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Strategies for lactase immobilization and delivery to relieve lactose intolerance

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ABSTRACT

Background: Approximately 70% of adults worldwide are affected by lactose malabsorption. Symptomatic lactose malabsorption is also known as lactose intolerance characterized by indigestion symptoms such as bloating, abdominal pain, and diarrhea. While many lactase-based approaches to relieve lactose intolerance are emerging, there has been a lack of timely and comprehensive evaluation of these approaches.

Scope and approach: Here, we summarize the application of lactase to relieve lactose intolerance. Specifically, we first introduce the classification of lactose intolerance and its harm, and then describe traditional methods to relieve lactose intolerance in which lactase is immobilized and used to eliminate lactose in food. Finally, we summarize the methods used to immobilize lactase.

Key findings and conclusions: However, lactose-free diet can cause harm to human health, and lactase delivery as a dietary supplement can better address the nutritional and health needs of lactose-intolerant individuals. We then summarize the challenges associated with these new lactase preparations and the development of oral lactase delivery systems. Finally, we discuss the novel methods for lactase delivery, possibilities of improving its targeted delivery, and the remaining challenges. This review is expected to help rational design of effective oral lactase delivery platforms.

1. Introduction

Lactose is the main carbohydrate in mammalian milk and dairy products, whose concentration is approximately 7 mg/g in human milk, 4.7–4.8 mg/g in cow and goat milk, and about 2%–8% (w/w) in dairy products (Fig. 1A). Lactose cannot be directly absorbed by human body. In the small intestine, it is broken down into glucose and galactose by β-galactosidase (β-Gal). β-Gal is commonly known as lactase (Szilagyi & Ishayek, 2018), whose deficiency can cause lactose malabsorption. Approximately 65% of the world's population has insufficient ability to break down lactose after childhood due to a decline in β-Gal level, resulting in lactose malabsorption in 70% of total population (Catanzaro, Sciuto, & Marotta, 2021). Typically, individuals with symptomatic lactose malabsorption (also known as lactose intolerance) often exhibit indigestion symptoms such as bloating, abdominal pain, and diarrhea due to the entry of undigested lactose into the colon (Szilagyi & Ishayek, 2018). Moreover, lactose-intolerant individuals may have difficulty in absorbing calcium and other minerals, which can affect the physical development of children and cause rickets or osteoporosis in adults (Catanzaro et al., 2021). Lactose intolerance has an apparently uneven global distribution, affecting approximately 50% of the population in the United States, 70% in Asia, and almost 100% in Africa

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(Fig. 1B). Considering the prevalence of lactose intolerance, it is urgent to develop appropriate treatment strategies.

As a matter of fact, a number of treatment strategies for lactose intolerance have been developed (Fig. 1C) (Damin, Kovalski, Fischer, Piccin, & Dettmer, 2021; Mesa, 2020). Considerable research has been focused on the immobilization of lactase to break down lactose in dairy products to obtain low-lactose or lactose-free dairy products at an industrial scale (Grosova, Rosenberg, & Rebroš, 2008; Harju, Kallioinen, &

