https://dergipark.org.tr/tr/pub/food
OPEN ACCESS JOURNAL

ISSN 2667-5803

FHTI Journal of Food Health and Technology Innovations
December Vol 2, No 6 (2019)

DergiPark AKADEMİK

Review Article

Technological Aspects for the Production of Beta-Galacto-Oligosaccharides (ß-GalOS) and its Physiological Properties for Health Benefits.

Osama Ibrahim[1*]

ABSTRACT

Beta-Galacto-oligosahaarides (β-GalOS) are naturally occurred in milk at low concentration, and are found in all dairy products. ß-GalOS are commercially produced enzymatically by trans glalactosylation of milk sugar lactose as a substrate using the microbial enzyme ß-galactosidase (EC 3.2.1.23) derived from bacteria, yeasts or molds. ß-GalOS are one of functional oligosaccharides and are known by different names as oligogalactosyllactose, oligogalactose, oligolactose, tranas-galactosylated oligo- saccharide, and transgalacto-oligosaccharide. Chemical structures of ß-GalOs are chain of galactose units that arise through consecutive transgalactosylation enzymatic reactions, linked together into terminal glucose unit in the disaccharide lactose. The degree of galactose polymerization in ß-GalOS are vary and ranging from 2 to 8 galactose units. This degree of polymerization depends on the property and source of the enzyme ß-galactosidase used in the process. ß-GalOS are pass undigested into the colon where increase bowel mass and act as prebiotics (growth factor) for good bacteria (probiotics) in the colon. ß-GalOS are commonly used for stomach disorders such as constipation and preventing from allergies specially for infants. It is also, reported that ß-GalOS can prevent consumers from colon cancer and rectal cancer.

Keywords: Beta-glacto-oligiosaccharides, Glacto-oligosaccharides, GalOS, ß-GaloS, GOS, ß-transglucosidase, lactase, probiotics, prebiotics, synbiotics.

TÜBİTAK ULAKBİM

1 Consultant Biotechnology Kraft Foods Technolgy Center, New York Medical College, S.B.Penick & Company N.J. USA
*Corresponding Author: Osoma Ibrahim. E-mail: bioinnovation04@yahoo.com

Introduction

ß-GalOS consist of variable number (2 to 8) of galactose units linked together by glycosidic bonds of ß-(1-6) and ß-(1-4) and linked together to a terminal glucose unit through an α- (1-4) glycosidic bond (figure 1). ß-GalOS present at low concentration in yogurt and in other fermented milk as a result of the presence of the enzyme ß-galactosidase in starter culture. This enzyme transforms some of lactose in milk into ß-GalOS at very low concentration [1]. ß-GalOS is commercially produced enzymatically from lactose as a substrate using microbial enzyme ß-galactosidase that are produced commercially from either bacteria , yeasts , or molds such as *Streptococcus thermophiles*, *Kluyveromyces lactis, Bacillus circulans,* or *Aspergillus oryzae* respectively [2]. The degree of galactose polymerizations is in the range of two to eight units depends on different factors. These factors are: enzyme property, enzyme activity, lactose concentration as a substrate, enzymatic reaction time, enzyme optimum conditions (pH, temperature, etc.), and enzymatic process method (free or immobilized enzymes) [3]. The endpoints from this enzymatic reaction are ß-GalOS, in addition to low concentrations of lactose, free galactose, and free glucose. The presence of free galactose and glucose as byproducts inhibit the enzymatic reaction causing low yield of the main product of ß-GalOS. To improve the enzymatic reaction and ß-GalOS yield, the released free galactose and glucose units as byproducts are continuously removed from the enzymatic reaction by using cross flow ultrafiltration membrane, while retaining the enzyme in the production process [4].

ß-GalOS are commercially available as a polymer of galactose with different degree of polymerization (DP), and are used as low cariogenic as a substitute for high cariogenic sugars such as, sucrose, glucose, or fructose, with properties of having pleasant taste, providing texture, mouth feeling, moisture retaining capacity to foods, and thermostable during food processing. In addition, ß-GalOS have health benefits such as, prebiotic properties [5], and have neglectable impact on blood glucose level due to, the lack of ß-GalOS digestible enzymes in the digestive system. ß-GalOS are identified as dietary fibers due to the non-digestible characteristic. The legal definition of dietary fibers is nondigestible carbohydrates polymers with three or more monomeric units.

