Chapter 14
Digestive Enzymes: Industrial Applications in Food Products

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Abstract Digestion is a very complex process involving many different enzymes expressed by the human cells and by the microbial community in the digestive tract. Digestive problems such as lactose intolerance and poor digestion of vegetable oligosaccharides affect a great part of the human population, causing discomforts due to their fermentation by gas-producing microorganisms. Although the treatment may involve the supplementation with digestive enzymes, such as lactase (beta-galactosidase) and alpha-galactosidase, respectively, the industrial processing of food products is another alternative. Gluten intolerant and celiac individuals could potentially be benefited by the administration of peptidases or the consumption of peptidase-treated food, however, this is not yet considered a treatment option that substitutes the complete avoidance of gluten. Enzymes have been applied in food processing for various purposes including the removal of undesired components. Besides the galactosidases that remove specific saccharides, another example is the use of L-asparaginase to avoid the formation of acrylamide, a possible carcinogen. In this chapter, the application of digestive enzymes in food bioprocessing will be reviewed, from traditional applications of alpha- and beta-galactosidases, to potential applications of proteases and lipases. Examples of commercial products and of the most recent and relevant patents in this area will be included.

Keywords Alpha-galactosidase · Beta-galactosidase · Peptidase L-asparaginase

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14.1 Introduction

Digestive disorders and poor digestion are health problems that affect a great part of the human population, their prevalence is difficult to estimate. Some of them are of genetic origin and most of them are affected by the lifestyle, especially by the diet and eating habits. Lactose intolerance as a result of congenital lactase deficiency is rare, however, the reduced ability to digest lactose is a problem that affects around 65% of the human adult population. Also, the consumption of non-digestible oligosaccharides present in legumes such as soybean may cause discomforts due to their fermentation by gas-producing microorganisms. The use of digestive enzymes such as beta-galactosidases to hydrolyze lactose and alpha-galactosidases to break the alpha-1,6 bonds of stachyose, raffinose and melibiose, the non-digestible oligosaccharides of vegetable origin, either as supplements or in the processing of food products, is the most effective strategy to avoid digestion problems associated to the consumption of dairy products and some vegetables such as soybean. Some processes are well established industrially for such purposes.

The celiac disease (CD), a chronic inflammatory intestinal disease triggered by the ingestion of gluten, affects around 70 million people globally. It is the most common disease in the digestive system of genetic origin, characterized by the abnormal response of the body's immune system to a group of proteins. The main amino acids present in gluten composition (proline and glutamine, called the prolamins) are responsible for the immune response in CD and gluten intolerance because the high proline content makes these proteins resistant to digestive enzymes. As a result, oligopeptides can reach the small intestine where they elicit an autoimmune response in susceptible individuals. A gluten-free diet is the only effective treatment for CD available, however, this problem could possibly be alleviated by using gluten-specific peptidases for gluten protein degradation into small or nonimmunogenic peptide fragments before they transit across the small intestinal mucosa.

In this chapter, the application of digestive enzymes in food bioprocessing will be reviewed, from traditional applications of alpha- and beta-galactosidases, to potential applications of peptidases, lipases, and L-asparaginases. The examples of commercial products and of the most recent and relevant patents in this area will be included.

14.2 Alpha-Galactosidases

Alpha-galactosidases are enzymes that catalyze the hydrolysis of simple and complex oligosaccharides and polysaccharides. Some food or feed products, e.g., soy-based products, are composed of galacto oligosaccharides and pectic polysaccharides, which are associated with some indigestibility problems (Kien 2008). Soy-based foods present some health benefits such as hypolipidemic,

anticholesterolemic, and antiatherogenic properties, and reduced allergenicity (Trindade et al. 2001), mainly because of their composition that includes lipids, vitamins, minerals, free sugars, isoflavones, flavonoids, saponins, and peptides of therapeutic value (Donkor et al. 2005; Sanjukta and Rai 2016). Besides, soy milk appears as an alternative to dairy products for lactose-intolerant people. Soybeans are abundant and less expensive than bovine milk and do not contain cholesterol (Hati et al. 2014). However, the beany flavor and the high levels of raffinose and stachyose which may cause gastrointestinal discomfort problems to consumers restrict the consumption of soybean and its products (Tsangalis and Shah 2004). The absence of alpha-galactosidase in the human intestinal mucosa makes the consumption of raffinose and stachyose, from soybean products, very difficult. Non-hydrolyzed oligosaccharides pass directly into the lower intestine and are then metabolized by alpha-galactosidase-producing bacteria, resulting in the production of gases (Tsangalis and Shah 2004). The use of alpha-galactosidase, or an organism that possesses high alpha-galactosidase activity, can minimize flatulence that is caused by some products composed by these oligosaccharides (Scalabrini et al. 1998). This fact could improve the nutritional quality of soy-based foods. In this way, there is great interest in the use of alpha-galactosidase pre-treated products, which may diminish gastric distress that is caused by carbohydrates fermentation in the large intestine. A promising solution for the elimination of these oligosaccharides in soy milk is the employment of alpha-galactosidases (Carević et al. 2016; LeBlanc et al. 2005).

