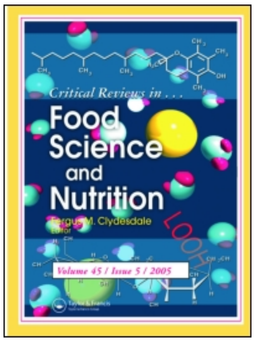

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Alpha-Galactosides: Antinutritional Factors or Functional Ingredients?

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This review focuses on updated information about α-galactosides, their chemical structure, biosynthesis, plant physiological functions, occurrence in foods, positive and negative physiological effects in animals, changes during food processing, and their potential application as prebiotics in the food industry. Although α-galactosides are considered as the main flatus-causing factors, they are also involved in several important functions during plant and seed development and beneficially stimulate the growth and activity of living bifidobacteria and lactobacilli in the human colon. We focus here also on legumes as a source of this kind of prebiotics as potential health promoters.

Keywords α-galactosides, flatulence, prebiotics, legumes

ALPHA-GALACTOSIDES: CHEMICAL STRUCTURE, BIOSYNTHESIS, AND FUNCTION

Chemical Structure

α-Galactosides, also known as the raffinose family of oligosaccharides (RFO), are low molecular weight non-reducing sugars that are soluble in water and water-alcohol solution (Dey, 1980). They are the most abundant soluble sugars and rank next to sucrose in their distribution in the plant kingdom. α-Galactosides occur only at low concentrations in plant leaves and accumulate in storage organs during the development process (Dey, 1990; Frias et al., 1996a).

The best-known α-galactosides are raffinose, stachyose, verbascose, and ajugose. These compounds are α(1→6) galactosides linked to the C-6 of the glucose moiety of sucrose. A less well-researched group of α-galactosides corresponds to the galactosyl cyclitols (Ganter et al., 1991; Obendorf et al., 1998). Ciceritol is one of the best-known galactosyl cyclitols that was first isolated from chick peas (Cicer aurenticum) (Quemener and Brillouet, 1983) and later from lentils (Lens culinaris) (Bernabé et al., 1993).

Chemically, α-galactosides are considered to be derivatives of sucrose since they are made up of combinations of D-galactose units linked to the D-glucose moieties group of the sucrose molecule, giving rise to a number of oligosaccharides (Dey, 1980). Their IUPAC (International Union Pure and Applied Chemistry) names are listed below.

- Raffinose α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1→2)-β-D- fructofuranoside
- Stachyose α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside
- Verbascose α-D-galactopyranosyl-(1→6)-[α-D-galactopyranosyl-(1→6)]$_2$-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside
- Ajugose α-D-galactopyranosyl-(1→6)-[α-D-galactopyranosyl-(1→6)]$_3$-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside
- Ciceritol α-D-galactopyran osyl-(1→6)-α-D-galactopyranosyl-(1→2)-4-O-methyl-*quiro*-inositol

Structures of these oligosaccharides are shown in Fig. 1.

Biosynthesis

Sucrose is formed as a major product of photosynthesis in higher plants and tends to translocate from leaves to other organs. It is the major carbohydrate-storage material that provides a ready source of D-glucose and D-fructose.

The synthesis of α-D-galactosyl derivatives of sucrose is catalyzed by the enzyme galactinol synthase (UDP-α-D-galactose:1-L-myo-inositol-*O*-α-galactopyranosyltransferase, EC 2.4.1.123) generating galactinol from UDP-galactose and

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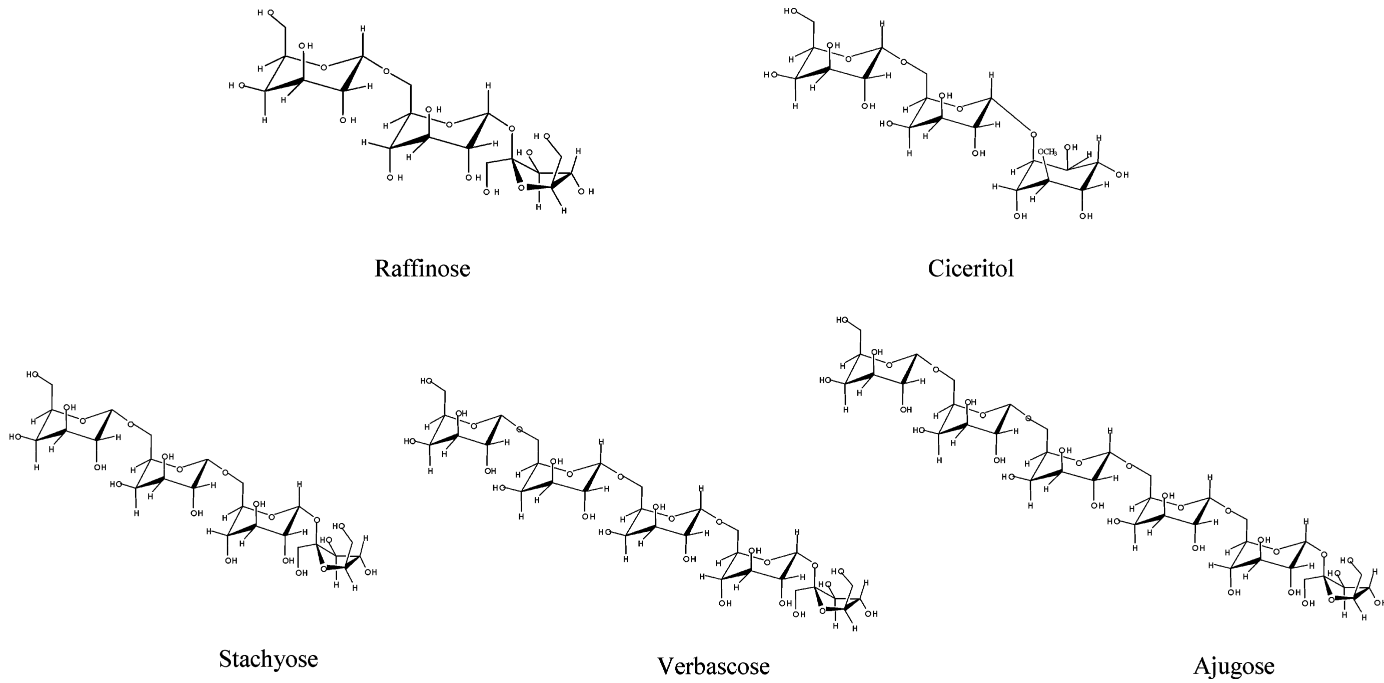

Figure 1 Chemical structure of α-galactosides.

myo-inositol (Fig. 2). Galactinol synthase seems to be the key enzyme in the biosynthesis pathway of α-galactosides (Sarawitz et al., 1987; Lowell and Kuo, 1989; Keller, 1992) and its activity increases when the seed begins to dry off (Castillo et al., 1990).

