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LACTOSE HYDROLYSED MILK AND ITS APPLICATION: A REVIEW

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ABSTRACT

Over 70 % of the world's population suffers from lactose intolerance, with the majority of instances happening in developing countries. Asians (more than 90 %), Africans (80–100 %), Native Americans (more than 90 %) and Southern Europeans are all affected by lactose intolerance (more than 80 %). Due to a deficiency of β-D galactosidase, around 53% of Indians are unable to consume lactose or lactose-containing foods. Lactose intolerance has been linked to a genetically programmed decrease in lactase activity in the small intestine as people grow older. Lactose content of milk can be reduced to solve this problem. Lactose intolerance can be managed by consuming lactose-free meals, hydrolyzing lactose in foods before eating, or using lactase supplements, which increase lactose hydrolysis in the gastrointestinal tract. Lactose hydrolysis in milk imparts better digestibility and sweet taste, leading to a recent rise in the demand for lactose hydrolysed dairy products. Glucose is a source of energy and galactose plays a vital function in brain development. Galacto-oligosaccharides are formed during lactose hydrolysis which increases technical and sensory features of dairy goods. Lactose hydrolysis technology has a wide range of nutritional, technological, and environmental uses.

Keywords: Lactose Free Milk , Lactose Hydrolysed, Lactose Free Product.

I. INTRODUCTION

β-galactosidase enzyme may be found in a wide range of natural sources, including microbes, plants, and mammals. The hydrolysis of terminal β-glycosidic bonds in polysaccharides can be catalysed by β-galactosidase, but its principal activity is to hydrolyse the β-glycosidic bonds in lactose to create glucose and galactose [1]. Lactose intolerance can result from a lack of lactase in the body, which can cause bloating, stomach pain, flatulence, diarrhoea, and nausea . Lactose intolerance can be managed by consuming lactose-free foods, hydrolysing lactose in foods before eating, or using lactase supplements, which increase lactose hydrolysis in the gastrointestinal system. Enzymatic lactose hydrolysis is often preferred over chemical lactose hydrolysis because it does not result in undesirable changes in food quality or nutritional profile. The lactase activity of the enzyme β-D -galactosidase is widely employed in the food sector, notably in dairy products [2].

In general, there are two approaches for employing β-D-galactosidase to hydrolyse milk. The soluble enzyme is employed in batch procedures, whereas the immobilised enzyme may be used continuously and ultrafiltration with reverse osmosis could be adopted during food production. Because they are quickly denatured at high temperatures, have a short lifespan, are unstable, the use of free enzymes has been restricted [3]. Immobilization of enzymes within porous matrices have been utilised for many years to improve their stability and recovery. Enzymes can be trapped within porous matrices using several approaches, including physical entrapment, physical bonding, and/or covalent bonding [4].

II. β-D- GALACTOSIDASE

β-D- galactosidase is a member of the hydrolase family and belongs to the saccharide converting enzymes group. β-Galactosidases have animal, vegetable, or microbial (bacterium, fungus, and yeast) origins, but the microbial enzymes show a higher productivity, resulting in cost reduction. The β-galactosidases used in industrial scale must come from Generally Recognized as Safe (GRAS) microorganisms. Enzymes obtained from fungi (Aspergillus oryzae and Aspergillus niger) and yeasts (Kluyveromyces lactis and Kluyveromyces fragilis) show great commercial potential [5]. The choice of the β-galactosidase source usually depends on the hydrolysis reaction conditions. The ones from fungi present an optimum pH between 2.5 and 5.4, being usually used for acid whey hydrolysis. The ones obtained from yeasts present optimum pH between 6.0 and 7.0, and its use is more adequate for milk and sweet whey lactose hydrolysis. This enzyme breaks down lactose in milk into two monosaccharides, glucose and galactose, which have a sweeter flavour, are more soluble, and are easier to digest [6].

