64% and 4.93%), respectively, compared to control (C) sample. At the beginning time of the process, there is a minor difference between the control (C) and enriched P0.5 samples. Different percent of sea buckthorn pomace improved pH in sea buckthorn juice during storage time. During storage time, enriched P1 and P2 of pH is more stable than the control (C) sample.

Pomace treatment, storage time and the two's interaction had a significant effect on the pH of buckthorn juice (Table 9. and Annex.2.). Pomace treatment, $F (3, 64) = 118.930$, $P<0.001$ and storage time, $F (7, 64) = 66.279$, $P<0.001$. Interaction between pomace treatment and storage on the pH of the juice had, $F (21, 64) = 6.461$, $P = 0.001$.

Our results are consistent with the finding by Cecilia and Maia (2003), which did not observe significant differences in pH at 5% between a zero times of 350 days for the hot filling process. Our results agree with the finding of Rehman et al. (2012), who observed a decrease in pH from 3.10 to 2.06 during 90 days on the storage of mango-sea buckthorn blended juice. After 2-year storage between 3.23 and 4.29 (Elisabeta et al., 2014).

4.3. Colour parameters

4.3.1. L* (lightness)

The results of the determination of the L* value can be seen in Table 10. The value of L* was reduced with the addition of pomace, thus the samples became less dark. The amount of pomace added affected the lightness factor (L*). The degree of darkening was increased addition of pomace, and the samples containing pomace became less dark compared to control (C) sample. The colour parameter L* (lightness) between all samples of SB juice had a slight difference. Of the experiment, enriched P0.5, P1 and P2 samples retained 0.02%, 0.60% and 0.15% darker (lower L* value), than the control (C) sample.

Therefore, the content of colour parameter L* (Lightness) in sea buckthorn juice ranged from 47.64 enriched P1 sample to 47.93 in control (C) sample in the initial day.

Effect of storage

The changes in lightness of the control (C) sample, an enriched sample of sea buckthorn juice (P0.5, P1 and P2) are illustrated in Figure 16.

Table 10. Effect of pomace treatment and storage time (months) on L* colour value of sea buckthorn juice

month	Control	P0.5	P1	P2
Initial	47.93±0.79^{eA}	47.92±0.05^{bA}	47.64±0.02^{eA}	47.86±0.01^{dA}
2	48.09±0.20^{deA}	47.51±0.39^{abA}	47.22±0.07^{dA}	47.45±0.56^{cdA}
4	47.35±0.34^{cdeB}	46.84±0.46^{abAB}	46.48±0.17^{cAB}	45.90±0.80^{abA}
6	46.10±0.77^{bcA}	46.37±0.76^{abA}	46.08±0.12^{bcA}	46.41±0.02^{bcA}
8	46.61±0.15^{bcdA}	45.40±1.88^{aA}	45.59±0.11^{bA}	44.93±0.54^{aA}
10	46.60±0.14^{bcB}	46.37±0.64^{abB}	45.53±0.28^{bAB}	44.96±0.52^{aA}
12	45.95±0.57^{bB}	45.47±0.69^{aB}	43.74±0.33^{aA}	45.27±0.26^{abB}
14	42.61±0.15^{aA}	45.45±0.53^{aC}	43.44±0.27^{aB}	45.75±0.15^{abC}

Superscripts with mall case letters indicate significance difference by time along the rows. Superscript with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

The colour parameter L* or lightness values of all samples of sea buckthorn juice on initial day was 47.93 (C), 47.92 (P0.5), 47.64 (P1) and 47.86 (P2), which were gradually decreased to 42.65 (C), 45.45 (P0.5), 43.44 (P1) and 45.72 (P2) during 14 months of storage, respectively. The L* values of the control (C) sample decreased over time (except the first 2 months) while the values were almost stable with a slight decrease for enriched P0.5 sample.

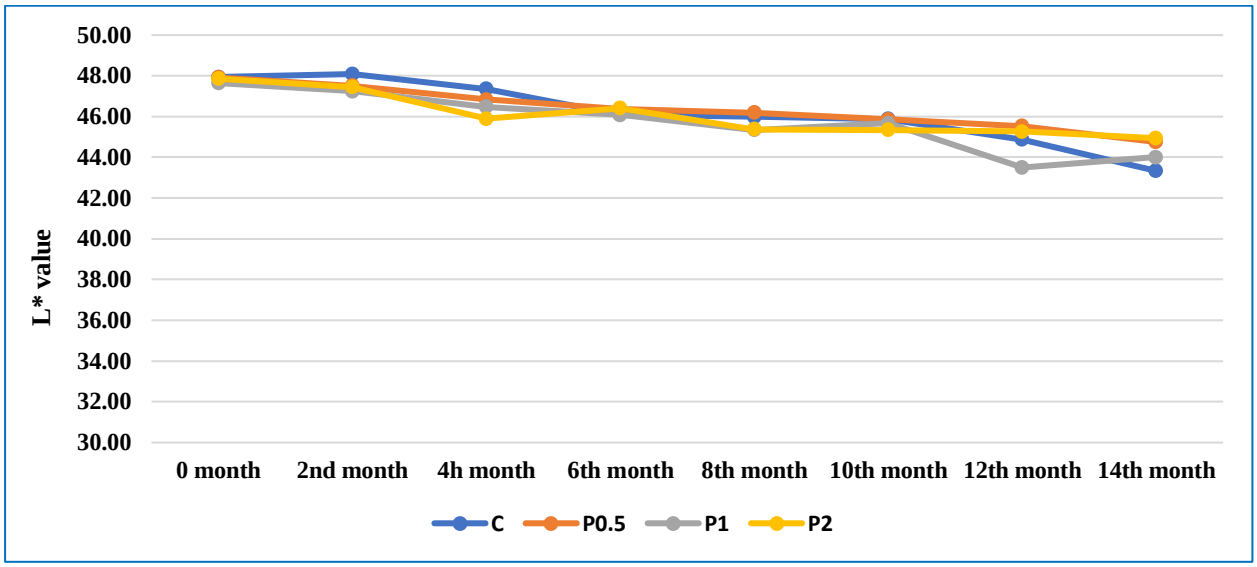


Figure 16. Change in colour parameter lightness values of sea buckthorn juice samples during 14 months storage at room temperature

Control sample, after 2 months, had 1.81% and 1.33% lighter than the enriched P1 and P2 samples, respectively, while the enriched P0.5 sample only 0.61% and 0.12% lighter than the

enriched P1 and P2 samples. However, in the enriched P1 and P2 samples, lightness decreased but slower compared to the control (C) sample.

After 14 months, enriched P0.5, P1 and P2 samples retained 3.25%, 1.55% and 3.69% lighter, respectively, compared to the control (C) sample. The end of storage, lowest colour parameter L* or lightness values was observed for the control (C) sample. Roopesh et al. (2014) observed browning and a decrease in L* value after 4 weeks (2.5%) and 8 weeks (5%) storage in UV-C treated pears, in comparison to treated pears (control).

Results showed that storage time and sample juices significantly affected the level of L* values (Table 10. and Annex.2.) of sea buckthorn juice. Both pomace treatment and storage time had a significant effect on the L* colour value of buckthorn juice. Pomace treatment, $F(3, 64) = 9.131$, $P<0.001$ and storage time, $F(7, 64) = 58.407$, $P<0.001$. Moreover, there was significant interaction between pomace treatment and storage on the L* colour value of the juice, $F(21, 64) = 5.907$, $P = 0.001$.

Usually, a decrease in colour parameter lightness value indicates browning process (Rico et al. 2007). In this study, the colour parameter lightness values of each samples of sea buckthorn juice decreased, while *a**(redness) and *b**(yellowness) decreased during the storage, indicating browning activities. This gradual darkening observed could be due to non-enzymatic browning in an aqueous environment with proteins and reducing sugars (Theodosiou et al., 2010).

4.3.2. Colour parameter a* (redness)

The colour parameter redness (a*) describes the colour of juices in which a positive a* value indicates a reddish tone while a negative number shows a greenish tone. Control (C) sample contained the most colour parameter redness all sea buckthorn juice samples. The results of the determination of the a* value (redness) can be seen in Table 11. Therefore, the content of colour parameter a* (redness) in sea buckthorn juice ranged from 14.57 (P2) to 17.19 (C) sample in the initial day. There were slight (but not significant) differences between the control C sample and the enriched P0.5. The colour parameter redness (a*) of control (C) and enriched P0.5 samples were significantly higher than enriched samples of sea buckthorn juice (P1 and P2) for all storage time.

In our experiment enriched samples of sea buckthorn juice (P0.5, P1 and P2) retained 0.47%, 9.31% and 15.24% less reddish (lower a* value), respectively, compared to the control (C) sample. In addition, the different content of sea buckthorn pomace in sea buckthorn juice aggravated the less redness in sea buckthorn juice sample. Ercisli et al. (2007) reported the colour parameter a* (redness) sea buckthorn berries were from 9.67 to 30.94. Therefore, Tiitinen et al. 2005 reported, the colour parameter a* (redness) was 4.0 ± 2.7 (‘Oranzhevaya’) to 18.5 ± 1.0

('Trofimovskaya').

Table 11. Effect of pomace treatment and storage time (months) on a* colour value of sea buckthorn juice

month	Control	P0.5	P1	P2
Initial	17.19±0.26^{bcC}	17.11±0.05^{cC}	15.59±0.04^{bcB}	14.57±0.02^{bA}
2	18.04±0.07^{cC}	16.44±0.54^{bcB}	16.53±0.64^{cB}	14.53±0.52^{bA}
4	16.68±1.09^{bcB}	15.16±1.08^{abAB}	15.27±0.37^{abAB}	13.40±0.47^{aA}
6	16.96±0.72^{bcB}	15.73±0.64^{abcAB}	15.25±0.91^{abAB}	13.95±0.36^{abA}
8	16.75±0.03^{bcD}	15.46±0.19^{abC}	15.07±0.05^{abB}	13.54±0.08^{aA}
10	16.71±0.11^{bcD}	15.45±0.20^{abC}	14.91±0.22^{abB}	13.75±0.19^{abA}
12	16.23±0.47^{bC}	14.98±0.45^{abB}	14.10±0.31^{aAB}	13.84±0.07^{abA}
14	14.09±0.49^{aA}	14.96±0.12^{aB}	14.38±0.19^{abAB}	14.25±0.23^{abAB}

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

Effect of storage

Colour change in redness of control and enriched samples of sea buckthorn juice are presented the Figure 17. The colour parameter redness (a*) values decreased significantly ($p<0.05$) with time from 17.19, 17.11, 15.59 and 14.57 to 14.09, 14.96, 14.38 and 14.25 in sea buckthorn juice samples C, P0.5, P1 and P2 respectively, during storage time.

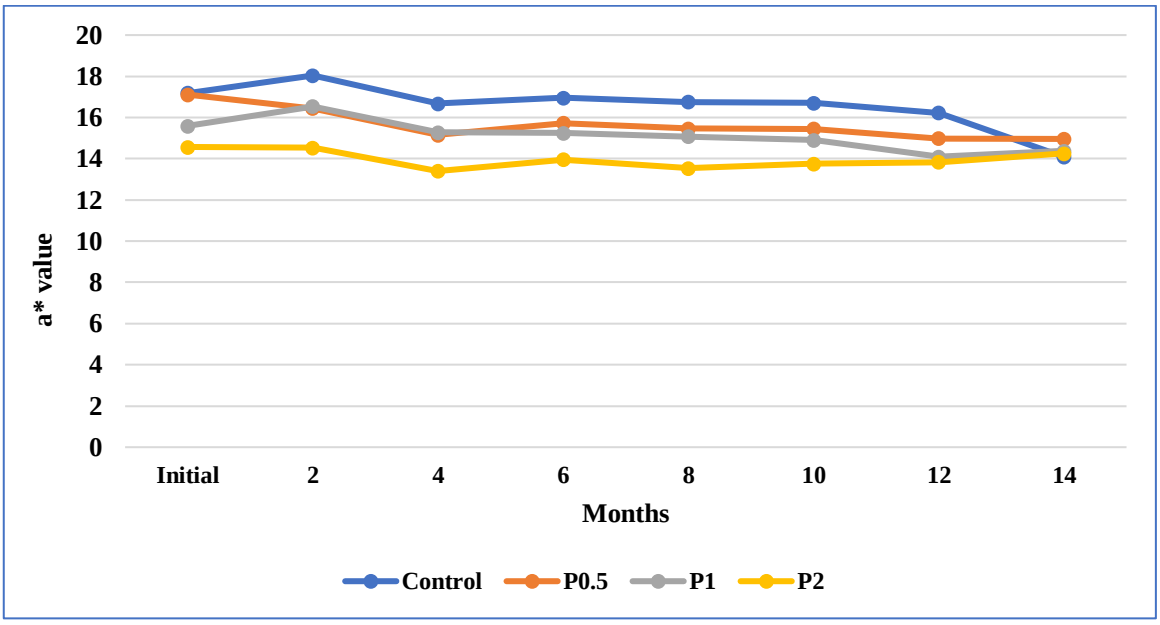


Figure 17. Change in colour parameter redness values of sea buckthorn juice samples during 14 months storage at room temperature

First 2 months control (C) and enriched P1 samples were significantly increased by 4.95% and 6.03% in storage and decreased after that.

The lowest colour parameter redness value was observed for the enriched P2 sample during storage time. After 4 month the a* value was 13.4 and was increased during the remaining storage period.

In the control (C) sample, maximum colour parameter redness 18.04 was recorded in first 2 months. At 14 months it comes down to 14.09. At the end of storage period, the control (C) sample was lowest in a*. Control (C) sample contained the most colour parameter redness other sea buckthorn samples (P0.5, P1 and P2). Different sea buckthorn pomace content in sea buckthorn juice declined reddish in sea buckthorn juice during storage time.

Results showed that storage time and sample juices significantly affected the level of a* values (Table 11. and Annex.2.). Both pomace treatment and storage time had significant effect on a* colour value of buckthorn juice. Pomace treatment, $F (3, 64) = 135.654$, $P<0.001$ and storage time, $F (7, 64) = 24.190$, $P<0.001$. Moreover, there was significant interaction between pomace treatment and storage on the a* colour value of the juice, $F (21, 64) = 4.424$, $P = 0.001$

4.3.3. Colour parameter b* (yellowness)

The sea buckthorn juice is yellow; the high amount of carotene is responsible for this yellow colouration. The presence of some of the other pigment also contributes to the colour. Granules or clumps are embedded in the juice, which is actually the containing spherical droplets that are yellow-brown (Beveridge and Harrison, 2001). The oil droplets also contribute to the yellow colouration. A positive colour parameter b* value indicates a yellowish tone while a negative value indicates a bluish tone. 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 et al., 2002, Beveridge et al., 1999).

One of the most important measurements between the colour parameter is b* (yellowness). The results of the determining the value of the b* (yellowness) can be seen in Table 12. The content of colour parameter b* in sea buckthorn juice ranged from 39.72 control sample (P1) to 45.21 in enriched P0.5 sample in the initial day. Thereover, enriched P1 and enriched P2 samples retained 3.17%, and 1.44% less yellowish (lower b* value), respectively, compared to control (C). The P0.5 more yellowish by 10.21% than the control (C) sample.

The yellowness of the sea buckthorn juice was significantly different ($p<0.05$) among the four sea buckthorn juice samples. Ercisli et al. (2007) reported, the Colour parameter b* (yellowness) sea buckthorn berries were from 37.44 to 57.79. Therefore, Tiitinen et al. 2005

reported, the colour parameter b* were 22.8 ± 5.6 (‘Oranzhevaya’) to 45.5 ± 1.5 (‘Raisa’).

Table 12. Effect of pomace treatment and storage time (months) on b* colour value of sea buckthorn juice

month	Control	P0.5	P1	P2
Initial	$41.02±0.25^{bC}$	$45.21±0.04^{bD}$	$39.72±0.03^{bcA}$	$40.43±0.02^{abB}$
2	$46.08±0.42^{cBC}$	$43.83±0.83^{abB}$	$48.38±0.54^{dC}$	$41.14±1.13^{bA}$
4	$41.00±3.27^{bA}$	$40.93±0.97^{aA}$	$41.76±0.97^{cA}$	$37.56±1.76^{aA}$
6	$42.29±1.99^{bcA}$	$42.36±1.34^{abA}$	$40.86±2.02^{cA}$	$39.52±1.24^{abA}$
8	$42.61±0.56^{bcC}$	$42.35±1.12^{abC}$	$40.24±0.12^{cB}$	$38.97±0.35^{abA}$
10	$42.93±0.19^{bcB}$	$42.51±0.89^{abB}$	$40.23±0.41^{cA}$	$38.87±1.32^{abA}$
12	$41.10±2.17^{bB}$	$41.00±1.87^{aB}$	$36.78±1.14^{aA}$	$39.34±0.62^{abAB}$
14	$33.62±1.55^{aA}$	$41.45±0.42^{aC}$	$37.19±0.20^{abB}$	$40.34±0.38^{abC}$

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey’s test, $p<0.05$)

Effect of storage

Changes in yellowness of samples are showing the Figure 18. Yellowness or b* values decreased significantly ($p<0.05$) with storage time and each sample. First 2 months control (C) sample and enriched P1 and P2 samples were significantly increased by 12.34%, 21.80% and 1.76% in storage and decreased after that. P1 sample, after 2 months, had 4.99%, 10.38% and 17.59% more yellowish than the control and enriched samples of sea buckthorn juice (P0.5 and P2), respectively.

The colour parameter b* values of the enriched samples of sea buckthorn juice (P0.5, P1 and P2) were between 45.21 to 41.45, 39.72 to 37.19 and 40.43 to 40.34 respectively, at end of storage period. On the initial day, change in colour parameter yellowness (b*) values of the control (C) sample was in the range of 41.02 to 33.62 at the end of storage period. Of colour parameter b* (yellowness) in samples of sea buckthorn juice (C, P0.5, P1 and P2) decreased by 18.03%, 8.37%, 6.80% and 0.22% at the end of storage period.

At the end of storage period, the control (C) was lowest in yellowness than other samples of sea buckthorn juice. Moreover, the enriched P1 of colour parameter yellowness was more correlated with a control (C) sample of than enriched P0.5 and P2 samples.

There was a significant interaction ($p<0.05$) between time and samples (Table 12. and Annex.2.). During storage time, enriched P2 of colour parameter yellowness is more stable than

the control (C) sample and enriched P0.5 sample. Yellowness of the sea buckthorn juice was significantly different ($p<0.05$) among the four sea buckthorn juice samples.

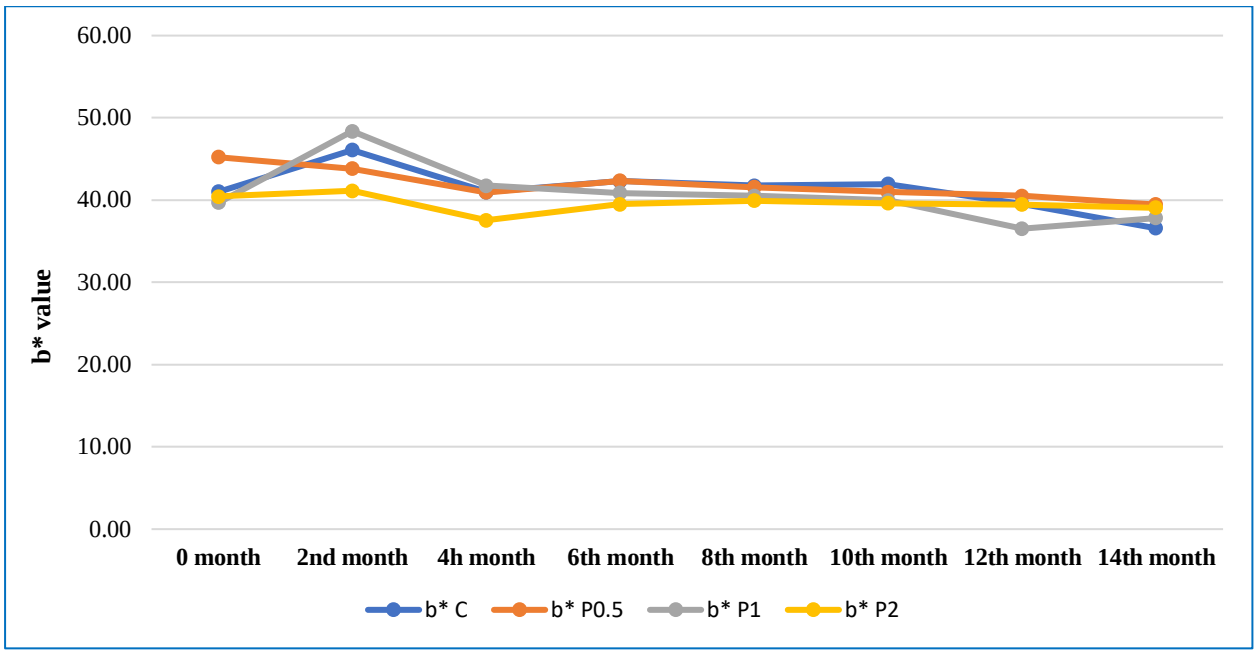


Figure 18. Change in colour parameter yellowness values of sea buckthorn juice samples during 14 months storage at room temperature

Both pomace treatment and storage time had significant effect on b* colour value of buckthorn juice. Pomace treatment, $F(3, 63) = 24.494$, $P<0.001$ and storage time, $F(7, 63) = 28.288$, $P<0.001$. Moreover, there was significant interaction between pomace treatment and storage on the b* colour value of the juice, $F(21, 63) = 8.776$, $P = 0.00$

4.3.4. Hue and Chroma measurements

Chroma

The chroma value shows saturation of colour and is proportional to the strength of the colour with higher values, corresponding to higher colour brilliance.

