Pectinesterase Activity and Proximate Analyses of Sea Buckthorn Juices

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ABSTRACT: Three varieties of sea buckthorn fruit were harvested and pressed to obtain juices. These were analyzed for pectin methylesterase activity, moisture, nitrogen, oil, pH, total acid, and °Brix. Yields of press juice for Hippophae rhamnoides ssp. rhamnoides L. varieties Luchistaya, Prozrachnaya, and ssp. mongolica Rousi cv. Indian Summer were 68%, 69%, and 66% w/w, respectively. Differences in juice composition were found to be in moisture content, °Brix, pH, and in total acid with the Luchistaya variety having the highest acid levels and lowest pH. Pectin methylesterase (PME) was present in all sea buckthorn juices with initial activity levels of 6.8 to 14.0 μ equivalents per min per 100 g of juice at pH 8 and 23 °C. Activity was pH-dependent with little PME activity at pH 3 to 5 and the highest activity at pH 8. The cream, pellet, and serum layers of centrifuged juices all contained PME. Heat treatment reduced PME activity in the juices by 1 decimal reduction or less.

Keywords: pectinesterase, sea buckthorn, juice, proximate analyses, centrifuge, pectin methylesterase, Hippophae rhamnoides

Introduction

Sea buckthorn is a nutritious fruit that is commercially processed and marketed as juice and juice blends in China, Russia, India, Germany, Pakistan, and Nepal (Li 2005). Generally sea buckthorn juice yields are 56% to 80%, with the higher levels obtained by adding enzymes to the fruit mash (Beveridge and others 1999, 2002). Aromas of strawberry, peach, mango, apricot, papaya, tropical, and citrus were frequently selected as descriptors of sea buckthorn juice (Tang and others 2001; Beveridge and others 2004), but sourness was the dominant flavor characteristic in both studies. Typically the juice looks similar to orange juice, is orange in color, opaque, and contains oil, nutrients, and insoluble solids. On standing the juice separates into an oil-rich cream layer above a cloudy/opalescent juice, with a sediment of large and dense particles. Centrifugation hastens and improves the separation, providing a compact cream layer above an opalescent juice or serum and a bottom pellet that contains cellular debris, trapped oil, and heavy particulate (Beveridge and Harrison 2001). The reasons for the separation are due to particle size, density, and possibly pectin methylesterase (PME) activity. PME, native in many fruit juices, reduces the pectin to pectic acid, which may then bind with calcium, precipitate, and thus reduce the amount of suspended particles in the juice (Whitaker 1994). No information on the presence or activity of PME in sea buckthorn juice has been encountered, but the total esterase activity (TEA) in developing fruit was documented by Skuridin and Harrison (1994) and Harrison and Beveridge (2001). The reasons for the separation are due to particle size, density, and possibly pectin methylesterase (PME) activity. PME, native in many fruit juices, reduces the pectin to pectic acid, which may then bind with calcium, precipitate, and thus reduce the amount of suspended particles in the juice (Whitaker 1994). No information on the presence or activity of PME in sea buckthorn juice has been encountered, but the total esterase activity (TEA) in developing fruit was documented by Skuridin and Privalov (1978) who found the fruit pulp levels decreased during fruit growth and development. Because the cloud stability of juice is very important from a marketing viewpoint (Beveridge 2002), the possibility that PME may destabilize sea buckthorn juices requires consideration.

Pectinesterase removes methoxyl groups from methylated pectin and releases a proton when each ester bond is hydrolyzed (Whitaker 1994). This causes the pH of the solution to decrease as pectinesterase attacks the pectin, thus the reaction may be followed by maintaining the pH at a set level, and recording the amount of base consumed over time. Plant PME, beta-pectinesterase, is most active between pH 6 and 8 and 30 °C, removes methoxyl groups in blocks and is capable of demethylating pectin to 20% to 30% of methyl groups (Benen and others 2003). The activity of PME is stimulated by NaCl and KCl, and may be reduced by phenolics, which bind with the enzymes and inactivate them (Whitaker 1994; Benen and others 2003). Recent studies of PME activity in fruit juices have measured the PME activity of orange juice between pH 7 and 8 and temperatures of 22 °C to 30 °C (van den Broeck and others 2006; Nienaber and Shellhammer 2001a; Lee and others 2003), oranges and limes at pH 7.0 and 25 °C (Evans and McHale 1978), soursop juice at pH 7.0 and 30 °C (Arbaisah and others 1997), and acerola juice at pH 8.3 and 27 °C (de Assis and others 2000).