14.2.1 Alpha-Galactosidase Classification and Action

Alpha-galactosidase, also called α-D-galactosidegalactohydrolase (E.C. 3.2.1.22), melibiase or alpha-D-galactopyranoside galacto hydrolase (IUBMB 2018) catalyzes the hydrolysis of α-(1,6)-galactosidic bonds causing the release of α-D-galactose (Anisha and Prema 2007; Du et al. 2013; Naumov 2004). Alpha-galactosidases are classified according to their substrate specificity or amino acid sequence similarity. According to Garman (2007), alpha-galactosidases can be divided into Group I and Group II. Group I is composed of enzymes that act on oligosaccharides such as the raffinose family oligosaccharides (RFOs). Group II includes enzymes that act on polysaccharides such as galacto(gluco)mannans. Alpha-galactosidases have been classified into more than 100 glycoside hydrolase (GH) families, including GH 4, 27, 36, 57, 97, and 110, which belong to the CAZy database (http://www.cazy.org/). The GH family 27 consists of the eukaryotic alpha-galactosidases. GH family 36 includes predominately bacterial alpha-galactosidases.

Some oligosaccharides, such as stachyose, melibiose, and raffinose present α-(1,6)-galactosidic links (Gote et al. 2004; Naumov 2004). They are also found in branched polysaccharides such as galactomannans and galacto (gluco) mannans (Naumov 2004; Ademark et al. 2001). For this reason, the enzyme hydrolyzes galactosides, including galactose oligosaccharides, galactomannans, and

galactolipids (IUBMB 2018). Alpha-galactosidases act on stachyose, releasing one molecule of galactose and raffinose, and on raffinose releasing galactose and sucrose, which in turn can be hydrolyzed by invertase for different industrial applications (Anisha and Prema 2007; Du et al. 2013; LeBlanc et al. 2005; Naumov 2004).

14.2.2 Microbial Production of Alpha-Galactosidases

Different sources and substrates were reported for alpha-galactosidase production. Animals, plants, bacteria, and fungi were described as alpha-galactosidase producers. However, microbial production is the most employed. Extracellular alpha-galactosidases are generally produced by *Aspergillus* (Liu et al. 2007), *Trichoderma* (Savel'ev et al. 1997) and *Penicillium* (Shibuya et al. 1995) species. In an industrial point of view, bacteria and fungi are potentially more economic alpha-galactosidases producers (Jin et al. 2001) (Table 14.1). Lower amounts of the enzyme are produced by plants and animals which represent higher levels of separation and purification.

Table 14.1 Alpha-galactosidase production from different microbial sources and conditions

Microbial source	Fermentation	Medium	Optimum activity	Source
Lactobacillus fermenti NRRL B-585	SmF	MRS broth	1.1 U/mL or 4.3 U/mg	Mudgettt and Mahoney (1985)
Bifidobacterium breve 203	SmF	PYF broth (peptone/ yeast extract/Fildes solution)	Glucose-grown: 61 U/g Raffinose-grown: 2000 U/g	Xiao et al. (2000)
Bacillus stearothermophilus NCIM—5146	SmF	–	2.0 U/mL	Gote et al. (2004)
Lactobacillus fermentum	SmF	MRS	5 U/mL	LeBlanc et al. (2004)
Lactobacillus agilis LPB 56	SmF	Vinasse	11.07 U/mL	Sanada et al. (2009)
Aspergillus sojae ATCC11906	SmF	Modified YpSs medium	10.4 U/mL	Gurkok et al. (2011)
Humicola sp. NCIM 1252	SSF	Soya flour	44.6 U/g	Kotwal et al. (1998)

(continued)

Table 14.1 (continued)

Microbial source	Fermentation	Medium	Optimum activity	Source
Aspergillus foetidus ZU-G1	SmF	Soybean meal, 2% wheat bran, 0.1% KH_2PO_4 , and 0.05% $FeSO_4{\cdot}7H_2O$	64.75 U/mL	Liu et al. (2007)
Humicola sp. NCIM 1252	SmF	Soya flour	13.9 U/g	Kotwal et al. (1998)
Aspergillus oryzae	SSF	RGPW—red gram plant waste	3.4 U/g	Shankar and Mulimani (2007)
Aspergillus oryzae	SSF	WB—wheat bran	2.7 U/g	Shankar and Mulimani (2007)
rAgas2 from Hermetia illucens (Escherichia coli)	SmF		Specific activity 128.37 U/mg	Lee et al. (2018)
Debaryomyces hansenii UFV-1	SmF	Lactose-based medium 0.62 g/L KH_2PO_4, 2.0 g/L K_2HPO_4, 1.0 g/L $(NH_4)_2SO_4$, 0.1 g/L $MgSO_4{\cdot}7H_2O$, 5.0 g/L yeast extract	1.10 U/mL	Baffa Júnior et al. (2018)
Thielavia terrestris (designated TtGal27A) *Pichia pastoris*	SmF	See Invitrogen's manual	402.1 U/mL	Liu et al. (2018)
Aspergillus foetidus NRRL 341	SmF	1.4 g/L $(NH_4)_2SO_4$, 2.0 g/L KH_2PO_4,0.3 g/L $MgSO_4{\cdot}7H_2O$, 0.4 g/L $CaCl_2{\cdot}2H_2O$, 0.3 g/L urea, 1.0 g/L proteose peptone, 0.2 g/L Tween 80, 20 g/L soybean hull, 0.005 g/L $FeSO_4{\cdot}7H_2O$, 0.0016 g/L $MnSO_4{\cdot}H_2O$, 0.0014 g/L $ZnSO_4{\cdot}7H_2O$, and 0.002 g/L $CoCl_2{\cdot}2H_2O$	5.22 U/mL	Li et al. (2018)

SmF Submerged fermentation; *SSF* Solid-state fermentation

Bacterial alpha-galactosidases are generally produced by submerged fermentation (SmF), using specific conditions of pH, temperature, and agitation according to the applied strain. Alpha-galactosidases activities range from 1.11 U/mL, by *Bifidobacterium bifidum* MB239 (Mudgettt and Mahoney 1985), to 11.07 U/mL, by *Lactobacillus agilis* LPB 56 (Sanada et al. 2009). Solid-state fermentation (SSF) or submerged fermentation (SmF) are usually employed for fungal alpha-galactosidases. In SSF activities range from 2.7 to 44.6 U/g, which are normally higher than in SmF.

