JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 511–517 **511**

Free Galactose Concentrations in Fresh and Stored Apples (*Malus domestica*) and Processed Apple Products

CHRISTINE H. SCAMAN,*,† VICKIE JIN WAI JIM,† AND CAROL HARTNETT‡

Food, Nutrition, and Health, 6650 Northwest Marine Drive, University of British Columbia, Vancouver, V6T 1Z4 British Colombia, Canada, and British Columbia's Children's and Women's Hospital, 4480 Oak Street, Vancouver, V6H 3V4 British Colombia, Canada

Gas chromatography was used to quantitate free galactose in Braeburn, Fuji, Red Delicious, and Spartan apples during cold storage, after thermal processing of apple slices and in juice produced using clarification and/or liquifaction enzymes. Spartan had significantly higher galactose levels as compared to Red Delicious apples, but changes in galactose in all varieties during 9 months of cold storage were insignificant. Blanching and canning decreased galactose levels, but doubling the thermal processing during canning increased the free galactose concentration detected in plant tissue. An enzymatic liquefaction aid used to prepare apple juice dramatically increased the free galactose content while a clarification aid caused only a slight increase due to its selective action on soluble pectin. These findings provide useful information for dietitians to base diet recommendations for galactosemic patients.

KEYWORDS: Galactose; galactosemia; apple; storage; variety; thermal processing; juice; enzyme; pectinase; cellulase; trimethylsilyl derivative; *Malus domestica*

INTRODUCTION

Galactosemia is an inherited syndrome associated with an inability to metabolize galactose due to defects in any of several enzymes (*1*). It is an autosomal recessive disorder, occurring in the United States with a variable frequency of between 1 in 18 000 and 1 in 70 000 (*2*). Data from a Taiwan screening program suggested an incidence of 1:400 000 in the Chinese population (*3*), and a similar study from Saudi Arabia showed an incidence of 1:8300 (*4*). The incidence in British Columbia has been shown to be 1:36 200 (*5*). Despite early diagnosis and dietary therapy, chronic complications such as cataracts, gynecologic failure, speech and language delays, neurologic impairment, and failure to thrive in infants are reported. Outcome studies have reported deficits of cognitive function that were variable and not related to the age at diagnosis or compliance with the classical galactose free diet (*6*). Fifty-four percent of the treated galactose patients in Oregon were reported to have the specific speech disorder verbal dyspraxia (*7*). As well, a review of 20 years of screening and patient outcomes in Ireland reported that out of 32 children who were diagnosed early and followed the classical low galactose diet, only 13 had no detectable complications. Nineteen were found to have either one or a combination of the reported complications (*8*).

There is no cure or drug therapy for galactosemia. The only recourse available to patients is diet modification to restrict intake of galactose while providing sufficient nutrients and energy for normal growth and development. In the past, patients were encouraged to include large amounts of fruits and vegetables in their diet, as these items were considered to contribute negligible amounts of galactose. However, several studies have shown that fruits and vegetables can contribute significant amounts of galactose (*9*−*11*). Estimates of galactose intake from fruits and vegetables on a traditional galactose-restricted diet range from 100 mg per day (*10*) to greater than 500 mg per day (*9*). However, because galactose does not contribute significantly to the caloric value of foods, there is little information available on the concentration of galactose in fruits and vegetables and virtually no information on the effect of variety, storage, or processing on galactose concentrations. Cultivar difference might affect the amount of free galactose in produce since it is known that different cultivars of a same product such as apples have different amounts and compositions of major sugars such as fructose and glucose (*12*−*14*). Galactose in fruits and vegetables is mainly found bound to the side chains of cell wall polysaccharides such as hemicellulose and pectin. During ripening, storage, and processing, these polysaccharides can be enzymatically and chemically solubilized and degraded (*15*−*17*). However, it is not known if the galactose that is liberated from pectic polysaccharides remains in the plant tissue in free form, is lost, or is further metabolized. This lack of information makes it very difficult for dietitians to make dietary recommendations to galactosemic patients.

This study was intended to determine how variety, storage, thermal processing, and juice production techniques affect galactose concentrations in apples and apple products. Apples

* To whom correspondence should be addressed. Tel: 604-822-1804. Fax: 604-822-3959. E-mail: cscaman@interchange.ubc.ca.
† University of British Columbia.
‡ British Columbia's Children's and Women's Hospital.

10.1021/jf034643k CCC: $27.50
Published on Web 01/14/2004

are a common dietary item in North America and are widely available throughout the year and in a variety of processed forms. The specific information on the galactose content of apple and apple products is of interest to dietitians. As well, this is the first study to systematically examine the effects of aging and processing on galactose levels in apples and products, and the results may serve as a model for other fruits and vegetables. This information will allow dietitians to give better recommendations to galactosemic patients in selecting the appropriate food items.

METHODS AND MATERIALS

Storage Study. Four varieties of apple, Braeburn, Spartan, Fuji, and Red Delicious, were harvested in October 2000, at optimum maturity, and stored in fiber board cartons in a 4 °C walk-in chamber immediately after harvest. Four samples were taken during a 9 month storage period on October 26, 2000, and then at 3 month intervals in January 2001, April 2001, and July 2001. At each sampling period, 4−5 apples from each cultivar, free from defects, were randomly removed from storage. These were peeled, cored, diced into approximately 0.5−1 cm^3 sections, and mixed, and three 10 g fresh weight (gfw) samples were taken for galactose analysis as described below.

