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MINI REVIEW

Galacto-oligosaccharides as functional foods and their properties

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Abstract

Galacto-oligosaccharides (GOS) are classified as prebiotics due to their indigestible nature. GOS are regarded as resistant to salivary digestion and are not utilized by the mouth. Thus, they can be utilized as substitutes for low-cariogenic sugar. The structure of GOS mixtures is determined by the differentiation between the transferase reaction and hydrolysis. Transgalactosylation can be achieved intramolecularly when the glycosidic bond in lactose cleaves and reassembles itself on another glucose molecule. Microbes can be used as a source for the β-galactosidase enzyme or as agents to produce GOS units. Commercial β-galactosidase enzymes likewise do have a great potential for their use in GOS synthesis. These transgalactosyl reactions could find useful applications in dairy. Since 70% of the world's population cannot tolerate lactose, lactose utilization could only be enhanced through hydrolysis into its components, the monomer sugars of D-glucose and D-galactose. GOS can be produced industrially using whey or lactose as the substrate. Trisaccharide (β1-4 or β1-6 galactosyllactose) are generally the major products of this process. In comparison with lactose and other saccharide molecules, GOS has been shown to have low carcinogenicity, low calorific value, and low sweetness. This review focuses on GOS production, and the physicochemical characteristics, physiological effects, and applications of these prebiotics are summarized.

1. Introduction

Galacto-oligosaccharides (GOS), also referred to as oligogalactosyllactose, oligo-galactose, oligo-lactose or transgalacto-oligosaccharide (TOS), are classified as prebiotics due to their indigestible nature. Prebiotics are defined as nondigestible dietary ingredients that have a beneficial effect on the host by boosting the viability and growth of useful bacteria in the colon. The stability of GOS enables them, in addition to their use in infant foods (Vandenplas *et al*., 2015), to be added to other foods such as beverages (fruit juices and acidic drinks), meal replacements, fermented and flavoured dairy products, and pastry products (Sangwan *et al*., 2011). GOS have a good taste, which may enhance the texture and mouth sensation in foods offering saccharose-like bulk properties. GOS are regarded as resistant to salivary digestion and are not utilized by the microbiota of the mouth. Thus, they can be utilized as substitutes for low-cariogenic sugar (Splechtna *et al*., 2006). Furthermore, research has shown that GOS offers multiple benefits, including enhancing immunity, improving mineral absorption, and supporting cardiovascular health. Functional oligosaccharides mainly refer to GOS, fructo-oligosaccharides, etc., which cannot be digested and absorbed by the body, but directly enter the intestinal tract to be utilized by *Bifidobacteria* (Ibrahem *et al.,*2022).

The purpose of this mini-review is, therefore, to analyze and summarize the functional properties and their applications in the food industry of Galacto-oligosaccharides as one of the ingredients possessing health value, prebiotic functions, and their linked possibilities of health promotion and mechanisms.

2. Transgalactosylation reaction

GOS is regarded as a combination blend of galactose -containing oligosaccharides delivered due to the transgalactosylation of a β-galactosidase as D-galactosyl moieties are carried onto the D-galactose component of lactose (Torres *et al*., 2010; Vera *et al*., 2011). There are three main steps in GOS synthesis: (i) hydrolysis, (ii) creation of an intermediary galactosyl-enzyme and (iii) synthesis of GOS (Díez-Municio *et al*., 2014). During

