25 Industrial Applications of Galactooligosaccharides

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25.1 Introduction

Galactooligosaccharides (GOS) are a relatively new class of functional ingredients that are used in a number of dietary applications. The use of GOS in nutritional applications has grown significantly since the early 2000s. Alongside the GOS market trends, this chapter describes the nutritional benefits that are the main drivers of the use of GOS. In recent years, substantial nutritional evidence has been gained in this area. A number of application-related topics associated with GOS –legislative and safety aspects, compositions, physico-chemical properties and stability – are also discussed.

25.2 Global market development for galactooligosaccharides

Consumers started to embrace products with prebiotic benefits in the early 2000s. Figure 25.1 shows the number of global product launches and their developments from 1999 to present. It clearly shows a significant growth of products in this category, whereby products with prebiotic benefits containing GOS are the fastest growing group of prebiotics since 2003.

Figure 25.2 (a) is an overview of launches of all products that contain prebiotic ingredients over a ten-year period (2001 to 2011). Figure 25.2 (b) is a similar overview of products formulated with GOS. The corresponding tables include the absolute numbers of such products launched in the most important countries worldwide. Some 57% of all products were launched in the Asia-Pacific region (APAC) which has been the most active area with regard to products with GOS. The EU comes in with 38%. The dominance of APAC can be attributed to the fact that infant and toddler nutrition is still an important market for GOS. The infant formula market in APAC is characterized by a relatively high number of market players, and consequently a relatively large number of product launches. In contrast, the US and Latin America are heavily dominated by only a handful of big market players. A second factor is that GOS have been an approved ingredient in the EU and APAC for many years, whereas it only received GRAS (Generally Recognized as Safe) status in the US much later.

Figure 25.3 depicts the number of GOS products launched by both existing players and new players and visibly indicates who is driving the growth of GOS. Clearly, more and more companies have started using GOS in their recipes. On top of that, companies who have gained experiences using GOS, tend to extend its use in new product introductions.

Figure 25.4 shows the growth with respect to the cumulative number of companies introducing GOS in their recipes. During the 1990s, Japanese companies like Yakult were true pioneers in introducing GOS to the market. Then from

Food Oligosaccharides: Production, Analysis and Bioactivity, First Edition. Edited by Dr. F. Javier Moreno and Dr. María Luz Sanz.

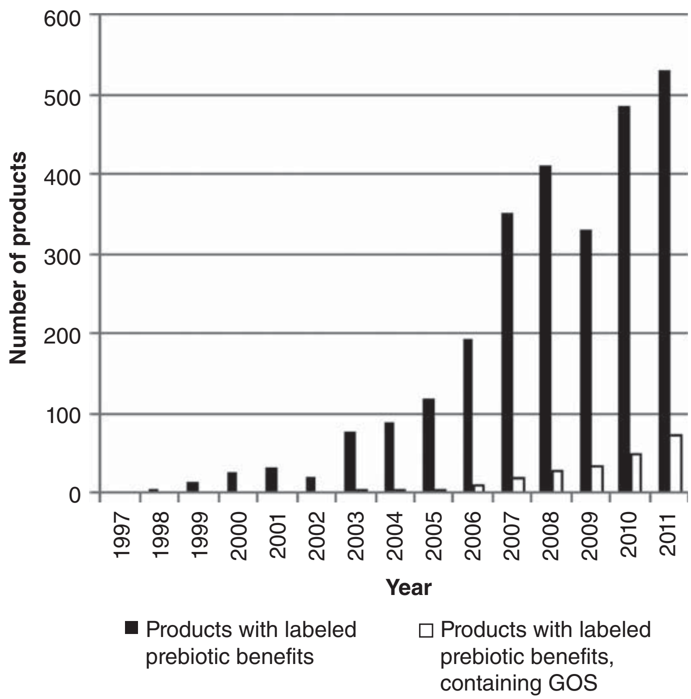
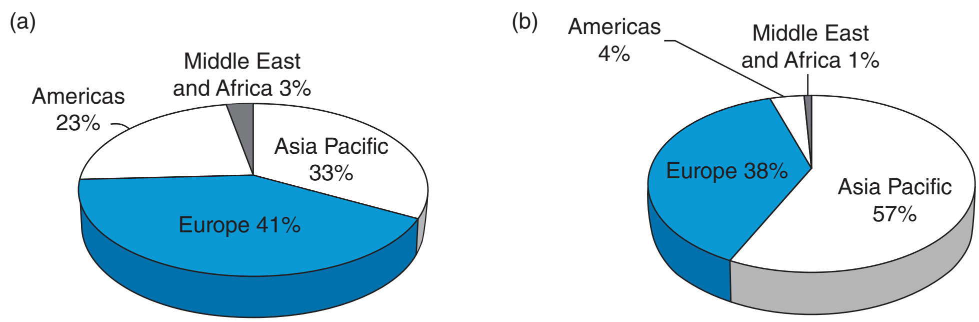


Figure 25.1 Market development of both prebiotic products and products containing GOS.



Top 5 countries	Number of product launches
China	516
USA	372
Germany	259
UK	220
Canada	183

Top 5 countries	Number of product launches
China	104
Japan	51
Vietnam	42
Netherlands	40
Germany	25

Figure 25.2 (a) Products formulated with prebiotics. (b) Products formulated with GOS. In both cases this data corresponds to a ten-year period (2001 to 2011).

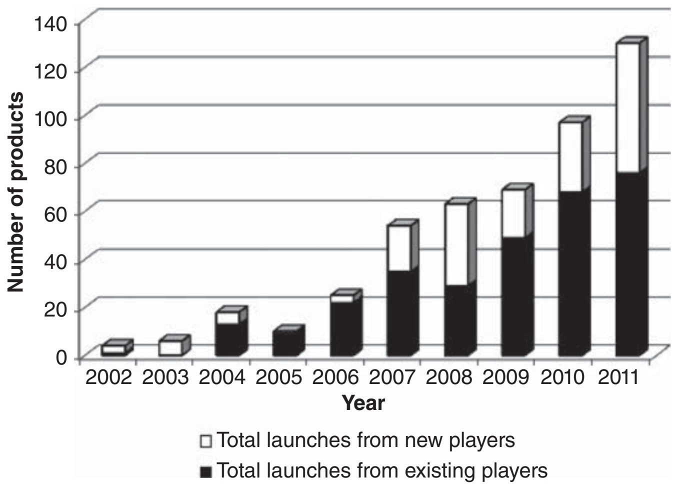


Figure 25.3 Launches of products with GOS.

2001, with the aim of formulating products that more closely approximated human milk, Danone introduced GOS in its branded infant products and advertised their immunity enhancement. This encouraged other players to include GOS in infant and toddler nutrition products.

Launches of infant formula and baby food still make up the majority of introductions of products containing GOS. However, there has been an increase in launches in other markets too, as seen in Figure 25.5.

To date, a significant number of infant and toddler nutrition producers are using GOS in their formulations. The use of GOS is most common in Asia and Europe, where respectively 36% and 27% of all infant formula producers use GOS in their products. In North and South America one in five producers uses GOS in their infant formulas.

Based on the number of newly launched products, product life cycles analysis suggests that GOS is still in its growth phase (see Mintel GNPD, www.mintel.com/gnpd, accessed 4 November 2013).

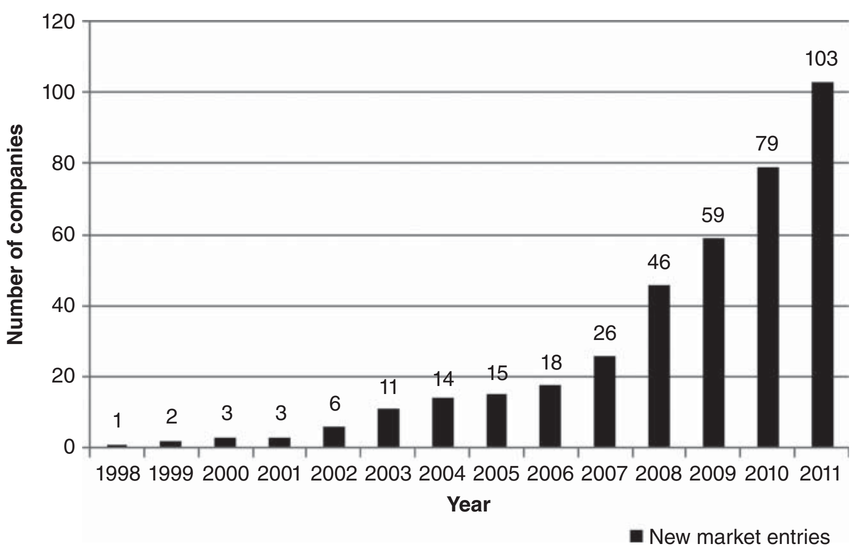


Figure 25.4 Cumulative number of companies that introduce GOS in their recipes.

