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# Influence of Harvest Time on the Quality of Oil-Based Compounds in Sea Buckthorn (*Hippophae rhamnoides* L. ssp. *sinensis*) Seed and Fruit

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The effect of the harvest time on oil-based bioactive compounds in sea buckthorn berries (*Hippophae rhamnoides* L. ssp. *sinensis*) was investigated. Sea buckthorn berries were collected at early maturity (September), maturity (November), and postmaturity (January) during the 2003–2004 harvest year. Whole berries were analyzed for physical characteristics, and fruit and seed fractions were analyzed for bioactive content. November-harvested berries yielded the highest values for berry sizes, CIELab factor *a**, and total carotenoid content in the fruit fraction ($p < 0.05$). September yielded significantly higher ($p < 0.05$) levels of major compounds, α-tocopherol and β-sitosterol, in the fruit fraction. Seed characteristics and bioactive compounds did not vary significantly with respect to the harvest time ($p > 0.05$). These results have identified the most suitable level of maturity for the optimization of certain compounds and the losses that may occur with winter harvest, commonly practiced in cold climates.

**KEYWORDS: Bioactive; carotenoids; fatty acids; harvest; phytosterols; sea buckthorn; tocopherols; tocotrienols**

## INTRODUCTION

Sea buckthorn (*Hipphophae rhamnoides* L.), a winter hardy, fruit-bearing shrub, has been used in Canada as a landscape and prairie shelterbelt plant for at least 20 years (*1*). This plant has been viewed as a possible diversification crop for the Canadian prairies, because of its hardiness and abundance of healthy berries. The oils of the pulp, peel, and seeds of the berry contain carotenoids, fatty acids, tocols, and phytosterols (*2–4*). Health benefits cited for sea buckthorn berry products include anti-inflammation, antimicrobial action, pain relief, promotion of tissue regeneration, boosting of the immune system, and protection against degenerative diseases, such as cancer and cardiovascular disease (*5*).

Sea buckthorn berries contain fruit acids, sugars, protein, amino acids, elements, flavonoids, vitamins [i.e., A, C, and E (tocols)], carotenoids, fatty acids, and phytosterols (*6*). Yang and Kallio (*3*) reported that a characteristic property of sea buckthorn berry pulp/peel oil is the high content of C16:1*n*7 (palmitoleic acid), 20–33% of total fatty acids. However, the commercial value of sea buckthorn oil is commonly based on levels of carotenoids (*2*) and tocols (*4*). Tocol content represented by tocopherols (α-T, β-T, γ-T, and δ-T) and tocotrienols (α-T3, β-T3, γ-T3, and δ-T3) is higher in pulp/peel oil than in seed or juice oil (*2*). Phytosterol content of berry oil is attributed to fruit-coat lipids (∼50%), pulp (∼20%), and seeds (∼30%) (*7*). β-Sitosterol is the major sterol present in pulp/peel and seed fractions (*8*). The content of individual biologically active (bioactive) compounds within the oils is influenced by climatic conditions, geographical location, berry variety, species, and maturity (*2*).

The harvest of sea buckthorn berries is complicated by the softness of mature fruit and lack of abscission layer (*9*). Berries generally ripen toward the end of August to late September in the Canadian prairies. Because of the lack of the abscission layer, berries persist on the shrubs all winter. In the Canadian prairies, berries are harvested at postmaturity at winter temperatures below −20 °C. In Europe and China, berries are normally harvested from the end of August to the middle of September and from the end of September to the end of November, respectively (*3, 10, 11*).

The content and quality of individual compounds for application in nutraceuticals and functional foods is dependent upon the maturity level at harvest. The variation in content and quality of fatty acids, carotenoids, tocols, and sterols during the ripening stage of sea buckthorn berries has been documented (*8, 10–12*); however, there is a lack of information on berries harvested months after ripening. Postmature berries are exposed to a variety of climatic conditions. Fluctuations and freeze/thaw cycles associated with late fall and winter temperatures cause extensive crystal formation and physical change, increasing the possibility of oxidation, a common cause of oil degradation (*13*). Oxidation is influenced by many other factors including light, oxygen, moderate water activity (0.5–0.8), pro-oxidants (i.e., metals), and enzymes. The presence of antioxidants within the

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product (i.e., vitamin C, tocols, and carotenoids) can reduce the rate of oxidation of fatty acids (*14*). However, many compounds including tocols, carotenoids, and phytosterols are susceptible to oxidation and can serve as initiators of oxidative reactions (*15*).

The objective of this research is to determine the effects that harvest time has on characteristics (i.e., berry color, berry and seed size, moisture content, and seed content) and oil-based bioactive compounds (i.e., carotenoids, fatty acids, tocols, and phytosterols) of sea buckthorn berries (*H. rhamnoides* L. ssp. *sinensis*). The purpose is to identify the losses that may occur with the winter harvest, commonly practiced in cold climates, such as the Canadian prairies, and to contribute to the international research performed on individual oil-based compounds. The discussion includes a comparison with research reported on *sinensis* berries unless otherwise noted.

## MATERIALS AND METHODS

**Harvest and Postharvest Handling.** Sea buckthorn berries ssp. *sinensis* were manually harvested from 5-year-old shrubs at St. Claude, Manitoba, Canada, during the 2003–2004 harvest year. Undamaged berries from 20 shrubs were collected in 200 g lots to form a representative sample from the orchard. The three harvest periods included early maturity (September); maturity (early November), and postmaturity (January). The first severe frost (−24.2 °C), occurring on November 7, was selected as the point at which berry development may have ceased.

Bagged berries were frozen in a single layer in a −40 °C freezer storage, within 4 h of being harvested. Once completely frozen, a minimum of 24 h, the berries were mixed to form one homogeneous pool for each harvest period. The berries were bagged and kept frozen until required. Berries were thawed at 4 °C for 5 h prior to testing. The literature reported that quick freezing and controlled thawing resulted in less ice-crystal formation and physical change to the berry structure (*16, 17*).

**Temperature Monitoring.** The temperature was recorded at 15 min intervals from September 4, 2003 to January 25, 2004, using a temperature data logger (ACR JR-1000 Series, model 01-0192, ACR Systems, Inc., Surrey, Canada). The data logger was suspended in an instrument shelter located in a row of shrubs in the orchard.