Table 13. The effect of storage time on the chroma of sea buckthorn juice samples

	Control	P0.5	P1	P2
Initial	7.63	7.894	7.437	7.416
2	8.007	7.763	7.711	7.461
4	7.616	7.489	7.552	7.139
6	7.595	7.622	7.491	7.312
8	7.636	7.527	7.454	7.32
10	7.648	7.479	7.406	7.314
12	7.444	7.438	7.104	7.302
14	7.175	7.34	7.197	7.255

Chroma of sea buckthorn juice samples control (C), P0.5, P1 and P2 presented in Table 13. Chroma measurements of the control (C) and enriched P0.5 sample were higher than the enriched P1 and P2 sample.

The time sample interaction was statistically significant. The chroma values samples of sea buckthorn juice control (C) and P0.5samples slowly decreased throughout storage.

Hue

Hue is the attribute by which we recognise and therefore describe the colour as red, orange, yellow, green, blue or violet.

The results of the determination of Hue value can be seen in Table 14. The ratio of yellowness and redness (hue degree) was the highest on enriched P0.5 and enriched P2 samples. Hue angle values were significantly greater in the enriched P2 sample.

The initial hue values were control (C) (1.174), P0.5 (1.209), P1 (1.197) and P2 (1.225).

Table 14. The Hue of sea buckthorn juice samples

	Control	P0.5	P1	P2
Initial	1.174	1.209	1.197	1.225
2	1.198	1.212	1.203	1.231
4	1.187	1.216	1.22	1.228
6	1.222	1.215	1.214	1.231
8	1.194	1.223	1.216	1.242
10	1.195	1.221	1.214	1.234
12	1.189	1.221	1.206	1.233
14	1.184	1.221	1.217	1.237

Upon storage of sea buckthorn juice samples, the hue angle increased as a function of storage time and temperature showing a deepening of the red colour with storage. The difference in hue was not significantly different at P0.5 and P2 during storage time but there was significant difference between (C) and P2.

4.3.5. Total colour difference

Total colour difference is a colourimetric parameter that is extensively used to characterise the variation of colour in food as compared to the initial samples. Total colour difference of sea buckthorn juice samples presented Figure 19. The change in the parameters of the sample or its deviation from a given colour pattern can be characterized by the spatial distance between the two-colour points, the total colour difference (ΔE^*).

During these investigations the parameters measured 14 months of all samples were compared with the initial ones, so the ΔE* values were determined with respect to the starting colour coordinates.

Total colour difference increased with time and samples and the increase was significantly different among the samples. The total colour difference value of 2 would be a noticeable difference in visual perception of many products. After14 months of storage enriched P2 sample, the total colour difference had surpassed 23, while control (C) and enriched P0.5 samples it took about 14 months to exceed 5.

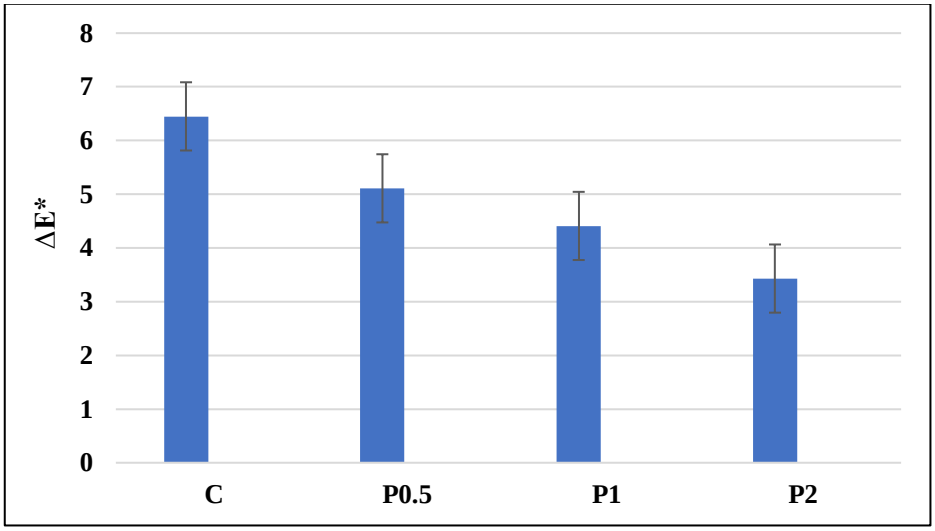


Figure 19. The colour difference parameters (ΔE*) after the storage period

The colour change of the pomace-containing pulps was 'clearly visible' at the end of storage, since ΔE* values were between 3.0 and 6.0. In the control sample, ΔE* was greater than 6.0, thus the difference was large. As the amount of pomace increased, the colour difference decreased, including a negative correlation (r =-0.9681). Within the Microsoft Excel data analysis program, the correlation was determined by Pearson's correlation, which measures the strength of the linear relationship between two variables, and the correlation coefficient (r) can be between -1 and 1 Earlier studies (Krebbers et al. 2003) reported little dependence of changes in lycopene concentration and L*, a*, b* colour values in samples subjected to thermal and combined pressure-temperature treatments.

4.4. Total polyphenolic content

Polyphenolic compounds are very important and beneficial to human health as they play a significant role in controlling the risk of many physiological and degenerative diseases in the human body. Total polyphenolic content changes of the control (C) sample; enriched samples of

sea buckthorn juice (P0.5, P1 and P2) are demonstrated in Table 15.

The content of total polyphenolic content in sea buckthorn juice ranged from 1400.99 µg GAE mL^{-1} in enriched sample P2 to 1750.59 µg GAE mL^{-1} enriched P0.5 sample. Mendelova et al. (2016) experimented 11 cultures of sea buckthorn juice. The highest total polyphenolic content was found in sea buckthorn juice 1566.5µg GAE mL^{-1}. Leposava et al. (2018) reported, TPC of apple juice determined between 255.9 and 441.9 µg GAE mL^{-1}.

Table 15. Effect of pomace treatment and storage time on total polyphenol content of sea buckthorn juice (µg GAE mL^{-1})

month	Control	P0.5	P1	P2
Initial	1649.98 ± 165.59^{Aa}	1750.59±31.02^{Aa}	1439.55±43.81^{Aa}	1400.99±37.46^{Ab}
2	1605.45±40.37^{Aa}	1607.65±40.96^{Aab}	1302.34±27.06^{Babc}	1408.53±25.50^{Bb}
4	1331.93±33.59^{Ab}	1441.76±43.21^{Abc}	1049.16±34.56^{Bd}	1068.88±65.04^{B}
6	1277.75±6.55^{Abc}	1143.61±18.19^{ABde}	1032.53±23.80^{Bd}	1097.22±30.69^{Bc}
8	1095.20±16.85^{Ac}	1083.29±38.74^{Ae}	1212.57±28.98^{Bcd}	1320.16±18.99^{Cbc}
10	1191.12±32.75^{Abc}	1276.63±28.70^{Acd}	1433.27±43.33^{Bab}	1459.26±35.74^{Bab}
12	1135.79±23.57^{Ac}	1286.97±29.35^{BCcd}	1229.13±63.85^{ABbcd}	1374.44±29.05^{Cb}
14	1142.21±36.11^{Abc}	1207.05±21.74^{Ade}	1286.18±105.07^{Aabc}	1624.41±42.95^{Ba}

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

According to Ercisli et al. (2007), who studied Turkish genotypes, their TPC value varies between 213.1 and 553.8 mg GAE 100 g^{-1}. Even an 11-fold difference (from 964 to 10704 mg GAE 100 g^{-1}) was measured by Korekar et al. (2014) in the TPC content of different Indian populations (trans-Himalayan region). The polyphenol content of sea buckthorn berries cultivar is affected by their genetic background and the growing and climatic conditions (Sabir et al., 2005; Tiitinen et al., 2005; Ercisli et al., 2007). The fruits of the studied SB cultivars also had significantly different ($p<0.005$) TPC values (1.86–3.81 mg GAE g^{-1}). The fruit of 'Askola' had the lowest TPC value, while the fruit of 'Orangeveja' had the highest. The different genetic materials can clearly explain the statistically significant difference in the TPC values of the cultivated varieties under the same climatic conditions.

Effect of storage

The total polyphenolic content of enriched and control sample of sea buckthorn juice (C, P0.5, P1 and P2) during storage was estimated every 2 months. Total polyphenolic content changes

of the control (C) sample; enriched samples of sea buckthorn juice (P0.5, P1 and P2) are demonstrated the Figure 20.

Generally, the total polyphenolic content values in all samples of sea buckthorn juice decreased during storage. The total polyphenolic content values of the enriched (P0.5, P1 and P2) samples were in the range of 1750.59 µg mL^{-1} to 1207.05 µg mL^{-1}, 1439.55 µg mL^{-1} to 1286.18 µg mL^{-1} and 1400.99 µg $mL^{-1}$to 1624.41 µg mL^{-1} on 14 months, respectively.

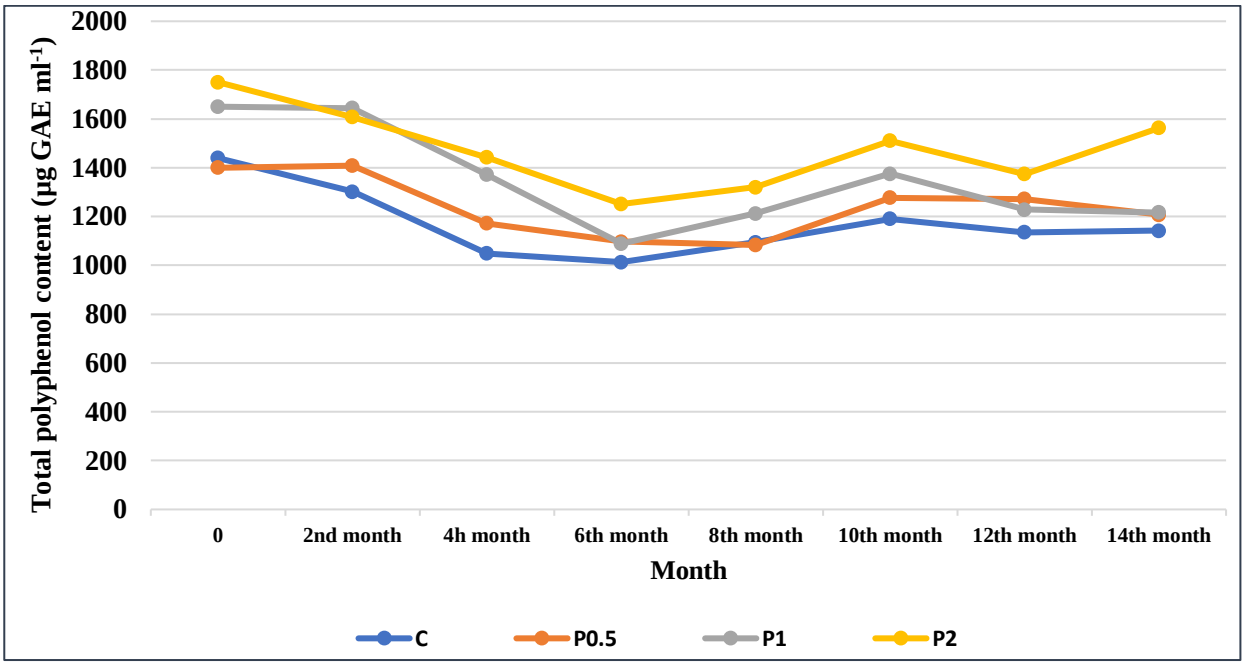


Figure 20. Total polyphenolic content of sea buckthorn juice samples during 14 months storage at room temperature

Considering the recommended polyphenols intake of *ca*. 1 g/day (Baghurst et al. 2006) effective route to increasing the antioxidant intake in our daily diet. The total polyphenolic content values of the control (C) sample was in the range of 1649.98.6 to 1142.21µg mL^{-1} end of storage (14 months). This increase might be attributed to the release of the bound form of total polyphenolic contents due to breakage of the cell wall by the cavitation-related activities. It could also be due to hydroxyl groups produced by sonication, and their interactions with aromatic rings of total polyphenolic compounds.

Results showed that storage time and sample juices significantly affected the level of total polyphenolic content (Table 15.) of all samples of sea buckthorn juice. Only enriched P2 and control (C)showed not significant change in the first 2 months.

First 6 months, all samples of sea buckthorn juice were sharply decreased 22.55% (C), 53.07% (P0.5), 39.42% (P1) and 27.68% (P2), respectively. The lowest (1049.16 µg mL^{-1}) TPC

value was observed for the control (P1) sample in the 4 months. At 8 months, it comes up to 1212.57 µg mL^{-1}. Therefore, from 8 to 14 months, enriched P2 sample contained the most TPC among all samples of sea buckthorn juice.

Samples of sea buckthorn juice (C, P0.5, P1 and P2), between 8 and 10 months increased the TPC 8.76% (C), 17.85% (P0.5), 13.41% (P1) and 14.44% (P2). Initial-day of TPC in all samples of sea buckthorn juice (C, P0.5, P1) decreased by 44.45%, 45.03%, and 11.92% the end of storage at room temperature, expect P2 sample, which TPC content increased by 15.95%. After 14 months, enriched P0.5, P1 and P2 samples retained 5.68%, 12.60%, and 42.27% more TPC compared to the control (C) sample.

In this study, there was a significant decrease in control (C), P0.5 and P1 sea buckthorn juice samples, expect P2. The increase in TPC during storage had previously observed and reported by other authors (Klimczak et al., 2007; Piljac-Žegarac et al., 2009).

Storage time, pomace treatment, and their interaction had significant effects on total polyphenol content (Table 15. and Annex.2.). Pomace treatment $F (3, 190) = 9.65$, $P<0.001$, storage time $F (7, 190) = 42.47$, $P<0.001$ and interaction of pomace treatment and storage time was also significant $F (21, 190) = 15.20$, $P<0.001$.

Other authors suggested other compounds formed during storage may react with Folin-Ciocalteu reagent. The decrease in TPC could be due to the total polyphenolic compounds reacting with sugars and sugar metabolites present in the juice (Agbenorhevi and Marshall, 2012). These results agree with the findings of Castro-López et al. (2016) who reported a decrease in TPC in fruit juice preserved with chemical preservatives (Castro-López et al., 2016). Lipowski et al.(2009) reported that mixed juices were observed in the orange-sea buckthorn juice, carrot-apple-sea buckthorn juice and tomato-sea buckthorn juice was richest in carotenoids. For the preservation of the total antioxidant activity, which is an indicator of the content of bioactive components, the highest value was found in the carrot- apple-sea buckthorn juice. After 10 months of storage the loss was merely 6%, while in the tomato-sea buckthorn juice a loss of 40% was noted. The losses in total polyphenols in all mixed juices were much lower and ranged from 3% to 24% and from 10% to 15%, respectively, depending on the juice composition.

4.5. Flavonoids

An experiment was initially conducted by the chromatographic method of separation of selected analyses, namely rutin, quercetin and dihydroxybenzoic acid. In addition, three flavonoid components were detected by HPLC. We found that the main flavonoid of the sea buckthorn samples was the quercetin (0.0456 – 0.0748 mg mL^{-1}), followed by dihydroxybenzoic acid (0.0125 – 0.0620 mg mL^{-1}) and then rutin (0.0073 – 0.0309 mg mL^{-1}).

4.5.1. Quercetin

Since quercetin is one of the most common flavonols and one of the most powerful antioxidants, it is important to have a simple, precise and accurate method for the determination of quercetin in different samples. Quercetin results of enriched and control samples of sea buckthorn juice presented Table 16.

The values of quercetin in enriched P1 and P2 sample were not significantly higher than in the control (C) sample. The experiment enriched P1 and P2 sample retained 3.07% and 1.64% more quercetin, respectively, compared to the control (C) sample.

Table 16. Effect of pomace treatment and storage time on total quercetin content of sea buckthorn juice (mg mL^{-1})

Interval storage Months	Quercetin (mg mL^{-1})			
	C	**P0.5**	**P1**	**P2**
Initial	$0.0644^{c,d,e,f,A}$	$0.0623^{b,A}$	$0.0664^{a,A}$	$0.0655^{a,e,A}$
2nd	$0.0604^{b,c,g,A}$	$0.0642^{b,B}$	$0.063^{a,A,B}$	$0.0677^{a,b,f,i,A,B}$
4th	$0.0594^{b,c,f,A,C}$	$0.0617^{b,A,B}$	$0.0656^{a,B,C,D}$	$0.0713^{a,b,c,i,D}$
6th	$0.0579^{b,i,A}$	$0.0620^{b,A}$	$0.0639^{a,A}$	$0.0650^{a,g,h,A}$
8th	$0.049^{a,d,g,h,i,j,A}$	$0.0538^{a,b,c,A,B}$	$0.0663^{a,A,B}$	$0.0672^{a,i,A}$
10th	$0.0456^{a,e,A,C}$	$0.0481^{c,A,D}$	$0.0716^{a,B,C,D}$	$0.0749^{c,f,g,B}$
12th	$0.0497^{a,f,k,A}$	$0.0534^{a,A}$	$0.0718^{a,B}$	$0.0736^{b,c,e,h,i,B}$
14th	$0.0563^{b,c,j,k,A}$	$0.0614^{a,b,c,A,C,D}$	$0.0708^{a,B,C}$	$0.0703^{a,b,c,B,D}$

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

Different percent content, sea buckthorn pomace slightly compared to quercetin of sea buckthorn juice samples. Thus, the highest quercetin values were observed for enriched P1 sample (0.0664 mg mL^{-1}) and the lowest quercetin value was observed for enriched P0.5 sample (0.0623 mg mL^{-1}). Quercetin was identified as the major flavonols in the SB berries. Raffo (2004) reported of quercetin of sea buckthorn juice Leikore between 30 and 37 mg kg^{-1} (from 54 to 66.6 µg mL^{-1}).

Quercetin in control and enriched samples of sea buckthorn juice were about 60 times more than that in quercetin of Negi (2013). Foods rich in isoquercitrin include apples, grapes, red wine, and some fruit juices. Quercetin is the other flavonoid detected in apple juice between 750 and 790 µg mL^{-1} (Tenore et al., 2012).

Effect of storage

The quercetin changes of the control and enriched samples of sea buckthorn juice (C, P0.5, P1 and P2) are on Figure 21.

The quercetin value of the enriched samples P1 and P2 on the initial day was 0.0664 and 0.0655 mg mL^{-1}, which were gradually increased to 0.0708 and 0.0703 mg mL^{-1}, respectively, during 14 months of storage. However, control (C) and enriched P0.5 samples on initial day was 0.0644 and 0.0623 mg mL^{-1}, which were significantly degraded to 0.0563 and 0.0614 mg mL^{-1} respectively during 14 months of storage.

The lowest value of quercetin was observed for a control (C) sample (0.049 mg mL^{-1}) and higher value of quercetin was observed for an enriched P2 sample (0.0749 mg mL^{-1}). Between 8 and 10 months, maximum increase was observed enriched P2 sample (11.38%) followed by enriched P1 sample (7.97%), while between 4 and 6 months, the minimum increase was observed in enriched P0.5 sample (0.45%). In the control (C) sample, the level of quercetin decreased by 7.4%.

