J. Inher. Metab. Dis. 14 (1991) 253–258

Fruits and Vegetables are a Source of Galactose: Implications in Planning the Diets of Patients with Galactosaemia*

K. C. GROSS[1] and P. B. ACOSTA[2]
[1]*United States Department of Agriculture, Agricultural Research Service, Product Quality and Development Institute, Horticultural Crops Quality Laboratory, Beltsville, MD 20705, USA*; [2]*Ross Laboratories, 625 Cleveland Avenue, Columbus, OH 43215, USA*

Summary: It has become apparent that removing dairy products from the diets of patients with galactosaemia does not sufficiently diminish the deleterious signs. We have determined the amount of soluble monomeric galactose in 45 fruits and vegetables using capillary gas chromatography and selective ion monitoring. Galactose contents ranged from less than 0.1 mg per 100 g of tissue in artichoke, mushroom, olive, and peanut to 35.4 mg per 100 g in persimmon. Fruits and vegetables with over 10 mg per 100 g included date, papaya, bell pepper, tomato and watermelon. These results will provide important data for planning the diets of patients with galactosaemia.

INTRODUCTION

Poor outcomes in patients who have been treated early for galactose-1-phosphate uridyltransferase (EC 2.7.7.10; GALT) deficiency (McKusick 23040) provide reason for concern (Buist *et al.*, 1988, 1989). The major causes of poor outcomes in these patients are unclear, but several factors may be contributory, including damage to the fetus *in utero*, damage after birth but before initiation of treatment, incorrect diet management (Donnell *et al.*, 1969), self-intoxication from continuous *de novo* galactose-1-phosphate synthesis (Gitzelman *et al.*, 1974), and most recently, UDP-galactose deficiency (Ng *et al.*, 1987).

Results of a number of studies since the 1950s support the hypothesis that the diet of patients with galactosaemia is not strict enough, potentially allowing for galactose toxicity due to the presence of galactose in cereals (Pomeranz, 1973), fruits (Jermyn and Isherwood, 1956; Gross and Sams, 1984) and vegetables (Shallenberger and Moyer, 1961; Weier and Benson, 1967; Wood and Siddiqui, 1972; Fry, 1982; Gross, 1983; Gross and Sams, 1984). It is clear that galactose is present in grains in the form

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MS received 27.7.90 Accepted 30.11.90

of raffinose saccharides, but it is doubtful whether these α-linked galactosyl residues would be available because they are not hydrolysed by human gut enzymes. However, it has become evident that plants, including many fruits and vegetables, contain free monomeric galactose as well as β-1,4-linked galactosyl residues in chloroplast membranes of green plant tissues (galactolipid) and in the cell wall (galactan). These cell wall and galactolipid-associated galactosyl residues would be available after hydrolysis by β-galactosidase (EC 3.2.1.23) in the gut.

Because of the lack of realization that many plant-related foodstuffs contain significant amounts of bound and free galactose, children who are presently considered to be on a galactose-free diet could in fact be ingesting significant amounts. Daily ingestion of significant amounts of galactose by patients with GALT deficiency may possibly be involved in precipitating symptoms of mental retardation, neurological disorders, ovarian failure and growth inhibition. Studies on galactose bioavailability in edible plant tissues such as fruits and vegetables will therefore provide useful information for the management of patients with GALT deficiency.

The objective of this study was to quantify the amount of soluble, digestible galactose available in various fruits and vegetables, to estimate the amount of galactose potentially available in the cell walls of fruits and vegetables, and to make some inferences relative to the diet management of patients with GALT deficiency.

MATERIAL AND METHODS

Plant material

For soluble galactose determination, fruits and vegetables were purchased at a local market at the horticulturally mature stage, i.e. the stage of maturity at which they are most commonly eaten. Plant material was rinsed with double distilled water and dried with a soft cloth.