**Berry and Seed Sizing.** Berry sizing was represented by the mass of a randomly selected batch of 100 thawed berries (g % berries), in triplicate (*18*). The seed size was measured as the mass of 100 seeds (g % seeds) after air drying at room temperature (25 °C) for 2 weeks, in triplicate (*18*).

**Moisture Content.** Moisture content [% (w/w) wet basis (wb)] was determined by the standard vacuum oven method according to the American Organization of Analytical Chemists (AOAC) official method 920.151 (*19*).

**Seed Content in Berries.** The seed content was the mass of seeds in a sample of berries [% (w/w) wb] (*8*). This evaluation was performed on nine 56 g fruit samples per harvest time.

**Color Analysis.** Color measurements were conducted on berries from each harvest time, in triplicate, with a Minolta Chroma Meter (model CR-410, Minolta Co. Ltd., Osaka, Japan). The Commission Internationale d'Eclairage (CIE) laboratory color system was followed (*20*). The CIELab scale measured the degrees of lightness ($L^*$), redness/greenness ($\pm a^*$), and yellowness/blueness ($\pm b^*$) in a sample. The unit was calibrated for white.

**Sample Preparation for Bioactive Testing.** The seeds, pulp, juice, and skin each contained different levels of bioactive compounds. To simplify the process, the fresh berries were separated into two fractions: seed and fruit (skin, pulp, and juice), according to AOAC method 920.149 (*19*). This preparation was performed in triplicate with three subsamples to yield nine 15 g fruit and three 10 g ground seed fraction samples per harvest period. A triple extraction based on the Folch method (*21*) using a chloroform/methanol solution (1:2, v/v) was employed for the fruit and seed fractions. Extracted oil samples were diluted and stored in hexane, yielding concentrations of 100 and 500 mg/mL hexane for the fruit and seed fractions, respectively.

**Carotenoids.** The determination of total carotenoids was based on a method proposed by Gao et al. (*10*). Solutions of fruit and seed fraction oil in hexane, 0.5 and 10 mg/mL hexane, respectively, were prepared to achieve an absorbance within 0.2 and 0.8. Total carotenoids were measured at 460 nm using a Spectronic spectrophotometer (model 3000 ARRAY, Milton Roy, Ivyland, PA). Quantification of amounts of carotenoids was based on calibration with a $\beta$-carotene standard (type II synthetic, Sigma-Aldrich Canada Ltd., Oakville, Canada). Total carotenoids were expressed in mg/100 g of oil, $\beta$-carotene equivalents.

**Fatty Acids.** Fatty acid composition determination was conducted through analysis of fatty acid methyl esters (FAMEs) prepared according to the American Oil Chemists Society method Ce 1-62 (*23*). FAMEs were analyzed with a gas chromatograph (GC) (model 17AAF, Shimadzu Corporation, Kyoto, Japan) equipped with a programmed split/splitless injector and flame ionization detector (FID). A fused silica capillary column DB-23 ($L$ = 30 m; i.d. = 0.25 mm; $d_f$ = 0.25 $\mu$m; J&W Scientific, Folsom, Canada) was used. The linear velocity of the carrier gas, hydrogen, was 0.5 m/s, with a split valve ratio of 1:80. The column temperature program included maintaining 155 °C for 2 min, increasing at a rate of 2 °C/min to 215 °C, and then holding for 1 min. FAMEs were identified by a comparison with the retention data of a standard mixture 461 (NuChek Prep, Elysian, MN). The fatty acid composition was expressed as a mass percentage (%, w/w) of the total fatty acids.

**Tocopherol and Tocotrienol Analysis.** Tocopherol and tocotrienol levels were determined following International Standards Organization procedure 9936 (*24*). These compounds were analyzed using normal-phase high-performance liquid chromatography with a Shimadzu 10AD apparatus, a Shimadzu SIL-10A auto-injector, and RF-10AXL fluorescence detector (Shimadzu Corporation, Kyoto, Japan). The excitation and emission wavelengths were set at 290 and 335 nm, respectively. A 5 $\mu$m silica column ($L$ = 250 mm; i.d. = 3.2 mm; $d_f$ = 5 $\mu$m; Phenomenex, Torrance, CA) was used for separation with 5% methyl *tert*-butyl ether (MTBE) in hexane as the mobile phase. The oil–hexane solution injection volume was 10 $\mu$L at a flow rate of 0.8 mL/min for a 25 min run time. The identification of individual tocol isomer peaks was performed by a comparison with retention data of standards (catalog number MT1072, MT1071, MT1073, and MT1790; $^{MJS}$Biolynx, Brockville, Canada). Quantification of tocol isomers was correlated to an external calibration. Individual isomers were expressed as % (w/w) of the total and in mg/100 g of oil.

**Identification of Sterols.** Sterols were analyzed in saponified oil samples following a method proposed by Yang et al. (*8*). Samples containing the internal standard 5α-cholestane (Sigma-Aldrich Canada Ltd., Oakville, Canada) dissolved in MTBE were saponified at room temperature with 2 mL of 1 N methanolic potassium hydroxide (KOH) solution for 18 h. Water (2 mL) was added to the saponified samples, and the unsaponified portion was triple-extracted with hexane. Upon removal of all water and solvent through nitrogen evaporation, the residue was dissolved in 1 mL iso-octane and analyzed for composition.

Sterols were analyzed using a Shimadzu GC (model 17AAF, Shimadzu Corporation, Kyoto, Japan), with a DB-5 capillary column ($L$ = 30 m; i.d. = 0.25 mm; $d_f$ = 0.25 $\mu$m; Restek, Bellefonte, PA). The column temperature program included maintaining 60 °C for 1 min, increasing at a rate of 40 °C/min to 240 °C, holding for 1 min, increasing at a rate of 2 °C/min to a final temperature of 300 °C, and holding for 2 min. Hydrogen was the carrier gas (2.2 mL/min), with the injector and FID temperatures set at 275 and 320 °C, respectively. Sterols were identified by a comparison with retention data for standards of campesterol (Sigma-Aldrich Canada Ltd., Oakville, Canada), $\beta$-sitosterol (SRL, Milan, Italy), stigmasterol (SRL, Milan, Italy), cholesterol (Chemservice, Inc., West Chester, PA), and 5α-cholestane and quantified using the internal standard. Individual sterols were expressed as % (w/w) of total sterols and in mg/100 g of oil.