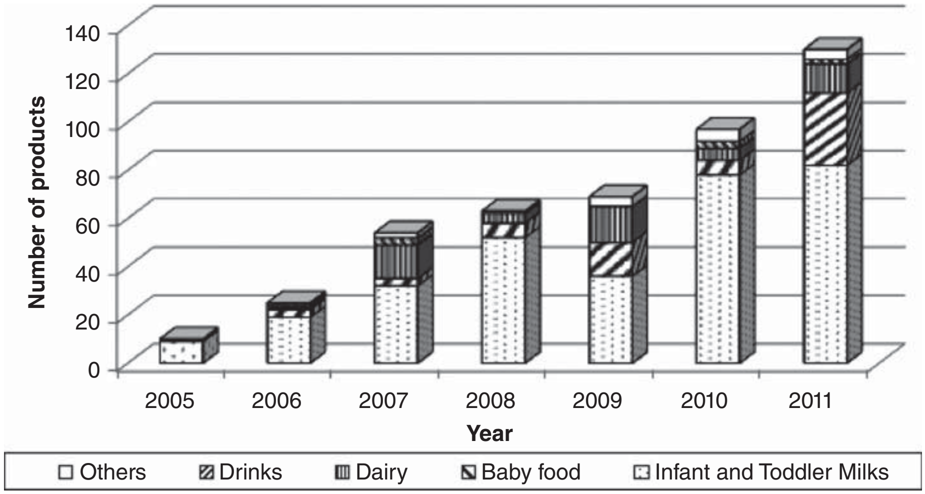


Figure 25.5 Launches of products containing GOS in different markets.

25.3 Nutritional benefits of galactooligosaccharides for infants and young children

As indicated in the previous section, the application of GOS in infant nutrition is by far the most important. The main impetus for applying GOS in infant food products is their structural and/or functional similarities to the oligosaccharides that are naturally present in human milk. The main benefits for newborns and young children are summarized below. Please note that there is no guarantee that the studies cited represent a complete list. They can therefore be considered as examples to illustrate possible benefits.

25.3.1 Gut microbiota in breastfed and bottle-fed infants

When infants are born they could literally be considered sterile because the abundant microbiota in the gut of an adult is not present. In addition, they also have an immature immune system; their initial immunity is derived from the mother. Massive and rapid colonization of gut microbiota occurs when the infant is exposed to maternal and environmental bacteria during delivery. The colonization of the intestine after birth depends on the bacterial load of the environment, the composition of the maternal microbiota, the mode of delivery, nutrition during the first weeks of life, and medication. Early bacterial colonization plays a crucial role in the development of the innate and adaptive immune system (Boehm *et al.* 2004; Veereman-Wauters *et al.* 2011).

The human intestine contains about 300–500 different species of bacteria, with total numbers that are 10 times larger than the number of human body cells. It can be expected that this amount of bacteria will lead to a constant interaction between the host and its microbial guest. The microbiota in the gut can be divided into bacteria that are believed to be health promoting (e.g. bifidobacteria and lactobacilli) and those that might become harmful if they proliferate (e.g. Clostridia) (Figure 25.6) (Gibson 1998; Guarner and Malagelada 2003). It is vital that the microbiota is in balance. When the pathogenic or harmful micro-organisms proliferate, problems can occur including illness and disease (Veereman-Wauters *et al.* 2011).

Breastfed infants have a gut microbiota that is dominated by bifidobacteria, whereas formula-fed infants have a more heterogeneous composition, with comparatively lower levels of bifidobacteria (Mackie *et al.* 1999). This difference is attributed to the presence of bifidogenic growth factors in human milk, such as oligosaccharides (Boehm *et al.* 2004; Newburg *et al.* 2005). Human milk oligosaccharides are thought to have multiple benefits for the infant.

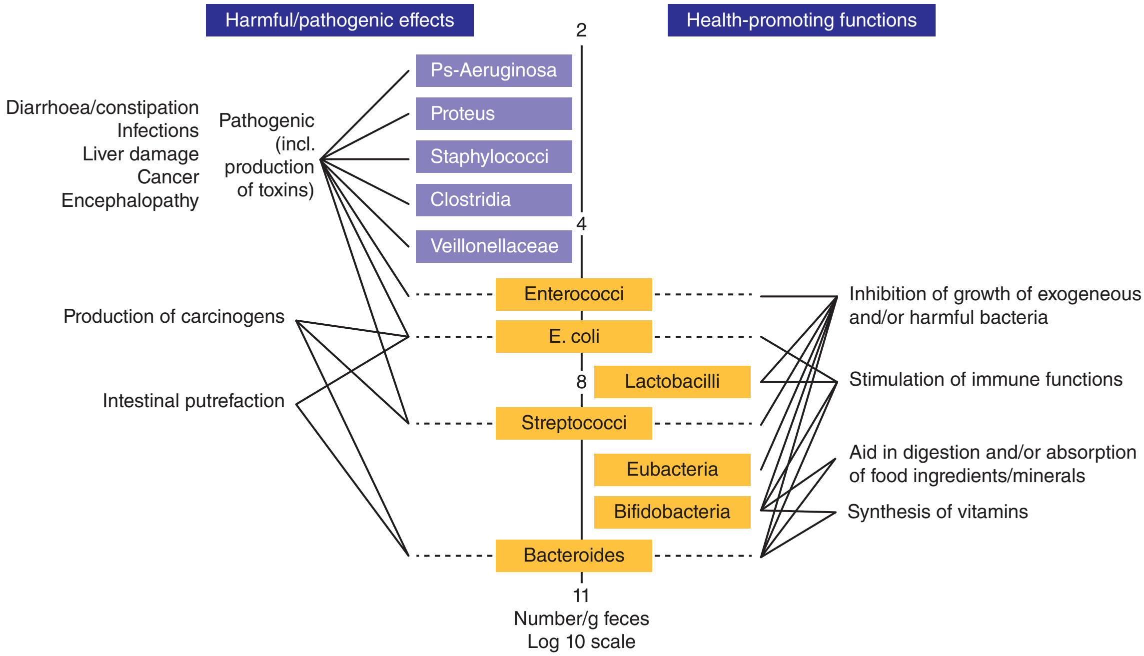


Figure 25.6 Generalized diagram of the composition and effects of predominant human fecal bacteria. (Gibson & Roberfroid 1995. Reproduced with permission of American Society for Nutrition.)

The oligosaccharide fraction is a major component in human milk with contents of about 0.7–1.2 g/100 mL. It contains a large variety of oligosaccharides, consisting of five major building blocks: galactose, glucose, fucose, sialic acid and *N*-acetylglucosamine (Macfarlane *et al.* 2008; Boehm and Stahl 2002; Bode and Jantscher-Krenn 2012).

Human milk oligosaccharides are food sources for beneficial bacteria in the gut and promote growth of these important micro-organisms. By the second year of life, the composition of the gut microbiota approximates that of adults (Gibson 1998).

Nondigestible oligosaccharides, such as GOS and long chain fructooligosaccharides (lc-FOS), are currently added to infant formulas in an attempt to mimic the intestinal microbiota of breastfed infants in bottle-fed infants (Boehm *et al.* 2002; Bode and Jantscher-Krenn 2012).