Yeast sources of β -D-galactosidases are often used to hydrolyse lactose in milk and sweet whey. Kluveromyces species have become a significant source of β -D-galactosidase . Kluveromyces species has a pH of 6.5-7.0, which is suitable for lactose breakdown in milk. The enzyme's source, which comprises of appropriate pH, temperature, and stability, determines the enzyme's properties, specificity, and structure [7].

1. LACTOSE

Bovine milk contains 4.4 to 5.2 % lactose, with an average of 4.8 % anhydrous lactose. The lactose concentration (g/100 ml) of the following mammalian milk is cow : 3.7–5.1; buffalo: 4.5–5.5, goat : 3.6–4.8, human: 6.2–7.5, sheep: 3.7–4.8, camel: 3.3–4.8, yak: 4.17–5.6.This is a big difference when compared to the bovine 7% present in human milk. Lactose (4-0-β-galactopyranosyl β-D -glucopyranose, $C_{12}H_{22}O_{11}$) is a disaccharide composed of one glucose molecule linked to one galactose molecule. The lactose content (g/100 ml) of some conventional dairy products are skim milk, 4.3–5.7; buttermilk, 3.6–5.0; cream, 0.1; yogurt, 4.70–4.76; ice cream, 3.6–8.4. Lactose cannot be absorbed in its natural state and must be digested in the colon by lactase. Lactose serves a variety of biological activities, including increasing calcium absorption, promoting the growth of bifidobacteria, and supplying galactose, which is required for the formation of cerebral galactolipids. In the guts of mammals, lactose is digested by β-galactosidase [8]. In human small intestines, glucose is absorbed by the villi and used as energy in the body. Galactose is also absorbed by the villi but is transferred to the liver to form glycoproteins and glycolipids. Glycoproteins are crucial in cell development since they function as antigens. Glycolipids attach to the cell membrane and cause the cell to be hydrophilic, keeping it from dissolving in an aqueous solution. The disaccharide has vital biological activities, such as boosting bifidobacteria development by delivering galactose, which is necessary for galactooligosaccharide synthesis. Lactose keeps the osmotic pressure between milk and blood constant. Lactose, the sugar found in milk, is sweetened, and different carbohydrate types in food are perceived differently. Saccharose is the standard (relative sweetness 100), whereas fructose is sweet (relative sweetness 63) and lactose is one of the least sweet carbohydrates (relative sweetness 0) [9].

2. LACTOSE INTOLERANCE

Lactose intolerance affects more than 70% of the world's population, with the majority of instances occurring in underdeveloped nations. Lactose intolerance affects a large percent of Asians (more than 90 %), most Africans (80–100 %), Native Americans (more than 90 %), and Southern Europeans (more than 80 %) (Roginski et al., 2003). However, roughly 53% of Indians are unable to ingest lactose or lactose-containing foods due to a lack of β-D galactosidase. Lactose intolerance has also been linked to a genetically programmed decrease in lactase activity in the small intestine with age. This issue can be solved by lowering the lactose level in milk [10].

The lactase-phlorizin hydrolase (LPH) enzyme, which is generated by epithelial cells near the small intestine's brush boundary, digests lactose in human milk. Lactose intolerance is caused by a deficiency of lactase in the highly specialised epithelial cells of the small intestine. This enzyme is required for the hydrolysis of lactose into glucose and galactose, which is critical for the survival of newborn animals which rely on milk for nutrition. The lactase enzyme's inability to hydrolyse lactose into glucose and galactose results in a variety of issues, including stomach pain and diarrhoea [11]. Lactose intolerance is caused by a shortage of the β–D-galactosidase enzyme in the colon, making it difficult to digest milk and dairy products.