**Statistical Analysis.** Statistical analysis was conducted using JMP IN Statistical Discovery Software (SAS Institute, Inc., Cary, NC). Simple statistical parameters [mean and standard deviation (SD)] were estimated for each of the physical characteristics and bioactive compounds studied. Group differences on these traits were established with one-way analysis of variance (ANOVA) for equal variances and

**Table 1.** Mean, Maximum, and Minimum Temperatures (°C) for the 2003–2004 Harvest Period

| | month | | | | |
|---|---|---|---|---|---|
| | September | October | November | December | January |
| mean | 11.7 | 6.2 | −7.0 | −8.6 | −16.3 |
| maximum | 34.0 | 27.3 | 9.2 | 4.6 | −5.4 |
| minimum | −3.4 | −10.0 | −24.2 | −30.8 | −35.2 |

**Table 2.** Physical Characteristics of Sea Buckthorn Berries for Different Harvest Times[a]

| | | harvest month (2003–2004) | | | | | |
|---|---|---|---|---|---|---|---|
| | | September | | November | | January | |
| characteristic | *n* | mean | SD | mean | SD | mean | SD |
| berry size (g % berries) | 3 | 15.6 a | 1.0 | 19.4 b | 0.6 | 17.9 b | 0.9 |
| seed size (g % seeds) | 3 | 1.00 | 0.03 | 0.97 | 0.01 | 0.99 | 0.02 |
| moisture content [% (w/w) wb] | 2 | 77.8 | 0.0 | 75.8 | 2.1 | 75.8 | 0.1 |
| seed content in berries [% (w/w) wb] | 9 | 7.0 a | 0.3 | 5.9 b | 0.1 | 6.6 c | 0.3 |

[a] Means along a row with similar letters are not significantly different at $p = 0.05$ (Tukey–Kramer HSD).

using nonparametric methods (Wilcoxon/Kruskal–Wallis) for unequal variances. Significant differences among means detected by ANOVA were compared using the Tukey–Kramer Honestly Significant Difference (HSD) test ($p = 0.05$).

## RESULTS AND DISCUSSION

**Temperature.** The three harvest periods occurred during September 4–8, 2003, November 9–12, 2003, and January 18–20, 2004. The mean dry bulb ambient temperatures from September 4, 2003 to January 25, 2004 ranged from 11.7 to −16.3 °C, with temperatures ranging from 34.0 to −35.2 °C (**Table 1**).

September berries were firm, easily removed from the branches, and at various stages of ripening. Berry development and ripening continued throughout the mild fall until early November. The November harvest was conducted at temperatures of ∼ −5.0 °C, which slowed the collection of intact whole berries. Freeze/thaw effects on the berry structure, ripeness, and lack of abscission layer caused tearing of the peel of many berries. Berries that remained on the shrubs for 10 additional weeks through winter conditions were exposed to freeze/thaw cycles, temperature fluctuations, precipitation, wind, and sunlight. The January harvested berries were collected at temperatures below −20 °C, resulting in easy removal of the frozen berries.

**Berry and Seed Sizing.** The effect of the harvest time on berry size was significant ($p < 0.05$) (**Table 2**). The berry size for September (15.6 g %) was the lowest ($p < 0.05$) because of berry immaturity. Berries increased in size as ripening progressed from September to November (19.4 g %), with an insignificant decrease to 17.9 g % in January ($p > 0.05$). Differences in seed sizes were considered insignificant ($p > 0.05$), possibly because of early seed development and protection from the elements by the berry structure. Berry sizes were lower than the 21.8–34.2 g % reported by Tang and Tigerstedt (*18*). As with berry sizes, Tang and Tigerstedt reported consistently larger seed sizes of 1.4 and 1.5 g % as compared to 1.0 g % (**Table 2**). Variations between studies throughout this discussion may have been attributed to differences in the geographical location, climate, environment, harvest period, and berry maturity.

**Moisture Content.** Moisture contents for September, November, and January were 77.8, 75.8, and 75.8%, respectively (**Table 2**). The differences between harvest times were insignificant ($p > 0.05$) possibly because of the large variation (SD = 2.1%) in berries encountered for November. Values compared well in magnitude with the 74% measured by Ma and Cui (*25*); however, they are lower than those reported by Tang and Tigerstedt (*18*) of 82.2–87.5%. A larger size of the latter berries may have contributed to the higher moisture content.

**Seed Content in Berries.** Seed contents varied significantly ($p < 0.05$) between harvest times (**Table 2**). The seed content was highest at 7.0% for the September berries, with a decrease to 5.9% by November and an increase to 6.6% by January ($p < 0.05$). The seed content trend across harvest times was opposite that for the berry size. This relationship was confirmed by the increasing berry and consistent seed sizes. The seed contents were within the ranges of 3.9–9% and 3.6–8.4%, reported by Yang and Kallio (*3*) and Yang et al. (*8*), respectively.

**Color Analysis.** The effect of the harvest time on the CIELab factor $a^*$ was significant ($p < 0.05$) (**Table 3**). Positive color factors $a^*$ and $b^*$ for each harvest period confirmed red and yellow values in the fruit, respectively. Visually, a portion of the September berries had a green coloring represented by lower values of $a^*$. Factor $a^*$ (+20.2) was significantly higher ($p < 0.05$) for November, possibly because of the processes involved with ripening: disappearance of chlorophylls, major biosynthesis of carotenoids, and esterification of xanthophylls with fatty acids (*26*). Lightness factor, $L^*$ was highest in January samples, at 47.2, and was significantly different ($p < 0.05$) from September samples, at 45.2. The lightening of the fruit may have been due to carotenoid degradation (*13*).