The first reference to prebiotic concept was initiated in the year 1954, when a researcher by the name Gyorgy reported that a component of human milk (N-acetyl-glucosamine) promoted the growth of a strain from the genus *Bifidobacterium* [6]. The recent definition of prebiotics is nondigestible food ingredients that target selected groups of healthy human colonic microflora, thus enhancing colonization of those microorganisms that offering health benefits to the host, such as *bifidobacteria* and *lactobacill species*. These prebiotics that enhance the growth of beneficial bacteria are known by the name bifidus factor or growth factor.

ß-GalOS are generally recognized as safe (GRAS) by FDA in the Unites States, and recognized as safe by other worldwide regulatory agencies as Novel Food (NF) status in the European Union , and as foods for specific health use (FOSHU)status in Japan [7]. These worldwide safety recognitions are due to the presence of ß-GalOS naturally at very low concentrations in human milk, yogurt, and in both human and animals' intestinal tracks from milk lactose fermentation by resident intestinal microflora. The recommended daily intake of ß-GalOS for human consumption is in the range of 0.3 to 0.4 gram / kilogram body weight, and the only known side effect of ß-GalOS intake is transient osmotic diarrhea that occurred when consumed at higher dose. [8]

Properties of ß-GalOS

Physiochemical properties of the marketed food-grade ß-GalOS are transparent syrups or white powders containing a mixture of ß-GalOS with different degree of polymerization (DP). plus containing low concentration of lactose and monomer sugars of glucose and galactose [9]. Purified ß-GalOS with more than 90% purity are also available in the market for some applications. Physiochemical properties such as solubility, osmolarity, viscosity, heat stability, freezing point, humectant property, and sweetness are shown in (Table 1). Other properties for ß-GalOS are crystal formation ability, and Miallard reactions. These physiochemical properties are slightly varied depending on the degree of galactose polymerization which in turn determine ß-GalOS proper applications. Physiological properties of ß-GalOS intake (consumed) are the indigestibility and stability to the hydrolysis by digestive enzymes and more that 90% of ß-GalOS passes into the colon [10]. Because of these physiochemical properties the caloric value of ß-GalOS has been estimated to be 1 to 2 Kcal/g [11&12].

Table (1): Summary of the commonly physicochemical properties of ß-GalOS

Solubility	Water-soluble, about 80% (w/w)
Appearance	Translucent/colorless
Viscosity	Similar to that of high-fructose corn syrup
Heat stability	Stable to 160 °C for 10 min at pH 7; stable to 100 °C for 10 min at pH 2; stable to 37 °C at pH 2 for several months
Freezing point	Reduces the freezing point of foods
Humectant properties	High moisture retaining capacity preventing excessive drying
Sweetness	Typically, 0.3 to 0.6 times that of sucrose

Prebiotics properties of ß-GalOS

Prebiotics is nondigestible food ingredients that target selected groups of the healthy human colonic microflora, thus enhancing colonization of *bifidobacteria* and *lactobacilli species* that offering health benefits to the host, and ß-GalOS are falling under this prebiotic's category.

The human intestinal tract harbors a mixture of prokaryotic and eukaryotic bacteria, fungi (molds), Archaea, viruses and bacteriophages. These mixture of microflora in the intestinal tracts is referred to intestinal microbiota. In human intestinal tract, it is estimated a total of 10^{14} microbiota cells that are mainly present in the colon. These intestinal microbiota are colonized immediately in human intestinal tract after birth and lasts for lifetime [13]. These prebiotics such as ß-GalOS selectively enhance the growth of healthy bacteria of *Lactobacillus* and *Bifidobacterium* species resulted in the inhibition of harmful microbiota in the colon. The enhancement of good bacteria and the inhibition of bad bacteria is known by the name competitive exclusion [14]. That said, most of the prophylactic health effect to the host (human or animals) from prebiotics such as ß-GalOS is due to the selective consumption of these prebiotics by *lactobacillus* and *Bifidobacterium* species as a fermentative substrate producing organic acids, short chain fatty acids, bacteriocins, and other metabolites. These generated metabolites in the colon protecting the host against enteric infection. In addition, the intake of ß-GalOS as dietary supplements increase mineral absorption, stimulate immunomodulation for the prevention of allergies and gut inflammatory conditions [15]. Other trophic effects from the intake of ß-GalOS are providing the host with healthy colonic epithelial cells, prevent constipation symptoms by increasing fecal bulking, and may reduce risk factors for colon cancer by reducing the toxicogenic and carcinogenic generated from intestinal microflora (microbiota) metabolism [16].

