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Free Galactose Content in Selected Fresh Fruits and Vegetables and Soy Beverages

HYUN-OCK KIM,[†] CAROL HARTNETT,[‡] AND CHRISTINE H. SCAMAN*,[†]

Food, Nutrition and Health, 2205 East Mall,University of British Columbia, Vancouver V6T 1Z4, and British Columbia's Children's and Women's Hospital, 4480 Oak Street, Vancouver V6H 3V4, Canada

The free galactose content was determined in three soy beverages, and 34 selected fruits and vegetables purchased at different times of the year and/or local markets in British Columbia, Canada. The objective of the work was to provide additional information on the free galactose content of foods to assist individuals with galactosemia in making dietary decisions. Free galactose contents in the selected plant materials ranged from 2.0 $\pm$ 0.1 mg/100 g in red potato to 39.7 $\pm$ 1.9 mg/100 g in red pepper. Different time of the season, variety, and storage of the product affected the free galactose contents in most of the plant materials measured in this study. Free galactose levels in kiwi, green seedless grapes, and bell peppers were found to be higher than previous reports, whereas the amount of free galactose in three varieties of tomatoes was significantly lower than previously reported. An evaluation of the change of galactose in Roma tomatoes during ripening showed that free galactose levels increased linearly over time, and storage at 4 °C significantly increased free galactose levels in tomatoes. Soy beverages made from soy protein isolate contained less free galactose (1.3 $\pm$ 0.2 mg /100 g) compared to the samples made from whole soybeans (4.8 $\pm$ 1.9 and 5.3 $\pm$ 1.7 mg/100 g). This study provides additional information on the range of free galactose in fruits and vegetables which will allow individuals with galactosemia to make more informed dietary choices.

KEYWORDS: Free galactose; fruit; vegetables; dietary recommendations; soy beverage

INTRODUCTION

Galactosemia is an inherited disorder associated with an inability to metabolize galactose due to genetic mutations in one of several enzymes, including galactose-1-phosphate uridyltransferase, galactokinase, and uridine diphosphate galactose-4-epimerase. Despite early diagnosis and dietary therapy, complication have been reported in greater than 50% of individuals with galactosemia, including cataracts, gynecologic failure, speech and language delays, neurological impairment, and growth inhibition in infants (*1–5*). Currently dietary restriction of galactose is the only treatment option for individuals with galactosemia; however, there are two factors which make diet selection difficult for these persons and their caregivers.

First, there is little information available on the free galactose content in foods in general, and most of the published data has been obtained from foods purchased in the U.S. market. This includes data on free galactose content in selected fruits and vegetables (*6*, *7*), baby food fruits and vegetables (*7*, *8*), baby food cereals and juices (*9*), and baby food meats (*10*). There is no specific information on the free galactose content in different varieties of a single product, or whether products purchased from different localities or at different times of the year affect free galactose levels. Very little has been reported on the effects of processing or sample preparation on free galactose levels.

Second, the metabolic significance of the small amounts of free galactose from fruits and vegetables is not clear (*11–13*). On one hand, fruits and vegetables, if consumed freely, could potentially represent a significant source of dietary galactose of up to 500 mg free galactose per day (*6*). However, recently the clinical significance of the reduction in free galactose consumption by limiting fruits and vegetables with a free galactose greater than 20 mg/100 g on long term outcome is questionable when compared to reported daily fluctuations in the endogenous production of galactose in individuals with galactosemia (*14*, *15*).

In this study, free galactose content was determined in soymilk and some selected fruits and vegetables purchased at different times of the year and/or with different varieties available at a local food market in British Columbia, Canada. An evaluation of galactose content of Roma tomatoes at different stages of ripeness was evaluated as well. Fresh tomatoes and tomato products are often restricted in the diets of individuals with galactosemia because of the reported high levels of free galactose (*6*); however, more recently, relatively low levels of galactose were noted in Roma tomatoes (*16*).

The information on the free galactose content in fruit and vegetable products provided in this work will provide more

* Corresponding author.
[†] University of British Columbia.
[‡] British Columbia's Children's and Women's Hospital.