**Carotenoids.** Means of total carotenoids for the fruit fraction for the three harvest periods were significantly different ($p < 0.05$) (**Table 4**). The values ranged from a low in September of 498.1 mg/100 g of oil to a high in November of 817.8 mg/100 g of oil. A marked decrease in carotenoids occurred from November to 616.8 mg/100 g of oil by January. Redness $a^*$ and carotenoid content followed the same trend. The 64% increase from September to November was comparable to the 62% increase for whole berries (ssp. *botanitjetskaja*, *trofimovskaja*, and *aromatnaja*) obtained by Gao et al. (*10*). The total carotenoids compared well with the 500–1000 mg/100 g of oil range (variety not known) reported by Xin et al. (*27*).

Carotenoid levels in the seed fraction did not significantly change between harvest times (**Table 4**). The seed carotenoid levels of 24.4, 25.6, and 27.6 mg/100 g of oil fell into the lower range of 20–85 mg/100 g of oil measured by Xin et al. (*27*).

**Fatty Acids.** Three major fatty acids, C16:0 (palmitic), palmitoleic, and C18:1*n*9 (oleic), accounted for approximately 32.2, 26.5, and 18.7% of the total fatty acids in the fruit fraction, respectively (**Table 5** and **Figure 1**). Yang and Kallio (*3*) obtained similar results with palmitoleic and oleic acids, accounting for 27.2 and 17.1% of the total fatty acids, respectively. The compound that accounted for 8.1% was identified by other researchers as vaccenic (*3*, *11*) or *cis*-vaccenic acid (*4*, *28*).

The fatty acid profile remained relatively stable between harvest times in the fruit fraction. Significant changes included a 1% increase ($p < 0.05$) in oleic acid from November to January, while minor (≤0.6%) differences ($p < 0.05$) occurred for C18:3*n*3 (α-linolenic) between all harvest times (**Table 5**). Zadernowski (*29*) reported that once berries (variety not known) turned a yellow orange (mid-September), palmitic, palmitoleic,

**Table 3.** Color Analysis of Sea Buckthorn Berries for Different Harvest Times[a]

| | | harvest month (2003–2004) | | | | | |
|---|---|---|---|---|---|---|---|
| | | September | | November | | January | |
| CIELab factor | *n* | mean | SD | mean | SD | mean | SD |
| lightness (*L**) | 3 | 45.2 a | 0.8 | 45.5 a,b | 1.0 | 47.2 b | 0.5 |
| (+)red/(−)green (*a**) | 3 | +14.2 a | 0.5 | +20.2 b | 0.4 | +17.4 c | 0.5 |
| (+)yellow/(−)blue (*b**) | 3 | +35.7 | 1.6 | +36.7 | 3.1 | +39.7 | 0.7 |

[a] Means along a row with the same letter are not significantly different at $p = 0.05$ (Tukey–Kramer HSD).

**Table 4.** Total Carotenoids in Sea Buckthorn Fruit and Seed Fractions for Different Harvest Times, Expressed in mg/100 g of Expressed Oil[a]

| | | harvest month (2003–2004) | | | | | |
|---|---|---|---|---|---|---|---|
| | | September | | November | | January | |
| fraction | *n* | mean | SD | mean | SD | mean | SD |
| fruit | 9 | 498.1 a | 37.4 | 817.8 b | 77.5 | 616.8 c | 35.4 |
| seed | 3 | 24.4 | 1.6 | 25.6 | 4.2 | 27.6 | 3.5 |

[a] Means along a row with similar letters are not significantly different at $p = 0.05$ (Tukey–Kramer HSD).

**Table 5.** Fatty Acid Composition in Sea Buckthorn Fruit and Seed Fraction Oils for Different Harvest Times, Expressed in Mass Percentage (%, w/w)[a]

| | | harvest month (2003–2004) | | | | | |
|---|---|---|---|---|---|---|---|
| | | September | | November | | January | |
| fatty acid | retention time (min)[b] | mean | SD | mean | SD | mean | SD |
| | | Fruit Fraction ($n = 9$) | | | | | |
| C16:0 | 7.88 | 32.1 | 0.2 | 32.2 | 0.6 | 32.2 | 1.1 |
| C16:1*n*7 | 8.09 | 26.2 | 0.5 | 26.8 | 0.5 | 26.5 | 0.8 |
| C18:0 | 10.16 | 1.4 a | 0.1 | 1.2 b | 0.0 | 1.3 a | 0.2 |
| C18:1*n*9 | 10.36 | 18.8 a,b | 0.6 | 18.2 a | 0.5 | 19.2 b | 1.0 |
| unknown | 10.46 | 8.1 | 0.2 | 8.0 | 0.2 | 8.1 | 0.4 |
| C18:2*n*6 | 10.84 | 6.9 | 0.3 | 6.8 | 0.3 | 6.6 | 0.3 |
| C18:3*n*3 | 11.52 | 2.6 a | 0.3 | 2.3 b | 0.1 | 2.0 c | 0.1 |
| | | Seed Fraction ($n = 3$) | | | | | |
| C16:0 | 7.88 | 8.6 | 0.1 | 8.5 | 0.2 | 8.4 | 0.2 |
| C16:1*n*7 | 8.09 | 0.8 | 0.0 | 0.6 | 0.1 | 0.7 | 0.1 |
| C18:0 | 10.16 | 2.3 | 0.0 | 2.3 | 0.1 | 2.3 | 0.2 |
| C18:1*n*9 | 10.36 | 19.3 | 0.2 | 19.0 | 0.7 | 19.9 | 0.6 |
| unknown | 10.46 | 2.3 | 0.0 | 2.3 | 0.1 | 2.3 | 0.0 |
| C18:2*n*6 | 10.84 | 36.2 a | 0.0 | 36.6 b | 0.1 | 36.4 a,b | 0.1 |
| C18:3*n*3 | 11.52 | 28.9 | 0.3 | 28.9 | 0.8 | 28.2 | 1.1 |

[a] Means along a row with similar letters are not significantly different at $p = 0.05$ (Tukey–Kramer HSD). [b] Retention times correlate with profiles provided in **Figures 1** and **2**.

C18:2*n*6 (linoleic), and C18:3*n*3 (α-linolenic) were fully synthesized and remained relatively constant for the remainder of the maturation period. Another study in which berries were collected at 2 week intervals reported up to 12% variation in levels of oleic and palmitoleic acids in mid-October in whole berry oil (*11*). Linoleic and α-linolenic acids were also reported to be negatively correlated (*3*).

