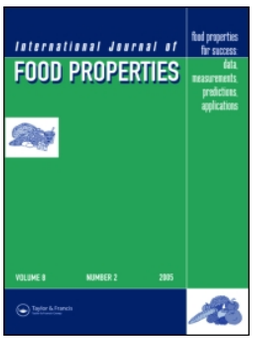

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Bioactive compounds and color of sea buckthorn (*Hippophae rhamnoides* L.) purees as affected by heat treatment and high-pressure homogenization

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ABSTRACT

Bioactive compounds and physical properties of sea buckthorn purees at production and after storage at 2°C and 18°C for 3 months were investigated. Heat treatment of the berries prior to sieving increased yield of the purees from 80% to 84%, and gave purees with 27-65% higher concentrations of total carotenoids, but did not affect vitamin C and total phenolics (TP). High-pressure homogenization (HPH) reduced the size of the particles in the purees, leading to lighter and more yellow purees. Vitamin C, total carotenoids, and TP were not affected by HPH. Storage explained most of the variation in vitamin C, TP, and chromaticity, i.e. they decreased during storage, especially at the highest storage temperature. To obtain higher yield and products with higher concentrations of bioactive compounds, heat treatment prior to further processing is recommended. HPH can be used to alter the physical properties and color of the product.

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Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a hardy, deciduous shrub native to Europe and central Asia. The plants are now cultivated both for soil and water conservation and for utilization of the fruits (berries). The berries are rich in both hydrophilic and lipophilic phytochemicals, including ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids (pro-vitamin A), essential fatty acids. and various phenolic compounds.[1–12] The high contents of these bioactive compounds, shown to have health promotive properties, make sea buckthorn berries very interesting from a health perspective.[13–15] The berries are juicy and have a distinctive flavor and an attractive, bright yellow-orange color, caused by the carotenoids present. Sea buckthorn berries contain both xanthophylls, carotenes, and esterified carotenoids, with the esterified carotenoids being the most abundant, and β-carotene being the most abundant of the free carotenoids.[2,8,12,16] The berries have high contents of organic acids, mainly malic and quinic acids, which are the main contributors to their sour and astringent taste.[17–19] Because of the sour and astringent taste, sea buckthorn berries are very seldom consumed as is but are processed into products such as juices and purees with the addition of sweeteners to balance the taste.[20] Processing and subsequent storage will influence chemical composition and thereby sensory and health-related quality of the berries. Processing leads to disruption of cell structures and initiation of enzymatic and non-enzymatic oxidation of bioactive compounds and other constituents in the berries.[21] Loss of bioactive compounds also occurs when fractions of the berries rich in these compounds, i.e. seeds and skins, are discarded when the berries are pressed or sieved to obtain juices or purees. Yields of juice and release of bioactive compounds into the juice depend on several factors including how the berries are treated prior to pressing and

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the equipment used. Pectinolytic mash treatments and heating of the berries before pressing are shown to increase juice yield.[22,23] However, extensive heating is shown to have negative effects on odor and flavor of sea buckthorn.[24]

In opposition to most fruits and berries, flesh of sea buckthorn berries contains lipids (ca. 3%).[7,9,25] The sea buckthorn juice contains insoluble solids and oil droplets suspended in the aqueous phase.[24] On standing, the juice separates in three phases; an upper floating creamy phase, a center fairy clear phase, and a particulate sediment at the bottom. This phase separation is not desirable and sea buckthorn juice requires stabilization to be acceptable for the consumer. The lipid phase and the sediment at the bottom can be removed by centrifugation or by treatment with carbohydrate hydrolyzing enzymes.[24] However, with these treatments, health-promoting constituents in the berries are lost. Homogenization has been suggested to delay phase separation of the juice.[24] High-pressure homogenization (HPH) has been used to improve the physical stability of emulsions and other plant-based products.[26–28] During homogenization, the material is subjected to mechanical forces, such as shear forces or local pressure fluctuations, leading to disruption of cells and droplets.[29] This leads to the reduction of particle size, which will increase the suspension of the particles, and increase the stability of the product.

While there are numerous papers demonstrating high concentrations of bioactive compounds in sea buckthorn berries, and recently some reports on both *in vivo* and *in vitro* health effects of sea buckthorn[30–32], scientific publications on the effects of processing and storage on bioactive compounds in sea buckthorn are scarce. It is important to understand how different processing factors affect the chemical composition and sensorial quality of sea buckthorn berries to obtain products where the desired properties are preserved also after storage. We wanted to study some factors with supposed effect on bioactive compounds and sensorial quality of sea buckthorn products, i.e. heating of the berries prior to pressing or sieving which may increase yield and extraction of desired compounds, but may also lead to degradation of these compounds, and HPH that will alter physical properties of the products. The aims of this study were thus to determine how 1) heating sea buckthorn berries prior to sieving to obtain puree and 2) HPH of the purees affected bioactive compounds (vitamin C, carotenoids, total phenolics), particle size distribution, and color of the purees at production and after storage at 2°C and 18°C for 3 months.

Materials and methods

Chemicals and reagents

Dehydro-L-(+)-ascorbic acid dimer, β-carotene, tris[2-carboxyethyl]-phosphine (TCEP), butylated hydroxytoluene (BHT), trizmahydrochloride (Tris-HCl) and *meta*-phosphoric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-(+)-ascorbic acid (AA), n-dodecyltrimethylammonium chloride, and disodium-EDTA (Na_2-EDTA) were obtained from Merck KGAa (Darmstadt, Germany). All chemicals and solvents were of analytical or HPLC grade and water was of Milli-Q quality (Millipore Corp., Cork, Ireland).

Fruit material

Berries of sea buckthorn used in the experiments were of the cultivars 'BHi 32415 hona' (LOTTA®), which was selected among seedlings from open pollination of 'BHi 31929′, a plant selected from a native Swedish population of sea buckthorn (*Hippophae rhamnoides* L. ssp *rhamnoides*), and 'BHi 10726′ (SOL®), selected after open pollination of the Russian cultivar 'Omskaja-27′. In both cases, open pollination of female parents was in a cultivar collection at Balsgård, Kristianstad, Sweden, with sea buckthorn of Nordic, Baltic, Russian, and Asian origin.

Ripe berries of LOTTA and SOL were harvested from the experimental field at Njøs Fruit and Berry Center, Leikanger in Western Norway (61° 11" N and 06° 51" E, altitude approx. 20 m a.s.l.) in

September 2014. The berries were frozen in plastic boxes at −20°C within 6 h after harvest and were stored frozen prior to analysis and processing in June 2015.

Berry weight was determined on 20 berries. For determination of distribution of skin, seed, and flesh in the berries, the skins were removed from partially thawed berries with the aid of a razor blade. The berries were cut in two and the seeds were separated manually from the flesh. The values are averages of 20 berries.

Processing and storage

The sea buckthorn berries were processed into purees per the flow chart shown in Figure 1. Frozen berries of each cultivar (4 kg) were rinsed in hot water (85°C for 20–30 s). One portion of the berries was heated at 80°C for 10 min in a steam oven (Electrolux-air-o-steam, Electrolux Professional GmbH, Germany) prior to sieving. The berries were processed into a puree using an automatic sieve (C 80, Robot-Coupe, France) with a medium-mesh screen (1.0 mm). One portion of the puree was subjected to HPH at 1000 bar (100 MPa) in a Panda PLUS 2000 (GEA Mechanical Equipment, GEA Niro Soavi S.p.A., Parma, Italy). All the samples were pasteurized (80°C for 10 s) in a saucepan. The purees were stored in glass jars (ca. 100 g) in the dark at 2°C and 18°C. The purees were analyzed at production and after 3 months of storage. Measurements of color and particle size were performed directly after processing and storage, while samples for other analyses were frozen at −20°C prior to analyses. The samples were analyzed or freeze-dried for carotenoid analyzes within 6 months of frozen storage.