Applications of ß-GalOS

ß-GalOS are available commercially as a polymer of galactose with different degree of polymerization (DP), and are used as low cariogenic as a substitute for high cariogenic sugars such as, sucrose, glucose, or fructose, with properties of having pleasant taste, providing texture, mouth feeling to foods, having moisture retaining capacity, and thermostable during food processing[16]. In addition, providing the host with health benefits such as, neglectable impact on blood glucose level due to, the lack of ß-GalOS digestible enzymes in the digestive system.

In addition to prebiotic properties and as dietary supplement, ß-GalOS are used in wide varieties of adult foods such as, backed goods, beverages, and are commonly used in infant formula at the range of 6.0 to 7.2 grams per liter together with fructo-oligosaccharides (FOS) at the range of 0.6 to 0.7 grams per liter[17].

Because of the prebiotic property of ß-GalOS, its application is not limited to human but also to animals. The application of ß-GalOS in animal feeds is to improve the health and growth of farm animals, and to reduce antibiotics use as a way to minimize the concern of immerging antibiotics resistance strains of microbial pathogens that effect both human and animals [18]. In addition, the incorporation of ß-GalOS in animal feeds have positive impact on environment by reducing methane gas emission from ruminates animals [19], and reducing fecal odor from animals' farms due to the non-fermentable properties of ß-GalOS [20].

Other ß-GalOS applications are in cosmetics [21], agriculture chemicals, and in pharmaceutical products.

Discussion

Functional oligosaccharides including Beta-Galcto oligosaccharides (ß-GalOS) are low molecular weight carbohydrates with different degrees of polymerization (DP) of monosaccharides in the range of two to eight units. They are intermediate chemical structures in molecular weight between monosaccharides, and polysaccharides. They are present naturally in small quantities in different natural sources such as yogurt, and dairy products as in the case of ß-GalOS.

Due to, increasing demands of these functional oligosaccharides for health benefits, functional oligosaccharides are produced commercially by enzymatic processes at higher yield and lower costs comparing to extraction methods from natural sources. Carbohydrates are the main substrates for microbial enzymes in the production of these functional oligosaccharides at large scale operations. In the case of ß-GalOS production on large scale, several microbial glycosides hydrolyses enzymes of ß-glactosidase that also known by the name lactase are capable to hydrolyze the disaccharide sugar lactose into D-glucose and D-galactose, and in the same time having the ability to catalyze transgalactosylation activity for the production of ß-GalOS from lactose as a substrate (figure 3). These microbial enzymes of ß- galactosidase are produced commercially from bacteria, yeasts, and molds (fungi). The end products from this enzymatic process using lactose as substrates are ß-GalOS mixture with different degree of polymerization (DP), plus low concentrations of lactose, glucose, and galactose as by products. The ratio of ß-GalOS mixture with degree of polymerization (DP) in the end product of this enzymatic process are variable depends on the microbial source and properties of ß-galactosidase enzyme used in the bioconversion process for the production of ß-GalOS.

ß-GalOS are non-digestible dietary fibers with prebiotic properties, that are gaining consumers demand for health benefits. The definition of prebiotics are non-digestible compounds that promote in the colon the growth of healthy endogenous or oral intake live beneficial bacteria [22]. These healthy bacteria (probiotics) with the help of ß-GalOS as prebiotics multiply in the colon at high growth rate that inhibit the growth of harmful pathogenic bacteria via competitive exclusion. Plus, producing metabolites that help maintaining healthy colon. These generated metabolites are short chain fatty acids such as butyric acid that might prevent the host from colon cancer and rectal cancer. It is important to highlight that the relationship between probiotics (beneficial bacteria), and prebiotics such as ß-GalOS is referred to synbiotics [23]. Synbiotics concept was first introduced as a mixture of probiotics and prebiotics that beneficially effect the gastrointestinal tract for the host (human or animals). This synbiotic formulation that contains both probiotics and prebiotics are manufactured by using microencapsulation technology to control release live probiotics in the colon and are marketed in the form of capsules, or tablets. Plus incorporated in healthy foods and beverages.