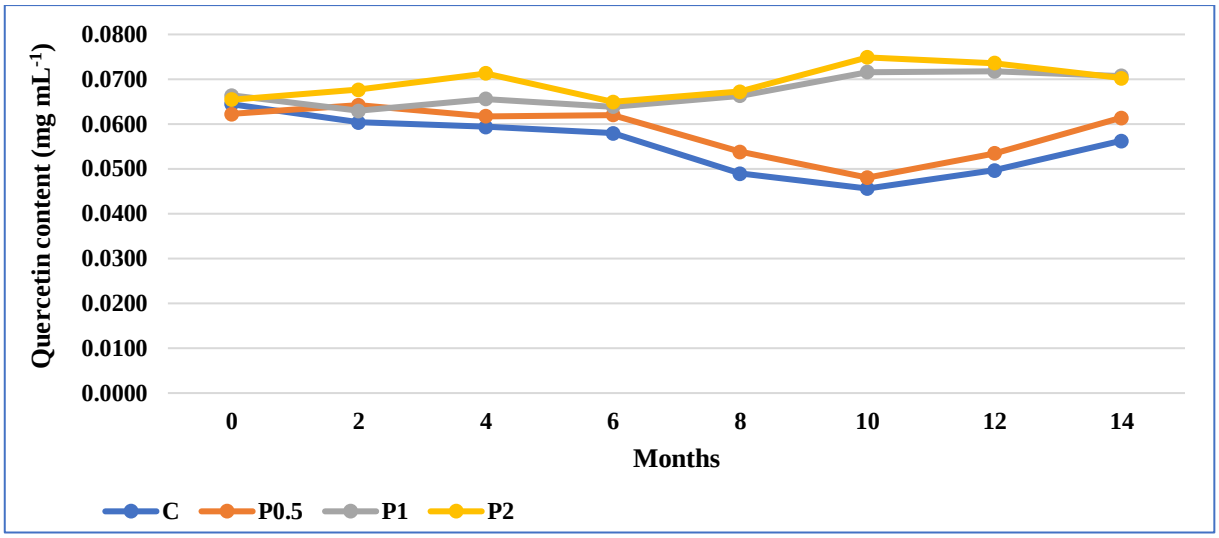


Figure 21. Quercetin content of sea buckthorn juice samples during 14 months storage at room temperature

Control (C) sample the level of quercetin reduced by 12.66%, during storage. Although, in reduction, the level of quercetin was 1.44% enriched P0.5 sample. Moreover, the control (C) sample of quercetin was more correlated with an enriched P0.5 sample than enriched P1 and P2 samples. From 8 to 14 months, enriched P1 and P2 samples of quercetin was slowly increased by 7.84% and 14.34% to 6.58 and 7.31%.

During storage time, enriched samples of sea buckthorn juice (P1 and P2) have more stability than other sea buckthorn juice samples (C and P0.5). After 14 months, enriched samples

of sea buckthorn juice (P0.5, P1 and P2) retained 9.14%, 25.82% and 24.92% more quercetin, respectively, compared to the control (C) sample. The quercetin was significantly higher in the enriched samples than the control (C) sample during storage, indicating that quercetin in enriched P1 and P2 samples were more effective than the control (C) sample. Percentage gain of quercetin was lower compared to that of dihydroxybenzoic acid. However, quercetin content in a sample of sea buckthorn juice was less than that in dihydroxybenzoic acid.

Both pomace treatment and storage time had a significant effect on quercetin values of buckthorn juice. Pomace treatment, $F (3, 141) = 40.93$, $P<0.001$ and storage time, $F (7, 141) = 2.616$, $P<0.05$. Moreover, there was a significant interaction between pomace treatment and storage on the quercetin value of the juice, $F (21, 141) = 3.294$, $P = 0.001$.

4.5.2. Rutin

Rutin is a citrus flavonoid glycoside, which is a low molecular weight polyphenolic compound. There are various physiological functions of rutin and related flavonoids in the human body and other species, including plants. Rutin is one of the best natural antioxidants in the known natural class (Patel and Patel, 2019).

Table 17. Effect of pomace treatment and storage time on rutin content of sea buckthorn juice (mg mL^{-1})

Interval storage Months	Rutin (mg mL^{-1})			
	C	**P0.5**	**P1**	**P2**
Initial	$0.0204^{b,d,c,A}$	$0.0221^{c,A,B}$	$0.0251^{b,c,e,C}$	$0.0295^{c,d,e,f,A,B,C}$
2nd	$0.0201^{b,d,A}$	$0.0223^{c,D}$	$0.0257^{b,c,B}$	$0.0307^{d,C}$
4th	$0.0207^{c,d,B}$	$0.0219^{b,A}$	$0.0261^{b,c,d,e,A,B,C}$	$0.0309^{d,g,C}$
6th	$0.0226^{d,f,A,C}$	$0.0247^{c,A,B}$	$0.0282^{c,f,B,C}$	$0.0288^{b,c,g,h,i,A,B}$
8th	$0.0171^{e,g,A}$	$0.0179^{b,A}$	$0.0206^{d,f,B}$	$0.0222^{b,e,C}$
10th	$0.0178^{e,f,B,C,D}$	$0.0183^{b,B,E}$	$0.0077^{a,e,f,A,C,E}$	$0.0093^{a,e,h,A,D}$
12th	$0.0091^{a,A}$	$0.0118^{a,B}$	$0.0079^{a,f,A}$	$0.0073^{a,A,B}$
14th	$0.0102^{a,g,A}$	$0.0111^{a,A}$	$0.0094^{a,f,A}$	$0.0084^{a,f,i,A}$

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

The rutin changes of the control (C) sample, enriched P0.5, P1, and P2 samples are illustrated in Table 17. Thus, the lowest rutin values were observed for the control (C) sample (20.41 µg mL^{-1}) and the highest rutin value was observed for enriched P2 sample (29.47 µg mL^{-1})

in the initial day. At the beginning of the experiment enriched P0.5, P1 and P2 sample retained 8.08%, 23.18%, and 44.39% more rutin, respectively, compared to the control (C) sample. A different percentage of sea buckthorn pomace improved the value of the rutin in the sea buckthorn juice (Table 17.). This effect was in direct proportion to the concentration of pomace added to the juice.

In enriched samples P0.5, P1 and P2, the content of rutin is reported to be lower Negi et al. (2013), but not lower than that of control sample control (C). We suppose different results depend on the sea buckthorn culture, location (soil and weather) and harvest time. Versari et al., (2008) reported, quercetin of sea buckthorn juice detected 2.41 μg mL^{-1}. Rutin is a naturally occurring flavonoid in many foods, especially buckwheat, apricots, cherries, grapes, grapefruit, plums, and oranges. Rutin, a flavonoid glycoside, is reported as the most dominant flavanol in apricot juice (15.5-2.83μg mL^{-1}). Rutin of sour cherry juice remarked between 15 and 59 41 μg mL^{-1} (Bonerz et al., 2007).

Effect of storage

Observations for the rutin in the storage of enriched and control samples of sea buckthorn juice were carried out every 2 months. The rutin changes of the control (C) sample, enriched sea buckthorn juice P0.5, P1 and P2 samples are illustrated in Table 17.

The rutin value of the all sample of sea buckthorn juice C, P0.5, P1 and P2 on the initial day was 0.0204, 0.0221, 0.0251 and 0.0295 mg mL^{-1}, which were rapid decreased to 0.0102, 0.0111, 0.0094 and 0.0084 mg ml^{-1}, respectively, during 14 months of storage. The rutin of enriched samples (P0.5 and P1) and control (C) remained constant or improved during the first 6 months in storage and decreased thereafter (between 6 and 8 months). Between 4 and 6 months, control (C) sample and enriched samples (P0.5 and P1) were increased to maximum score. Maximum increase was observed enriched P1 sample (12.01%) followed by enriched P0.5 sample (11.96%) and control (C) sample (10.95%).

In the first 4 months, enriched P2 sample was not significantly increased by 4.99% at storage temperature, but between 4 and 12 months enriched P2 sample sharply decrease by 96.327%. For all samples, between 6 and 8 months, there is a rapid decrease by 24.32%, 27.53%, 26.91% and 22.67% of all sample of sea buckthorn juice of total mass (Figure 22.). From 6 to 14 months, there is parallel minor difference between use of enriched P1 and P2 samples.

In enriched P2 sample a maximum rutin value 0.031 mg mL^{-1} was recorded in 4 months. Therefore, a minimum rutin value 0.0073 mg mL^{-1} was observed for the by enriched P2 sample in 12 months.

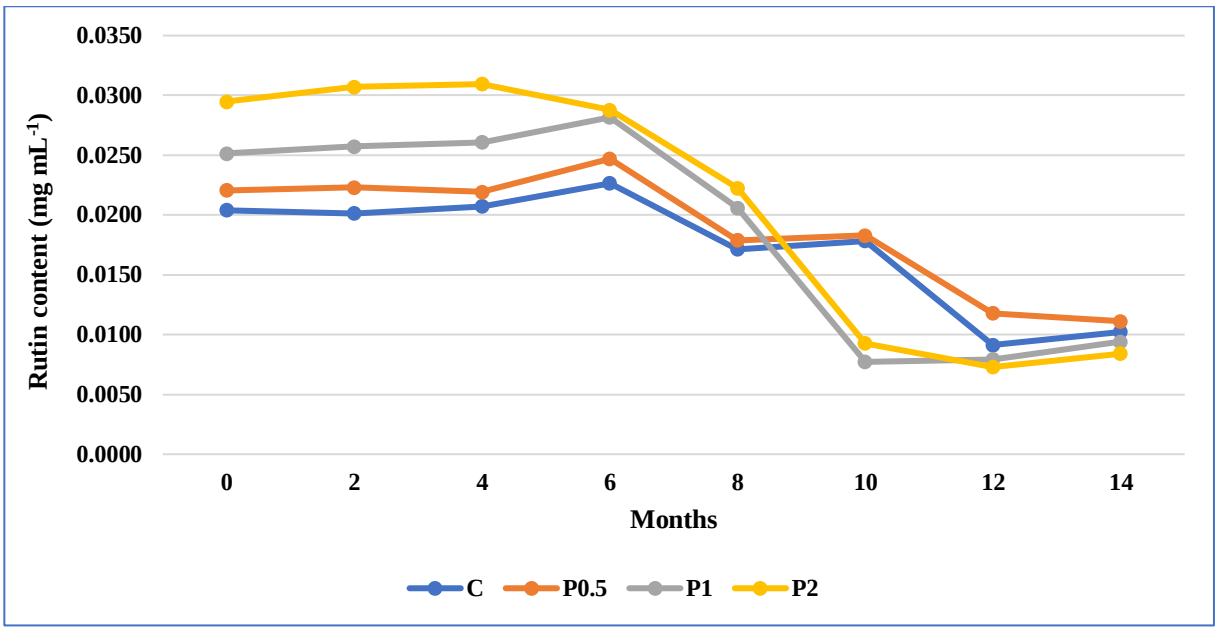


Figure 22. Rutin content of sea buckthorn juice samples during 14 months storage at room temperature

Results showed that enriched P1 and P2 samples retained 8.11% and 17.91% less rutin, respectively, compared to control (C) sample. The enriched P0.5 sample retained more rutin than control (C) and enriched (P1 and P2) samples. During storage time, enriched P0.5 sample retained 7.95%, 10.66 and% 24.42% more rutin, respectively, compared to the control (C) sample and enriched P1 and P2 samples. After 14 months, the detected level of rutin in enriched P2 sample was lower than that in control (C) and enriched (P0.5 and P1) samples. Moreover, the control (C) sample of rutin was more correlated with an enriched P0.5 sample than enriched (P1 and P2) samples. However, after 14 months, enriched P1 (62.55%) and P2 (71.53%) resulted in much higher rutin degradation than the control (C) sample (50%) and enriched P0.5 (49.57%).

Both pomace treatment and storage time had significant effects on rutin levels of the buckthorn juice. Pomace treatment, $F (3, 117) = 10.049$, $P<0.001$ and storage time, $F (7, 117) = 165.706$, $P<0.001$. Moreover, there was a significant interaction between pomace treatment and storage time on the rutin of the juice, $F (21, 117) = 11.182$, $P = 0.001$.

4.5.3. Dihydroxybenzoic acid

Structurally, hydroxylbenzoic acids are common metabolites of flavonoids and several hydroxycinnamic acids (e.g. chlorogenic acid) and contain four hydroxyl groups surrounding a single benzene ring (C6). The dihydroxybenzoic acid changes of the control (C) and enriched samples (P0.5, P1 and P2) are presented in Table 18.

As a rule, all sea buckthorn juice samples increase significantly during storage. The dihydroxybenzoic acid values of all samples of sea buckthorn juice (C, P0.5, P1 and P2) on the initial day was 0.0166, 0.0148, 0.0137 and 0.0203 mg mL^{-1}, which were gradually increased to 0.0587, 0.0529, 0.0378 and 0.0388 mg mL^{-1} during 14 months of storage, respectively.

Table 18. Effect of pomace treatment and storage time on dihydroxybenzoic acid content of sea buckthorn juice (mg mL^{-1})

Interval Months	Dihydroxybenzoic acid (mg mL^{-1})			
	C	**P0.5**	**P1**	**P2**
Initial	$0.0166^{a,b,A}$	$0.0148^{a,A}$	$0.0137^{a,A,B}$	$0.0203^{a,B}$
2nd	$0.0125^{a,f,A}$	$0.0208^{a,b,A}$	$0.0345^{b,c,B}$	$0.0408^{b,c,B}$
4th	$0.0294^{b,c,f,A}$	$0.0269^{a,b,e,A}$	$0.0320^{b,c,A}$	$0.0480^{d,f,B}$
6th	$0.0418^{c,d,g,h,B}$	$0.0276^{a,b,d,e,A}$	$0.0300^{b,c,A}$	$0.0523^{b,d,B}$
8th	$0.0543^{d,e,i,B}$	$0.0300^{b,c,e,A}$	$0.0261^{b,A}$	$0.0467^{d,g,h,B}$
10th	$0.0488^{d,e,C}$	$0.0334^{b,e,A}$	$0.0331^{b,c,B}$	$0.0418^{b,c,e,g,B}$
12th	$0.0620^{g,i,C}$	$0.0425^{c,d,f,B}$	$0.0359^{b,c,A}$	$0.0460^{d,e,g,B}$
14th	$0.0587^{c,h,i,C}$	$0.0529^{d,B,C}$	$0.0378^{c,A}$	$0.0388^{b,e,f,g,A,C}$

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

In enriched P2 sample a maximum DHBA value 0.0203 mg mL^{-1} was recorded in the initial day. In enriched P0.5 sample, the content of DHBA is resulted in a high enriched P1 sample, but not higher than that of control (C) and enriched P2 samples. Therefore, a minimum DHBA value 0.0137 mg mL^{-1} was observed for the enriched P1 sample. The experiment enriched P2 sample retained 22.29%, more DHBA, respectively, compared to the control (C) sample. There is a parallel minor difference between the use of enriched P0.5 and control (C) samples. Different percent content, sea buckthorn pomace improved, enriched P2 sample by DHBA of sea buckthorn juice sample. However, sea buckthorn pomace did not affect enriched P0.5 and P1 samples concentrations significantly.

Ranjith (2009) reported a considerable amount of p-hydroxybenzoic acid (4.0 mg 100 g^{-1}) in sea buckthorn pulp, seed coat (2.6 mg 100 g^{-1}) and kernel (25.6 mg 100 g^{-1}). Törrönen et al. (2012) reported p-hydroxybenzoic acid and 3,4-DHBA of blackcurrant juice fortified with crowberry was 0.68 and 5.66 mg 100 g^{-1}. Therefore, Zadernowski et al. (2005) determined, six cultivars of Sea buckthorn berries researched 2,5-DHBA were between 1.08 and 7.93 mg 100 g^{-1}

and p-hydroxybenzoic acid were between 0.37 and 2.0 mg 100 g^{-1}.

Effect of storage

In control (C) sample of dihydroxybenzoic acid decreased from 0.0166 mg mL^{-1} at the initial day to 0.0125 mg ml^{-1} after 2 months in storage to increase after that to a maximum of 0.062 mg mL^{-1} and reached 0.0587 mg L^{-1} at the end of the storage period (Figure 23.). The control (C) sample, between 8 and 10 months, retained 5.32% less dihydroxybenzoic acid than between 6 and 8 months. First 2 months, dihydroxybenzoic acid of control (C) sample was lower than that of the enriched P0.5 (39.9%), P1 (63.77%) and P2 (69.36%). Between 8 and 10 months, control (C) samples had 31.56%, 32.17% and 14.34% more dihydroxybenzoic acid than the enriched samples of sea buckthorn juice (P0.5, P1 and P2), respectively.

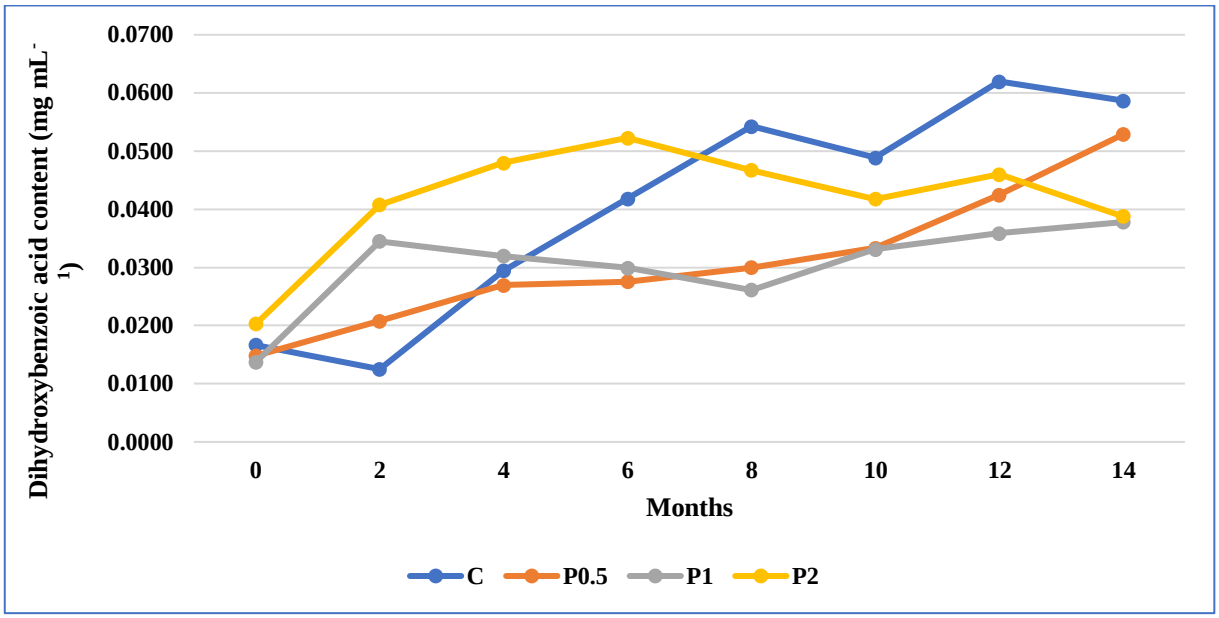


Figure 23. Dihydroxybenzoic acid content of sea buckthorn juicesamples during 14 months storage at room temperature

A constant increase was observed during storage time in enriched P0.5 samples and more stability than other sea buckthorn juice samples (C, P1 and P2). First 2 months, dihydroxybenzoic acid of enriched P1 sample was increased approximately 40.54% than initial day and decreased after that. The enriched P1 sample between 2 and 8 months retained 7.25% to 13% less dihydroxybenzoic acid compared to between the initial day and 2 months.

In first 6 months dihydroxybenzoic acid of enriched P2 sample increased from 100.99% to 157.64% and between 6 and 10 months retained 10.71% to 10.49% less dihydroxybenzoic acid compared to between 4 and 6 months. After 14 months, enriched samples of sea buckthorn juice

(P0.5, P1 and P2) retained 9.88%, 35.61% and 33.9% less dihydroxybenzoic acid, respectively, compared to the control (C) sample.

Both pomace treatment and storage time had significant effects on the dihydroxybenzoic acid of the sea buckthorn juice. Pomace treatment, $F (3, 141) = 37.602$, $P<0.001$ and storage time, $F (7,141) = 43.736$, $P<0.001$. Moreover, there was a significant interaction between pomace treatment and storage on the dihydrobenzoic acid of the juice, $F (21, 141) = 11.529$, $P<0.001$.

The percentage gain of dihydroxybenzoic acid was higher compared to that of quercetin. However, the dihydroxybenzoic acid content in samples of sea buckthorn juice was less than that in quercetin. These results are believed that the formation of certain compounds of dihydroxybenzoic acid group during storage involves anthocyanin degradation products. The generation of protocatechuic and dihydroxybenzoic acid descends from the B-ring of the anthocyanidin aglycon from cyaniding-3-glucoside and pelagonidin-3-glucoside, respectively (Sun et al., 2011). These observations are supported by Rødtjer et al. (2010) , suggesting that the formation of some phenolic acids during the storage, especially dihydroxybenzoic acid and protocatechuic acid, is caused by anthocyanidin degradation. After 60-day, purple corn of dihydroxybenzoic acid increased during storage at 30°C (Kapcum and Uriyapongson, 2018).

We found less correction between quercetin, rutin and DHBA in enriched sample P2. The enriched samples of sea buckthorn juice improved both flavonoid measurements more than the control (C) sample. Therefore, the enriched P1 sample was increased by measuring quercetin and rutin, but on DHBA the measurement result was lower than that of the control (C) sample.

4.6. Antioxidant activity (FRAP)

Sea buckthorn berry is a rich source of antioxidants and bioactive components beneficial for human health. Previous studies had also shown that the fruits of SB cultivars have a strong antioxidant effect, but there is considerable variance among the cultivars (Gao et al., 2000; Korekar et al., 2014). The measurement of antioxidant activity of food extracts is important to evaluate the nutritive value (Apak et al., 2016).