3. MEASUREMENT OF ENZYMATIC ACTIVITY

The most common methods for evaluating lactose hydrolysis are enzymatic and chromatographic approaches. A chromatographic approach was used to provide quantitative data for sugars such as lactose, glucose, galactose, sucrose, and maltose, among others, for carbohydrate analysis. Analyzing a succession of low molecular weight carbohydrates requires a lot of precision [12]. Analyzing all low molecular weight carbohydrates, on the other hand, is a complex task that requires the use of high performance liquid chromatography (HPLC) or thin layer chromatographic techniques. As a result, owing of its sensitivity and simplicity, the enzymatic technique is preferred. ONPG (O-nitrophenyl- β-D-galactopyranoside) can also be used as a substrate for -D-galactosidase, using O-nitrophenol as a chromogen to monitor the reaction. Enzyme activity is determined by mixing a diluted enzyme sample with O-nitrophenyl-β-D-galactopyranoside (ONPG). The quantity of O-nitrophenol liberated from ONPG is determined spectrophotometrically, and lactase activity is expressed as the amount of enzyme that liberated one mole O-nitrophenol per gram of materials [13].

4. LACTOSE HYDROLYSIS

During the process of making dairy products. Lactose hydrolysis causes a variety of changes in milk. Another application for the hydrolysis process aims at adding value to whey, improper disposal poses a serious environmental problem. The number of microorganisms capable of metabolizing lactose as a source of carbon is smaller than the number of microorganisms capable of metabolizing glucose and galactose [14]. Therefore, whey lactose enzymatic hydrolysis makes the bioremediation processes and biomolecules and biomass productions viable with the use of these kinds of whey as cultivation media [15]. Lactose hydrolysed milk prevents lactose crystallisation in frozen and condensed milk products and accelerates the acidification process in yoghurt and cheese manufacturing. As a result, lactose hydrolysis offers a wide range of nutritional, technological, and environmental applications.

5. ALTERNATIVE PROCESSES FOR LACTOSE HYDROLYSIS

Lactose is a disaccharide made up of glucose and galactose that is linked by a β-(1-4)- glycosidic bond that may be degraded chemically or with enzymes.

5.1 ENZYMATIC HYDROLYSIS

When compare to chemical hydrolysis, enzyme hydrolysis offers several benefits. There is no by-products, no additional flavour, odour, or colour, and no component deterioration in dairy products. Moreover, when milk is treated with β-D-galactosidase, the milk's nutritional value is retained [16]. Disaccharides, trisaccharides, and higher oligosaccharides are produced through Transgalactosylation [17]. As a result, the use of β-D-galactosidase for lactose hydrolysis is critical in dairy, culinary, and pharmaceutical applications. Free or immobilised β-D-galactosidase can be used in the enzymatic hydrolysis of milk lactose.

5.2 HYDROLYSIS THROUGH FREE ENZYME

Lactose hydrolysis is a simple process that does not require any additional equipment in dairy plants. For lactose breakdown, β-D- galactosidase is only utilised once. Temperature, pH, reaction time, and enzyme activity are only a few things to consider. Lactase enzyme can be added to dairy products and maintained at 35-45°C for a period of time to save money. However, this usually leads to an increase in the development of bacteria in milk. [18]. When immobilised Bacillus circulans lactase was used to hydrolyse skim milk at temperatures ranging from 20 to 50°C, microbial growth was unavoidable at higher temperatures, and enzyme inactivation was considerable. A soluble lactase enzyme produced by bacteria is employed in the majority of cases.

