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THE COMPARISON OF COMMERCIALLY AVAILABLE β-GALACTOSIDASES FOR DAIRY INDUSTRY: REVIEW

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Abstract
β-Galactosidase (EC 3.2.1.23) is one of the widely used enzymes for lactose-free milk production and whey permeate treatment. Enzymes can be obtained from microorganisms, plants and animals. Nowadays, microorganisms are becoming an important source for production of commercially available enzymes, which are of great interest and offer several advantages such as easy handling and high production yield. The aim of this review was to summarize findings of research articles on the application of commercially available β-galactosidase preparates in dairy industry, to analyse and compare the most suitable β-galactosidase commercial preparates for lactose hydrolysis. The results showed that the main factor to choose an appropriate β-galactosidase for lactose hydrolysis was reaction condition. Enzymes from microorganisms contain a wide range of optimal pH from 4.0 (*Penicillium simplicissimum* and *Aspergillus niger*) to 8.5 (*Bacillus subtilis*). The greatest commercial potential have enzymes obtained from fungi (*Aspergillus oryzae* and *Aspergillus niger*) and yeasts (*Kluyveromyces lactis* and *Kluyveromyces fragilis*). Fungal origin enzymes are more suitable for the hydrolysis of lactose in acid whey due to its acidic pH but yeasts origin enzymes for milk and sweet whey. In the study, commercial preparates from different suppliers with the purpose to analyse their lactose hydrolysis potential and give more detailed characteristics of each preparate advantages and drawbacks were also summarized.
Key words: β-Galactosidase, lactose hydrolysis, commercial preparates.

Introduction

The scientists worldwide are searching for new biotechnological tools which promote the use of dairy by-products (whey, buttermilk and other) for obtaining compounds with new perspective or products with high added value (De Jesus *et al*., 2015).

Enzymes are protein molecules which are working as catalysts for chemical reactions (Chaudhary *et al*., 2015) and are entirely involved to change and develop the nutritional, functional and sensory properties of ingredients and products, and have found extensive application in processing and production of food products (Oort, 2010). Important segment of food enzymes industry are dairy enzymes, which are used for the developing and improving sensory characteristics (aroma, flavour and colour) and functional properties of milk products (Singh *et al*., 2016). Dairy industry is one of the largest markets for commercial enzymes application (Kelly & Fox, 2012) and one of the highly used enzymes is β-galactosidase (EC 3.2.1.23) (Grosová, Rosenberg, & Rebroš, 2008). β-Galactosidase is known as lactase, which hydrolyses lactose into glucose and galactose (Panesar, Kumari, & Panesar, 2010). The enzyme is used to produce lactose-free products for people who are lactose-intolerant (Shu *et al*., 2014). Lactose is a disaccharide and major sugar in milk. This sugar may affect flavours and odours absorption and leads to many faults in refrigerated foods such as formation of crystals, deposit and sandy or gritty texture. There is another reaction where β-galactosidase can be used and it is in transglycosylation of lactose for galacto-oligosaccharides (GOSs) synthesis. These are the nondigestible oligosaccharides, which in the upper intestinal tract are not hydrolyzed or absorbed (Panesar, Kumari & Panesar, 2010). β-Galactosidase has animal, plant and microbial (bacteria, fungi, and yeasts) origin; for reducing production costs, mostly microbial enzymes, which represent a higher activity than others are used. Enzyme used in food production must come from microorganisms which have been approved by Generally Recognized as Safe (GRAS) (Dutra Rosolen *et al*., 2015). GRAS provides unhampered usage of their enzymes without any profuse and expensive purification (Carević *et al*., 2015). The greatest commercial potential shows enzymes which are obtained from fungi and yeasts (Dutra Rosolen *et al*., 2015).

The aim of this review was to summarize findings of research articles on the application of commercially available β-galactosidase preparates in dairy industry, to analyse and compare the most suitable β-galactosidase commercial preparates for dairy production.

Materials and Methods

Monographic method was used in this study. The review summarizes the research findings on commercially available β-galactosidase that is used for the hydrolysis of lactose. Enzyme properties and suitability for dairy products production, commercial preparates for industrial and research tasks, as well as lactose hydrolysis potential has been studied.

