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Galactose Content of Legumes, Caseinates, and Some Hard Cheeses: Implications for Diet Treatment of Classic Galactosemia

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ABSTRACT: There are inconsistent reports on the lactose and/or galactose content of some foods traditionally restricted from the diet for classic galactosemia. Therefore, samples of cheeses, caseinates, and canned black, pinto, kidney, and garbanzo beans were analyzed for free galactose content using HPLC with refractive index or pulsed amperometric detection. Galactose concentrations in several hard and aged cheeses and three mild/medium Cheddars, produced by smaller local dairies, was <10 mg/100 g sample compared to 55.4 mg/100 g sample in four sharp Cheddars produced by a multinational producer. Galactose in sodium and calcium caseinate ranged from undetectable to 95.5 mg/100 g sample. Free galactose level in garbanzo beans was lower than previously reported at 24.6 mg/100 g sample; black beans contained 5.3 mg/100 g, and free galactose was not detected in red kidney or pinto beans. These data provide a basis for recommending inclusion of legumes, caseinate-containing foods, and some aged hard cheeses that had been previously restricted in the diet for individuals with galactosemia.

KEYWORDS: *galactose, caseinate, legumes, Cheddar, Emmentaler, Parmesan, classic galactosemia, galactose-1-phosphate uridyltransferase deficiency*

INTRODUCTION

Galactosemia (OMIM 230400) is an inherited disorder in the metabolism of galactose. There are three distinct disorders in this pathway, but the most common and most severe form, classic galactosemia, is caused by an enzymatic deficiency of galactose-1-phosphate uridyltransferase (GALT). In GALT deficiency, galactose-1-phosphate (Gal-1-P) and UDP-glucose are not converted to glucose-1-phosphate and UDP-galactose. The cellular accumulation of Gal-1-P and other galactose metabolites can damage the hepatic, hematologic, ovarian, renal, ocular, and nervous systems.[1]

Treatment for classic galactosemia is to limit the dietary intake of galactose. The major dietary source of galactose is the disaccharide lactose, and elimination of milk and milk products is the backbone of the galactose-restricted diet.[1] However, despite strict dietary adherence and good control of Gal-1-P concentrations in red blood cells, the majority of individuals with classic galactosemia exhibit long-term complications including intellectual impairment, speech defects, tremor, and, in females, ovarian failure. The etiology of these complications and the role of diet adherence remain unclear.[2,3]

Whereas avoidance of dairy products is recommended for life, there is little consensus among professionals about the degree of galactose restriction required beyond infancy. The controversy is complicated by various factors, including the role of endogenous galactose production[4,5] and the widespread presence of galactose in nondairy foods. Galactose is found in a variety of fruits and vegetables, soy products, and legumes and may appear either as free galactose or in various galactose-containing oligosaccharides, proteins, or lipids, often referred to as "bound" galactose.[6−12] Humans do not have the intestinal enzymes required to digest most sources of bound galactose,[13] yet the presence of free galactose in some of these foods continues to prevent consensus about allowing them in the galactose-restricted diet.

Studies to date have shown that legumes may contain more free galactose than fruits and vegetables; in 1991, Acosta and Gross reported that the free galactose content ranged from 42.4 mg/100 g in pinto beans to 444 mg/100 g in garbanzo beans.[7] Thus, some metabolic clinics have eliminated or limited legumes, especially garbanzo beans, from the diet.[14] Because total, reducing, and nonreducing sugars can leach into soaking and cooking water,[15] one aim of this current study is to analyze commercially canned legumes, separating beans and canning water, to better delineate whether some or all of the legumes can be allowed in the diet for classic galactosemia.