14.2.3 Applications of Alpha-Galactosidases

The most important industrial applications of alpha-galactosidases occur in sugar beet and soybean food processing industries and as therapeutic use for humans (Gote et al. 2007). In the first case, raffinose is the major impurity in sugarbeet, accounting for about 1% (w/w), and has a strong effect on both morphology and growth rate of sucrose crystals. The raffinose content is unstable and declines during storage. So, the use of alpha-galactosidase to remove raffinose is carried out to avoid this problem (Martin et al. 2001; Nakata et al. 2012). In the soybean industry, alpha-galactosidases have been used to reduce the oligosaccharide content in soybean meal, soy protein concentrate, soybean milk, and other soybean-based foods (Ju et al. 2018).

Concerning therapeutic use, alpha-galactosidases are administered orally to enhance the digestion of legumes such as peas, beans, especially soybeans, that have a high content of oligosaccharides. In soybean, there is a great amount of raffinose and stachyose that are carbohydrates of low molecular weight, non-metabolizable in the human intestine by the lack of α-galactosidase, thus causing flatulence. Raffinose ingestion also suppresses serum IgE level that is associated with some allergic diseases such as atopic dermatitis, allergic rhinitis, and asthma (Nagura et al. 2002; Nakata et al. 2012). Another application of alpha-galactosidase is for Fabry's disease treatment, which is related to α-galactosidase A's deficiency (Francesco et al. 2013) which causes immune system irregularities (Castaneda et al. 2008). Immune cells from affected patients display a constitutive proinflammatory pattern of cytokine expression (Francesco et al. 2013). Male patients, with little or no alpha-galactosidase activity, exhibit the "classic form" of Fabry disease (Togawa et al. 2012), which causes pain in the peripheral extremities, hypohydrosis, angiokeratomas, corneal opacities, and renal, cardiac and cerebrovascular involvement. For this problem, an enzyme replacement therapy with recombinant alpha-galactosidase is recommended (Schiffmann et al. 2006; Weidemann et al. 2009).

14.2.4 Commercial Products Containing Alpha-Galactosidases

Some commercial dietary supplements containing alpha-galactosidase are presented in Table 14.2. The cost of capsules can be $0.08–$0.58/capsule or $0.13–$0.22/g. They are employed as digestive supplements or for pancreatic problems.

14.2.5 Patents on Alpha-Galactosidases

Alpha-galactosidases were used in food products to manufacture a soy protein product having modified sugar profile (high sucrose and monosaccharide content and low indigestible oligosaccharides), rich in isoflavones and no galactinol content, resulting in a product with improved flavor and functional properties. The product was prepared from defatted soybean material, by the treatment with enzyme, removing the fiber before or after this process to achieve the requisite protein content, and inactivating the enzyme (Monagle, WO200215712-A2).

Tzortzis et al. (WO2007071987-A2) claimed a new alpha-galactosidase DNA and amino acid sequences with transgalactosylating activity isolated from

Table 14.2 Commercial supplements containing alpha-galactosidase

Name	Brand	Enzymes and complements	References
Alpha-galactosidase	Nutriteck® Canada/ United States	Alpha-galactosidase	Nutriteck (2018)
Alpha-galactosidase from *Aspergillus niger*	Bean-Zyme® United States	Alpha-galactosidase	Bean-Zyme® (2018)
Alpha-galactosidase —enzyme tablets	Beano® United States	Alpha-galactosidase	Beano® (2018)
Digestive enzymes	Baseline Nutritionals® United States	Alpha-galactosidase	Baseline Nutritionals (2018)
Digestive health supplement	Elle Belle UK® England	Digestive enzymes blend (amylase, cellulase, caseine protease, invertase, phytase, lipase, penthosanase (hemi cellulose), alpha-galactosidase)	ElleBelleUK® (2018)
Gas enzyme alpha-galactosidase	Vitacost® United States	Alpha-galactosidase	Vitacost® (2018)
Pancreatic enzymes and alpha-galactosidase	AOR Zymes® Canada	Alpha-galactosidase	Supplete (2018)

Bifidobacterium bifidum. The enzyme is useful for converting melibiose to alpha-galactobiose disaccharides that can be incorporated into food and feed products that promote the growth of bifidobacteria in the gut, thus improving the health.

Alpha-galactosidase was also used to increase the yield of sucrose from beet molasses by reducing the content of raffinose. The enzyme was added to beet juice with a concentration of 10–60° Brix and withdrawn before the sugar-boiling and centrifugal separation process, thereby hydrolyzing raffinose into sucrose and galactose (Suzuki et al. US3992260-A).

A patent with 28 citations describes an invertase-free alpha-galactosidase preparation by culturing *Saccharomyces cerevisiae* NRRL-Y-12533 in a medium containing enzyme-inducing substance (e.g., glucose, melibiose, or galactose), and recovering the enzyme; this preparation can be used to remove raffinose from soybean meal or sugar beet. A method for degrading oligosaccharides (stachyose and raffinose) in soybean milk comprises contacting the milk with cells of *S. cerevisiae* NRRL-Y-12533, with extracts of the cells, or with alpha-galactosidase purified from the extracts. The enzyme can also be used to hydrolyze raffinose in sugar beets without hydrolyzing sucrose (Olivieri et al. EP81262-A).

