The influence of harvest time on levels of bioactive compounds in sea buckthorn berries
(*Hippophaë rhamnoides* L. ssp. *sinensis*)

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Abstract

Sea buckthorn berries (*Hippophae rhamnoides* L. ssp. *sinensis*) were collected at early maturity (September), maturity (November), and post-maturity (January) for the 2003-2004 harvest year. Each sample was analysed to determine the influence of harvest time on physical characteristics and biologically active (bioactive) compounds. Correlating with the early maturity level, the September berries had the lowest berry size 15.62 g/100 berries (g%) (P<0.05), fruit carotenoid content 506.58 mg/100g (P<0.05), and redness a* = 14.19 (P<0.05). In comparison, the berries collected in November had the highest berry size 19.42 g% berries, redness a* = 20.18 (P<0.05), and total levels of fruit carotenoids, 836.75 mg/100g (P<0.05). A decrease in fruit carotenoids to 650.74 mg/100g (P<0.05) was accompanied by a significant change in colour (increase in lightness L* = 47.21 (P<0.05) and yellowness b* = 36.69 (P<0.05) with a reduction in redness a* = 17.44 (P<0.05)), for January. Seed characteristics and bioactive compounds did not vary significantly with respect to harvest time (P<0.05). The three main fatty acids in the fruit fraction were: C16:0 palmitic (32.18% w/w); C16:1n7 palmitoleic (26.16% w/w); and C18:1n9 oleic (18.80% w/w) and in the seed fraction were: C18:2 linoleic (36.16% w/w); C18:3n3 α-linolenic (28.91% w/w), and C18:1n9 oleic (19.30% w/w).
INTRODUCTION

The development of the functional food and nutraceutical industries is based upon evidence that certain products rich in biologically active (bioactive) compounds can be used in the prevention against certain degenerative diseases such as cancer, cardiovascular disease, and diabetes, as well as other diet related illnesses (Oomah and Mazza 2000). To ensure final product quality, the bioactive compounds must be preserved at every stage of the value-added cycle.

As with many crops, the level of maturity at which it is harvested can influence the characteristics and quality of the product. Harvesting at optimal maturity is not always possible due to climatic and environmental conditions, or feasibility of harvest methods. This paper focuses upon the “optimal time of harvest” for a specialty crop, sea buckthorn berries (*Hippophae rhamnoides* L. *ssp. sinensis*). The Canadian sea buckthorn industry has not yet been fully established due to barriers in the harvesting side of production.

Sea Buckthorn

Originating in Asia and Eastern Europe, sea buckthorn (*H. 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 (Li 2003). In recent years this plant has been viewed as a possible diversification crop for the Canadian Prairies, due to its hardiness and abundance of valuable berries.

Berry characteristics Ripe sea buckthorn berries range in colour from yellow, orange, or red, are spherical in shape, and range in size between 3 to 8 mm in diameter (Li 2003).
The waxy skinned berries contain a single sheathed seed and a juice filled cellular structure (Beveridge et al. 2002). Depending on the variety, sea buckthorn berries generally consist of pulp (68%), seed (23%), and peel (7.75%) (Zadernowski et al. 1997 as cited by Oomah 2003; Yang and Kallio 2001).

The limited data for moisture content and berry and seed size is due to a lack of published English research for *sinensis* berries. Tang and Tigerstedt (2001) determined mean berry sizes of 18.08 and 23.15 g/100 berries (g% berries) for consecutive years. Seed size showed a narrower range of 1.35 and 1.54 g/100 seeds (g% seeds). Moisture contents are high for these berries at 86.74 and 83.43%, as compared to 74% determined by Ma and Cui (1987 as cited by Tang and Tigerstedt 2001). Seed content in fruit ranged from 3.9 to 9.0 %w/w wet basis (wb) (Yang and Kallio 2001).

**Harvest challenges** Sea buckthorn berries are a challenge to harvest and store as are most soft fruit. The harvesting aspect is complicated by thorny branches, soft fruit, and lack of abscission layer (Beveridge 2003). Due to the lack of this layer the berries persist on the shrubs all winter. Certain cultivars are more difficult to harvest than others (i.e. *H. rhamnoides* ssp. *sinensis* versus *H. rhamnoides* ssp. *indian summer*). A variety of harvesting methods are utilized worldwide, ranging from manual to semi-automated harvesters.