Galactinol acts as a transfer-intermediate for galactose in the biosynthesis of RFOs and although it is thought to have no other role within the plant (Keller, 1992) some hypotheses suggest it to be involved in the synthesis of galactomanans

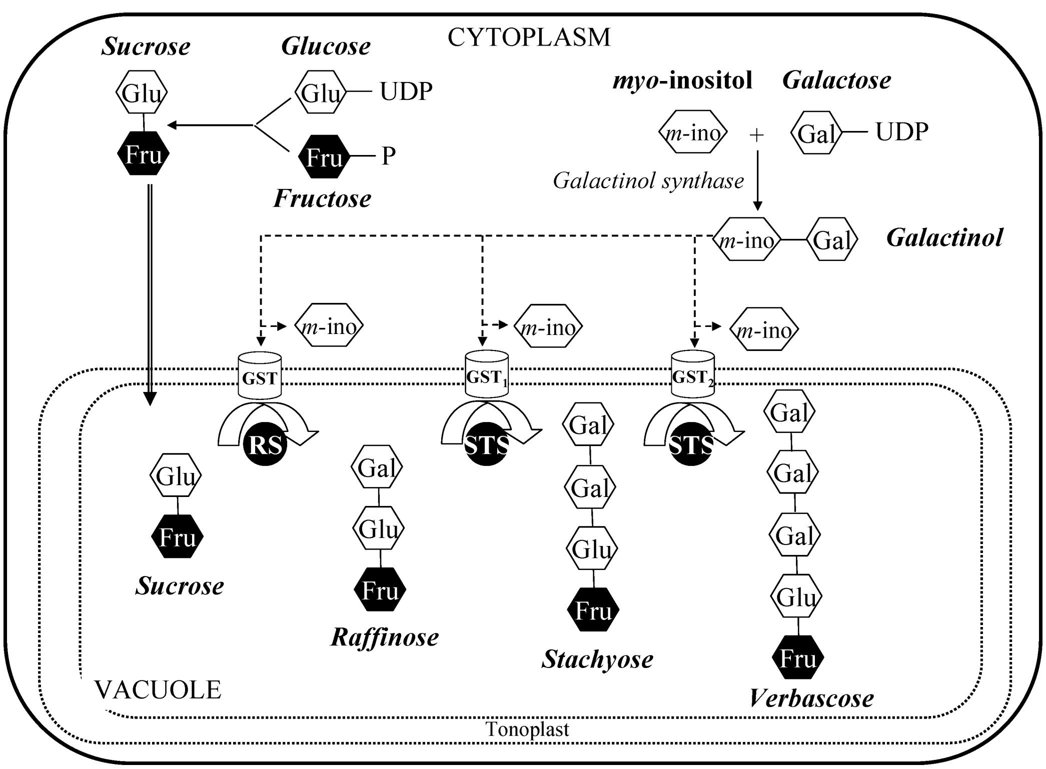

Figure 2 α-Galactoside biosynthesis. GST: galactosyl transferase; RS: raffinose synthase; STS: stachyose synthase.

(Reid, 1995). Sucrose is the acceptor of the galactosyl moiety from galactinol, synthesizing raffinose with the release of myo-inositol, a reaction catalyzed by the galactosyl transferase raffinose synthase (EC 2.4.1.82, Fig. 2). In a further step, raffinose acts as the substrate to produce the next oligosaccharide, stachyose, by transferring the α-D-galactosyl moiety from galactinol to the C-6 of the non-reducing α-D-galactose moiety with regeneration of myo-inositol by action of the enzyme stachyose synthase (EC 2.4.1.67) (Tanner and Kandler, 1966; Gaudreault and Webb, 1981). In addition, some other papers describe other reactions associated with this enzyme like the production of galactopinitol (Schweizer et al., 1978), galactosylononitol (Peterbauer and Richter, 1998) galactosyl pinitol A (Hoch et al., 1999) and verbascose (Frias et al., 1999), this latter oligosaccharide is produced from the addition of galactinol to stachyose (Tanner et al., 1967). Finally, Bachmann and Keller (1995) reported that biosynthesis of the high molecular weight raffinose family oligosaccharide could occur by a galactinol-independent galactosyl-transferase activity.

Physiological Function in the Plant

Generally, seeds accumulate sucrose in the early stages of development, and α-galactosides are synthesized and deposited in storage organs, such as seeds, roots, and tubers of many leguminous and other plants (Dey, 1980; Andersen et al., 2005). Raffinose only occurs at low concentrations in plant leaves, but accumulates, together with its higher homologous, in the storage organs during the developmental process (Frias et al., 1996a; Lowell and Kuo, 1989; Horbowicz and Obendorf, 1994; Górecki et al., 1996, 1997) where they perform protective physiological functions associated with the onset of desiccation tolerance and frost resistance (Clegg et al., 1982; Obendorf et al., 1998).

By in vitro experiments, it has been shown that protoplasmic membranes of plant cells are damaged in frost and drought and can be protected from this by some oligosaccharides. Santarius (1973) showed that the trisaccharide raffinose was more effective in chloroplast membrane stabilization during temperature and water stress than sucrose or glucose. Blackman et al. (1991) and Blackman et al. (1992) reported that soybean seeds naturally develop desiccation tolerance associated with other physiological changes such as the loss of green colour in the embryonic axis tissues, the accumulation of raffinose family oligosaccharides in the axis tissues, the accumulation of LEA (late-embriogenesis-abundant) proteins and the hydrolysis of starch. Soybean seeds in the early stages of maturation could germinate but did not resist rapid desiccation. However, when slowly dried these seeds tolerated desiccation and amounts of raffinose and stachyose were detected in their embryonic axes. The α-galactoside content recorded was almost 3 times higher than those found when axes mature naturally. In contrast, when seed maturation was performed at 100% relatively humidity, an absence of stachyose and desiccation tolerance was shown in soybean.

The mechanism by which α-galactosides confer seed desiccation tolerance has been studied in a large number of publications (Bruni and Leopold, 1991; Horbowicz and Obendorf, 1994; Vertucci and Farrant, 1995). The authors agree about a role for α-galactosides as membrane protecting agents by mechanisms of water replacement and glass formation. The first mechanism is based on the replacement of water by carbohydrate hydroxyl groups, providing the required hydrophilic interaction that stabilizes cellular membranes and proteins (Bryant and Wolfe, 1992; Vertucci and Farrant, 1995). The second mechanism explains that α-galactoside synthesis prevents the crystallization of sucrose during desiccation and enhances the formation of a stable glassy state (Koster and Leopold, 1988; Sun and Leopold, 1993; Leopold et al., 1994).

Oligosaccharides and galactosyl cyclitols also exert a protective role during desiccation tolerance because the monosaccharide content decreases as a consequence of their synthesis. A low monosaccharide content may inhibit respiration, which is a source of free radicals (Vertucci and Farrant, 1995). In addition, a low monosaccharide content may partially prevent consequences of Maillard's reaction, which is destructive to proteins (Wettlaufer and Leopold, 1991).

On the other hand, α-galactosides have been involved in providing seed storability (Horbowicz and Obendorf, 1994), which depends on the ratio of sucrose to total α-galactosides. Sucrose:oligosaccharide ratio <1.0 confers a seed storability with a half-viability period >10 years, whereas ratios >1.0 give a seed storability with a half-viability period <10 years.

A limited number of experiments reported that α-galactosides also play some role in the temperature stress response of maturing seeds. α-Galactoside content and composition during seed maturation is affected by temperature. Górecki et al. (1996) reported a higher dry matter accumulation of Lupinus albus seeds matured at 28°C than those matured at 13°C, whereas small changes in the α-galactoside content were found. However, pinitol and the galactose-containing pinitols were 2-fold higher when seed maturation was performed at 28°C. In contrast with these results, Lupinus luteus seeds matured at 18°C showed that stachyose and verbascose increased by two-fold compared with seeds matured at 25°C (Górecki et al., 1997).

Moreover, commonly accumulation of sucrose and α-galactosides in leaves and seeds may contribute to cold acclimatization and the protection of membrane proteins from denaturization towards dormancy. In vitro experiments have demonstrated that α-galactosides exerts a protective role of membrane tilacoids to frost, and raffinose seems to be the most effective oligosaccharide (Santarius, 1973).