GOS contain two of the building blocks of human milk oligosaccharides: galactose and glucose. Human milk oligosaccharides as well as GOS resist digestion in the upper part of the gastro-intestinal tract. This means they are virtually intact when reaching the colon, where they can influence the intestinal habitat allowing specific changes in both composition and activity of the gut microbiota (Nauta *et al.* 2010). These changes in the gut microbiota can provide benefits for infants and young children, including supporting natural defenses, increasing calcium absorption and relief of constipation (Nauta and Schoterman 2009).

A large number of studies with bottle-fed infants have shown that infant formulas enriched with oligosaccharides, particularly GOS, result in a significant increase in bifidobacteria and lactobacilli to a level similar to that of breastfed infants (Nauta *et al.* 2010).

Ben *et al.* (2004, 2008) found that a dosage of 0.24 g of GOS per 100 mL of infant formula during a three- to six-months feeding period resulted in significantly higher levels of bifidobacteria and lactobacilli, as compared to infants fed with the standard infant formula without GOS. The results also showed that the infants fed with a formula containing GOS showed a gastrointestinal microbiota more closely resembling that of breastfed infants.

Fanaro *et al.* (2009) studied changes in bifidobacteria after six weeks using a follow-on formula containing 0.5 g of GOS per 100 mL and a placebo. Both groups showed an increase in fecal bifidobacteria, but the increase was greater in infants fed the GOS supplemented formula. The results showed that GOS can also increase beneficial bifidobacteria in children age six to twelve months.

Other studies in infants have been carried out with a combination of 90% GOS and 10% lc-FOS in amounts of 0.4 up to 1 g/100 mL formula (Rigo *et al.* 2001; Boehm *et al.* 2002; Moro *et al.* 2002; Schmelze *et al.* 2003; Knol *et al.* 2005; Desci *et al.* 2005; Costalos *et al.* 2008). In these studies, an increase in bifidobacteria was seen in comparison to the control group receiving a standard formula without oligosaccharides. Scholtens *et al.* (2006) showed that the addition of GOS/lc-FOS to solid foods for infants aged four-six months induces an increase in bifidobacteria as well.

Instead of determining the bifidogenic effect of GOS, Haarman and Knol (2006) investigated the fecal differences in variable lactobacilli species between breastfed children, children fed with an infant formula enriched with GOS/lc-FOS, and children given a standard infant formula. The results indicate that infants who were fed the oligosaccharide formula showed a significant increase in lactobacilli in comparison to both standard formula-fed infants and breastfed infants.

Similar results were shown in a study by Moro *et al.* (2002, 2003), in which 90 infants were fed either a standard formula or a formula enriched with GOS/lc-FOS. The results of the differences in intestinal microbiota showed an increase of both bifidobacteria and lactobacilli in the group given a formula enriched with the oligosaccharide mixture.

25.3.2 Natural defenses

25.3.2.1 Pathogens, toxins and infections

Gastrointestinal disorders are a significant global public health problem. An inadequate balance in intestinal microbiota is thought to play a crucial role in its pathogenesis. Human milk has been identified as an effective way to protect children under age five from gastrointestinal infection, thanks to several defense factors it contains, for example, oligosaccharides (Jones *et al.* 2003).

Modulation of the microbiota by consuming nondigestible oligosaccharides might increase the resistance to invading pathogens through different mechanisms. Examples are lowering the gut pH through production of short-chain fatty acids (SCFAs) to levels at which pathogens are not able to compete effectively, and blocking the adherence of pathogens to the host gastrointestinal epithelial cells (Guarner and Malagelada 2003; Tuohy *et al.* 2005; Shoaf *et al.* 2006).

Animal studies have shown that the consumption of prebiotics could be implicated in prevention and treatment of diarrhea. Studies in healthy infants also indicate that the consumption of oligosaccharides can decrease the incidence of fever, infections and pathogens, although the number of studies in this field is still limited (Dominiquez-Vergara *et al.* 2009).

Ofek *et al.* (2003) showed that GOS are able to inhibit *in vitro*, the adhesion of enteropathogenic *E. coli* to HEp-2 and Caco-2. This was also found by Shoaf *et al.* (2006), who investigated the adherence of *E. coli* to tissue culture cells. In this study, other oligosaccharides were tested as well (FOS, inulin, lactulose and galactose). In comparison to the other substances, GOS showed the largest adherence inhibition (Shoaf *et al.* 2006).

Another *in vitro* trial demonstrated that GOS inhibit the adherence of cholera toxin (an enterotoxin produced by *Vibrio cholera*) to human colonocytes (Sinclair *et al.* 2009).

These studies support a possible antiadhesive functionality of GOS, whereby the GOS function mimics for the cell-surface pathogen and toxin receptors.

A study in preterm infants fed an infant formula with 1 g GOS/lc-FOS per 100 mL demonstrated that stimulation of bifidobacteria reduces the presence of clinically relevant pathogens (e.g. *Staphylococcus aureus*, *Enterobacter*, *Streptococcus* group B, *Clostridium difficile* and *Bacillus subtilis*) in the fecal flora (Knol *et al.* 2005). Another study from Costalos *et al.* (2008) determined the changes in stool microbes of children fed infant formula with or without the addition of GOS/lc-FOS. In addition to an increase in bifidobacteria, it showed a decrease in the 'harmful' bacteria *E. coli* and *Clostridium.* Arslanoglu *et al.* (2008) determined the impact of an infant formula containing 0.8 g GOS/lc-FOS on the preventive effect and occurrence of infections during the first six months of life in a randomized, double-blind, placebo-controlled trial. The results showed that the occurrence of upper respiratory tract infections was significantly reduced after consumption of the GOS/lc-FOS formula. Arslanoglu *et al.* (2008) studied whether these protective effects lasted beyond the intervention period. At the age of 2 years, children in the GOS/lc-FOS group still had fewer episodes of upper respiratory tract infections and were prescribed fewer antibiotics.

To determine the clinically relevant effect of GOS in infant formula, Bruzzese *et al.* (2009) compared a standard formula to a formula containing 0.8 g GOS/lc-FOS over a period of twelve months. They studied the incidences of acute diarrhea and upper respiratory tract infections as well as the length of time they needed antibiotics. The clinical data indicate that the addition of the oligosaccharide mixture to infant formula results in a decrease in the incidence of antibiotic use and the occurrence of acute diarrhea. In addition, a trend was shown of a reduction in upper respiratory tract infections.

In summary, these studies suggest that GOS have the potential to decrease certain pathogenic bacteria and toxins in the human intestine, and to play a role in protecting against infections.

25.3.2.2 Immunomodulation

The intestinal mucosa is the main interface between the immune system and the external environment. The gut-associated lymphoid tissue (GALT) contains the largest pool of immune competent cells in the human body. The microbial colonization of the gastrointestinal tract affects the composition of the gut-associated lymphoid tissue. Changes in the intestinal microbiota that occur after consumption of oligosaccharides may potentially mediate immune changes.

In a murine influenza vaccination model, it was shown that a mixture of GOS/lc-FOS can stimulate delayed-type hypersensitivity (DTH) responses (marker of T-helper 1 (Th1) immunity). In one study, this effect was accompanied by a reduction in T-helper 2 (Th2) cytokine production by splenocytes *in vitro*. These results suggest that the presence of oligosaccharides in infant formulas are beneficial for the development of the infant's immune system and provide an opportunity to inhibit infections and Th2-related immune disorders in humans, such as allergies (Vos *et al.* 2006).

Bakker-Zierikzee *et al.* (2006) and Scholtens *et al.* (2008) showed a trend towards higher fecal SIgA levels after consumption of an infant formula with 0.6 g GOS/lc-FOS per 100 mL. SIgA has a protective role against the adherence and invasion by harmful bacteria and viruses.

The results of another infant study indicated that consumption of oligosaccharides, including GOS, can modulate postnatal development of the immune system by altering the gut microbiota (Moro *et al.* 2006).

25.3.2.3 Allergy

The oligosaccharides can influence the activity of the immune system. Consequently, they are thought to play a role in the prevention of allergies in infants.