Results and Discussion

Characterisation of β-galactosidase

The commercially available β-galactosidase is derived from a variety of microorganisms which are

Table 1

Characterisation of β-galactosidase

Source	Microorganism	pH optimum	Temperature optimum, ◦C	References
Fungi	*Aspergillus niger*	4.0 – 4.5	60	Sykes *et al*., 1983
	Aspergillus oryzae	5.0	50 – 55	Park, Santi, & Pastore, 1979
	Penicillium simplicissimum	4.0 – 4.6	55 – 60	Cruz *et al*., 1999
Bacteria	*Bacillus subtilis*	8.0 – 8.5	35	El-Kader *et al*., 2012
	Bacillus licheniformis	6.5	50	Juajun *et al*., 2011
	Bacillus circulans	6.0	60	Yin *et al*., 2017
	Bacillus stearothermophilus	7.0	70	Chen *et al*., 2008
	Escherichia coli	7.0	55	Nakanishi *et al*., 1983
	Arthrobacter psychrolactophilus	8.0	10	Nakagawa *et al*., 2007
Yeast	*Kluyveromyces lactis*	7.0	40	Bosso *et al*., 2016
	Kluyveromyces fragilis	6.6	37	Jurado *et al*., 2002
	Kluyveromyces marxianus	7.5	40	Brady *et al*., 1995

of great interest and offer a number of benefits such as easy handling and high production yield (Panesar, Kumari, & Panesar, 2010).

Summary about optimal activity conditions of β-galactosidase from various research works is given in Table 1.

Feature impacts on β-galactosidase characteristics (Iliev, 2012), such as molecular properties, metal ion and substrate specificity, as well as the temperature and pH conditions necessity is very important for optimal activity of enzyme (Rampelotto, 2016). It is set that out of the total enzymes which are used for industrial use, over 50% are obtained from yeast and fungal sources and one third is extracted from bacterial sources (Panesar, Marwaha, & Chopra, 2010).

The application of β-galactosidase in industrial processes mainly depends on the hydrolysis reaction conditions (Dutra Rosolen *et al*., 2015). β-Galactosidase is used for dairy products which have pH differences (Walsh, 2007), for example, sweet whey and bovine milk pH 6.0 – 7.0 and acid whey pH have less than 5.0 (Dutra Rosolen *et al*., 2015). Enzymes are extremely sensitive to the pH of the reaction medium and therefore each β-galactosidase has an optimal pH in which it reaches maximal activity (Scopes, 2002). β-Galactosidase from *Penicillium simplicissimum* and *Aspergillus niger* have pH optimum of about 4.0 – 5.0 while from *Bacillus subtilis* has pH optimum of 8.0 – 8.5 (Table 1). This shows that β-galactosidase from microorganisms contain a wide range of optimal pH condition and are able to used for different kind of medium.

Cruz and co-authors (1999) noted that *Penicillium simplicissimum* β-galactosidase optimal pH for hydrolysis was in the range 4.0 – 4.6. The enzyme reached great results, hydrolysed 67% lactose from milk and 84% lactose from milk whey which show a potential of using for food production (Cruz *et al*., 1999). El-Kader and co-authors (2012) discovered that at pH 8.0 the activity of *Bacillus subtilis* increased rapidly till 1278 UI·mL^{-1} at 30 minutes, while enzyme activity at pH 8.5 rise to 1387 UI·mL^{-1} at 10 minutes (El-Kader *et al*., 2012). For industrial production, it is a great opportunity to choose an appropriate β-galactosidase for product with a certain pH. Another important property is enzymatic thermostability and activity at low temperature (Dutra Rosolen *et al*., 2015). Cold-active β-galactosidase is of high biotechnological interest as a food industrial enzyme, because it is intended for hydrolysis of lactose in milk and dairy products at low temperature (Pandey, Negi, & Soccol, 2017). Nakagawa and co-authors (2007) showed that the β-galactosidase which is obtained from *Arthrobacter psychrolactophilus* strain F2 could provide 70% lactose hydrolyse at 10 °C in 24 h. That shows the enzyme has a great potential as a cold-active enzyme in the industrial field (Nakagawa *et al*., 2007).

Furthermore, thermostable β-galactosidase has high reaction velocity, diminished risk of contamination, long half-lives, reduced product inhibition, high solubility and yields (Pandey, Negi, & Soccol, 2017). *Bacillus stearothermophilus* β-galactosidase has the highest enzyme activity at 70 °C (Table 1), showing that this thermostable enzyme has great industrial potential of promoting product production at high temperatures (Chen *et al*., 2008). Chen and co-authors (2008) stated that enzyme *Bacillus stearothermophilus* showed the highest activity at 70 °C and saved 80% of its activity even at 75 °C. Providing necessary condition of thermostable β-galactosidase, which optimal pH is neutral, enzyme would be a great choice for the lactose hydrolysis in

milk. This kind of enzyme would be able to be used for application in low-lactose milk production during milk pasteurization (Chen *et al*., 2008). It shows that thermostable β-galactosidase could be a potential alternative for dairy products processing, which are treated to high temperature for reducing microbial contamination.