In addition to roles described above, α-galactosides store and transport carbon skeletons during seed germination (Dey, 1980; Górecki et al., 1997) and levels of raffinose, stachyose, and verbascose decreased gradually during legume sprouting (Reddy and Salunkhe, 1978; Vidal-Valverde and Frias, 1992; Frias et al., 1996b). Reddy and Salunkhe (1978) reported a decrease in α-galactoside content during germination which correlated with a rise in α-galactosidase activity and monosaccharides (fructose, glucose, and sucrose) in soybean cotyledons.

ALPHA-GALACTOSIDES IN FOODS

Differences in the α-galactoside contents in different plant species have been widely described in the literature and the content range for individual and total α-galactosides is listed in Table 1. The occurrence of individual α-galactosides seems to depend on the species and the genotype (Dey, 1980; Reddy et al., 1984; Salunke and Kadam, 1989; Frias et al., 1994, 1996a, 1999) and several chemotaxonomic studies have been carried out to evaluate the authenticity/falsification of feed and food additives when RFO-containing plants are used (Andersen et al., 2005; Martínez-Villaluenga et al., 2005a).

Total α-galactoside content reported in different pea varieties varied between 2.3–9.6% (Reddy et al., 1984; Troszynska et al., 1995; Vidal-Valverde et al., 2003) (Table 1). Raffinose (0.4%) and stachyose (0.3–5.5%) were present in all pea varieties tested and verbascose was detected only in 24 varieties.

According to the information found in the literature for several lupin varieties (Trugo et al., 1988; Ruiz-López et al., 2000; Andersen et al., 2005; Martínez-Villaluenga et al., 2005a), these legumes seeds are the richest source of oligosaccharides and the total α-galactoside content in the seeds studied ranged between 5.1 and 16.1% (Table 1) and a wide variation in content and composition among lupin species was observed. L. luteus showed a remarkably high content of total α-galactosides (11.0–16.1%) which was about twice that recorded in other lupin cultivars. The highest amount of raffinose was detected in L. mutabilis (1.9%), whereas the raffinose content in the remaining Lupinus species ranged from 0.6–1.2% in L. angustifolius cultivars, 0.5–0.6% in those of L. luteus and 0.3–0.6% in the cultivars of L. albus. Stachyose was always the major α-galactoside present in lupin seeds. L. luteus and L. albus cultivars presented the highest level of stachyose (6.1–8.6% and 5.0–7.2%, respectively) while the L. angustifolius cultivars ranged from 3.6 to 5.2%. The largest variation for individual α-galactosides was found for verbascose, which was not detected in 4 L. albus seeds, while L. luteus, L. angustifolius, and L. mutabilis varieties presented amounts from 2.8–3.5%, 0.8–2.5%, and 1.0%, respectively. Ajugose was present exclusively in lupin seeds and was only detected in 14 varieties of all the Lupinus species. L. albus and L. mutabilis contained the lowest ajugose levels (0.2–0.5% and 0.2%, respectively) followed by L. angustifolius (1.7–2.6%) and, finally, L. luteus (0.6–4.6%).

According to information recorded from earlier studies in different soybean varieties (Reddy et al., 1984; Naczk et al., 1997; Hollung et al., 2005) the main oligosaccharides in seeds are the α-galactosides raffinose (1.0–2.0%) and stachyose (2.2–4.9%), accounting for a total α-galactoside content of 6.0–8.0%. Verbascose was in very low amount or not detected and ajugose was not detected in the soybean varieties studied (Table 1).

The total α-galactoside content of nine varieties of bean (Phaseolus vulgaris) (Rackis, 1975; Reddy et al., 1984; Salunkhe et al., 1989; Vidal-Valverde et al., 1993a; Troszynska et al., 1995), ranged broadly from 0.4–8.0% and the individual α-galactoside content from 0.2–2.5%, 0.2–4.2% and 0.1–4.0% for raffinose, stachyose, and verbascose, respectively (Table 1).

Large differences in the range of α-galactoside content (1.8–7.5%) were described for several varieties of lentil seeds (Reddy et al., 1984; Vidal-Valverde et al., 1992a; Vidal-Valverde and Frias, 1992; Vidal-Valverde et al., 1993a; Frias et al., 1994; Troszynska et al., 1995). Regarding the amount of individual α-galactoside, ciceritol was found in all varieties ranging from 0.2 to 2.1%. Raffinose was also present in all lentil varieties with quantities ranging from 0.1 to 1.0%. Stachyose was the most abundant α-galactoside, ranging between 1.1 and 4.0%. Verbascose, however, was not present in some lentil varieties while in others levels reached up to 6.4% (Table 1).

Studies in several chickpea varieties (Rackis, 1975; Reddy et al., 1984; Rossi et al., 1984; Saini and Knights, 1984; Chavan et al., 1989; Vidal-Valverde et al., 1993a, Frias et al., 2000; Alajaji and El-Adawy, 2006) showed a wide range in α-galactoside content. Some varieties showed traces of raffinose and verbascose whilst others presented levels of 2.4 and 4.5% for these sugars, respectively. Ciceritol was present in considerable amount in most of the species studied (from 1.2 to 3.1%) and stachyose ranged from 0.4 to 2.6% (Table 1).

Total α-galactoside content of different varieties of faba bean (Reddy et al., 1984; Kozlowska et al., 1992; Freijnagel et al., 1997; Vidal-Valverde et al., 1998) ranged from 1.0–4.5%. However, the raffinose content was lower than for other legumes such as beans and peas, and stachyose content was the lowest among the grain legumes reported (Table 1).

There are few data on α-galactoside contents in other plants since, generally, relevant amounts have only been found in legume seeds. Nevertheless, Andersen et al. (2005) reported individual α-galactoside content in Brassica species and barley. The Brassica varieties contained a low RFO content compared with legumes with contents ranging from 0.9–2.1% with the exclusive detection of raffinose and stachyose (Table 1). B. campestris and B. nigra contained 0.2% and 0.6% of raffinose, respectively, whereas B. napus contained 0.2–0.4%. In the Brassica species, the stachyose content was considerably lower compared with legumes, ranging from 0.7 to 1.7% of the seed weight. Barley (Hordeum vulgare L.) contained 0.5% raffinose, which was the only RFO component present in the seeds.

PHYSIOLOGICAL EFFECTS OF ALPHA-GALACTOSIDES

Negative Effects

High levels of α-galactosides have been reported to present several negative nutritional effects including:

- Flatulence due to the anaerobic fermentation of α-galactosides in the hindgut.

Table 1 α-Galactoside content (range as % dry matter) of various seeds of Leguminosae, Brassica, and barley species