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the first two reaction steps, the glycosidic linkages of lactose molecules are hydrolysed by β-galactosidase, yielding an intermediate galactosyl-enzyme covalent. A subsequent reaction resulting from the intermediate can take place either when it interacts with water (in which case hydrolysis occurs) or when it reacts with a glycoside molecule (like galactose, glucose or lactose), in which case transgalactosylation occurs (Díez-Municio *et al*., 2014). Hydrolysis and transgalactosylation occur simultaneously (Vera *et al*., 2011), and the monosaccharides and oligosaccharides produced can be hydrolysed and transgalactosylated further. Two mechanisms contribute to the transgalactosylation reaction, that is, transgalactosylation can occur intermolecularly or intramolecularly. Transgalactosylation can be achieved intramolecularly when the glycosidic bond in lactose cleaves and reassembles itself on another glucose molecule. In intermolecular reactions, transgalactosylation can be accomplished externally; in order to form long-chain oligosaccharide (tri-, tetra- and higher) GOS, galactose molecules are transferred to another acceptor (Splechtna *et al*., 2006). When the concentration of lactose is high at the start of the reaction, a galactosyl donor molecule may be readily available along with a greater likelihood of lactose being the donor; this, in turn, favours the transgalactosylation reaction and increases the GOS synthesis rate. Moreover, water activity is lowered by increasing lactose concentration, leading to a decrease in the lactose hydrolysis rate and formation of new GOS (Lopez *et al*., 1989). Thus, a GOS mixture is composed of glucose, galactose, lactose, disaccharides free of lactose (glycosidic bonds differentiate between galactose and galactose or glucose and galactose) and oligosaccharides of varying length. In this manner, the ultimate composition of a GOS mixture is identified by the differentiation between the transferase reaction and hydrolysis, which depends on the conditions of the response and the enzyme utilized within the reaction. To illustrate this, the exitance of particles of magnesium was shown to improve the response of transgalactosylation (Bráa *et al*., 2010), whereas a raised concentration of lactose, in the beginning, preferred a stabile enzyme, decreased activity of water, and high availability of galactosyl or other acceptors of saccharide and thus encouraged the production of more GOS (Mahoney, 1998; Urrutia *et al*., 2013). The parameters that change the hydrolysis to transgalactosylation ratio could include the source of the enzyme (Saqib *et al*., 2017), the pH (Oh *et al*., 2017) and access to the active site in addition to the components of the buffer, time and temperature of the reaction (Boon *et al*., 2000). Therefore, optimization efforts always benefit GOS production conditions.

3. Galacto-oligosaccharides as functional foods

Since 70% of the world's population cannot tolerate lactose, it is only possible to enhance lactose utilization through hydrolysis to its components, namely the monomer sugars of D-glucose and D-galactose (Ugidos-Rodríguez *et al.,* 2018). A lack of lactose-hydrolysing enzymes results in lactose intolerance, which causes gastrointestinal complications in lactose-intolerant individuals (Ugidos-Rodríguez *et al*., 2018). In comparison to lactose, D-galactose and D-glucose are regarded as relatively sweet and easy to digest. GOS can be produced industrially using whey or lactose as a substrate. Using β-galactosidase and following the unit operations as illustrated in the graphical scheme, it is possible to synthesize GOS under optimized conditions. GOS production starts with the lactose substrate, which may be derived in any form and should undergo transgalactosylation through β-galactosidase. Based on the enzyme source, different types of linkages and degrees of polymerization may form in GOS. In many cases, decolourization caused by other reactions like the Maillard reaction occurs, particularly when the reaction takes place at high temperatures. Food-grade decolourizers can be used to eliminate these off-colours. Dairy-based sources of synthesized GOS frequently contain calcium, which is useful for bone well-being, although it may be eliminated to upgrade the product's purity. Thereafter, filtration is done based on molecular sizes and frequently includes UF methods. Evaporation is used to concentrate the filtered liquor to obtain GOS syrup; alternatively, GOS powder is produced via freeze-drying. Trisaccharides (β1-4 or β1-6 galactosyllactose) are generally the major products of this process along with another longer oligosaccharide composed of four or more monosaccharide units. As reported by Mussatto and Mancilha (2007), these reactions also produce significant amounts of transgalactosylated disaccharides (TD). In comparison with lactose and other saccharide molecules, GOS show low carcinogenicity, low calorific value and a low sweetness. Therefore, GOS are preferable as functional components. GOS have ideal physicochemical properties, such as stability in acidic conditions and raised temperatures. Hence, they are regarded as appropriate candidates for supplementation in prepared foods like bread. GOS can impart excellent flavour and consistency in bread (Sako *et al*., 1999). Moreover, they can remain stable at room temperature and under acidic conditions for long periods. Additionally, it is well known that the stability of GOS is preferable to that of fracto-oligosaccharides (FOS).