In the Canadian Prairies, due to the absence of a commercial harvesting system suitable for all varieties, it is common practice to harvest the berries frozen, during the winter (i.e. temperatures below 20°C) (C. Robert, Producer, Branching Out Orchards, St. Claude, MB). The berries are removed by striking pruned branches on a solid surface or running a hand along the frozen berry laden branches, catching the berries in a tarp.
Berry maturity usually depends on the fruit cultivar, geographical location, and climatic and weather conditions (Yang et al. 2001). In Europe, berries are normally harvested from the end of August to the middle of September. In China, harvesting period lasts from the end of September to the end of November. Berries generally ripen toward the end of August to late September in the Canadian Prairies.

Potential health benefits linked to sea buckthorn berries

According to Schroeder and Yao (1995), “sea buckthorn berries are among the most nutritious and vitamin-rich fruits found in the plant kingdom”. The berries are rich in carbohydrates, protein, water, and fat soluble vitamins including the antioxidants (i.e. vitamins C and E, β-carotene, and lycopene), essential fatty acids, amino acids, phytosterols, and flavonoids, in addition to at least 24 chemical elements (i.e. iron, calcium, etc.) (Prescriber's Letter and Pharmacists's Letter 2002; (Zhang et al. 1989; Mironov 1989) as cited by Beveridge et al. 1999).

Although, used for centuries in its native Europe and Asia, this plant has recently gained worldwide attention, mainly for its nutritional and medicinal value. Some of the health benefits cited for sea buckthorn products include: anti-inflammation, antimicrobial action, pain relief, the promotion of tissue regeneration, boosting of the immune system, and protection against cancer and cardiovascular disease (Li et al. 2003). These health benefits are due to the many compounds within the leaves, bark, and fruit. The oil from the fruit (pulp/peel and seeds) is especially valuable due to its contents of fatty acids, carotenoids (mainly in pulp/peel), phytosterols, and tocopherols and tocotrienols (Li et al. 2003).
Oil content of sea buckthorn fruit

Generally, the oil from the pulp/peel fraction is combined due to the difficulty involved with separation. Yang and Kallio (2001) measured the oil content of the seeds (7.3% w/w dry basis (db)), pulp/peel (1.7% w/w db), and whole berry (includes seed) in wild *sinensis* berries (2.1% w/w db).

Climatic conditions influence oil accumulation in the berries (Schapiro 1989 as cited by Oomah 2003). Dry, warm periods during the spring increase oil content production. Humid conditions, extended wet and cold weather, and shortened periods of sunshine results in low oil content. Other parameters that affect oil content, include genetics, pollen origin, growing altitude, and time of harvest (Oomah 2003). Seed oil accumulates at a very fast rate with the onset of maturation to a maximum and remains constant, or begins to decrease as the fruit matures and ripens. Pulp oil, rises slowly over the maturation process and levels remain constant as the fruit reaches maturity, and ripens.

Yang and Kallio (2002) also investigated whether time of harvest and annual climatic conditions have an effect on seed size and oil concentration. No significant differences were found between years of harvest on weight of seed with respect to berry weight. Any differences noted may have been due to weather conditions, affecting the size and water content of the berries.

Oil quality of Sea Buckthorn fruit

The oils of the pulp, peel, and seeds contain varying levels of carotenoids (i.e. β-carotene) and fatty acids (i.e. oleic acid, palmitic, palmitoleic, α-linolenic) as well as vitamin E and phytosterols (Oomah 2003; Yang and Kallio 2001; Kallio et al. 2002).
The content of the various compounds in the oils is also influenced by climatic conditions, geographical location, berry variety and species, and berry maturity (Oomah 2003).

**Carotenoids**

Carotenoid content is the main parameter by which sea buckthorn oil is traded commercially (Beveridge et al. 1999). Carotenoids vary widely depending on the source of the oil, ranging from 50 to 2139 mg/100g. Pulp and fruit oils are a good source of carotenoids as can be seen by their rich colours, at about 5 to 10 g/kg (Xin et al. 1995 as cited by Oomah 2003). Seed oil usually contains low levels of carotenoids at about 20 to 85 mg/100g.