Genus	Species	Raffinose	Ciceritol	Stachyose	Verbascose	Ajugose	Total	References
Pisum	sativum	0.4–2.3	ND	0.3–5.5	0–4.3	ND	2.3–9.6	Reddy et al., 1984; Troszynska et al., 1995; Vidal-Valverde et al., 2003
Lupinus	albus	0.3–0.6	ND	5.0–7.2	ND–0.9	0.2–0.5	5.5–8.1	Trugo et al., 1988; Ruiz-López et al., 2000; Andersen et al., 2005; Martínez-Villaluenga et al., 2005a
	luteus	0.5–0.6	ND	6.1–8.6	2.8–3.5	0.6–4.6	11–16.1	Trugo et al., 1988; Ruiz-López et al., 2000; Andersen et al., 2005; Martínez-Villaluenga et al., 2005a
	angustifolius	0.6–1.2	ND	3.6–5.2	0.8–2.5	1.7–2.6	6.7–11.5	Trugo et al., 1988; Ruiz-López et al., 2000; Andersen et al., 2005; Martínez-Villaluenga et al., 2005a
	mutabilis	1.9	ND	2.3	1.0	0.2	5.1	Trugo et al., 1988; Ruiz-López et al., 2000; Andersen et al., 2005; Martínez-Villaluenga et al., 2005a
Glycine	max	1.0–2.0	ND	2.2–4.9	ND–0.3	ND	6.0–8.0	Reddy et al., 1984; Naczk et al., 1997; Hollung et al., 2005
Phaseolus	vulgaris	0.2–2.5	ND	0.2–4.2	0.1–4.0	ND	0.4–8.0	Rackis, 1975; Reddy et al., 1984; Salunkhe et al., 1989; Vidal-Valverde et al., 1993a; Troszynska et al., 1995
Lens	culinaris	0.1–1.0	0.2–2.1	1.1–4.0	ND–6.4	ND	1.8–7.5	Reddy et al., 1984; Valverde and Frias, 1992; Vidal-Valverde et al., 1992; Vidal-Valverde et al., 1993a; Frias et al., 1994; Troszynska et al., 1995
Cicer	arietinum	ND –2.4	1.2–3.1	0.4–2.6	ND–4.5	ND	2.0–7.6	Rackis, 1975; Reddy et al., 1984; Rossi et al., 1984; Saini and Knights, 1984; Chavan et al., 1989; Vidal-Valverde et al., 1993a; Frias et al., 2000; Alajaji and El-Adawy, 2006
Vicia	faba	0.1–1.5	ND	0.2–2.4	1.1–2.4	ND	1.0–4.5	Reddy et al., 1984; Kozlowska et al., 1992; Reddy et al., 1984; Freijnagel et al., 1997; Vidal-Valverde et al., 1998
Brassica	campestris	0.2	ND	0.7	ND	ND	0.9	Andersen et al., 2005
	napus	0.2–0.4	ND	0.7–1.7	ND	ND	0.9–2.1	Andersen et al., 2005
	nigra	0.6	ND	1.3	ND	ND	1.9	Andersen et al., 2005
Hordeum	vulgare	0.5	ND	ND	ND	ND	0.5	Andersen et al., 2005

ND = Not detected.

- Decreased dietary net energy contributions due to a higher proportion of hindgut fermentation.
- Osmotic effects of oligosaccharides in the intestine.
- Interference with the digestion of other nutrients.

Flatulence

Ingestion of large amounts of legumes is known to cause flatulence in humans and monogastric animals. Accumulation of flatus in the intestinal tract results in discomfort, abdominal rumblings, cramps, pain, and diarrhea. The α-galactosides (raffinose, stachyose, and verbascose) from legumes have been identified as one of the important contributors to flatus and one of the main reasons that deters people from eating more pulses (Murphy et al., 1972; Cristofaro et al., 1973; Price et al., 1988). Members of the raffinose family of oligosaccharides are not digested by man because the intestinal mucosa lacks the hydrolytic enzyme α-galactosidase and these sugars themselves are unable to pass through the intestinal wall (Cristofaro et al., 1974; Rackis, 1975). The microflora in the lower intestinal-tract then metabolize the raffinose family oligosaccharides and produce large amounts of carbon dioxide, hydrogen, and small quantities of methane and short chain fatty acids, and the pH is lowered (Cristofaro et al., 1974; Rackis, 1975; Anderson et al., 1979; Olson et al., 1981; Rackis, 1981; Naczk et al. 1997). Bacterial gases make almost three-fourths of the flatulence (Kurbel et al., 2006) and the consumption of a legume-containing diet is commonly associated with this abdominal discomfort (Price et al., 1988; Cristofaro et al., 1974; Rackis, 1981; Kurbel et al., 2006).

The first evidence of the non-nutritional effect of α-galactosides was published by Kuriyama and Mendel (1917) who fed rats with raffinose, which resulted in severe diarrhoea with evidence of raffinose residues in the feces. In 1966, Steggerda et al. (1966) demonstrated that soybean fractions containing low-molecular weight carbohydrates were the predominant flatulence-causing factors for the gas-producing potential in human subjects. Years later, Fleming (1981) reported a significant positive correlation between hydrogen production and the content of α-galactosides in legume seeds.

Reddy et al. (1984) reviewed the flatulence problem and the oligosaccharides causing it and reported that mung beans and green lentils are less flatulent than navy, kidney, red kidney, chickpea, and peas. Gumbmann and Williams (1971) found that the amount of hydrogen evolved by rats increased with increasing consumption of beans and, similarly, Reddy et al. (1980) showed a typical dose-response curve between the cooked bean cotyledons and hydrogen produced by rats. These authors suggested that this relationship could be used to predict the flatus potential of legumes and legume products.

Tomomatsu (1994) demonstrated that daily ingestion of 100 ml of a carbonated soft drink containing 3 g of α-galactosides for 2 weeks did not provoke negative physiological effects.

According to these studies, high doses of α-galactosides can produce flatulence whilst the consumption of low amounts do not cause discomfort and can even provide health benefits, as it will be shown further.

Reduction of Dietary Net Energy

Studies in vivo demonstrated that extensive fermentation occurred in the lower gastrointestinal tract when animals were fed with α-galactoside-containing diets. Müller et al. (1989) described lower net energy values of legume seeds rich in α-galactosides compared with those obtained for glucose which is absorbed in the upper gut. Coon et al. (1990) showed losses of 20% of the true metabolizable energy in diets containing 5.3% α-galactosides on a dry weight basis compared with a diet containing only 1% of these carbohydrates. This observation was justified on the basis of laxative properties of the raffinose family of oligosaccharides.

Further experiments performed in chickens and adult cockerels showed a high apparent digestibility coefficient (CDA) for α-galactosides, associated with extensive microbial fermentation in the lower gut of birds (Carré et al., 1991, 1995). However, in the case of pigs, microbial fermentation of α-galactosides especially occurs in the large intestine (Krause et al., 1994).

Seve et al. (1989) pointed out that in the practical feeding of pigs, the α-galactoside content does not exceed 2% of the feed dry weight and it has been demonstrated that a low α-galactoside content in the pig's diet did not show negative effects on growth performance, feed efficiency, nitrogen digestibility, and retention. However, a raffinose content above 0.45% in diets of leghorn roosters decreased the true metabolizable energy and digestibility (Leske et al., 1995).

In an attempt to increase the intestinal digestion of α-galactosides, many studies have been carried out with exogenous α-galactosidase supplements (Brenes et al., 1992; Gdala et al., 1997; Viana et al., 2005; Urbano et al., 2007). The addition of this enzyme to diets tends to improve growth and the apparent metabolizable energy.

Osmotic Effects of Oligosaccharides in the Intestine and Interference with Other Nutrients

A high raffinose content in the diet ($>$6.7%) results in osmotic pressure imbalance with small losses of raffinose before this can be fermented by the microorganisms (Wagner et al., 1976). The increase in fermentable carbohydrates in the lower gut leads to a microbial imbalance, causing diarrhoea (Veldman et al., 1993). A strategy to follow in animal feeding to alleviate these problems is the addition of 7.1 U g^{-1} of α-galactosidase to diets containing 2.75% of α-galactosides (Veldman et al., 1993).

Osmotic pressure imbalance generated in the small intestine by the raffinose family oligosaccharides reduces its absorption capacity (Wiggins, 1984; Zdunczyk et al., 1999). There was experimental evidence for a lower intestinal absorption of glucose, methionine, and water when α-galactosides (4–8%) from lupin seeds were incorporated in the perfusion fluid.