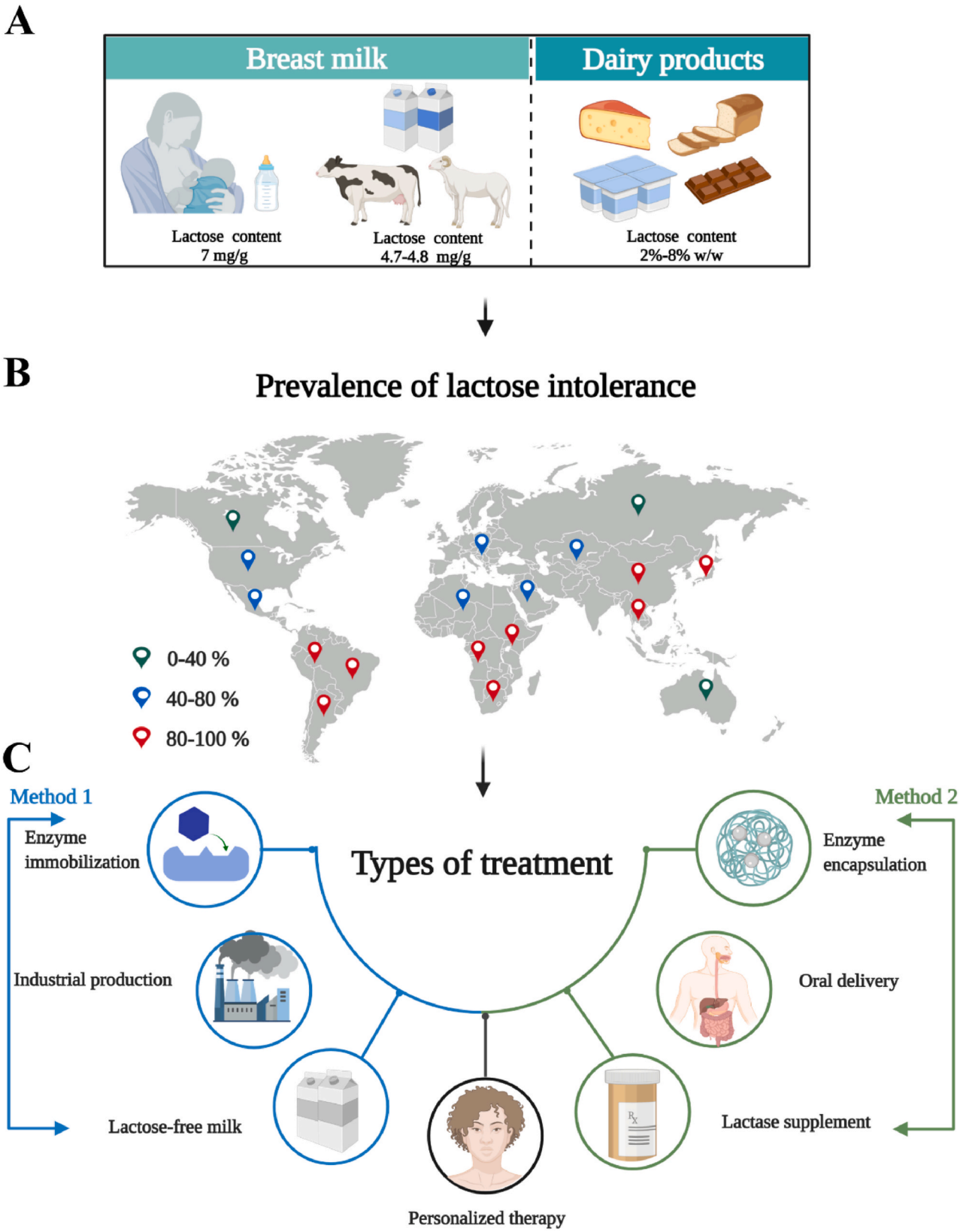

Fig. 1. (A) Lactose content in common foods; (B) Global prevalence of lactose intolerance; (C) Treatment methods for lactose intolerance.