5.3 BATCH PROCESS

5.3.1 PRE-PROCESS

Milk that has been hydrolyzed before to pasteurisation and is used in combination with high (HTST) pasteurisation is known as pre-process hydrolysis. Because the optimum temperature for β-galactosidase is between 35°C and 65°C, it is used at a low temperature to minimise microbial deterioration, which slows down the process. Depending on the individual enzyme and environmental change, incubation time at low temperature (4°C - 8°C) normally takes 12–24 hours. To make the milk more pleasant to customers, some glucose and galactose may be filtered during processing. The milk is then HTST pasteurised and can be kept in the refrigerator for 2–3 weeks after hydrolysis and filtration. The delayed hydrolysis time, energy expense of retaining all the milk at a low temperature, and difficulty to recover the enzyme after production are all disadvantages of the batch process. Another disadvantage is that the enzyme's interaction with other substances is uncontrollable, which might result in off-flavors in the milk. The enzyme is not entirely suppressed by HTST pasteurisation of milk, which causes issues during storage. To inactivate all of the enzymes, pre-process hydrolysed milk can be pasteurised at a higher temperature and for a longer period. As a result, difficulties arise due to protein denaturation, browning (perhaps due to the Maillard reaction), and the formation of off-flavors in milk [19].

5.3.2 POST-PROCESS

Ultra-high-temperature (UHT) pasteurisation of milk is used for post-process lactose hydrolysis. Milk is UHT treated before hydrolysis, which is a pre-process hydrolysis in which milk is pasteurised after enzyme addition. Under sterile circumstances, the enzyme is added to the milk and left at room temperature for three days. This procedure allows the product to be used immediately after the milk has been kept in storage to finish the hydrolysis process. The milk has a storage life of 3–6 months at 37 °C since it is UHT pasteurized [20]. Due to the ingredients required to deliver the enzyme, the post-process of lactose-hydrolyzed milk has a high start-up cost. Larger amounts are more difficult to make since the product must be kept in storage for at least three days before being consumed. Lactose hydrolyzed milk that has been ultra-heated is more prone to discolouration and lysine blockage, although it may be kept at temperatures higher than room temperature (22°C). The discoloration of pre-hydrolyzed UHT milk versus post-hydrolyzed UHT milk was measured over a 12-week period at various storage temperatures. When comparing pre- and post-hydrolyzed UHT milk stored for 12 weeks at 5°C, 22°C, 30°C, and 45°C, E increased more in the pre-hydrolyzed milk than the post-hydrolyzed milk by 0.6, 0.3, 4.0, and 2.1, respectively, indicating that there is a high probability of pre-hydrolyzed UHT milk developing an off-color regardless of storage temperature [21].

5.4 RECENT PATENTS

As the global market becomes more of lactose hydrolyzed and lactose-free milk, companies such as Valio and Fairlife have developed complex filtration technologies to optimize and scale lactose hydrolysed milk. In contrast, companies such as Royal DSM have patented a technology regarding enzyme engineering for a better-quality lactose hydrolysed milk. Two of the patents regarding the production of lactose hydrolyzed milk that Valio has developed are a process for preserving low-lactose or lactose-free milk product [22] which describes the production of low-lactose, lactose-free, or carbohydrate-free milk products. This is characterized by fractionating the carbohydrate and proteins present in the milk, thermally pretreating them, altering or removing the carbohydrates, hydrolysing the lactose if desired, then recombining them back into a product. The second patent, a process for producing a lactose-free milk product described the separation of milk components via ultrafiltration, nanofiltration, and reverse osmosis, followed by hydrolysis of the lactose-reduced milk (ultrafiltered retentate) and the optional addition of water or whey salts. Royal DSM filed a patent, intending to produce higher quality milk using recombinant gene engineering. The patent, enzyme preparation that produces a pure flavour [22] describes a method to produce lactase using recombinant gene manipulation techniques that produce aryl sulfatase, an enzyme that breaks down sulfatides. This newly engineered lactase preparation allows lactose hydrolysed milk production without the risk of the enzyme interacting with other milk compounds to produce sulfate, minimising the off-flavor of the hydrolysed milk.