Alpha- and beta-galactosidases can also be added as components in food products. Zanarotti et al. (US2016303206-A1) patented a pharmaceutical or nutritional composition containing alpha-galactosidase and beta-galactosidase and excipient or carrier. The composition is beneficial to individuals with gastroesophageal reflux disease and allows digestion of food rich in both lactose and oligosaccharide.

14.3 Beta-Galactosidases—Lactases

Beta-galactosidases, also called lactases, are one of the most important enzyme groups used in food processing (Mahdian et al. 2016). They are also employed in technological and environmental applications. Milk and dairy products have protein, calcium, phosphorous, magnesium, and other micro- and macronutrients in their composition. Almost 70% of the world adult population is discouraged in consuming milk due to lactase deficiency (Mattar et al. 2012; Lomer et al. 2008). In this way, the consumption of dairy products by this group of people became possible with the use of lactases for lactose hydrolysis into glucose and galactose (Husain 2010). Besides, additional characteristics of the products could be ameliorated such as sweetness. Additionally, de-lactosed milk or whey products amplify the value and possibilities of using whey, which is a highly polluting waste (Ansari and Satar 2012; Panesar et al. 2016).

The industrial-scale production of microbial beta-galactosidases is established, even though there are some obstacles in large-scale production that are still discussed. Some points of investigation are related to the discovery of novel microbial sources of beta-galactosidases with different characteristics and applications.

Recombinant beta-galactosidases of bacterial, fungal, and yeast origin were also reported (Ansari and Satar 2012; Anisha 2017).

14.3.1 Beta-Galactosidases' Classification and Action

Beta-galactosidases (EC 3.2.1.23) catalyze the hydrolysis of lactose (β-D-galacto-pyranosyl-(1-4)-D-glucopyranose) releasing D-glucose and D-galactose as an end product. Lactases are also involved in transgalactosylation reactions producing galactooligosaccharides (GOS) (Gosling et al. 2010; Mussatto and Mancilha 2007), which are consumed by bifidobacteria as growth-promoting substrates in human intestine (Kamran et al. 2016; Ansari and Satar 2012).

14.3.2 Microbial Production of Beta-Galactosidases

Beta-galactosidases or lactases can be produced in high yields by bacteria, fungi, yeasts. They also occur in plants (almonds, peaches, apricots, and apples) as well as in animal tissues (Husain 2010; Mahdian et al. 2016). Some examples of microbial sources and substrates for beta-galactosidases are presented in Table 14.3.

Table 14.3 Beta-galactosidases produced from different microbial sources and substrates

Microorganism	Medium	Activity	Reference
Bacillus sp.	Lactose (1%) and $MgCl_2$	50 U/mL	Kamran et al. (2016)
Bacillus sp. MPTK	Lactose and $MgCl_2$	~65 U/mL	Kumar et al. (2012)
Bacillus safensis	Lactose and glucose (3:1–39:1)	~0.14 U	Nath et al. (2014)
Streptococcus thermophilus	Acid whey	7.76 U/mL	Princely et al. (2013)
Kluyveromyces lactis NRRL Y8279	Lactose medium	14.10 U/mL	Dagbagli and Goksungur (2008)
Kluyveromyces marxianus	Lactose medium	31 U/mL	Cortes et al. (2005)
*Kluyveromyces marxianus*CCT 7082	Lactose medium	8.5 U/mL	Manera et al. (2008)
Aspergillus tubengensis GR1	Czapeck Dox Lactose	169 U/mL	Raol et al. (2015)
Trichoderma acidotherma AIU BGA-1	Lactose (2%)	54.6 U	Yamada et al. (2016)

14.3.3 Applications of Beta-Galactosidases

Beta-galactosidases are mainly employed in food and beverages, dietary supplements, and pharmaceuticals. Dairy and pharmaceutical industries are the major users of lactases. In the case of condensed milk and frozen dairy products, for example, they can be used to reduce the effect of lactose crystallization. The enzyme is also employed in the hydrolysis of whey components from the cheese processing industry, to decrease water pollution (Kamran et al. 2016; Husain 2010). The main application of beta-galactosidases is in the food industry. The use of lactases in whey hydrolysis, for example, can release sugar substitutes for cooking, confectionery, and nonalcoholic beverages (Panesar et al. 2016), which is another alternative for reducing the environmental impact of the sub-product (Marwaha and Kennedy 1988; Mahdian et al. 2016). Beta-galactosidases are also employed in transgalactosylation reactions to produce GOS (Gosling et al. 2010; Mussatto and Mancilha 2007) that are non-digestible oligosaccharides. They can be consumed as prebiotic food ingredients to improve the growth of bifidobacteria population, which is benefic for health as these bacteria reduce cholesterol level, producing different essential vitamins and also having anticarcinogenic properties (Grosová et al. 2008; Kamran et al. 2016).

14.3.4 Commercial Products Containing Beta-Galactosidases

The world's lactase market is continuously growing at 3.7% from 2017 to 2025 reaching around US$1,235 Mn in 2017 and it is expected to rise to over US$1,647 Mn by the end of 2025 (Persistence Market Research 2018). *Kluyveromyces lactis, Kluyveromyces fragilis, Kluyveromyces marxianus, Candida kefyr, Saccharomyces cerevisiae,* and the fungi *Aspergillus niger* and *Aspergillus oryzae* are the main lactase producers (Ansari and Satar 2012). Novozymes, Merck KGaA (Sigma-Aldrich), Sternenzyme, Amano Enzyme Inc., Calza Clemente, Senson, Natural Factors Inc., Specialty Enzymes and Biotechnologies, Nature's Way Products, LLC, Chr. Hansen A/S, Advanced Enzyme Technologies Limited, DuPont de Nemours and Company, DSM Chemicals and others are the main lactase industrial manufacturers. Liquid and solid enzymatic commercial formulations can be found (Table 14.4).