Three major fatty acids, linoleic, α-linolenic, and oleic accounted for approximately 36.4, 28.7, and 19.4% of the total fatty acids in seed fraction oil, respectively (**Table 5** and **Figure 2**). In sharp contrast to the fruit fraction, the seed fraction contained approximately 8.5% palmitic and 0.7% palmitoleic acids. Harvest time had a negligible effect ($p > 0.05$) upon fatty acid concentration in the seed fraction. Seed fatty acid proportions were in close agreement with the results reported by Yang and Kallio (*3*). However, as with whole berries (*11*), variations in oleic acid content in the seed fraction were reported (*3*).

**Table 6.** Major Tocopherol (T) and Tocotrienol (T3) Concentrations in Sea Buckthorn Fruit and Seed Fraction Oils for Different Harvest Times, Expressed in mg/100 g of Oil)[a]

| | | harvest month (2003–2004) | | | | | |
|---|---|---|---|---|---|---|---|
| | | September | | November | | January | |
| tocol | retention time (min)[b] | mean | SD | mean | SD | mean | SD |
| | | Fruit Fraction ($n = 9$) | | | | | |
| α-T | 6.83 | 388 a | 42 | 338 b | 43 | 272 c | 27 |
| β-T | 8.87 | 10 a | 1 | 12 b | 2 | 10 a | 1 |
| γ-T | 10.84 | 9 a | 2 | 4 b | 1 | 2 c | 1 |
| δ-T | 15.62 | 18 a | 4 | 15 a | 2 | 9 b | 2 |
| α-T3 | 7.85 | 9 a | 2 | 12 b | 2 | 10 a,b | 2 |
| β-T3 | 10.16 | 40 a | 5 | 39 a | 4 | 31 b | 5 |
| γ-T3 | 12.77 | 10 | 2 | 9 | 2 | 8 | 1 |
| δ-T3 | 18.56 | 1 | 0 | 1 | 0 | 1 | 0 |
| total | | 485 a | 53 | 430 a | 53 | 343 b | 34 |
| | | Seed Fraction ($n = 3$) | | | | | |
| α-T | 6.82 | 158 | 5 | 139 | 28 | 156 | 4 |
| β-T | 8.85 | 12 | 0 | 11 | 2 | 14 | 3 |
| γ-T | 10.81 | 68 | 1 | 57 | 11 | 63 | 2 |
| δ-T | 15.65 | 10 | 2 | 7 | 2 | 7 | 0 |
| P-8 | 10.13 | 3 | 0 | 3 | 0 | 3 | 0 |
| γ-T3 | 18.56 | 11 | 19 | nd | | nd | |
| total | | 262 | 19 | 217 | 42 | 243 | 5 |

[a] Means along a row with similar letters are not significantly different at $p = 0.05$ (Tukey–Kramer HSD). nd = not detected. [b] Retention times correlate with profiles provided in **Figures 3** and **4**.

**Tocopherols and Tocotrienols.** Total tocol concentrations ranged from 485 to 343 mg/100 g of oil and from 217 to 262 mg/100 g of oil for fruit and seed fractions, respectively (**Table 6**). A significant loss ($p < 0.05$) of total tocols in the fruit fraction occurred by January. Kallio et al. (*12*) reported that the range of tocols in the *sinensis* fruit fraction, 400–700 mg/100 g of oil, was 2–3 times higher than ssp. *rhamnoides* and *mongolica*. They also reported total tocol contents of 100–300 mg/100 g of oil in *sinensis* seeds.

A major proportion (91.2%) of fruit fraction oil tocols was comprised of α-T (79.2%), β-T3 (8.8%), and δ-T (3.2%). Significant changes ($p < 0.05$) in proportions included a 1.6% decrease from September to November (α-T), a 0.9% decrease from September to January (δ-T), and a 0.9% increase from September to November (β-T3). Similar proportions were reported by Kallio et al. (*12*), with changes in α-T up to 10% within a period from August to November.

September fruit fraction had significantly higher ($p < 0.05$) levels of α-T, with concentration (mg/100 g of oil) losses of 13.1 and 29.8% by November and January, respectively (**Table 6** and **Figure 3**). Significant losses ($p < 0.05$) were also noted from September to January for γ-T, δ-T, and β-T3. The consistent decreasing trend from September to January did not occur for minor isomers, β-T and α-T3, which had slightly higher levels in November ($p < 0.05$). Maximum amounts of α-, γ-, and δ-T were also reported for the oil of whole berries (ssp. *nadbaltycka*) harvested in September (*30*). The berries were of an olive–yellow coloring, denoting under-ripeness as in this study.