3.1 Health benefits of galacto-oligosaccharides

Oligosaccharides act as growth factors for the native *Bifidobacterium* in the colon. Increasing the amounts of

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Bifidobacteria restricted the activity of putrefactive bacteria such as *Clostridia* sp. and, in turn, reduced the generation of toxic fermentation products (Mussatto *et al*., 2007). GOS enhance the health of the intestine by preventing harmful bacteria like *Escherichia coli* from adhering to the intestinal wall. They achieve this by mimicking the intestinal walls such that *E. coli* attach themselves to the GOS rather than the intestine and are then flushed out via the intestinal tract. GOS also make it possible for the body to better absorb many minerals, including calcium. Thus, GOS supplementation could also prevent the development of osteoporosis. GOS and useful bacteria reduce the severity and diversity of allergic reactions in the body, assist in the maintenance of intestinal integrity, and prevent vaginal and urinary tract infections. Additionally, GOS assist in the alteration of bowel movements and relieve constipation. GOS and useful bacteria additionally assist in relieving dermatitis and other skin conditions. Studies also showed that GOS appreciably reduced colorectal cancer (CRC)-related risk factors.

3.2 Oligosaccharide benefits anti-flocculant (negative interaction)

Biological systems cover a wide range of regulated cell adhesions, including the development of ordered cell structures such as fungal, viral, and bacterial infections or biofilms in microbiology. Cell adhesion is generally defined as cell aggregation to another cell, surface or substrate that is generally an organic matrix (Roach *et al*., 2010). The *Saccharomyces cerevisiae* strain S288C family of adhesive proteins can be divided into two classes. The first class of proteins is encoded by genes such as FLO1, FLO5, FLO9 and FLO1054. Morphogenic events (aggregation, filament formation and invasive growth) are tightly regulated (Goossens and Willaert, 2010). Flocculation often occurs when sugar is deficient in the late stages of exponential phase or stationary phase growth (Goossens and Willaert, 2010). Mannan residues form the outer and inner cores of the cell wall and are bound to the asparagine residues of the protein component bound by the B- (1 → 4) glycosidic bond (Domingo *et al*., 2003).

4. Physicochemical properties of galacto-oligosaccharide

GOS food-grade syrup formulations are generally clear and sticky, with high solubility. GOS physicochemical properties can vary to different extents depending on the mixture components. Its sweetness equals 40% of that of a sucrose solution (Torres *et al*., 2010). Many *in vitro* studies showed that GOS is less likely to be cariogenic (Ambrogi *et al*., 2021), reflected as a reduction in rat caries when GOS was used instead of 50% sucrose, further confirmed by observation (Ooshima and Hamada, 1983). GOS, FOS and inulin differ in their structure and physicochemical characteristics. For instance, many researchers have illustrated significant hydrolysis of FOS and inulin upon exposure to pH lower than 5 at high temperatures (Mensink *et al*., 2015). On the contrary, GOS preparations (after the removal or inactivation of β-galactosidase) are stable at pH 2 (acidic conditions) and retain their structure even after exposure to temperatures up to 160°C. Studies measuring the stability of commercial FOS, inulin and GOS upon exposure to a low pH showed that GOS remained unaffected, whereas FOS and inulin were adversely affected by a pH of 3. These properties, along with their ability to retain moisture, make GOS a suitable ingredient for use in functional foods like baked products, in addition to infant formula, to prevent excessive undesirable drying and improve texture and taste (Sangwan *et al*., 2011; Martins *et al*., 2019). Functional food is defined as manufactured or plain food that, when routinely eaten in appropriate amounts as part of a variety of diets, has potential health benefits beyond nourishment (Granato *et al*., 2017). Although health demands are done for specific foods around the world, the selection criteria for functional foods need in vitro, in vivo and clinical trials to boost their health attributes (Granato *et al*., 2017).