Vitamin C

Vitamin C, comprising L-ascorbic acid (AA) and the oxidized form dehydroascorbic acid (DHAA), was determined with a method previously described.[33] Briefly, ascorbic acids in the samples (5 g) were extracted with 5% *meta*-phosphoric acid containing 1 mM disodium-dihydrogen-EDTA (25 mL). DHAA in the extracts (100 µL) was reduced to AA with 5 mM TCEP, pH 9.0 (50 µL) and diluted with mobile phase adjusted to pH 4.2 (350 µL). Separation and detection of AA in the

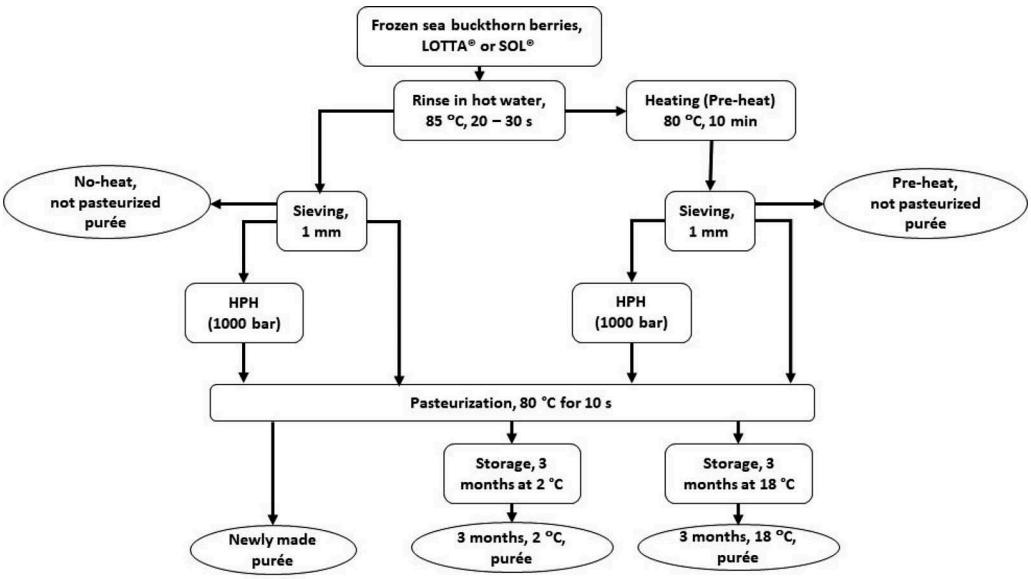

Figure 1. Flow sheet to produce purees of sea buckthorn berries.

extracts (15 µL) and the reduced samples (15 µL) were performed on an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) with a monolithic Chromolith® Performance RP-18e column (100 mm × 4.6 mm i.d., Merck KGaA, Darmstadt, Germany). Mobile phase consisted of 2.5 mM $Na_2H_2PO_4 \square 2H_2O$, 2.5 mM *n*-dodecyltrimethylammonium chloride, 1.0 mM Na_2-EDTA, and 2% acetonitrile, pH 4.7. AA was detected at 264 nm and quantified using external standard with a calibration curve. DHAA was calculated by subtracting the AA concentration from the total ascorbic acid concentration determined after reduction of the DHAA present. The contents of ascorbic acids were expressed as milligrams per 100 g of fresh weight (fw). The samples were extracted and analyzed in duplicate.

Carotenoids

Purees and berries were freeze-dried for 3 days (Gamma 1–16, Christ GmbH, Osterode am Harz, Germany). Seeds from the freeze-dried berries were removed. The freeze-dried purees and berry soft parts were milled in a mortar. Milled samples (100 mg) were extracted with hexane/acetone/ethanol (2:1:1, v/v/v) (2 mL) containing 0.1% BHT by shaking (350 rpm) at 4°C for 30 min (Incubator Shaker, Innova 40, New Brunswick Scientific, Edison, New Jersey, USA). The extracts were centrifugated (3000 *g* at 4°C for 10 min, Heraeus Multifuge 4Kr centrifuge, Thermo Electron Corporation, Germany) and the supernatants collected. The insoluble plant materials were re-extracted with the extraction solvent (2 mL). The solvent was removed from pooled extracts by a nitrogen flow at 37°C (Labconco Rapidvap Vertex Evaporator, Kansas City, MO, USA) and the samples were dissolved in 5 mL THF/methanol (1:4, v/v) containing 0.1% BHT.

Filtered samples (15 µL) were analyzed on an Agilent 1290 UPLC system (Agilent Technologies) equipped with a diode array detector. Separation was performed on an Acquity UPLC RP18 column (1.7 µm particle size, 100 mm x 2.1 mm i.d, Waters, Milford, MA, USA) with mobile phases consisting of A; acetonitrile:methanol:0.1 M tris-HCl pH 8.0 (72:8:3, v/v/v) and B; methanol:ethyl acetate (68:32, v/v) and the following gradient: 0% B for 3 min, 0–100% B for 5 min, 100% B for 3 min, 100–0%B for 0.5 min and 0% B for 3.5 min. The solvent flow rate was 0.3 mL/min. Identification of β-carotene was done by spiking and comparing UV-Vis spectra with authentic standard. Carotenoids eluting earlier and later than β-carotene (6.8 min) were assigned as free and esterified carotenoids, respectively, based on elution profiles of carotenoids in sea buckthorn previously published.[2,8] The carotenoids were detected at 452 nm and quantified as β-carotene equivalents by external standard curves of β-carotene. Concentrations of β-carotene, total esterified carotenoids, and total carotenoids were given as equivalents of β-carotene and expressed as µg per g of dry weight (dw). The samples were extracted and analyzed in duplicate.

Total phenolics (TP)

TP was determined in methanolic extracts of the samples. Purees (5 g) or homogenized berries (10 g) were added methanol (10 or 20 mL) and homogenized at 28 000 rpm for 30 s (Ultra Turrax, PT3100 Polytron, IL, USA). The mixture was centrifuged at 4°C for 10 min at 39200 *g* (Avanti J-26 XP, Beckman Coulter Inc., USA) and the supernatant collected. The pellet was re-extracted with 70% methanol in water (10 or 20 mL). The supernatants were combined, and 70% methanol added to total volume 25 or 50 mL (concentration 0.2 g fw/mL).

TP was determined using the Folin–Ciocalteu colorimetric method as previously described.[33] Appropriately diluted extracts (0.2 mL) were mixed with Folin–Ciocalteu reagent (1.0 mL) and incubated for 3 min before the addition of 7.5% sodium carbonate (0.8 mL). The mixture was incubated for 30 min at 20–22°C in the dark before absorbance was measured at 765 nm (Agilent 8453 spectrophotometer, Agilent Technologies). TP content was expressed as gallic acid equivalents (GAE) in mg per 100 g fw. All samples were extracted and analyzed in duplicate.

Color

Surface color was measured using a digital color imaging system (DigiEye, VeriVide Ltd., Leicester, UK). The samples were mixed and transferred to plastic cups and placed in a light-box with standardized daylight (CIE D65) with diffuse lighting and photographed with a calibrated digital camera (Nikon D7000, 35 mm lens, Nikon Corp., Japan). Color measurements of the pictures were made in the CIE color (L*a*b* values) with DigiPix software (version 2.63). L* describes lightness. Hue angle (arctan (b*/a*)) designates color shade where low values (Hue = 0°) indicates a red-bluish color and high values (Hue = 90°) a yellow color. Chroma $((a^{*2} + b^{*2})^{1/2})$ shows transition from gray (low values) to pure color (high values).