There is also evidence that the α-galactosides found in soybean can have a negative effect on protein utilization. Van Barneveld (1999) showed that the extraction of these oligosaccharides significantly improved the digestion of all amino acids from lupin by swine. This supports the hypothesis that galacto-oligosaccharides could interfere with the digestion of other nutrients when fed to swine and suggests that the α-galactoside content of lupins may also influence the nutritional value of its own protein. Findings of a study performed by Glencross et al. (2003) provided good support for the assumption that lupin oligosaccharides reduce the protein digestibility and, hence, the nutritional value of lupin meals in rainbow trout.

Positive Effects

The Prebiotic Effect

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995; Swennen et al., 2006). This implies that they resist hydrolysis by digestive enzymes and/or are not absorbed in the upper part of the gastrointestinal tract and pass into the large bowel and promote the growth of Bifidobacterium and Lactobacillus (Roberfroid, 2002). α-Galactosides are among this group of compounds.

The right balance of intestinal bacterial flora is important for human health. In particular, the growth of Bifidobacterium and Lactobalillus to dominate pathogenic organisms and thus invigorate human health is facilitated by certain oligosaccharides (Salminen et al., 1998; Roberfroid, 2002). Furthermore, it is known that diseases and ageing cause decay or a significant decrease in intestinal bifidobacteria. Based on these facts, a lively interest has arisen in food additives that enhance human health. Some reports deal with the presence of specific prebiotic oligosaccharides in human or animal nutrition that improve health by encouraging the growth of bifidobacteria and, therefore, positively affect intestinal cells and the immune system (Roberfroid, 1998; Swennen et al., 2006).

The beneficial influence of non-digestible oligosaccharides, mainly fructooligosaccharides, on monogastric organisms has been investigated by many scientists (Tomomatsu, 1994; Van-Loo et al., 1999) and recently reviewed by Swennen et al. (2006). However, relatively less is known about the beneficial effects of legume α-galactosides. Moreover, only in vivo data are of real value to demonstrate a prebiotic effect.

Human studies have shown an increase in bifidobacteria resulting from the ingestion of soybean α-galactosides and a daily dose of 3 g increased not only bifidobacteria but also bacteroides and eubacteria (Hayakawa et al., 1990; Wada et al., 1991). Earlier studies demonstrated that lupin and pea galacto-oligosaccharides injected into 19 day-old chicken embryos with a dose of 0.18–0.88 mg/egg significantly increased the number of faecal bifidobacteria in 2 day-old chickens (Villaluenga et al., 2004) and this effect is maintained in 6-week-old chickens (Pilarski et al., 2005). Gulewicz et al. (2002) have reported that the administration of 15 mg of lupin and pea α-galactosides per 100 g of body weight to Wistar rats increased the amount of fecal bifidobacteria, while fecal and total coliforms decreased. Bifidobacteria prevent the growth of exogenous pathogenic microbes and excessive growth of indigenous detrimental microflora results in the production of short-chain fatty acids (SCFA), mainly acetic acid and lactic acid at a 3:2 mole ratio and an ability to produce some antibiotic materials (Roberfroid, 2002). The growth-inhibiting and destructive effects of acetic and lactic acids on undesirable bacteria are known (Rasic and Kurmann, 1983) and the suppressive effects of these acids against Salmonella (Chung and Goepfert, 1970) and E. coli (Tamura, 1983) have also been reported. Bifidin, an antibiotic produced by Bifidobacterium bifidum, is effective against Shigella dysenteriae, Salmonella typhosa, Staphylococcus aureus, E. coli and other non-desirable bacteria (Anand et al., 1984, Anand et al., 1985).

The effects of SCFAs on colonic physiology are reviewed by Topping and Clifton (2001). They either directly or indirectly affect the proliferation of enterocytes, inflammation, colorectal carcinogenesis, mineral availability, colonization by pathogens, enzyme activities, and the production of nitrogenous metabolites.

The fermentation product butyrate is the main energy source for epithelial cells (Bugaut and Bentenjac, 1993) and is believed to protect against colonic cancer by promoting cell differentiation (Kim et al., 1982). Recent preclinical studies support the assumption that butyrate might be chemopreventive in carcinogenesis (Scheppach and Weiler, 2004), although direct evidence for protection is still not available. In addition to butyrate, propionate can also have anti-inflammatory effects on colon cancer cells (Nurmi et al., 2005).

The production of SCFAs and the following acidification of the colonic content affect mineral availability. The possible mechanisms involved in the stimulation of mineral absorption by prebiotics are summarized by Scholz-Ahrens et al. (2001). Lower pH causes an increased solubility of some minerals, particularly of calcium and magnesium, which consequently increases passive absorption down a chemical gradient (Scholz-Ahrens et al., 2001). Prebiotics might also stimulate active calcium absorption at the mucosa (Yanahira et al., 1997) which may be particularly relevant for postmenopausal women and the elderly, to prevent or postpone osteoporosis and anemia in these groups (Scholz-Ahrens et al., 2001).

Prebiotics may also affect the lipid metabolism. Several hypothetical mechanisms may explain these effects. The first mechanism assumes that the type of SCFAs produced could modify liver lipogenesis. In studies with rat hepatocytes, acetate acts as a lipogenic substrate whereas propionate inhibits lipogenesis (Demigne et al., 1995). The second hypothesis confirms that prebiotics might also decrease lipogenic enzyme activity in the liver since prebiotics reduce peak-levels of blood-glucose after a meal (Delzenne and Kock, 1998). Finally, the third mechanism proposes that serum cholesterol is reduced because of precipitation and excretion of bile acids from the intestine, which requires the liver to use cholesterol for further bile acid synthesis (Pedersen et al., 1997).

Recent results suggest that selected prebiotics can modulate the immune response. Altering the composition and/or activity of the microbiota can also influence the immune system (Schley and Field, 2002), as is most clearly illustrated in germ-free animals that have an immature and poorly-developed immune system (Norin and Midtvedt, 2000). However, such effects are not always observed in humans (Bunout et al., 2002). This indicates subject-to-subject variation and the fact that an optimal functioning immune system might not respond to prebiotics. Moreover, different prebiotics are likely to influence the microbiota, and thereby the immune system, in different ways. According to the first way, lactic acid bacteria or its cell wall or cytoplasmic constituents can penetrate the intestinal epithelial cells leading to activation of the gut associated lymphoid tissue(Manning and Gibson, 2004). The second way proposes that SCFAs produced by fermentation of prebiotics influence the immune system by their immunomodulatory and anti-inflammatory properties (Kelly-Quagliana et al., 2003).

The reduction of toxic metabolites by the ingestion of oligosaccharides alleviates the detoxifying load of the liver. Dosages of 3 g/day of soybean oligosaccharides to a liver cirrhosis (non-A, non-B) patient age sixty-nine, who had hepatic comatose and constipation symptoms improved both symptoms in about five days (Takasoye et al., 1990). In addition, soybean oligosaccharides were recently observed to increase the urinary excretion of polychlorinated biphenyls (Kimura et al., 2004). The mechanism behind this is not clear, but possibly a prebiotic-induced change in the microbiota composition or activity leads to an alteration in xenobiotic metabolism or could interfere with the enterohepatic cycling of xenobiotics.

Ingestion of soybean oligosaccharides is reported to reduce blood pressure. Administration of 3 g/day of soybean galacto-oligosaccharides for a week to six healthy adult males aged 28–48 decreased average diastolic blood pressure by 6.3 mm Hg. There was a significant negative correlation between the diastolic blood pressure and the ratio of fecal bifidobacteria to total bacteria counts (Masai et al., 1987).