Thermal Processing. Fuji apples stored for 9 months were peeled, cored, and sliced into 0.5 cm wide strips and blanched in boiling water in a steam kettle for 2 min. Three blanching sessions were done, and three samples were taken from each blanching session for galactose analysis as described below.

After blanching, the samples were drained with a household sieve and 330 g of apple pieces was hand-filled into a no. 2 (307 × 409) "C" enamel can (Wells Can, Burnaby, BC). Blanching water was used to fill the cans, leaving 10 mm of headspace, and the cans were sealed immediately with a hand-operated can sealer. Ecklund needle type rigid thermocouples were inserted 1 in. from the bottom of the can, and temperature data were collected and recorded using a Data Taker DT 100F (Data Electronics Australia Pty. Ltd.) with Decipher version 1.02 (Data Electronics Australia Pty. Ltd.). Cans were processed to achieve commercial pasteurization (Po = 10.7 ± 0.51 min) and twice commercial pasteurization (Po = 21.6 ± 0.43 min), calculated by the Improved General Method developed by Ball using a T_r of 121 °C and z of 10 °C (*18*). Triplicate canning sessions of six cans were carried out for each product, and three samples were analyzed for galactose from each session. Canned products were stored at 4 °C for 2 weeks prior to sugar extraction.

Juice Production. Juice was produced from Red Delicious apples, bought from a local market in October 2001. For all juices, apples were first peeled and pureed with a food processor. Following the various treatments noted below, juices were centrifuged at 16 300*g* for 15 min at 20 °C, followed by pasteurization in a boiling water bath until the juice reached 95 °C for 1 min. Juices were stored at −25 °C until sugar analysis was carried out.

Four different protocols were used for juice production. Treatment 1 had no addition of enzymes. A 400 g sample of apple puree was spread evenly on a sieve (mesh no. 100), and the juice was collected by gravity at room temperature for 30 min. Treatment 2 involved the addition of Pectinex Ultra-SPL, an enzyme preparation used for liquifaction of plant tissue. Pectinex Ultra-SPL (0.044% (v/w)) (Novo Industries AS, Copenhagen, Denmark) was added to 400 g of apple puree. The puree was heated to and held at 50 °C for 2 h with constant stirring, prior to collection and pasteurization. For treatment 3, Ultrazym 100 (Novo Industries AS), a clarification aid, was added at 100 mg/L to the collected juice. The juice was heated to and held at 40 °C for 20 min with constant stirring. The fourth treatment included addition of both Pectinex Ultra-SPL and Ultrazym 100 as described above. Each treatment was carried out in triplicate.

Sugar Extraction. Free galactose present in food materials was extracted using established protocols (*9*, *19*). Blanched and canned apple slices were drained to remove excess processing liquids with a household sieve for 30 min before extraction.

Ten gram samples of fresh, blanched, and thermal-processed apples were taken, and each was placed in 30 mL of 80% ethanol. Samples were then placed in a boiling water bath for 10 min, stored at −18 °C for 16 h, and then homogenized with a Polytron (Brinkmann Instruments) at speed 7. The homogenates were vacuum filtered through Whatman no.1 filter paper. Residues were rinsed with 3 mL of 80% ethanol, and the filtrates were combined and centrifuged at 20 000*g* for 15 min. Supernatants were collected and brought up to 30 mL total volume with 80% ethanol. A 10 mL aliquot of each sample was then passed through a C_{18} Sep-Pak cartridge (Waters Corporation), the cartridge was then rinsed with 2 mL of water, and the filtrates were pooled. Three 0.3 mL aliquots from each extract were taken to dryness in a vial with a stream of nitrogen at 45 °C. Dried samples were stored at −25 °C until derivatization for gas chromatography was carried out.

For apple juice, 0.3 mL of each type of apple juice was used directly for analysis. Before derivatization, the samples were lyophilized using a SC110 SpeedVac concentrator (Savant Instruments, Inc.).

Sugars Derivatization and Gas Chromatography Quantification. Sugars extracted from fruits and vegetables were converted to trimethylsilyether/ester (TMS) and TMS-oxime derivatives (*20*−*21*). The sugars and 1 mL of pyridine, containing 1.25 g of hydroxylamine hydrochloride 100 mL^{-1} and 0.1 mg/mL phenyl-β-D-glucopyranoside (internal standard), were mixed and heated for 30 min at 75 °C. The cooled samples were then trimethylsilylated with a mixture of 1.8 mL of hexamethyldisilazane (HMDS) and 0.2 mL of trifluoroacetic acid (TFAA) for 60 min at 100 °C. Thereafter, the solutions were transferred to 4 mL glass vials and were evaporated to dryness under nitrogen at 45 °C. One milliliter of a solution of HMDS:TFAA = 9:1 was then added to each vial to dilute the sample prior to chromatographic analysis.

Complete separation of galactose from other soluble sugars was accomplished on an SBP-1 column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Supelco Inc., Toronto, ON, Canada) under the following conditions: carrier gas, helium; flow rate, 1 mL/min; injector port temperature, 250 °C; detector temperature, 300 °C; and injection volume, 1 μL. The oven temperature program consisted of an initial temperature of 180 °C held for 5 min that was increased to 200 °C at 5 °C/min, held for 11 min, and increased to 270 °C at 10 °C/min and held at 270 °C for 13 min. The total analysis time for each injection was 40 min. Flow rates of the helium makeup gas and the hydrogen gas were set at 30 mL/min and for air at 60 mL/min. The head pressure of the column was set at 15 psi, and the flow rate of the helium carrier gas was 1.7 mL/min. Peak integration was performed with the JCL 6000 Chromatography Data System for PC (Jones Chromatography, Lakewood, CO).