Soluble galactose extraction

The most commonly eaten portion of each fruit or vegetable was excised from at least two samples, diced into approximately 0.5–1 cm^3 sections, mixed, and three subsamples of 10 g fresh weight (gfw) were each placed in 30 ml of 80% aqueous ethanol. Samples were then placed in a boiling water bath for 10 min, stored at −20°C for 16 h, and homogenized using a Polytron (Brinkmann Instruments) at speed 4 for 1 min. Homogenates were filtered through four layers of cheesecloth, the residues rinsed with 3 ml of 80% ethanol, and the filtrates centrifuged at 20 000 *g* for 15 min. Supernatants were removed and brought up to a total volume of 30–40 ml with 80% ethanol. A 10 ml aliquot of each sample was then passed through a C_{18} Sep-Pak cartridge (Waters), the cartridge was washed with 2 ml of 80% ethanol, and three 3 ml aliquots from each extract were taken to dryness in a vial with a stream of nitrogen at 45°C and placed in a vacuum oven at 35°C until derivatization for gas chromotography/mass spectrometry was carried out.

Galactose derivatization

Soluble galactose in the dried ethanolic extracts was made into its alditol acetate derivative using a method similar to that of Blakeney and colleagues (1983), and 100 μl of 1 mol/L ammonium hydroxide containing 100 μg of allose as an internal standard was added to each sample vial. After adding 500 μl of dimethylsulphoxide containing 10 mg of sodium borodeuteride, $NaBD_4$, the vials were sealed with teflon screw caps and incubated at 45°C with occasional mixing. After 90 min the samples were neutralized with 100 μl of glacial acetic acid, and 100 μl of 1-methylimidazole was added. After the addition of 500 μl of acetic anhydride, the samples were mixed vigorously and allowed to stand at 22°C for 10 min. Samples were purified by partitioning twice with 1.5 ml of water and 1 ml of methylene chloride. The methylene chloride phases were combined and taken to dryness with a stream of nitrogen at 40°C. Samples were redissolved in 10–20 μl of methylene chloride and injected.

Galactose analysis

Complete separation of galactose from other soluble sugars was accomplished using capillary gas chromatography. Detection and quantification of galactitol acetate were performed using a mass selective detector by selective ion monitoring of m/e 212 after elution of glucitol acetate.

Chromatography was performed on a 5% phenylmethylsilicone column (30 m × 0.2 mm i.d.) under the following conditions: carrier gas, He; flow rate, 1 ml/min; injection port temperature, 225°C; detector temperature, 230°C; split ratio, 40:1; injection size, 1 μl. The oven temperature programme consisted of an initial temperature of 160°C, increasing to 190°C at 2°C/min, at which time the rate was increased to 4°C/min until a temperature of 210°C was reached. The rate was then increased to 10°C/min until a final temperature of 250°C was reached.

The GC/MS system was equipped with a computer which automatically performed peak integration and internal standard calculations, and determined individual response factors. A seven point calibration curve was used which showed a linear response up to 5 ng galactose. The amount of galactose in all samples injected onto the column was within this range.

RESULTS AND DISCUSSION

A survey of 45 fruits and vegetables revealed a wide range of soluble galactose content, ranging from less than 0.1 mg/100 gfw in artichoke, mushroom, olive, and peanut to 35.4 mg/100 gfw in persimmon (Table 1). Since patients with GALT deficiency may need to limit their intake of galactose to less than 125 mg daily (Bower and Smallpiece, 1955), it is clear that fruits and vegetables are an important source of soluble galactose and consideration should be given to this fact when planning the diet of such patients.

In addition to soluble galactose, fruits and vegetables contain substantial amounts of bound galactose in the cell wall, primarily as pectic-associated β-1,4-galactan. This galactose-containing polysaccharide is hydrolysed by β-galactosidase, an exo-type hydrolytic enzyme which liberates galactose from the non-reducing end of the galactan

Table 1 Soluble galactose content (mg/100 g fresh weight ± SE) of various fruits and vegetables