A study by Schouten *et al.* (2009), using a mouse model of orally induced cow's milk allergy, showed that when animals were fed a diet containing 2% GOS/lc-FOS, ear swelling (a marker for atopic dermatitis) was reduced significantly. When the probiotic strain *B. breve* M16V was added to the diet, the animals showed a significant decrease in anaphylaxis and mast-cell degranulation (histamine release). In this study, no significant effect was seen in the probiotic group alone, indicating that the use of a combination of pre- and probiotics (synbiotic) may be more effective in the battle against this allergic manifestation.

Moro *et al.* (2006) studied infants at risk of suffering from atopy. They compared the effect of an infant formula containing 0.8 g GOS/lc-FOS per 100 mL infant formula with a formula containing maltodextrin (placebo) on the occurrence and development of atopic dermatitis. Ten infants in the prebiotic group and 24 infants in the placebo group developed atopic dermatitis. Although this effect requires further investigation, these results provide initial indications for a potential role of oligosaccharides in allergy prevention.

Arslanoglu *et al.* (2008) studied whether these protective effects were lasting beyond the intervention period (until the children were two years of age). As in Moro's study, during this period infants in the GOS/lc-FOS group showed a significantly lower incidence of allergic manifestation.

25.3.3 Calcium absorption

The mineral calcium (Ca) is important for bone mineralization. It is known that 75% of the Ca in human milk is absorbed by infants, while calcium in milk-based infant formulas is only up to 20% absorbed (Baker *et al.* 1999). Adequate bioavailability of minerals is critical in achieving optimal peak bone mass and modulating the rate of bone loss associated with ageing. If the calcium intake is not sufficient during infancy and/or adolescence, acquired skeletal mass cannot be maintained. This leads to a suboptimal peak bone mass and osteoporosis in later life (Kalkwarf *et al.* 2003).

Galactooligosaccharides can contribute to ensuring sufficient levels of calcium in the body as it stimulates its absorption through a number of potential mechanisms. One of these mechanisms is facilitating paracellular transport due to increased levels of SCFAs in the colon. This leads to a decrease in pH, which consequently increases the solubility of calcium in the colon (Bongers and van den Heuvel 2003).

Several animal studies have demonstrated that administration of GOS results in more efficiently absorbed calcium in comparison with a control diet. In some cases, this effect was accompanied by higher bone ash weight and tibia calcium content, which are indications of the prevention of bone loss (Chonan *et al.* 1995; Chonan and Watanuki 1995, 1996).

Pérez-Cónesa *et al.* (2006, 2007) studied the effects of GOS-enriched infant formulas on calcium absorption and bone mineralization in rats. It was found that these formulas increased calcium, magnesium and phosphorus absorption, and their concentrations (especially calcium and magnesium) increased in both the femur and tibia.

Weaver and Martin (2011) investigated the potential of GOS in improving the mineral balance and bone properties in rats. The dose response effect of GOS was examined in this study using 0, 2, 4, 6 and 8% GOS by weight for eight weeks. Supplementation of GOS in the diet of growing rats showed beneficial effects on net calcium and magnesium absorption and retention, increased uptake of labeled isotope femur ^{45}Ca (increased concentration of labeled isotope calcium-45 in femur bone), bone strength and bone mineral density. These effects were attributed to changes in cecal pH, cecal content and wall weight and number of bifidobacteria.

In a recent study of young girls aged 10–13, the effect of consumption of GOS on calcium absorption was determined by means of a dual stable isotope technique. In addition, the effects of GOS on gut microbiota were measured. Daily consumption of two drinks containing 2.5 g GOS had a positive effect on fractional calcium absorption and the number of bifidobacteria. No dose response relation was seen. The authors concluded that consumption of 5 g of GOS per day improved calcium absorption and may increase peak bone mass accrual during adolescence by influencing microbial communities in the lower gut (Whisner *et al.* 2013). More studies are needed to demonstrate this effect in children and infants.

25.3.4 Stool consistency and frequency

Breastfed infants are known to have softer stools than their bottle-fed counterparts. This can be attributed to the occurrence of human milk oligosaccharides in breast milk. Studies have shown that the addition of nondigestible oligosaccharides to infant formula can also influence stool characteristics, such as stool consistency.

Fanaro *et al.* (2009) demonstrated that consumption of 0.5 g of GOS per 100 mL follow-on formula resulted in softer stools than the control group without GOS. And a study in China showed that stool characteristics were influenced by supplementation with GOS. In comparison to the control group receiving an infant formula without GOS, stool frequency was significantly increased in infants fed with a GOS supplemented formula as well as in breastfed infants (Ben *et al.* 2004, 2008).

In addition, several studies of infants demonstrate that stool consistency among those consuming a formula with a GOS/lc-FOS mixture is softer compared to control groups and closer to that of breastfed groups (Moro *et al.* 2002, 2003; Schmelze *et al.* 2003).

25.4 Legislative aspects and safety of galactooligosaccharides

Different regulations are applicable to food and nutritional products containing GOS.

25.4.1 Food applications

In several countries around the world, including Austria, Finland, Italy, Belgium, the Netherlands and Japan, GOS is used as a food ingredient, for example in dairy products, fruit drinks, confectionery and cereal bars.

25.4.1.1 European Union

In the EU, GOS was used as a food ingredient before the Novel Foods Regulation (258/97/EC) went into effect in May 1997. In 1996, the Dutch Ministry of Health, Welfare and Sport approved GOS for the use in food products. Based on the approval and use of GOS before 1997, GOS can be used as a non-Novel Foods ingredient in food products in all EU member states.

25.4.1.2 United States

Galactooligosaccharides are considered GRAS (Generally Recognized As Safe) by the US Food and Drug Administration (FDA) for different intended uses, e.g. infant formulas, dairy products, fruit drinks, waters and cereals (GRAS file Vivinal GOS GRN 236, 2007; GRAS file Oligomate, GRN 334, 2010; GRAS file GTC Nutrition, GRN 285, 2009). As a result, GOS can be used in the US as an ingredient in a broad range of food categories.

25.4.1.3 Other countries

Galactooligosaccharides have also received official approval from a number of other authorities for the use of GOS in food products in China, Brazil, Canada, and Japan.

25.4.2 Infant nutrition applications

Galactooligosaccharides are broadly used in standard and premium types of infant formulas, follow-on formula and growing-up milk. They are used in many countries, for example, the European countries, South Korea, Thailand, Indonesia, China, Singapore, Malaysia, the Philippines, India, Pakistan, Vietnam, Taiwan, Israel, Saudi Arabia, the United States, Mexico, Russia, Australia, New Zealand, Argentina, Brazil, Chile. Related legislative issues are described per geographical area below.

25.4.2.1 European regulation

In the EU, GOS and FOS can be added to infant formula and follow-on formula in amounts up to 0.8 g of GOS per 100 mL and the combination of 90% GOS and 10% FOS (Commission Directive 2006/ *141/ EC,* Annex I, point 9 and Annex II, point 7 on infant formulae and follow-on formulae and amending. Directive 1999/21/EC. OJ L 401). Other combinations and other maximum levels of GOS and FOS can be used in accordance with the provisions of Articles 5 and 6 of EU Directive 2006/141/EC on Infant Formulae (IF) and Follow-on Formulae (FOF). This implies suitability should be demonstrated by generally accepted scientific data and notification when used in IF. Based on this, the usage of 100% GOS in both IF and FOF is in alignment with the directive.

25.4.2.2 United States

As commented before, GOS is considered GRAS by the US FDA. As a result, GOS can be used in the US as an ingredient in infant formula (GRAS files: Vivinal GOS GRN 236, Oligomate GRN 334, GTC Nutrition GRN 286).

25.4.2.3 Australia and New Zealand

According to the first review report of the Food Standards Australia New Zealand (on Proposal P306 Addition of Inulin/FOS and GOS to Food of 11 November 2008), the addition of GOS is permitted to infant formula products, infant foods and formulated supplementary foods for younger children.

25.4.3 Claims

For legislation regarding claims, distinction should be made between EU legislation and legislation in the rest of the world.