A 10 point standard curve ranging from 0.001 to 0.06 mg/mL galactose and an internal standard (phenyl-β-D-glucopyranoside) concentration of 0.1 mg/mL was performed to determine the linear range and response factor of galactose peak area with reference to the internal standard. Each sample was run in triplicate. The ratio of the arabinose and sucrose peaks as compared to the internal standard was used as an indicator of the relative concentration of these sugars in some samples.

Statistical Analysis. Data were statistically analyzed using Minitab statistical software (Minitab release 13.30, Minitab Inc., PA). Two way analysis of variance (ANOVA) with replication was used for the analysis of the effect of storage and cultivar differences on the galactose content of apples. One way ANOVA and Tukey's multiple comparison test were used to analyze the individual effect of time and cultivar, the effect of heat treatment, and the effect of different enzymatic preparations on amounts of galactose in apples and apple juice, respectively. All treatments were considered to be significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

TMS-Oxime Derivatives. A chromatogram showing 0.05 mg/mL galactose and 0.1 mg/mL phenyl-β-D-glucopyranoside derivatized into TMS-oxime and TMS derivatives, respectively, is shown in **Figure 1**. With this method, two peaks are formed for each reducing sugar, representing the syn and anti isomers. The inset shows the chromatogram obtained after derivatizing

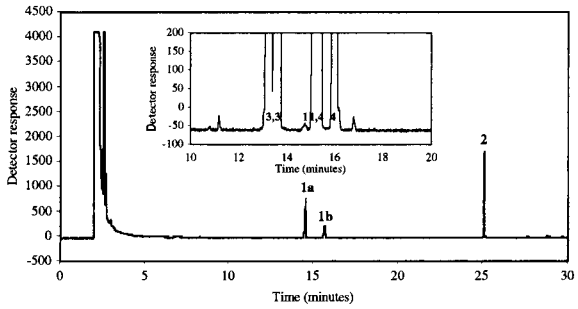

Figure 1. Separation of TMS-oximes and TMS derivatives of sugars by gas chromatography. Peak identification: 1, galactose (0.001 mg/mL); 2, phenyl-β-D-glucopyranoside (internal standard) (0.10 mg/mL). Insert: enlarged view of gas chromatogram of a mixture of TMS-oximes of standard sugars. Peak identification: 1, galactose (0.001 mg/mL); 3, fructose (1.00 mg/mL); and 4, glucose (1.00 mg/mL).

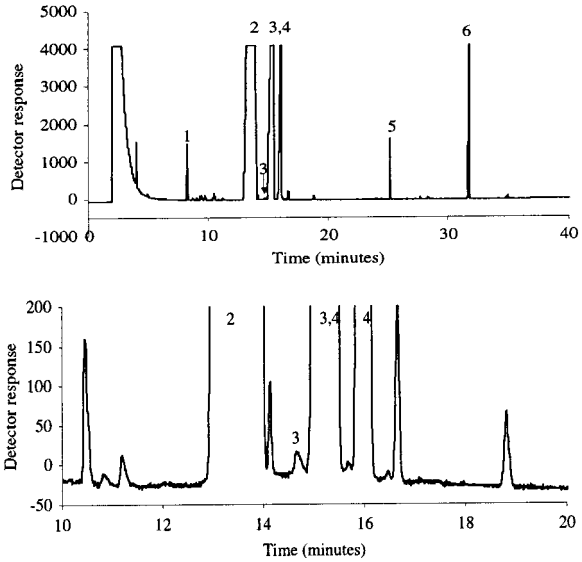

Figure 2. (**A**) Gas chromatogram of sugars extracted from Fuji apples stored for 9 months at 4 °C. Sugars were derivatized into TMS-oxime and TMS derivatives. Peak identification: 1, arabinose; 2, fructose; 3, galactose; 4, glucose; 5, phenyl-β-D-glucopyranoside (internal standard); and 6, sucrose. (**B**) Enlarged view of panel **A**. Peak identification as stated in panel **A**.

0.01 mg/mL galactose in a mixture with 1 mg/mL of each fructose, glucose, and sucrose, which is representative of sugar levels present in apple tissue. This concentration of galactose is equivalent to 0.001 mg/mL galactose after derivatization and was considered the detection limit used in this work. This concentration produced a sharp peak for the major galactose peak that was quantified by peak integration. The minor peak for galactose was coeluted with the major peak of glucose. Similar resolution of the galactose peak was observed when sugars extracted from the apple samples were derivatized (**Figure 2**). A 10 point standard curve of concentrations from 0.001 to 0.06 mg/mL galactose produced a linear response (response factor = 1.2307) with a high r^2 value of 0.9906.

Recovery of galactose was determined to be 53.6 ± 1.31% for samples spiked with 0.0−20.0 mg/100 gfw of galactose added before extraction. The major loss of soluble sugars may have occurred at extraction, since the peak area of the internal standard, added either after extraction or before derivatization, remained constant. Recovery may have been improved by repeating the extraction procedure. However, the percent recoveries for the different added galactose concentrations were not significantly different from each other ($p < 0.05$); therefore, this extraction and derivatization method was reliable.