Sources of β-galactosidase

Fungi. β-Galactosidase from fungal sources are thermostable, however, are mainly sensitive by galactose (Husain, 2010). They are usually used to hydrolyse lactose from products which has an optimum pH between 2.5 and 5.4, such as acid whey (Dutra Rosolen *et al*., 2015). β-Galactosidase from *Aspergillus, Penicillinum* and *Paecilomyces spp.* have the major interest from industrial field, they have extracellular character, higher and better thermal tolerance and lower pH of activity (Gargova, Pishtijski, & Stoilova, 1995). The most used β-galactosidases are from *Aspergillus niger* and *Aspergillus oryzae*, because of their extracellular nature, high activity, GRAS status (Hu *et al*., 2010) higher thermal stability and for the lactose hydrolysis at low pH (Hatzinikolaou *et al*., 2005).

Acidic pH is the optimal medium for *Aspergillus niger* β-galactosidase and it can be an ideal candidate for lactose hydrolysis in acid whey permeate (Hatzinikolaou *et al*., 2005). Although the enzyme can be inhibited by galactose, it is quite complicated to get complete lactose hydrolysis (Soccol *et al*., 2012).

Aspergillus oryzae is highly accepted as source of enzyme which is used for food and feeds (Nizamuddin, Sridevi, & Narasimha, 2008). The optimal temperature and pH of enzyme action at 60 °C, pH 4.75 can be used for lactose hydrolysis in whey. Benefit of this β-galactosidase from a technological viewpoint is its comparative stability at high temperature what is very important for controlling and preventing microbial risk during the hydrolysis process (Cruz *et al*., 1999).

Bacteria. Bacterial β-galactosidase has great properties of fermentation, hydrolysis activity and stability, and usually used for the lactose hydrolysis. Lactic acid bacteria (diverse group of lactococci, streptococci and lactobacilli) and bifidobacterium have been used as good sources of β-galactosidase, particularly for functional food production and more important is that organisms need to be accepted by GRAS (Princely *et al*., 2013). Lactobacilli isolated from fermented Ragi have remarkable industrial potential. They can provide better yields at high temperatures and avoid microbial contamination in milk processing (Mozumder *et al*., 2012).

Bacillus licheniformis is a soil-dwelling endospore forming microorganism. Juajun and co-authors (2011) analysed activity and stability of enzyme at numerous pH and temperature conditions, and results showed that β-galactosidase from *Bacillus licheniformis* is stable and has good perspectives compared to other industrially used enzymes. This enzyme is most active between pH 5 and 9 at 37 °C and up to 42 °C, at pH 6.5. In food manufacturing, the hydrolytic effect of β-galactosidase from *Bacillus licheniformis* opens new perspectives for lactose hydrolysis, or for improving quality and properties of dairy products by rising their solubility and sweetness level. However, it is mainly inhibited by the hydrolysis products – glucose and galactose (Juajun *et al*., 2011).

Commercial β-galactosidase from *Bacillus circulans* exerts at least four isoforms, but the most used is the so-called β-galactosidase-I (Torres & Batista-Viera, 2012). Enzyme optimal condition is at 60 °C and pH 6.0, which pH is similar to sweet whey (Yin *et al*., 2017). This enzyme also can catalyse the formation of a large amount of oligosaccharides. Enzyme optimal condition properties are suitable for the hydrolysis of lactose in milk and whey, and for oligosaccharide production (Torres & Batista-Viera, 2012).

β-Galactosidase of *Escherichia coli* have been carried out numerous times and in various ways (Huber *et al*., 1979). β-Galactosidase from *Escherichia coli* is the most extensively studied and is not suitable for industrial use, because it is regarded as not safe for food applications. Nonetheless, this enzyme is commercially available for analytical researches (Pandey, Negi, & Soccol, 2017). This enzyme hydrolyses lactose into glucose and galactose, using this sugar as a substrate for heterotrophic bacterial growth. This enzyme is as a reporter protein or molecular sensor in microbiology and molecular and cell biology (Feliu & Villaverde, 1998).

Yeast. From an industrial point of view yeast is an important source of β-galactosidase (Panesar *et al*., 2006). The food industry interest of this enzyme production by yeast might have started since this enzyme is used for the production of products with low lactose content (Husain, 2010). Yeast enzymes are commonly used for products with neutral pH which have optimum pH 6.0 – 7.0 such as milk and sweet whey (Dutra Rosolen *et al*., 2015).