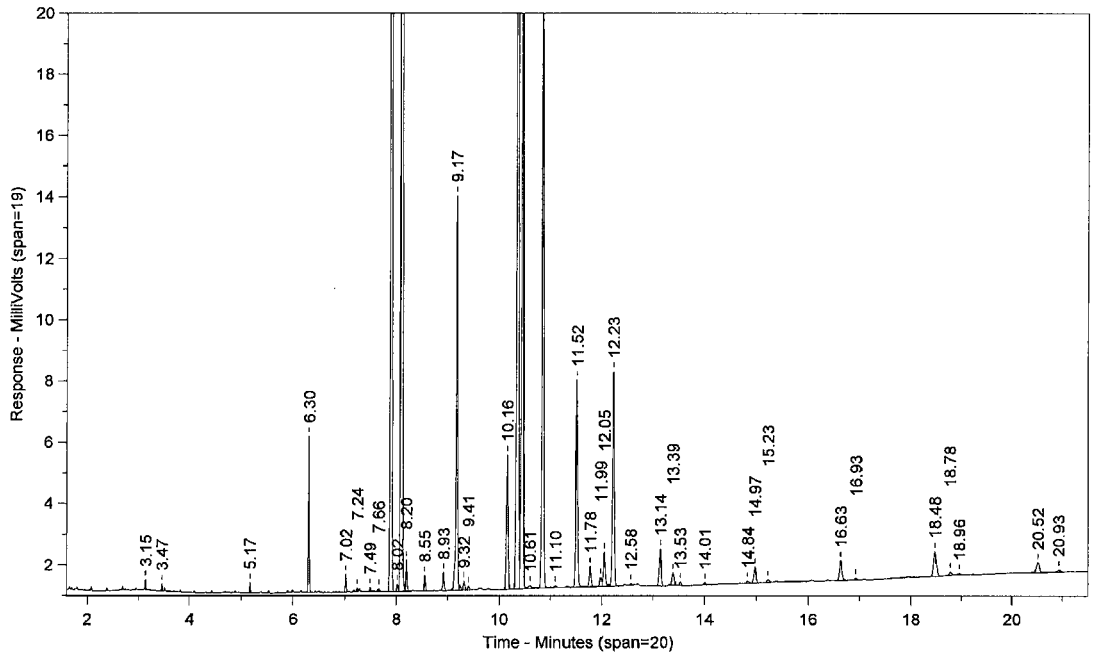


**Figure 1.** Fatty acid profile for the sea buckthorn fruit fraction oil (November harvest). Major fatty acids with respective retention times: C16:0, 7.89 min; C16:1*n*7, 8.10 min; C18:0, 10.16 min; C18:1*n*9, 10.37 min; C18:2*n*6, 10.85 min; and C18:3*n*3, 11.52 min.

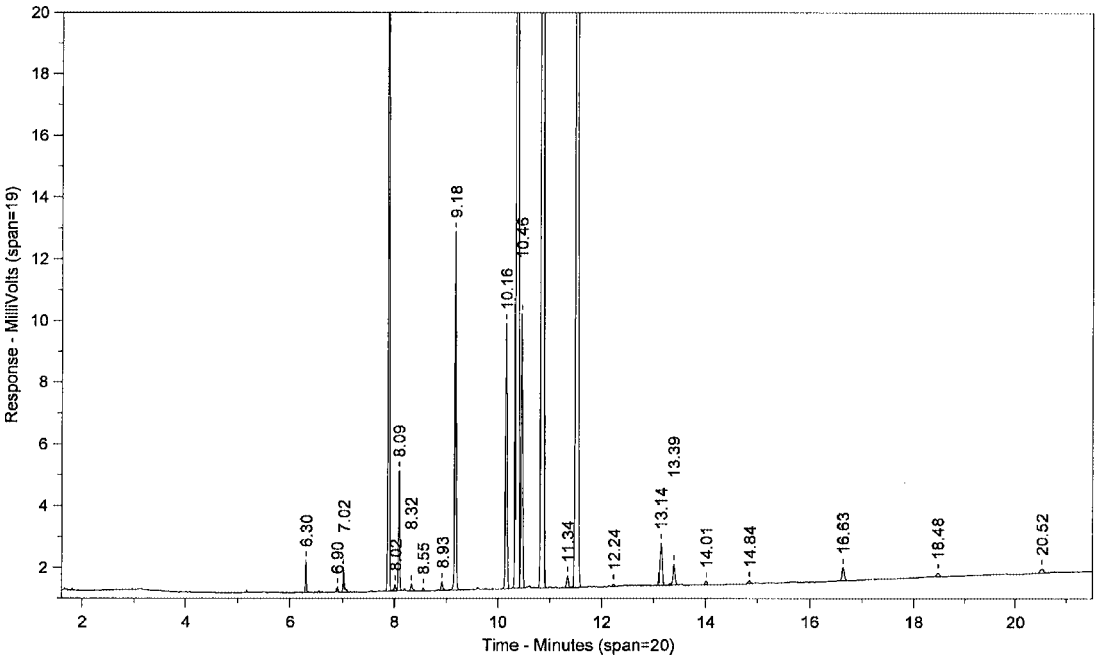


**Figure 2.** Fatty acid profile for the sea buckthorn seed fraction oil (November harvest). Major fatty acids with respective retention times: C16:0, 7.89 min; C16:1*n*7, 8.09 min; C18:0, 10.16 min; C18:1*n*9, 10.36 min; C18:2*n*6, 10.84 min; and C18:3*n*3, 11.52 min.

The isomers, α-T, $\beta$-T, $\gamma$-T, $\delta$-T, $\gamma$-T3, and plastochromanol 8 (P-8) were detected in the seeds. A major proportion (94.1%) of the seed fraction oil tocols was comprised of α-T (62.8%), $\gamma$-T (26.1%), and $\beta$-T (5.2%) (**Table 6** and **Figure 4**). Effects of the harvest time were insignificant ($p > 0.05$) for the isomer concentration in seeds. Lower proportions of α-T (30–50%) were reported by Kallio et al. (*12*), with changes in α-T and $\gamma$-T approaching 20% within a period from August to November.

**Phytosterols.** Over 20 phytosterols and terpenes have been identified in the oils of sea buckthorn fruit and seed fractions (*31*, *8*). Phytosterols identified in this study included cholesterol, campesterol, stigmasterol, and $\beta$-sitosterol (**Table 7** and **Figure 5**). Other peaks were detected but were not identifiable because of limited standards and incomparable spectra presented in other studies. A major proportion of the identified phytosterols in the fruit and seed fractions was comprised of $\beta$-sitosterol.

Concentrations of $\beta$-sitosterol in the fruit fraction were significantly ($p < 0.05$) higher in September (928 mg/100 g of oil), with lowest levels occurring in November (693 mg/100 g of oil). Similarly, a decrease was reported in levels of $\beta$-sitosterol from August to November paired with a slight increase of campesterol (*8*). Reported values of the fruit fraction total sterol concentration ranged from 1030 to 2870 mg/100 g of oil (ssp. *sinensis* and *rhamnoides*) (*8*) and 771 mg/100 g of oil (ssp. *sinensis*) (*32*). The content of $\beta$-sitosterol ranged from 61 to 83% (ssp. *sinensis* and *rhamnoides*) (*8*) and 85% (ssp. *sinensis*) (*32*).