Storage Study of Different Varieties of Apples. Storage of apples in a chilled environment after harvest is a common practice to delay ripening so that fruits can be marketed at later times**.** Before storage, the galactose concentration of the apples ranged from 4.84 mg/100 gfw for Red Delicious apples to 7.26 mg/100 gfw for Spartan apples (**Table 1**). During the 9 month storage period, minor changes in galactose were observed for each cultivar, generally with a slight increase in galactose for the first 3 or 6 months of storage, followed by a slight decrease. However, the changes in galactose concentration over time were not significant, both statistically and in practical terms for galactosemic patients (**Table 2**). Currently, there is controversy within the metabolic dietician community regarding the amount of dietary restriction needed for fruits and vegetables. However, most clinics have recommended limiting the intake of food items containing more than 20 mg galactose/100 g (*22*−*23*).

There were significant cultivar effects, with Red Delicious (5.80 mg/100 gfw) being significantly lower in galactose than Spartan apples (10.36 mg/100 gfw). Again, in terms of recommendations to galactosemic patients, these differences were not of practical significance. The previously reported concentration of galactose in apples is 8.3 mg/100 gfw (*9*). The values obtained in this work for the different varieties at different physiological ages are similar to the reported values, suggesting that dietitians do not need to consider these factors in making recommendations to galactosemic patients.

Although soluble and insoluble pectin content and composition were not analyzed in this research, losses of galactose and arabinose from pectin side chains in apples during storage have been very well-documented (*15*, *17*, *24*). In this study, it was found that arabinose content, similar to galactose levels, increased during the first 6 months of storage and then decreased, whereas sucrose decreased throughout storage (data not shown). Therefore, it is likely that galactose and arabinose were liberated from pectin side chains during storage due to the action of cell wall hydrolases whose activities increase during fruit ripening and storage. However, as the storage time progresses, the activities of cell wall hydrolases are reported to decrease (*25*, *26*). This might explain why during the latter part of storage, the level of galactose and arabinose decreased. As the liberation of galactose and arabinose from pectin side chains slows, the utilization of these sugars by the tissue as fuel to maintain respiration and other functions may result in a net loss.

Another factor that may have contributed to the decrease in galactose concentration during the latter part of storage is the action of cell wall synthesizing enzymes whose activities are highest during the latter part of storage (*26*). Galactose liberated from pectin side chains by cell wall hydrolases are incorporated into other polysaccharide chains by these cell wall synthesizing enzymes; therefore, the galactose may have become bound again.

Thermal Treatments of Apples. The galactose content in blanched and canned apples was compared to untreated apples (**Table 3**). Blanching reduced the amount of galactose from 8.66 mg/100 gfw in fresh Fuji apples to 2.78 mg/100 gfw. Canning the apples to commercial pasteurization further reduced the amount of galactose in the apples to an undetectable level (<1 mg/100 gfw). While thermal processing of commercial foods is usually kept to the minimum required to maintain product quality, if there is any doubt about the lethality of the process, additional thermal treatment is given to the product. Therefore, in this work, apples were also thermally processed to achieve

Table 1. Free Galactose Content of Four Different Apple Cultivars Stored for 9 Months at 4 °C[a]

	galactose (mg/100 gfw) storage time (months)				
apple cultivar	0	3	6	9	cultivar mean
Braeburn	6.96 ± 0.24 (3.4)	7.85 ± 0.30 (3.8)	7.57 ± 0.23 (3.0)	7.28 ± 0.34 (4.7)	7.42 ± 0.38^{xy}
Spartan	7.26 ± 0.23 (3.1)	10.80 ± 0.24 (2.2)	10.99 ± 0.32 (2.9)	12.40 ± 0.17 (1.4)	10.36 ± 2.19^{y}
Fuji	5.05 ± 0.15 (2.9)	5.65 ± 0.21 (3.7)	9.18 ± 0.27 (2.9)	8.66 ± 0.27 (3.1)	7.14 ± 2.09^{xy}
Red Delicious	4.84 ± 0.12 (2.5)	6.98 ± 0.22 (3.2)	6.72 ± 0.17 (2.5)	4.64 ± 0.16 (3.4)	5.80 ± 1.23^{x}
mean	6.03 ± 1.26^{z}	7.82 ± 2.18^{z}	8.62 ± 1.88^{z}	8.25 ± 3.23^{z}	

[a] Nine subsamples were done for each cultivar at each sampling period, and results are presented as means ± standard deviation (coefficient of variation). ^{x,y}Mean cultivar values denoted by different superscripts are significantly different from each other ($p < 0.05$). ^{z}Mean values over time are not significantly different from each other ($p > 0.05$).

Table 2. Two Way ANOVA of Galactose Content in Four Apple Cultivars Stored at 4 °C for 9 Months

	degrees of freedom		free galactose content
cultivar	3	F ratio	8.13
		P value	0.006
time	3	F ratio	2.92
		P value	0.093
error (cultivar × time)	9	MS	5.44
subsample	2	F ratio	0.11
		P value	0.90
subsample error	30	MS	0.024

twice the usual commercial pasteurization. This process caused galactose to increase to 3.45 mg/100 gfw. A similar trend was observed for arabinose content. On the other hand, sucrose decreased as the severity of the heat treatment increased, although there was no significant difference ($p < 0.05$) between canning to commercial pasteurization and canning to double the commercial pasteurization.