ß-GalOS are stable in wide pH range and temperatures that are suitable for several applications in infant formulas, foods, feeds, pharmaceuticals, cosmetics, and agriculture chemicals industries. Furthermore, the low sweetness attribute and low caloric values of ß-GalOS makes it them useful as bulking agents in low calories food formulations. This bulking property of ß-GalOS, stimulates healthy bowel movements, preventing constipation, and diarrhea symptoms [24]. In addition, ß-GalOS are humectants due to its high moisture retaining capacity without increasing foods water activity. This humectants property of ß-GalOS has multiple applications in baked goods, other foods, animal feeds, pharmaceuticals, cosmetics, and agriculture chemicals formulations.

ß-GalOS are mainly used in infant milk and food formulae. These infant formulae are usually containing between 6 to 7.2 gram per liter galacto oligosaccharides (ß-GalOS) in addition to about 0.6 to 0.8 gram per liter fracto-oligosaccharides (FOS). Furthermore ß-GalOS are also incorporated into wide variety of adult foods such as fruit juices, other acid drinks, fermented milk, flavored milk, bread, and other backed goods. ß-GalOS are suitable in backed goods because are not fermentable by yeast and not consumed or hydrolyzed during dough fermentation. plus providing Maillard reactions (browning) and thermostable in baking processes [25].

In food applications ß-GalOS are nondigestible, have a pleasant taste and improve texture and mouthfeel of foods providing bulk properties similar to sugar. Therefore ß-GalOS can be used as food ingredient for diabetes as low cariogenic sugar substitute with neglectable impact on blood sugar [26]. In addition, ß-GalOS are suitable for the application in specialized food to elderly and hospitalized patients.

ß-GalOS received Generally Recognized As safe (GRAS) status in the United States, Novel Food (NF) status in the European Union, foods for specific health use (FOSHU) status in Japan. These worldwide safe status of ß-GalOS are due to their presence naturally in human milk, yogurt. It is important to highlight that ß-GalOS are well-tolerated up to intake (consumption) levels of 20 grams per day.

In addition, ß-GalOS also have wide applications in livestock feed, poultry feed, aquaculture feed, and pet food industries to improve the health and growth of farm animals, poultry and fish for meat production. Plus, minimizing antibiotics use, and reduce farm animals' fecal odor [27]. ß-GalOS have been also applied to suppress methane gas production by ruminants' animals [28]. Suppress methane gas produced from ruminates have positive impact in environment by mitigating global warming.

Conclusion

Galacto-oligosaccharide (ß-GalOS)are commercially manufactured enzymatically from lactose. ß-GalOS have unique properties, and health benefits, with wide varieties of applications, in food and feed industries as food additives. in Pharmaceutical industry ß-GalOS is used as expedients for drugs formulation. other applications are in cosmetics, and agriculture chemicals. ß-GalOS are being approved for human consumption and are regulated in United States, Europe, Japan and in other countries

References

1. **Montira Intanon, Sheryl Lozel Arreola, Ngoc Hung Pham, Wolfgang Kneifel, Dietmar Haltrich, and Thu-Ha Nguyen. 2014** Nature and biosynthesis of galacto-oligosaccharides related to oligosaccharides in human breast milk. *FEMS Microbiol Lett*. **353** (2): 89–97

2. **Jennifer Loveland, Kevin Gutshal, Jodie Kasmir, P. Prema, and Jeane E. Brenchley: 1994** Characterization of Psychrotrophic Microorganisms producing B-Galactosidase activities. *Applied Microbiology* 60 (10 12-18).

3. **Daniel Obed Otieno.2010** Synthesis of ß-galactooligosaccharides from lactose using microbial ß-galactosidases. *Comprehensive reviews in food science and food safety*. **(9)** 5:471-482

4. **Suwimol Chockchaisawasdee, Vasileios I. Anthanasopoulos, Keshavan Niranjan, and Robert A. Rastal. 2005** Synthesis of galcto-oligosaccharides from lactose using ß from *Kluyveromyces lactis*: studies on batch and continuous UF membrane filtered bioreactor. *Biotechnology and bioengineering* 89 (4) 434-443

5. **Lamsal PB. 2012** Production, health aspects, and potential food uses of dairy prebiotic galacto-oligosaccharides. *J. Sci. Food Agric*. 92 (10) 2020-2028