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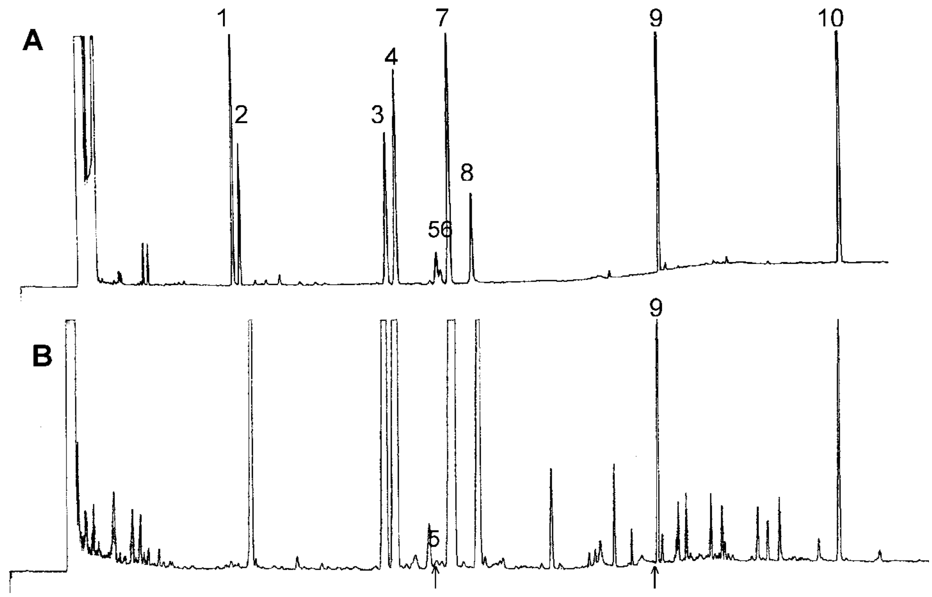

Figure 1. Gas chromatogram of (**A**) TMS-derivatives of phenyl-β-D-glucopyranoside (internal standard) and standard sugars showing the *syn*- and *anti*-oxime isomers of each reducing sugar; 1/2, arabinose; 3/4, fructose; 5/8, galactose; 6/8, mannose; 7/8, glucose; 9, phenyl-β-D-glucopyranoside; 10, sucrose; (**B**) TMS-derivatized Roma tomato extract with arrows indicating the peaks of galactose (5) and internal standard (9) used for quantitation.

objective information on the free galactose content of foods in the British Columbia marketplace and assist individuals with galactosemia in making more informed dietary choices.

MATERIALS AND METHODS

Materials. Fruits and vegetables were purchased at local markets at typical maturity. The first set of samples was obtained from two local produce/grocery stores on May 2 and 3, 2004. The second set of samples were obtained from five different stores on July 6 and 7, 2004. The third set of samples was obtained from one store on October 13, 2004. Fruits and vegetables were washed with water and dried with paper towel. The edible part of each fruit and vegetable was diced into approximately 1 cm^3, mixed, and three 10 g samples were taken for free galactose analysis.

Roma tomatoes used to monitor free galactose during ripening were obtained from a local commercial greenhouse and were picked from the top branch of each plant to ensure that all of the tomatoes were at the same stage of development. Tomatoes were stored at 25 °C for 13 days, and sampled every second day. Equal amounts of three tomatoes were combined, and from the mixture, three replicates were taken. At each sampling date, a tomato was placed at 4 °C for 7 days, and then analyzed again for free galactose.

Three soy beverages were purchased locally. Two samples were made from whole soybeans, whereas the third was made from soy protein isolate.

Sugar standards, phenyl-β-D-glucopyranoside (internal standard), hexamethyldisilazane (HMDS), trifluoroacetic acid (TFAA), hydroxylamine HCl, and pyridine were obtained from Sigma Chemical Co. (St. Louis, MO). The SPB-1 gas chromatography column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was purchased from Supelco Inc. (Toronto, Canada).

Moisture contents of samples were determined by vacuum oven drying at 70 °C for 20 h.

Extraction. Free galactose was extracted from plant materials using the procedure described by Gross and Acosta with modification (*6*). Ten gram of fresh samples were placed in 30 mL of 80 % ethanol, heated in boiling water bath for 10 min, cooled, and then stored at −18 °C for 16 h. Samples were homogenized with an Ultra Turrax at 11,000 rpm. The homogenates were vacuum filtered through Whatman No. 1 filter paper, and the residues were rinsed with 3 mL of 80 % ethanol. The filtrates were combined and centrifuged at 20000*g* for 15 min. Supernatants were collected and brought up to a total volume of 30–40 mL with 80 % ethanol. A 3 mL aliquot of each sample was then passed through C_{18} Sep-Pak cartridge (Waters) and the cartridge was washed with 2 mL distilled–deionized water. A one mL aliquot from each extract was dried in SC 110 Speedvac concentrator (Savant Instrument Inc.) at 45 °C and stored at −18 °C until derivatization.