Blanching and canning may have decreased galactose, arabinose, and sucrose due to diffusion of these water soluble sugars into the blanching water. These sugars were detected in the blanch water (data not shown). Leaching of sugars into processing water was also reported by others (*27*−*30*). However, blanching causes minimal modification to the structure of pectic substances (*27*, *28*, *30*, *31*). Therefore, it is likely that during blanching only the free galactose and arabinose that were already present in the produce were leached from the tissue.

It is difficult to determine if the loss of galactose and arabinose from plant tissues observed during canning to commercial pasteurization in this study was originally in the free form or whether they were hydrolyzed from pectin side chains. Many studies have examined the changes in cell wall polysaccharide composition and structure after canning. It has been well-established that pectin is partially solubilized and lost into the processing water during canning (*32*, *33*), whereas cellulose and hemicellulose were not soluble to any large extent. Loss of galactose and arabinose from the pectin side chains that accompanies pectin solubilization has been observed in both low pH products such as tomatoes (*34*) and high pH products such as carrots (*30*, *35*) and green beans (*29*). Therefore, during canning, both free galactose and arabinose and the sugars released from pectin side chains were likely leached into the processing water. The net result was that the recovered galactose and arabinose in the plant tissues was lower than the blanched samples.

When apples were subjected to the more severe heat treatment (double pasteurization), the galactose and arabinose contents increased whereas sucrose continued to decrease. Therefore, it is evident that some changes took place in the plant polysaccharides that caused the additional release and entrapment of galactose and arabinose. Hemicellulose is reported to be degraded under more rigorous thermal treatment (*30*). Therefore, galactose and arabinose attached to the hemicellulose polymers might also be released under these conditions. However, the galactose and arabinose were retained in the plant tissue instead of being released into the processing water. Cellulose and hemicellulose are bonded together to form a network around the cell (*36*), and the galactose and arabinose that are released during hemicellulose degradation might remain inside the cellulosic matrix. This effect was not observed in products canned to commercial pasteurization due to the fact that pectin degradation, mainly in the middle lamella, occurs outside the cell wall, thereby allowing the galactose and arabinose to be solubilized into the processing water.

Enzymatic Aids Used in Juice Production. The total soluble solids, expressed as °Brix, pH, and the yield of the four different types of apple juice produced, are presented in **Table 4**. The control juice obtained without enzyme additions had a °Brix value of 11.7 and pH of 3.9, which are close to other reported values of juice made from Red Delicious apples of 11.4 °Brix and pH of 3.7 (*37*) and 12.0 °Brix and pH of 4.1 (*38*). Juice produced from the addition of Ultrazym 100 was characterized with a °Brix value of 11.8 and pH of 4.0, which was not significantly different from the control juice. Clear apple juice produced from the addition of Pectinex Ultra SPL had higher refraction, 12.9 °Brix, and lower pH value of 3.7, and higher yield. These results are in accordance with the results presented by others (*37*). The increase in refraction and decrease in pH when compared to control juice with no enzyme preparation added and juice with clarification enzymes added indicated that more sugars and other soluble solids such as soluble pectins and other fragments of cell wall were present in the juice due to increased depolymerization of cell wall materials during liquefaction. Furthermore, liquefaction produced juice that was higher in acidity due to the release of galacturonic acids from pectin chains. Addition of both enzyme preparations produced juice that had similar °Brix and pH to that produced with the addition of only Pectinex Ultra SPL. The addition of Pectinex Ultra SPL increased the amount of juice extracted from the same amount of apple puree due to the degradation of cell wall materials, facilitating juice release.

The production of clarified apple juice is an industry almost totally dependent on the use of added enzymes (*39*). The addition of Ultrazym 100, the clarification enzyme preparation, caused a slight increase in galactose concentration from 3.98 to 4.86 mg/100 mL, whereas the addition of Pectinex Ultra-SPL caused a dramatic increase in galactose content in the clear apple juice produced to 18.60 mg/100 mL (**Table 5**). Amounts of arabinose in the four types of apple juice showed the same trend as galactose. With the apple juice produced with both

Table 3. Galactose, Arabinose, and Sucrose Concentrations in Untreated, Blanched, and Canned Fuji Apples[a]

	heat treatment			
sugar	untreated	blanched	commercial pasteurization	double commercial pasteurization
free galactose[b]	$8.66^{w} \pm 0.27$ (3.1)	$2.78^{x} \pm 0.08$ (3.0)	undetectable	$3.45^{y} \pm 0.17$ (4.8)
free arabinose[c]	$1.60^{w} \pm 0.06$ (3.6)	$0.962^{x} \pm 0.032$ (3.3)	$0.734^{y} \pm 0.035$ (4.8)	$0.803^{z} \pm 0.036$ (4.8)
sucrose[d]	$5.44^{w} \pm 0.25$ (4.6)	$3.36^{x} \pm 0.15$ (4.6)	$2.47^{y} \pm 0.092$ (3.7)	$2.36^{y} \pm 0.083$ (3.5)

[a] Three replicates with three subsamples each were analyzed for each treatment, and results are presented as means ± standard deviation (coefficient of variation). [w–z]Treatments denoted by different superscripts are significantly different ($p < 0.05$) from each other within each row. [b] Galactose concentrations in mg/100 gfw. [c] Arabinose concentration calculated as ratio of arabinose peak area:internal standard peak area. [d] Sucrose concentration calculated as ratio of sucrose peak area:internal standard peak area.