**Optimal harvest time** Gao et al. (2000) determined the change in levels of carotenoids with respect to the sampling period between August 6 and 25, 1997 in southern Sweden. Carotenoid levels increased in two out of three cultivars (5.1 to 11.5 mg/100g and 8.2 to 13.3 mg/100g) during the period. This may be due to some of the berries being slightly under ripe (greenish) at the beginning of the period.

**Fatty Acids**

Yang and Kallio (2001) determined a characteristic property of sea buckthorn berry pulp/peel oil is the high content of palmitoleic acid (C16:1n7) (20 to 30% of total fatty acids). Fruit pulp/peel oil consists of 36% saturated fat (mainly palmitic acid (C16:0)) and 64% unsaturated fat (28% palmitoleic (C16:1n7), 18% oleic (C18:1n9), 13% linoleic (C18:2), and 7% α-linolenic acids (C18:3n3)).
Although, high in concentration in fruit pulp/peel, palmitoleic acid is low in seed oil. Seed oil is characterized by high C18 unsaturated fatty acids (41% linoleic (C18:2), 27% $\alpha$-linolenic (C18:3n3), and 20% oleic (C18:1n9)) and lower saturated fat content (8.7% palmitic (C16:0) and 2.5% stearic (C18:0)) (Yang and Kallio 2001).

**Optimal harvest time** The end of September (early stage of maturity), was determined to be the optimal time of harvest for total levels of fatty acids in whole berries harvested during the period from late August to late November (Yang and Kallio 2002). Berries collected at different harvest dates, were not all from the same bushes or picked during the same year. Within the whole berries, the proportion of palmitoleic acid (C16:1n7) peaked in the middle of October (1.5 times higher than in mid-September). The levels of oleic acid (C18:1n9) presented a completely opposite trend.

The proportion of linoleic acid (C18:0) in the total fatty acid profile for seeds, showed a slight but steady increase (7%) from late September to late November (Yang and Kallio 2002). This was accompanied by a slight decrease (4%) in the proportion of $\alpha$-linolenic (C18:3n3) acid. The relative level of saturated fatty acids (i.e. palmitic (C16:0), stearic (C18:0)) in seeds remained fairly constant, whereas the proportion of oleic acid (C18:1n9) varied to a greater extent.

The objective of this research is to determine the effects harvest time has upon berry characteristics (i.e. berry colour, berry and seed size, moisture content, seed content, and oil content in berries and seeds) and bioactive compounds (i.e. carotenoids and fatty acids) in sea buckthorn berries (*H. rhamnoides* L. ssp. *sinensis*).
METHODS

Harvesting and post harvest handling

Sea buckthorn berries (*H. rhamnoides* L. ssp. *sinensis*) were manually harvested from five year old shrubs from the Branching Out Sea Buckthorn Orchard, St. Claude, MB, during the harvest year 2003-2004. Undamaged berries from 20 shrubs were collected in 200 g samples to allow for a representative sample from the orchard. The three harvest periods include: early maturity (September); maturity (early November), and post-maturity (January).

The bagged berries, were frozen in a thin layer using a method similar to the IQF (individually quick frozen) method (Feng et al 1999). Once completely frozen (a minimum of 24 h) the berries from each harvesting period were mixed to form three homogeneous pools of berries. The berries were bagged and kept frozen at -40 °C, until required. To prepare the berries for testing, they were thawed in a thin layer. The literature proved this post-harvest handling technique resulted in less damage to the berry structure (Feng et al 1999).

Temperature monitoring

Temperature was recorded at 15 minute 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, B.C.). The data logger was suspended in a Stevenson screen located in a row of shrubs in the orchard.
**Colour analysis**

Colour measurements were conducted on a sample of 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) L*a*b* colour system is followed (Francis 2003). The CIE scale measures the degrees of lightness (L*), redness or greenness (+/-a*), and yellowness or blueness (+/-b*) in a sample. The unit had been calibrated for white.

**Berry sizing**

Berry sizing was represented by the mass of a randomly selected batch of 100 thawed berries (g%) in triplicate (Tang and Tigerstedt 2001). Seed size was measured as the mass of 100 seeds (g%) in triplicate after air drying at room temperature (25°C) for 2 weeks (Tang and Tigerstedt 2001).