Triplicate samples of soy beverages were diluted with four volumes of distilled–deionized water, centrifuged at 20000*g* for 15 min, and one mL aliquots were used directly for derivatization.

Sugars Derivatization. Sugars were converted to trimethylsilyl (TMS) ether/ester and TMS-oxime derivatives by the procedure described by Scaman et al., with modification (*17*). Dried samples were dissolved in 0.5 mL of 95 % methanol and then centrifuged at 13000*g* for 2 min. A 0.1 mL aliquot of supernatant was transferred to 4 mL vial and dried under nitrogen at 35 °C. To the dried sugars, 0.5 mL of pyridine with hydroxylamine HCl (12.5 mg/mL) and phenyl-β-D-glucopyranoside (0.1 mg/mL) was added and then the mixture was heated at 75 °C for 30 min. The cooled samples were then trimethylsilylated with 1.0 mL mixture of HMDS:TFAA (10:1, v/v) at 100 °C for 60 min. Clear supernatant of the derivatized solution was evaporated to dryness under nitrogen at 35 °C and diluted with a mixture of HMDS and TFAA (10:1, v/v) prior to chromatographic analysis.

Galactose Analysis by Gas Chromatography. Samples were analyzed for free galactose content using a Hewlett-Packard (HP) 5890 gas chromatograph equipped with flame ionization detector and an SPB-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Chromatography conditions were as follows: carrier gas, helium; flow rate, 1.5 mL/min; injector temperature, 250 °C; detector temperature, 300 °C; split ratio, 30:1. Flow rates of helium make-up gas, hydrogen, and air were 35 mL/min, 35 mL/min, and 400 mL/min, respectively. The column temperature program consisted of an initial temperature of 180 °C, that was increased to 200 °C at 5 °C/min, held for 11 min., increased to 270 °C at 10 °C/min., held for 13 min., and then increased to 280 °C at 10 °C/min, held for 5 min. Integration of data collected was performed using a HP 3396A integrator.

A calibration curve was obtained, regressing weight ratios against peak area ratios of the standard galactose (0.002–0.070 mg/mL) and internal standard (phenyl-β-D-glucopyranoside), 0.1 mg/mL.

Statistical Analysis. Statistical analysis was performed using Minitab version 13.30 program (Minitab Inc., PA). One-way analysis of variance followed by Tukey test was used to compare means. Significance of difference was defined at $p < 0.05$.

Table 1. Free Galactose Content (mg/100 g Fresh Weight, Mean ± Standard Deviation of Triplicate Determinations) of Fruits and Vegetables Purchased on Three Dates