Kluyveromyces lactis is one of the important sources of commercial β-galactosidase which is used in the dairy industry (Teles De Faria *et al*., 2012) because of its environmental habitat and great lactose hydrolysis productivity, enzyme main disadvantage is thermostability (Chen *et al*., 2008). Another possibility to use it is for whey processing for lactose hydrolysis to get glucose and galactose (Teles De Faria *et al*., 2012).

β-Galactosidase from *Kluyveromyces fragilis* and *Kluyveromyces lactis* are mostly the same (Jazairi,

Table 2

Commercial sources of β-galactosidase

Enzyme source	Trade name	Activity	Lactose hydrolysis, %	Supplier	References
Yeast					
Kluyveromyces sp.	Enzeco Lactase NL	NM*	95	EDC, New York, US	Horner *et al*., 2011
Kluyveromyces lactis	GODO-YNL2	5000 NLU·g^{-1}	99	Danisco A/S, Denmark	
	Maxilact® LX5000	5000 NLU·g^{-1}	100	Sedim Cedex, France	Bosso *et al*., 2016
	Maxilact-L/2000	2000 NLU·g^{-1}	90	Gist-Brocades	Jurado *et al*., 2004
	Lactozym 2600L	2600 LAU·g^{-1}	NM*	Novozymes	Reddy, Nath, & Reddy, 2016
	Lactomax Pure	NM*	75	Prozyn, Brazil	Dutra Rosolen *et al*., 2015
	Lactozym pure 6500 L	1320 U·mL^{-1}	95	Novozymes	Rodriguez-Colinas *et al*., 2014
	Ha-Lactase 5200	8040 LAU·g^{-1}	90	Chr. Hansen, Denmark	Hendriksen *et al*., 2010
Kluyveromyces fragilis	Lactozym 3000L HPG	3000 LAU·mL^{-1}	72	Novo Nordisk	Jurado *et al*., 2004; Volpato Fernanda, & Souza, 2016
Fungal					
Aspergillus oryzae	Lactomax F30	NM*	50	Prozyn, Brazil	Dutra Rosolen *et al*., 2015
	Bio-Cat	5000 NLU·g^{-1}	41	INC/USA	Bosso *et al*., 2016
Bacteria					
Bacillus circulans	Biolactase NTL	553 U·mL^{-1}	50	Biocon, Spain	Rodriguez-Colinas *et al*., 2014

*NM- not mentioned

Ghorrah, & Bakri, 2014). Matioli, Farias de Moraes, & Maria Zanin (2001) stated that the β-galactosidase enzyme from *Kluyveromyces fragilis* is highly active at 45 °C and pH 6.0 (Matioli, Farias de Moraes, & Maria Zanin, 2001). With pH 7 enzyme is well appropriate for hydrolysis of lactose in milk (Panesar *et al*., 2006). Ladero and co-authors (2002) stated that the immobilized β-galactosidase from *Kluyveromyces fragilis* can be useful for frozen dairy products production and to refrain lactose crystal formation process and to increase the intake and flavour of such products (Ladero *et al*., 2002).

Transgalactosylation activity of β-galactosidase

Oligosaccharides have attracted the attention of researchers to investigate possible transferase activities by β-galactosidase which produce a number of carbohydrates (Panesar *et al*., 2006). Research results show that the highest producer of GOS of all fungal sources is *Aspergillus oryzae* compared with *Aspergillus niger* (Prenosil, Stuker, & Bourne, 1987) from yeasts *Sterigmatomyces elviae* CBS8119 (Onishi & Tanaka, 1998). Nakanishi and co-authors (1983) showed that *Bacillus circulans* is more perspective compared to *Escherichia coli* and yeasts (Nakanishi *et al*., 1983). The proportion between hydrolysis of lactose and GOS synthesis depends significantly on the concentration of lactose, the temperature of the process and the intrinsic enzyme properties (Rodriguez-Colinas *et al*., 2016).