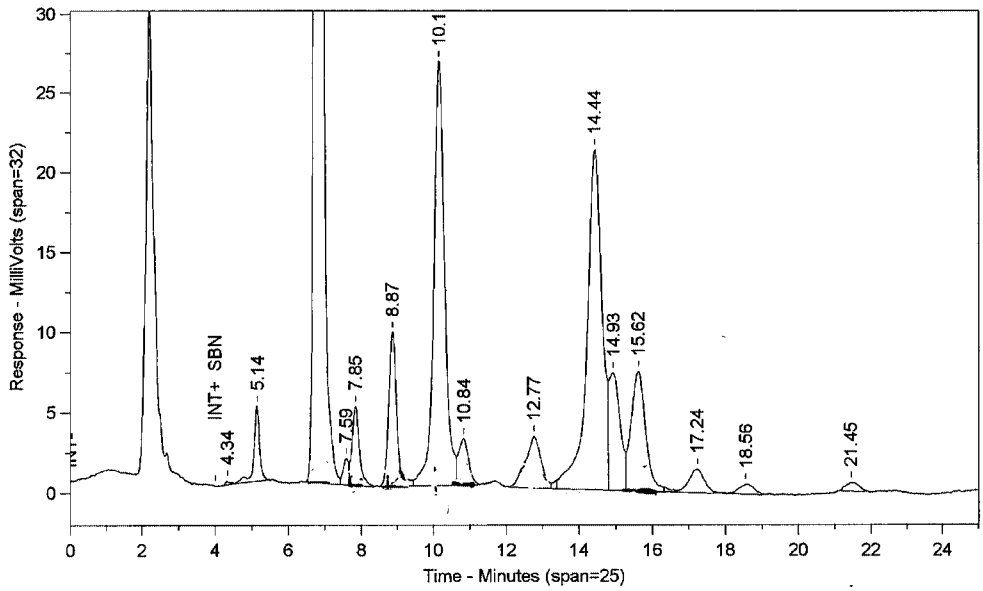


**Figure 3.** Tocol profile for the sea buckthorn fruit fraction oil (November harvest). Major tocols with respective retention times: α-tocopherol, 6.83 min; β-tocopherol, 8.87 min; γ-tocopherol, 10.84 min; δ-tocopherol, 15.62 min; α-tocotrienol, 7.85 min; β-tocotrienol, 10.16 min; γ-tocotrienol, 12.77 min; and δ-tocotrienol, 18.56 min.

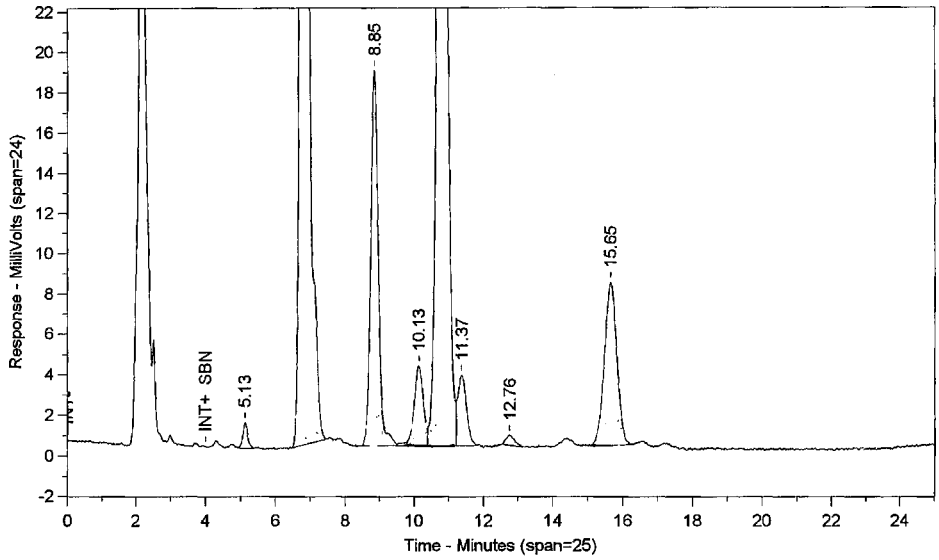


**Figure 4.** Tocol profile for the sea buckthorn seed fraction oil (November harvest). Major tocols with respective retention times: α-tocopherol, 6.82 min; β-tocopherol, 8.85 min; γ-tocopherol, 10.81 min; δ-tocopherol, 15.65 min; plastochromanol 8 (P-8), 10.13 min; and γ-tocotrienol, 18.56 min.

Concentrations (mg/100 g of oil) of β-sitosterol in the seed fraction ranged insignificantly ($p > 0.05$) from September (521) to January (567) (**Table 7** and **Figure 6**). No significant effects ($p > 0.05$) of harvest time occurred for total sterols and β-sitosterol in the seed fraction, as similarly reported by Yang et al. (*8*), for a harvest time between August and November. Reported values of the total sterol concentration ranged from 1240 to 2300 mg/100 g of oil (ssp. *sinensis* and *rhamnoides*) (*8*) and from 1022 to 1298 mg/100 g of oil (ssp. *sinensis*) (*32*). The content of β-sitosterol ranged from 57 to 76% (ssp. *sinensis* and *rhamnoides*) (*8*) and 74% (ssp. *sinensis*) (*32*). Contrary to this study, total phytosterol concentrations have been reported as being higher in the seed versus fruit fraction (*8, 31, 32*).

**Effects of Harvest Time.** Changes that occurred with respect to the harvest time varied depending upon the physical characteristics and oil compound. The individual mechanisms by which these changes occurred are complex and were not identified in this study. However, these mechanisms could have been divided into two main groups: those related to (1) fruit

**Table 7.** Sterol Concentrations in Sea Buckthorn Fruit and Seed Fractions, Expressed in mg/100 g of Oil)[a]

| | | harvest month (2003–2004) | | | | | |
|---|---|---|---|---|---|---|---|
| | | September | | November | | January | |
| phytosterol | retention time (min)[b] | mean | SD | mean | SD | mean | SD |
| | | Fruit Fraction ($n = 9$) | | | | | |
| cholesterol | 16.10 | 4 | 6 | 6 | 4 | 2 | 3 |
| campesterol | 18.10 | 19 a | 4 | 14 b | 2 | 16 a,b | 2 |
| stigmasterol | 18.79 | 7 | 4 | 4 | 3 | 8 | 9 |
| β-sitosterol | 19.89 | 928 a | 196 | 693 b | 105 | 723 b | 106 |
| | | Seed Fraction ($n = 3$) | | | | | |
| cholesterol | 16.10 | 2 | 2 | nd | | 3 | 2 |
| campesterol | 18.10 | 13 | 1 | 12 | 2 | 14 | 1 |
| stigmasterol | 18.79 | nd | | nd | | 1 | 1 |
| β-sitosterol | 19.89 | 521 | 8 | 528 | 52 | 567 | 27 |

[a] Means along a row with similar letters are not significantly different at $p = 0.05$ (Tukey–Kramer HSD). nd = not detected. [b] Retention times correlate with profiles provided in **Figures 5** and **6**.