Particle size distribution

The particle size distribution of the purees was measured using a laser diffraction sensor, HELOS/KR, with the wet dispersing system, QUIXEL, for liquid samples (Sympatec GmbH, Clausthal-Zellerfeld, Germany). The software WINDOX 5 (Release 8.2.1) was used for data recording. The samples were dispersed in water at 30°C after sonication for 30 s and 10 s pause. The optic concentration was 18–26% (ca. 10 drops of sample in 250 mL water). The measurements were done in the range 0.5–1750 µm. Particle size analyses were done four times for each sample. The particle diameters (µm) for which 10%, 50%, and 90% of the particles in the samples had diameters less than these diameters, were designated d(0.1), d(0.5), and d(0.9), respectively.

Statistical analysis

Statistical analysis was performed using Minitab® Statistical Software (version 18, Minitab Ltd., Coventry, UK). Analysis of variance (ANOVA), a general linear model, was performed to determine the effects of the experimental factors Cultivar, Pre-Heat, HPH and Storage, and their two-factor interactions. Explained variances of the experimental factors were calculated as the sum-of-squares as % of total sum-of squares.

Results and discussion

Puree yield

Sea buckthorn berries were processed into purees by sieving. The purees were liquid, as juice, but had high contents of suspended solids and were opaque. The puree yields were the same for LOTTA and SOL, i.e. 80% for non-heated berries and 84% for pre-heated berries. The residues were mainly seeds and skin, in line with the distribution in the berries. The berries contained on average about 5%, 11%, and 84% of seeds, skin, and flesh, respectively (Table 1). The seed contents were in

Table 1. Berry weight, berry distribution (seed, skin, flesh), total ascorbic acid (Vitamin C), β-carotene, total carotenoids, and total phenolics (TP) in berries of LOTTA and SOL[a].

	LOTTA	SOL
Berry weight (mg)	507 ± 88	528 ± 99
Seed (%)	4.5	4.9
Skin (%)	11.3	10.6
Flesh (%)	84.2	84.5
Vitamin C (mg/100 g fw)	74 ± 9	102 ± 3
β-carotene (µg/g dw)	116 ± 2	104 ± 27
Total carotenoids (µg/g dw)	945 ± 25	826 ± 112
TP (mg GEA/100 g fw)	137 ± 0	195 ± 1

[a]All values are means ± standard deviation of two samples, except for berry weight (n = 20) and weight distribution, which is the average of 20 berries.

accordance with reports on sea buckthorn berries containing 4–10% seeds[9,25], while higher weight percentage of seeds (23%) and lower weight percentage of skins (8%) were found in another study.[34] Juice yield obtained by conventional pressing was found to be only 56–68% for non-enzymed berries but increased to 70–80% after enzymatic treatment of the berry mash.[22] In another study, juice yields after pressing were in the range 65 – 95%, depending on pressing techniques.[23] The highest yield was obtained, as in our study, when the berries were heated prior to pressing.

Vitamin C

Total ascorbic acid (vitamin C) contents in berries of LOTTA and SOL were 74 and 102 mg/100 g fw, respectively (Table 1). These numbers are in accordance with previous studies of sea buckthorn spp. *rhamnoides* and *mongolica*.[7,11,17] The concentrations of vitamin C in newly made purees of LOTTA and SOL were like that in berries, that is, about 60 and 100 mg/100 g fw, respectively (Figure 2), illustrating that the greater part of the water-soluble vitamin C was preserved in the purees. The concentrations of DHAA in newly made purees were low, and DHAA contributed on average 4.3% to vitamin C contents in newly made purees. Pre-heating of the berries had significant, but minor influence on the vitamin C contents in the purees with 0.8% explained variance (Table 2). AA and total ascorbic acid concentrations in purees made of pre-heated berries were on average 9% lower than in purees made of non-heated berries (Figure 2). HPH had no effect on vitamin C contents in the purees. Neither had pasteurization

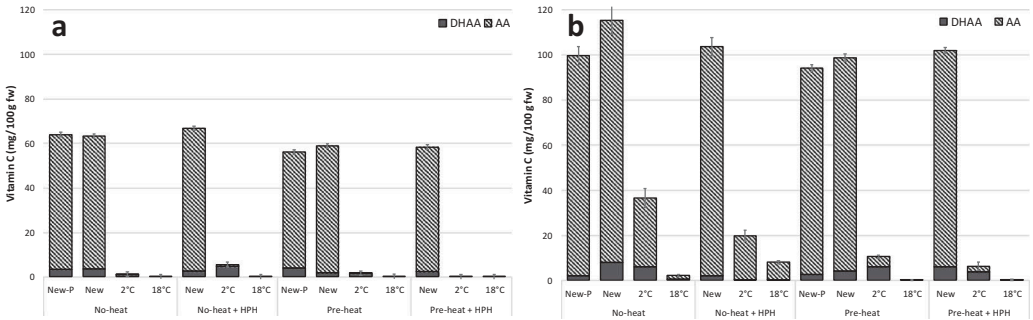

Figure 2. Vitamin C, i.e. L-(+)-dehydroascorbic acid (DHAA), and L-(+)-ascorbic acid (AA), in purees of LOTTA (a) and SOL (b) at production (New) and after storage for 3 months at 2°C and 18°C. New-P; newly made puree prior to pasteurization. The standard deviations (bars) are of total ascorbic acid (vitamin C) (n = 2).

Table 2. ANOVA[a] of L-(+)-ascorbic acid (AA), dehydro-L-(+)-ascorbic acid (DHAA), total ascorbic acid (Vitamin C), β-carotene, total carotenoids, total phenolics (TP), and color parameters (L*, °Hue, Chroma) in sea buckthorn purees. The numbers are explained variances (%) and the significant levels are represented as stars[b].

Source	DF[c]	AA	DHAA	Vitamin C	β-carotene	Total carotenoids	TP	L	°Hue	Chroma
Cultivar	1	6.2***	10.9	6.8***	3.8*	12.1*	6.3***	0.4**	0.0	2.9**
Pre-heating	1	0.8**	0.2	0.8**	65.2***	58.6***	0.1	4.0***	2.1***	0.2
HPH	1	0.0	3.8	0.0	3.8*	1.3	0.1	58.9***	94.6***	9.4***
Storage	2	87.3***	43.0*	86.5***	3.8*	6.0	90.5***	32.1***	0.0	77.1**
Cultivar x Pre-heating	1	0.4*	2.9	0.3	11.8***	5.0	0.1	1.2***	2.0***	0.0
Cultivar x HPH	1	0.0	6.2	0.1	0.8	4.4	0.2	0.4**	0.5***	0.5
Cultivar x Storage	2	4.4***	4.7	4.6***	4.4*	0.5	1.9**	1.2***	0.0	2.3*
Pre-heating x HPH	1	0.0	2.0	0.0	1.2	0.4	0.0	0.0	0.0	2.9**
Pre-heating x Storage	2	0.2	0.1	0.2	1.0	0.6	0.0	0.0	0.1	0.0
HPH x Storage	2	0.1	1.3	0.1	0.9	0.2	0.4	1.5***	0.5***	3.4**
Residual (Error)	2	0.1	3.1	0.1	3.3	11.0	0.0	0.0	0.0	0.0
R^2 (adjusted)		0.99	0.64	0.98	0.92	0.72	1.00	1.00	1.00	0.99

[a]Analysis of variance; main effects and their two-factor interactions. The factors were Cultivar (LOTTA or SOL), Pre-heating (No or Yes), HPH (No or Yes) and Storage (Newly made, 3 months at 2°C or 3 months at 18°C). [b]Explained variances are the sum-of-squares as % of total sum-of squares. Significant levels: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. [c]Degrees of freedom.