Summary of α-Galactoside Physiological Effects

α-Galactosides act as antinutritional factors or beneficial compounds (Fig. 3) depending on the doses at which the α-galactosides are consumed. It could be suggested that the

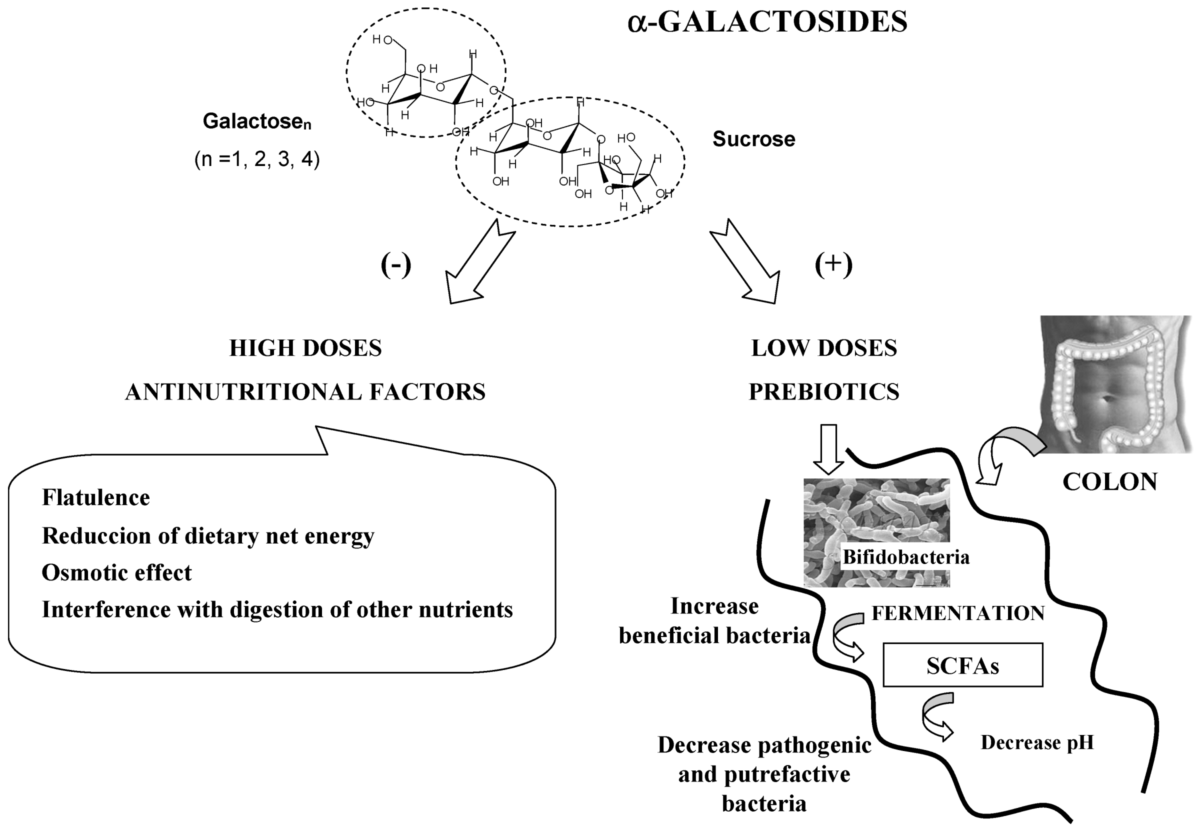

Figure 3 Physiological effects of α-galactosides.

effective daily doses of α-galactosides for obtaining health benefits are 3 g/day while higher doses could cause the above-mentioned problems.

EFFECT OF PROCESSING ON LEGUME ALPHA-GALACTOSIDE CONTENT

Various approaches have been suggested in order to decrease the flatulence-causing factors of food legumes and these are represented in Fig. 4.

Raffinose, stachyose and verbascose are all water-soluble. Therefore, soaking seeds in water or saline solutions and then discarding the water will remove most of these α-galactosides (Vidal-Valverde et al., 1992, 1993a, 1998). The effectiveness of the soaking process is affected by several factors such as the type of legume and the variety, time, temperature, seed/water ratio, and the presence of salts in water (Jood et al., 1985; Vijayakumari et al., 1996). Upadhay and García (1988) reported that individual α-galactoside solubility and diffusion coefficient could influence carbohydrate losses during the soaking process. Only 1–10% of these losses have been explained by leaching into the soaking solution, whereas the remaining losses seem to be due to metabolic processes in the imbibed seed resulting in release of the monosaccharides and disaccharides (Vidal-Valverde et al., 1992, 2002a).

Usually, the most traditional procedure for food purposes is to soak the seed before cooking. In this way, losses of α-galactosides can reach between 20 and 100% of the untreated bean (Vidal-Valverde et al., 1993a; Vijayakumari et al., 1996;Vidal-Valverde et al., 1998; Frias et al., 2000; Alajaji and El-Adawy, 2006) and the amounts depend on the cooking conditions and time. These losses were primarily due to the solubilization and ulterior leaching of carbohydrates not only into the soaking solution but also into the water during boiling. There are, however, some reports of increases in the oligosaccharide content after cooking (Rao and Belavady, 1978; Revilleza et al., 1990) which can be explained because the process water is not discarded or because of the release of oligosaccharides bound to proteins or to other macromolecules or to the hydrolysis of high molecular weight polysaccharides.

Germination is one of the best-known methods for decreasing the α-galactoside content of legumes in order to minimize flatulence potential. This process totally eliminates raffinose, ciceritol, stachyose, and verbascose (Reddy and Salunkhe, 1978; Vidal-Valverde and Frias, 1992; Urbano et al., 1995; Frias et al., 1996b; Lahuta et al., 1997; Torres et al., 2007) and the α-galactoside changes are mainly determined by germination conditions.

The degradation of these sugars in germinating seeds takes place during seed imbibition and proceeds more intensively in the embryonic axis and cotyledons. The α-galactosides are lost

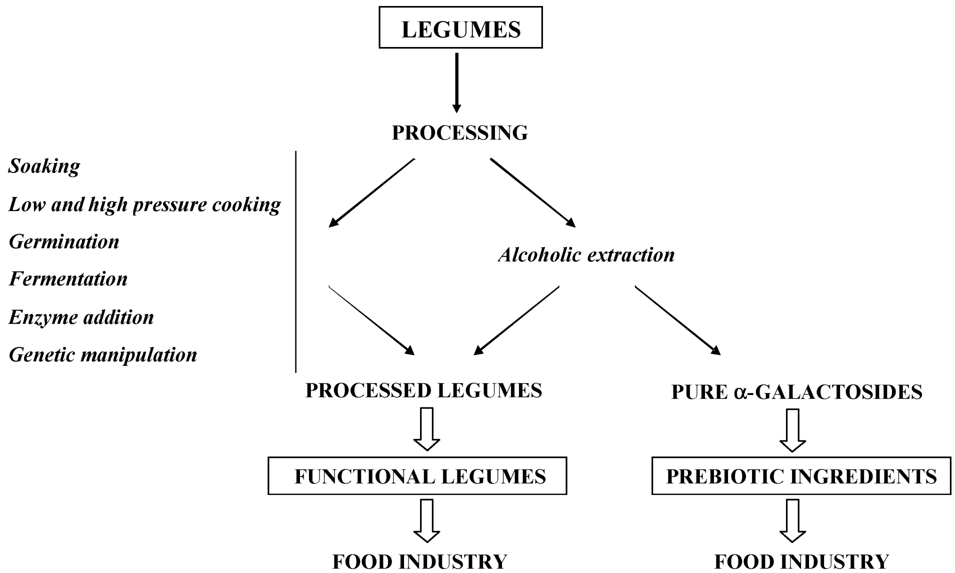

Figure 4 Products from processed legumes.

from the axis during the first two days of soybean, pea, and lupin germination, whereas in cotyledons oligosaccharide hydrolysis is prolonged for 4–6 days (Górecki and Obendorf, 1997; Lahuta et al., 1997; Górecki et al., 1997). Verbascose, stachyose, and raffinose are steadily degraded by α-galactosidase, while the level of monosaccharides increases gradually as germination progresses (Reddy and Salunkhe, 1978; Vidal-Valverde and Frias, 1992; Frias et al., 1996b; Urbano et al., 1995; Górecki and Obendorf, 1997). On the other hand, germination in darkness seems to decrease α-galactoside content more rapidly than with light (Vidal-Valverde et al., 2002b).