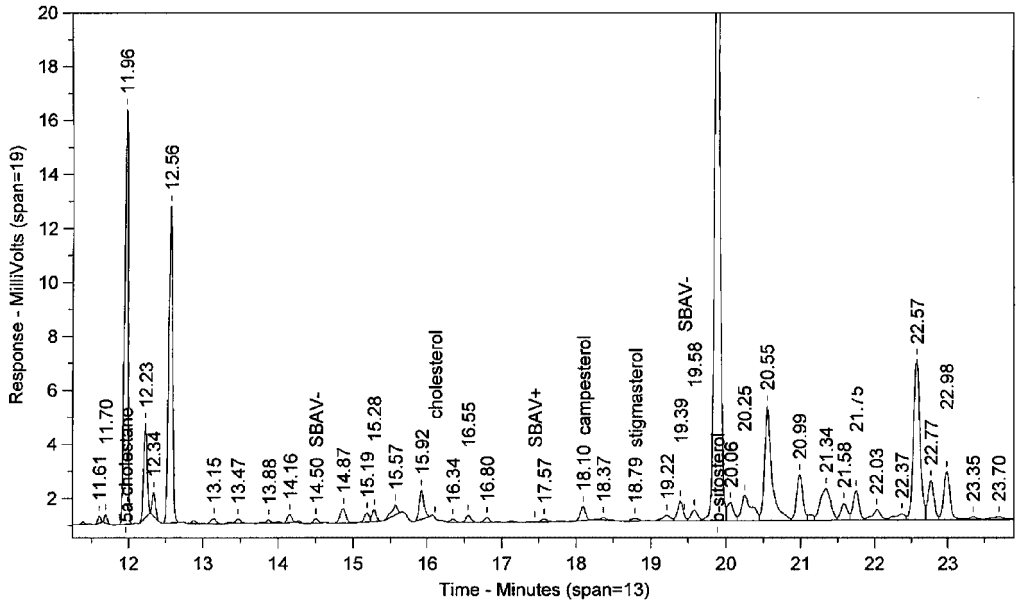


**Figure 5.** Sterol profile for the sea buckthorn fruit fraction oil (November harvest). Major sterols with respective retention times: cholesterol, 16.10 min; campesterol, 18.10 min; stigmasterol, 18.79 min; and $\beta$-sitosterol, 19.89 min.

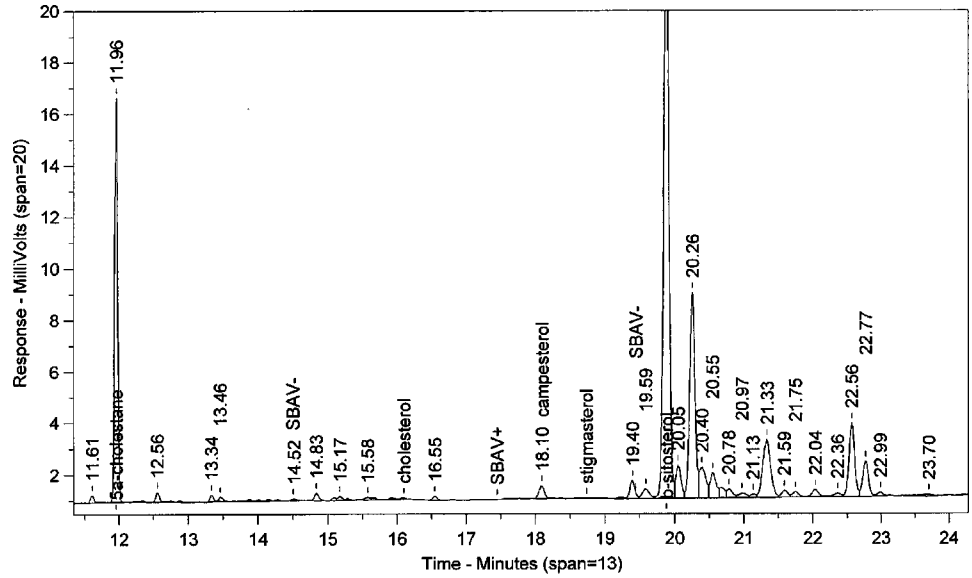


**Figure 6.** Sterol profile for the sea buckthorn seed fraction oil (November harvest). Major sterols with respective retention times: cholesterol, 16.10 min; campesterol, 18.10 min; stigmasterol, 18.79 min; and $\beta$-sitosterol, 19.89 min.

development and ripening and (2) degradation because of postmaturity and climatic conditions.

Varying rates of biosynthesis and metabolic pathways attributed to the differences in trends and levels between individual compounds during the fruit development and ripening stage (from September to November). The berry size and seed content reflected the growth and development that occurred. Major compounds, such as $\alpha$-T and $\beta$-sitosterol, were at their highest in under-ripe berries, with losses occurring during the ripening stage. Conversely, biosynthesis of carotenoids represented by concentration and color factors increased during the same period.

Extended exposure to temperature fluctuations including freeze/thaw conditions, environmental conditions, such as precipitation, light, and wind, and inherent pro-oxidants would have occurred between November and January. This combined with the cessation of chemical and physical reactions related to fruit development was associated with significant losses in total tocols, individual isomers (i.e., $\alpha$-T, $\beta$-T, $\gamma$-T, $\delta$-T, and $\beta$-T3), total carotenoids, and color (i.e., $a^*$) in the fruit fraction oil. The antioxidant activity of tocols and carotenoids in the berry may have contributed to both their respective losses as well as stability of the fatty acid profile. On the basis of this study, harvesting during the postmaturation stage resulted in a lower quality of fruit fraction oil. Compound degradation was limited to the fruit fraction because no significant changes occurred in the seed fraction oil.

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