GOS are also suitable for beverage formulation because they are stable in acidic solutions and have only a minor effect on taste (Sako *et al*., 1999). In addition to their use in juices and soft drinks, GOS has gained the interest of the dairy industry due to their potential for application in products such as yoghurt and butter (Čurda *et al*., 2006). With the addition of GOS (2%), the functional characteristics of yoghurt during its production and storage (improved survival rate of lactic acid bacteria strains, shortened fermentation, increased acetic acid level and increased proteolysis) were comparable with those of the control sample (Prasad *et al*., 2013).

The addition of GOS to ice cream has also been reported to have a positive effect on its physicochemical, optical and sensory properties. Increases in hardness and decreases in the melting rate were especially noteworthy, which led to the increased stability of the final product (Balthazar *et al*., 2015). Even though GOS has been utilized primarily in infant formula, its physicochemical properties and reported bioeffects have attracted considerable interest in the food industry for the development of new functional foods.

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5. Application of galacto-oligosaccharides

5.1 Dairy products and *beverages*

GOS can be found in a wide range of products, including dairy products, processed food, sparkling water and juice (Lamsal, 2012). Apart from their nutritional advantages, their effect on the stability of the product and its organoleptic characteristics have a significant impact on these applications. A trained panel assesses a product's sensory profile and the impact of an ingredient using a technique known as quantitative descriptive analysis (QDA). It is applied to assess the effects of adjustments to composition or manufacturing methods on the general organoleptic characteristics of food products through the calculation of the depth of applicable organoleptic characteristics (do Prado *et al*., 2018). The sensory characteristics of plain yoghurt and yoghurt with GOS syrup may be considered as an illustration. Yoghurt containing GOS is regarded as sweeter and creamier (possibly because of the existence of glucose in GOS); additionally, the mouthfeel is warmer compared with GOS-free yoghurt. Because of the viscosity of GOS, a low concentration (1–5%) is utilized in manufactured liquid foods so there is no significant effect on the formulation viscosity. At these concentrations, GOS would also not have a thickening effect in juices.

5.2 Medical nutrition

The conventional enteric formula has been fibre-loose because of the hazard of nutrition-tract blocking and the fact that relaxed intestines benefit healing. Specific fibres may be protected within the formula without blocking the intestinal tract. Moreover, the interplay of fibres and the products of their fermentation in addition to short-chain fatty acids (SCFA) may be utilized as a source of energy for the mucosa, thereby retaining intestinal body structure and enhancing intestinal tolerance, which leads to constipation and diarrhoea prevention.

Furthermore, enteral nutrition using fibre impacts lipid retention and glycaemia. Various types of fibres may be used depending on the underlying illness, although the category of the most useful fibre is no longer recognized (Chen and Peterson, 2009). Fermentable fibres have been shown to help in lowering diarrhoea in severely ill patients and after surgery. Gum (guar-gum) and pectin have been studied and appear to be better than soy-polysaccharides. A combination of bulk and fermentable fibre appears to be the optimum option for non–intensive-care unit (ICU) patients or patients who require intestinal feeding for an extended duration (Volkert *et al*., 2006). Diarrhoea and constipation are two of the most common side effects of enteral tube feeding. Diarrhoea is a major problem in the acute care setting, causing dehydration, increasing the risk of infection, negatively impacting resource costs, and reducing the quality of life. Constipation has a less obvious impact but could lead to a lower quality of life and requires care and medical intervention. Although the exact causes of both conditions are unknown, a lack of fibre in enteral meals has been linked to gastrointestinal problems.