Tossavainen, 2012; Saqib, Akram, Halim, & Tassaduq, 2017). However, these products are approximately three times sweeter than non-treated dairy products due to lactose hydrolysis, resulting in an undesirable taste (Dekker, Koenders, & Bruins, 2019). Furthermore, recent studies have demonstrated that a lactose-free diet can cause some nutritional problems, such as deficiencies in calcium, phosphorus, and vitamin D, which may lead to insufficient bone mineralization (Mesa, 2020). In recent years, lactase has become an increasingly popular dietary supplement with better patient compliance because it does not adversely change the food quality or nutritional status of the diet (Ferreira-Lazarte, Moreno, & Villamiel, 2018; McClements, 2018). However, it remains technically challenging to maintain lactase activity during manufacturing, storage, and passage through the gastrointestinal tract. Some unfavorable conditions, such as freeze-drying, temperature changes, and highly acidic and protease-rich environment of the stomach, can significantly alter the specific three-dimensional structure of lactase, thereby reducing its bioactivity (Dan, Samanta, & Almoazen, 2020; Liu, Yao, Rao, Lu, & Gao, 2017; Perry & McClements, 2020; Raeisi Estabragh, Bami, Ohadi, Banat, & Dehghannoudeh, 2021). Hence, lactase must be encapsulated to effectively protect its activity during processing, transport, storage, and delivery into the small intestine. The encapsulation vehicle must be formulated with food-grade ingredients (such as proteins, polysaccharides, lipids, surfactants, and mineral oils). The manufacturing of encapsulated lactase requires food-grade and cost-effective processing operations, and the product must be sufficiently stable for commercial application (McClements, 2018; Perry & McClements, 2020). To date, numerous types of vehicles have been developed for lactase, such as nanoparticles (Deng, Zhu, Li, Zhou, & Zhang, 2020; Dong & Zhong, 2019; Zhang, Yu, Mei, Zhang & Deng, 2018), microparticles (Ahn, Lee, & Kwak, 2019; Bertoni, Albertini, Dolci, & Passerini, 2018; Deng, Pei, et al., 2019; Deng, Wang, et al., 2020; Deng, Wang, et al., 2021; Homayun & Choi, 2020), emulsions (Souza, Comunian, Kasemodel, & Favaro-Trindade, 2019; Zhang & Zhong, 2017, 2018), and gels (Deng, Li, et al., 2021; Deng, Pei, et al., 2020; Facin, Moret, Baretta, Belfiore, & Paulino, 2015; Li et al., 2020; Nichele, Signoretto, & Ghedini, 2011; Nussinovitch, Chapnik, Gal, & Froy, 2012; Silva, Trevisan, & Garcia, 2020; Traffano-Schiffo, Aguirre Calvo, Castro-Giraldez, Fito &.Santagapita, 2017; Traffano-Schiffo, Castro-Giraldez, Fito, & Santagapita, 2017; Wang, Chen, An, Chang, & Song, 2018; Zhang, Zhang, Chen, & McClements, 2016; Zhang, Zhang, & McClements, 2017), with each vehicle having advantages and disadvantages. Selection of the most efficacious oral delivery system for a specific application depends on a thorough understanding of the factors affecting the loading, retention, stability, and release of the protein in that specific system.

This review summarizes the critical factors for the development of food-grade nanoparticles and microparticles as oral delivery systems for β-Gal. Specifically, we highlight the drawbacks, challenges, development, and opportunities of these delivery systems to serve as additives, excipients, or matrices for the controlled delivery of β-Gal to the intestine.

2. Lactose intolerance

Lactose malabsorption refers to failure to digest and/or absorb lactose in the small intestine, which can be symptomatic or asymptomatic. Symptomatic lactose malabsorption, also known as lactose intolerance, is characterized by symptoms such as abdominal pain, bloating, or diarrhea in individuals after ingestion of lactose (Catanzaro et al., 2021; Szilagyi & Ishayek, 2018). When lactase activity is insufficient, the undigested lactose will pass from the small intestine to the large intestine, where it is fermented by microorganisms to produce a gaseous mixture of hydrogen, carbon dioxide, and methane, causing bloating and abdominal pain (Szilagyi & Ishayek, 2018). Furthermore, undigested lactose and the fermentation products can raise the osmotic pressure in the large intestine, increasing the amount of water flowing into the intestine to cause diarrhea (Catanzaro et al., 2021).

2.1. Types of lactose intolerance

Lactose intolerance is generally divided into four types: primary, secondary, developmental, and congenital lactose intolerance (Szilagyi & Ishayek, 2018).

Primary lactose intolerance: Individuals with primary lactose intolerance have normal lactase activity at birth, but the activity will gradually decrease with age to 5%–10% of the initial level (Szilagyi & Ishayek, 2018). It is the most common type of lactose intolerance, accounting for more than 70% of lactose-intolerant individuals (Katoch, Nain, Kaur, & Rasane, 2022). Primary lactose intolerance is caused by the absence of an allele that enables lactase persistence in the body. This genetic pattern differs significantly among races and regions (Fassio, Facioni, & Guagnini, 2018), whose incidence rate is 90%–100% in Africa, approximately 70% in Asia, and under 15% among white populations in Northern Europe, North America, and Australia (Catanzaro et al., 2021).

Secondary lactose intolerance: In secondary lactose intolerance, the small intestine is infected with viruses, bacteria, or parasites, resulting in a decrease in lactase activity; however, the lactase activity can be restored to normal level after the small intestine recovers (Katoch et al., 2022).

Developmental lactose intolerance: This type of intolerance mainly occurs in premature infants of less than 34 weeks in gestational age (Szilagyi & Ishayek, 2018). The low lactase activity in the intestine may be due to the immature organ development of premature infants. However, the lactase activity can gradually rise over time (Fassio et al., 2018).