sample	sampling date May 2004	July 2004	October 2004	previously published values[a]
bean, green		5.5 ± 1.0		5.0 (*27*)
bitter melon	6.8 ± 0.9[b]	7.7 ± 1.6[b]		
bok choy/baby	3.1 ± 0.9[b]	4.3 ± 0.7[b]		
cucumber,				4.0 ± 0.3 (*6*)
Japanese		4.3 ± 1.3		
Persian	6.0 ± 0.6[b]	7.0 ± 0.6[b]		
gai-lan	4.0 ± 0.9[b]	7.3 ± 1.5[c]		
grape				100 (*29*); 400 (*30*) (European grapes)
green/seedless	11.9 ± 1.1[b]	12.4 ± 1.3[b]		2.9 ± 0.1 (*6*)
red/globe	14.5 ± 0.4[b]	7.6 ± 0.8[c,i]	5.9 ± 0.2[d,j]	
red/seedless	15.9 ± 0.8[b,g]	6.6 ± 1.7[c]	5.7 ± 1.4[c,j]	
kiwi	27.1 ± 1.9[b]	26.5 ± 1.2[b]		9.8 ± 0.4 (*6*)
lettuce				3.1 ± 0.3 (*6*)
butter	2.6 ± 0.6[b,e]	2.2 ± 0.7[b,e]		
green leaf	7.8 ± 0.6[b]	7.1 ± 1.0[b]		
iceberg	6.4 ± 0.9[b,j]	4.5 ± 0.8[c]		
romaine	7.7 ± 0.8[b]	4.2 ± 0.7[c]		
onion, boiler	15.3 ± 1.0[b]	11.6 ± 0.7[c]		5.1 ± 0.3 (*6*)
pea				4.9 ± 0.8 (*6*); 161 (*5*)
English		11.8 ± 0.8		
snap	15.7 ± 3.4[b,h]	12.7 ± 1.4[b]		
snow	13.7 ± 0.9[b,h]	17.0 ± 0.4[c]		
pear				12 (*29*); 7.3 ± 1.4 (*6*)
Asian	13.4 ± 0.4			
Shisheki		10.1 ± 0.5[c]		
pepper				10.2 ± 0.4 (*6*)
green	12.1 ± 0.8[b]	11.8 ± 1.3[b]	19.4 ± 1.1[c,j]	
orange	18.5 ± 1.0[b]	10.4 ± 2.1[c]	13.9 ± 1.1[d,f]	
red	39.7 ± 1.9[b]	13.6 ± 1.4[c]	12.5 ± 1.8[c,f]	
yellow	14.5 ± 1.0[b]	15.4 ± 1.5[b]		
potato				
red	10.7 ± 0.6[b]	2.0 ± 0.1[c]	2.2 ± 0.3[c,j]	
white	10.8 ± 0.9[b]	3.5 ± 0.7[c]	3.0 ± 0.6[c,e]	1.2 ± 0.3 (*7*)
yelllow	10.4 ± 0.5[b]	3.3 ± 0.5[c]		
siu choy	6.5 ± 0.5[b]	3.6 ± 1.1[c]		
spinach	5.5 ± 0.8[b]	2.3 ± 0.8[c]		<1 (*27*); 0.1 ± 0.1 (*6*)
strawberry		10.7 ± 0.5		4.6 ± 0.3 (*7*)
tomato				8 (*29*); 23.0 ± 2.0 (*6*)
beefsteak	15.5 ± 2.5[b]	11.6 ± 1.4[c]	7.4 ± 0.6[d,f]	
cherry	10.8 ± 0.7[b]	11.0 ± 1.4[b]		
Roma	12.8 ± 1.1[b]	5.7 ± 1.0[c]	5.6 ± 0.5[c,j]	
yam		16.3 ± 1.5[b]	7.6 ± 1.3[c,j]	12 (*30*); 7.7 ± 0.7 (*7*) (sweet potato)

[a] Previously published galactose levels in mg/100 g fresh weight (*5–7, 27, 29, 30*) Refer to literature cited. [b-d] Within each product, samples with different letters are significantly different, $p < 0.05$. [e] From BC. [f] From BC Hothouse. [g] From Chile. [h] From China. [i] From Mexico. [j] From U.S.

RESULTS AND DISCUSSION

Analysis of Standard Sugars. The typical gas chromatogram of TMS derivatives of standard sugars and phenyl-β-D-glucopyranoside, and a chromatogram of the extract obtained from Roma tomatoes is shown in **Figure 1**. Each sugar peak was identified on the basis of retention time and coelution with the individual TMS-derivatives. As previously reported (*18, 19*) two peaks, representing the *syn*- and *anti*-oxime isomers for each reducing sugars were obtained. Phenyl-β-D-glucopyranoside and sucrose as nonreducing sugars, yielded a single peak. Since the ratios of the TMS *syn*- and *anti*-oximes are stable and independent of the amounts to be determined (*19*), the major *syn*-isomer galactose peak, eluting before the glucose peak, was used for quantification. The minor galactose peak coeluted with the minor peak of glucose as previously reported (*17*, *20*, *21*). A standard curve of concentrations from 0.002 to 0.07 mg/mL galactose produced a linear response, with a response factor of 1.1325 ($r^2 = 0.9986$).

Galactose Content in Fruits and Vegetables. The galactose content of selected fruits and vegetables is shown in **Table 1**. Previous reports of free galactose content of various fruits and vegetables gave no indication of the time of the season for purchasing, the origin of the product, nor the variety. In this study, plant material was bought from different local food markets in the vicinity of Vancouver, British Columbia, Canada at three different times of the year.

Some products showed consistency in free galactose content between sampling periods in this study and published values (if available), suggesting that the mature products may not vary much in free galactose content. These include bitter melon, bok choy, Persian cucumber, green leaf lettuce, butter lettuce, snap peas, yellow pepper, and cherry tomatoes. Similarly, it has been reported that the galactose level of apples did not change significantly over 9 months of storage (*17*).