(New-P vs. New in Figure 2). These results are in accordance with previous studies, showing low losses of vitamin C even during industrial juice production, confirming that vitamin C in sea buckthorn is stable during processing in opposition to other berry species, such as strawberries.[23,35] The factors explaining most of the variations in vitamin C contents in the purees were Storage (86.5%), Cultivar (6.8%), and the Cultivar x Storage interaction (4.6%) (Table 2). After 3 months of storage, hardly any vitamin C was left in purees made of LOTTA, while on average 17.8% and 2.5% of vitamin C was left in purees made of SOL, stored at 2°C and 18°C, respectively (Figure 2). Ascorbic acid is an antioxidant and several factors such as oxygen concentration, metal catalysts, and temperature play a role in its degradation.[36] In a study of a model juice, AA degradation was found to follow first-order kinetic when the initial oxygen headspace concentration was higher than 0.63%.[37] Increasing oxygen concentration gave fast, exponential degradation of AA, and when O_2 was 20.9% (air), AA was almost completely broken down after 10 days at 22°C. In the current study, the initial oxygen headspace concentration was 20.9% and it is likely that the high oxygen availability was the main cause of the fast decay of ascorbic acid during storage of the purees. To our knowledge, there are no reports on the preservation of vitamin C and other bioactive compounds in sea buckthorn products during extended storage. In a short time storage experiment, it was shown that the vitamin C content already had decreased ca. 20% after 7 days storage at ambient temperature.[35] When the juice was stored cool (6°C), the loss was 11–12%.

Carotenoids

β-Carotene was the most abundant carotenoid in the sea buckthorn samples in the current study, in accordance with previous findings.[2,8,11,12] Berries of LOTTA and SOL contained 116 and 104 μg/g dw of β-carotene and 945 μg/g dw and 826 μg/g dw of total carotenoids, respectively (Table 1). Dry matter of the berry soft parts and the purees was about 14.5%. The levels of β-carotene and total carotenoids were in the ranges previously reported for sea buckthorn.[2,8,11] Varying with cultivar and treatment, 32–73% of β-carotene and 54–100% of total carotenoids present in the berries were recovered in the newly made purees. Pre-heating of the berries prior to sieving was the experimental factor influencing the concentration of β-carotene and total carotenoids in the purees the most, with 65% and 59% explained variance, respectively (Table 2). It was also a significant Cultivar x Pre-heating interaction, i.e. pre-heating had more effect on the release of carotenoids from SOL than of LOTTA. This could be due to differences in the physical properties of the berries. The berries had the same distribution of seeds, skins, and flesh (Table 1), but the berries of LOTTA were more fragile than SOL (observation). Compared with purees made from not-heated berries, pre-heating gave in average 24% and 114% increase in β-carotene in newly made purees of LOTTA and SOL, respectively (Figure 3a and b), while total carotenoid content had increased 27% and 65% in purees of LOTTA and SOL, respectively (Figure 3c and d). Also previously it was found that the carotenoid content was higher in juice made of heated berries.[23] The heat treatment supports the release of carotenoids from the soft part of the berries, probably mainly due to increased liberation of carotenoids from the skin. The skin of the sea buckthorn berries had a darker orange color than the flesh (Picture in Supplementary Material). However, the concentration of carotenoids in different parts of berries was not measured, and as far as we know, carotenoids are previously only been determined in the seeds and/or in flesh and skin combined, not in the skin alone. HPH had a minor effect on β-carotene in the purees. Similar to our results, HPH did not have any effect on total β-carotene in carrot purees[38] or on lycopene and β-carotene in tomato puree.[39] Storage affected β-carotene in LOTTA negatively, while total carotenoids were not significantly affected by neither HPH nor storage. In average, 78% and 69% of carotenoids in the not-heated and pre-heated samples were esterified, respectively. There was no change in this proportion during storage. The high stability of carotenoids in sea buckthorn purees during storage may be explained by several factors. First, the purees were pasteurized, thus degrading enzymes affecting the stability of carotenoids (mainly lipoxygenase) are supposed to be inactivated. Second, the purees were stored in the dark, and illumination are known to greatly accelerate the degradation of carotenoids.[40,41] Furthermore,

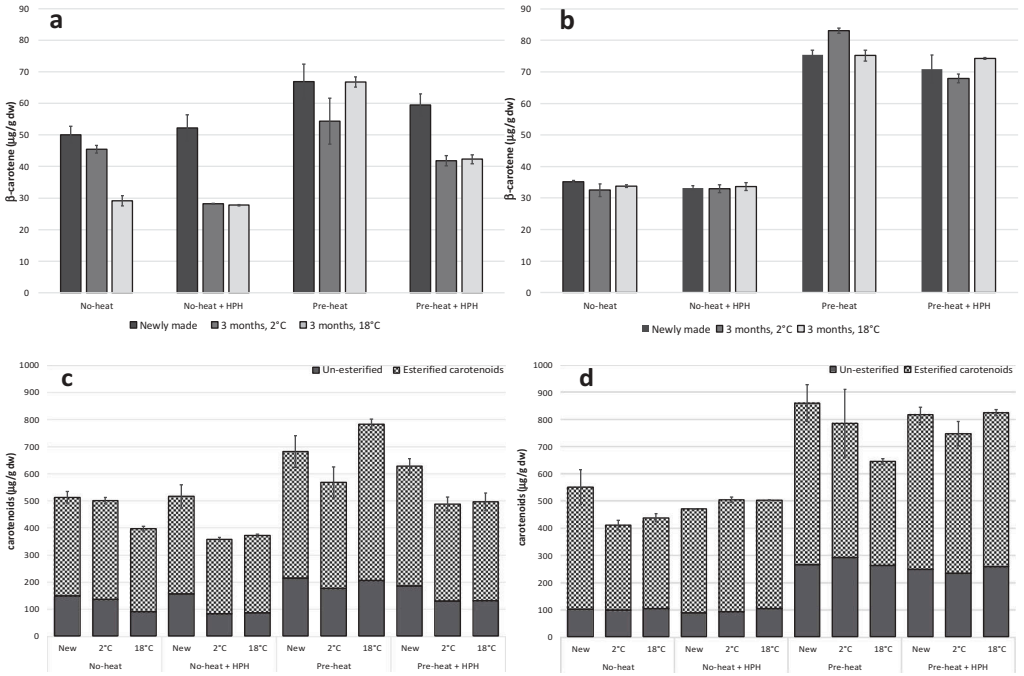

Figure 3. Carotenoids in the purees at production (New) and after storage for 3 months at 2°C and 18°C; β-carotene in LOTTA (a) and SOL (b), un-esterified and esterified carotenoids in LOTTA (c) and SOL (d). The standard deviations (bars) are of total carotenoids (n = 2).

ascorbic acid present in the sea buckthorn berries may have acted as an antioxidant against oxidation of carotenoids.[42] Other constituents in the purees may also exert protective effects, as shown for β-carotene in carrot juice.[41] In line with our results, no degradation of carotenes was found in pumpkin puree after storage in the dark for 180 days[43] and in a study of storage stability of bioactive compounds in a beverage of rosehip and sea buckthorn, the carotenoid levels were not changed after 35 days storage at 4°C.[44]

Total phenolics (TP)

TP contents in berries of LOTTA (137 mg GAE/100 g fw) and SOL (195 mg GAE/100 g fw) (Table 1) were in the same range as found in sea buckthorn grown in the Slovak republic (70–362 mg GAE/100 g/fw)[12] and in Hungary (186–381 mg GAE/100 g fw)[45], but much lower than found in some other studies, e.g. TP in wild sea buckthorn from trans-Himalaya was reported to be from 1000 to 10 000 mg GEA/100 g.[46] TP measured with the Folin-Ciocalteu's assay is a measure of all reducing compounds in a sample, not only the phenolic compounds, and is as such a measure of total antioxidant activity of the sample.[47] In accordance with this, it was found that ascorbic acid (vitamin C) was the major antioxidant in sea buckthorn juice, contributing approximately 75% to total antioxidant activity of the juice.[48] Carotenoids were shown to have no reducing ability when measured in an assay with similar principles as the Folin-Ciocalteu's assay.[47,49] Vitamin C, present in high concentrations in berries and newly made purees in the current study, is thus expected to be the main contributor to TP in these samples. TP was higher in SOL than in LOTTA, which could be explained by the higher concentration of vitamin C in SOL. Storage was the factor influencing TP the most with 90.5% explained variance (Table 2), which is in accordance with the decrease in vitamin C during storage. TP had decreased about 30% and 50% after 3 months of storage at 2°C and 18°C, respectively (Figure 4). In line with the high stability of vitamin C during processing, TP was not influenced by pre-heating, HPH, or pasteurization.