A review of the literature (Elia and Stratton, 2008) showed that fibre in intestinal formulas is well tolerated and has therapeutic effects on patients. Many medical findings were obtained using physiological indicators, such as watery stool and diarrhoea, in patients and healthy volunteers. As sturdy useful impact of fibre on the occurrence of diarrhoea is excessive amongst groups with an excessive occurrence of diarrhoea and lower or absent amongst people with a few occurrences of diarrhoea. The basic mechanism underlying the role of fibre in decreasing diarrhoea is the stimulation of water and electrolyte absorption in the colon with the aid of SCFA.

SCFA are thought to be produced through fibre fermentation. SCFA impact the intestinal microbial flora that may be useful in decreasing diarrhoea. Therapeutic nutritional products frequently incorporate fibres to offer an abdominal feature as near as feasible to everyday meals and to prevent constipation or diarrhoea. Both soluble fibres and insoluble fibres are used in such products. GOS constitute a supply of indigestible oligosaccharides that may be utilized in such formulas (Blaauw, 2010). GOS are appropriate for use in various nutrient formulations, such as tube, sip feed and powdered supplements. The stability of the product is vital for aqueous formulations. Furthermore, GOS utilized for tube feeding needs to be lactose-free because patients frequently display elevated lactose intolerance, for example, after surgery.

Conflict of interest

The authors declare no conflict of interests.

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References

Balthazar, C.F., Silva, H.L.A., Celeguini, R.M.S., Santos, R., Pastore, G.M., Conte Junior, C.A.,

Freitas, M.Q., Nogueira, L.C., Silva, M.C. and Cruz, A.G. (2015). Effect of galactooligosaccharide addition on the physical, optical and sensory acceptance of vanilla ice cream. *Journal of Dairy Science*, 98(7), 4266-4272. https://doi.org/10.3168/jds.2014-9018

Blaauw, R. (2010). The use of specialised enteral formulae for patients with diabetes mellitus. *South African Journal of Clinical Nutrition*, 23(1 SUPPL.), 55-57. https://doi.org/10.1080/16070658.2010.11734272

Boon, M.A., Janssen, A.E.M. and Van't Riet, K. (2000). Effect of temperature and enzyme origin on the enzymatic synthesis of oligosaccharides. *Enzyme* and *Microbial Technology*, 26(2-4), 271-281. https://doi.org/10.1016/S0141-0229(99)00167-2

Bráa, N.F., Fernandes, P.A. and Ramos, M.J. (2010). QM/MM studies on the β-galactosidase catalytic mechanism: Hydrolysis and transglycosylation reactions. *Journal of Chemical Theory* and *Computation*, 6(2), 421-433. https://doi.org/10.1021/ct900530f

Chen, Y. and Peterson, S.J. (2009). Enteral nutrition formulas: Which formula is right for your adult patient? *Nutrition in Clinical Practice*, 24(3), 344-355. https://doi.org/10.1177/0884533609335377

Čurda, L., Rudolfová, J., Štětina, J. and Dryák, B. (2006). Dried buttermilk containing galactooligosaccharides-process layout and its verification. *Journal of Food Engineering*, 77(3), 468–471. https://doi.org/10.1016/j.jfoodeng.2005.07.016

Díez-Municio, M., Herrero, M., Olano, A. and Moreno, F.J. (2014). Synthesis of novel bioactive lactose-derived oligosaccharides by microbial glycoside hydrolases. *Microbial Biotechnology*, 7(4), 315-331. https://doi.org/10.1111/1751-7915.12124

do Prado, D.Z., Capoville, B.L., Delgado, C.H.O., Heliodoro, J.C.A., Pivetta, M.R., Pereira, M.S., Zanutto, M.R., Novelli, P.K., Francisco, V.C.B. and Fleuri, L.F. (2018). Nutraceutical food: Composition, biosynthesis, therapeutic properties and applications. In Holband, A.M. and Grumezescu, A.M. (Eds.) Alternative and replacement foods, p. 95-140. USA: Academic Press. https://doi.org/10.1016/B978-0-12-811446-9.00004-6