Destruction of PME in fruit juices is usually achieved by heat treatment, high pressures, or combinations of both. The rate of enzyme destruction is often described by the decimal reduction time (D-value) and its temperature dependence (z-value) (Eagerman and Rouse 1976; Arbaisah and others 1997; de Assis and others 2000). Eagerman and Rouse (1976) found 2 decimal reductions or 99% destruction of orange juice PME to 10⁻⁴ PEu or 1.3 micro equivalents/min/100 g juice, based on 12.8 °Brix, produced a commercially stable juice. Other authors prefer to use 1st-order rate constants, k values, and activation energies (Ea), to predict and describe the percent destruction of PME at different temperatures, pressures, and times. The D and z values may also be calculated from the rate constants by equation (Nienaber and Shellhammer 2001a; Anthor and others 2002). Regardless of the method of calculation of thermal reduction, it is extremely important to report the initial enzyme activity found in the food system, as this value is required for comparison purposes and allows researchers and processors to determine how many decimal reductions are needed to produce a stable juice or juice blend, and decide whether certain juice blends are likely to create PME-related stability problems.
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Thermal reduction curves are complicated when working with cloudy juices, which are complex food systems that contain many components and often more than 1 type of PME. Nienaber and Shellhammer (2001b) found PME attached to the cell walls of orange sacs caused an increase in activity remaining after heat and pressure treatments. Versteeg and others (1980) separated 3 pectinesterases from navel oranges and obtained linear enzyme reduction curves for the pure enzymes, but found when a mix of the crude enzymes were heated in orange juice, the heat stability curves were nonlinear due to the presence of more than 1 enzyme. Orange, grapefruit, lime, tomato, souros, and acerola fruit all contain at least 2 pectinesterases with different heat stability profiles (Evans and McHale 1978; Arbaisah and others 1997; de Assis and others 2004). Moisture was determined by weight loss after freeze-drying (Kalinina and Panteleyeva 1987) and Beveridge and others (2002). The moisture content is in the range reported by Beveridge and others (2002) and higher than values reported by Tang and others (2001). The total acid was calculated as quinic acid as that was the major acid found in sea buckthorn fruit (Lu 1990; Beveridge and others 1999, 2002). The pH of all 3 varieties was 3.0 to 3.3, which is in the range reported by Beveridge and others (2002) and higher than values reported by Tang and others (2001). The total acid was calculated as quinic acid as that was the major acid found in sea buckthorn fruit by high-performance liquid chromatography (Beveridge and others 2002). For comparison with other literature values, the titratable acidity may be converted to 1.12% to 1.71% malic acid by dividing the table values by 2.86 (Beveridge and others 2002). The total acid values are at the low end of the range reported by Kalinina and Panteleyeva (1987) and Beveridge and others (2002) and are 22% to 72% of the values reported by Tang and others (2001) and Beveridge and others (2004). The moisture content was 15% to 19% higher than the values reported by Beveridge and others (2004). The oil was in the range of juice oil contents reported by Beveridge and others (1999), whereas protein was between

Materials and Methods

**Juice production**

Sea buckthorn (Hippophae rhamnoides ssp. rhamnoides L. varietiess Luchistaya, Prozrachnaya, and ssp. mongolica Rousi cv. Indian Summer) fruit were harvested from experimental plots at the Sumnerland Pacific Agri-Food Research Centre, September 9, 2003, with a suspension shaker harvester (Beveridge 2003a). The leaves were removed from the collection trays by air classification and the fruit was transferred to clear plastic bags and stored at 0 °C overnight. Fruit were crushed between counter-rotating cylinders set 1.4 mm apart (Beveridge 2003b) and pressed by rack and cloth at 21 MPa (Beveridge and Harrison 2005). Juice was pooled by variety, mixed well, dispensed into containers, and frozen at −25 °C.

**Proximate analyses**

Degrees Brix were determined with an Abbe Mark II refractometer (AO Scientific Instruments, Buffalo, N.Y., U.S.A.); pH and total acids were determined by the methods of Beveridge and others (2004). Moisture was determined by weight loss after freeze-drying and nitrogen and oil content were determined on freeze-dried powder utilizing the LECO nitrogen analyzer for nitrogen (LECO Instruments Ltd, Mississauga, Ont., Canada) and the Goldfisch fat extraction apparatus for oil (Labconco, Kansas City, Mo., U.S.A.), as described in Beveridge and others (2004).