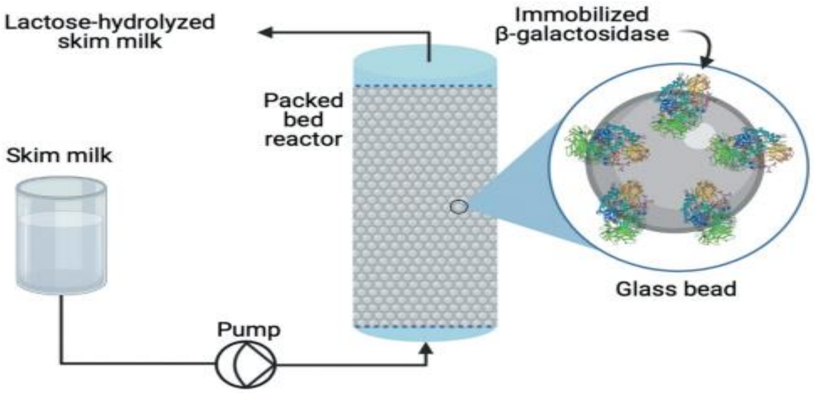

Fig 1: Diagram of a packed bed reactor with immobilized β-galactosidase bound onto glass beads [23].

5.5 ALTERNATIVE TECHNOLOGIES

In addition to traditional methods of batch hydrolysis using free enzymes and continuous hydrolysis using immobilized enzymes with packed bed reactor, there are other novel technologies being developed to push these boundaries. Technologies employing surfactant-free micromotors, enzymatic hydrolysis coupled with membrane technology and acid hydrolysis are promising techniques to advance the boundaries of lactose hydrolysis in milk.

5.5.1 MICROMOTORS

Recent developments in macromaterials have led to the development of carbon nanofibers based micromotors. This new technology employs catalytic materials such as Pt, Pd, Ag, Au, or MnO2 bound inside of on a fibril carbon-nano fiber. The high catalytic activity allows for the propulsion of the micromotor.[35] This novel technology has recently been used to develop surfactant-free β - galactosidase micromotors.[24] This technology works two-fold. First, the enzyme is immobilized onto the carbon allotrope-based micromotors surface through the functionalization of its carboxylic group. Second, the micromotor inner portion is coated with nickel-platinum nanoparticles as the catalytic activity source, allowing self-propulsion in the milk. Regarding the kinetics of lactose hydrolysis within this system, the dynamic micromotor movement enhanced the enzyme's affinity toward lactose [25].

5.5.2 ENZYMATIC HYDROLYSIS WITH MEMBRANE FILTRATION

Enzymatic membrane bioreactors function by optimizing GOS production through either continuous immobilized enzyme hydrolysis or batch hydrolysis with membrane filtration. This process allows GOS, or other value-added products, to be filtered out of the system. An example of an enzymatic membrane bioreactor is shown in Figure 1.

A batch reactor with free β-galactosidase from A. niger with an ultrafiltration membrane. This process used high concentrations of lactose (470 g/L), and by varying transmembrane-pressure, cross flow-velocity, and temperature, they were able to achieve 2.44-fold higher GOS synthesis than batch hydrolysis of lactose. The optimal condition of this process was found to be transmembrane-pressure (4.38 bar), cross flow velocity (7.35 m/s), and temperature (53.1°C) [26].

A novel ceramic membrane to be used in scaling up GOS production from lactose. In this case, soluble K. lactis was used in batch hydrolysis with a tubular ceramic ultrafilter membrane made of AL2O3/TiO2 with MWCO of 20,000 DA. Aqueous lactose concentration 10–30 % (w/w). The highest yield of GOS was found to be 38 (%, w/w) when using 30 % (w/w) lactose concentration at a membrane pressure of 2 bar. Although the final yield of the ceramic membrane was lower than the other enzymatic membrane bioreactor systems, it has the capability to be utilized in a scale-up operation. Technology such as chemically enhanced backflush (CEB) can be utilized to easily clean ceramic membranes that may foul. [27]