Congenital lactose intolerance: This type of lactose intolerance arises from a genetic mutation in the coding region of the lactase gene; and the absence of a recessive allele on the autosome causes the inability of the individual to secrete lactase (Szilagyi & Ishayek, 2018). The typical feature of congenital lactose intolerance is the absence of lactase at birth, despite morphologically normal microvilli of the small intestine.

2.2. Harm of lactose intolerance

The typical symptoms of lactose intolerance comprise abdominal pain, bloating, flatulence, diarrhea, abdominal pain, nausea, and vomiting, and vary considerably among individuals depending on the lactase activity in their small intestine and ingestion amount of lactose (Katoch et al., 2022).

2.2.1. Harm of lactose intolerance to infants

Lactose is an important energy source for infants, as 1 g of lactose can produce approximately 16.75 kJ of energy. It accounts for nearly 40% of the total energy intake of exclusively breastfed infants (Fassio et al., 2018). The lactose in breast milk is broken down by lactase into galactose and glucose. Under the action of lactase transglycosylation, some galactose forms galactooligosaccharides, which are the only oligosaccharide source derived from breast milk. Galactooligosaccharides are beneficial for the proliferation of intestinal bifidobacteria and can promote the proliferation of small intestinal villus epithelial cells, thereby increasing the amount of lactase-secreted “lactase-galactooligosaccharide-bifidobacteria” microecological cycle that maintains the microecological balance in the intestine (Catanzaro et al., 2021). Lactase deficiency will decrease the galactooligosaccharide content and amount of bifidobacteria in infants; moreover, after entering the colon, undigested lactose will promote the growth of harmful bacteria (Catanzaro et al., 2021), resulting in an unbalanced microbial ecology in the intestine. Galactose promotes the formation of cerebrosides and mucopolysaccharides, two important compounds for the intellectual development of infants (Catanzaro et al., 2021). Moreover, lactose can

form complexes with calcium and zinc. When lactose is digested to produce lactic acid, the intestinal pH will decrease, thereby promoting the absorption of calcium and zinc. The main harms of lactose intolerance to infants include chronic diarrhea, calcium malabsorption, rickets, low weight, growth retardation, and particularly delayed brain development (Szilagyi & Ishayek, 2018).

2.2.2. Harm of lactose intolerance to adults

Individuals with lactose intolerance suffer from not only gastrointestinal discomfort (diarrhea, nausea, bloating, and abdominal pain) but also extraintestinal symptoms, such as memory loss, headache, musculoskeletal pain, arrhythmia, depression, and anxiety (Ratajczak, Rychter, Zawada, Dobrowolska, & Krela-Kazmierczak, 2021). The pathogenesis of these extraintestinal symptoms has not been elucidated yet. In addition to symptoms directly related to changes in lactose metabolism, lactose-intolerant individuals are at higher risk of certain diseases (Catanzaro et al., 2021). For example, because lactose can promote calcium absorption, its malabsorption tends to cause higher risks of osteoporosis and fractures in women (Ratajczak et al., 2021). In addition, the glycemic index of lactose is low in humans. Consumption of 50 g lactose will increase the blood sugar only by 74 mg/100 mL, which is much lower than that of glucose (by 140 mg/100 mL). Some studies have shown that consuming low-glycemic index foods can reduce the risk of type II diabetes (Catanzaro et al., 2021).

3. Relief of lactose intolerance

In recent decades, various methods have been developed to meet the needs of individuals with lactose intolerance. The traditional method is to eliminate dietary lactose by immobilizing lactase to break down lactose in dairy products, which can be used industrially to produce low-lactose or lactose-free dairy products (Grosova, Rosenberg, Rebroš, Sipocz, & Sedlackova, 2008; Harju et al., 2012; Husain, 2010; Saqib et al., 2017). In recent years, direct ingestion of lactase as a dietary supplement has become a research focus to ensure balanced nutrition for lactose-intolerant individuals (Ferreira-Lazarte et al., 2018; McClements, 2018; Mesa, 2020).