**Heat treatments**

Juices (40 g) were heated to 50 °C, 70 °C, 80 °C, 90 °C, and 99 °C in 125-mL Erlenmeyer flasks with ground glass stoppers in a covered reciprocal shaking water bath (Precision Scientific, Winchester, Va., U.S.A.) at speed 150. Thermocouples (Omega, Stamford, Conn., U.S.A.) were inserted in the water bath and in the center of juice samples to monitor temperature every 10 s. Samples reached target temperatures within 2 min and cooled to 4 °C within 2 min of removal when mixed in an ice bath. Heat treatment time was measured as the time the sample reached and remained at the target temperature. Samples (30 g) were titrated for pectin methyl-esterase (PME) activity at pH 8.0 and 23 °C or 30 °C as noted.

**Juice centrifugation**

Thawed juice samples (30 g) were centrifuged in pre-weighed straight-wall centrifuge tubes in a refrigerated centrifuge (model RC 5B plus; Sorvall, Guelph, Ont., Canada) at 38000 × g and 5 °C for 16 min. The juice serum was decanted from the cold tube into a beaker while holding back the cream layer with a frozen spatula. The cream layer was scooped out and weighed. The side walls of the tube were then cleaned of any traces of cream and the weight of the pellet was obtained by difference. Juice serum was titrated for PME at pH 8.0 and 30 °C as described subsequently. The pellet and cream samples were suspended in 30 mL of 1% malic acid (BDH; VWR Intl., Mississauga, Ont., Canada) and titrated for PME activity at pH 8.0 and 30 °C. All analyses were repeated in triplicate.

Pectin methylesterase activity and pH effects

Pectinesterase activity was determined by the method of van den Broeck and others (2000) with some modifications. Sodium chloride solution, 10 mL of 0.125 N NaCl (Sigma; Sigma-Aldrich Canada Ltd., Oakville, Ont., Canada), was added to 30 g of juice, water, buffer, or centrifuged sample. The pH was adjusted to the target pH plus 0.01 pH unit with dilute NaOH or HCl, then the pH was maintained by auto-titration with 0.1 N NaOH (719 S autotitrator; Metromh, Herisau, Switzerland) for at least 10 min to obtain blank or baseline activity. Pectin solution (10 mL of 1% apple pectin [Fluka; Sigma-Aldrich Canada Ltd.] dissolved in 0.125 N NaCl) was then added to the sample, pH was readjusted, and the sample re-titrated to determine PME activity. Pectinesterase standards (Sigma, P-5400, P-1889) were diluted to 101 nominal units of activity/mL in volumetric flasks, dispensed into 1-mL Eppendorf vials, and frozen at −5 °C until used. Buffers of 1% malic acid in Milli-Q water were used to determine blank and standard activities. Thawed PME standard (200 μL) was added to the titrated pectin plus water or buffer blanks and the titration continued at 23 °C or 30 °C as noted. Effects of pH were determined at pH 3 to 11 and 23 °C. Pectin esterase activity was determined as micro equivalents of activity per min per 100 g of sample (PMEu):

\[
PMEu = \frac{(mL \text{NaOH} \times \text{molarity} \times 1000)}{\text{time (min) \times sample (g)}} \times 100
\]

Results and Discussion

**Juice composition**

The 3 varieties of sea buckthorn yielded similar quantities of juice, 66% to 69% w/w (Table 1), which was in the range of juice recovery expected from sea buckthorn fruit (Lu 1990; Beveridge and others 1999, 2002). The “Brix of 6% to 10% was in the range of average values for sea buckthorn reported by Beveridge and others (1999). The Indian Summer cultivar had the highest “Brix, which was consistent with values reported for Indian Summer by Beveridge and others (2002). The pH of all 3 varieties was 3.0 to 3.3, which is in the range reported by Beveridge and others (2002) and higher than values reported by Tang and others (2001). The total acid was calculated as quinic acid as that was the major acid found in sea buckthorn juice by high-performance liquid chromatography (Beveridge and others 2002). For comparison with other literature values, the titratable acidity may be converted to 1.12% to 1.71% malic acid by dividing the table values by 2.86 (Beveridge and others 2002). The total acid values are at the low end of the range reported by Kalinina and Panteleyeva (1987) and Beveridge and others (2002) and are 22% to 72% of the values reported by Tang and others (2001) and Beveridge and others (2004). The moisture content was 15% to 19% higher than the values reported by Beveridge and others (2004). The oil was in the range of juice oil contents reported by Beveridge and others (1999), whereas protein was between
Pectinesterase activity in sea buckthorn . . .