Table 14.4 Beta-galactosidases producers and suppliers

Enzyme source	Product name	Supplier
Bacteria		
Bacillus sp.	Novozym 231	Novozymes A/S, Bagsvaerd, Denmark
Escherichia coli	β-Galactosidase	Sigma Aldrich, UK
Yeast		
Candida pseudotropicallis	Neutral lactase	Pfizer, Milwaukee, USA
Kluyveromyces sp.	Lactase NL	Enzyme Development Corporation
Kluyveromyces lactis	Maxilact	DSM Food Specialties, Delf, The Netherlands
	β-Galactosidase	SNAM Projetti, Italy
	Lactase	Sigma Aldrich, UK
Kluyveromyces marxianus	Lactozyme	Novozymes A/S, Bagsvaerd, Denmark
Saccharomyces fragilis	β-Galactosidase	Sigma Aldrich, UK
Fungi		
Aspergillus niger	Sumylact	Sumitomo Chemical, Japan
	Lactase	Vallio Laboratory, Finland
	Aspergillus galactosidase	AmanoEnzyme Inc., Europe
Aspergillus oryzae	Astrolac	Calza Clemente, Italy
	Fungal lactase	
	Biolacatase	
	Lactase 2214C β-Galactosidase	

Source Modified from Panesar et al. (2010)

14.3.5 Patents on Beta-Galactosidases

The most recent patents on the use of lactase in food products are related to methods for lactose hydrolysis in milk products. These processes usually involved lactase hydrolysis and filtration procedures.

Within these processes, Doering et al. (EP3251515-A1) developed a method for preparing lactose-free dairy products by ultrafiltering the starting milk (lactose content of 3–5 wt%), performing reverse osmosis of the first permeate, mixing the first retentate with the second permeate and milk minerals in order to obtain a standardized milk product, and hydrolyzing the dairy product with lactase, so that the remaining amount of lactose still contained in the product (0.45–0.55 of the initial lactose value) was completely cleaved into glucose and galactose.

Knights (US2013287892-A1) invented a process for making low-lactose milk protein concentrate useful to provide protein, calcium, and other nutrients to food

compositions. It comprised heating a liquid milk composition of initial solids including whey protein, caseins, and lactose (9–25 wt%), so that the whey protein adhered to the caseins producing aggregates; cooling; membrane filtering; retaining protein aggregates, and adding lactase to reduce the concentration of lactose to less than 2 wt% relative to the total protein content. This method reduced gel formation in a milk product during storage, avoiding the whey protein crosslinking. The milk protein concentrate had a total protein content of 70 wt% of the retained solids, and the whey protein was present at 15–25 wt% of the solids.

A patent written by Stevens (US4853246-A) had 39 citations, it described the production of sweetened, high protein, low fat, and reduced lactose milk obtained by ultraheat-treating low-fat milk and adding lactase. The content of total milk solids was increased in an initial low-fat milk. The product contained 10–35% wt. total milk solids, less than ca. 2% wt. milk fat and less than ca. 1 wt% lactose.

Choi and Lee (KR2004103818-A) described the production of low-lactose milk by hydrolyzing milk with lactase and concentrating the hydrolyzed lactose milk by nanofiltration to partially remove glucose and galactose. Water was added to remove the sweetness.

Low-lactose dried milk powders of high lysine content were prepared by mixing separately dried protein retentate and lactase hydrolyzed permeates obtained by ultrafiltration or diafiltration, as described by Uiterwaal (EP108838-A). The milk protein was separated as a retentate by ultrafiltration or diafiltration, while the lactose was separated in the permeate, which was treated with lactase. These two fractions were dried and mixed. Lysin content was preserved by the prevention of Maillard reaction through the separate drying of protein and reducing sugars.

Shi et al. (CN101317599-A) claimed a method for producing lactose-free whole milk preparation by heating raw milk and degreasing by centrifugal separation, cooling skimmed milk, applying ultrafiltration, applying nanofiltration to the permeated filtrate of the ultrafiltration treatment, mixing, and homogenizing the fractions of interest (cream powder, permeated filtrate of nanofiltration and trapped fluid of ultrafiltration), pasteurizing, applying lactase enzymolysis, and finally ultrahigh temperature sterilization.

Lange (WO200045643-A1) claimed a method to produce lactose-free milk by hydrolyzing lactose to glucose and galactose, without altering the taste, i.e., without conferring a sweet taste to the product. Ultrafiltration and diafiltration were used to adjust protein and lactose concentrations to the ratio of about 1:1. The reduction of the lactose ratio was a key feature that allows its conversion into monosaccharides with an unnoticeable change in sweetness.

Finally, a method for the processing of acid whey to generate a product (acid whey solution, acid whey powder or texturized acid whey) with at least 40% less lactose was developed by Onwulata et al. (US2015150275-A1). The process involved treating the acid whey solution with alpha-galactosidase and beta-galactosidase, filtering the acid whey solution to form a retentate containing proteins and a permeate containing lactose and residual proteins, recovering lactose from the permeate and optionally drying the retentate to form acid whey powder.

Other processes to reduce the lactose content involving alternative procedures, novel lactases, and immobilization were developed. A process to produce lactose-free sour milk product, e.g., yogurt was developed by Silfverberg et al. (FI118115-B1). It involved performing lactase pre-hydrolysis on milk raw material, inactivating the enzyme by heating, and hydrolyzing the residual lactose simultaneously with the souring of the product. The resulting milk product contained less than 0.01% to less than 1% lactose.