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Another area of controversy is the acceptability of hard cheeses in the diet management of galactosemia. Bacterial fermentation and aging of some cheeses substantially reduces the galactose content.[16] Negligible amounts of lactose and galactose were found in European sources of Gruyere, Emmentaler, Jarlsberg, grated and block Italian Parmesan (Parmigiano Reggiano, Grana Padona), and Cheddar cheese aged for >1 year produced by the UK West Country Farmhouse Cheese Makers Association in England.[16] Minibel Emmental (Laughing Cow), a mild processed Emmentaler cheese produced in France, did contain various amounts of residual lactose, with an average of 6 mg galactose/100 g.[17] A study of 30 8−12-month-aged Cheddar cheeses reported undetectable concentrations of lactose and galactose using HPLC with refractive index detection,[18] whereas others have reported variable amounts of galactose in hard cheeses.[19−22] Analysis of additional sources of these cheeses has not been completed in recent years; thus, it is not known whether these aged cheeses can be universally recommended for those with galactosemia. In addition, caseinates may contain negligible galactose and need to be further evaluated.[23] Understandably, addition of milk-based products has been especially difficult to accept by families who are hesitant to include these foods without adequate assurance regarding their safety. Thus, a second aim of this current study is to determine the galactose content of these same hard cheeses produced in the United States and Canada and to analyze sources of food-grade caseinates to provide additional data to help establish dietary recommendations for classic galactosemia.

■ MATERIALS AND METHODS

Various types of cheeses (n = 19) and sodium and calcium caseinate (n = 6) were purchased between June and August 2011 in different locations in North America. These products were analyzed at a commercial food analysis laboratory (Covance, Inc., Madison, WI, USA). Each sample was ground to ensure consistent composition, and a 2 g aliquot was extracted with room temperature water. The sample was shaken for ~1 min with 10 volumes of chloroform to remove lipids and then centrifuged for 5 min. The aqueous fraction was filtered through a 0.45 μm filter. The quality control sample used for the analysis was a cereal product that had ranges determined on 60 replicates of the material.

Total galactose content was determined from both lactose and galactose quantitated by a high-performance anion exchange chromatograph equipped with a pulsed amperometric detector (HPAEC-PAD).[24] Each standard curve had an r^2 > 0.995. The individual standard concentrations for each analyte were within 10% of the theoretical concentrations. For any sample containing lactose, 53% of total lactose was considered galactose, on the basis of the molecular weights of 342 for lactose and 180 for galactose. All galactose concentrations are expressed as milligrams per 100 g product. For dairy products, a galactose content <5.1 mg/100 g product was considered below quantitation limits. The published method reported carbohydrate recoveries of 94.9−105% and a coefficient of variation of 3.3%.[24]

For legume analysis, commercially canned garbanzo beans (kabuli chickpeas), black beans, red kidney beans, and pinto beans were selected. One can of three national brands of each product were purchased in Madison, WI, USA, in December 2012. From each sample can, liquid was drained and weighed, and beans were briefly rinsed with distilled water to ensure that all canning liquid was collected. Whole beans were macerated in a food processor, and both the liquid (38−51 g sample) and beans (61−82 g sample) were freeze-dried separately. Sugar extraction and analysis were completed in Food, Nutrition and Health at the University of British Columbia in Vancouver using the method of Sanchez-Mata et al.[25] with an extraction temperature of 60 °C. Duplicates of each freeze-dried bean and liquid sample were extracted in 80% ethanol before analysis of galactose and galacto-oligosaccharides using an Agilent 1100 series HPLC system with an aminopropyl Zorbax carbohydrate column (4.6 × 250 mm, 5 μm particle size, 30 °C). Sugars were detected using an Agilent 1200 series refractive index detector (Agilent Technologies, Canada, Inc., Mississauga, ON, Canada) regulated at 35 °C. The isocratic mobile phase was premixed 75:25 acetonitrile/water, at 2.0 mL/min. Sample aliquots of 100 μL were injected. Total galactose, free galactose, melibiose, raffinose, ciceritol, and stachyose were quantitated and expressed in milligrams sugar per 100 g product. Commercial standards for all compounds except ciceritol were used to construct standard curves for quantitation (r^2 > 0.99). The galactose, melibiose, and stachyose standards were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada), and raffinose was purchased from Fisher Scientific Canada (Ottawa, ON, Canada). All sugar standards were of 98% purity or higher. Ciceritol concentrations were estimated using the raffinose standard curve with a correction for the molecular weight.

■ RESULTS AND DISCUSSION

Addressing the galactose content of caseinates, hard cheeses, and legumes will provide further data to address the inclusion or restriction of these foods and ingredients in the diet for classic galactosemia.

Caseins, a complex group of proteins comprising α_{s1}, α_{s2}, β, and κ gene products, are present in milk in the range of 2.4−2.9 g/100 mL.[27] The isolated proteins, termed caseinates, are used as food ingredients in a wide range of products for emulsification, stabilizing, water binding, film formation, and nutritional purposes. Therefore, restricting caseinate in the galactosemia diet eliminates many foods that could add dietary and nutritional variety.