**Moisture content**

Moisture content (% w/w wb) was determined by the standard vacuum oven method according to the AOAC official method 934.06 for dried fruits (Boland 1995). Fresh fruits were prepared as per official method 920.149 (Boland 1995). A 5 g sample was dried at 70°C under vacuum (≤100mm Hg or 13.3 kPa) for 6 h. Duplicate determinations should agree within 0.2%.
**Fruit Preparation for carotenoid and fatty acid determination**

The seeds, pulp, juice, and skin each contain different levels of bioactive compounds. To simplify the process, the fresh berries were separated into two fractions: the seed fraction and the fruit fraction (skin, pulp, and juice).

**Fresh fruit** Pre-weighed thawed fresh whole berries were crushed in a blender (Sunbeam Model #4072, Oster, Sunbeam Corporation, Boca Raton, FL) on grind for approximately 2 min. This method allows for the total separation of the seed from its transparent coating and the fruit pulp without damaging the seed. The crushed mixture was strained to remove the juice, which was reserved. The seeds were removed from the pulp using forceps. Seeds were frozen until extraction was performed. The juice was added back into the seeded pulp to make the fresh fruit fraction. This preparation was performed in triplicate with three sub-samples.

**Seeds (fresh)** Seeds are collected and ground in a coffee grinder for 30 s. Of the final ground product approximately 10 g was used. This collection was performed in triplicate.

**Oil Extraction**

A triple extraction method using a chloroform:methanol solution in a ratio of 1:2 volume:volume (v:v) was employed for the fruit and seed fractions. The method is a modified version of the Folch method, established for removal of lipid from animal tissue, which states the chloroform:methanol solution should be in a 2:1 v:v ratio (Folch et al as cited by Dobson 2002). The change to increase the methanol fraction was made due to the high water content in the fruit.
The sample was homogenized (Heidolph Diax 900, Heidolph Instruments LLC, Cinnaminson, NJ) with the chloroform:methanol solution and filtered through a funnel. Distilled water in a ratio of 1:2 v:v to methanol was used to separate the lipid and aqueous layer. The layers were left to separate overnight.

A rotary evaporator (Yamato RE200, Yamato, Orangeburg, NY) and water bath (Yamato BM100, Yamato, Orangeburg, NY) set at 55°C were used to evaporate the lipid solution to dryness. To provide complete removal of the solvent, 5 to 8 mL of 2-propanol/iso-propanol was added in the final stages of evaporation. Final oil samples were stored in 5 mL and 10 mL hexane for the seed and fruit fraction oil, respectively.

**Carotenoids**

The determination of total carotenoids is based upon a method proposed by Gao et al. (2000). The fruit fraction oil:hexane solution (1 g/10 mL) produced in the extraction phase was diluted with hexane in a two step process: 100 µL oil/hexane:4.9 mL hexane and 500 µL oil/hexane:1.5 mL hexane. The seed oil/hexane solution (1 g/5 mL) was diluted with hexane in a concentration of 100 µL oil/hexane:1.9 mL hexane. The dilutions selected are based upon achieving an absorbance within 0.2 and 0.8. The total carotenoids were measured at 460 nm using a spectrophotometer (Spectronic, model 3000 ARRAY, Milton Roy, Ivyland, PA). Beta-carotene (type II: synthetic) is used as a standard. Total carotenoids are expressed in mg/100 g of oil, β-carotene equivalents.

**Fatty Acids**

The fatty acid compositional analysis using gas chromatography (GC) is adapted from the method proposed by Yang and Kallio (2001).
The esterified GC sample was prepared in the following manner. Hexane was evaporated using nitrogen, from a sample of lipid solution to yield 50 mg of lipids with an accuracy of 0.0001 g. A volume of 1 mL of an iso-octane solution (contains 1 mg of internal standard heptadecanoic acid, methyl ester (C17:1)) was added to the lipids and mixed to achieve a monophase system. A solution containing 12 mL 2% H₂SO₄ and 98 mL methanol solution was added to the lipid solution and mixed on a vortex (Vortex Maxi Mix I, model M16700, 120 V, 60 Hz, 0.5 A, Barnstead/Thermolyne, Dubuque, IA).