Havlik et al. (US2016143305-A1) patented a yogurt composed by cultured milk containing milk solids and non-fat or solute chosen from glucose, galactose, lactic acid derived from milk solids from milk and lactase. The enzyme was added to hydrolyze the lactose in the milk during the culturing step performed by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

Ernst et al. (WO2009071539-A1) developed a method for the treatment of milk to reduce the content of lactose to very low levels using the lactase from *Bifidobacterium bifidum*. The enzyme was active at high temperatures (52 °C) and improved the quality of the milk. Hydrolysis occurs during fermentation and in the final dairy product.

Finally, the problem of enzyme cost could be handled by immobilizing lactase for reutilization during hydrolysis. In this sense, Ottofricke et al. (DE3310430-A) developed a process for lactose hydrolysis in whey catalyzed by lactase immobilized on carrier particles in solid bed periodically cleaned with aqueous solution accompanied by mechanical fluidization. The whey was passed through a solid bed of enzyme immobilized on carrier particles, and an aqueous solution was periodically passed in countercurrent to clean the system. The operation could be carried out continuously for long periods (around 3 months) without additional clarification of the whey, and the degree of hydrolysis remained high (80–185%) at the end of the operation.

14.4 Peptidases

Peptidases are enzymes that degrade proteins by the hydrolysis of peptide bonds (Barrett and McDonald 1986), and many of them are applicable in the food industry. Hydrolyzed proteins could enhance the protein digestibility, decrease protein allergy, and alter the sensory quality due to modifications in the matrix. In some cases, bioactive peptides may be liberated by enzyme action (Stover and Mehta 2017; Tavano et al. 2018). Although various proteases are currently under investigation for the processing of food, this topic refers only to digestive peptidases and their influence in human health and life quality.

Proteins found in grains (wheat, rye, barley, and oats) are related to a chronic inflammatory intestinal disease, called celiac disease (CD). These proteins are generally called gluten, a component with unique viscoelastic properties and widely used in different food products. The CD triggered by the ingestion of gluten affects around 70 million people globally. It is the most common disease of the digestive

system of genetic origin, characterized by the abnormal response of the body's immune system to a group of proteins (Palabiyik et al. 2016; Ribeiro et al. 2018).

The main amino acids present in gluten composition (proline and glutamine, called the prolamins) are responsible for the immune response in CD and gluten intolerance, because the high proline content makes these proteins resistant to digestive enzymes action in the gastrointestinal tract. Proline is the only amino acid whose side-chain links to the backbone, thus avoiding the protein cleavage by most proteases. In susceptible individuals, the oligopeptides elicit an autoimmune response in the small intestine and an inflammatory injury of the intestinal mucosa (Colgrave et al. 2017; Ribeiro et al. 2018). This inflammatory process leads to mal-absorption of different nutrients and the clinical manifestations vary according to the patient, including malnutrition, diarrhea, growth retardation, anemia, fatigue, and other diseases (e.g., adenocarcinoma, neurological, and hormonal disorders) (Ribeiro et al. 2018; Vaquero et al. 2018).

A gluten-free diet is the only effective treatment for CD yet, however, this very restrictive diet is not easy and it is costly. The patients usually give up the diet due to the difficulties in changing the lifestyle and avoiding any food containing gluten. For all these reasons, various alternatives of nondietary and dietary therapeutic procedures have been investigated (Jnawali et al. 2016; Rey et al. 2016; Ribeiro et al. 2018; Vaquero et al. 2018). Various dietary therapies (e.g., wheat genetic engineering and probiotic treatments) are currently under investigation to avoid the CD complications. This problem could be alleviated avoiding the gluten transit across the small intestinal mucosa, by a previous gluten protein degradation into a small or nonimmunogenic peptide. The gluten-specific peptidases are important enzymes that could be used for this purpose.

14.4.1 Peptidase' Classification and Action

Peptidases catalyze the hydrolysis of peptide bonds and show preference for small peptides as substrates. Some peptidases attack the peptide bonds in different positions, while others are selective by attacking the bonds between specific amino acids. These diverse specificities determine their classification and possible uses. Considering the position that the enzymes attack, endopeptidases are those that act in the middle of the polypeptide chain and exopeptidases are those that cleave at the end of the chain (Barrett and McDonald 1986; Tavano et al. 2018).

14.4.2 Microbial Production of Peptidases

Peptidase enzymes produced by various microorganisms (*Flavobacterium meningosepticum*, *Sphingomonas capsulate*, *Myxococcus xanthus,* and *Aspergillus niger*) are prolyl endopeptidases (PEP). The PEP is capable of degrading

proline-containing peptides (Shan et al. 2004; Stepniak et al. 2006). However, most of these enzymes are irreversibly inactivated in the stomach by pepsin and acidic pH, thus failing to degrade gluten before it reaches the small intestine. Encapsulation of these PEP was proposed in order to protect them from the acid and enzymes present in the gastrointestinal tract (Caputo et al. 2010).

An endopeptidase from *Aspergillus niger* showed efficiency in the digestion of gluten proteins and could be administered orally for the treatment of CD. It was active at the pH of the stomach and of the duodenum and retained enzymatic activity in the small intestine of rats. *Aspergillus niger* is generally recognized as a safe microorganism (GRAS) and is adequate for industrial-scale use. This PEP might be suitable for oral administration to degrade gluten proteins in the gastrointestinal tract (Mitea et al. 2008).