Caseinates can be manufactured via acid precipitation or enzymatic (chymosin) precipitation. After precipitation, the caseinate is washed and dewatered. The isolated caseinate is subsequently solubilized by the addition of an alkali or polyphosphates and dried.[26] Because of the removal of whey via precipitation and extensive washing of the precipitated protein, the concentration of lactose, and therefore galactose, remaining in the final caseinate is expected to be low. Analysis of food grade caseinates in this study confirmed this observation. Residual lactose was detected in three of six caseinate samples with equivalent galactose contents ranging from 40 to 96 mg/100 g powder (Table 1). Typically, the amount of caseinates added to various foods ranges from 1 to

Table 1. Galactose Content of Sodium and Calcium Caseinate[a]

	country of origin	galactose (mg/100 g)
sodium caseinate	USA (Wisconsin)	95.5
	New Zealand	62.0
	The Netherlands	<5.1
	mean ± SD	54.2 ± 45.7
calcium caseinate	USA (Wisconsin)	<5.1
	New Zealand	40.3
	The Netherlands	<5.1
	mean ± SD	16.8 ± 20.4
	total mean ± SD	35.5 ± 37.7

[a]Values reported are from a single analysis.

25% by weight,[27] which suggests that foods containing caseinates are an insignificant dietary source of galactose.

Cheesemaking is a complex process, and there are multiple factors that may affect the residual galactose concentration in the final product. Reports of residual galactose in cheese vary within the same type of cheese and can be attributed to minor variations in the milk itself[28] but, more importantly, to the specific starter and the nonstarter (secondary) cultures, the processing steps used to separate the curd from the lactose-rich whey, and the conditions used for cheese aging.[29] Hard cheeses that are aged for extended periods of time may be expected to have lower residual galactose content due to the metabolic activity of the secondary cultures[30,31] and, in some cases, persistence of the starter culture.[32]

For all analyzed cheese samples, only one sample contained both residual lactose and galactose. For all other cheeses, lactose was below quantitation limits and only galactose was measurable. The data confirm that Gruyere, Emmentaler, and brick Parmesan cheeses produced in U.S. dairies contain negligible lactose or galactose, similar to results reported for these cheese varieties produced in Europe.[16] The galactose content was below quantitation limits in Emmentaler and Gruyere cheeses produced in Switzerland and Wisconsin (Table 2). In North America, Emmentaler cheese may be called "Swiss" cheese; one sample of Swiss cheese contained 7.4 mg galactose/100 g. Another study also reported the absence of lactose and galactose, determined enzymatically, for Swiss cheese manufactured in the United States.[33] The specific bacterial starter culture used may contribute to differences in the residual galactose in Emmentaler cheese. For example, *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus helveticus* are thermophilic starter species typically used for Emmentaler or Swiss cheese production. The presence of *L. helveticus* is essential to produce a low-galactose cheese, as only a fraction of *S. thermophilus* strains are able to metabolize galactose;[34] however, *L. helveticus* strains vary in their ability to deplete galactose during the initial stages of cheesemaking.[35]

One sample of block Parmesan cheese aged for >10 months contained galactose below quantitation limits (Table 2). Unlike grated Parmesan cheese from Italy found to contain negligible galactose by enzymatic analysis,[16,36] a commercial brand of grated cheese from the United States contained 23.6 mg galactose/100 g. Similarly, using HPLC-PAD, eight New Zealand-produced Parmesan cheese samples varying in age from 6 to 19 months were found to contain an average of 38 ± 21 mg galactose/100 g, whereas two samples aged for 7 and 20 months contained 378 and 156 mg galactose/100 g, respectively[22] As with Emmentaler cheese, the growth and metabolic activity of the specific *Lactobacillus* species in the starter culture and during aging may affect residual galactose. Together, the data suggest that Parmesan cheese produced using traditional methods and aging are likely to have the lowest free galactose content.