The vials were placed in an oven (1-2 h at 65-70°C) and mixed every 3 to 5 min for the first 20 min of heating or until a monophase system was achieved. Once cool, 6 ml each of iso-octane and distilled water were added to the vials and mixed by turning the vial upside down. A portion (1 mL) of the upper layer was transferred into a dry GC vial.

A 1.0 µL of solution was analyzed by using a GC (Hewlett Packard Gas Chromatograph system, model 5890, Palo Alto, CA) equipped with a programmed split/splitless injector and flame ionization detector. A Silica GC capillary column DB-23 (L=30 m; i.d.=0.25 mm; d_l=0.25 µm, J & W Scientific, Folsom, CA) was used. The linear velocity setting of the carrier gas (hydrogen) was 0.5 m/s (split valve ratio 1:80). The temperature program included maintaining a temperature of 155°C for 2 min then increasing at a rate of 2°C/min to 215°C and holding for 1 min. The fatty acid esters were identified by comparison with a standard mixture of known composition (461, NuChek Prep, Elysian, MN). The fatty acid composition was expressed as a mass percentage (% w/w in g/g) of the total fatty acids (mass of total fatty acid comprising total oil mass).
RESULTS and DISCUSSION

Temperature

The dry bulb ambient temperature for the Branching Out Orchard, St. Claude, Manitoba is presented in Fig. 1. The three harvest periods occurred during: September 4 to 8, 2003; November 9 to 12, 2003, and January 18 to 20, 2004. The mean, maximum, and minimum temperatures for September 4, 2003 through January 25, 2004 are provided in Table 1.

![Temperature graph](image)

**Fig. 1 Ambient field temperature for September 4, 2003 – January 25, 2004 (°C)**

**Table 1. The mean, maximum, and minimum temperatures for the 2003-2004 harvest period**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>11.66</td>
<td>6.16</td>
<td>-6.96</td>
<td>-8.63</td>
<td>-16.31</td>
</tr>
<tr>
<td>Maximum</td>
<td>34.01</td>
<td>27.34</td>
<td>9.15</td>
<td>4.58</td>
<td>-5.37</td>
</tr>
</tbody>
</table>
**Colour analysis**

Within an orchard there could be great variation in degree of ripeness of berries among neighbouring shrubs, resulting in some displaying the associated yellow to orange colour of ripeness, as well as those that have a slight greenish tinge of immaturity. The berry samples harvested in September did contain a percentage with a greenish tinge, while those harvested in November were fully ripe, bearing colours of yellow, orange, and red. The berries harvested in January continued to display the yellow, orange, and red colours seen in November, however, with less intensity. The colour analysis results reinforced the visual perception of the fruit samples.

The values of L*, a*, and b* are provided in Table 2. The results for September are L*= 45.23 ±0.66, a* = +14.19 ±0.46, and b* = +35.65 ±1.38. The colour values a* and b*, being positive confirm the yellow and red values in the fruit. The degree of redness was highest (P<0.05) at +20.18 ±0.38 for November, whereas the degree of yellowness was highest for the January samples (P<0.05) at 39.69 ±0.62. The degree of lightness L* was also highest in the January samples (P<0.05) at 47.21 ±0.40. The increase in lightness and yellowness as well as the decrease in redness for this harvest period may correlate with the loss of compounds such as carotenoids and vitamin C.

**Table 2: Colour analysis of sea buckthorn (H. rhamnoides L. sinensis) berries**

<table>
<thead>
<tr>
<th>CIELab Factor</th>
<th>Harvest month (2003-2004)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>September</td>
<td>November</td>
<td>January</td>
<td></td>
</tr>
<tr>
<td>Lightness, L*</td>
<td>Mean 45.23a ±0.66</td>
<td>Mean 45.46a ±0.85</td>
<td>Mean 47.21b ±0.40</td>
<td></td>
</tr>
<tr>
<td>Red/green, a*</td>
<td>Mean +14.19a ±0.46</td>
<td>Mean +20.18b ±0.38</td>
<td>Mean +17.44c ±0.40</td>
<td></td>
</tr>
<tr>
<td>Yellow/blue, b*</td>
<td>Mean +35.65a ±1.38</td>
<td>Mean +36.67a ±2.69</td>
<td>Mean +39.69b ±0.62</td>
<td></td>
</tr>
</tbody>
</table>

a – Means with the same letters are not significantly different at p=0.05 (Tukey’s test)
Berry and seed sizing

The berry size for September (15.62 g% ±0.99), as indicated in Table 3, was the lowest (P<0.05), possibly due to the lack of complete maturity in some berries. Berries continued to increase in size as ripening progressed from September to November (19.42 g% ±0.60) with a slight decrease to 17.93 g% ±0.92 in January. Differences in seed sizes were considered insignificant (P<0.05), ranging from 0.97 g% berries (November) to 0.99 g% (September and January).