Fermentation has been reported to cause a general improvement in the nutritional value of legumes and to bring about desirable changes in taste and texture (Zamora and Fields, 1979; Vidal-Valverde et al., 1993b; Frias et al., 1996c; Granito et al., 2002; Doblado et al., 2003; Granito et al., 2005; Torres et al., 2006). The fermentation process can be performed spontaneously with the only microorganisms present in the seed (natural fermentation) or fermentation can be induced using a microbial culture, generally comprised of lactic bacteria or moulds. Natural fermentation is a simple, widely accepted, and inexpensive processing method to reduce or eliminate oligosaccharides. It has been established that the preparation of the fermentative suspension has an important effect on the level of α-galactoside content (Frias et al., 1996c). Furthermore, Zamora and Fields (1979) studied natural fermentation of cowpea and chickpea and identified lactic acid bacteria as the main microorganisms involved in the reduction or elimination of α-galactosides since they use oligosaccharides for their metabolism. Many authors have concluded that after the natural fermentation of legumes, the α-galactoside content was sharply reduced or not detected in the fermented product depending on conditions of time, temperature, and concentration (Vidal-Valverde et al., 1993b ; Frias et al., 1996c; Granito et al., 2002; Doblado et al., 2003; Granito et al., 2005).

Controlled fermentation with lactic acid cultures reduces the raffinose and stachyose content in different ways, depending on the Lactobacillus strain used. Mital and Steinkraus (1975) tested a number of lactic cultures for α-galactosidase activity. They found this enzyme to be present in Lactobacillus bucheri, Lactobacillus brevis, Lactobacillus cellobiosis, Lactobacillus fermentum, and Lactobacillus salivarius subsp salivarius , and could be induced in Lactobacillus plantarum. Tewary and Muller (1992) found that, during the production of wari from black and soybean with Lactobacillus bulgaricus and Streptococcus thermophilus, the total concentration of raffinose, stachyose, and verbascose diminished from 4.4% to 0.6%. Duszkiewicz-Reinhard et al. (1994) reported that controlled fermentation of pinto bean and field pea flours with Lactobacillus fermentum and Lactobacillus plantarum for 72 h reduced the stachyose content by 27% and 43% respectively. Granito et al. (2003) found that natural fermentation of bean (Phaseolus vulgaris) brought about a gradual decrease in the content of raffinose, stachyose, and verbascose after 96 h, while controlled fermentation with ABT-4 (Lactobacillus acidophilus, Bifidobacterium, and Streptococcus thermophilus; CHR Hansen's DVS) commercial starter, was less effective at removing α-galactosides. These results are in agreement with those found by Doblado et al. (2003) where natural fermentation of beans led to a higher reduction of α-galactosides (95%) compared with controlled fermentation (87%) using Lactobacillus plantarum as a starter culture. Fermentation with Rhizopus oligosporus induced a depletion of the flatulence-causing sugars of lupin seeds of 30–50% (Chango et al., 1993). The last attempt has been to ferment soy products and soy milk with probiotic acid bacteria strains, not only to degrade seed α-galactosides but also to deliver these healthy microorganisms directly to the lower gut (Connes et al., 2004).

The addition of α-galactosidase enzyme from different sources has been suggested for potential use in the food industry to hydrolyse flatulence-causing sugars in processed food (Dey and del Campillo, 1984; Frias et al., 2003; Viana et al., 2005) and to produce sucrose from high-raffinose sugar beets (Wong-Leung et al., 1993). The enzyme α-galactosidase (EC 3.2.1.22) is widely distributed in nature and its physiological significance lies in its ability to hydrolyze the α-(1–6) linkages present between galactose moieties into the galacto-oligosaccharides (Dey and del Campillo, 1984). Almost complete removal of galacto-oligosaccharides has been reported by using crude or purified α-galactosidase from Aspergillus cladosporioides, Aspergillus oryzae or Aspergillus awamori (Cruz et al., 1981; Cruz and Park, 1982; Khare et al., 1994; Viana et al., 2005; Song and Chang, 2006) to obtain flatulence-free soy milk and soy bean products. Frias et al. (2003) reported that the addition of commercial α-galactosidase in lentil and pea flour suspensions brought about increases in fructose, glucose, and galactose and a higher reduction of sucrose, raffinose, stachyose, and verbascose that were more noticeable when incubation time was prolonged up to 90 min at 37°C in buffer pH 5.5. LeBlanc et al. (2005) reported the use of Lactobacillus fermentum CRL 722, lactic acid bacteria with elevated α-galactosidase activity, or a cell-free extract of this strain to feed animals and these authors suggested this protocol as a vehicle to safely transfer α-galactosidase in the small intestine of monogastric animals.

Alcohol extraction was first proposed as a possible alternative processing method to remove growth depressing factors in soybean meal (Borchers, 1981; Grant et al., 1983; Hancock et al., 1990). Tolman (1995) studied the effect of extraction performed at different temperatures using different concentrations of ethanol on certain antinutritional factors, protein denaturation, and functional properties in raw peas and pea protein isolates. Galacto-oligosaccharides are known to be extractable in 80% aqueous ethanol and their solubility could be improved by combining this with thermal treatment. The reduction in α-galactoside content seems to depend on the duration and temperature of the process (Sanz et al., 2001). Gulewicz et al. (2000) and Martínez-Villaluenga et al. (2004) developed a rapid and simple procedure to extract α-galactosides with 50% ethanol at 40°C in different legumes seeds. Some authors have used this procedure to obtain functional legumes (Torres et al., 2005; Martínez-Villaluenga et al., 2006). In addition, the α-galactoside extracts can be purified and used as prebiotic ingredients and extracted seeds provide a high nutritional value (Martínez-Villaluenga et al., 2005b) (Fig. 4).

Genetic manipulation has always been used by breeders. Several attempts have been made to reduce α-galactoside content in legume seeds (de Lumen, 1992; Jones et al., 1999; Frias et al., 1999) but, since these sugars play an important role as reserve carbohydrates for short and long-term purposes, for transport and cold acclimation, the crops obtained must be physiologically viable (de Lumen, 1992).

Kerr (1996) patented a nucleotide sequence of galactinol synthase (GS) genes from courgette and soybean. Suitable regulatory genes capable of transforming plants to produce GS at lower levels than that found in the target plant, have also been provided. There are also methods for varying the content of D-galactose-containing oligosaccharides of sucrose in plants to produce transformed plants and seeds. Another approach could be based on the use of genes encoding enzymes that degrade α-galactosides to produce legume plants with low levels of these oligosaccharides.

α-Galactosidases can be activated by external factors, preferably after seed harvesting. For this purpose it could, therefore, be of interest to obtain genes encoding thermostable enzymes from hyperthermophile bacteria (Thermotoga spp.). An α-galactosidase from Thermotoga napolitana has been used for the hydrolysis of guar (galactomannan) gum (McCutchen et al., 1996). This enzyme has an optimum temperature close to 100°C, and decreasing activity at lower temperatures. After harvesting, during seed heating processes, the enzyme could be activated at this temperature and hydrolyse α-galactosides.