The antioxidant capacity (FRAP) in sea buckthorn juice ranged from 1066.04 µg AA mL^{-1} in the control (C) sample to 1202.37 µg AA mL^{-1} enriched P2 sample in the initial day. No significant differences were found between the enriched P1 and P2 samples in the antioxidant capacity (FRAP) of different percentages of sea buckthorn pomace. Enriched samples of sea buckthorn (P0.5, P1 and P2) retained 7.07%, 9.26% and 12.79% more antioxidant capacity (FRAP) compared to the control (C) sample. Different percent content of sea buckthorn pomace sharply improved FRAP in sea buckthorn juice samples.

Korekar et al. (2014) found considerable differences between the antioxidant capacity (FRAP) values of different Indian SB populations (from to 180 to 1355µg mL^{-1}). Tukey's test proved statistically significant differences in total polyphenols content in the evaluated juice samples.

Effect of storage

The antioxidant capacity changes of the control and enriched C, P0.5, P1 and P2 samples are demonstrated in Table 19.

Table 19. Effect of pomace treatment and storage time (months) on antioxidant activity of sea buckthorn juice (µg AA mL^{-1})

month	Control	P0.5	P1	P2
Initial	1066.04 ± 12.52^{aA}	1141.45 ± 30.82^{aA}	1164.74 ± 27.34^{aB}	1202.37 ± 17.09^{aC}
2	991.05 ± 10.52^{bA}	957.33 ± 52.52^{bA}	1058.35 ± 31.00^{bB}	1194.75 ± 33.91^{bC}
4	929.40 ± 29.45^{bA}	910.89 ± 24.53^{bA}	1063.95 ± 29.92^{bB}	1152.20 ± 24.72^{bC}
6	955.98 ± 24.84^{bA}	976.37 ± 50.82^{bA}	1033.15 ± 19.10^{bB}	1165.93 ± 64.72^{bC}
8	825.25 ± 22.49^{cA}	881.02 ± 21.79^{cA}	940.12 ± 30.76^{cB}	1053.88 ± 27.36^{cC}
10	811.73 ± 24.05^{cA}	844.29 ± 23.51^{cA}	917.90 ± 24.37^{cB}	1035.72 ± 31.57^{cC}
12	679.64 ± 22.37^{dA}	709.60 ± 18.31^{dA}	773.66 ± 17.58^{dB}	890.83 ± 22.02^{dC}
14	598.50 ± 17.76^{dA}	648.22 ± 11.09^{dA}	801.56 ± 16.52^{dB}	859.44 ± 35.06^{dC}

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

Different percent of sea buckthorn pomace improved FRAP in sea buckthorn juice on the initial day. Generally, the antioxidant capacity values in all samples of sea buckthorn juice decreased during storage. The antioxidant capacity values of all samples of sea buckthorn juice (C, P0.5, P1 and P2) on the initial day was 1066.04 µg mL^{-1}, 1141.45 µg mL^{-1}, 1164.74 µg mL^{-1} and 1202.37 µg mL^{-1}, which were gradually decreased to 598.50 µg mL^{-1}, 648.22 µg mL^{-1}, 801.56 µg mL^{-1} and 859.44 µg mL^{-1} during 14 months of storage, respectively. Bonsi et al. (2005) also reported the stability of antioxidant capacity in clear apple juices over 6 months of storage (Figure 24.). The antioxidant capacities in pulpy juices decreased significantly at early storage (from 0 to 12 weeks), but there was no significant loss from 12 to 24 weeks storage. The FRAP values of enriched P1 and P2 samples were significantly higher than control (C) and P0.5 samples for during storage time.

The antioxidant capacity in control (C) sample was not significantly different from that of enriched P0.5 sample, but was lower than that of enriched P1 and P2 samples.

During storage time, enriched P2 sample had the highest antioxidant activity among other sea buckhorn juice samples (C, P0.5 and P1).

After 14 months, enriched samples of sea buckthorn juice (P0.5, P1 and P2) retained 8.31%, 33.92% and 43.60% more antioxidant activity compared to the control (C) sample.

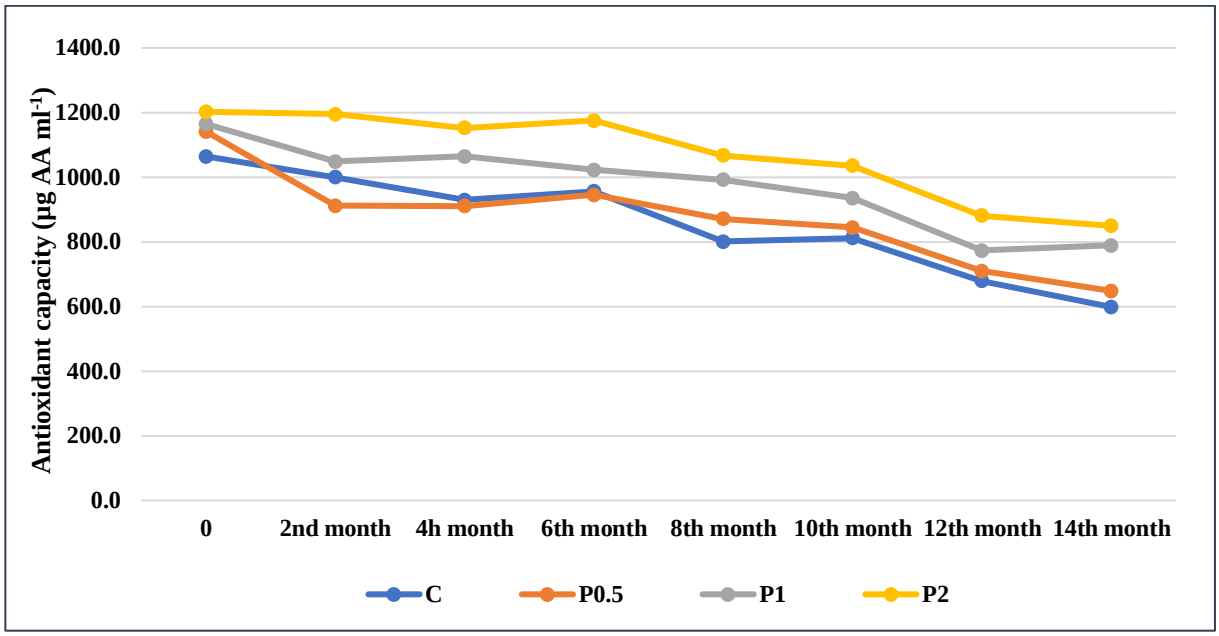


Figure 24. Antioxidant capacity of sea buckthorn juice samples during 14 months storage at room temperature

At the end of storage time, all samples of sea buckthorn juice had less antioxidant capacity (C, P0.5, P1 and P2) respectively, compared to the initial day (78.12%, 76.70%, 45.31% and 39.90%). Moreover, the enriched P2 sample of FRAP value was more related with an enriched P1 than enriched P0.5 and control (C) sample. This decrease might be attributed to a decrease in polyphenolic content as a result of rutin cavitation. In our tests, we did see a decrease in total polyphenolic content during storage in each samples of sea buckthorn. Previous studies also show that there is a positive relationship between total polyphenolic content and antioxidant activity in many plant species such as lime, orange, and carrots (Gulcin et al., 2004).

Results showed that storage time and sample of juices significantly ($p \leq 0.05$) affected the ferric reducing antioxidant power (FRAP) of the juice (Table 19. and Annex.2.). Both pomace treatment and storage time had significant effect on antioxidant activity of buckthorn juice. Pomace treatment, $F(3, 179) = 74.343$, $P<0.001$ and storage time, $F(7, 179) = 82.192$, $P<0.001$. Interaction of pomace treatment and storage time was not significant, $F(21, 179) = 0.833$, P =

0.677.

Other investigators reported that if antioxidant activity is increasing while phenolic compounds and vitamin C are decreasing, the increase in the antioxidant activity of stored juice may be ascribed to formation of non-enzymatic reaction products (Del Pino-Garcia et al., 2012). The great difference among sea buckthorn genotypes in terms of total phenolics and antioxidant activity is supposed to be largely due to the genotype because all plants were grown in the same ecological condition. It has also previously been reported that plant genotype (Scalzo et al., 2005) affects total phenolic content in berry species.

Our experiment showed improvement in the antioxidant capacity (FRAP) and total polyphenolic content (TPC) level enriched by pomace of sea buckthorn juice than a control sample of sea buckthorn juice. The evolution of FRAP values during storage was consistent with changes in total polyphenol content. Different sea buckthorn pomace content improved FRAP in sea buckthorn juice and helped to slowly decrease during storage.

There is a correlation between TPC and FRAP values, as also shown by Makovics-Zsohár et al. (2014), who found strong correlation between TPC and FRAP values. The closeness of the linear relationship between the two parameters was investigated by Pearson's correlation coefficient for each sample separately (Simple and Login, 2010), the results are shown in Table 20. The values of the correlation coefficient give a value close to 1, r = 0.8614 on average. This means that there is a positive correlation between total polyphenol content and antioxidant capacity based on the ability to restore iron; if one parameter increases the other increases. The relationship would be completely linear if r were 1 (or -1 for negative correlation). The correlation between FRAP and TPC indicates that a significant part of the antioxidant capacity of sea buckthorn is due to different polyphenols in the berry (Makovics-Zsohár, 2014). Overall, FRAP and TPC measurements confirm that the pomace has an antioxidant effect, because in each case higher values were obtained for the juice with higher pomace content.

Table 20. Values of Pearson's correlation coefficient between TPC and FRAP

Sampling	Correlation coefficient (R)
0	0.7851
2	0.6487
4	0.9293
6	0.9135
8	0.9502
10	0.9922
12	0.8635
14	0.8087

The correlation between the parameters of antioxidant capacity (TPC - FRAP) had been investigated in several research work. Some of these studies showed strong correlation between the antioxidant parameter (Velioglu et al., 1998), while others could not find strong correlation (Ercisli et al., 2007). Our results suggest strong correlation between the results of different antioxidant properties (antioxidant capacity and total polyphenolic content).

4.7. ß-carotene

The observations for beta-carotene of the storage enriched and control samples of sea buckthorn juice were taken on every 2 months. The beta-carotene changes of the control (C), enriched samples of sea buckthorn juice (P0.5, P1 and P2) are illustrated in Table 21.

In our experiment enriched sample of sea buckthorn juice (P0.5, P1 and P2) contained more beta-carotene, respectively, compared to the control (C) sample.

Table 21. Effect of pomace treatment and storage time (months) on β carotene of sea buckthorn juice (mg 100 g^{-1})

month	Control	P0.5	P1	P2
Initial	1.93 ± 0.22^{aA}	3.61 ± 0.11^{aB}	4.37 ± 0.00^{abB}	5.87 ± 0.33^{abC}
2	2.40 ± 0.22^{abA}	3.75 ± 0.15^{abB}	4.66 ± 0.05^{abC}	5.76 ± 0.14^{abD}
4	2.46 ± 0.15^{abA}	4.29 ± 0.16^{abcB}	4.29 ± 0.16^{aB}	5.59 ± 0.18^{abC}
6	3.04 ± 0.21^{bcA}	4.61 ± 0.10^{bcB}	4.98 ± 0.21^{acbB}	5.23 ± 0.21^{aB}
8	3.28 ± 0.09^{bcA}	4.99 ± 0.12^{cB}	5.26 ± 0.26^{abcB}	6.11 ± 0.04^{abC}
10	3.89 ± 0.03^{cA}	5.16 ± 0.13^{cB}	5.69 ± 0.05^{cbC}	6.24 ± 0.13^{cbD}
12	3.21 ± 0.24^{bcA}	4.35 ± 0.11^{abcAB}	6.03 ± 0.80^{cdBC}	5.89 ± 0.16^{abC}
14	3.71 ± 0.28^{cA}	4.82 ± 0.38^{acAB}	5.49 ± 0.35^{acbBC}	6.52 ± 0.47^{bdC}

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

Therefore, the content of beta-carotene in sea buckthorn juice ranged from 1.93 mg mL^{-1} in control (C) sample to 5.87 mg 100 mL^{-1} enriched P2 sample in the initial day. Different percent of sea buckthorn pomace improved by beta-carotene of sea buckthorn juice samples. In enriched P0.5, P1 and P2 samples, the content of beta-carotene is reported to be high Eccleston et al. (2002), and higher than that of control sample (C). In carrot juices, the beta-carotene contents ranged from 3.28 to 8.48 mg 100 mL^{-1}.

Sea buckthorn fruits are rich in carotenoids; the most active representative is β-carotene, whose average content was mentioned by Bajer (2014) 1.8-3.9 mg 100 g^{-1} fruit. In the study of

Raffo et al. (2004) in Germany varieties of sea buckthorn 'Leikora' a presence of beta-carotene (0.3-5 mg 100 g^{-1}). Eccleston et al. (2002) determined in sea buckthorn juice at the amount beta-carotene 3.3 mg 100 mL^{-1}. Yang and Kallio (2002a) state the total content of β–carotene content in sea buckthorn berries from 0.2 to 17.0 mg 100 g^{-1}. Kuruczek et al. (2012) analysed nine Russian varieties of sea buckthorn and the maximum levels of carotenoids were found in varieties Aromatnaya (28.97 mg 100 g^{-1} fresh weight), Arumnyj (21.51 mg 100 g^{-1}) and Botanicheskaya (14.2 mg 100 g^{-1}).

Effect of storage

The beta-carotene values of all samples of sea buckthorn juice (C, P0.5, P1 and P2) on the initial day was 1.93, 3.61, 4.37 and 5.87 mg 100mL^{-1}, which were gradually increased to 3.71, 4.82, 5.49 and 6.52 mg 100mL^{-1} during 14 months of storage, respectively (Figure 25.). The beta-carotene values of all enriched samples of sea buckthorn juice (P0.5, P1 and P2) were significantly higher than control (C) sample for all storage time, thus enriched P2 sample contained the most beta-carotene among all samples of sea buckthorn juice.

During storage maximum increase was observed control (C) sample (92.23%) followed by enriched P1 sample (25.62%), while minimum increase was observed in enriched P2 sample (11.07%) followed by enriched P0.5 sample (33.52%). In this study, there was a significant decrease in beta-carotene in between 10 and 12 months compared to the initial day.

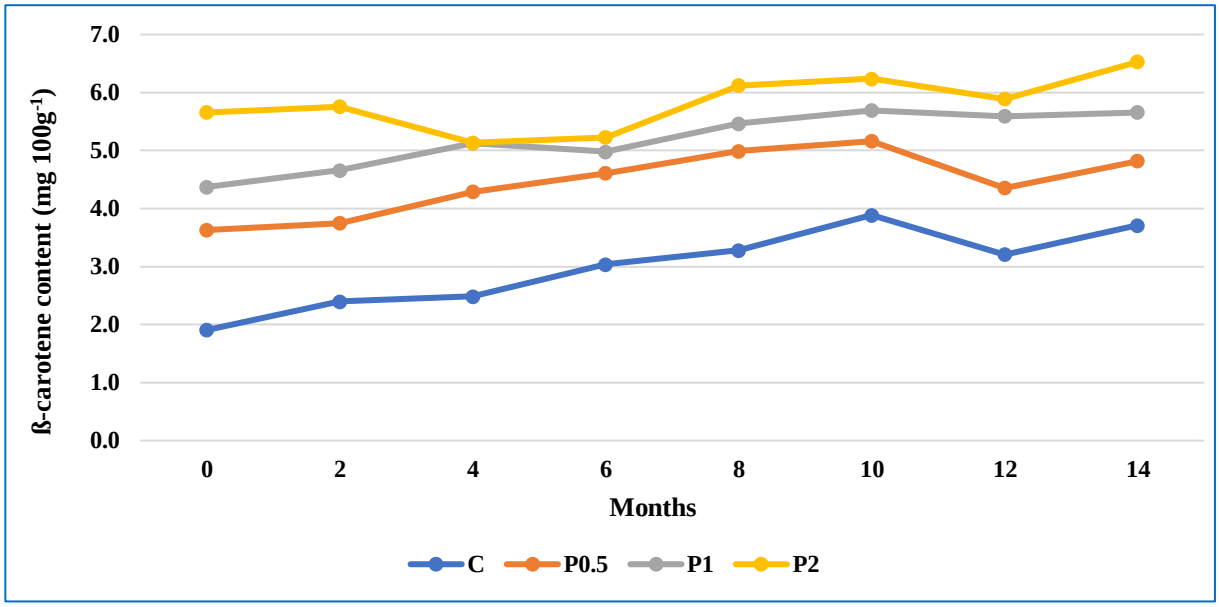


Figure 25. Beta-carotene content of sea buckthorn juice samples during 14 months storage at room temperature

After 14 months, enriched samples of sea buckthorn juice (P0.5, P1 and P2) retained 30.35%, 18.23% and 17.32% more beta-carotene, respectively, compared to the control (C)

sample. During storage time, enriched P1 and P2 samples of beta-carotene are more stable than the control (C) sample. In addition, the control (C) sample of beta-carotene was more correlated with an enriched P0.5 sample than enriched P1 and P2 samples. Beta-carotene constitutes 15–55% of total carotenoids, depending on the origin (Yang et al., 2001). Levels of beta-carotene from 0.2 to 17 mg 100 g^{-1} and total carotenoids (calculated as beta-carotene) from 1 to 120 mg 100 g^{-1} in fresh berries have been reported in the literature (Yang et al., 2001).

Both pomace treatment and storage time had significant effect on β carotene of buckthorn juice (Table 21. and Annex.2.). Pomace treatment, $F (3, 102) = 230.45$, $P<0.001$ and storage time, $F (7, 102) = 19.024$, $P<0.001$. Moreover, there was significant interaction between pomace treatment and storage on the β carotene content of the juice, $F (21, 102) = 2.214$, $P = 0.01$.

It is generally observed that beta-carotene is not or slightly degraded by heat treatment, but instead of isomerization occurs (Qiu et al., 2009) since naturally occurring trans-β-carotene it is very unstable and is easily converted to cis-isomer on effect of light and heat. The effect of processing and storage in fruits and vegetables is mainly 13-cis-β-carotene produce during geometric isomerization, and the light exposure results 9-cis-β-carotene (Schieber and Carle, 2005; Lozano-Alejo et al., 2007). In control (C) and enriched P0.5 samples, the β-carotene content in the pulp increased significantly, similar to data of Lessin et al. (1997) who examined the geometric isomers of carotenoids in processed fruits and vegetables.

Processing resulted increase (16-50%) in the total amount of carotenoids with provitamin A activity compared to fresh samples. The reasons for this increment include increased extraction efficiency, inactivation of carotenoid-degrading enzymes, and loss of soluble solids in the liquid heat treatment medium (Lessin et al., 1997).

4.8. Ascorbic acid

Ascorbic acid (AA) is an important component of our nutrition and an additive in many foods because of its antioxidant capacity (Burdurlu et al., 2006). Thus, if well retained, other quality attributes and nutritional value of foods are also retained (Marfil et al., 2008). The analysis of the content of vitamin C in the juice of *H. rhamnoides* berries, which is a biologically important photochemical substance for humans.

Vitamin C represents the major vitamin compound in sea buckthorn juices (Souci et al., 2000). Storage experiments and analysis of vitamin contents are used as instruments to predict the shelf life of sea buckthorn juices, particularly regarding loss of valuable nutrients. Moreover, kinetic models of thermal degradation are obligatory for designing new processes and providing optimal product quality (Riboh and Labuza, 1982; Karhan et al., 2004).

The observations for ascorbic acid of the storage enriched and control samples of sea

buckthorn juice were taken every two months. The ascorbic acid changes of the control (C), enriched samples of sea buckthorn juice (P0.5, P1 and P2) are illustrated in Table 22.

Vitamin C is one of the most important antioxidants. Sea buckthorn juice can contain from 28 to even 2,500 mg 100 g^{-1} (Beveridge et al., 2002). Sea buckthorn does not ascorbin-oxidase, so vitamin C is largely retained during processing (Artemova, 2001; Prokkola and Mäyrä, 2003).

In our results, the enriched P2 sample was higher ascorbic acid than other enriched samples (P0.5, and P1) and the control (C) samples of sea buckthorn juice. Our experiment enriched P0.5, P1, and P2 samples retained 6.42%, 16.31%, and 20.65% more ascorbic acid than the control (C) sample on the initial day.