25.4.3.1 European Union legislation

At the end of 2006, the EU adopted a new regulation on nutrition and health claims made on foods (Regulation EC 1924/2006). This regulation differentiates between nutrition and health claims. A nutrition claim states, suggests or implies that a food product has particular nutritional properties. A health claim states, suggests or implies a relationship between the food product and health status. Only products complying with a so-called nutrient profile may comprise a claim. This profile is defined by the EU.

For GOS, a nutrition claim can be made on the fiber content of the product. “Source of fiber” can be claimed when the food product contains 3 g of fiber per 100 g or 1.5 g of fiber per 100 kcal. To claim “High fiber content” this level needs to be 6 g of fiber per 100 g or 3 g of fiber per 100 kcal.

However, there is no uniform definition of fiber when comparing the EU and countries outside of Europe. For countries where carbohydrate polymers with a DP 3–9 are included in the scope of the dietary fiber definition, basically two-thirds of a GOS product can be declared as dietary fiber. This leaves a functional part of GOS undeclared or incorrectly declared as digestible carbohydrates with a caloric value of 4 kcal per gram.

According to EU Directive 2006/141/EC on IF and FOF, a nutrition claim for the addition of GOS and FOS can be made for infant formulae. In the EU, other nutrition and health claims related to oligosaccharides are not allowed for infant formula for infants from 0 to 6 months of age (Directive 2000/13/EC).

In the new EU regulation there are three types of health claims:

- Generally accepted health claims (“Article 13 and 13,5 claims”).
- Claims referring to children’s development and health (“Article 14 claims”).
- Disease risk reduction (“Article 14 claims”).

Despite the large amount of data indicating health promoting effects, to date no health claims can be made for applications containing GOS. According to the EFSA reviewing committee more solid substantiation is needed.

25.4.4 Safety aspects of galactooligosaccharides

25.4.4.1 Enzyme

In order to substantiate the safety of the enzyme ß-galactosidase, which is used for the production of GOS, extensive testing has been done to verify the absence of mycotoxins, antibacterial activity, toxicity and potential mutagenicity of the enzyme preparation. Based on this information various governments have approved the use of the ß-galactosidase, e.g. the Netherlands (Ministry of Health, Welfare and Sport), Canada (Health Canada), China (Ministry of Health), Australia/New Zealand (FSANZ), Japan (Ministry of Health and Welfare) and Brazil (ANVISA).

25.4.4.2 Galactooligosaccharides

Several studies have been carried out in order to demonstrate the safety of GOS. For example, in a 90-day oral study in male and female rats, GOS was administered by gavage in amounts of 2500 or 5000 mg/kg body weight per day for 90 days. Results showed no significant adverse toxicological effects attributable to the treatment. Based on the lack of toxicological effects the NOAEL (no-observable-adverse-effect level) for GOS is 5000 mg/kg body weight per day when administered for 90 consecutive days (Anthony *et al.* 2006).

Several studies with infants have shown good tolerance after consumption of GOS. Consumption of GOS in amounts up to 0.9 g/100 mL had no influence on the incidence of side effects (e.g. crying, regurgitation, vomiting) (Boehm *et al.* 2002; Moro *et al.* 2002; Schmelze *et al.* 2003; Ben *et al.* 2004; Knol *et al.* 2005; Ben *et al.* 2008; Rigo *et al.* 2001). Sawatzki *et al.* (2005) demonstrated that GOS have no negative effect on the water balance and growth parameters and can be considered as safe.

Apart from the studies described here, even before the safety of GOS has been proven, a number of commercial GOS products have already received the GRAS status from the FDA in US. The proposed use of GOS as an ingredient in foods and infant formulas has been determined to be safe on the basis of results from *in vitro* and *in vivo* studies as per the FDA’s scientific procedures (GRAS file Vivinal GOS GRN 236, 2007; GRAS file Oligomate, GRN 334, 2010; GRAS file GTC Nutrition, GRN 285, 2009).

25.5 Galactooligosaccharide products

As indicated in Chapter 9, GOS are produced from lactose using the catalytic activity of glycoside hydrolases mostly described as ß-galactosidases. The reaction is kinetically controlled by means of the competition between hydrolysis and

Table 25.1 Commercial GOS products.

GOS brand name	Producer	Country	Purity GOS (%)	Enzyme source	Reference
Cup oligo	Nissin Sugar	JP	70	*Crypcoccus laurentii*	Hartemink *et al.* (1997)
Oligomate 55N	Yakult Honsha	JP	55	*Sporobolomyces singularis* and *Kluyveromyces lactis*	GRAS file Oligomate Yakult (2010)
Bimuno	Classado Ltd.	UK	50	*Bifido bifidum*	Vulevic *et al.* 2008
Purimune	Corn Products Int.	KR	90	*Bacillus circulans*	GRAS file GTC Nutrition (2009)
King Prebiotics series;	New Francisco	CN		*Bacillus circulans*	website
GOS 570-S	Biotechnology		57		
GOS 700-S			70		
GOS 900-S			90		
Promovita GOS	First Milk	UK	35	*Aspergillus oryzae*	Sangwan *et al.* (2011); Larson and Poulson (2011)
Vivinal GOS	FrieslandCampina Domo	NL	60	*Bacillus circulans*	Coulier *et al.* (2009)

transgalactosylation. During this conversion, a complex mixture of various galactose-based di- and oligosaccharides of different structures is generated.

The yield of GOS synthesis using ß-galactosidase (EC 3.2.1.23) largely depends on (i) the specific nature of the biocatalyst used, (ii) applied reaction conditions, including the substrate concentration and related water activity, (iii) downstream processing technology available to remove the final product and/or reactants or remaining substrate from the reaction medium. For most of the GOS produced globally, batch processes are used and the yield lies between 30% and 77%, corresponding with a lactose conversion of, respectively, 45% and 95% (Torres *et al.* 2010).

These processes either use enzymes extracted from microbial cells, specific micro-organisms cultured in a lactose-containing media, or finally, resting cells of a lactose-utilizing micro-organism acting as a so-called enzyme bag (Dombou *et al.* 1992).

Various commercial GOS compositions are available. Their characteristics have been described in Table 25.1.

25.5.1 Characterization of galactooligosaccharides products

The GOS synthesized via enzymatic route is usually a complex mixture of oligosaccharides. Various types of sugar linkages and chain lengths are obtained. The exact composition depends, among other things, on the specific ß-galactosidase used for production and the reaction conditions applied (Torres *et al.* 2010). To get a preliminary indication of the sugar profile in GOS, High Performance Anion Exchange Chromatography combined with Pulsed Amperometric Detection (HPAEC-PAD) is used when applying a specific elution gradient. Unique peak patterns are observed for the various GOS products obtained with the corresponding enzyme. Two HPAEC-PAD fingerprints of GOS are illustrated and compared in Figure 25.7.

To quantify fractions with a specific degree of polymerization (DP) that are present in the oligosaccharide mixture size-exclusion chromatography (SEC) with refractive index (RI) detection is used. Figure 25.8 shows the GOS products illustrated above but analyzed using this HPLC-RI method.

More advanced mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) techniques are needed to unravel specific structures of the individual components. From the analysis of GOS derived from the ß-galactosidase from *B.*

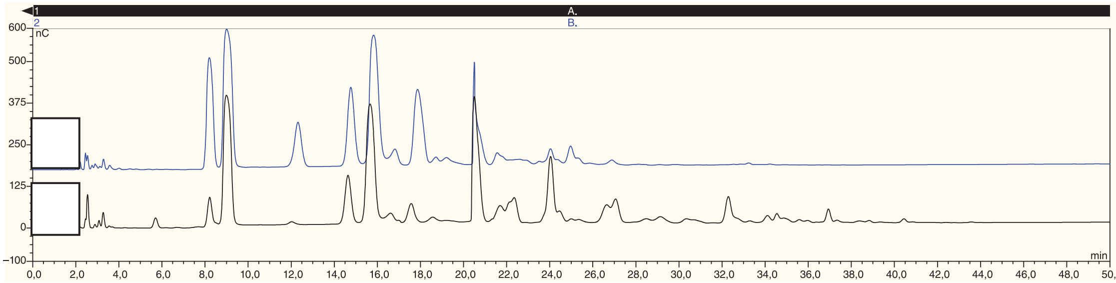


Figure 25.7 Fingerprints of GOS products produced via β-galactosidase derived from different strains; *B. circulans* (lower line) and *A. oryzae* (upper line).

circulans it has been determined that β-(1→4) Gal/Gal and Gal/Glc prevail. To a lesser extent β-(1→3), β-(1→6) and β-(1→2) linkages are present (Coulier *et al.* 2009). It is observed that the glucose moiety of GOS may also contain a β-(1→1) linked Gal moiety that can be further extended with Gal units. Such types of molecules have two terminal Gal units and two nonreducing ends (Fransen *et al.* 1998). In addition, branched oligosaccharide structures have been identified (Yanahira *et al.* 1995; Coulier *et al.* 2009).