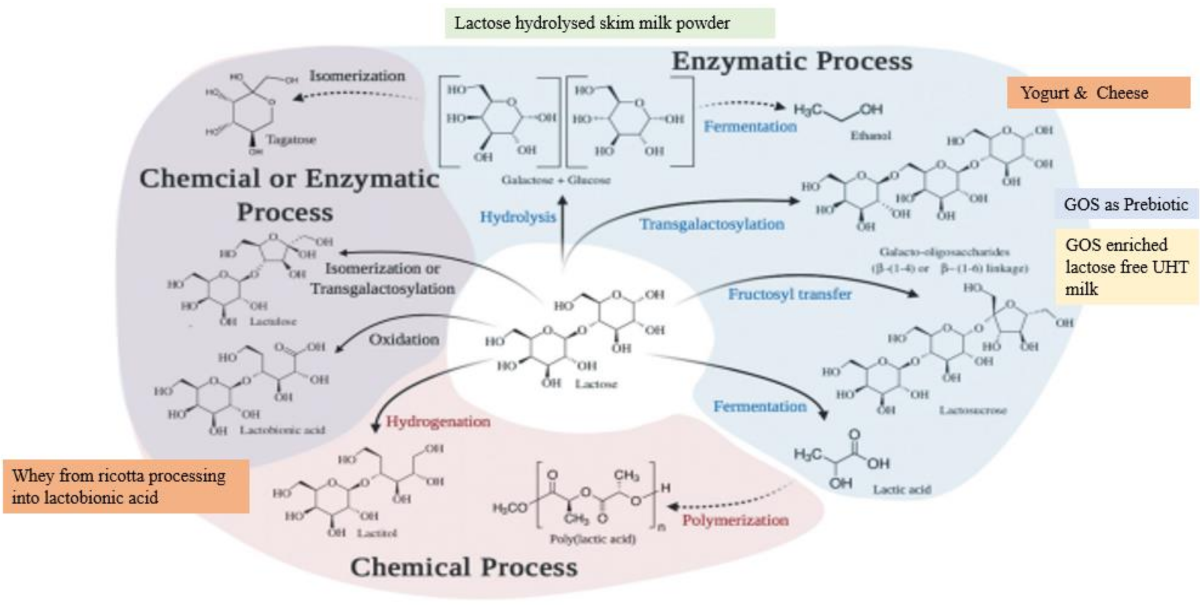

Fig 2: Conversion of lactose into various value-added products categorized by chemical or enzymatic process [27]

5.5.3 LACTOSE HYDROLYSED SKIM MILK POWDER

Skim milk powder (SMP) has a high amount of lactose, approximately 52 % by weight, and is available at a lower cost than whole milk powder, dry whey, buttermilk powder, and whey protein concentrate. This, combined with a large fraction of the world population being lactose intolerant, proves there is high potential to produce lactose hydrolysed skim milk powder. Milk powders can be very profitable when used as a protein source in a product.] The lactose hydrolysis products, galactose, and glucose are highly susceptible to Maillard browning, significantly more than lactose. A possible application to lower the high browning potential of LHSMP is to increase the production of GOS, produced by the transgalactosylation during enzymatic hydrolysis of lactose. When galactose, GOS, galactose plus glucose, and GOS plus galactose and glucose, all within a casein protein model, were analyzed for Maillard reaction during heating; GOS and GOS plus galactose and glucose were found to have a lower degree of browning when compared to galactose and glucose [28]. This product line includes Eila sweet lactose-free skimmed milk powder, produced by enzymatic hydrolysis, spray drying, and Eila pro lactose-free skimmed milk powder by an initial lactose filtration step followed by hydrolysis and spray drying, which contains fewer carbohydrates and higher protein than the Eila Sweet product [29] (Figure 2).