Domingo, G.J., Caivano, A., Sartorius, R., Barba, P., Bäckström, M., Piatier-Tonneau, D., Guardiola, J., De Berardinis, P. and Perham, R.N. (2003). Induction of specific T-helper and cytolytic responses to epitopes displayed on a virus-like protein scaffold derived from the pyruvate dehydrogenase multienzyme complex. *Vaccine*, 21 (13-14), 1502-1509. https://doi.org/10.1016/S0264-410X(02)00664-3

Elia, M. and Stratton, R.J. (2008). A cost-utility analysis in patients receiving enteral tube feeding at home and in nursing homes. *Clinical Nutrition*, 27(3), 416-423. https://doi.org/10.1016/j.clnu.2008.02.004

Goossens, K. and Willaert, R. (2010). Flocculation protein structure and cell-cell adhesion mechanism in *Saccharomyces cerevisiae*. *Biotechnology Letters*, 32(11), 1571-1585. https://doi.org/10.1007/s10529-010-0352-3

Granato, D., Nunes, D.S. and Barba, F.J. (2017). An integrated strategy between food chemistry, biology, nutrition, pharmacology and statistics in the development of functional foods: A proposal. *Trends in Food Science* and *Technology*, 62, 13-22. https://doi.org/10.1016/j.tifs.2016.12.010

Ibrahem, A.A., Al-Shawi, S.G. and Al-Temimi, W.K.A. (2022). The antagonistic activity of the synbiotic containing Lactobacillus acidophilus and pineapple residue FOS against pathogenic bacteria. *Brazilian Journal of Biology*, 84*,* e258277.

Lamsal, B.P. (2012). Production, health aspects and potential food uses of dairy prebiotic galactooligosaccharides. *Journal of the Science of Food* and *Agriculture*, 92(10), 2020-2028. https://doi.org/10.1002/jsfa.5712

Lopez, A., Paul, F. and Ramaud, M. (1989). Novel enzymatic synthesis of oligosaccharides. *Food Biotechnology*, 3(1), 11-29. https://doi.org/10.1080/08905438909549695

Mahoney, R.R. (1998). Galactosyl-oligosaccharide formation during lactose hydrolysis: A review. *Food Chemistry*, 63(2), 147–154. https://doi.org/10.1016/S0308-8146(98)00020-X

Mensink, M.A., Frijlink, H.W., van der Voort Maarschalk, K. and Hinrichs, W.L.J. (2015). Inulin, a flexible oligosaccharide. II: Review of its pharmaceutical applications. *Carbohydrate Polymers*, 134, 418-428. https://doi.org/10.1016/j.carbpol.2015.08.022

Mussatto, S.I. and Mancilha, I.M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers*, 68(3), 587-597. https://doi.org/10.1016/j.carbpol.2006.12.011

Oh, S.Y., Youn, S.Y., Park, M.S., Kim, H.G., Baek, N.I., Li, Z. and Ji, G.E. (2017). Synthesis of β-galactooligosaccharide using bifidobacterial β-galactosidase purified from recombinant *Escherichia coli*. *Journal of Microbiology* and *Biotechnology*, 27 (8), 1392-1400. https://doi.org/10.4014/jmb.1702.02058

Ooshima, T., Izumitani, A., Sobue, S. and Hamada, S. (1983). Cariostatic effect of palatinose on experimental dental caries in rats. *Japanese Journal of Medical Science and Biology*, 36(4), 219-223. https://doi.org/10.7883/yoken1952.36.219

Park, A.R. and Oh, D.K. (2010). Galacto-oligosaccharide production using microbial β-galactosidase: Current state and perspectives. *Applied Microbiology* and *Biotechnology*, 85(5), 1279-1286. https://doi.org/10.1007/s00253-009-2356-2