25.5.2 Physico-chemical properties of galactooligosaccharides

The composition of GOS determines the physico-chemical properties of these ingredients. These, in turn, determine the suitability of GOS for various food applications and the processing conditions.

Table 25.2 provides an overview of the most relevant physical parameters of GOS syrup with 75% dry matter (DM) with a GOS content of 57%.

Galactooligosaccharide products are mostly colorless and water-soluble up to 800 g per liter product with a viscosity similar to that of high-fructose corn syrup.

Apart from pH, the water activity is directly correlated with the microbial stability of a product (*internal results FrieslandCampina Domo*). Despite high water content (25%) GOS demonstrated a relatively long shelf life; exceeding more than 18 months without any microbial spoilage. In this case, the relatively low water activity of 0.74 is combined with a high osmotic value of the solution, which is predominantly caused by the presence of a high number of small solutes, in particular the monosaccharides, which comprise approximately 20% of the dry matter.

From a processing perspective this high content of monosaccharides makes it difficult to (spray) dry these type of products. Products with high levels of monosaccharides show a high sensitivity for stickiness during spray drying caused

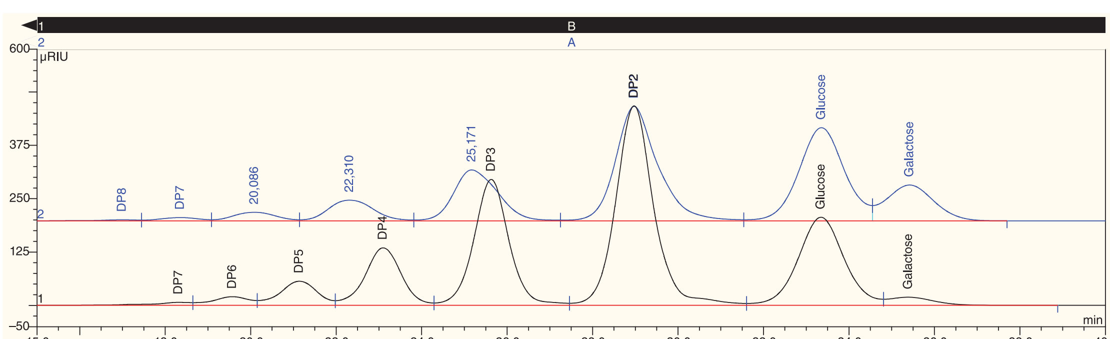


Figure 25.8 DP distributions of GOS products produced via β-galactosidases derived from different strains; *B. circulans* (lower line) and *A. oryzae* (upper line) on a Phenomenex Rezex RSO-Oligosaccharide column and a RI detector.

Table 25.2 Overview of a number of physico-chemical and nutritional properties of GOS syrup with 75% DM; consisting of 60% GOS, 19% lactose, 20% glucose and 1% galactose.

Parameter	Value	Relevant for	Method	Reference
Solubility	>80%	Stability	Visual	Lamsal (2012)
Appearance	Colourless	Application	Visual	
Sweetness	0.3–0.6 of sucrose	Application	Sensory	Torres *et al.* (2010)
Viscosity (20 °C)	2700 mPa.s	Product stability and processing	Rheometry	Erickson *et al.* (1966)
Water activity	0.74	Microbial stability	See reference	Roos (1995)
Heat capacity (Cp)	2.5 J/g/°C	Evaporation	DSC	Gill *et al.* (2010) Brown (1998)
Density (20–40 °C)	1.37–1.38	Blending	Pycknometer	Viana *et al.*, (2002)
Boiling point	106 °C	Evaporation	Condensation	
Freezing point	−15 °C	Freeze drying	Osmometer	Baer and Czmowski (1985)
Glass transition for GOS as powder	37 °C	Spray drying	DSC	Roos and Karel (1990) Torres *et al.* (2011)

Table 25.3 Moisture absorption of powdered GOS, dried with different carriers.

	Percentage absorbed moisture after 24 h at 20 °C	
Percentage relative humidity	GOS with WPC as carrier (protein: 17% and GOS: 28%)	GOS with MD as carrier (maltodextrin: 48% and GOS: 28%)
50	11.3–12.5	5.0–10.0
75	24.4–28.5	20.5–22.5

by a phase transition of the sugars into a rubbery-glass state (Roos *et al.* 1990). Food products in general, and sugars in particular, can be found in an amorphous state which is very sensitive to changes in temperature and moisture content (Torres *et al.* 2011). For this reason, nonpurified GOS can only be dried together with carriers such as whey protein concentrate (WPC) or maltodextrin (MD). However, mono- and disaccharides in these blended products also negatively affect the stability of these powders. GOS blends with WPC and MD, respectively, have a high tendency to absorb water (hygroscopic) as is shown in Table 25.3. By absorbing water the products lose their amorphous character, which destabilizes the product. Storage under dry conditions is therefore recommended.

The viscosity of GOS syrup with 75% dry matter is approximately 2700 mPa.s at 20 °C. As shown in Figure 25.9, the viscosity is independent of the applied shear rate at a product temperature between 4 °C and 20 °C with values of 2000 Pa.s and 1200 mPa.s, respectively. At 50 °C, a shear-thinning effect is observed.

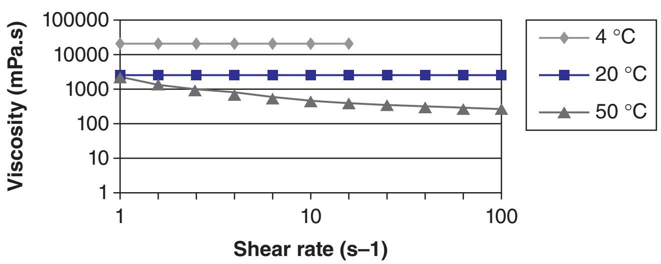


Figure 25.9 Viscosity of GOS syrup (75% w/w) at different temperatures and shear rates.

Table 25.4 Different options for calculation of caloric value of GOS depending on fiber definition (Sako *et al.* 1999).

Definition fiber	Calculated with	Caloric value GOS
Valid for total GOS	100% GOS is 2 kcal/g	2.9 kcal per gram dry matter
Only valid for DP ≥3	67% GOS is 2 kcal/g	3.2 kcal per gram dry matter
Only valid for DP ≥10	All GOS is considered as carbohydrate	4.0 kcal per gram dry matter

25.5.3 Physiological properties of galactooligosaccharides

25.5.3.1 Caloric value

The caloric or energy value of GOS is relatively low because the oligosaccharides resist digestion in the upper part of the gastrointestinal tract. However, part of the energy is salvaged during fermentation and absorption of short-chain fatty acids in the colon (Elia and Cummings 2007).

The average value for GOS is estimated to be between 1 and 2 kcal/g but for food labeling purposes in Europe GOS should be given a caloric value of 2 kcal/g. Depending on the fiber definition that is applied different labeling could be needed, set out in Table 25.4.

25.5.3.2 Glycemic index (GI)

Glycemic index (GI) describes the blood glucose response after consumption of a carbohydrate containing test food relative to a carbohydrate containing reference food, typically glucose or white bread. So a low GI food will cause a small increase, while a high GI food will trigger a large increase. The GI of foods can be divided into three categories: foods with a high GI (>70), foods with a medium GI (55 to 70) and foods with a low GI (<55). There are indications of a positive relation between a low GI, diabetes, weight management and cardiovascular diseases (Venn and Green 2007).