3.1. Lactose-free products

3.1.1. Free β-gal

According to the United States Food and Drug Administration, lactase (β-galactosidase, β-Gal) derived from fungi and yeast is safe to be used in the food industry, and is therefore widely applied in the dairy industry (Saqib et al., 2017). Since β-Gal first became commercially available in 1970, various low-lactose dairy products have been developed (Harju et al., 2012). Compared with common milk, lactose-free milk can avoid causing the symptoms of lactose intolerance, and its high galactooligosaccharide content promotes beneficial intestinal flora and reduces the production of harmful substances. Moreover, it has been demonstrated that adding β-Gal to milk after thermal processing can reduce the amount of β-Gal needed and the cost than addition of β-Gal before thermal processing (Tossavainen & Kallioinen, 2008). It can also reduce the Maillard reaction in the milk, thereby preventing the loss of nutrients. Therefore, it is recommended to add β-Gal after thermal processing. Although β-Gal can be used to produce lactose-free milk, many factors should be considered, such as the substrate nature, enzyme characteristics, production conditions, and costs and profits (Harju et al., 2012).

3.1.2. Immobilized β-gal

There are still problems with the use of free β-Gal to hydrolyze lactose, such as poor stability, lower reusability, high cost, and residual enzymes in the product (Grosova et al., 2008). Compared with free enzymes, immobilized enzymes typically have higher thermal stability, acid-base stability, storage stability, and resistance to proteases and inhibitors (Gonçalves et al., 2019). Furthermore, they can retain their activity for a long time and be used repeatedly to reduce the cost of catalysis (Bashir, Sood, & Bandral, 2020).

Immobilized β-Gal can continuously hydrolyze lactose in dairy products, thereby significantly improving the efficiency of lactose decomposition (Nath, Mondal, Chakraborty, Bhattacharjee, & Chowdhury, 2014). However, the application of immobilized β-Gal to hydrolyze lactose in milk is faced with the following challenges. First, the neutral pH of milk promotes microorganism growth; and second, the proteins in milk are readily adsorbed to the surface of immobilized β-Gal (Damin et al., 2021; Grosova et al., 2008; Husain, 2010). Moreover, the choice of method for enzyme immobilization is critical because the enzyme performance is affected by interactions between the carrier and the enzyme. β-Gal has been successfully immobilized through various methods, including physical adsorption, encapsulation, crosslinking, covalent bonding, or a combination of these methods (Fig. 2). Many studies have reported various immobilized β-Gal systems to prepare low-lactose milk, each of which has both advantages and disadvantages (Damin et al., 2021). We will introduce them in detail below.

3.1.2.1. Adsorption. The adsorption method is based on the physical interaction between the enzyme and the carrier. The interactions may be van der Waals interactions, ionic interactions, hydrophobic interactions, hydrogen bonds, or their combination, through which the enzyme is immobilized on the carrier surface (Nisha, Karthick, & Gobi, 2012). The immobilization efficiency depends on several factors such as the characteristics of the enzyme and the pH, ionic strength, and temperature of the reaction (Nisha et al., 2012). β-Gal has been immobilized on porous cellulose acetate fibers through adsorption. It was the first immobilized β-Gal to hydrolyze lactose in milk industrially to produce low-lactose milk. Harju further optimized this method, successfully reducing the residual amount of β-Gal in the final product (Harju, 2004). β-Gal has been successfully immobilized on different carriers by physical adsorption. This method has the advantages of simple process, mild conditions, a wide range of candidate carriers, and reproducibility, while the disadvantage is that the immobilized β-Gal is easily affected by environmental factors during use, such as changes in temperature, pH, and ionic strength, which can cause the desorption of β-Gal. For successful adsorption, the carrier must satisfy certain conditions, among which the affinity between β-Gal and the carrier is essential. Recently, some research has been focused on the development of appropriate carriers for β-Gal. For example, β-Gal was immobilized on hydroxypropyl methylcellulose film through hydrophobic interactions and hydrogen bonds, which could reduce the lactose content in milk by approximately 80% (Silva, Stevanato, Garcia, & Silva, 2020). β-Gal also was immobilized on a modified nanosilver-reduced graphene oxide (Ag@rGO) nanocomposite, which could improve β-Gal stability under adverse conditions (Fig. 3A). Compared with free β-Gal, immobilized β-Gal has higher activity due to its enhanced affinity with the substrate. In a recent study, immobilized β-Gal was found to retain 85% of its activity after 10 repeated uses, and could reduce the lactose content in milk by 89% (Shafi, Ahmed, & Husain, 2021).