6. **Mary O'Connell Motherway, Frances O'Brien, Tara O'Driscoll, Patrick G. Casey, Fergus Shanahan, and Douwe van Sinderen. 2018** Carbohydrate Syntrophy enhances the establishment of *Bifidobacterium breve* UCC2003 in the neonatal gut. *Scientific report*. **8:** 10627

7. **Tzortzis G, Vulevic J. 2009** Galacto-oligosaccharide prebiotics. In: Charalampopoulos D, Rastall RA, editors. *Prebiotics and probiotics science and technology*. New York: Springer Link. 207 - 244

8. **Leena Niittynen, Kajsa Kajander, and Riitta Korpela. 2007** Galacto-oligosaccharides and bowel function. *Scand. J. Food Nutr*. **51**(2): 62–66

9. **Playne MJ, and Crittenden RG. 2009** Galacto-oligosaccharides and other products derived from lactose. *In: McSweeney PLH, Fox PF, editors.Water, salt,Advanced Dairy Chemistry, water, salts and minor constituents*. 3rd ed. New York: Springer. 121 – 201

10. Van Loo J, Cummings J, Delzenne N, Englyst H, Franck A, Hopkins M, Kok N, Macfarlane G, Newton D, Quigley M, Roberfroid M, Van Vliet T, and Van Den Heuvel E. 1999 Functional food properties of non-digestible oligosaccharides*:* a consensus report from the ENDO project (DGXII AIRII-CT94-1095). *Br J Nutr* 81(2):121–132

11. Roberfroid M, Gibson GR, and Delzenne N. 1993 The biochemistry of oligofructose, a nondigestible fiber-an approach to calculate its caloric value. *Nutr. Rev.* **51** (5):137-146

12. Cummings JH, Roberfroid MB, Andersson H, Barth C, FerroLuzzi A, Ghoos Y, Gibney M, Hermonsen K, James WPT, Korver O, Lairon D, Pascal G, and Voragen AGS. 1997 A new look at dietary carbohydrate: chemistry, physiology and health. *Eur. J. Clin. Nutr.* **51**(7):417–423

13. Juan Miguel Rodríguez, Kiera Murphy, Catherine Stanton, R. Paul Ross, Olivia I. Kober, Nathalie Juge, Ekaterina Avershina, Knut Rudi, Arjan Narbad, Maria C. Jenmalm, Julian R. Marchesi, and Maria Carmen Collado. 2015 The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb. Ecol. Health Dis.* **26**: 10

14. Callaway TR, Edrington TS, Anderson RC, Harvey RB, Genovese KJ, Kennedy CN, and Venn DW, Nisbet DJ. 2008 Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. *Anim. Health Res Rev.*;**9** (2):217-225

15. G.T. Macfarlane ,H. Steed, and S. Macfarlane. 2008 Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *J. of Applied Microbiology*.**104** (2) 305-324

16. Leena Niittynen, Kajsa Kajander, and Riitta Korpela. 2007 Galacto-oligosaccharides and bowel function. *Scand. J. Food Nutr.* **51**(2): 62–66

17. Bakker Zierikzee AM, Alles MS, KNOJ J, Kok FJ, Tolboom JJ, and Bindels JG. 2005 Effect of infant formula containing a mixture of galacto-oligosaccharides and fructo-oligosaccharides or viable *Bifidobacterium animalis* on the intestinal microflora during the first 4 months of life. *Br. J. Nutr.* **94** (5) 783-790 (2005).

18. Gerad Huyghebaert, Richard Ducatelle, and Filip Vanlmmerseel. 2011 An update on alternative to antimicrobial growth promoters for boilers. *The Veterinary J.* **187** (2) 182-188

19. Muhammad Farooq Iqbal, Yan Fen Cheng, Wei Yun Zhu, and Basil Zeshan. 2008 Mitigation of ruminant methane production: Current strategies, constraints and future options *World Journal of Microbiology and Biotechnology* v.(12) 2747-2755

20. G. Q. Yana, Y. Yin, H.Y. Liue, and G. H.Liu.2016 Effects of dietary oligosaccharide supplementation on growth performance, concentrations of the major odor-causing compounds in excreta, and the cecal microflora of broilers. *Poultry science* 95: 2342 -2351

21. Jean-Pierre H. G. Lamothe,Yves G. Marchenay,Pierre F. Monsan,Francois M. B. Paul, and Vincent Pelenc. 1996 Cosmetic compositions containing oligosaccharides. United States patent number 5518733A