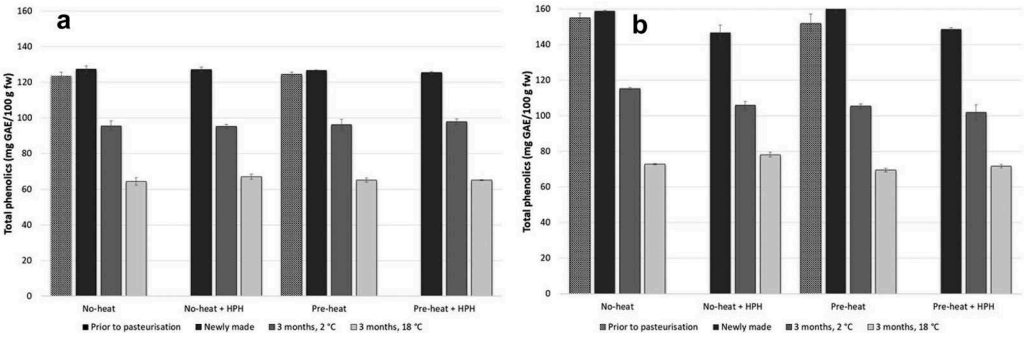

Figure 4. Total phenolics in the purees of LOTTA (a) and SOL (b) at production and after storage.

Particle size distribution

As illustrated in Figure 5, particle size distribution in the purees changed with processing. Not HPH treated purees made of not pre-heated berries (No-heat), had a bi-modal particle size profile, with one peak with particles size below 50 μm and second peak consisting of particles with about 500 μm diameter. In total, 90% of the particles in the purees of LOTTA and SOL had diameters below 572 and 515 μm, respectively (Table 3). In purees made of pre-heated berries (Pre-heat), this second peak was reduced and 90% of the particles now had a particle size below ca. 250 μm. The reason for this might be that some of the bigger particles in sea buckthorn were solubilized during processing of the heat-treated berries. After HPH, the second peak was considerably reduced and the average particle size in the purees were reduced, especially in purees made of not pre-heated berries. The decrease in particle size with pressure, due to mechanical forces is anticipated[29] and are previously found after HPH of for example tomato pulp[27], tomato juice[28], tomato and pepper emulsions,[50] and rosehip nectar.[51] The size of particles, or droplets, is important, as it can determine the bioaccessibility of bioactive compounds, in addition to stability, viscosity, color, and possibly taste of the product.[29] It was shown that reduced size of particles in orange juice lead to increased bioaccessibility of carotenoids[52] and that HPH of carrot products increased bioaccessibility of β-carotene measured with *in vitro* assays.[26,38] Decreased bioaccessibility of carotenoids after HPH has, however, also been found, as HPH attributes to the fiber network and increases the viscosity of the product.[27]

Color

Light absorbance and light scattering are the two phenomena contributing to color of a material.[53] In the purees of sea buckthorn, carotenoids are the pigments absorbing light, while variation in particle

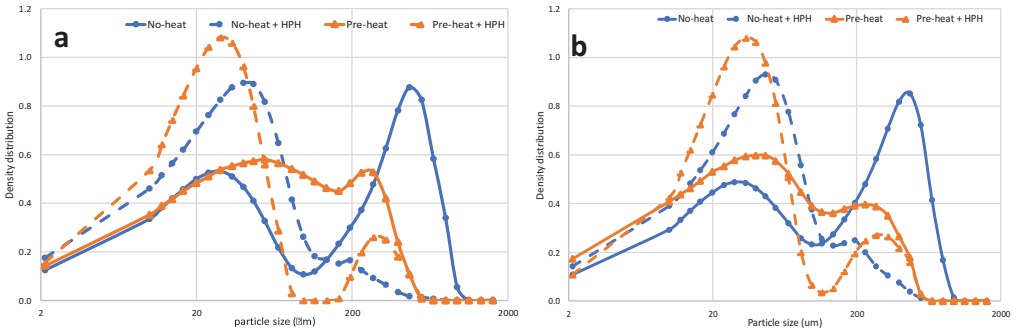

Figure 5. Density distribution of particles in newly made purees of LOTTA (a) and SOL (b). The curves are average of four measurements.

Table 3. Particle size distribution of newly made sea buckthorn purees[a].

Sample		*d*(0.1) (µm)	*d*(0.5) (µm)	*d*(0.9) (µm)
No-heat	LOTTA	6.0 ± 0.4	52.4 ± 2.2	572 ± 6
	SOL	7.0 ± 0.5	73.0 ± 1.5	515 ± 6
No-heat + HPH	LOTTA	4.4 ± 0.5	26.4 ± 3.7	92 ± 20
	SOL	5.2 ± 0.2	32.8 ± 1.8	124 ± 20
Pre-heat	LOTTA	5.2 ± 0.1	42.9 ± 0.5	253 ± 5
	SOL	4.4 ± 0.1	33.3 ± 0.8	242 ± 2
Pre-heat + HPH	LOTTA	4.9 ± 0.1	22.8 ± 0.2	69 ± 2
	SOL	6.9 ± 0.2	29.3 ± 1.1	187 ± 35

[a]*d*(0.1), *d*(0.5), and *d*(0.9) are the particle diameters (µm) for which 10%, 50%, and 90% of the particles in the sample had diameters less than these diameters. Means ± standard deviations of four measurements.

size leads to differences in light scattering. HPH was the factor influencing L* and °Hue of the purees the most, with 59% and 95% explained variance, respectively (Table 2). L* and °Hue increased after HPH (Figure 6a-d), i.e. the purees became lighter and more yellow, which is also illustrated in the pictures (Supplementary Material). HPH reduced the size of the particles in the purees. Smaller particles have larger particle surfaces which increase light scattering, resulting in a lighter color.[53] Also in tomato juice and rosehip nectar increases in L* after HPH were found.[28,51] However, in these juices, in opposition to the current study on sea buckthorn, a* increased and °Hue was unaffected by HPH. The increase in a* was explained by the release of lycopene, the main carotenoid present, from the cells when the cells, membranes, and the chromoplast carotenoid-protein complexes were disrupted by HPH. The reason for the different color hue when homogenizing sea buckthorn might be that other carotenoids than lycopene are responsible for the color of sea buckthorn, but the explanation may also be that the higher fat content in sea buckthorn (ca. 3%) than in tomato juice and rosehip nectar (ca. 0.2%), resulted in an oil-in-water emulsion when homogenized. In line with our results, L* and b*increased and a* decreased after homogenization of diluted avocado puree containing 6% fat.[54] In the sea buckthorn puree, Chroma was influenced by HPH (9.4% explained variance), but Storage was the factor influencing Chroma the most (77% explained variance) (Table 2). There were minor changes in color of the purees stored at 2°C for 3 months. Storage at 18°C, on the other hand, resulted in decrease in L* and Chroma values, i.e. darker and duller colors of the purees (Figure 6). The reduction in Chroma is most probably due to the degradation of carotenoids. There were small but significant effects of the HPH x Storage interaction on the color parameters, that is, the color was better preserved in HPH purees, e.g. after 3 months at 18°C, there were in average 16% and 25% decrease in Chroma of HPH and non-HPH purees, respectively. The higher carotenoid content in purees made of preheated berries was reflected in a decrease in L* and a small decrease in Hue* for purees made of SOL. Cultivar explained less than 3% of the variation in color of the purees (Table 2). That is, overall there were minor differences in color of the purees made of LOTTA and SOL. Of the two factors contributing to the color of the purees, the particle size was found to be the most determinantal for L* and °Hue, while carotenoid concentration and composition might be the most important for Chroma.