Table 22. Effect of pomace treatment and storage time (months) on vitamin C of sea buckthorn juice (mg 100 g^{-1})

month	Control	P0.5	P1	P2
Initial	$308.57 \pm 1.89^{e,f,A}$	$328.11 \pm 0.44^{f,B}$	$357.83 \pm 0.1.63^{f,C}$	$372.81 \pm 1.89^{e,D}$
2	$273.30 \pm 9.36^{d,e,g,A,B}$	$258.29 \pm 5.48^{e,A}$	$273.27 \pm 5.17^{e,A,B}$	$299.76 \pm 13.59^{b,d,e,f,B}$
4	$218.72 \pm 8.70^{c,d,h,A}$	$235.46 \pm 1.70^{d,e,A}$	$265.14 \pm 5.67^{d,e,B}$	$266.76 \pm 3.35^{c,d,B}$
6	$192.85 \pm 28.53^{b,c,f,g,i,A}$	$191.24 \pm 8.75^{d,A}$	$230.44 \pm 2.70^{d,A}$	$244.49 \pm 9.19^{c,f,g,A}$
8	$128.70 \pm 7.82^{a,b,A}$	$125.01 \pm 4.45^{c,g,h,A}$	$155.91 \pm 1.02^{c,g,h,B}$	$183.50 \pm 0.63^{b,g,h,C}$
10	$110.63 \pm 7.65^{a,i,A}$	$119.61 \pm 1.82^{b,c,i,A,B}$	$132.27 \pm 4.09^{b,c,i,B}$	$129.60 \pm 1.19^{a,A,B}$
12	$101.22 \pm 18.79^{a,h,i,A}$	$113.98 \pm 9.29^{a,b,g,A}$	$107.63 \pm 6.41^{a,b,g,A}$	$117.66 \pm 3.56^{a,A}$
14	$77.55 \pm 3.93^{a,i,A}$	$77.82 \pm 6.14^{a,h,i,A}$	$97.92 \pm 9.40^{a,h,i,A}$	$98.84 \pm 10.42^{a,h,A}$

Superscripts with mall case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, $p<0.05$)

Therefore, the ascorbic acid content in sea buckthorn juice ranged from 308.57 ± 1.89 mg mL^{-1} in the control (C) sample to 372.81 ± 1.89 mg 100 mL^{-1} enriched P2 sample in the initial day. Different percent of sea buckthorn pomace improved by ascorbic acid of sea buckthorn juice samples. In experiments of other authors, fruits of three German cultivars ('Askora', 'Hergo' and 'Leikora') contained 180-370 mg 100 g^{-1} ascorbic acid (Mörsel et al., 2014; Zadernowski et al., 2007).

The ascorbic acid values of all enriched samples of sea buckthorn juice (P0.5, P1 and P2) were significantly higher than the control (C) sample after 14 months (Fig. 26), thus enriched P2 sample contained the most ascorbic acid among all samples of sea buckthorn juice during storage time (only between 8 and 10 months enriched sample P2 was less than the enriched sample P1).

During storage, the maximum decrease was observed the enriched P0.5 sample (34.63%)

followed by the control (C) sample (33.26%), while a minimum decrease was observed in enriched P2 sample (29.37%) followed by the enriched P1 sample (32.34%). In this study, there was a significant decrease in ascorbic acid between 6 and 10 months compared to the initial day.

After 14 months, enriched samples of sea buckthorn juice (P0.5, P1 and P2) retained 0.35%, 26.25%, and 27.44% more ascorbic acid than the control (C) samples. During storage time, enriched P1 and P2 samples of beta-carotene are more stable than the control (C) sample. In addition, the control sample (C) of ascorbic acid was more correlated with an enriched P2 sample than enriched P0.5 and P1 samples. Moreover, thermally treated products may be stable during storage due to the inactivation of derivative enzymes such as ascorbic acid oxidase, polyphenol oxidase and peroxidase (Awuah et al., 2007; Igual et al., 2010).

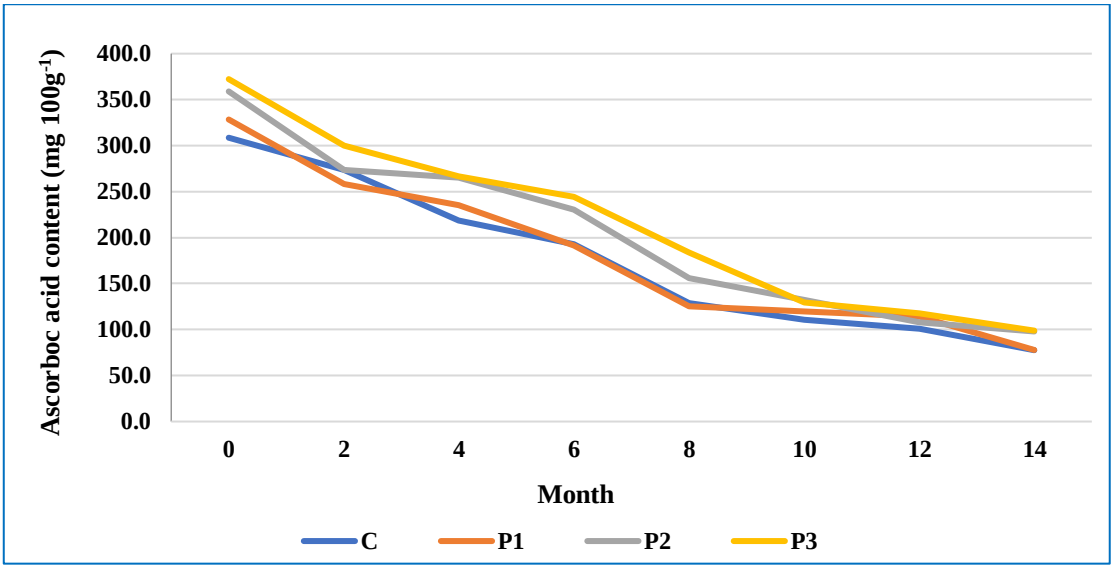


Figure 26. Ascorbic acid content of sea buckthorn juice samples during 14 months storage at room temperature

At the end of the experiment, all samples (C, P0.5, P1, and P2) lost ascorbic acid by 74.86%, 76.3%, 72.71%, and 73.45% on the initial day.

Both pomace treatment and storage time significantly effects on the vitamin C concentration of the sea buckthorn juice. Pomace treatment, $F(3, 64) = 34.589$, $P<0.001$ and storage time, $F(7, 64) = 445.269$, $P<0.001$. Moreover, there was a significant interaction between pomace treatment and storage time on the vitamin C of the juice, $F(21, 64) = 1.756$, $P<0.05$.

After 35 days, some stability was reached, after which the reduction was more regular. By then, the content of ascorbic acid had decreased by 13–22% depending on the storage temperature and composition of the model beverages (Rumpunen and Andersson, 2012). Also, to respond to this question, Cortes etal. (2005) evaluated the effect of frozen conditions on the

contents of carotenoids and the ascorbic acid content inorange-carrot juice. After storing the juice at −40 °C for 132 days, they concluded that ascorbic acid decreased by 4.1% during storage; however, vitamin A (ascorbic acid) activity increased.

Other authors reported about ascorbic acid, longer storage time and higher temperature influence loss of ascorbic acid. For instance, Remini et al. (2015) reported that 20% of the initial ascorbic acid was lost after 28 days of storage of pasteurized blood orange juice at 4°C, and a complete degradation was observed at higher storage temperatures. The loss of ascorbic acid in pasteurised juice during storage is likely attributed to non-enzymatic pathways, since enzymatic degradation is eliminated during processing (Burdurlu and Karadeniz, 2003).

4.9. New scientific results

1. A significant decrease in the antioxidant activity and TPC were recorded for each sample of sea buckthorn juice during storage time. The added pomace has a protective effect for the antioxidant activity and TPC of the juices. The antioxidant activity decreased to a lesser extent in the pomace-containing juice (P1, 1% pomace added, 45.31%; P2, 2% pomace added, 39.90%), than in the control sample (C, without pomace added, 78.12%). Antioxidant capacity was shown to be directly correlated with TPC.
2. We found that the main flavonoid of the sea buckthorn samples was the quercetin (0.0456 – 0.0748 mg mL^{-1}; between 44.64-69.73%), followed by dihydroxybenzoic acid (0.0125 – 0.0620 mg mL^{-1}; between 19.11-36.95%) and the rutin (0.0073 – 0.0309 mg mL^{-1}; between 11.16-18.41%).
3. After 14 months storage at room temperature, P1 (1% pomace added) (62.55%) and P2 (2% pomace added) (71.53%) resulted in much higher rutin degradation than a control (C, without pomace added) (50%) and P0.5 (0.5% pomace added) (49.57%).
4. A significant increase in the dihydroxybenzoic acid was recorded for each sample of sea buckthorn juice during storage time. After 14 months storagethe control (C, without pomace added, 0.0587 mg mL^{-1}) sample showed a significant ($P<0.05$) higher dihydroxybenzoic acid than the P1 (1% pomace added, 0.0378 mg mL^{-1}) and P2 (2% pomace added, 0.0388 mg mL^{-1}) samples.
5. After 14 months storage, a significant increase in the quercetin was recorded for P1 (1% pomace added) (6.6%) and P2 (2% pomace added) (7.3%), while, in the control (C, without pomace added) (12.6%) and P0.5 (0.5% pomace added)(1.44%) were degraded. During 14 months storage time, the quercetin of sea buckthorn juice was more stable than hydroxy-benzoic acid.

6. A significant increase in the beta-carotene was recorded for each sample of sea buckthorn juice during storage time. After 14 months, enriched samples of sea buckthorn juice (P0.5, P1 and P2) retained 30.35%, 18.23% and 17.32% more beta-carotene, respectively, compared to the control (C) sample.
7. The ascorbic acid content decreased in all samples. The losses of about 74.87%, 76.3.45%, 72.72% and 73.45% of vitamin C were observed in control samples (C) and enriched samples (P0.5, P1 and P2) after 14 months storage.
8. A significant increase in the pH was recorded for all sea buckthorn juice samples during 14 months of storage. During storage time, the pH values of P2 (2% pomace added) sample were more stable than the control (C, without pomace added), P0.5 (0.5% pomace added) and P1 (1% pomace added). The soluble solid content of control (C, without pomace added), was significantly lower compared to the other sea buckthorn juice samples (P0.5, P1, P2) after 14 months storage period.

CHAPTER 5 - CONCLUSIONS AND RECOMMENDATIONS

The aim of this research was to set up a technological process to obtain high-value biologically active extracts from sea buckthorn pomace, thereby helping to reduce waste from the juice industry. On one hand, these could be retrieved using the proper method, and used again by the food industry. These new aspects concerning the use of the pomace as by-products for further exploitation on the production of food additives with high nutritional value, them recovery may decrease quantity of a waste of valuable components and may be economically attractive.

In our research work the conditions in which the samples were stored were in a cool, dry place. We wanted to represent the normal, average circumstances of a department store during storage. During the experiment, someone measured weekly the ambient temperature and ranged from 18 to 23°C, depending on the season.

In summary, the results from this study indicate that P1 and P2 retained more antioxidant capacity, beta-carotene, TPC, rutin, quercetin, hydroxy-benzoic acid, SSC and ascorbic acid, and less colour parameters compared to the control (C) on the initial day.

P1 and P2 significantly altered chemical parameters such as TPC, FRAP, SSC, beta-carotene, pH-value, quercetin and ascorbic acid. A significant increase in the beta-carotene was recorded for P1 and P2 during storage time. The P1 had quality parameters close of P2 and significantly better than the control (C).

During 14 months storage, the SB control (C) juice lost some ofthe quercetin, rutin, TPC, FRAP and ascorbic acid content, respectively. We have successfully recovered and recycled the antioxidant compounds from pomace of SB to produce more valuable and SB juice (P0.5, P1, and P2). These new aspects concerning the use of the pomace as by products for further exploitation on the production of food additives with high nutritional value, their recovery may decrease quantity of a waste of valuable components and may be economically attractive.

Experiments have shown that sea buckthorn has a remarkable antioxidant effect, which can neutralize free radicals and helps maintain health. Our results clearly demonstrated that the addaed pomace amount significantly influenced the phytochemical composition and antioxidant capacity of the enriched samples of sea buckthorn juice studied.

This work in the field of studying the effect of sea buckthorn pomace is only the initial stage, which offers many other possibilities for further research:

- In order to avoid wastage of significant quantities of nutrients by treating the pomace as waste, it would be worth to use it not only for feeding purposes, but also in functional

foods for human consumption.

- In this study, sea buckthorn pomace was used in heat-treated juices, but it would be worthwhile to investigate the effect of pomace on the antioxidant component and shelf life of freshly pressed non-heat-treated juice, as well.
- It would also be worthwhile to study the antimicrobial effect of sea buckthorn pomace in various food products (combine the heat treatment and pomace added) in order to reduce the negative effect of heat treatment. A detailed study is required to investigate the inhibitory effect of sea buckthorn on certain microorganisms
- Further studies are needed on the use of sea buckthorn pomace in other products, e.g. in jams and jellies. More than 2% pomace content could be usedand investigated the antioxidant and rheological properties of jams during storage.
- I consider it necessary to identify additional flavonoid compounds and to monitor their changes in the products due to different technological effects and during storage.

CHAPTER 6 – SUMMARY

Sea buckthorn (SB) juice is rich in biologically active compounds. It has considerable health benefits; thus, it can serve as functional food ingredient. The commercial production of sea buckthorn (*Hippophae rhamnoides*) juice results in a large amount of pomace, which is one of the by-products of SB and end product of SB berries.

In the past few years, treating waste from the food industry has become a remarkably important issue due to environmental and economic reasons. Recycling waste should mean a satisfactory alternative, particularly if we consider the amount of valuable components remaining in the waste of certain plants. On the one hand, these could be retrieved using the proper method, and used again by the food industry.

The present work has been carried out as an alternative to use of SB pomace to open new opportunities. The study was then designed in two parts: (a) Samples enriched SB juice compared to control sample of SB juice. (b) Samples of enriched and control SB juice were monitored for changes in individual parameters for 14 months in storage temperature and the sampling took place every two months.

The aim of our experimental work was to investigate the effect of SB pomace on antioxidants compounds in SB juice. The berries of the SB (*Hippophae rhamnoides* L.) cultivar 'Leikora' was collected from a commercial orchard (North latitude 47° 10′ 29″, East longitude 20° 11′ 47″) near Szolnok. Berries were collected during October in 2017 at the stage of commercial maturity, as judged by juiciness and appearance. During the research, four different samples were made as control sample of sea buckhorn juice (C), sea buckthorn juice 99.5% + pomace from sea buckthorn 0.5% (P0.5), sea buckthorn juice 99% + pomace of sea buckthorn 1% (P1) and sea buckthorn juice 98% + pomace of sea buckthorn 2% (P2). The parameters examined were as follows: colour parameters (L*, a*, b*), beta-carotene content, pH value, soluble solid content, flavonoids (rutin, quercetin and hydroxybenzoic acid), antioxidant capacity (FRAP), total polyphenol and ascorbic acid content.

In our study, we have successfully recovered and recycled the antioxidant compounds from the pomace of SB to produce more valuable SB juice. The beta-carotene and polyphenol content, as well as the antioxidant capacity of the samples, increased with the growth of the pomace content. During storage, degradation occurred in the polyphenol content and antioxidant capacity, but the beta-carotene content increased. The smaller reduction in antioxidant compounds was, the higher the sample of pomace content is. However, the smaller the beta-carotene content increase during storage was, the more pomace is in the sample. The FRAP and total polyphenol values

measured during storage confirm that the pomace has an antioxidant effect. There is a close correlation between the two parameters, including a positive correlation (r = 0.8614), which indicates that a significant part of the antioxidant capacity of buckthorn is due to the presence of different polyphenols. During storage time, each sample of SB juice of quercetin is more stable than hydroxy-benzoic acid and P1 and P2 more stable than control (C) and P0.5.

These new aspects concerning using of the pomace as by-products for further exploitation on the production of food additives with high nutritional value, their recovery may decrease the quantity of a waste of valuable components and may be economically attractive. The results from this study can be used to update recommendations concerning optimal enriched SB juice.

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ANNEX 1

References

1. AGBENORHEVI, J.K. and MARSHALL, L.J. (2012): Investigation into the total phenols and antioxidant activity during storage of fruit smoothies. *Journal of Food Science and Engineering*. 2 72-79
2. AGRAWALA, P.K. and GOEL, H.C. (2002): Protective effect of RH-3 with special reference to radiation induced micronuclei in mouse bone marrow. *Indian Journal. Exp. Biol*, 40 525-530. p.
3. ALEXANDRAKIS, Z., KYRIAKOPOULOU, K., KATSAROS, G., KROKIDA, M. and TAOUKIS, P. (2014): Selection of process conditions for high pressure pasteurization of sea buckthorn juice retaining high antioxidant activity. *Food and Bioprocess Technology*. doi: 10.1007/s11947-014-1299-5.
4. ALI, J and AHMAD, B. (2015): Comparative antitumor and anti-proliferative activities of *Hippophae rhamnoides* L. leaves extracts. *Journal of Coastal Life Medicine*. 3(3) 228-232. p.
5. ANSARI, A. S. (2003): "Sea buckthorn (*Hippophae* spp.) - A Potential Resource for Biodiversity Conservation in Nepal Himalayas" paper presented at International Workshop on Underutilized Plant Species. 06 to 08 May 2003, Leipzig, Germany.
6. APAK, R., ÖZYUREK, M., GUCLU, K. and CAPANOTLU, E. (2016): Antioxidant activity/capacity measurement. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. *Journal of Agricultural and Food Chemistry*. 64(5) 997–1027. p.
7. ARIMBOOR, R., KUMAR., KS. and ARUMUGHAN, C. (2008): Simultaneous estimation of phenolic acids in sea buckthorn (*Hippophae rhamnoides*) using RP-HPLC with DAD. *J Pharm Biomed Anal*, 47(1), 31–38. p.
8. ARIMBOOR, R., VENUGOPALAN, V. V., SARINKUMAR, K., ARUMUGHAN, C and Sawhney, C. (2006): Integrated processing of fresh Indian sea buckthorn (*Hippophae rhamnoides*) berries and chemical evaluation of products, *Journal Science Agriculture*, 88 2345–2353. p.https://doi.org/10.1002/jsfa
9. ARTEMOVA, A. (2001): Oblepiha iscelajuscaja i omolozivajuscaja. Sanktpeterburg. DILA (in Russian), pp. 27-39.
10. AWUAH, G. B., RAMASWAMY, H. S. and ECONOMIDES, A. (2007): Thermal processing and quality: Principles and overview. *Chemical Engineering and Processing: Process Intensification*, *46*(6), 584-602.
11. BAJER, J. (2014): Sea buckthorn. 1st ed. Prague: Mladá fronta, 156 p. ISBN 978-80-204-3385-5
12. BAL, L.M, MEDA, V., NAIK, S.N. and Satya, S. (2011): Sea buckthorn berries: a potential source of valuable nutrients for neutraceuticals and cosmoceuticals. *Food Res Int*, 44 1718–1727. p.
13. BEKKER, N.P. and GLUSHENKOVA, A.I. (2001): Components of Certain Species of the Elaeagnaceae Family. *Chemistry of Natural Compounds*, 37(2) 97-116. p. DOI: 10.1023/A:1012395332284
14. BENZIE, I. F., STRAIN, J. J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1) 70-76. p.
15. BEVERIDGE, T.H.J. (2003): Post-harvest handling and storage. In Sea Buckthorn (*Hippophae rhamnoides* L.): Production and Utilization, eds. pp 69-17. Ottawa, ON: NRC Research Press.
16. BEVERIDGE, T. and HARRISON, J.E. (2001): Microscopic structural components of sea buckthorn (*Hippophae rhamnoides* L.) Juice prepared by centrifugation. *Lebensm.-Wiss. u. -Technol*, 34: 458-461.
17. BEVERIDGE, T., HARRISON, J. E. and DROVER, J. (2002): Processing effects on the composition of sea buckthorn juice from *Hippophae rhamnoides* L. *cv*. Indian Summer. *Journal of Agricultural and Food Chemistry*, 50 113 - 116.p.
18. BEVERIDGE, T., LI, TS. C., OOMAH, B. D. and SMITH, A. (1999): Sea buckthorn products:

manufacture and composition. *Journal of Agricultural and Food Chemistry*, 47(9) 3480–3488. p.
19. BISWAS, A., BHARTI, V.K., ACHARYU, S., PAWAR, D.D. and SINGH, S.B. (2010): Sea buckthorn: New feed opportunity for poultry in cold arid Ladakh region of India. *Worlds Poult. Sci. J*, 66 707-714. p.
20. BONERZ, D., WURTH, K., DIETRICH, H. and WILL, F. (2007): Analytical characterization and the impact of aging on anthocyanin composition and degradation in juices from five sour cherry cultivars. *Eur. Food Res. Technol*, 224 355–364. p.
21. BONSI, I. A. (2005): Effects of Processing on Quality of Apple Products: Apple Juice, Cider and Sauce. PhD Thesis. Cornell University.
22. BURDURLU, H. S., KOCA, N. and KARADENIZ, F. (2006): Degradation of vitamin C in citrus juice concentrates during storage. *Journal of food engineering*, *74*(2), 211-216.
23. BUSHWAY, R. J. (1986): Determination of alpha and beta-carotene in some raw fruits and vegetables by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 34(3) 409-412. p.
24. CAKIR, A. (2004): Essential oil and fatty acid composition of the fruits of *Hippophae rhamnoides* L. (sea buckthorn) and *Myrtuscommunis* L. from Turkey. *Biochemical Systematics and Ecology*, 32(9) 809-816. p.
25. CAPRIOLI, G., IANNARELLI, R. and CIANFAGLIONE, K. (2016): Volatile profile, nutritional value and secretory structures of the berry-like fruits of *Hypericum androsaemum* L. *Food Research International*, 79, 1–10. p.
26. CASTRO-LÓPEZ, C., SANCHEZ-ALEJO, E.J., SAUCEDO, SAUL., MOLINA, R.R., ARANDA-RUIZ, J., and MARTINEZ, G.C. (2016): Fluctuations in phenolic content, ascorbic acid and total carotenoids and antioxidant activity of fruit beverages during storage. *Heliyon*, 2(9) e00152. DOI: 10.1016/j.heliyon. 2016.e00152
27. CECILIA, M.O., MAIA, G.A., RAIMUNDO, W.F., MOREIRA, M.S. and BRASIL, I.M. (2003): Storage stability of cashew apple juice preserved by hot fill and aseptic processes. *Ciênc. Tecnol. Aliment*, 23 106-109. p. https://doi.org/10.1590/S0101-20612003000400020
28. CENKOWSKI, S., YAKIMISHEN, R., PRZYBYLSKI, R. and MUIR, W. E. (2006): Quality of extracted sea buckthorn seed and pulp oil. *Canadian Biosystems Engineering*, 48 3.p.
29. CHAKRABORTY, M., KARMAKAR, I., HALDAR, S., NEPAL, A. and HALDAR, P.K. (2015): Anticancer and antioxidant activity of methanol extract of *Hippophae salicifolia* in EAC induced Swiss albino mice. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(8) 180-184. p.
30. CHANDRA, S., ZAFAR, R., DWIVEDI, P., SHINDE, L. P. AND PRITA, B. (2018). Pharmacological and nutritional importance of sea buckthorn (*Hippophae*). *The Pharma Innovation*, *7*(5, Part D), 258.
31. CHAUHAN, A.S., NEGI, P.S. and RAMTEKE, R.S. (2007): Antioxidant and antibacterial activities of aqueous extract of sea buckthorn (*Hippophae rhamnoides*) seeds. *Fitoterapia*, 78 590-592. p.
32. CHAWLA, R., ARORA, R., SINGH, S., KUMAR, R., SAGAR, R. K., SHARMA, A., GUPTA, M.L., SINGH, S., PRASAD, J., KHAN, H.A., SWAROOP, A., SINHA, A.K., GUPTA, A.K., and TRIPATHI, R.P. (2007). Radioprotective and antioxidant activity of fractionated extracts of berries of *Hippophae rhamnoides*. *J. Med. Food*, 1 101-109. p.
33. CHEN, C., ZHANG, H., XIAO, W., YONG, Z. P. and BAI, N. (2007): High-performance liquid chromatographic fingerprint analysis for different origins of sea buckthorn berries. *J Chromatogr A*, 1154(1-2) 250-259. p.
34. CHEN, J.H. (1991): Effect of the immunomodulating agents (BCG) and the juice of HRL on the activity of splenic NK cell and LAK cells from tumour bearing mice. *Chinese J. Microbiol. Immunol*, 11 105–108. p.
35. CHEN, Y., ZHONG, X., LIU, T. and GE, Z. (2003): The study on the effects of the oil from *Hippophae rhamnoides* in hematopoiesis. *Zhong yaocai Zhongyaocai Journal of Chinese medicinal materials*, 26(8) 572-575. p.

36. CHENG, J., KONDOA, K., SUZUKI, Y., IKEDA, Y., MENG, X. and UMEMURA, K. (2003): Inhibitory effects of total flavones of *Hippophae rhamnoides* on thrombosis in mouse femoral artery and in vitro platelet aggregation. *Life Science*, 72 2263-2271. p.
37. CHIRILA, E., OANCEA, E. and OANCEA, I. A. (2014): Physico-chemical characterisation of sea buckthorn extracts for cosmetic use. *Ovidius University Annals of Chemistry*, 25 (2) 75-80. p. http://dx.doi.org/10.2478/auoc-2014-0014
38. CHRISTAKI, E. (2012): *Hippophae rhamnoides* L. (Sea Buckthorn): A Potential Source of Nutraceuticals. *Food and Public Health*, 2(3) 69-72. p. DOI: 10.5923/j.fph.20120203.02
39. Codex Alimentarius 558/93/EEC, Commission Regulation (EEC) No 558/93 of 10 March 1993 on the refractometry method of measuring dry soluble residue in products processed from fruit and vegetables.
40. CORTES, C., ESTEVE, J. M., FRIGOAL, A. and TORREGROSA, F. (2005): Changes in carotenoids including geometrical isomers and ascorbic acid content in orange-carrot juice during frozen storage. *European Food Research Technology*, *221*(1–2), 125–131.
41. CRIZEL DE MORAES, T., JABLONSKI, RIOS DE OLIVEIRA, A., RECH, R. and FLÔRES HICKMANN, S. (2013): Dietary fiber from orange by-products as a potential fat replacer. LWT-*Food Sci. Technol*, 53 9–14. p.
42. DEKA, B. C. (2000): Preparation and storage of mixed fruit juice spiced beverage, Ph.D Thesis, IARI, New Delhi
43. DEL PINO-GARCÍA R., GONZÁLEZ-SAN JOSÉ, M.L., RIVERO-PÉREZ, M.D. and MUÑIZ, P. (2012): Influence of the degree of roasting on the antioxidant capacity and geno protective effect of instant coffee: contribution of the melanoidin fraction. *J Agric Food Chem*, 60 10530-10539. p. doi:10.1021/jf302747v.
44. DENG, Q., ZINOVIADOU, K.G., GALANAKIS, C.M., ORLIEN, V., GRIMI, N., VOROBIEV, E., LEBOVKA, N. and BARBA, F.J. (2015): The effects of conventional and non-conventional processing on glucosinolates and its derived forms, isothiocyanates: extraction, degradation and applications. *Food Engineering Reviews*, 7(3) 357-381. p.
45. DHYANI, D., MAIKHURI, R. K., RAO, K. S., KUMAR, L., PUROHIT, V. K. and SUNDRIYAL, M. (2007): Basic nutritional attributes of sea buckthorn (*Hippophae rhamnoides*) populations from Uttarakhand Himalaya, India. *Current Science*, 92(8) 1148-1152. p.
46. ECCLESTON, C., BAORU, Y., TAHVONEN, R., KALLIO, H., RIMBACH, G.H. and MINIHANE, A.M. (2002): Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. *J. Nut. Biochem*, 13 346-354. p. doi.org/10.1016/S0955-2863(02)00179-1
47. EDRAKI, M., AKBARZADEH, A., HOSSEINZADEH, M., TANIDEH, N., SALEHI, A. and KOOHI-HOSSEINABADI, O. (2014): Healing effect of sea buckthorn, olive oil, and their mixture on full-thickness burn wounds. *Food Chem Toxicol*, 47(6) 1146-53. p. DOI: 10.1016/j.fct.2009.02.002.
48. ELISABETA. C., ELENA. O and IOANA A. O. (2014): Physico-chemical characterisation of sea buckthorn extracts for cosmetic use. Volume 25, Number 2, pp. 75-80. doi: 10.2478/auoc-2014-0014
49. ER, J. (2003): The important role of sea buckthorn development in improving ecological environmentof West China. *The global Sea buckthorn Research and Development*, 2 1-2. p
50. ERCISLI, S. and ORHAN, E. (2007): Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morusnigra*) mulberry fruits. *J. Food chemistry*, 103 (4) 1380-1384. p. https://doi.org/10.1016/j.foodchem.2006.10.054
51. FATIMA, T., SNYDER, C. L., SCHROEDER, W. R., CRAM, D., DATLA, R., WISHART, D., WESELAKE J.R. and KRISHNA P. (2012): Fatty acid composition of developing sea buckthorn (*Hippophae rhamnoides* L.) berry and transcriptome of the mature seed. *PloS One*, 7(4) 1-18. p. https://doi.org/10.1371/journal.pone.0034099.
52. FERNÁNDEZ-LÓPEZ, J., FERNÁNDEZ-GINÉS, J.M., ALESON-CARBONELL, L., SENDRA, E., SAYAS-BARBERÁ, E. and PÉREZ-ALVAREZ, J.A. (2004): Application of

functional citrus byproducts to meat products. *Trends Food Sci. Tech*, 15(3-4), 176-185. p.
53. FICZEK, G., MÁTRAVÖLGYI, G., FURULYÁS, D., RENTSENDAVAA, C., JÓCSÁK, I., PAPP, D., SIMON, G., VÉGVÁRI, GY. and STÉGER-MÁTÉ, M. (2019): Analysis of bioactive compounds of three sea buckthorn cultivars (*Hippophae rhamnoides* L. *'Askola', 'Leikora', and 'Orangeveja'*) with HPLC and spectrophotometric methods. *European Journal of Horticulture Science*, 84 (1) 31-38. p. https://doi.org/10.17660/eJHS.2019/84.1.5
54. FURULYÁS, D., RENTSENDAVAA, C., KIS, F., BÍRÓ, K., STÉGER-MÁTÉ, M. and STEFANOVITS-BÁNYAI, É. (2018): Preliminary study of optimal extraction of biologically active compounds from sea buckthorn (*Hippophae rhamnoides* L.*)* pomace In: *Alapi, T; Ilisz, I (szerk.) Proceedings of the 23rd International Symposium on Analytical and Environmental Problems Szeged*, 392-396. p. https://doi.org/10.14232/analecta.2018.1.1-7
55. GALANAKIS, C. M. (Ed.). (2015): *Food waste recovery: processing technologies and industrial techniques*. Academic Press.
56. GALANAKIS, C. (2012): Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. *Trends in Food Science and Technology*, 26 (2). 68-87. p. http://dx.doi.org/10.1016/j.tifs.2012.03.003
57. GAO, X., OHLANDER, M., JEPPSSON, N., BJO, L. and TRAJKOVSKI, V. (2000): Changes in Antioxidant Effects and Their Relationship to Phytonutrients in Fruits of Sea Buckthorn (*Hippophage rhomboids L.*) during Maturation, 1485–1490. p. https://doi.org/10.1021/jf991072g
58. GAO, Z., GU, X., CHENG, F. and JIANG, F. (2003): Effect of sea buckthorn on liver fibrosis: a clinical study. *W. J. Gastroenterol*, 9 1615-1617. p.
59. GEETHA, S., SAIRAM, M., SINGH, V., ILAVAZHAGAN, G. and SAWHNET, R.C. (2002): Anti-oxidant and immunomodulatory properties of sea buckthorn (*Hippophage rhomboids*). An *in vitro* study. *J. Ethnopharmacology*, 79 37–378. p.
60. GIL-IZQUIERDO A. and GIL MI, FERRERES F. (2002): Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds. *J Agric Food Chem*, 50. 5107–5114. p.
61. GOEL, H.C., PRASAD, J., SINGH, S., SAGAR, R.K., KUMAR, I.P. and SINHA, A.K. (2002): Radioprotection by herbal preparation of *Hippophage rhomboids. RH-3. against whole body lethal irradiation in mice. Phytomedicine*, 9. 15-25. p.
62. GORBATSOVA, J., LOUGAS, T., VOKK, R. and KALJURAND, M. (2007): Comparison of the contents of various antioxidants of sea buckthorn berries using CE. *Electrophoresis*, 28 (22). 4136-4142. p.
63. GÓRECKA, D., PACHOŁEK, B., DZIEDZIC, K. and GÓRECKA, M. (2010): Raspberry pomace as a potential fiber source for cookies enrichment. *Acta Sci. Pol. Technol. Aliment*, 9 451–460. p.
64. GULCIN, I., KUFREVIOGLU, O. I., OKTAY, M. and BUYUKOKUROGLU, M. E. (2004): Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urticarious L.*). *Journal of Ethnopharmacology*, 90205–215. p.
65. GUPTA, R. and FLORA, S. J. S. (2006): Protective effects of fruit extracts of *Hippophae rhamnoides* L. against arsenic toxicity in Swiss albino mice. *Human & Experimental Toxicology*, 25 (6). 285-295. p.
66. GUPTA, V. N. (2000): An Ecological Assessment of Sea buckthorn (*Hippophae* spp.) Resource in North-West Nepal. Tree Improvement and Silviculture Component, Hattisar, Kathmandu, Nepal.1-45.p.
67. GUTZEIT D, BALEANU G, WINTERHALTER P. and JERZ G. (2007a): Determination of processing effects and of storage stability on vitamin K1 (Phylloquinone) in Sea buckthorn berries (*Hippophae rhamnoides* L. ssp. *rhamnoides*) and related products. *J Food Sci*, 72 (9). 491–497. p.
68. GUTZEIT D. and WINTERHALTER P, JERZ G. (2007b): Application of preparative high-speed counter-current chromatography/electrospray ionization mass spectrometry for a fast screening and fractionation of polyphenols. *J ChromatogrA*, 1172 (1). 40–46. p.

69. GUTZEIT, D., BALEANU, G., WINTERHALTER, P. and GEROLD, J. (2008): Vitamin C Content in Sea Buckthorn Berries (*Hippophae rhamnoides* L. *ssp rhamnoides*) and Related Products. A Kinetic Study on Storage Stability and the Determination of Processing Effects. *Journal of Food Science*, 73 (9). C615-20. p. DOI: 10.1111/j.1750-3841.2008.00957.x
70. HAKKINEN, S. H., KARENLAMPI, S. O., HEINONEN, M., MYKKANEN, H. M. and TORRONEN, A. R. (1999): Content of flavanols quercetin, myricetin and kaempferol in 25 edible berries. *Journal of Agricultural and Food Chemistry*, 472274 - 2279. p.
71. HARRISON, J. E. and BEVERIDGE, T. (2002): Fruit structure of *Hippophae rhamnoides* cv. Indian Summer (sea buckthorn). *Canadian Journal of Botany*, 80 (4). 399–409. p.
72. HELLSTRÖM, J., PIHLAVA, J., MARNILA, P., MATTILA, P., and KAUPPINEN, S. (2013): Sea buckthorn leaves–processing waste rich in valuable phytochemicals. In Poster session presentation at the meeting of the 6th conference of the international sea buckthorn association, SBT-a fresh look at technology, health and environment, Potsdam, Germany. Sea buckthorn-Research for a Promising Crop. BoD Print Norderstedt, 101-103.p.
73. HENG, W.W., XIONG, L.W., RAMANAN, R.N., HONG, T.L., KONG, K.W., GALANAKIS, C.M. and PRASAD, K.N. (2015): Two level factorial design for the optimization of phenolics and flavonoids recovery from palm kernel by-product. *Ind. Crop. Prod*, 63 238–248. p.
74. HÖHNE, F. (2015): Overview of cultivation technologies and their challenges. In: Sanna, K., Ekaterina, P., eds. Producing Sea Buckthorn of High Quality. Natural Resources and bioeconomy studies 31/2015. Proceedings of the third European Workshop on Sea buckthorn Euro Works 2014. 14-16 October 2014, Naantali, Finland. 31-35. p.
75. HUFF NK, AUER AD, GARZA F, KEOWEN ML, KEARNEY MT, MCMULLIN RB. and ANDREWS FM. (2012): Effect of Sea Buckthorn Berries and Pulp in a Liquid Emulsion on Gastric Ulcer Scores and Gastric Juice PH in Horses. *J Vet Intern Med*, 261186-1191.p.
76. IGUAL, M. G. M. E., GARCIA-MARTINEZ, E., CAMACHO, M. M., and MARTINEZ-NAVARRETE, N. (2010): Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. *Food Chemistry*, *118*(2), 291-299.
77. JIA, D. and BARTISH, I. V. (2018): Climatic Changes and Orogeneses in the Late Miocene of Eurasia: The Main Triggers of an Expansion at a Continental Scale? *Frontiers in plant science*, 9 1–15. p. https://doi.org/10.3389/fpls.2018.01400
78. JOHANSSON, A. K., KORTE, H., YANG, B., STANLEY, J.C. and KALLIO, H.P. (2000): Sea buckthorn berry oil inhibits platelet aggregation. *J. Nutr. Biochem*, 11. (10). 491 – 495. p.
79. KALLIO, H., YANG, B. and PEIPPO, P. (2002): Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (*Hippophae rhamnoides*) berries. *Journal of Agricultural and Food Chemistry*, 50.(21). 6136–6142. p.
80. KALLIO, K., YANG, B.R., TAHVONEN, R. and HAKALA, M. (1999): Composition of sea buckthorn berries of various origins. Proceeding of International Symposium Sea buckthorn (*Hippophae rhamnoids* L.). Beijing, China. 17 - 23. p.
81. KALT, W. (2005): Effects of production and processing factors on major fruit and vegetable antioxidants. *Journal of Food Science*, 70 11-19. p.
82. KAPCUM, C. and URIYAPONGSON, J. (2018): Effects of storage conditions on phytochemical and stability of purple corn cob extract powder. *Food Sci. Technol*, 38 (2) 1-5. p. http://dx.doi.org/10.1590/1678-457x.23217
83. KARAKAYA, S. (2004): Bioavailability of Phenolic Compounds. *Critical Reviews in Food Science and Nutrition*, 44 453-464. p.
84. KARHAN, M., AKSU, M., TETIK, N. and TURHAN, I. (2004): Kinetic modeling of anaerobic thermal degradation of ascorbic acid in rose hip (*Rosa canina* L) pulp. *Journal of Food quality*, 27 (5). 311-319. p.
85. KAUSHAL, M. and SHARMA, P.C. (2011): Nutritional and antimicrobial property of sea buckthorn (*Hippophae* sp.) seed oil. *J Sci Indust Res*, 70 1033-1036. p.
86. KEE, H.J., RYU, G.H. and PARK, Y.K. (2000): Preparation and quality properties of extruded snack using onion pomace and onion. *Korean J. Food Sci. Technol*, 32578–583. p.

87. KHAN, BA, AKHTAR, N. and MAHMOOD, T. (2010): A comprehensive review of a magic plant *Hippophae rhamnoides. Pharmacogn J*, 1658-61. p.
88. KIM MW. (2013): Effect of Sea Buckthorn Leaves on Hepatic Enzyme Levels in Streptozotocin Induced Diabetic Rats. *Journal of the Korean Society of Food Science and Nutrition*, 42. (1). 40-45. p. DOI: 10.3746/jkfn.2013.42.1.040
89. KLIMCZAK, I., MALECKA, M., SZLACHTA, M. and ANNA, G.S. (2007): Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis*, 20(3-4). 313-322. p. http//www.doi.org/ 10.1016/j.jfca.2006.02.012
90. KOREKAR, G., DOLKAR, P., SINGH, H., SRIVASTAVA, R.B. and STOBDAN, T. (2014): Variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of Sea buckthorn (*Hippophae rhamnoides* L.) from trans-Himalaya. *LWT- Food Science and Technology*, 55. (1). 157-162. p. DOI: 10.1016/j.lwt.2013.09.006.
91. KREBBERS, B., MATSER, A.M., HOOGERWERF, W.S., SANNE. W., MOEZELAAR, R., MONIC. M.M. and ROBERT.T. W. VAN DEN BERG. (2003): Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innovative Food Science and Emerging Technologies*, 4(4) 377-385 DOI: 10.1016/S1466-8564(03)00045-6
92. KREJCAROVÁ, J., STRAKOVÁ, E., SUCHÝ, P. and KARÁSKOVÁ, K. (2015): Sea buckthorn (*Hippophae rhamnoides* L.) as a potential source of nutraceutics and its therapeutic possibilities - A review. *Acta Veterinaria Brno*, 84. (3). 257-268. p.
93. KRISHNA, J.G. and CHANDRASEKARAN, M. (2012): Biochemical and nutritional aspects of food processing byproducts. Valorization of food processing by-products. ISBN 978-1-4398-4885-2
94. KUMAR, R., KUMAR, G.P., CHAURASIA, O.P. AND SINGH, S.B. (2011): Phytochemical and pharmacological profile of Sea buckthorn oil: a review. *Res J Med Plant*, 5 491-499. p.
95. KUMAR, S. and SAGAR, A. (2007): Microbial associates of *Hippophae rhamnoides (Sea buckthorn)*. *Plant Pathol J*, 6 299-305. p.
96. KURUCZEK, M., SWIDERSKI, A., MECH-NOWAK, A. and KRÓL, K. (2012): Antioxidant capacity of crude extracts containing carotenoids from the berries of various cultivars of Sea buckthorn (*Hippophae rhamnoides* L.). *Acta Biochemica Polonica*, 59 (1). 135-137. p.
97. LAAKSONEN, O., KNAAPILA, A., NIVA, T., DEEGAN, K.C. and SANDELL, M. (2016): Sensory properties and consumer characteristics contributing to liking of berries. *Food Quality and Preference*. 53 117–126. p.
98. LADANIYA, M.S. (2008): Citrus fruit biology. Technology and Evaluation. Elsevier, Amsterdam. Le Concorde Hotel, Quebec City, Quebec, Canada, 12–16 Aug, 5–8. p.
99. LEPOSAVA, P., SNEŽANA, U., MILENA, J., DANIELA, Đ. and PREDRAG, Đ. (2018): Determination of Flavonoids and Total Polyphenol Contents in Commercial Apple Juices. *Food Analysis, Food Quality and Nutrition. Czech J. Food Sci*. 36(3). 233–238. p. https://doi.org/10.17221/211/2017-CJFS.
100. LESSIN, W. J., CATIGANI, G. L. and SCHWARTZ, S. J. (1997): Quantification of cis-trans Isomers of provitamin a carotenoid in fresh and processed fruits and vegetables. *Journal of Agricultural and Food Chemistry*. 45(10). 3728-3732. p.
101. LI, T.S.C. (2002): Product development of sea buckthorn. In Trends in New Crops and New Uses. *Alexandria, VA: ASHS Press*. 393-398. p.
102. LI, T. S. C. and BEVERIDGE, T. H. J. (2003). Sea buckthorn (*Hippophae rhamnoides* L.) production and utilization. NRC Research Press.
103. Li, T. S. C. and SCHROEDER, W. R. (1996): Sea buckthorn (*Hippophae rhamnoides* L.). a multipurpose plant. Hort Technology. 6. (4). 370–380. p.