Berry weight losses between November and January could be attributed to a loss of moisture, compounds, or change in structure due to freeze/thaw conditions. Berry sizes were similar to those reported by Tang and Tigerstedt (2001) at 18.08 g% for the year 1997 and 23.15 g% for the year 1998. Based on the large difference that occurred between growing years in the Chinese grown berries, yearly climatic and environmental conditions also appear to have an affect upon berry size. As with berry sizes, Tang and Tigerstedt (2001) reported consistently larger seed sizes of 1.35 and 1.54 g%.

Table 3. Characteristics of sea buckthorn (*H. rhamnoides* L. *sinensis*) berries

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Harvest month (2003-2004)</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>November</td>
<td>January</td>
</tr>
<tr>
<td>Berry size g%</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>15.62 a</td>
<td>0.99</td>
<td>19.42 b</td>
<td>0.60</td>
</tr>
<tr>
<td>Seed size g%</td>
<td>0.99</td>
<td>0.02</td>
<td>0.97</td>
<td>0.01</td>
</tr>
<tr>
<td>Moisture content (MC) (% w/w wb)</td>
<td>77.82</td>
<td>0.03</td>
<td>75.84</td>
<td>2.06</td>
</tr>
<tr>
<td>Seed content in fruit fraction (% w/w wb)</td>
<td>7.03 a</td>
<td>0.27</td>
<td>5.92 b</td>
<td>0.14</td>
</tr>
<tr>
<td>Oil content in fruit fraction (% w/w wb)</td>
<td>1.25</td>
<td>0.11</td>
<td>1.22</td>
<td>0.12</td>
</tr>
<tr>
<td>Oil content in seeds (% w/w wb)</td>
<td>10.74</td>
<td>0.26</td>
<td>9.41</td>
<td>0.72</td>
</tr>
</tbody>
</table>

*– Means with the same letters are not significantly different at p=0.05 (Tukey’s test)*
**Moisture content**

The moisture content results for September, November, and January were: 77.82% ±0.03; 75.84% ±2.06, and 75.82% ±0.06, respectively, as indicated in Table 3. The differences in moisture content between harvest dates are considered as insignificant (P<0.05) possibly due to the large standard deviation encountered for November. This standard deviation could imply that a larger sampling size may be required for testing due to the variability between berries. Overall, these results compare well with the 74% measured by Ma and Cui (1987) as cited by Tang and Tigerstedt (2001), however are lower than those determined by Tang and Tigerstedt (2001), 86.74% and 83.43%.

**Seed content in fruit**

The seed contents, as indicated in Table 3, varied significantly (P<0.05) between all harvesting months. Seed content was 7.03% ±0.27 for September, with a decrease to 5.92% ±0.14 by November, and return to a value in January of 6.62% ±0.31. The low seed content for November correlates well with the larger berry size and moisture content results. A higher content of moisture, pulp, and peel would result in a lower percentage of weight associated with the seed. The highest values encountered in September also correlate well with the berry size results. Due to incomplete ripeness of the fruit, the pulp, peel, and juice portions of the fruit would not have yet fully developed resulting in a larger mass percentage of seed. The seed content difference between November and January, may be due to a change in moisture content or other components within the berry. The values from this research fall into the range proposed by Yang and Kallio (2001), 3.9 to 9.0 %w/w wb).
Oil content in fruit and seed fractions

Oil content in the fruit fraction did not vary considerably (P<0.05) as indicated in Table 3. The values for September, November, and January were 1.25% ±0.11, 1.22% ±0.12, and 1.34% ±0.12, respectively. The slight increase in oil concentration for January, correlates with the loss of moisture that occurred between November and January. The oil content in seeds changed insignificantly (P<0.05) for the three harvest periods: Sept (10.74% ± 0.26), November (9.41% ± 0.72), and January (9.86% ± 0.68).