5.5.4 GOS ENRICHED LACTOSE FREE UHT MILK

GOS synthesis by transgalactosylation increases with reaction temperature. However, increasing temperature in milk becomes problematic due to microbial growth. In post-process hydrolysis, UHT milk is sealed aseptically to prevent microbial growth allowing the product to be stored at room temperature. Therefore, aseptic packaging milk containing β -galactosidase can be stored at elevated temperatures without spoilage to increasing the rate of GOS production by transgalactosylation. Generally, GOS has a relatively low sweetness, 30– 60% that of sucrose. For example, B. bifidum can hydrolyse lactose and produce GOS [30]. The ability to lower the relative sweetness by increasing the DP, by selective choice of enzyme sources, could allow additional lactose to be added to the milk before hydrolysis. This would increase GOS synthesis due to the direct relationship between lactose concentration and the rate of transgalactosylation. Thus, producing a high yield of GOS without adding excess sweetness or to the milk products. More research should be done on post-process hydrolysis during the storage period. If the temperature rises too high, Maillard reaction can occur and increase furosine concentration, discoloration, and lysine blockage. In the case of GOS, when temperatures rise to the point of Maillard browning, glycosidic linkages start to break, decreasing the degree of polymerization, producing an undesirable product [31].

5.6 MARKET STATUS OF LACTOSE HYDROLYSED MILK

Milk consumption, as well as demand for milk products, has grown massively in Asian countries in recent years. However, because lactose intolerance affects the majority of Asia's population, demand for lactose hydrolyzed milk products has increased. Ultrafiltration reduces the lactose content in milk from 4.8 % to 0.01 %, followed by the addition of β-D-galactosidase, which hydrolyzes the lactose to glucose and galactose. The advantages of lactose hydrolyzed products have improved the functional and nutritional qualities of dairy products. The lactose hydrolyzed milk process and its effects on the production of various milk products such as American cheese, cottage cheese, and ice cream had been the subject of research. Lactose-free dairy is the fastest-growing segment of the dairy business. Lactose-free dairy is expected to bring in €9 billion by 2022, outperforming overall dairy growth (7.3% vs. 2.3 %). The most popular lactose-free dairy product is potable milk, which accounts for two-thirds of the market and drives the category's total growth. In recent years lactose-free products have been popular in food markets. The Euromonitor analysis of the market development for lactose-free dairy products estimated a compound annual growth rate (CAGR) of +7.3% from 2017 to 2022. In particular, a 6.8% growth of lactose-free yogurt, a 7.6% growth in milk, an 8.6% growth in cheese, and a 5.3% growth in other dairy products [32]. There are two ways to produce a lactose-free product, either by lactose hydrolysis or filtration. Lactose hydrolyzed dairy products have a higher product yield than filtration as they do not produce any waste product. Lactose hydrolysis also makes the product sweeter; lactose has a sweetness of 20% that of sucrose, where the relative sweetness of glucose is 75% and galactose is 60% that of sucrose. In nanofiltered lactose-free products, lactose is filtered out, leaving rest of the dairy product constituents. When lactose is removed, there is approximately a 52% lower yield of milk solids due to this process. Another benefit of lactose hydrolysis is the production of GOS. GOS are non-digestible prebiotics that contains a long chain of monomers containing one glucose molecule and anywhere from 2– 20 galactose molecules. GOS increases Bifidobacteria and Lactobacilli growth in the gut of humans [33].

III. CONCLUSION

Consumers growing awareness of lactose-hydrolyzed dairy's nutritional benefits and the vast scope of individuals who suffer from lactose intolerance are promising indicators for the lactose-hydrolyzed dairy market. These products provide excellent nutritional properties where their dairy-free counterpart fall short. The industry demand for lactose-hydrolyzed products has driven companies to make headway in separation technology and enzymatic technologies to produce better sensory quality products. Whereas academic interest in lactose hydrolyzed dairy products is more focused on immobilized pack bed reactors, focusing on enzymatic recovery and the production of rare sugars and value-added compounds. Within this rapidly growing industry, consumers expect to see many different new products, which may include lactose hydrolyzed skim milk power, GOS enriched milk.

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