Commercial preparate

The main features what make enzymes appropriate catalysts in the diversity of applications are the variety of catalytic function and the capability to work under optimal conditions. Industrial enzymes can be prepared differently as partly purified or 'bulk' enzymes, and highly purified for analytical or diagnostic use (Oort, 2010). The industrial applications of β-galactosidase make use of either free enzyme or immobilized enzyme. The use of free enzyme is technically simpler, its main drawback – the enzyme

in solution cannot be reused and increases the cost of repeated production of enzyme preparations (Pandey, Negi, & Soccol, 2017). Immobilized β-galactosidase offers the possibility of repetitive and continuous use of enzyme with good operational stability (Husain, 2010). It should be noted that the company which first used the commercial production of lactose-free milk by immobilized β-galactosidase from *Kluyveromyces lactis* was Centrale del Latte of Milan (Italy) galactose (Panesar, Kumari, & Panesar, 2010). Company Drouin Cooperative Butter Factory (Australia) used immobilized *Aspergillus oryzae* β-galactosidase for manufacturing market milk and hydrolyzed whey. This enzyme was developed by Sumitomo Chemical (Japan) (Pandey, Negi, & Soccol, 2017).

Summary about commercial sources used for milk or whey lactose hydrolysis of various studies is given in the Table 2.

The main features which regulate the technology and costs of the lactose hydrolysis process is enzyme characteristics and price (Grosová, Rosenberg, & Rebroš, 2008). Now the main commercial source of β-galactosidase is *Kluyveromyces lactis* because of dairy environmental habitat (Mozumder *et al*., 2012). Studies carried out by Adalberto and co-authors (2010) identified that enzymatic preparation Lactozym 3000L HPG is poorly stable. Sensitivity to pH and heat are greater in the absence of metal ions, showing that metal ions have an influence on enzymatic stability (Adalberto *et al*., 2010).

Bosso and co-authors (2016) analysed enzyme activity in UHT and skimmed milk at variable concentration, temperature, and pH. The best results were detected at 40 °C and pH 7.0 for the enzyme Maxilact® LX5000, and at 55 °C and pH 5.0 from Bio-Cat. Bio-Cat enzyme was able to hydrolyze 41% of lactose in UHT milk and 32.7% in skimmed milk. Furthermore, Maxilact® LX5000 hydrolyses 100% of lactose in UHT milk and 92% in skimmed milk. Considering enzyme thermal stability, it showed that the enzyme from Bio-Cat had better heat resistance than Maxilact® LX5000. Knowing properties of β-galactosidase from *Kluyveromyces lactis* and *Aspergillus oryzae* allows using it more competently in many industrial productions (Bosso *et al*., 2016). Dutra Rosolen and co-authors (2015) evaluated the influence of enzyme concentration, temperature, and reaction time in milk, cheese whey, and whey permeate using two commercial preparates Lactomax F30 and Lactomax Pure. At the same enzyme condition, during 2 h of reaction, the Lactomax Pure presented higher hydrolysis efficiency 75% than the Lactomax F30 50%. The results display that a higher lactose hydrolysis of milk and whey can be achieved using the Lactomax Pure and in the permeate using Lactomax F30 (Dutra Rosolen *et al*., 2015). In the studies by Jurado and co-authors (2004) pointed out that activity of both commercial β-galactosidase preparates Lactozym 3000L HP-G and Maxilact-L/2000 have similar action in different condition and similar kinetic parameter values (Jurado *et al*., 2004). Horner and co-authors (2011) tested food-grade β-galactosidase enzymes to evaluate their potential for use in hydrolysis process. Results showed that in pasteurized whole milk in 24 h, using supplier's recommended dosage GODO-YNL2 hydrolyzed on average 99% of the lactose and Enzeco Lactase NL was able to hydrolyze 95%, added a double dose. Lactose hydrolysis can be achieved also with less enzyme if the reaction is allowed to proceed for a longer time. In pasteurized whole milk GODO-YNL2 achieved 95% average hydrolysis in 72 h while using one-fourth the recommended dosage and 99% hydrolysis under the same conditions using one-half the recommended dosage. Instead enzymes Enzeco Lactase NL accomplished approximately 99% hydrolysis on average in 72 h by using the recommended dosage. By using a smaller dose and a longer time for lactose hydrolysis, enzyme cost for a liter of lactose-free milk could be minimized, potentially reducing the cost of lactose-free milk (Horner *et al*., 2011). It shows that each preparate has advantages and drawbacks for choosing the best one for lactose hydrolysis.

Conclusions

1. The use of β-galactosidase in industrial processes mainly depends on the hydrolysis reaction conditions. Each enzyme preparate has optimal parameters in which it can reach the highest activity to hydrolyse lactose.
2. There are significant parameters such as concentration of lactose, the temperature of the reaction and the enzyme properties which impact transferase activities by β-galactosidase causing GOS synthesis.
3. A large number of scientific researches and industrial utilization showed that the greatest commercial potential has enzymes, which are gained from *Aspergillus oryzae*, *Aspergillus niger* and *Kluyveromyces lactis*, *Kluyveromyces fragilis* due to their productivity.

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