14.4.3 Applications of Peptidases

The high content of prolamins in the primary structure of gluten proteins is the main obstacle to their total hydrolysis in the gastrointestinal tract. Then, for the complete degradation of gluten and to avoid the immune response, gluten-specific peptidases with high specificity or selectivity are necessary. In vitro experiments showed good results of gluten degradation using a PEP obtained from *Aspergillus niger* (Stepniak et al. 2006). The PEP addition into the gastrointestinal tract is being considered as a therapeutic strategy since the digestive enzymes could degrade and prevent the accumulation of these glutamine and proline-rich peptides (Vaquero et al. 2018). The oral administration of PEP has some limitations, whereas the enzymes and bile acids present in the gastrointestinal tract could degrade them. For this purpose, the enzymes must be resistant and active under acidic conditions and be specific to gluten proteins (Lähdeaho et al. 2014; Rey et al. 2016; Ribeiro et al. 2018). The oral administration of an enzyme has the advantage of avoiding the loss of the original and unique viscoelasticity of the gluten network. In the case of enzyme addition to a food product, the hydrolytic mechanisms and the ingredients composition would have to be carefully studied and optimized.

14.5 Lipases

Obesity and overweight have serious health consequences and affect about 1.9 billion adults worldwide. It is estimated that 3 billion people will be overweight in 2030 (WHO 2015). The consumption of high-calorie foods and sedentary lifestyle are the main causes of increased obesity (Tchernof and Despres 2013; Kushner 2014). Facing this problem and the growing public awareness of the negative effects of fat intake on health, a variety of functional less-caloric foods has appeared on the market (Dupont et al. 2018). Designing new food structures and

using carbohydrate or fat substitutes are some of the strategies to reduce calories in food formulations. In another perspective, novel foods and additives capable of modulating the metabolism of nutrients in the gastrointestinal tract may be included. Concerning specifically the digestion of the fats, some studies have looked for compounds that interfere in the activity of the digestive enzymes (Tan and Chang 2017).

14.5.1 Lipases Classification

Triacylglycerol lipase (E.C. 3.1.1.3) or triacylglycerol ester hydrolases are a group of enzymes that catalyze the hydrolysis of fats in aqueous media. The hydrolysis of triacylglycerols to glycerol, diacylglycerols, monoglycerols, and free fatty acids occur in an oil–water interface(Treichel et al. 2010). In addition, lipases are also involved in the interesterification, alcoholysis, acidolysis, and esterification (Yu et al. 2016). Considering their specificities, lipases are classified into three categories: (i) substrate-specific, (ii) regioselective and (iii) enantioselective (Sarmah et al. 2018).

14.5.2 Microbial Production of Lipases

Lipases can be produced by yeasts, fungi, bacteria, animals, and plants. Recent findings also relate the enzymes from marine sources (Navvabi et al. 2018). Microbial lipases find wide applicability in many industries due to properties such as high stability, selectivity, and vast substrate specificity (Treichel et al. 2010). They are extensively used in food, dairy, flavor, pharmaceutical, biofuels, leather, cosmetic, detergent, and chemical industries. The microbial lipases represent the most widely used class of enzymes in biotechnological applications and organic chemistry. Microbial enzymes may catalyze the reaction by different mechanisms even when they belong to the same class. Nonspecific lipases act independently of the position, catalyzing the complete hydrolysis of triacylglycerols into fatty acids and glycerol in a random way (e.g., lipases produced by *Candida cylindracea*, *Staphylococcus aureus*, and *Pseudomonas* spp.). Specific 1, 3 microbial lipases catalyze reactions only at the outer positions of the glycerol backbone (e.g., lipases from *Aspergillus niger*, *Rhizopus delemar*, *Rhizopus oryzae*, *Candida lipolytica*, and *Penicillium roquefortii*) (Sharma and Kanwar 2014).

14.5.3 Applications of Lipases

Lipases are very versatile catalysts. In the food industry, they are related to the production of esters and specialty fats, improvement of food texture, flavor modification, and in the taste of butter, cheeses, and margarine. They have been applied to decrease the time of cheeses ripening, in the fermentation of vegetables, in the curing of meat products, in the processing of fish, in the modification of soybean milk, and in flavor improvement of alcoholic beverages. They can also promote changes in the lipids of flour used in the manufacture of bread and act in the synthesis of structured lipids for infant foods. In addition, lipases are useful in increasing the titer of polyunsaturated fatty acids in vegetable oils and improving the digestibility of natural lipids (Aravindan et al. 2007; Ferreira-Dias et al. 2013). Prospects for the use of lipases are great not only in food processing but also in pharmaceutical products. A variety of lipases from diverse sources such as animal gastric, microbial and plant lipases have been tested for replacement therapy (Fieker et al. 2011). Lipase activators and inhibitors have been either derived from natural sources (plants/animals/microbes) or have been artificially synthesized (Jawed et al. 2018). Colantuono (2018) showed different classes of polyphenols with different inhibitory capacities for lipase, α-amylase, and α-glucosidase and that these differences were linked to characteristic chemical structures of the enzyme. Microbial lipases of fungal or bacterial origin are of potential interest because of their acid and protease-stable properties and their activity at pH 3 to 10 (Borowitz et al. 2006; Aloulou et al. 2007).

In order to be used in industrial processing or as food additives, lipases need to be stable against proteolytic action, against thermal processing and also against oxidative compounds and detergent ingredients. Even with the aid of genetic engineering tools, specific and desired characteristics for lipases can still be developed. The development of lipases with application in novel foods, food additives or therapeutic supplements is an area with a prospect of expansion, given the projections of increased cases resulting from diseases related to inadequate diets and the population's concern for life quality.