Table 4. Total Soluble Solids (°Brix), pH, and Yield of Apple Juice Produced by the Addition of Different Enzymatic Preparations[a]

	enzymatic preparations added			
parameter	none	Pectinex Ultra SPL	Ultrazym 100	Pectinex Ultra SPL and Ultrazym 100
°Brix at 20 °C	$11.7^{y} \pm 0.42$ (3.5)	$12.9^{z} \pm 0.31$ (2.4)	$11.8^{y} \pm 0.49$ (4.2)	$13.1^{z} \pm 0.44$ (3.3)
pH at 20 °C	$3.9^{y} \pm 0.06$ (2)	$3.7^{z} \pm 0.06$ (2)	$4.0^{y} \pm 0.06$ (2)	$3.7^{z} \pm 0.06$ (2)
yield[b]	$81.7^{y} \pm 3.1$ (3.8)	$155^{z} \pm 6.1$ (4.0)	$84.3^{y} \pm 4.0$ (4.8)	$161^{z} \pm 5.0$ (3.1)

[a] Three replicates with three subsamples each were analyzed for each treatment, and values are expressed as means ± standard deviation (coefficient of variation). [y,z]Treatments denoted by different superscripts are significantly different ($p < 0.05$) from each other within each row. [b] Yield was expressed as amount of juice produced (mL) per 400 g of puree.

Table 5. Free Galactose, Arabinose, and Sucrose Concentrations in Apple Juice Produced with the Addition of Different Enzymatic Preparations[a]

	enzymatic preparations added			
sugar	none	Pectinex Ultra SPL	Ultrazym 100	Pectinex Ultra SPL + Ultrazym 100
galactose[b]	$3.98^{w} \pm 0.12$ (3.4)	$18.6^{x} \pm 0.33$ (4.4)	$4.86^{y} \pm 0.17$ (3.9)	$19.3^{z} \pm 0.20$ (3.3)
arabinose[c]	$1.50^{w} \pm 0.056$ (3.8)	$1.83^{x} \pm 0.076$ (4.2)	$1.69^{y} \pm 0.055$ (3.3)	$2.04^{z} \pm 0.072$ (3.5)
sucrose[d]	$50.3^{w} \pm 1.9$ (3.7)	$49.8^{w} \pm 1.6$ (3.1)	$50.3^{w} \pm 1.9$ (3.7)	$50.5^{w} \pm 1.4$ (2.7)

[a] Three replicates with three subsamples each were analyzed for each treatment, and values are expressed as means ± standard deviation (coefficient of variation). [w–z]Treatments denoted by different superscripts are significantly different ($p < 0.05$) from each other within each row. [b] Galactose concentrations in mg/100 gfw. [c] Arabinose concentration calculated as ratio of arabinose peak area:internal standard peak area. [d] Sucrose concentration calculated as ratio of sucrose peak area:internal standard peak area.

Pectinex Ultra-SPL and Ultrazym 100 added, an additive effect of each enzyme preparation was observed for both galactose and arabinose content. As expected, the amount of sucrose was not affected by the enzymatic preparations.

Although the specific enzymatic activities present in the commercial preparations are not available, other studies using Ultrazym 100 have reported the presence of pectinesterases, polygalacturonases, and pectin lyases (*38*, *40*). Ultrazym 100, a clarification aid, only hydrolyzes dissolved pectin in the juice; therefore, galactose and arabinose, which are attached to insoluble pectin and other cell wall polysaccharides, are not affected. The slight increase in galactose content in the juice, therefore, is likely due to enzymatic release of the galactose that was attached to the soluble pectin only.

The addition of Pectinex Ultra SPL caused a large increase in galactose content in the juice because it contains enzymatic activities that can hydrolyze both soluble pectin and insoluble protopectin (*40*). In addition, hemicellulases and cellulases are present that can further solubilize and partially depolymerize pectin and other cell wall polysaccharides (*41*). As a result, the concentrations of galactose and arabinose were increased significantly in the juice.

The use of the two enzyme preparations gives some indication about the position and availability of arabinose and galactose in cell wall materials. Pectinex Ultra-SPL increased the concentration of arabinose 8% and galactose 285%, as compared with Ultrazym 100. This may indicate that most of the arabinose exists in the dissolved portion of the pectin, while more galactose was found attached to insoluble pectin and hemicellulose.

Implications for Dietary Management for Galactosemic Patients. Cold storage was shown to result in a small change in galactose content in apples during fruit ripening and softening. Red Delicious apples were lower in galactose than Spartans, but the varietal differences observed would have a minimal impact on the dietary recommendations made to galactosemic patients. Levels of galactose found in fresh and stored apples in this work corresponded well to concentrations reported elsewhere (*9*). Therefore, it appears that orchard management practices, cultivar, weather, and such factors may not have a significant effect on galactose concentration in apples.

The main effect of galactose release from cell wall polysaccharides was seen during thermal treatment and juice production. The increased galactose in fruits and vegetables may be due to the release of galactose from pectin and hemicellulose from degradation of cell wall polymers by either enzymes in vivo, exogenous sources of cell wall degrading enzymes, or heat treatment. Although blanching and pasteurization of apples decreases the galactose concentration compared to the fresh fruit, galactosemic patients should be advised that raw apples or

processed apple products are acceptable dietary choices, as they all contain less than 20 mg galactose/100 g.

Blanching was shown to reduce the amount of galactose in apples due to leaching of soluble components into processing water. Therefore, blanching in boiling water might be a beneficial routine cooking practice for galactosemic patients. However, other produce might have a different response to blanching.