Prasad, L.N., Sherkat, F. and Shah, N.P. (2013). Influence of Galactooligosaccharides and Modified Waxy Maize Starch on Some Attributes of Yogurt. *Journal of Food Science*, 78(1), 77-83. https://doi.org/10.1111/j.1750-3841.2012.03004.x

Roach, P., Parker, T., Gadegaard, N. and Alexander, M.R. (2010). Surface strategies for control of neuronal cell adhesion: A review. *Surface Science Reports*, 65(6), 145-173. https://doi.org/10.1016/j.surfrep.2010.07.001

Sako, T., Matsumoto, K. and Tanaka, R. (1999). Recent progress on research and applications of non-digestible galacto-oligosaccharides. *International Dairy Journal*, 9(1), 69-80. https://doi.org/10.1016/S0958-6946(99)00046-1

Sangwan, V., Tomar, S.K., Singh, R.R.B., Singh, A.K. and Ali, B. (2011). Galactooligosaccharides: Novel Components of Designer Foods. *Journal of Food Science*, 76(4), R103-R111. https://doi.org/10.1111/j.1750-3841.2011.02131.x

Saqib, S., Akram, A., Halim, S.A. and Tassaduq, R. (2017). Sources of β-galactosidase and its applications in food industry. *3 Biotech*, 7(1), 79. https://doi.org/10.1007/s13205-017-0645-5

Splechtna, B., Nguyen, T.H., Steinböck, M., Kulbe, K.D., Lorenz, W. and Haltrich, D. (2006). Production of prebiotic galacto-oligosaccharides from lactose using β-galactosidases from *Lactobacillus reuteri*. *Journal of Agricultural* and *Food Chemistry*, 54(14), 4999-5006. https://doi.org/10.1021/jf053127m

Torres, D.P.M., Gonçalves, M.do P.F., Teixeira, J.A. and Rodrigues, L.R. (2010). Galacto-Oligosaccharides: Production, properties, applications and significance as prebiotics. *Comprehensive Reviews in Food Science* and *Food Safety*, 9(5), 438–454. https://doi.org/10.1111/j.1541-4337.2010.00119.x

Ugidos-Rodríguez, S., Matallana-González, M.C. and Sánchez-Mata, M.C. (2018). Lactose malabsorption and intolerance: a review. *Food* and *Function*, 9(8), 4056-4068. https://doi.org/10.1039/c8fo00555a

Urrutia, P., Rodriguez-Colinas, B., Fernandez-Arrojo, L., Ballesteros, A.O., Wilson, L., Illanes, A. and Plou, F.J. (2013). Detailed analysis of galactooligosaccharides synthesis with β-galactosidase from *Aspergillus oryzae*. *Journal of Agricultural* and *Food Chemistry*, 61(5), 1081-1087. https://doi.org/10.1021/jf304354u

Vandenplas, Y., Zakharova, I. and Dmitrieva, Y. (2015). Oligosaccharides in infant formula: More evidence to validate the role of prebiotics. *British Journal of Nutrition*, 113(9), 1339-1344. https://doi.org/10.1017/S0007114515000823

Vera, C., Guerrero, C., Illanes, A. and Conejeros, R. (2011). A pseudo steady-state model for galacto-oligosaccharides synthesis with β-galactosidase from *Aspergillus oryzae*. *Biotechnology* and *Bioengineering*, 108(10), 2270-2279. https://doi.org/10.1002/bit.23201

Volkert, D., Berner, Y.N., Berry, E., Cederholm, T., Coti Bertrand, P., Milne, A., Palmblad, J., Schneider, S., Sobotka, L., Stanga, Z., Lenzen-Grossimlinghaus, R., Krys, U., Pirlich, M., Herbst, B., Schütz, T., Schröer, W., Weinrebe, W., Ockenga, J. and Lochs, H. (2006). ESPEN Guidelines on Enteral Nutrition: Geriatrics. *Clinical Nutrition*, 25(2), 330-360. https://doi.org/10.1016/j.clnu.2006.01.012