Conclusion

Heat treatment of sea buckthorn berries prior to sieving gave higher yield of the purees and purees with higher concentrations of carotenoids, and lower L* and Hue, but did not affect vitamin C and TP. HPH reduced the size of the particles in the purees leading to lighter and more yellow purees. Vitamin C, total carotenoids, and TP were not affected by HPH. Storage explained most of the variation in vitamin C, TP, and Chroma, that is, vitamin C, TP, and Chroma decreased during storage, especially at the highest storage temperature. To obtain higher yield and products with higher concentrations of bioactive compounds, heat treatment prior to further processing is recommended. HPH can be used to alter the physical properties and color of the product.

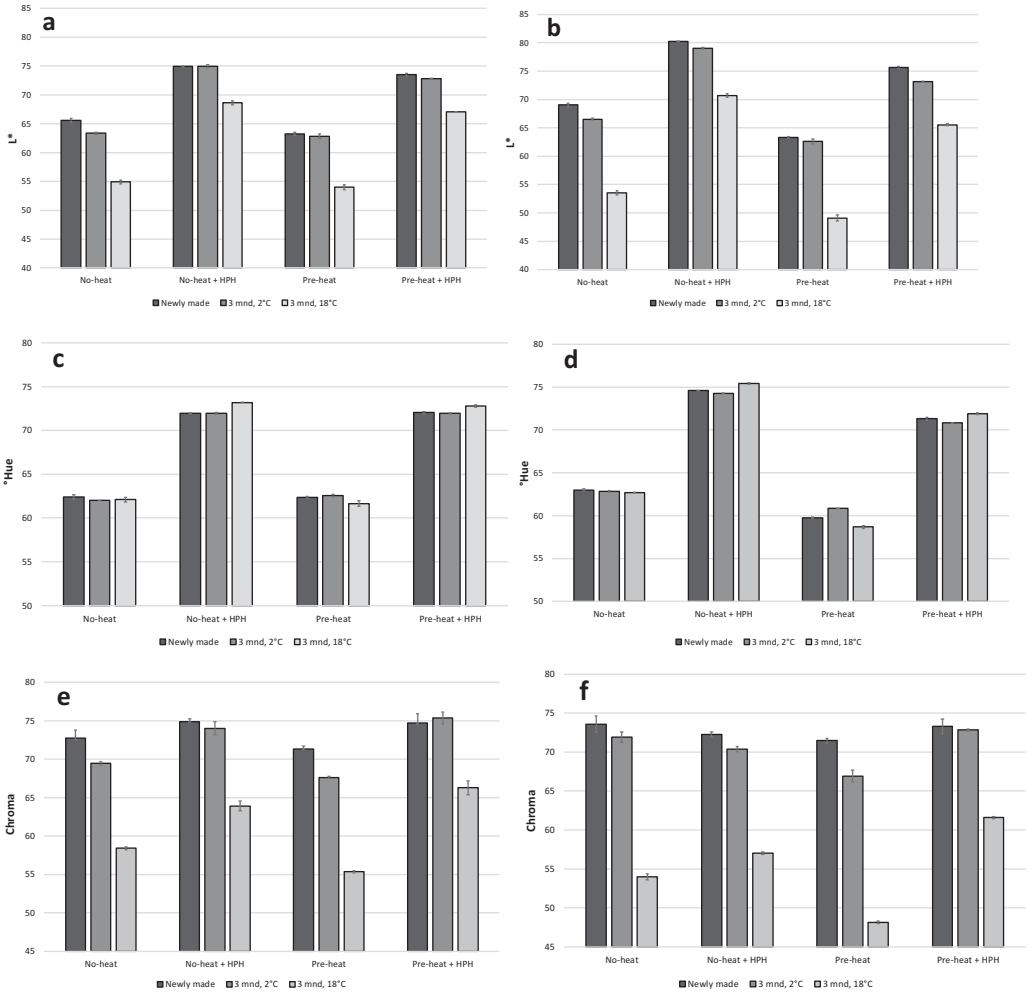

Figure 6. Color of the purees at production and after storage; L* of LOTTA (a) and SOL (b), °Hue of LOTTA (c) and SOL (d) and Chroma of LOTTA (e) and SOL (f).

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Conflicts of interest

There are no conflicts of interest to declare.

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References

[1] Andersson, S. C.; Rumpunen, K.; Johansson, E.; Olsson, M. E. Tocopherols and Tocotrienols in Sea Buckthorn (*Hippophae Rhamnoides* L.) Berries during Ripening. *J. Agric. Food Chem.* **2008**, *56*(15), 6701–6706. DOI: 10.1021/jf800734v.

[2] Andersson, S. C.; Olsson, M. E.; Johansson, E.; Rumpunen, K. Carotenoids in Sea Buckthorn (*Hippophae Rhamnoides* L.) Berries during Ripening and Use of Pheophytin a as a Maturity Marker. *J. Agric. Food Chem.* **2009**, *57*(1), 250–258. DOI: 10.1021/jf802599f.

[3] Yang, B.; Halttunen, T.; Raimo, O.; Price, K.; Kallio, H. Flavonol Glycosides in Wild and Cultivated Berries of Three Major Subspecies of *Hippophaë Rhamnoides* and Changes during Harvesting Period. *Food Chem.* **2009**, *115*(2), 657–664. DOI: 10.1016/j.foodchem.2008.12.073.

[4] Yang, W.; Laaksonen, O.; Kallio, H.; Yang, B. Proanthocyanidins in Sea Buckthorn (*Hippophaë Rhamnoides* L.) Berries of Different Origins with Special Reference to the Influence of Genetic Background and Growth Location. *J. Agric. Food Chem.* **2016**, *64*(6), 1274–1282. DOI: 10.1021/acs.jafc.5b05718.

[5] Arimboor, R.; Kumar, K. S.; Arumughan, C. Simultaneous Estimation of Phenolic Acids in Sea Buckthorn (*Hippophae Rhamnoides*) Using RP-HPLC with DAD. *J. Pharm. Biomed. Anal.* **2008**, *47*(1), 31–38. DOI: 10.1016/j.jpba.2007.11.045.

[6] Kallio, H.; Yang, B. R.; Peippo, P. Effects of Different Origins and Harvesting Time on Vitamin C, Tocopherols, and Tocotrienols in Sea Buckthorn (*Hippophaë Rhamnoides*) Berries. *J. Agric. Food Chem.* **2002**, *50*(21), 6136–6142. DOI: 10.1021/jf020421v.

[7] Tiitinen, K. M.; Hakala, M. A.; Kallio, H. P. Quality Components of Sea Buckthorn (*Hippophaë Rhamnoides*) Varieties. *J. Agric. Food Chem.* **2005**, *53*(5), 1692–1699. DOI: 10.1021/jf0484125.

[8] Pop, R. M.; Weesepoel, Y.; Socaciu, C.; Pintea, A.; Vincken, J.-P.; Gruppen, H. Carotenoid Composition of Berries and Leaves from Six Romanian Sea Buckthorn (*Hippophae Rhamnoides* L.) Varieties. *Food Chem.* **2014**, *147*, 1–9. DOI: 10.1016/j.foodchem.2013.09.083.

[9] Yang, B.; Kallio, H. Fatty Acid Composition of Lipids in Sea Buckthorn (*Hippophaë Rhamnoides* L.) Berries of Different Origins. *J. Agric. Food Chem.* **2001**, *49*(4), 1939–1947. DOI: 10.1021/jf001059s.

[10] Yang, B.; Kallio, H. Composition and Physiological Effects of Sea Buckthorn (*Hippophaë*) Lipids. *Trends Food Sci. Technol.* **2002**, *13*(5), 160–167. DOI: 10.1016/S0924-2244(02)00136-X.