Cheddar cheese is classified by the length of aging; mild Cheddar is aged for 2−3 months, medium Cheddar for 4−6 months, and sharp Cheddar, for >9 months. Nine samples of sharp Cheddar cheese were analyzed, averaging 28.9 mg galactose/100 g. One sample of medium Cheddar cheese and two samples of mild Cheddar cheese contained negligible galactose. Interestingly, sharp Cheddar cheeses produced in smaller regional dairies contained 7.8 ± 4.0 mg/100 g compared to 55.4 ± 37.2 mg/100g found in sharp Cheeses produced by a larger multinational producer (Kraft Foods Global, Northfield, IL, USA). This suggests that a more consistent product may be produced in smaller dairies. A similar conclusion was reached in analysis of sharp cheeses produced in England; cheeses produced at the UK West Country Farmhouse Cheese Makers Association contained negligible amounts of galactose determined enzymatically, whereas less consistency was found from other manufacturers.[16,17] It may be speculated that smaller producers are more consistent in the use of a traditional Cheddar process, whereas larger plants may adapt various modifications to reduce costs and/or increase efficiency of the operation. For example, high residual galactose concentrations were reported in a Cheddar cheese made using a 42 °C cook temperature to decrease production time.[30] Cheddar cheese is usually made with mesophilic *Lactococcus lactis* subsp. *cremoris* and/or *lactis* starter culture and a cook temperature of 38 °C. However, at the higher temperature, *L. lactis* was not effective in metabolizing galactose and was not active during cheese ripening due to a high salt sensitivity and extensive autolysis. In these experimental Cheddar cheeses, lactose was undetectable after 3−5 months of aging, but galactose was slower to decrease and was present in some samples at approximately 170 mg/100 g cheese after 6 months.[30]

Table 2. Galactose Content of Various Types of Cheese[a]

type	brand/manufacturer	galactose (mg/100 g)
Cheddar, sharp		
sharp, aged 1 year	Brennan's Cellars/Brennan's, New Glarus, WI	6.6
seriously sharp	Cabot Vermont/Creamery Cooperative, VT	<5.1
naturally sharp	Cracker Barrel/Kraft Foods Global, IL	60.4
extra sharp white	Cracker Barrel/Kraft Foods North America, IL	16.5
sharp	Tillamook/Tillamook County Creamery Association, OR	14.7
sharp	Kraft/Kraft Foods Global, IL	104.3
sharp	JS Brands/JS Brands of Wisconsin, WI	7.4
old Cheddar	Cracker Barrel/Kraft Canada, ON	40.3
old Cheddar	Best Buy/Lucerne Foods, AB	<5.1
	mean ± SD	28.93 ± 34
Cheddar, mild and medium		
medium, aged 6 months	Brennan's Cellars/Brennan's, New Glarus, WI	<5.1
mild, aged 3 months	Brennan's Cellars/Brennan's, New Glarus, WI	5.6
mild, yogurt base	Cabot Vermont/Cabot Creamery Cooperative, VT	<5.1
Emmentaler	Whole Foods Market/Emmi Ltd., Switzerland	<5.1
	Brennan's Cellars/Brennan's, New Glarus, WI	<5.1
Swiss	Crystal Farms/Crystal Farm, WI	7.4
Gruyere	Whole Foods Market/Emmi Ltd., Switzerland	<5.1
	Brennan's Cellars/Brennan's, New Glarus, WI	<5.1
Parmesan		
powder	Kraft/Kraft Foods Global, IL	23.6
block, aged 10 months	Brennan's Cellars/Brennan's, New Glarus; WI	<5.1

[a]Values reported are from a single analysis.

Table 3. Free Galactose, Bound Galactose in Four Oligosaccharides, and Total Galactose in Commercially Canned Legumes and Canning Liquid[a]