Canning to commercial pasteurization further reduced galactose contents in apples, and these products can be safely consumed when processing water is drained off. However, canned products can sometimes be reprocessed to ensure commercial sterility, and this would increase the galactose concentration as seen in produce that were processed to double pasteurization. It might be appropriate to advise patients to avoid lower grade canned fruits and vegetables, since overprocessing can be one reason for downgrading canned products.

Enzyme preparations added during apple juice production increased the galactose content in the juices produced. Addition of clarification enzymes caused a small but significant increase in galactose content, while liquefaction enzymes caused a large increase. However, it is not possible to know what enzymes are added during production of commercial apple juices; therefore, consumption of commercial apple juices should be limited by galactosemic patients. Home cold press juicing may be a reasonable alternative.

Only apple products were evaluated in this study, and the galactose levels of other produce may respond differently to processing. Therefore, additional work with other products and processes is being undertaken.

ACKNOWLEDGMENT

We thank Dr. Sam Lau of the Pacific Agri-Food Research Center, Summerland, BC, for providing the apples used in the storage study.

LITERATURE CITED

(1) Novelli, G.; Reichardt, J. K. V. Molecular basis of disorders of human galactose metabolism: past, present, and future. *Mol. Genet. Metab*. **2000**, *71*, 62−65.

(2) Elsas, L. J.; Acosta, P. B. Nutrition support of inherited metabolic disease. In *Modern Nutrition in Health and Disease*, 8th ed.; Shils, M. E., Olson, J. A., Shike, M., Eds.; Lea & Febiger: Philadelphia, 1994; pp 1147−1206.

(3) Cheung, K. L.; Tang, N. L.; Hsiac, K. J.; Law, L. K.; Wong, W.; Ng, P. C.; Pang, C. P.; Applegarth, D. A, Fok, T. F.; Hjelm N. M. Classical galactosaemia in Chinese: A case report and review of disease incidence. *J. Paediatr. Child. Health* **1999**, *35*, 399−400.

(4) Moammar, H.; Ratard, R.; Cheriyan, G.; Mathew, P. Incidence and features of galactosaemia in Saudi Arabs. *J. Inherited Metab. Dis*. **1996**, *19*, 331−334.

(5) Applegarth, D. A.; Toone, J.; Lowry, R. B. Incidence of Inborn Error of Metabolism in British Columbia, 1969−1996. *Pediatrics* **2000**, *105*, e10.

(6) Kaufman, F. R.; McBride-Chang, C.; Manis, F. R.; Wolff, J. A.; Nelson, M. D. Cognitive functioning, nuerologic status and brain imaging in classical galactosemia. *Eur. J. Pediatr*. **1995**, *154* (7, Suppl. 2) S2−S5.

(7) Nelson C. D.; Waggoner, D. D.; Donnell, G. N.; Tuerck, J. M.; Buist, N. R. Verbal dyspraxia in treated galactosemia. *Pediatrics* **1991**, *88*, 346−350.

(8) Badawi, N.; Cahalane, S. F.; McDonald, M.; Mulhair, P.; Begi, B.; O'Donohue, A.; Naughten, E. Galactosaemia−a controversial disorder. Screening and outcome Ireland 1972−1992. *Irel. Med. J*. **1996**, *89*, 16−17.

(9) Gross, K. C.; Acosta, P. B. Fruits and vegetables are a source of galactose: Implications in planning the diets of patients with galactosemia. *J. Inherited Metab. Dis.* **1991**, *14*, 253−258.

(10) Berry, G. T.; Palmieri, M.; Gross, K. C.; Acosta, P. B.; Henstenburg, J. A.; Mazur, A.; Reynolds, R.; Segal, S. The effect of dietary fruits vegetables on urinary galactitol excretion in galactose-1-phosphate uridyltransferase deficiency. *J. Inherited Metab. Dis.* **1993**, *16*, 91−100.

(11) Gropper, S. S.; Weese, S. J.; West, P. A.; Gross, K. C. Free galactose content of fresh fruits and strained fruit and vegetable baby foods: More foods to consider for the galactose-restricted diet. *J. Am. Diet. Assoc.* **2000**, *100*, 573−575.

(12) Fuleki, T.; Pelayo, E.; Palabay, R. B. Sugar composition of varietal juices produced from fresh and stored apples. *J. Agric. Food Chem.* **1994**, *42*, 1266−1275.

(13) Sharma, D.; D'Souza; McConnel, M.; Mazza, G. Identification and quantification of sugars in winter-hardy apples by high performance liquid chromatography. *Can. Inst. Food Sci. Technol. J*. **1988**, *21*, 435−437.

(14) Fourie, P. C.; Hanamann, C. F.; Oberholzer, H. M. Sugar content of fresh apples and pears in South Africa. *J. Agric. Food Chem.* **1991**, *39*, 1938−1939.

(15) Redgwell, R. J.; Fischer, M.; Kendal, E.; MacRae, E. A. Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. *Planta* **1997**, *203*, 174−181.

(16) Kitagawa, Y.; Kanayama, Y.; Yamaki, S. Isolation of β-galactosidase fractions from Japanese pear: activity against native cell wall polysaccharides. *Physiol. Plant.* **1995**, *93*, 545−550.

(17) Gheyas, F.; Blankenship, S. M.; Young, E.; McFeeters, R. Dietary fibre composition in developing apple fruits. *J. Hortic. Sci. Biotechnol.* **1998**, *73*, 631−639.