[11] Teleszko, M.; Wojdylo, A.; Rudzinska, M.; Oszmianski, J.; Golis, T. Analysis of Lipophilic and Hydrophilic Bioactive Compounds Content in Sea Buckthorn (*Hippophae Rhamnoides* L.) Berries. *J. Agric. Food Chem.* **2015**, *63*(16), 4120–4129. DOI: 10.1021/acs.jafc.5b00564.

[12] Sytarova, I.; Orsavova, J.; Snopek, L.; Mlcek, J.; Byczynski, L.; Misurcova, L. Impact of Phenolic Compounds and Vitamins C and E on Antioxidant Activity of Sea Buckthorn (Hippophae Rhamnoides L.) Berries and Leaves of Diverse Ripening Times. *Food Chem.* **2020**, *310*. DOI: 10.1016/j.foodchem.2019.125784.

[13] Olas, B. Sea Buckthorn as a Source of Important Bioactive Compounds in Cardiovascular Diseases. *Food Chem. Toxicol.* **2016**, *97*, 199–204. DOI: 10.1016/j.fct.2016.09.008.

[14] Suryakumar, G.; Gupta, A. Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.). *J. Ethnopharm.* **2011**, *138*(2), 268–278. DOI: 10.1016/j.jep.2011.09.024.

[15] Guo, X. F.; Yang, B.; Cai, W. W.; Li, D. Effect of Sea Buckthorn (*Hippophae Rhamnoides* L.) On Blood Lipid Profiles: A Systematic Review and Meta-analysis from 11 Independent Randomized Controlled Trials. *Trends Food Sci. Technol.* **2017**, *61*, 1–10. DOI: 10.1016/j.tifs.2016.11.007.

[16] Giuffrida, D.; Pintea, A.; Dugo, P.; Torre, G.; Pop, R. M.; Mondello, L. Determination of Carotenoids and Their Esters in Fruits of Sea Buckthorn (*Hippophae Rhamnoides* L.) By HPLC-DAD-APCI-MS. *Phytochem. Anal.* **2012**, *23*(3), 267–273. DOI: 10.1002/pca.1353.

[17] Zheng, J.; Yang, B.; Trepanier, M.; Kallio, H. Effects of Genotype, Latitude, and Weather Conditions on the Composition of Sugars, Sugar Alcohols, Fruit Acids, and Ascorbic Acid in Sea Buckthorn (*Hippophaë Rhamnoides* Ssp. *Mongolica*) Berry Juice. *J. Agric. Food Chem.* **2012**, *60*(12), 3180–3189. DOI: 10.1021/jf204577g.

[18] Yang, B.;. Sugars, Acids, Ethyl beta-D-glucopyranose and a Methyl Inositol in Sea Buckthorn (*Hippophaë Rhamnoides*) Berries. *Food Chem.* **2009**, *112*(1), 89–97. DOI: 10.1016/j.foodchem.2008.05.042.

[19] Ma, X. Y.; Yang, W.; Laaksonen, O.; Nylander, M.; Kallio, H.; Yang, B. R. Role of Flavonols and Proanthocyanidins in the Sensory Quality of Sea Buckthorn (*Hippophaë Rhamnoides* L.) Berries. *J. Agric. Food Chem.* **2017**, *65*(45), 9872–9880. DOI: 10.1021/acs.jafc.7b04156.

[20] Geertsen, J. L.; Allesen-Holm, B. H.; Giacalone, D. Consumer-led Development of Novel Sea-buckthorn Based Beverages. *J. Sens. Stud.* **2016**, *31*(3), 245–255. DOI: 10.1111/joss.12207.

[21] Terefe, N. S.; Buckow, R.; Versteeg, C. Quality-related Enzymes in Fruit and Vegetable Products: Effects of Novel Food Processing Technologies, Part 1: High-pressure Processing. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*(1), 24–63. DOI: 10.1080/10408398.2011.566946.

[22] Beveridge, T.; Harrison, J. E.; Drover, J. Processing Effects on the Composition of Sea Buckthorn Juice from *Hippophae Rhamnoides* L. Cv. Indian Summer. *J. Agric. Food Chem.* **2002**, *50*(1), 113–116. DOI: 10.1021/jf010369n.
[23] Seglina, D.; Karklina, D.; Ruisa, S.; Krasnova, I. The Effect of Processing on the Composition of Sea Buckthorn Juice. *J. Fruit Ornamental Plant Res.* **2006**, *14*(Suppl. 2), 257–264.
[24] Beveridge, T.; Li, T. S. C.; Oomah, B. D.; Smith, A. Sea Buckthorn Products: Manufacture and Composition. *J. Agric. Food Chem.* **1999**, *47*(9), 3480–3488. DOI: 10.1021/jf981331m.
[25] Ranjith, A.; Kumar, K. S.; Venugopalan, V. V.; Arumughan, C.; Sawhney, R. C.; Singh, V. Fatty Acids, Tocols, and Carotenoids in Pulp Oil of Three Sea Buckthorn Species (*Hippophae Rhamnoides, H-salicifolia*, and *H-tibetana*) Grown in the Indian Himalayas. *J. Am. Oil Chem. Soc.* **2006**, *83*(4), 359–364. DOI: 10.1007/s11746-006-1213-z.
[26] Liu, J. N.; Bi, J. F.; Liu, X.; Zhang, B. Q.; Wu, X. Y.; Wellala, C. K. D.; Zhang, B. Effects of High Pressure Homogenization and Addition of Oil on the Carotenoid Bioaccessibility of Carrot Juice. *Food Funct.* **2019**, *10*(1), 458–468. DOI: 10.1039/C8FO01925H.
[27] Panozzo, A.; Lemmens, L.; Van Loey, A.; Manzocco, L.; Nicoli, M. C.; Hendrickx, M. Microstructure and Bioaccessibility of Different Carotenoid Species as Affected by High Pressure Homogenisation: A Case Study on Differently Coloured Tomatoes. *Food Chem.* **2013**, *141*(4), 4094–4100. DOI: 10.1016/j.foodchem.2013.06.099.
[28] Kubo, M. T. K.; Augusto, P. E. D.; Cristianini, M. Effect of High Pressure Homogenization (HPH) on the Physical Stability of Tomato Juice. *Food Res. Int.* **2013**, *51*(1), 170–179. DOI: 10.1016/j.foodres.2012.12.004.
[29] Stang, M.; Schuchmann, H.; Schubert, H. Emulsification in High-pressure Homogenizers. *Eng. Life Sci.* **2001**, *1*(4), 151–157. DOI: 10.1002/1618-2863(200110)1:4<151::AID-ELSC151>3.0.CO;2-D.
[30] Boca, A. N.; Ilies, R. F.; Saccomanno, J.; Pop, R.; Vesa, S.; Tataru, A. D.; Buzoianu, A. D. Sea Buckthorn Extract in the Treatment of Psoriasis. *Exp. Ther. Med.* **2019**, *17*(2), 1020–1023. DOI: 10.3892/etm.2018.6983.
[31] Zhang, G. K.; Liu, Y. F.; Liu, P. Active Components from Sea Buckthorn (*Hippophae Rhamnoides* L.) Regulate Hepatic Stellate Cell Activation and Liver Fibrogenesis. *J. Agric. Food Chem.* **2018**, *66*(46), 12257–12264. DOI: 10.1021/acs.jafc.8b05306.
[32] Mortensen, M. W.; Spagner, C.; Cuparencu, C.; Astrup, A.; Raben, A.; Dragsted, L. O. Sea Buckthorn Decreases and Delays Insulin Response and Improves Glycaemic Profile following a Sucrose-containing Berry Meal: A Randomised, Controlled, Crossover Study of Danish Sea Buckthorn and Strawberries in Overweight and Obese Male Subjects. *Eur. J. Nutr.* **2018**, *57*(8), 2827–2837. DOI: 10.1007/s00394-017-1550-8.
[33] Aaby, K.; Wrolstad, R. E.; Ekeberg, D.; Skrede, G. Polyphenol Composition and Antioxidant Activity in Strawberry Purees; Impact of Achene Level and Storage. *J. Agric. Food Chem.* **2007**, *55*(13), 5156–5166. DOI: 10.1021/jf070467u.
[34] Zadernowski, R.; Nowak-Polakowska, H.; Lossow, B.; Nesterowicz, J. Sea-buckthorn Lipids. *J. Food Lipids.* **1997**, *4*(3), 165–172. DOI: 10.1111/j.1745-4522.1997.tb00090.x.
[35] Gutzeit, D.; Baleanu, G.; Winterhalter, P.; Jerz, G. Vitamin C Content in Sea Buckthorn Berries (*Hippophaë Rhamnoides* L. Ssp *Rhamnoides*) and Related Products: A Kinetic Study on Storage Stability and the Determination of Processing Effects. *J. Food Sci.* **2008**, *73*(9), C615–C620. DOI: 10.1111/j.1750-3841.2008.00957.x.
[36] Gregory, J. F., III;. Vitamins. In *Food Chem.*, Fourth ed.; Damodoran, S., Parkin, K. L., Fennema, O. R., Eds.; CRC Press: Boca Raton, FL, USA, **2008**; pp 467–476.
[37] Van Bree, I.; Baetens, J. M.; Samapundo, S.; Devlieghere, F.; Laleman, R.; Vandekinderen, I.; Noseda, B.; Xhaferi, R.; De Baets, B.; De Meulenaer, B. Modelling the Degradation Kinetics of Vitamin C in Fruit Juice in Relation to the Initial Headspace Oxygen Concentration. *Food Chem.* **2012**, *134*(1), 207–214. DOI: 10.1016/j.foodchem.2012.02.096.
[38] Knockaert, G.; Lemmens, L.; Van Buggenhout, S.; Hendrickx, M.; Van Loey, A. Changes in β-carotene Bioaccessibility and Concentration during Processing of Carrot Puree. *Food Chem.* **2012**, *133*(1), 60–67. DOI: 10.1016/j.foodchem.2011.12.066.
[39] Pérez-Conesa, D.; García-Alonso, J.; García-Valverde, V.; Iniesta, M.-D.; Jacob, K.; Sánchez-Siles, L. M.; Ros, G.; Periago, M. J. Changes in Bioactive Compounds and Antioxidant Activity during Homogenization and Thermal Processing of Tomato Puree. *Innov. Food Sci. Emerg. Technol.* **2009**, *10*(2), 179–188. DOI: 10.1016/j.ifset.2008.12.001.
[40] Lin, C. H.; Chen, B. H. Stability of Carotenoids in Tomato Juice during Storage. *Food Chem.* **2005**, *90*(4), 837–846. DOI: 10.1016/j.foodchem.2004.05.031.
[41] Pesek, C. A.; Warthesen, J. J. Kinetic-model for Photoisomerization and Concomitant Photodegradation of β-carotenes. *J. Agric. Food Chem.* **1990**, *38*(6), 1313–1315. DOI: 10.1021/jf00096a004.
[42] Delgado-Vargas, F.; Jimenez, A. R.; Paredes-Lopez, O. Natural Pigments: Carotenoids, Anthocyanins, and Betalains - Characteristics, Biosynthesis, Processing, and Stability. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*(3), 173–289. DOI: 10.1080/10408690091189257.
[43] Provesi, J. G.; Dias, C. O.; Amante, E. R. Changes in Carotenoids during Processing and Storage of Pumpkin Puree. *Food Chem.* **2011**, *128*(1), 195–202. DOI: 10.1016/j.foodchem.2011.03.027.