As nondigestible carbohydrates, such as those present in GOS, resist digestion in the small intestine, an increase of blood glucose is not to be expected after consumption. The glycemic index of GOS has been estimated to be negligible. The GI of GOS syrup will be somewhat higher, due to the presence of digestible carbohydrates (i.e. glucose, lactose). Nevertheless, when taking into account all the carbohydrates present in GOS syrup, its glycemic index can still be considered as low and has been estimated to be about 30.

25.6 Applications of galactooligosaccharides

Apart from infant nutrition, GOS can also be incorporated into a wide variety of other foods, e.g. dairy-based medical nutrition products and/or beverages, bakery products and feed for animals (Bockmühl *et al.* 2007; Burr *et al.* 2008; Torres *et al.* 2010; Lamsal 2012).

25.6.1 Application of galactooligosaccharides in infant nutrition

Galactooligosaccharides are mainly used in infant nutrition, follow-on formulas, and growing-up milks (Playne and Crittenden 2009). The nutritional benefits, as described above are the most important driver for using GOS in infant nutrition. GOS are used in infant food products as human milk oligosaccharides mimicking fractions. HMOs are abundantly present in breast milk and are responsible for a number of physiological effects that are important for the development of newborns.

Infant formulas typically contain 6 to 8 g of GOS per liter of formula. Sometimes GOS are applied in combination with other oligosaccharides, e.g. FOS and/ or pectin-derived oligosaccharides (Rastall 2006). Figure 25.10 illustrates a typical composition of an infant formula product stage 1 (for babies of 0 to 6 months of age).

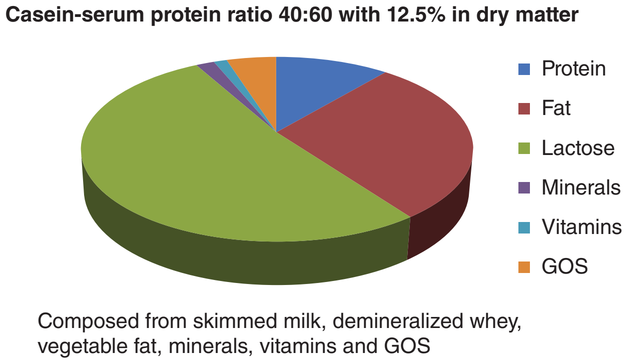


Figure 25.10 Typical composition of an infant formula with GOS. (*For color details please see color plate section.*)

Galactooligosaccharide syrup is suitable for wet blending processes and liquid products. Powdered GOS ingredients, on the other hand, are developed for dry-blending processes. Of the powdered GOS alternatives, the GOS blend with WPC should not be used in hypoallergenic infant formula due to its extremely low (intact) protein level requirements.

25.6.2 Application of galactooligosaccharides in medical nutrition

Traditionally enteral formulae were fiber-free due to a high risk of feeding-tube obstructions and the fact that the bowel should rest to benefit recovery. But this approach has since changed. Specific fibers can be included in the formulae without obstruction of the tube. Furthermore, the interaction of fiber and its fermentation products as well as short-chain fatty acids (SCFAs) can be used as energy for the mucosal cells, thus maintaining gut physiology and improving gut tolerance, which prevents diarrhea and constipation. Furthermore fiber in enteral nutrition affects the glycemic and lipid control. Specific fibers can be used depending on the underlying disease, however, classification of the optimal fiber is still not known (Chen and Peterson 2009).

Fermentable fiber has shown to be effective in reducing diarrhea after surgery and in critically ill patients. Studies have been done with gum (guar-gum) and pectin, which seems to be superior to soy-polysaccharides.

For non-ICU (intensive-care unit) patients or patients requiring enteral nutrition for a long period, a mixture of bulk and fermentable fiber would appear to be the best approach (Volkert *et al.* 2006).

Diarrhoea and constipation are two frequent symptoms associated with enteral tube feeding. Diarrhoea is a significant issue in the acute care setting: dehydration, increased infection risk and the associated negative impact on resource costs as well as a decrease in quality of life. In the case of constipation the impact is less overt but can lead to impaired quality of life and needs nursing and pharmaceutical interventions.

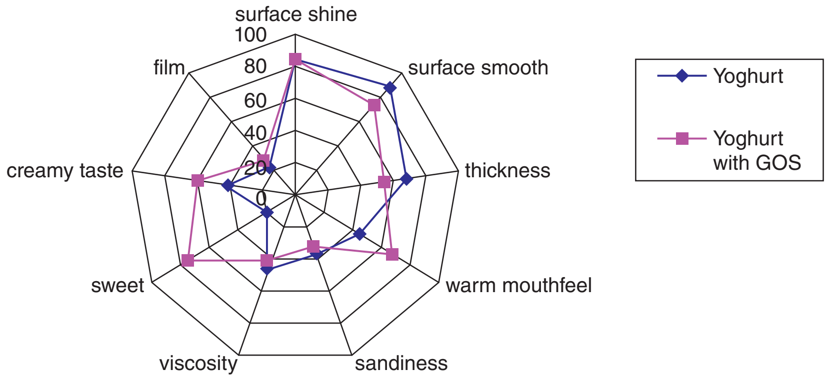
Although the causes of both symptoms are poorly understood, the absence of fiber in enteral feeds has been implicated in these bowel impairments.

A literature review (Elia *et al.* 2008) demonstrates that fiber in enteral formulae is well tolerated and has clinical benefits in patients. The clinical findings are observed by objective physiological parameters in both patients and healthy volunteers; liquid stools, diarrhea score, and so forth.

As strong beneficial effect of fiber on the incidence of diarrhea is high among groups with a high incidence of diarrhea and smaller or absent among those with a low incidence of diarrhea. The underlying mechanism for the effect of fiber in reducing diarrhea is stimulation of colonic water and electrolytes absorption by SCFA. The hypothesis is that SCFA is produced by the fermentation of fiber. SCFA affects the gut microbiota, which would be beneficial in reducing diarrhea.

Clinical (medical) nutrition products often contain fibers to provide an intestinal function as close as possible to normal food and to prevent constipation or diarrhea. Both insoluble and soluble fibers are used for this. GOS form a source of nondigestible oligosaccharides that can be used in such formulas (Blaauw 2010).

GOS are suitable for use in different types of medical nutrition concepts, including tube- and sip feed and powdered supplements. Product stability is extremely important for liquid formulas. Additionally, the GOS used for tube feeding must be lactose-free due to the fact that patients often show increased lactose intolerance, for example after surgery.



Typical composition of fermented beverage:

Component	Percentage in dry matter
Skimmed milk	91
WPC35	3
GOS syrup	6
Lactic acid starter	

Figure 25.11 Sensory analysis via "spider-web" description of yoghurt with GOS syrup compared to plain yoghurt. (*For color details please see color plate section.*)

25.6.3 Application of galactooligosaccharides in dairy products and beverages

Galactooligosaccharides can be used in a broad range of applications such as dairy products, refreshing water and fruit juices (Lamsal 2012). For these applications, aside from the nutritional benefits, the impact on product stability and the sensory profile becomes increasingly important.

The sensory profile of a product and the impact of an ingredient is assessed by a trained panel doing the so-called quantitative descriptive analysis (QDA). This method is used to verify the effects of changes in ingredients or production process on the overall sensory profile of a food product by scoring the intensity of relevant sensory attributes (Brinkman 2006).

In Figure 25.11, the sensory profile is shown for yoghurt with and without GOS syrup as an example. Statistical processing of the data demonstrates that the yoghurt with GOS is perceived as creamier, sweeter (most likely due to the presence of glucose in GOS) and the mouth feel is experienced as warmer than yoghurt without GOS.

Due to the viscosity of GOS, at the relatively low concentrations (1–5%) used in liquid food formulations, no substantial impact is observed on the viscosity of the formulation. This means GOS will not have any thickening and/ or bulking effect when applied in beverages.