The concentration of free galactose in several products did not closely correspond to the values previously reported. Green grapes were found to have approximately 4 times the free galactose, kiwi almost 3 times the free galactose, and onions and strawberries approximately double the free galactose previously reported. However, all three grape varieties analyzed had substantially lower levels of free galactose compared to two reports (*29, 30*). The level of galactose in all the pea samples

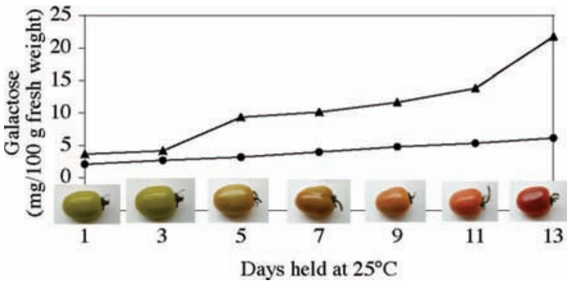

Figure 2. Free galactose concentration in Roma tomatoes. Circles, samples held at 25 °C for up to 13 days (mean of triplicate samples, standard deviations ranged from 0.08 to 0.31). Pictures show the ripeness of the tomatoes used at each sampling date. Triangles, samples held at 25 °C, followed by 1 week at 4 °C (single determinations).

ranged from approximately 2 to 3 times that reported by ref *6*. The free galactose in the three potato varieties examined were higher than the published value on all three sampling dates. The values for the samples obtained in May were especially high, and may reflect the altered physiological state of the potatoes after being held in storage since the previous fall. For some produce, variety, season, maturity, and storage have been shown to affect the levels of major sugars such as glucose, fructose, and sucrose (*22–26*). Our results indicate that these factors may also affect the free galactose content of some fruits and vegetables.

There were some differences in free galactose content among different varieties of produce as well; for example, butter lettuce had approximately one third the free galactose of leaf lettuce. For most products, the differences between varieties were of a similar magnitude as the differences between sampling dates.

It is important to note that the range of free galactose concentrations found in the samples may have limited metabolic significance to individuals with galactosemia (*12, 13, 15*). When counseling, dietitians have previously often restricted fruits and vegetables with greater than 20 mg galactose /100 g (*27*). In this study of 34 fruits and vegetables, only kiwi and red peppers (one sample) were in this category.

Typically, tomatoes have been included in this restricted consumption category, however beefsteak, cherry, and Roma tomatoes were all found to have free galactose content less than 20 mg galactose/100 g. This corresponds to the value of free galactose in an unspecified tomato variety previously reported (*29*). Roma tomatoes were monitored from the hard green stage to the soft red ripe stage over a 13 day period (**Figure 2**). During this time galactose levels increased linearly from 2.1 to 6.2 mg/100 g. An increase was expected because free galactose is liberated with the enzymatic break down of pectin during ripening (*28*). Tomatoes from each sampling date were stored at 4 °C for 1 week and subject to reanalysis. Galactose release continued and was accelerated under refrigeration conditions. Levels of free galactose of samples held at 25 °C for 5 or more days, and then stored at 4 °C were considerably higher than those that were maintained at 25 °C until fully ripened. The free galactose concentration of 22 mg/100 g noted after refrigeration of the 13 day sample approached the level that has previously been reported for tomatoes (*6*). These results would suggest that tomatoes may be included in the diet of individuals with galactosemia, but cold storage should be avoided.

The free galactose contents of three soy beverages were determined. The two samples produced from whole soybeans had 4.8 ± 1.9 and 5.3 ± 1.7 mg free galactose/100 g while the sample prepared from soy protein isolate had 1.3 ± 0.2 mg free galactose/100 mL. The steps involved in production of soy protein isolate, including washing the beans, is likely responsible for the reduced free galactose in the beverage made from this material. Infant formulas made from soy protein isolate have been reported to contain <5 mg free galactose/100 g (*27*). Therefore, to help minimize consumption of free galactose, it may be preferable to consume soy beverages made from soy protein isolate rather than whole soy beans.

It is important to note that the galactose content of all fruits and vegetables analyzed was still under 40 mg/100 g, which is mimimal when compared to the galactose content of dairy products. Assuming that the galactose in dairy products is 50% of the lactose concentration, milk contains 2000–2500 mg galactose/100 g, while cheddar cheese can contain up to 1000 mg/100 g (*31*). Further work is needed to establish the metabolic significance of these small amounts of free galactose, particularly in the first few years of life, when endogenous production is highest. Until this can be established, the data presented here will provide dietitians and individuals with galactosemia with objective information on the range of free galactose in fruits and vegetables available in the marketplace, so that they may plan diets with less uncertainty.

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