Frias et al. (1999) suggested an alternative way to reduce α-galactoside content, such as the presence of additional pathways enhancing the synthesis of related compounds such as ciceritol. It is believed that these compounds play an important role in protecting plants and seeds against drought and frozen stress (Keller and Ludlow, 1993). Increasing levels of this sugar may have the additional advantage of reducing the nutritional problem associated with α-galactosides. There is evidence that ciceritol is more slowly hydrolyzed than other raffinose family oligosaccharides by α-galactosidases, which might result in a relative decrease in the flatus potential of seeds with decreased α-galactosides and an increased level of ciceritol (Quemener and Brillouet, 1983).

α-GALACTOSIDES AS FUNCTIONAL INGREDIENTS. POTENTIAL APPLICATION IN FOOD INDUSTRY

In spite of the fact that α-galactosides originate flatulence, these oligosaccharides selectively stimulate the growth of bifidobacteria in the colonic microbiota and they are model type "prebiotics." Therefore, α-galactosides are obvious candidates to be recognized as "functional food ingredients" for which "health claims" may become authorized (Tomomatsu, 1994).

EU Concerted Action on Functional Food Science in Europe (FUFOSE) coordinated by ILSI Europe developed and reached a Consensus on "Scientific Concepts of Functional Foods" that was published in 1999 (Diplock et al., 1999). The unique features of "functional food" are:

- Being a conventional or every day food to be consumed as part of the normal/usual diet.
- Composed of natural (as opposed to synthetic) components sometimes in increased concentration of present in foods that would not normally supply them.
- Having a positive effect beyond nutritive value/basic nutrition that may enhance well-being and health and/or reduce the risk of disease or provide health benefit so as to improve the quality

of life including physical, psychological and behavioural performances,
- Have scientifically based claims.

Recently, the concept has moved towards food additives exerting a positive effect on the gut microbiota, introducing pro- and prebiotics (Ziemer and Gibson, 1998).

α-Galactoside Production

Methods for the isolation of α-galactosides have recently been developed (Nakakuki, 1993; Crittenden and Playne, 1996; Muzquiz et al., 1999; Gulewicz et al., 2000; Kim et al., 2003; Martínez-Villaluenga et al., 2004) and it is likely that the addition of α-galactosides to foods and drinks will increase their added value within the next few years in the Western countries.

Muzquiz et al. (1999) extracted and purificated oligosaccharides from defatted soybean meal using 10% ethanol-water solution at 50°C followed by ultrafiltration, and they obtained a mixture composed by fructose, sucrose, raffinose, and stachyose (38.6, 51.4, 54.2, and 52.6%, respectively). Gulewicz et al. (2000) developed a simple method based on several alcoholic extractions and further purification of legume seeds. This procedure permits α-galactoside preparations with 75% purity to be obtained, but considerable amounts of sucrose (20%) were detected. Furthermore, Martínez-Villaluenga et al. (2004) reported an improved purification procedure obtaining an α-galactoside preparation with 99% purity. There are five stages in the improved method of isolation and purification of α-galactosides from legumes: (i) imbibition of seeds, (ii) extraction of RFOs, (iii) α-galactoside precipitation, (vi) purification on diatomaceous earth and charcoal, and (v) cation-exchange chromatography.

In the purification stage with diatomaceous earth and charcoal (iv), percolation of α-galactosides with water used in the method described by Gulewicz et al. (2000) was not efficient for the total removal of monosaccharides, sucrose, and other impurities. Using 6% ethanol solution all the sucrose can be eluted. Finally, a higher concentration of ethanol (70%) resulted in the total elution of pure α-galactosides.

In addition, soybean oligosaccharides may be extracted from the soybean whey, a by-product from the production of soy protein isolates and concentrates (Crittenden and Playne, 1996). These extracted sugars are concentrated to produce soybean oligosaccharide syrup (SOS), which is commercially available by Japanese and Chinese companies such as The Calpis Food Industry Co. (Tokyo) and Pine Agritech Limited (Bancheng Town), respectively, (SOS production in 2005 reached 10,400 tons).

Food Application of α-Galactosides

The oligosaccharides market is already substantial and continues to expand rapidly. At present, Japanese companies still dominate worldwide oligosaccharide production, as well as research and development activity. However, European interest in oligosaccharides is increasing with several companies currently producing, or planning to produce, oligosaccharide products. Oligosaccharides provide several manufacturing and health benefits, which make their use as food ingredients particularly attractive. The specific physicochemical and physiological properties of food-grade oligosaccharide products vary depending on the type of mixture purchased. However, some properties are common to all oligosaccharide products, including α-galactosides.

α-Galactosides are water soluble and mildly sweet, typically 0.3–0.6 times as sweet as sucrose. The sweetness of the product depends on the chemical structure and the molecular mass of the oligosaccharides present, and the levels of mono- and disaccharides in the mixture. Their relatively low sweetness is useful in food production when a bulking agent with reduced sweetness is desirable to enhance other food flavors (Crittenden and Playne, 1996). Compared with mono- and disaccharides, the higher molecular weight of α-galactosides provides increased viscosity, leading to improved body and mouthfeel. They can also be used to alter the freezing temperature of frozen foods, and to control the amount of browning due to Maillard reactions in heat-processed foods. The raffinose family oligosaccharides provide a high moisture-retaining capacity, preventing excessive drying, and a low water activity, which is useful to control microbial contamination (Nakakuki, 1993; Crittenden and Playne, 1996).

Although α-galactosides possess these useful physicochemical characteristics, most of the interest in their use as food ingredients stems from their many beneficial physiological properties. The currently available food-grade oligosaccharides are not used by mouth microflora to form acids or polyglucans. Hence, α-galactosides can be used as low-cariogenic sugar substitutes in confectionery, chewing-gums, yogurts, and drinks. Moreover, α-galactosides are not digested by humans and this property makes them suitable for use in sweet, low-calorie diet food, and for consumption by individuals with diabetes. In the case of very sweet foods, they may be used as bulking agents in conjunction with intense artificial sweeteners such as aspartame, phenylalanine, or sucralose, since they could mask the aftertastes produced by some of these intense sweeteners.

The major use of α-galactosides is in beverages. "OligoCC," produced by the Calpis Food Industry Co., was launched in 1989 and was one of the first commercially successful functional food products. This soybean-oligosaccharide drink had estimated sales of 80 million bottles (Nakakuki, 1993). α-Galactosides could have many uses in confectionary like other oligosaccharides which are incorporated in desserts such as jellies and ice creams; bakery products including biscuits, breads, and pastries; spreads such as jams and marmalades; and infant milk formulas.

The current high interest in the application of bifidobacteria to improve colonic health has made the bifidogenic property of α-galactosides one of their strongest marketing points. Symbiotic health food products containing both probiotic bifidobacteria and prebiotic oligosaccharides are emerging to take advantage of this. Martínez-Villaluenga et al. (2005b) reported the effects of adding lupin oligosaccharides as bifidogenic factors in the manufacture of a probiotic fermented milk. Fermentation time was shortened due to lupin oligosaccharide enhanced probiotic growth and activity. In addition, the final symbiotic product contained high numbers of probiotic bacteria (>8 cfu/g) and the remaining amounts of α-galactosides.

There are thus many new potential areas of application for α-galactosides as prebiotics. The mechanisms behind these applications and their possible health benefits are, in most cases, mediated through the intestinal microbiota but more scientific evidence is needed to establish health claims.

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