25.7 Stability of galactooligosaccharides

Ingredients like GOS should be stable under the applied storage and processing conditions. Depending on the specific application, parameters like pH, ionic strength, heat load, and so forth, are important.

Various analytical methods are available to study the effect of certain conditions on GOS as such, or in specific formulations. Currently there is no direct analytical method to assess the GOS content in the ingredient and/or product in which GOS are applied. In order to determine the GOS content, an indirect method has been developed by de Slegte (2002) that is accredited by AOAC. This method indirectly measures the GOS content by quantifying the release of extra galactose units by a complete enzymatic hydrolysis which are derived from the GOS. The GOS content is calculated from the amount of released galactose units corrected by the blank value of galactose which were already present in the sample including the fraction from lactose. For the quantification of sugars standard HPAEC-PAD equipment is used.

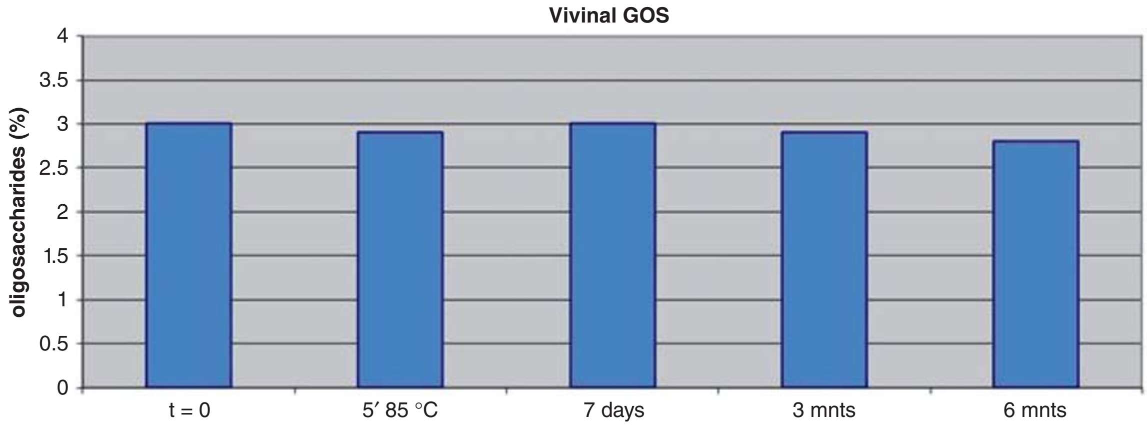


Figure 25.12 Stability of GOS (Vivinal GOS 75% w/w) at pH 2 after heat treatment and storage.

The AOAC method also can be used for quantifying GOS in food products with a detection level of at least 2%. However, the accuracy of this AOAC method is negatively affected when a product containing GOS (e.g. infant food) also has a high content of lactose or galactose. A substantial part of the lactose (80%) has to be removed using an ethanol extraction. If galactose from sources other than GOS is present in the application, it is difficult to quantify GOS. This occurs, for instance, in yoghurt-based products as GOS is partly degraded by the β-galactosidase activity of the lactic acid bacterial strain during fermentation.

Galactooligosaccharides are very stable under acidic conditions, even in combination with high temperatures, as demonstrated in shelf life tests. GOS (Vivinal GOS syrup) were added to a model soft drink with a dry matter content of 10%, respectively 3% GOS, and a pH of 3.0. After mixing and pH stabilization, the soft drink was pasteurized for 5 min at 85 °C (Figure 25.12). The oligosaccharides content in the model soft drink system was analyzed by using ion exchange chromatography at different times: before pasteurization (t = 0), after pasteurization and after storage at room temperature for seven days, three and six months, respectively. Overall, no significant losses in GOS content were observed.

In another setup, no losses of intact GOS were shown when heated for 10 min at pH 2 at temperatures between 100 and 160 °C and subsequently storage at 37 °C for several months (Lamsal 2012). Therefore, it can be suggested that GOS can be applied in fruit juices and/or beverages with low pH; e.g. fermented products. GOS ingredients and in particular GOS syrup (75%, w/w), were stable during long-term storage. Both the oligosaccharide content and the composition remain unchanged (no degradation) for at least 18 months. In addition, GOS blended with WPC or maltodextrin remained stable when stored for 18 months.

During this length of storage some changes in the color of the GOS syrup may be observed, whereby it turns slightly yellow depending on the storage temperature. As can be seen in Figure 25.13, the absorption increases significantly faster at 45 °C and 60 °C in comparison to temperatures ranging from 5 °C to 30 °C (Gillett *et al.* 1949). The temperature-induced color formation could be the result of conjugation with specific protein or nonprotein nitrogen in the product.

During processing (wet or dry) GOS may react with the food-matrix components. These reactions could affect the functionality or the product appearance (e.g. color).

For safety reasons, food products and in particular infant and toddler nutrition products are heat treated to ensure that no microbial contamination of potential pathogenic bacteria is left in the product. Infant and follow-on formulas are more prone to thermally induced degradation reactions than regular milk products, as a consequence of their special composition. Reactions that occur during milk processing comprise the glycation of lysine residues with lactose (called lactosylation), yielding the Amadori product lactulosyllysine, the formation of advanced glycation end products (AGEs) and protein-free sugar degradation products, as well as protein or lipid oxidation (Pischetsrieder and Henkle 2012).

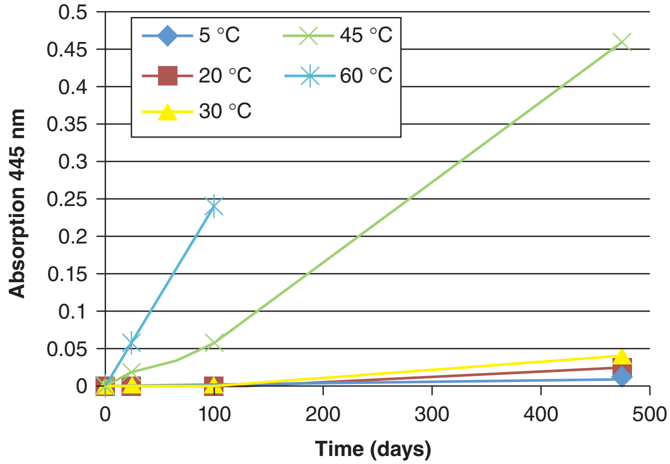


Figure 25.13 Color development in GOS syrup (75% w/w) during storage at different temperatures. (*For color details please see color plate section.*)

Meanwhile, the formation of Maillard-derived conjugates between GOS and proteins can lead to an improved behavior in the gut. This was extensively studied by Sanz *et al.* (2007). The stability of the glycated ß-lactoglobulin to *in vitro* simulated gastrointestinal digestion was also described and compared with that of the nonglycated protein. The yield of digestion products of glycated ß-lactoglobulin was lower than that observed for the nonglycated protein. The conjugation of prebiotic carbohydrates to stable proteins and peptides could open new routes of research in the study of functional ingredients.

25.8 Concluding remarks and future developments

Galactooligosaccharides have shown a great potential in providing beneficial health effects and therefore many research efforts are still ongoing to study the relationship between the obtained oligosaccharide structures and their nutritional and physico-chemical properties. There are strong indications that the supposed (prebiotic) properties of GOS are dependent on the combination of linkage type between monosaccharides, the length of the oligomers and level of branching. These properties are determined not only by the source of the β-galactosidase used, but the reaction conditions that are present during processing (Iqbal 2011).

When more solid substantiation is obtained, specific claims could be granted, thus opening new avenues of application. This would stimulate GOS use.

Whether GOS will become successful in other markets beside infant and toddler nutrition will also depend on production costs. So far, the overall yield for the conversion of lactose into GOS is still too low to become an attractive ingredient for other markets. The challenges for the GOS producers are to develop enzymes and processes resulting in a higher yield with lower conversion costs.

Research activities focusing on cost-efficient processing routes should start with the biocatalyst. Enzymes with optimized substrate conversion rates and GOS yields form good alternatives to the enzymes currently used. Another improvement could be obtained by using immobilized enzymes and/or specific reactor concepts that enable more efficient processing and reuse of the enzymes.

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