Fruit/vegetable	*Genus/species*	*Content*
Apple	*Malus domestica* Borkh.	8.3 ± 0.7
Apricot	*Prunus armeniaca* L.	1.1 ± 0.6
Artichoke	*Cynara scolymus* L.	ND
Asparagus	*Asparagus officinalis* L.	1.2 ± 0.6
Avocado	*Persea americana* Mill.	< 0.5
Banana	*Musa* × *paradisiaca* L.	9.2 ± 0.8
Bean sprouts, green	*Phaseolus vulgaris* L.	4.3 ± 0.2
Beet, red	*Beta vulgaris* L.	0.8 ± 0.2
Broccoli	*Brassica oleracea* var botrytis L.	6.8 ± 0.7
Brussels sprouts	*Brassica oleracea* var gemmifera DC	9.2 ± 0.7
Cabbage, common	*Brassica oleracea* var capitata L.	3.3 ± 0.2
Cantaloupe melon	*Cucumis melo* L.	4.3 ± 0.2
Carrot	*Daucus carota* Arcang.	6.2 ± 0.4
Cauliflower	*Brassica oleracea* var botrytis L.	4.3 ± 0.3
Celery	*Apium graveolens* Mill.	2.4 ± 0.1
Corn, sweet	*Zea mays* L.	3.7 ± 0.3
Cucumber	*Cucumis sativus* L.	4.0 ± 0.3
Date	*Balanites aegyptiaca* (L.) Del	11.5 ± 0.6
Eggplant (aubergine)	*Solanum melongena* L.	4.7 ± 0.2
Grape, green	*Vitis amurensis* Rupr.	2.9 ± 0.1
Grapefruit	*Citrus* × *paradisi* Macfad.	4.1 ± 0.1
Kale	*Brassica oleracea* var acephala DC	2.3 ± 0.2
Kiwi	*Actinidia chinensis* Planchon	9.8 ± 0.4
Lettuce, garden	*Lactuca sativa* L.	3.1 ± 0.3
Mushroom, common	*Agaricus campestrus* L.	ND
Olive, green	*Olea europaea* L.	ND
Onion, yellow	*Allium cepa* L.	5.1 ± 0.3
Onion, bunching	*Allium fistulosum* L.	6.1 ± 0.3
Orange, sweet	*Citrus sinensis* (L.) Osbeck	4.3 ± 0.4
Papaya	*Carica papaya* L.	28.6 ± 1.9
Pea, sweet	*Lathyrus odoratus* L.	4.9 ± 0.8
Peanut	*Arachis hypogaea* L.	ND
Pear	*Pyrus communis* L.	7.3 ± 1.4
Pepper, bell	*Capsicum annuum* L.	10.2 ± 0.4
Pepper, cayenne	*Capsicum frutescens* L.	9.7 ± 4.0
Persimmon, American	*Diospyros virginiana* L.	35.4 ± 2.5
Potato, white	*Solanum tuberosum* L.	1.2 ± 0.3
Potato, sweet	*Ipomoea batatas* (L.) Lam	7.7 ± 0.7
Pumpkin	*Cucurbita maxima* Duchesne	9.9 ± 2.5
Radish, red	*Raphanus sativus* L.	0.5 ± 0.3
Spinach	*Spinacia oleracea* L.	0.1 ± 0.1
Tomato	*Lycopersicon esculentum* Mill.	23.0 ± 2.0
Turnip	*Brassica rapa* L.	4.9 ± 0.6
Watermelon	*Citrullus lanatus* (Thunb.)	14.7 ± 2.0
Zucchini squash	*Cucurbita pepo* L.	3.3 ± 0.1

ND = Not detectable

chain. This galactose would then be potentially available for absorption in the gut.

A reanalysis of the data presented by Gross and Sams (1984) reveals that, of 17 fruits and vegetables surveyed, the potential amount of cell wall galactan available for absorption in the gut ranged from 8 to 110 mg/100 gfw in blackberry and plum, respectively (data not shown). For these calculations, it was assumed that among all fruits and vegetables the amount of cell wall material was a constant 1 g of dry cell wall per 100 gfw, and that 80% of the cell wall galactosyl residues were β-1,4-linked, i.e., labile to β-galactosidase action.

Ingestion of 100 g each of cucumber, pear, plum and tomato could potentially provide approximately 500 mg of total galactose (soluble + cell wall galactan). However, before recommendations can be made to restrict further low galactose diets for patients with galactosaemia, *in vivo* studies must be carried out to examine the effect of consuming fruits and vegetables high in galactose content. In addition, studies are needed to determine the maximum intake of galactose tolerated long term, to determine the availability of galactose in β-1,4-galactan, to determine the affinity of human β-galactosidase(s) for plant galactans, to determine the effect of food processing on the availability of galactose in foods, and to survey all fruits and vegetables, as well as other foodstuffs, for galactose and galactan content.

ACKNOWLEDGEMENT

The authors wish to acknowledge the excellent technical assistance of J. Norman Livsey.

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