Carotenoids

The means of total carotenoids for the fruit fraction for the three harvest periods were significantly different (P<0.05). A graphical representation is provided in Fig. 2. The values ranged from 506.58 mg/100g ±48.59 in September to the high in November of 836.75 mg/100g ±60.51. A marked decrease in carotenoids occurred between November and January resulting in 650.74 mg/100 g ±58.73. This data correlates well with the values measured for colour, berry size, and seed content.

Based on these physical characteristics, September berries were deemed as being under-developed, therefore the carotenoids would not have reached their peak. The 65% increase from September through to November is comparative with the work done by Gao et al. (2000), which showed increases in carotenoid levels of 125% and 62%, during the ripening stage. The total carotenoids compare well with the 500 to 1000 mg/100g (5 to 10 g/kg) proposed by Xin et al. (1995).
In comparison to the total carotenoid levels measured in the fruit fraction oil, the levels in the seed oil did not significantly change between harvest dates. Results for the seed fraction are shown graphically in Fig. 3. The seed carotenoid levels of 24.37, 27.80, and 26.24 mg/100g oil fall into the lower range of levels (20 to 85 mg/100g oil) proposed by Gao et al. (2000).
Fatty acids

The fatty acid composition in the Sea buckthorn berry fruit fraction is presented graphically in Fig. 4. The data upon which this chart is based is provided in Table A.1 in the Appendix. Three main fatty acids, C16:0 (palmitic), C16:1n7 (palmitoleic), and C18:1n9 (oleic) account for approximately 32, 27, and 18% of the total fatty acids, respectively. Yang and Kallio (2001) had similar results with C16:0 (palmitic), C16:1n7 (palmitoleic), and C18:1n9 (oleic) accounting for 36, 28, and 18% of the total fatty acids, respectively.

![Graph showing fatty acid composition](image)

**Fig. 4. Proportion of individual fatty acids in fruit fraction oil**

*mass fatty acid / mass of total fatty acids %*

The only significant difference (P<0.05) between harvest times for the major fatty acids occurred between November (18.17% ±0.5) and January (19.25% ±0.97) for oleic acid (C18:1n9). This 5.94% change in proportion is in conjunction with slight distribution changes of other minor fatty acids.
Within a period from August to November, Yang and Kallio (2001) noted distinct changes within levels of palmitoleic acid (C16:1n7) and oleic acid (C18:1n9). These changes may be due in part to the fact that Yang and Kallio (2001) harvested berries at many times during the maturity period and that there may have been distinct variation between berries due to the method of sampling.

The fatty acid composition in the seed fraction is presented graphically in Fig. 5. The data upon which this chart is based is provided in Table A.2 in the Appendix. Three main fatty acids, C18:2 (linoleic), C18:3n3 (α - linolenic), and C18:1n9 (oleic) account for approximately 36, 29, and 19% of the total fatty acids, respectively. These results are in close agreement (4% difference) with the work performed by Yang and Kallio (2001) in which the corresponding fatty acids accounted for 41, 27, and 20%, respectively.

![Fig. 5. Proportion of individual fatty acids in seed oil (mass fatty acid / mass of total fatty acids %)](image-url)
The only significant difference (P<0.05) between harvest times for these three fatty acids occurred between September (36.16% ± 0.02) and January (36.59% ± 0.12) for linoleic acid (C18:2). This change in proportion is matched with insignificant (P<0.05) distribution changes of the other fatty acids. Of the total fatty acids, the third major fatty acid, oleic acid (C18:1n11) accounts for approximately 18.74% in the fruit fraction and 19.41% in the seed fraction. In sharp contrast to the fruit fraction, the seed fraction contains approximately 8.49% palmitic (C16:0) and 0.71% palmitoleic acid (C16:1n7).
CONCLUSIONS

November harvested berries yielded the highest levels (P<0.05), for berry size (19.42 g%), carotenoid levels (836.75 mg/100g), and colour (redness a*= 20.18). Early harvested (September) berries had the lowest (P<0.05) berry size (15.62 g%), fruit carotenoid content (506.58 mg/100g), and colour (redness a*=14.19). Late harvested berries (November) also had significantly different (P<0.05) levels of lightness (L*=47.21) and yellowness (b*=39.69).