104. Li, T.S.C. (2003b): Taxonomy, natural distribution and botany. In Sea buckthorn (*Híppophae rhamnoides* L.): Production and Utilization, eds. T.S.C. Li and T.H.J. Beveridge, 7-I1. Ottawa, ON: NRC Research Press. ISBN:978-0-660-19007-5
105. LIPOWSKI, J., MARSZAŁEK, K. and SKĄPSKA, S. (2009): Sea buckthorn- an innovative Raw material for the fruit and vegetable processing industry. Journal of Fruit and Ornamental Plant Research location. *In: Proceedings of the 3rd international sea buckthorn association conference Loews*. 17. (2). 121-126. p. https://www.researchgate.net/publication/260243502
106. LIMA, M., HESKITT, B.F., BURIANEK, L.L., NOKES, SE. and SASTRY, SK. (1999): Ascorbic acid degradation kinetics during conventional and ohmic heating. *J Food Process Press*. 23 421–34. p.
107. LOZANO-ALEJO, N., CARRILLO, G. V., PIXLEY, K. and PALACIOS-ROJAS, N. (2007): Physical properties and carotenoid content of maize kernels and its nixtamalized snacks. *Innovative Food Science and Emerging Technologies.* 8 (3). 385–389. p.
108. LU, R. (1990): Sea buckthorn Resouces and its Unexploited Potential in the Himalayan Region. International Center for Integrated Mountain Development (ICIMOD). MFS Series, (12).
109. LU, R. (2003): Sea buckthorn: A Multipurpose Plant for Mountain People. Asian Case Study, Chinese Academy of Sciences, China.
110. LUNDEN, S., TIITINEN, K. and KALLIO, H. (2010): Aroma analysis of sea buckthorn berries by sensory evaluation, headspace SPME and GC-Olfactometry. *Expression of Multidisciplinary Flavour Science; Blank, I. Wust, M*. 490-493. p.
111. MA, X., LAAKSONEN, O., HEINONEN, J., SAINIO, T., KALLIO, H. and YANG, B. (2017a): Sensory profile of ethyl b-D-glucopyranoside and its contribution to quality of sea buckthorn (*Hippophae rhamnoides* L.). *Food Chemistry.* 233 263–272. p.
112. MA, X., YANG, W., LAAKSONEN, O.A., NYLANDER, M., KALLIO, H. and YANG, B. (2017b): Role of flavonols and proanthocyanidins in the sensory quality of sea buckthorn (*Hippophae rhamnoides* L.) Berries. *Journal of Agricultural and Food Chemistry*. 65 9871–9879. p.
113. MAKOVICS-ZSOHÁR, N., HEGEDŰS, A., STEFANOVITS-BÁNYAI, É., RÉDEI, R., and PAPP, N. (2014): The antioxidant capacity of sea buckthorn (*Hippophae rhamnoides* L.) berries depends on the genotype and harvest time. *International Journal of Horticultural Science*. 20 (3–4): 27–29. p.
114. MANEA, I. and BURULEANU, L. (2009): Mathematical Model for The Evaluation of the Sea-buckthorn juice preservation. *Ovidius University Annals of Chemistry*. 20(1), 83-86. p.
115. MARFIL, P. H. M., SANTOS, E. M. and TELIS, V. R. N. (2008): Ascorbic acid degradation kinetics in tomatoes at different drying conditions. *LWT-Food Science and Technology*, *41*(9), 1642-1647.
116. MENDELOVA, A., MENDEL, L., CZAKO, P. and MARECEK, J. (2016): Evaluation of carotenoids, polyphenols content and antioxidant activity in the sea buckthorn fruit. *Potravinarstvo*. 10 (1). 1-6. p. doi:10.5219/551
117. MICHEL, T., DESTANDU, E., LE, FLOCH, G., LUCCHESI, ME. and ELFAKIR, C. (2012): Antimicrobial, antioxidant and phytochemical investigations of sea buckthorn (*Hippophae rhamnoides* L.) leaf, stern, root and seed. *Food Chem*. (137). 754-760. p.
118. MILDNER-SZKUDLARZ, S., BAJERSKA, J., ZAWIRSKA-WOJTASIAK, R. and GÓRECKA, D., (2013): White grape pomace as a source of dietary fiber and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits. *J. Sci. Food Agric.* 93. (2). 389–395. p.
119. MIRABELLA, N., CASTELLANI, V. and SALA, S. (2014): Current options for the valorization of food manufacturing waste: a review. *Journal of cleaner production*. (65). 28-41. p. https://doi.org/10.1016/j.jclepro.2013.10.051
120. MIŠAN, A., ŠARIC´, B., NEDELJKOVIC´, N., PESTORIC´, M., JOVANOV, P., POJIC´, M., TOMIC´, J., FILIPCˇEV, B., HADNAĐEV, M. and MANDIC´, A. (2014): Gluten-free cookies enriched with blueberry pomace: optimization of baking process. *World Acad. Sci. Eng.*

Technol., Int. J. Biol., Veter. Agric. Food Eng. 8 330–333. p.
121. MISI, S.N. and FORSTER, S.F. (2002): Semi-continuous anaerobic co-digestion of agrowaste. *Environ Technol*. 23 445–451. p.
122. MÖRSEL, J. T., TSIRENOVA, E., and ZUBAREV, Y. A. (2014): 4. Differences in SBT between Russian and German varieties. *Sea buckthorn. Research for a promising crop: A look at recent developments in cultivation, breeding, technology, health and environment*, 23.
123. MUSAYEV, M.K. (2013): Agro-ecological characteristics of sea buckthorn (*Hippophae rhamnoides* L.) in Azerbaijan. *J Crop Weed*. 9 (11).114–120
124. NEGI, B. and GARGI, D. (2013): Effects of co-fermentation by Saccharomyces cerevisiae and Issatchenkiaorientalis on sea buckthorn juice. *International Journal of Food Sciences and Nutrition*. 64 (4). 508-513. p. DOI: 10.3109/09637486.2012.759182.
125. NERSESIAN, A.K., ZILFIAN, V.N., KUMKUMADZHIAN,V.A. and PROSHIAN, N.V. (1990): Antimutagenic properties of sea buckthorn oil. *Genetika*. 26 378-80. p.
126. NERSESYAN, A. and MURADYAN, R. (2004): Sea buckthorn juice protects mice against genotoxic action of cisplatin. *Experimental oncology*. 26(2). 153-155. p.
127. NUERNBERG, K., NUERNBERG, G., PRIEPKE, A. and DANNENBERGER, D. (2015). Sea buckthorn pomace supplementation in the finishing diets of pigs – Are there effects on meat quality and muscle fatty acids? Archives Animal *Breeding*. 58 107–113. p. DOI: 10.5194/aab-58-107-2015.
128. OOMAH, B. D., and MAZZA, G. (1999): Health benefits of phytochemicals from selected Canadian crops. *Trends in Food Science & Technology*. 10(6–7). 193–198. p.
129. OSAWA, K., CHINEN, C., TAKANAMI, S., KURIBAYASHI. and T. KUROKOUCHI, K. (1995): Studies on effective utilization of carrot pomace. II. Effective utilization to cake, dressings and pickles. *Research Report of the Nagano State Laboratory of Food Technology*. 23 15–18. p.
130. ÖTLES, S. AND CAGINDI, Ö. (2008): Carotenoids as natural colorants. In: Socaciu, C. (Ed.), Food Colorants: Chemical and Functional Properties. CRC Press, Boca Raton, pp. 51–70.
131. PADMAVATHI, B., UPRETI, M., SINGH, V., RAO, R.A., RANA P., SINGH, PRAMOD C. and RATH, P.C. (2005): Chemoprevention by *Hippophae rhamnoides*: Effects on Tumorigenesis, Phase II and Antioxidant Enzymes, and IRF-1 Transcription Factor. *Nutrition and Cancer*. 51 (1). 59-67. p. DOI: 10.1207/s15327914nc5101_9.
132. PANG, X., ZHAO, J., ZHANG, W. and ZHUANG, X. (2008): Antihypertensive effect of total flavones extracted from seed residues of *Hippophae rhamnoides* L. in sucrose-fed rats. *Journal of ethnopharmacology*.117 (2). 325-331. p. doi: 10.1016/j.jep.2008.02.002
133. PATEL, K., and PATEL, D. K. (2019): The beneficial role of rutin, a naturally occurring flavonoid in health promotion and disease prevention: a systematic review and update. In *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases* (pp. 457-479). Academic Press.
134. PATIAL, V., ASRANI, R.K. and PATIL R.D. (2013): Safety evaluation of sea buckthorn (*Hippophae rhamnoides*) leaves in Japanese quail. *Vet. World*. 6 596-600. p.
135. PAULI, H. (1976): Proposed extension of the CIE recommendation on "Uniform color spaces, color difference equations, and metric color terms. *Journal of the Optical Society of America*. 66 (8). 866-867. p. https://doi.org/10.1364/JOSA.66.000866
136. PERINO-ISSARTIER, S., ABERT-VIAN, M. and CHEMAT, F. (2011): Solvent free microwave-assisted extraction of antioxidants from sea buckthorn (*Hippophae rhamnoides*) food by-products. *Food and Bioprocess Technology*. 4 (6). 1020-1028. p.
137. PILJAC ZEGARAC, J. and BELSCAK, A. and PILJAC, A. (2009): Antioxidant Capacity and Polyphenolic Content of Blueberry (*Vaccinium corymbosum* L.) leaf Infusions. *Journal of medicinal food.* 12 (3). 608-14. p. http//www.dx.doi.org/ 10.1089/jmf.2008.0081
138. POP, R. M., WEESEPOEL, Y., SOCACIU, C., PINTEA, A., VINCKEN, J. and GRUPPEN, H. (2014): Carotenoid composition of berries and leaves from six Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. *Food Chemistry*. 147 1–9. p.

139. PROKKOLA, S. and MAYRA, K. (2003): Effect of processing on vitamin C of sea buckthorn. ISSA – Meeting Sea buckthorn – a Resource of Health, a Challenge to Modern Technology. Berlin, Germany, 14-18 September 2003, p. 47.
140. QIU, D., CHEN, Z. R., LI, H. R. (2009): Effect of heating on solid β-carotene. *Food Chemistry*. 112 (2). 344–349. p.
141. RAFALOWSKI, R., ZOFIA, Z., KUNCEWICZ, A. and ZBIGNIEW, B. (2008): Fatty acid composition, tocopherolsand beta-carotene content in Polish commercial vegetable oils. *Pakistan Journal of Nutrition.* 7 (2)6-12.p. DOI: 10.roop3923/pjn.2008.278.282
142. RAFFO, A., PAOLETTI, F. and ANTONELLI, M. (2004): Changes in sugar, organic acid, flavonol and carotenoid composition during ripening of berries of three sea buckthorn (*Hippophae rhamnoides* L.) cultivars. *European Food Research and Technology*. 219(4) 360-368. http://dx.doi.org/10.1007/s00217-004-0984-4
143. RANJITH, A. and ARUMUGHAN, C. (2009): Phytochemical investigations on sea buckthorn (*Hippophae rhamnoides* L.) berries. Doctoral dissertation. National Institute for Interdisciplinary Science and Technology.
144. REHMAN, U. K., SHAMSUR, R. A., MUHAMMAD, I., SHER, A.K., HAMIDA, A. and MUHAMMAD, S. (2012): Effect of different chemical preservatives on the storage stability of mango – sea buckthorn blended juice. *Pak. J. Biochem. Mol. Biol.* 45(1). 6-10. p.
145. REMINI,H.,MERTZ,C., BELHANI, A., ACHIR, N., DORNIER, M., MADANI, K. (2015): Degradation kinetic modelling of ascorbic acid and colour intensity in pasteurised blood orange juice during storage. *Food Chemistry*, 173 (15). 665-673. p.
146. RIBOH, D. L. and LABUZA, T. P. (1982): Effect of sine wave temperature cycling on thiamin loss in fortified pasta. *Journal of Food Processing and Preservation*, *6*(4), 253-264.
147. RICO, D., MARTÍN-DIANA, A. B., BARAT, J. B. and BARRY-RYAN, C. (2007): Extending and measuring the quality of fresh-cut fruit and vegetables; a review. *Trends in Food Science & Technology*. 18 (7). 373-386. p. http:// dx.doi.org/10.1016/j.tifs.2007.03.011.
148. RISHAVY, MA., HALLGREN, KW., WILSON, LA., USUBALIEVA, A., RUNGE, KW. and BERKNER KL. (2013): The vitamin K oxidoreductase is a multimer that efficiently reduces vitamin K epoxide to hydroquinone to allow vitamin K-dependent protein carboxylation. *J Biol Chem*.288 (44). 31556-31566. p.
149. RODRIGUEZ-AMAYA, D. B. (2016). Natural food pigments and colorants. *Current Opinion in Food Science*. 7 20-26. p.
150. RØDTJER, A., SKIBSTED, L. H., and ANDERSEN, M. L. (2010): The role of phenolic compounds during formation of turbidity in an aromatic bitter. *Food Chemistry*. 123 (4). 1035-1039. p. http://dx.doi.org/10.1016/j.foodchem.2010.05.056.
151. RONGSEN, LU. (2007). The correlation between sea buckthorn berry quality and altitudes of its growing location. In: *SEA BUCKTHORN ASSOCIATION CONFERENCE*. 5-8. p.
152. ROOPESH, M. S., SHARI, L. L., KANISHKA, B., SHYAM, S. S., FRANK, D., BARBARA, R., KAREN, K., AMIT, D. and CAROLYN, R. (2014): UV-C light inactivation kinetics of Penicillium expansum on pear surfaces: Influence on physicochemical and sensory quality during storage, *Postharvest Biology and Technology*. 87 27-32. p.
153. ROSCH, D., MUGGE, C., FOGLIANO, V. and KROH, L. W. (2004a): Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice, *Journal of Agricultural Food Chemistry*. 51(15). 4233-4239. p.
154. ROSCH, D., MUGGE, C., FOGLIANO, V. and KROH, L. W. (2004b): Antioxidant oligomeric proanthocyanidins from Sea buckthorn (*Hippophae rhamnoides*) pomace. *J. Agric. Food Chem*. 52 6712-6718. p. https://doi.org/10.1021/jf040241g
155. RUMPUNEN, K. and ANDERSSON, S. (2012). Bra stabilitet hos goda drycker med hög halt naturliga vitaminer och antioxidanter. https://pub.epsilon.slu.se/9370/17/rumpunen_et_al_130107.pdf

156. SABIR, S. M., MAQSOOD, H., HAYAT, M., KHAN, M.Q. and KHALIQ, A. (2005): Elemental and nutritional analysis of sea buckthorn (*Hippophae rhamnoides* ssp. *turkestanica*) berries of Pakistani origin. *J Med Food*. 8 518–522. p.
157. SALAHAT, M.A., FARAH, H.S. and AL-DEGS, Y.S. (2002): Importance of HDL cholesterol as predictor of coronary heart disease in Jordan population: the role of HDL-Sub fractions in reverse cholesterol transport. *Pak. J. Biol. Sci*. 5 1189-1191. p.
158. SAVELYEV, N., MAKAROV, V., VLAZNEVA, L. AND CHERENKOVA, T. (2007): Biologically active substances in sea buckthorn and production of functional foods. In: *Proceedings of the 3rd international sea buckthorn association conference Loews Le Concorde Hotel, Quebec City, Quebec, Canada*. 127–129. p. http://argousier.qc.ca/stock/eng/ISA2007_Proceedings_Web.pdf
159. SAYEGH, M., MIGLIO, C. and RAY, S. (2014): Potential cardiovascular implications of Sea buckthorn berry consumption in humans. *J Food Sci Nutr*. 65(5) 521-528. p. doi:10.3109/09637486.2014.880672.
160. SCALZO, J., POLITI, A., PELLEGRINI, N., MEZZETTI, B. and BATTINO, M. (2005): Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition*. 21 207–213. p.
161. SCHIEBER A. and CARLE R. (2005): Occurrence of carotenoid cis-isomers in food: Technological, analytical, and nutritional implications. *Trends in Food Science and Technology*. 16(9) 416–422. p.
162. SCHROEDER, W.R. and YAO, Y. (1995): Sea buckthorn: a promising multipurpose crop for Saskatchewan. *Prairie Farm Rehabilitation Administration, Agriculture & Agri-Food Canada*. 14.p.
163. SHARMA, H.S., OBEROI, G.S. and DHILLON. G.C. (2016): Fruit and vegetable processing waste: renewable feed stocks for enzyme production. *In Agro-Industrial Wastes as Feedstock for Enzyme Production*. 23-59. p.
164. SHENG, Y., ÅKESSON, C., HOLMGREN, K., BRYNGELSSON, C., GIAMAPA, V. and PERO, R. W. (2005): An active ingredient of Cat's Claw water extracts: Identification and efficacy of quinic acid. *Journal of Ethnopharmacology*. 96(3) 577–584. p.
165. SIMPLE, H. D. AND LOGIN, M. (2010). Analisis Pengaruh Migrasi Masuk terhadap Permintaan Rumah Sederhana di Kota Pekanbaru. *Sumber*, *21*(605), 26-092.
166. SINGH, V. (2005): Free radicals, diseases, anti-oxidants and anti-oxidant properties of sea buckthorn (*Hippophae rhamnoides* L.). *Sea buckthorn, Daya Publishing House, New Delhi, India*. 3–69.
167. SINGH, R.P. and HELDMAN, D.R. (2001): Introduction to Food Engineering, 3rd ed., London, UK: Academic Press, Inc. ISBN 0-12-676757-2
168. SINGLETON, V. L. and ROSSI, J. A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*. 16(3) 144-158. p.
169. SOLONENKO, L.P. AND PRIVALOV, G.F. (2005): Amino acids and other nutrients of Siberian sea buckthorn fruit. In Sea buckthorn (*Hippophae* L.): A Multipropose Wonder Plant. *Daya Publishing House, New Delhi, India*. 2 263-271. p.
170. SOUCI, S.W., FACHMANN, W., KRAUT, H. (2008). Food composition and nutrition tablet, 7th revised and completed edition. ISBN 13: 9780849341410 and ISBN 10: 0849341418
171. STOBDAN, T., CHAURASIA, O.P., KOREKAR, G., YADAV, A. and SINGH, S.B. (2010). Attributes of sea buckthorn (*Hippophae rhamnoides* L.) to meet nutritional requirements in high altitude. *Defense Science Journal*. 60, 226–230. p.
172. STOBDAN, T., YADAV, A., MISHRA, G.P., CHAURASIA, O.P., SHRIVASTAVA, R.P. (2011): Sea buckthorn: The Super Plant (Production, Characterization, Post harvest & Health Applications). *DIHAR, DRDO, Leh-Ladakh*, 1- 88.
173. SUBEDI, CK. and ADHIKARI, K. (2001): Propagation Techniques of Sea Buckthorn (*Hippophae* Linn.) in Manang and Mustang Districts. A Report Submitted to ISC, Hattisar,