Table 1—Yield and proximate analyses of sea buckthorn juices*

<table>
<thead>
<tr>
<th>Variety/cultivar</th>
<th>Juice yield</th>
<th>°Brix</th>
<th>pH</th>
<th>Total acid</th>
<th>Moisture</th>
<th>Oil</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian Summer</td>
<td>66.3</td>
<td>9.9 ± 0.2</td>
<td>3.3 ± 0.0</td>
<td>3.26 ± 0.15</td>
<td>88.50 ± 0.05</td>
<td>1.49 ± 0.08</td>
<td>0.85 ± 0.01</td>
</tr>
<tr>
<td>Luchistaya</td>
<td>68.3</td>
<td>6.7 ± 0.1</td>
<td>3.0 ± 0.0</td>
<td>4.89 ± 0.01</td>
<td>91.70 ± 0.04</td>
<td>1.42 ± 0.05</td>
<td>1.02 ± 0.02</td>
</tr>
<tr>
<td>Prozrachnaya</td>
<td>69.0</td>
<td>6.1 ± 0.1</td>
<td>3.21 ± 0.00</td>
<td>92.37 ± 0.02</td>
<td>1.47 ± 0.02</td>
<td>1.10 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

*aAverages and standard deviations of triplicate samples of pooled juice.
*bPercent of juice obtained from pooled fruit from 2 to 7 trees.
*cPercent total acid calculated as quinic acid.
*d(Total weight – dry weight) expressed as %.
*eOil calculated as % wet weight.
*fPercent protein calculated as (%nitrogen wet weight × 6.25).

Table 2—Pectin methylesterase (PME) activity at pH 8 and 30 °C in centrifuged juice fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Layer</th>
<th>Weight PME units/100 g initial juice</th>
<th>PME units/100 g of fraction (ww)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% ww Baseline Pectin added</td>
<td>Baseline Pectin added</td>
</tr>
<tr>
<td>Indian Summer</td>
<td>Cream</td>
<td>4.1 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Pellet</td>
<td>0.9 ± 0.0</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>94.4 ± 0.2</td>
<td>6.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>99.4 ± 0.2</td>
<td>7.3 ± 1.8</td>
</tr>
<tr>
<td>Luchistaya</td>
<td>Cream</td>
<td>3.1 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Pellet</td>
<td>0.7 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>95.9 ± 0.2</td>
<td>3.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>99.6 ± 0.0</td>
<td>4.0 ± 1.1</td>
</tr>
<tr>
<td>Prozrachnaya</td>
<td>Cream</td>
<td>3.0 ± 0.2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Pellet</td>
<td>0.4 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>95.8 ± 0.2</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>99.2 ± 0.1</td>
<td>5.7 ± 1.2</td>
</tr>
<tr>
<td>Malic acid buffer</td>
<td>Cream</td>
<td>98.5 ± 0.9</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Milli-Q water</td>
<td>99.6 ± 1.0</td>
<td>0.1 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
</tbody>
</table>

dthe ranges found in raw juice by Beveridge and others (1999, 2004). It appears the fruit was fully ripe as the pH was high and the total acid was low for sea buckthorn juices.

Pectin methylesterase activity and pH effects

The pectin methylesterase (PME) activity of sea buckthorn juice (Figure 1) was virtually undetectable at pH 3 to 5 for all 3 varieties of juice, with slight activity at pH 6, and definite activity between pH 7 to 9. The optimum pH for PME activity was pH 8 for varieties Luchistaya and Prozrachnaya and pH 8 to 9 for the cultivar Indian Summer, resulting in activities of 5.2 to 12.1 micro-equivalents of PME per min per 100 g of juice (PMEu) at 23 °C. These levels are 50 to 440 times lower than the 600 units/100 g of extracted limes and 2300 units/100 g of extracted oranges obtained by Evans and McHale (1978), and 20 to 100 times lower than the 246 to 512 PMEu (based on 12.8 °Brix) for Valencia orange juice (Rouse and Atkins 1952; Eagerman and Rouse 1976). Luchistaya and Prozrachnaya juice blanks, titrated before pectin addition, displayed little or no activity over the pH range of 3 to 9 (Table 2). The Indian Summer blank had detectable activity between pH 7 and 9, with no activity at pH 6 and below. This indicates that cv. Indian Summer contained pectin in the juice, whereas the other 2 varieties had little, if any, pectin in their juices. The pectin blank had little or no activity over the pH range of 2 to 9 but had very high activity at pH 11, which was likely due to auto hydrolysis of the pectin.