22. Seema Patel, and Arun Goyal. 2012 The current trends and future perspectives of prebiotics research: a review. *3 Biotech.* 2(2): 115–125

23. Paulina Markowiak, and Katarzyna Śliżewska. 2017 Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients*.; **9** (9): 1021

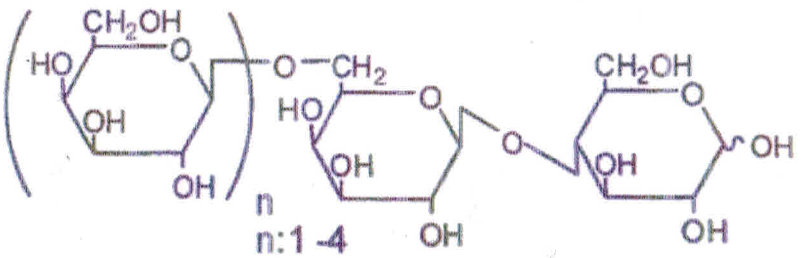
24. Leena Niittynen, Kajsa Kajande, and Riitta Korpela. 2007 Galacto-oligosaccharides and bowel function. *Scandinavian Journal of Food and Nutrition* **51** (2): 62-66

25. Duarte P.M. Torres, Maria do Pilar José A. F. Gonçalves Teixeira, . and Lígia R. Rodrigues .2010 Galacto-Oligosaccharides : Production, Properties, Applications, and Significance as Prebiotics, *Comprehensive review in food science and food safety* **9** (5) 438-454

26. Gonai M, Shigehisa A, Kigawa I, Kurasaki K, Chonan O, Matsuki T, Yoshida Y, Aida M, Hamano K, and Terauchi Y. 2017 Galacto-oligosaccharides ameliorate dysbiotic Bifidobacteriaceae decline in Japanese patients with type 2 diabetes. *Benef Microbes*. 13;8(5):705 -716

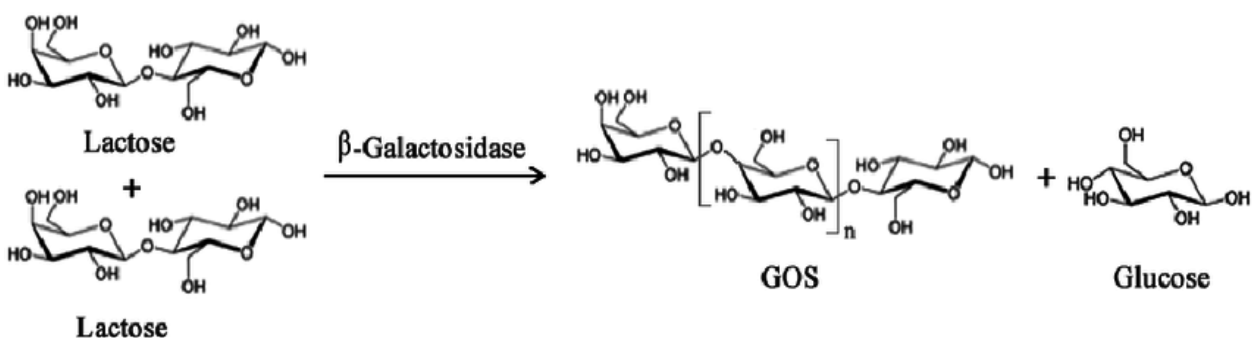
27. G. Q. Yang, Y. Yin, H. Y. Liu, and G. H. Liu. 2016 Effects of dietary oligosaccharide supplementation on growth performance, concentrations of the major odor-causing compounds in excreta, and the cecal microflora of broilers.*Poultry Science*, 95 (10), 2342–2351

28. B. Santoso, S. Kume1, K. Nonaka1, K. Kimura, H. Mizukoshi, Y. Gamo and J. Takahashi. 2003 Methane Emission, Nutrient Digestibility, Energy Metabolism and Blood Metabolites in Dairy Cows Fed Silages with and without Galacto-oligosaccharides Supplementation. *Asian-Australasian Journal of Animal Sciences* 16 (4) 534-540.



Galacto-oligosaccharides (ß-GalOS)

Figure 1: Composed of galactose chain linked together with ß (1-6) glycosidic bonds, and bound to glucose terminal by ß (1-3) or ß (1-4) glycosidic bonds.