(18) Lund, D. Heat Processing. In *Principles of Food Science. Part II: Physical Principles of Food Preservation*; Karel, M., Fennema, O. R., Eds.; Marcel Decker Inc.: New York, 1975; pp 31−92.

(19) Gross, K. C.; Jean Weese, S.; Johnson, J.; Gropper, S. S. Soluble galactose content of selected baby food cereals and juices. *J. Food Compos. Anal.* **1995**, *8*, 319−323.

(20) Li, B. W.; Andrews, K. W. Separation of trimethylsilylated oximes of monosaccharides by capillary gas−liquid chromatography. *Chromatographia* **1986**, *21*, 596−598.

(21) Molnar-Perl, I.; Morvai, M. Rapid method for the simultaneous GC quantitation of acids and sugars in fruits and vegetables. *Food Addit. Contam.* **1992**, *9*, 505−514.

(22) Hartz, L.; Pettis, K.; vanCalcar, S. *Understanding Galactosemia A Diet Guide*, 2nd ed.; Waisman Center on Mental Retardation and Human Development: Madison, Wisconsin, 2000.

(23) Acosta, P. B.; Yannicelli, S. *The Ross Metabolic Formula System Nutrition Support Protocols*, 4th ed.; Abbott Laboratories: Columbus, Ohio, 2001.

(24) Chun, J. P.; Hwang, Y. S.; Lee, J. C.; Huber, D. J. Cell wall component changes during maturation and storage in 'Tsugaru' and 'Fuji' apple fruits. *J. Korean Soc. Hortic. Sci.* **1999**, *40*, 705−710.

(25) Fischer, R. L.; Bennett, A. B. Role of cell wall hydrolases in fruit ripening. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **1991**, *42*, 675−703.

(26) Fry, S. C. Polysaccharide-modifying enzymes in the plant cell wall. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1995**, *46*, 497−520.

(27) Stolle-Smits, T.; Beekhuizen, J. G.; van Dijk, C.; Voragen, A. G. J.; Recourt, K. Cell wall dissolution during industrial processing of green beans (*Phaseolus vulgaris L.). J. Agric. Food Chem.* **1995**, *43*, 2480−2486.

(28) Nyman, E. M. G. L.; Nylander, T.; Asp, N.-G. L. Degradation of water-soluble fiber polysaccharides in carrots after different types of processing. *Food Chem*. **1993**, *47*, 169−176.

(29) Nyman, E. M. G. L.; Svanberg, S. J. M.; Asp, N.-G. L. Molecular weight distribution and viscosity of water-soluble dietary fiber isolated from green beans, brussel sprouts and green peas following different types of processing. *J. Sci. Food Agric.* **1994**, *66*, 83−91.
(30) Massiot, P.; Guiller, I.; Baron, A.; Drilleau, J. F. Cell wall polysaccharides modifications during heat treatment and enzymatic degradation of carrot tissues. *Food Sci. Technol.* **1992**, *25*, 559−563.
(31) Muller, S.; Kunzek, H. Material properties of processed fruit and vegetables. I. Effect of extraction and thermal treatment on apple parenchyma. *Food Res. Technol.* **1998**, 264−272.
(32) Voragen, A. G. J.; Pilnik, W.; Thibault, J. F.; Axelos, M. A. V.; Renard, C. M. G. C. Pectins. In *Food Polysaccharides and their Applications*; Stephen, A. M., Ed.; Food Science and Technology, Marcel Dekker: New York, 1995; Vol. 67, pp 287−339.
(33) Thakur, B. R.; Singh, R. K.; Handa, A. K. Chemistry and uses of pectin—a review. *Crit. Rev. Food Sci. Nutr.* **1997**, *37*, 47−73.
(34) Reinders, G.; Their, H. P. Nonstarch polysaccharides of tomatoes. II. Influence of thermal processing. *Eur. J. Food Res. Technol.* **1999**, *209*, 47−51.
(35) Plat, D.; Ben-Shalom, N.; Levi, A.; Reid, D.; Goldschmidt, E. E. Degradation of pectic substances in carrots by heat-treatment. *J. Agric. Food Chem.* **1988**, *36*, 362−365.
(36) Vincent, J. F. V. From cellulose to cell. *J. Exp. Biol.* **1999**, *202*, 3263−3268.
(37) Schols, H. A.; Veld, P. H.; van Deelen, W.; Voragen, A. G. J. The effect of the manufacturing method on the characteristics of apple juice. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 142−148.
(38) Ibarz, A.; Grijelmo, C.; Pagan, J. Depectination of apple juices. *Fruit Process.* **1997**, *7*, 460−474.
(39) Lae, A. G. H. Enzymes in the production of beverages and fruit juices. In *Enzymes in Food Processing*, 2nd ed.; Tucker, G. A., Woods, L. F. J., Eds.; Blackie Academic and Professional: Glasgow, England, 1995; Vol. No. 7, pp 223−249.
(40) Urlaub, R. Advantages of enzymatic apple mash treatment and pomace liquefaction. *Fruit Process.* **1996**, *6*, 399−406.
(41) Dongowski, G.; Sembries, S. Effects of commercial pectolytic and cellulolytic enzyme preparations on the apple cell wall. *J. Agric. Food Chem.* **2001**, *49*, 4236−4242.

Received for review June 17, 2003. Revised manuscript received October 27, 2003. Accepted December 8, 2003. V.J.W.J. acknowledges the financial support of NSERC through a postgraduate scholarship.

JF034643K