Thermal destruction

Thermal destruction of PME in sea buckthorn juice did not follow trends of linear or semi-log reductions of activity as time and temperature were increased (Figure 2). The 40-g juice samples required up to 2 min to achieve the desired temperature for heat treatment and the activity at zero time was much lower than the initial PME activity before heat treatment, so most of the heat inactivation curves were lost during the come-up times. Semi-log curves (Figure 2a) show that only the Prozrachnaya variety achieved 1 log reduction of activity, which occurred within 5 min at 70 °C. The PME activity in the Luchistaya and Indian Summer juices was not reduced by a full log with any of the heat treatments that ranged from 50 °C to 120 °C.
Pectinesterase activity in sea buckthorn...

Table 3—Comparison of initial pectinesterase activities for pH, temperature, and centrifugation effects

<table>
<thead>
<tr>
<th></th>
<th>pH effect*</th>
<th>Temperature effect*</th>
<th>Centrifugation effect*</th>
<th>Standards*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian Summer + pectin</td>
<td>9.7 ± 1.4</td>
<td>9.2 ± 0.6</td>
<td>13.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Indian Summer blank</td>
<td>3.6 ± 0.9</td>
<td>5.3 ± 0.2</td>
<td>7.3 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Luchistaya + pectin</td>
<td>5.2 ± 3.4</td>
<td>6.8 ± 0.5</td>
<td>10.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Luchistaya blank</td>
<td>0.4 ± 0.6</td>
<td>2.1 ± 0.8</td>
<td>4.0 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Prozrachnaya + pectin</td>
<td>10.7 ± 0.3</td>
<td>14.0 ± 0.8</td>
<td>18.3 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Prozrachnaya blank</td>
<td>0.0 ± 0.0</td>
<td>3.5 ± 0.4</td>
<td>5.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Pectin methyltransferase</td>
<td>12.9 ± 0.1</td>
<td>0.2</td>
<td>0.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Pectin blank</td>
<td>0.4 ± 0.6</td>
<td>0.2</td>
<td>18.7 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Milli-Q water no pectin</td>
<td>11.5 ± 0.8</td>
<td>31.9 ± 3.4</td>
<td>0.1 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Buffer + pectin + PME</td>
<td>11.5 ± 0.8</td>
<td>31.9 ± 3.4</td>
<td>0.1 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Buffer + pectin + PME</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Buffer only (1% malic acid)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

*Pectin methyltransferase activity units/100 g at pH 7.5 and 30 °C.
*Pectin methyltransferase activity units/100 g at pH 8 and 30 °C.
*Heat-stable PME, with the level obtained being inconsistent among heat treatments at and above 70 °C. This indicates that either the standard 1 with predicted activity of 67.25 units/100 g. 
*Standard 1 with predicted activity of 67.25 units/100 g.
*Standard 2 with predicted activity of 67.37 units/100 g.

To 99 °C for up to 4 h. The vertical axes of the curves in Figure 2 represent activity as PMEU to allow easy comparisons of the actual activities of the 3 juices. Heat treatment of the juices at 50 °C for 5 min caused 80% and 75% reductions of pectin esterase activity in Prozrachnaya and Luchistaya juices, respectively, while it only mildly affected the PME activity in Indian Summer juice. Heating the juice to 70 °C reduced the PME activity in Prozrachnaya by a further 10%, but did not reduce the activity in Luchistaya juice. Heat treatment at 80 °C for 0.2 to 15 min reduced the PME activity in Indian Summer juice by 60% to 70%. Heating the juices at 99 °C did not further reduce the PME activity. These results indicate there are at least 2 types of pectinesterase in sea buckthorn juice, 1 being heat-labile and the other heat-stable. The heat-labile PME was degraded at 50 °C for the Prozrachnaya and Luchistaya varieties and at 80 °C for Indian Summer juice. The heat-stable PME did not appear to degrade at temperatures of 99 °C or lower. Similar results were obtained with Guava (Labib 1999), which required 16 min at 95 °C to destroy the heat-stable PME and Acerola (de Assis and others 2000), which required 98 °C for 110 min or 106 °C for 5 min to destroy the heat-stable PME. The heat-stable PME in sea buckthorn juice was more heat-resistant than the heat-stable PME enzymes of acerola and guava. These results show that heat treatment alone will only reduce, and not completely destroy, the PME activity in sea buckthorn juices.