Enzymatic production of ß-GalOS from lactose

Figure (2): Trangalactosylation enzymatic process involving the substrate sugar lactose and the enzyme ß-Galactosidase for ß-GalOS production.

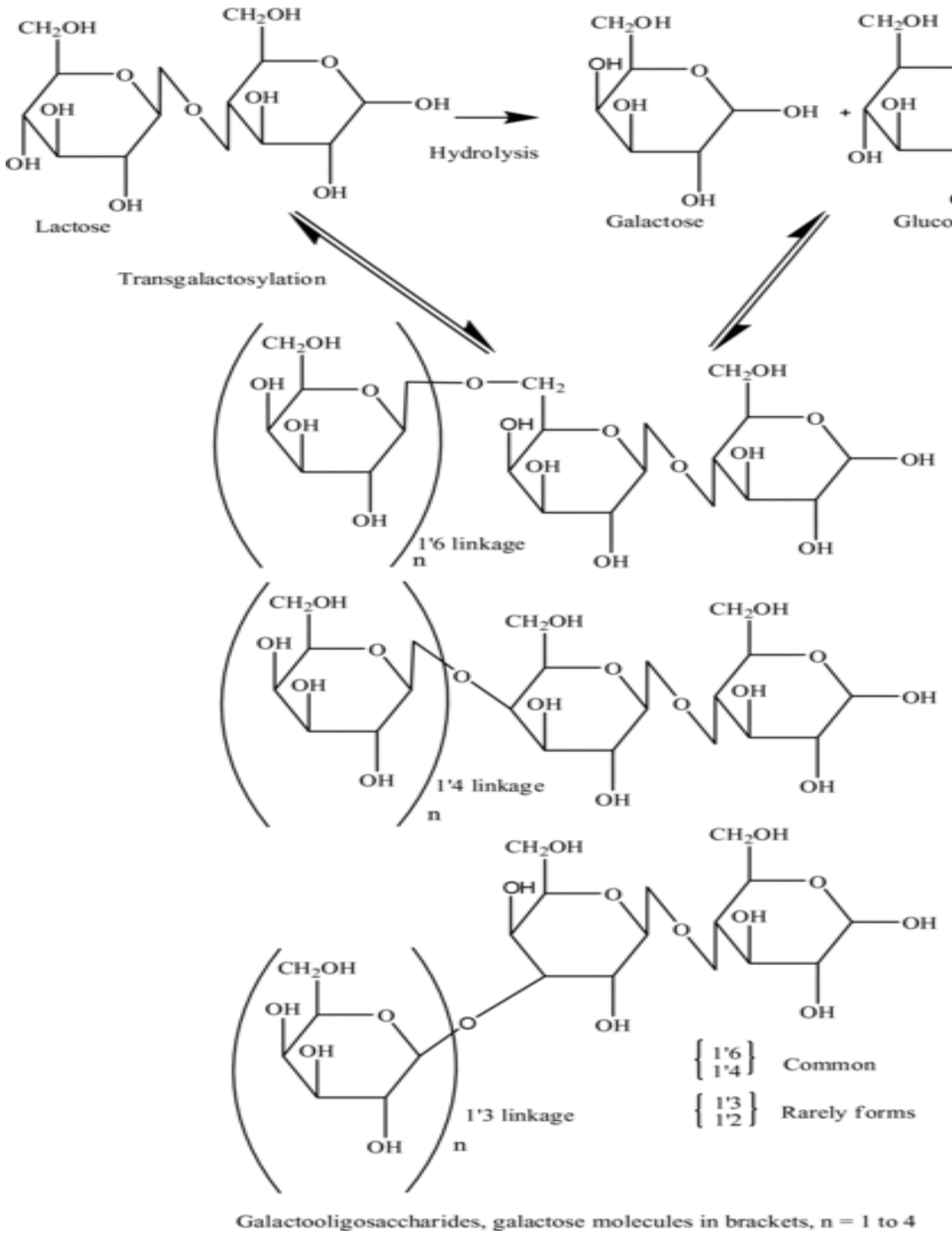


Figure 3: The enzyme ß-Galactosidase catalyzes two reactions; the first reaction is the hydrolysis of lactose into galactose and glucose, the second reaction is trans-galactocylation of galactose as a donor to the second lactose forming ß-GalOS with different degrees of galactose polymerization.