Kathmandu.
174. SUDHA, M.L., BASKARAN, V. and LEELAVATHI, K., (2007): Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *J Food Chem*. 104 686–692. p.
175. SULEYMAN, H., BUYUKOKUROGLU, M.E., KORUK, M., AKCAY, F., KIZILTUNÇ, A. and GEPDIREMEN, A. (2001a): The effects of *Hippophae rhamnoides* L. Extract on ethanol induced gastric lesion and gastric tissue glutathione level in rats: a comparative study with melatonin and omeprazole. *Indian J. Pharm*, 33 77-81. p.
176. SUN, J., BAI, W., ZHANG, Y., LIAO, X. and HU, X. (2011): Identification of degradation pathways and products of cyanidin-3-sophoroside exposed to pulsed electric field. *Food Chemistry*. 126(3) 1203-1210. p. http://dx.doi.org/10.1016/j.foodchem.2010.12.002.
177. SUNG, J., SUH, J.H., CHAMBERS, A.H., CRANE, J. and WANG, Y. (2019): The relationship between sensory attributes and chemical composition of different mango cultivars. *Journal of Agricultural and Food Chemistry*. 67 5177–5188. p.
178. SUOMELA, J.P., AHOTUPA, M., YANG. B., VASANKARI, T. and KALLIO, H. (2006): Absorption of flavonoids derived from sea buckthorn (*Hippophae rhamnoides* L.) and their effect on emerging risk factors for cardiovascular disease in humans. *J Agric Food Chem*. 54 7364-7369. p. https://doi.org/10.1021/jf061889r
179. SURYAKUMAR, G. and GUPTA, A. (2011): Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides* L.). *Journal of Ethnopharmacol*. 138 268-278. p.
180. TAIZ, L. AND ZEIGER, E. (2006). Plant Physiology. Sinauer Associates: Sunderland, MA, pp. 197–344. ISBN-13: 978-0878938568
181. TENG, B. S., LU, Y. H., WANG, Z. T., TAO, X. Y. and WEI, D. Z. (2006): In vitro anti-tumor activity of isorhamnetin isolated from *Hippophae rhamnoides* L. against BEL-7402 cells. *Pharmacol*. *Res*. 54 186-194. p.
182. TENORE, G.C., MANFRA, M., STIUSO, P., COPPOLA, L., RUSSO, M., GOMEZ MONTERREY, I.M. and CAMPIGLIA, P. (2012): Antioxidant profile and in vitro cardiac radical-scavenging versus pro-oxidant effects of commercial red grape juices (*Vitis vinifera* L. cv. *Aglianico* N.). *J. Agric. Food Chem*. 60 9680–9687. p.
183. THEODOSIOU, M., LAUDET, V. and SCHUBERT, M. (2010): From carrot to clinic: An overview of the retinoic acid signaling pathway. *Cellular and Molecular Life Sciences*. 67(9) 1423-1445. p.
184. TIAN, Y., LIIMATAINEN, J., ALANNE, A., LINDSTEDT, A., LIU, P., SINKKONEN, J. and YANG, B. (2017): Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chemistry*. 220 266–281. p.
185. TIE, J.K., JIN, D.Y., STRAIGHT, D.L. and STAFFORD, D.W. (2011): Functional study of the vitamin K cycle in mammalian cells. *J Blood*. 117(10) 2967-2974. p.
186. TIGONG, C., NI, M. K., LI, R., and JI, F. (1991): Investigation of the biological properties of central Asian sea buckthorn growing in the province of Kansu (China). *Chemistry of Natural Compounds*. 27(1) 119-121. p.
187. TIITINEN, K., VAHVASELKA, M., LAAKSO, S. and KALLIO, H. (2007): Malolactic fermentation in four varieties of sea buckthorn (*Hippophae rhamnoides* L.). *European Food Research and Technology*. 224(6) 725–732. p.
188. TIITINEN, K.M., HAKALA, M.A. and KALLIO, H.P. (2005): Quality component of sea buckthorn (*Hippophae rhamnoides*) varieties. *J. Agr. Food Chem*. 53 1692-1699. p. http://doi.org/10.1021/jf0484125.
189. TONG, JC., ZHANG, Z., ZHAO, Y. and YANG, T. K. (1989): The determination of the physical-chemical constants and sixteen mineral elements in raw sea buckthorn juice. *Proceedings of the International Symposium on Sea buckthorn* (*H. rhamnoides* L.), *Xian, China*. 132–137. p.
190. TÖRRÖNEN, R., SARKKINEN, E., NISKANEN, T., TAPOLA, N., KILPI, K., and NISKANEN, L. (2012): Postprandial glucose, insulin and glucagon-like peptide 1 responses to

sucrose ingested with berries in healthy subjects. *British Journal of Nutrition*. 107 1445-1451. p.
191. UPADHYAY. N.K., KUMAR, M.S.Y. and GUPTA, A. (2010): Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (*Hippophae rhamnoides* L.) leaves. *Food Chem Toxicol*, 48 3443-3448. p. https://doi.org/10.1016/j.fct.2010.09.019
192. VELIOGLU, Y.S., MAZZA, G., GAO, L. and OOMAH, B.D. (1998): Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *Journal of Agricultural and Food Chemistry*. 46(10) 4113-4117. p. http/www.dx.doi.org/10.1021/jf9801973
193. VERSARI, A., PARPINELLO, G., MATTIOLI, A. and GALASSI, S. (2008): Characterisation of Italian commercial apricot juices by high-performance liquid chromatography analysis and multivariate analysis. *Food Chem*.108 334–340. p.
194. WILDMAN, R.E.C. (2001). Classifying nutraceuticals. In Handbook of Nutraceuticals and Functional Foods. 13–30. p. CRC Press LLC, Boca Raton, FL. ISBN: 9780429195563
195. WILLS, R., MCGLASSON, B., GRAHAM, D. AND JOYCE, D. (2007). Postharvest, an introduction to the physiology and handling of fruit, vegetables and ornamentals 5th edition. Oxford shire, UK: CABI. ISBN-10: 0851992641
196. XIAO, Z., PENG, W., ZHU, B. and WANG, Z. (2003): The inhibitory effect of total flavonoids of *Hippophae* on the activation of NF-kappa B by stretching cultured cardiac myocytes. *Journal of Sichuan University. Medical science edition*. 34(2) 283-285. p.
197. XING, J., YANG, B., DONG, Y., WANG, B., WANG, J. and KALLIO, H. (2002): Effects of sea buckthorn (*Hippophae rhamnoides* L.) seed and pulp oils on experimental models of gastric ulcer in rats. *J Fitoterapia*. 73 644-650. p.
198. XU, X., XIE, B., PAN, S., LIU, L., WANG, Y. and CHEN, C. (2007): Effects of sea buckthorn procyanidins on healing of acetic acid-induced lesions in the rat stomach. *Asia PacJ Clin Nutr*. 16(1) 234-238. p.
199. YANG, B. and KALLIO, H. P. (2001): Fatty acid composition of lipids in sea buckthorn (*Hippophae rhamnoides* L.) berries of different origins. *Journal of Agricultural and Food Chemistry*. 49(4) 1939–1947. p.
200. YANG, B. and KALLIO, H. P. (2002a): Composition and physiological effects of sea buckthorn (*Hippophae*) lipids. *Trends in Food Science and Technology*. 13(5) 160–167. p.
201. YANG, B. and KALLIO, H. P. (2002b): Supercritical CO2-extracted sea buckthorn (*Hippophae rhamnoides*) oils as new food ingredients for cardiovascular health. *Proceedings of health ingredients of Europe*. 17 19.p.
202. YANG, B. and KALLIO, H.P. (2005): Lipophilic components of sea buckthorn (*Hippophae rhamnoides* L.) seeds and berries. Sea buckthorn (*Hippophae* L.) - *A multipurpose wonder plant. Indus Publishing Company*. 70-97. p.
203. YANG, B., KALIMO, K.O., TAHVONEN, R.L., MATTILA, L.M., KATAJISTO, J.K. and KALLIO, H.P. (2000): Effect of dietary supplementation with sea buckthorn (*Hippophae rhamnoides*) seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis. *J. Nutr. Biochem*. 11 338-340. p.
204. YANG, B., KARLSSON, R.M., OKSMAN, P.H. and KALLIO, H.P. (2001): Phyto-sterols in sea buckthorn (*Hippophae rhamnoides* L.) berries: identification and effects of different origin and harvesting time. *Journal of Agricultural and Food Chemistry*. 49 5620-5629. p.
205. YAN-JUN, X., MEERA, K., RESHVINDER, S.D., PARAMIJ, S.T. and NARANJAN, S.D. (2011): Health benefits of sea buckthorn for the prevention of cardiovascular diseases. *J. Funct. Food*. 3 2–12. p.https://doi.org/10.1016/j.jff.2011.01.001
206. ZADERNOWSKI, R., NACZK, M., CZAPLICKI, S., RUBINSKIENE, M. and SZALKIEWICZ. M. (2005): Composition of phenolic acids in sea buckthorn (*Hippophae rhamnoides* L.) berries. *Journal of the American oil chemists Society*. 82 175-179. p.
207. ZADERNOWSKI, R., SZALKIEWICZ, M. and CZAPLICKI, S. (2007): Skład chemiczny owoców rokitnika zwyczajnego (*Hippophae rhamnoides* L.). *ZESZ. PROBL. POST. NAUKROL.*, 468: 519-530.

208. ZEB, A. (2004): Chemical and Nutritional Constituents of Sea Buckthorn Juice. *Pakistan Journal of Nutrition*. 3(2). 99–106. p. https://doi.org/10.3923/pjn.2004.99.106
209. ZEB, A. and MEHMOOD, S. (2004): Carotenoids contents from various sources and their potential health applications. *Pak. J. Nutr*. 3 197-202. p.
210. ZHANG, W., YAN, J., DUO, J., REN, B. and GUO, J. (1989): Preliminary study of biochemical constitutions of berry of sea buckthorn growing in Shanxi province and their changing trend. In: *Proceedings of international symposium on sea buckthorn* (*Hippohae rhamnoides* L.). 96-105. p.
211. ZIELINSKA, A. and NOWAK I. (2017): Abundance of active ingredients in sea-buckthorn oil. *Lipids in Health and Disease*. 16(95). 1-11.p. DOI: 10.1186/s12944-017-0469-7
212. ZU, Y., LI, C., FU, Y. and ZHAO, C. (2006): Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. *Journal of Pharmaceutical and Biomedical Analysis*. 41 (3) 714-719. p. DOI: 10.1016/j.jpba.2005.04.052.

WEB resource

- BAGHURST, K. (2006): Fruit juice, nutrition and health - A review. A position paper for health professionals provided by the Australian fruit juice association. www.afja.com.au on 19.04.2010.
- csp_Ursula1964 -- https://www.fotosearch.com/CSP285/k2851802/
- FAO, 2014. Save food – global initiative on food loss and waste reduction. Available from: http://www.fao.org/docrep/015/i2776e/i2776e00.pdf.
- HCSO, 2016. Hungarian Central Statistical Office: http://www.ksh.hu, Accessed: 07/08/2016.
- https://www.astm.org/Standards/E1978.htm
- https://www.seabuckwonders.com/pages/what-is-sea-buckthorn
- NRCC. 2002. Canadian Sea buckthorn Technology Mission to Russia and Germany - Final Report. National Research Council Canada/ industrial Research Assistance Program, Newfoundland &. Labrador and Nunavut Region. https://nrc.canada.ca/en/certifications-evaluations-standards/calibration-laboratory-assessment-service/directory-accredited-calibration-laboratories/clas-certificate-number-2002-04
- Silkrute -- https://bazovo.ru/hu/metody-lecheniya/poleznye-svoistva-i-protivopokazaniya-k-primeneniyu/
- USDA food database: https://fdc.nal.usda.gov/fdc-app.html#/food-details/171704/nutrients

ANNEX 2

SSC

Levene's Test of Equality of Error Variances[a]

Dependent Variable: Soluble_solids

F	df1	df2	Sig.
,618	3	92	,605

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Treatment

Levene's Test of Equality of Error Variances[a]

Dependent Variable: Soluble_solids

F	df1	df2	Sig.
,538	7	88	,803

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Time

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for Soluble_solids	,073	96	.200*	,981	96	,194

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

ANOVA TABLE

Tests of Between-Subjects Effects

Dependent Variable: Soluble_solids

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	22.329[a]	31	,720	18,205	,000
Intercept	9394,116	1	9394,116	237431,251	,000
Treatment	19,677	3	6,559	165,777	,000
Time	1,640	7	,234	5,922	,000
Treatment * Time	1,012	21	,048	1,218	,268
Error	2,532	64	,040		
Total	9418,977	96			
Corrected Total	24,861	95			

a. R Squared = .898 (Adjusted R Squared = .849)

pH value

Levene's Test of Equality of Error Variances[a]

Dependent Variable: pH

F	df1	df2	Sig.
,610	3	92	,610

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a. Design: Intercept + Treatment

Levene's Test of Equality of Error Variances[a]

Dependent Variable: pH

F	df1	df2	Sig.
3,528	7	88	,002

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a. Design: Intercept + Time

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for pH	,098	96	,025	,984	96	,314

a. Lilliefors Significance Correction

ANOVA TABLE

Tests of Between-Subjects Effects

Dependent Variable: pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.358[a]	31	,012	30,853	,000
Intercept	571,546	1	571,546	1528367,064	,000
Time	,174	7	,025	66,279	,000
Treatment	,133	3	,044	118,930	,000
Time * Treatment	,051	21	,002	6,461	,000
Error	,024	64	,000		
Total	571,927	96			
Corrected Total	,382	95			

a. R Squared = .937 (Adjusted R Squared = .907)

L*

Levene's Test of Equality of Error Variances[a]

Dependent Variable: L_colour_value

F	df1	df2	Sig.
,633	3	92	,595

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Treatment

Levene's Test of Equality of Error Variances[a]

Dependent Variable: L_colour_value

F	df1	df2	Sig.
7,097	7	88	,000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Time

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for L_colour_value	,131	96	,000	,914	96	,000

a. Lilliefors Significance Correction

ANOVA table

Tests of Between-Subjects Effects

Dependent Variable: L_colour_value

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	162.901[a]	31	5,255	18,074	,000
Intercept	204469,606	1	204469,606	703260,449	,000
Treatment	7,964	3	2,655	9,131	,000
Time	118,871	7	16,982	58,407	,000
Treatment * Time	36,066	21	1,717	5,907	,000
Error	18,608	64	,291		
Total	204651,114	96			
Corrected Total	181,509	95			

a. R Squared = .897 (Adjusted R Squared = .848)

a*

Levene's Test of Equality of Error Variances[a]

Dependent Variable: a_colour_value

F	df1	df2	Sig.
2,789	3	92	,045

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Treatment

Levene's Test of Equality of Error Variances[a]

Dependent Variable: a_colour_value

F	df1	df2	Sig.
1,900	7	88	,079

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Time

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for a_colour_value	,146	96	,000	,945	96	,001

a. Lilliefors Significance Correction

ANOVA TABLE

Tests of Between-Subjects Effects

Dependent Variable: a_colour_value

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	139.738[a]	31	4,508	21,587	,000
Intercept	22586,309	1	22586,309	108164,425	,000
Treatment	84,980	3	28,327	135,654	,000
Time	35,358	7	5,051	24,190	,000
Treatment * Time	19,400	21	,924	4,424	,000
Error	13,364	64	,209		
Total	22739,412	96			
Corrected Total	153,102	95			

a. R Squared = .913 (Adjusted R Squared = .870)

b*

Levene's Test of Equality of Error Variances[a]

Dependent Variable: b_colour_value

F	df1	df2	Sig.
3,809	3	91	,013

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Treatment

Levene's Test of Equality of Error Variances[a]

Dependent Variable: b_colour_value

F	df1	df2	Sig.
1,564	7	87	,157

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Time

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for b_colour_value	,118	95	,002	,960	95	,006

a. Lilliefors Significance Correction

ANOVA table

Tests of Between-Subjects Effects

Dependent Variable: b_colour_value

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	654.535[a]	31	21,114	14,311	,000
Intercept	158781,893	1	158781,893	107618,089	,000
Treatment	108,415	3	36,138	24,494	,000
Time	292,154	7	41,736	28,288	,000
Treatment * Time	271,921	21	12,949	8,776	,000
Error	92,951	63	1,475		
Total	159724,974	95			
Corrected Total	747,486	94			

a. R Squared = .876 (Adjusted R Squared = .814)

TPC

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for tpc	0,028	222	.200*	0,993	222	0,347

Levene's Test of Equality of Error Variances[a,b]					
		Levene Statistic	df1	df2	Sig.
tpc	Based on Mean	0,322	3	218	0,809
	Based on Median	0,209	3	218	0,890
	Based on Median and with adjusted df	0,209	3	197,202	0,890
	Based on trimmed mean	0,280	3	218	0,840

Tests of Between-Subjects Effects						
Dependent Variable:	tpc					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	6937671.920[a]	31	223795,868	21,363	0,000	
Intercept	321594153,811	1	321594153,811	30697,950	0,000	
treatment	303762,078	3	101254,026	9,665	0,000	
time	3114414,211	7	444916,316	42,470	0,000	
treatment * time	3345953,226	21	159331,106	15,209	0,000	
Error	1990455,058	190	10476,079			
Total	375294069,029	222				
Corrected Total	8928126,977	221				

FRAP

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
Antioxidant_activity	Based on Mean	.311	3	207	.817
	Based on Median	.270	3	207	.847
	Based on Median and with adjusted df	.270	3	201.245	.847
	Based on trimmed mean	.297	3	207	.828

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Dependent variable: Antioxidant_activity

b. Design: Intercept + Treatment

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for Antioxidant_activity	.045	211	.200*	.994	211	.572

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Tests of Between-Subjects Effects

Dependent Variable: Antioxidant_activity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5089210.85[a]	31	164168.092	27.664	.000
Intercept	169278838.2	1	169278838.2	28525.013	.000
Treatment	1323539.162	3	441179.721	74.343	.000
Time	3414327.519	7	487761.074	82.192	.000
Treatment * Time	103817.565	21	4943.694	.833	.677
Error	1062257.611	179	5934.400		
Total	184542813.1	211			
Corrected Total	6151468.456	210			

a. R Squared = .827 (Adjusted R Squared = .797)

Rutin

Levene's Test of Equality of Error Variances[a,b]					
		Levene Statistic	df1	df2	Sig.
Rutin	Based on Mean	3,626	7	141	0,001
	Based on Median	1,269	7	141	0,270
	Based on Median and with adjusted df	1,269	7	78,131	0,276
	Based on trimmed mean	3,028	7	141	0,005

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for Rutin	0,132	149	0,000	0,908	149	0,000

ANOVA TABLE

Tests of Between-Subjects Effects

Dependent Variable: Rutin

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.008[a]	31	0,000	44,519	0,000
Intercept	0,049	1	0,049	8880,235	0,000
Time	0,006	7	0,001	165,706	0,000
Treatment	0,000	3	5,529E-05	10,049	0,000
Time * Treatment	0,001	21	6,153E-05	11,182	0,000
Error	0,001	117	5,502E-06		
Total	0,058	149			
Corrected Total	0,008	148			

a. R Squared = .922 (Adjusted R Squared = .901)

Quercetin

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for Quercetin	0,136	173	0,000	0,843	173	0,000

Homovar for time factor

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
Quercetin	Based on Mean	24,335	7	165	0,000
	Based on Median	9,030	7	165	0,000
	Based on Median and with adjusted df	9,030	7	53,478	0,000
	Based on trimmed mean	22,992	7	165	0,000

ANOVA TABLE

Tests of Between-Subjects Effects

Dependent Variable: Quercetin

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.011[a]	31	0,000	7,677	0,000
Intercept	0,643	1	0,643	14245,263	0,000
Time	0,001	7	0,000	2,616	0,014
Treatment	0,006	3	0,002	40,930	0,000
Time * Treatment	0,003	21	0,000	3,294	0,000
Error	0,006	141	4,514E-05		
Total	0,697	173			
Corrected Total	0,017	172			

Dihidroxibenzoic acid

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for Dihidrobenzoicacid	0,053	173	.200*	0,986	173	0,077

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
Dihidro-benzoic acid	Based on Mean	14,741	3	169	0,000
	Based on Median	9,536	3	169	0,000
	Based on Median and with adjusted df	9,536	3	115,078	0,000

ANOVAB TABLE

Tests of Between-Subjects Effects

Dependent Variable: Dihidro-benzoic acid

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.027[a]	31	0,001	22,490	0,000
Intercept	0,210	1	0,210	5421,928	0,000
Time	0,012	7	0,002	43,736	0,000
Treatment	0,004	3	0,001	37,602	0,000
Time * Treatment	0,009	21	0,000	11,529	0,000
Error	0,005	141	3,869E-05		
Total	0,273	173			

Ascorbic acid

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for VitaminC	0,132	96	0,000	0,919	96	0,000

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
Vitamin C	Based on Mean	0,279	3	92	0,841
	Based on Median	0,268	3	92	0,849
	Based on Median and with adjusted df	0,268	3	87,983	0,849
	Based on trimmed mean	0,287	3	92	0,835

ANOVA TABLE

Tests of Between-Subjects Effects

Dependent Variable: Vitamin C

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	717153.379[a]	31	23133,980	105,082	0,000
Intercept	3597809,420	1	3597809,420	16342,341	0,000
Treatment	22844,659	3	7614,886	34,589	0,000
Time	686190,455	7	98027,208	445,269	0,000
Treatment * Time	8118,264	21	386,584	1,756	0,044
Error	14089,768	64	220,153		
Total	4329052,567	96			
Corrected Total	731243,148	95			

a. R Squared = .981 (Adjusted R Squared = .971)