Prozrachnaya and Luchistaya juices were reduced to 1 to 3 PMEu at 70 °C to 99 °C, while Indian Summer juice was reduced to 2 to 5 PMEu at 80 °C to 99 °C (Figure 2b). These values are close to that of a stable orange juice, 1.3 PMEu (Rouse and Atkins 1976), but only the Prozrachnaya juice PME activity level was reduced to 1 PMEu, with the level obtained being inconsistent among heat treatments at and above 70 °C. This indicates that either the standard error in the heat treatments or titrations was high, or PME was released from components in the cloudy juice matrix. Release of PME from cellular components in the juice matrix upon heating is consistent with the findings of Nienaber and Shellhammer (2001b).

The PME activities of sea buckthorn juices were only 1% to 5% of the activity found in orange juice by Rouse and Atkins (1952) and 0.2% to 0.5% of the activity extracted from oranges by Evans and McHale (1978). The initial activity in sea buckthorn juice was further reduced 50% to 80% by mild heat treatment, much of which occurred in the initial come-up time. The heat-stable PME in sea buckthorn may not be a problem in juice blends, as Nienaber and Shellhammer (2001b) found orange juice with 3.9% residual PME activity had a stable shelf life of 3 mo at 4 °C. However, Versteeg and others (1980) found the heat-stable high-molecular-weight PME in orange juice, which accounted for only 5% of the PME activity, resulted in cloud instability at 5 °C in less than 1 wk. Sea buckthorn juices and blends should be tested for cloud stability before marketing to ensure that the low levels of enzyme present do not cause cloud instability problems.

Centrifuged juice components

The centrifuged juices were composed of 3% to 4% cream, 0.4% to 1.0% pellet, and 94% to 96% serum (Table 2). These results are similar to those obtained by Beveridge and Harrison (2005). The cream and serum components of all 3 varieties tested contained PME activity in both the layer only and in the layer plus pectin samples (Table 2). The pellet component showed activity in all varieties when pectin was added, but the activity in the Luchistaya and Prozrachnaya pellet samples without pectin added was close to the activity obtained with the water and buffer blanks. In all cases, except the Indian summer serum, the addition of pectin to the sample increased the activity. This indicates that pectin is a limiting factor in the reactions for all fractions but the Indian Summer serum. For the Luchistaya and Prozrachnaya varieties, most of the juice PME activity was found in the serum, the next-largest amount was found in the cream layer, and the lowest amount was found in the pellet layer. The Indian Summer serum and cream layers contained similar levels of PME activity whereas the pellet contained much less activity. When the PME activity was converted to units per 100 g of component layer (Table 2) the cream layer and pellet layers had similarly high levels of PME activity with the serum containing much lower levels of activity for all samples. These results show that the PME activity in the cream and pellet layers was not carried over from serum residing in these components, but was due to the matrix making up the pellet and cream layers. This is consistent with the thermal destruction data presented earlier, suggesting the release of PME from juice particulate due to heating may have contributed to increased PME activity (Figure 2). The concentration of PME activity in the matrix of both the cream and pellet layers was higher than that found in the serum layer, but because the serum was the major fraction of the juice components the PME activity of the serum accounts for most of the PME activity in the juices, except for Indian Summer in which the cream layer contains a similar level of activity to the serum. The total PME activity of the centrifuged samples at pH 8 and 30 °C (Table 2 and 3) was higher than the PME activity of juice at pH 8 and 23 °C in the pH and temperature trials (Table 3). This was also true of the stan-
Pectinesterase activity in sea buckthorn juice contained at least 2 forms of PME, 1 being heat-labile, the other very heat-stable. Centrifugation of all juice samples produced 3 layers, all of which contained PME activity. The PME activity in the cream and pellet layers was higher than that of the serum when calculated as PME units per 100 g of wet fraction, but because the juice was composed primarily of serum, the largest fraction of activity was in the serum for Luchistaya and Prozrachnaya varieties, and was in the serum and cream layers of Indian Summer juice.

Conclusions

The actual concentrations of PME in sea buckthorn juice are very low compared with orange juice so may not be a problem for cloud stability in these juices, or juice blends, but storage trials should be conducted before marketing of cloudy juice products containing sea buckthorn juice components.

References


Li T. 2005. Personal communication. Agriculture and agr-Food Canada, Pacific Agri-Food Research Centre, Summerland, B.C., Canada.