[44] Andersson, S. C.; Ekholm, A.; Johansson, E.; Olsson, M. E.; Sjoholm, I.; Nyberg, L.; Nilsson, A.; Rumpunen, K. Effect of Storage Time and Temperature on Stability of Bioactive Compounds in Aseptically Packed Beverages Prepared from Rose Hips and Sea Buckthorn Berries. *Agric. Food Sci.* **2015**, *24*(4), 273–288. DOI: 10.23986/afsci.49331.

[45] Ficzek, G.; Matravolgyi, G.; Furulyas, D.; Rentsendavaa, C.; Jocsak, I.; Papp, D.; Simon, G.; Vegvari, G.; Steger-Mate, M. Analysis of Bioactive Compounds of Three Sea Buckthorn Cultivars (*Hippophae Rhamnoides* L. 'Askola', 'Leikora', and 'Orangeveja') with HPLC and Spectrophotometric Methods. *Eur. J. Hort. Sci.* **2019**, *84*(1), 31–38. DOI: 10.17660/eJHS.2019/84.1.5.

[46] Korekar, G.; Dolkar, P.; Singh, H.; Srivastava, R. B.; Stobdan, T. Variability and the Genotypic Effect on Antioxidant Activity, Total Phenolics, Carotenoids and Ascorbic Acid Content in Seventeen Natural Population of Seabuckthorn (*Hippophae Rhamnoides* L.) From trans-Himalaya. *LWT-Food Sci. Technol.* **2014**, *55*(1), 157–162. DOI: 10.1016/j.lwt.2013.09.006.

[47] Huang, D. J.; Ou, B. X.; Prior, R. L. The Chemistry behind Antioxidant Capacity Assays. *J. Agric. Food Chem.* **2005**, *53*(6), 1841–1856. DOI: 10.1021/jf030723c.

[48] Rösch, D.; Bergmann, M.; Knorr, D.; Kroh, L. W. Structure-antioxidant Efficiency Relationships of Phenolic Compounds and Their Contribution to the Antioxidant Activity of Sea Buckthorn Juice. *J. Agric. Food Chem.* **2003**, *51*(15), 4233–4239. DOI: 10.1021/jf0300339.

[49] Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant Activity of Dietary Polyphenols as Determined by a Modified Ferric Reducing/antioxidant Power Assay. *J. Agric. Food Chem.* **2000**, *48*(8), 3396–3402. DOI: 10.1021/jf9913458.

[50] Kirkhus, B.; Afseth, N. K.; Borge, G. I. A.; Grimsby, S.; Steppeler, C.; Krona, A.; Langton, M. Increased Release of Carotenoids and Delayed in Vitro Lipid Digestion of High Pressure Homogenized Tomato and Pepper Emulsions. *Food Chem.* **2019**, *285*, 282–289. DOI: 10.1016/j.foodchem.2019.01.124.

[51] Saricaoglu, F. T.; Atalar, I.; Yilmaz, V. A.; Odabas, H. I.; Gul, O. Application of Multi Pass High Pressure Homogenization to Improve Stability, Physical and Bioactive Properties of Rosehip (*Rosa Canina* L.) Nectar. *Food Chem.* **2019**, *282*, 67–75. DOI: 10.1016/j.foodchem.2019.01.002.

[52] Stinco, C. M.; Fernandez-Vazquez, R.; Escudero-Gilete, M. L.; Heredia, F. J.; Melendez-Martinez, A. J.; Vicario, I. M. Effect of Orange Juice's Processing on the Color, Particle Size, and Bioaccessibility of Carotenoids. *J. Agric. Food Chem.* **2012**, *60*(6), 1447–1455. DOI: 10.1021/jf2043949.

[53] Hutchings, J. B.;. Food Color Mechanisms. In *Food Color and Appearance*, 2nd ed.; Hutchings, J. B., Ed.; Aspen Publishers, Inc: Gaithersburg, Maryland, USA, **1999**; pp 453–592.

[54] Bi, X. F.; Hemar, Y.; Balaban, M. O.; Liao, X. J. The Effect of Ultrasound on Particle Size, Color, Viscosity and Polyphenol Oxidase Activity of Diluted Avocado Puree. *Ultrason. Sonochem.* **2015**, *27*, 567–575. DOI: 10.1016/j.ultsonch.2015.04.011.