legume	mass ratio beans/liquid	free galactose	melibose	raffinose	ciceritol	stachyose	total
garbanzo beans	1.1	24.6	ND[b]	49.3	248.5	185.6	507.9
	1.0–1.3	0.0–52.5		29.1–64.6	167.9–295.2	102.3–236.7	320.5–636.0
canning liquid		39.5	2.0	113.7	539.3	474.8	1169.2
		9.0–56.3	0.0–5.9	106.4–125.9	463.9–653.1	427.2–519.1	1090.9–1307.0
kidney beans	2.7	ND	ND	47.0	4.2	391.8	443.0
	2.0–4.1			39.1–52.9	0.0–12.5	367.5–404.4	406.6–469.4
canning liquid		17.7	11.7	42.4	ND	506.0	577.9
		0.0–28.9	5.4–17.0	41.3–43.6		380.8–572.1	427.4–660.4
black beans	2.7	5.3	ND	66.7	ND	428.3	500.3, 453.4–529.0
	1.8–3.7	0.0–15.9		51.8–77.3		401.7–451.7	
canning liquid		ND	48.3	105.6	15.7	932.3	1101.8
			38.5–57.9	90.6–119.7	5.9–26.3	848.5–1091.4	1012.5–1274.3
pinto beans	2.7	ND	ND	40.4	ND	253.2	293.6
	1.4–3.7			36.8–43.7		181.4–349.1	222.1–392.8
canning liquid		ND	23.4	48.1	ND	411.8	483.3
			16.3–34.7	28.1–81.7		250.7–622.5	304.6–738.8

[a]Values are the average and range of three analyses. Carbohydrate values are in milligrams per 100 g beans or 100 mL liquid. [b]ND, not detected.

Free galactose is toxic to plant cells and is highly regulated, so it is a very minor component of most plants and often not reported.[37] Instead, the major source of galactose in legumes is galacto-oligosaccharides, which are thought to aid in seed tolerance to desiccation and to serve as an energy reserve for germination.[38,39] The most common linear oligosaccharides include raffinose, stachyose, and verbascose, with one, two, or three galactosyl residues, respectively, linked α-1–6 to the glucose residue of sucrose and α-1–6 to each other. Galactose can also be linked to inositols; for example, ciceritol, found in garbanzo beans, is the α-digalactoside of pinitol (α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→2)-4-*O*-methyl-*chiro*-inositol).

The effect of various legume preparation methods on galacto-oligosaccharides has been reported;[15,40–43] however, there is almost no information on the effect of these treatments on free galactose concentrations. Although soaking and cooking reduce the oligosaccharide content, it is not clear whether these compounds are simply lost from the tissue or whether they are subject to enzymatic or thermal degradation, which may release free galactose.

The galactose content of various commercially canned legumes and canning liquid is shown in Table 3 Free galactose was highest in garbanzo beans (24.6 ± 26.4 mg/100 g whole beans and 39.5 ± 26.5 mg/100 mL canning liquid) compared to the other varieties examined. Free galactose was also found in black beans (5.3 ± 9.2 mg/100 g) but was not detected in whole red kidney beans or pinto beans; however, free galactose was detected in the canning liquid of kidney beans (17.7 ± 15.5 mg/100 mL). Stachyose contributed the greatest amount of bound galactose in pinto, black, and kidney beans, whereas ciceritol was the highest source of bound galactose in garbanzo beans.

Free galactose found in the canned garbanzo beans and the canning liquid is equivalent to approximately 180 mg/100 g bean dry weight (calculated accounting for the weight of each portion). This value is substantially lower than the 444 mg/100 g reported by Acosta and Gross,[7] but similar to concentrations of 184, 110, and 80 mg/100 g reported by others.[44–46] The high concentration reported by Acosta and Gross may be due in part to a different variety of garbanzo bean or the different analytical technique used for quantitation. Although similar extraction methods were used in all studies, Acosta and Gross reduced and derivatized the sugars to alditol acetates for analysis by gas chromatography, which would exaggerate the galactitol peak with any galactitol naturally present in the beans. As well, the difference may be related to the bean preparation method. Soaking beans at room temperature prior to cooking as done by Acosta and Gross would initiate germination, which, in turn, initiates hydrolysis of galacto-oligosaccharides by α-galactosidase with possible liberation of free galactose.[47]

In addition, the higher free galactose concentration found in the canning liquid indicates that the free galactose content associated with the consumption of canned legumes can be significantly reduced if the canning or cooking liquid is discarded and any residual liquid on the legumes is removed by rinsing.

Although the maximum amount of dietary galactose safely tolerated by those with classic galactosemia remains unknown,[3,48] results from this study find that the galactose content of specific cheeses, caseinates, and legumes are within those found in currently allowed foods and ingredients and are low enough to be acceptable in the diet for classic galactosemia. Allowing some previously restricted foods in the diet could improve both adherence and nutrient intake of individuals who require chronic galactose restriction. These analytical findings may have important implications for patients with galactosemia and support a review of current dietary recommendations for classic galactosemia.

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