14.6 Enzymes Used to Remove Undesirable Compounds

Several enzymes are used for the elimination of compounds harmful to health in the different industries. In the processing of food products, L-asparaginase stands out presenting important industrial applications.

14.6.1 L-Asparaginase

L-asparaginase (L-asparagine amidohydrolase, E.C. 3.5.1.1) is a type of hydrolytic enzyme that catalyzes the conversion of L-asparagine to L-aspartic acid and ammonia under physiological conditions.

L-asparaginase is an agent commonly used as an alternative for the treatment of different cancers such as a malignant disease of the lymphoid system named ALL (acute lymphoblastic leukemia) and Hodgkin's lymphomas. L-asparagine can be synthesized in the human body, and lymphoblasts depend on the free L-asparagine available in the bloodstream for survival. Thus the addition of L-asparaginase into the blood performed with intravenous injection can lead tolymphoblasts' death (Meena et al. 2015; Horvat et al. 2016). L-asparaginase can be obtained from *Escherichia coli* and *Erwinia chrysanthemi* (Ebrahiminezhad et al. 2014; Shrivastava et al. 2016; Prabhu et al. 2017).

In the food industry, L-asparaginase can be used in the pre-treatment of baked or fried starchy foods, to reduce the formation of acrylamide by depleting the precursor asparagine (Hendriksen et al. 2009). Acrylamide (2-propenamide, C_3H_5NO) has long been considered a neurotoxin classified as potentially carcinogenic, discovered in foods in 2002. It is present in many food products, especially in baked and fried ones because of the reaction at high temperature between reducing sugars, formed by a process called Maillard reaction, and asparagine. Several potential strategies have been proposed to reduce acrylamide levels in foods. The use of the L-asparaginases has been reported, resulting in a decrease of acrylamide level of 30–97% (Mihooliya et al. 2017).

Different microorganisms have been reported as L-asparaginase producers including *Erwinia carotovora*, *Streptomyces*, *Escherichia coli*, *Aerobacter* spp., *Photobacterium* spp., *Serratia* spp., *Xanthomonas* spp., *Pseudomonas aeruginosa*, *Vibrio* spp., *Aspergillus tamari*, *Aspergillus niger*, *Aspergillus oryzae*, *Cladosporium* sp., *Beauveria bassiana*, *Bacillus* spp. (Qeshmi et al. 2018). Specifically for use in the reduction of acrylamide in foods, L-asparaginases from *Aspergillus oryzae*, *Thermococcus zilligii*, *Cladosporium* sp. and *Bacillus licheniformis* and recombinant strains of *Aspergillus oryzae* and *Aspergillus niger* have been studied (Shi et al. 2017; Dias et al. 2017).

Only recently L-asparaginase products have been made available to the food industry, thus their use is very expensive. These enzymes need to be stable to different forms of the degradation to which they are exposed under the conditions of food processing, be produced on a large scale and must not induce allergic effects. In this sense, additional studies are needed aiming at the economical enzyme production and efficient utilization. To enable enzyme reutilization, immobilization studies have been carried out with the use of several natural or synthetic carriers such as silica gel, dextran, alginate, gelatin, chitosan, and others (Agrawal et al. 2018).

Another strategy for L-asparaginase production is the substitution of submerged fermentation used industrially by the more economical solid-state fermentation with

agroindustrial residues as substrate. Soybean meal, red gram husk, coconut oil cake, rice bran are examples of substrates that have been used (Doriya and Kumar 2018). Nevertheless, factors as substrate source, control of pH, salinity, and temperature into the process significantly affect L-asparaginase production and need to be taken into account so that the process can be economically viable.

14.6.2 Beta-Glucanases

Beta-glucanases are hydrolytic enzymes, also called 1,3-1,4-β-glucanases (1,3-1,4-β-D-glucan-4-glucanohydrolase; GH family 17; EC 3.2.1.73), that are capable of releasing oligosaccharides by hydrolyzing high molecular weight glucans. Beta-glucans are linear polysaccharides consisting of cello oligosaccharide blocks united by β-1,3 linkages, normally present in the cell walls of many cereals like rice, oats, and wheat. The β-glucan content is an important parameter of quality in various food industries, for example, the barley β-glucans may result in higher viscosity in beer during the mashing process (Furtado et al. 2011). The use of 1,3-1,4-β-glucanases in brewing improves the process because they act in the reduction of viscosity and turbidity, which increases the rate of filtration and yield, producing a malt of the highest quality (Chaari et al. 2014).

Beta-glucanases are fiber-digesting enzymes that could be used to increase nutrients absorption. Beta-glucooligosaccharides (β-GOS) are produced by the cleavage of the β-(1 → 4) glycosidic linkage of glucans with the use of an endo-1,3-1,4-β-D-glucan-4-glucanohydrolase (EC 3.2.1.73). Beta-GOS as well as β-glucan promote different health benefits because they exhibit anti-oxidant and antibacterial activity, can reduce serum cholesterol and, for some probiotic bacteria, provide a selective substrate. Thus, this β-glucanase has shown promising applications as an additive in food, especially in the development of probiotic bacteria (Cho et al. 2018).

14.6.3 Others

Crosslinking enzymes can be used in the bioprocessing of dairy products in order to alter milk proteins' allergenicity and digestibility. The formation of molecular bonds between enzymes and proteins in combination with a mediator decreases the rate of digestion, which can result in elimination of allergens. In this sense, studies suggest a decrease in the potential for allergenicity because in the enzymatic crosslinking of β-casein using transglutaminase, tyrosinase, and laccase, also in combination with caffeic acid, highly polymerized caseins with increased digestion stability and high potential for IgE binding inhibition were obtained (Stanic et al. 2010).

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