Characteristics and compounds that did not change significantly (P<0.05) with harvest time include: seed size, moisture content, oil content, and carotenoid levels in seeds. Minor effects (1 to 6%) were demonstrated on the fatty acid distribution of both the fruit and seed fractions. Levels of the major fatty acids for the fruit fraction oil were: C16:0 palmitic (32.18%), C16:1n7 palmitoleic (26.16%), and C18:1n9 oleic (18.80%). The distribution of fatty acids was considerably different for seeds. Levels of C16:0 palmitic and C16:1n7 palmitoleic acids were 8.39% and 0.71%, respectively. The three major fatty acids for the seed fraction oil were: C18:2 linoleic (36.16%), C18:3n3 α-linolenic (28.91%), and C18:1n9 oleic (19.30%).

ACKNOWLEDGEMENTS

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REFERENCES


### APPENDIX

Table A.1 Fatty acids composition in sea buckthorn berry fruit fraction oil *(H. rhamnoides* L ssp. *sinensis)*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>September</th>
<th>November</th>
<th>January</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0 palmitic</td>
<td>32.10 ± 0.25</td>
<td>32.23 ± 0.60</td>
<td>32.21 ± 1.07</td>
</tr>
<tr>
<td>C16:1 palmitoleic</td>
<td>26.16 ± 0.50</td>
<td>26.82 ± 0.46</td>
<td>26.52 ± 0.75</td>
</tr>
<tr>
<td>C18:0 stearic</td>
<td>1.37 ± 0.05</td>
<td>1.20 ± 0.02</td>
<td>1.32 ± 0.15</td>
</tr>
<tr>
<td>C18:1n9 oleic</td>
<td>18.80 ± 0.60</td>
<td>18.17 ± 0.50</td>
<td>19.25 ± 0.97</td>
</tr>
<tr>
<td>C18:1n11 vaccenic</td>
<td>8.12 ± 0.15</td>
<td>8.04 ± 0.21</td>
<td>8.10 ± 0.38</td>
</tr>
<tr>
<td>C18:2 linoleic</td>
<td>6.90 ± 0.28</td>
<td>6.82 ± 0.26</td>
<td>6.64 ± 0.28</td>
</tr>
<tr>
<td>C18:3n3 α - linolenic</td>
<td>2.57 ± 0.26</td>
<td>2.26 ± 0.12</td>
<td>2.02 ± 0.12</td>
</tr>
</tbody>
</table>

* a – Means with the same letters are not significantly different at P<0.05 (Tukey’s test)

### Table A.2 Fatty acids composition in sea buckthorn berry seed fraction oil *(H. rhamnoides* L ssp. *sinensis)*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>September</th>
<th>November</th>
<th>January</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0 palmitic</td>
<td>8.57 ± 0.09</td>
<td>8.51 ± 0.09</td>
<td>8.38 ± 0.09</td>
</tr>
<tr>
<td>C16:1n7 palmitoleic</td>
<td>0.78 ± 0.03</td>
<td>0.64 ± 0.10</td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td>C18:0 stearic</td>
<td>2.30 ± 0.04</td>
<td>2.34 ± 0.14</td>
<td>2.31 ± 0.16</td>
</tr>
<tr>
<td>C18:1n9 oleic</td>
<td>19.30 ± 0.32</td>
<td>19.01 ± 0.32</td>
<td>19.92 ± 0.32</td>
</tr>
<tr>
<td>C18:1n11 vaccenic</td>
<td>2.30 ± 0.04</td>
<td>2.28 ± 0.05</td>
<td>2.25 ± 0.04</td>
</tr>
<tr>
<td>C18:2 linoleic</td>
<td>36.16 ± 0.02</td>
<td>36.59 ± 0.12</td>
<td>36.41 ± 0.13</td>
</tr>
<tr>
<td>C18:3n3 α – linolenic</td>
<td>28.91 ± 0.30</td>
<td>28.88 ± 0.78</td>
<td>28.23 ± 1.06</td>
</tr>
</tbody>
</table>

* a – Means with the same letters are not significantly different at P<0.05 (Tukey’s test)