3.1.2.2. Encapsulation. Encapsulation refers to the wrapping of enzymes with a synthetic or natural polymer network. As a typical polymer hydrogel, polyvinyl alcohol (PVA) has low toxicity, good mechanical properties and stability, and low biodegradability, and does not interfere with enzyme-catalyzed reactions. PVA has been used to immobilize β-Gal (Husain, 2010). For example, β-Gal isolated from *Aspergillus oryzae* was immobilized in PVA hydrogel. The activity of immobilized β-Gal was about 10% higher than that of free β-Gal, and remained almost unchanged after 35 repeated uses and storage at 4 °C for 14 months (Grosova et al., 2008). Compared with synthetic polymers, natural polymers such as alginate, carrageenan, pectin, chitosan, and gelatin are more suitable for preparing hydrogels to immobilize enzymes due to

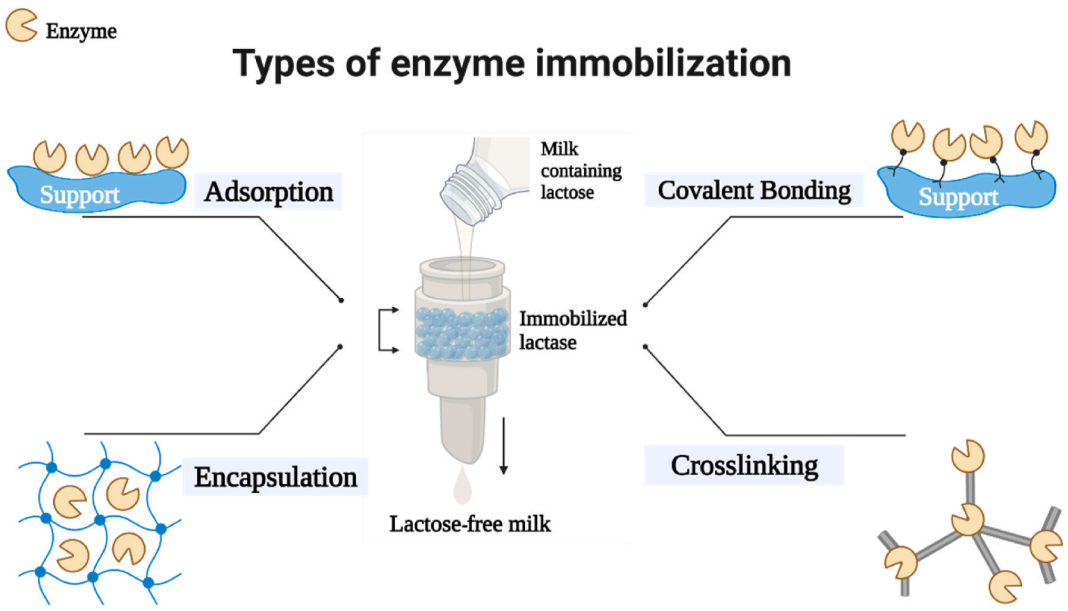

Fig. 2. Methods for immobilizing lactase to prepare lactose-free milk.

their non-toxicity and availability in the food industry (Estevinho, Damas, Martins, & Rocha, 2014). In another study, alginate and gelatin were combined to immobilize β-Gal, and the immobilized β-Gal showed high stability as the activity showed no decrease over 35 days; moreover, the immobilized β-Gal had broader optimum temperature and pH ranges than free β-Gal (Tanriseven & Doğan, 2002). In another study, the addition of chitosan was shown to improve the stability of alginate gel, and this combination was used to immobilize β-Gal (Won, Kim, Kim, Park, & Moon, 2005). The calcium alginate-chitosan system showed a β-Gal encapsulation rate of 60%, while that based on barium alginate-chitosan was 100% (Taqieddin & Amiji, 2004). In addition, compared with free β-Gal, the barium alginate-chitosan β-Gal encapsulation system could expand the optimum temperature and pH range of β-Gal. A previous study found that it took 12 h for free β-Gal but only 2 h for immobilized β-Gal to hydrolyze the same amount of lactose (Katrolia, Liu, Li, & Kopparapu, 2019). An alginate λ-carrageenan complex was also successfully used to immobilize β-Gal, and the activity of immobilized β-Gal was less affected by pH and temperature changes than that of free β-Gal (Souza, Garcia-Rojas, et al., 2019). A recent study used pectin gel to immobilize β-Gal (Fig. 3B), and added pine fibers to this system to increase β-Gal activity and reduce enzyme leakage (Cargnin, Gasparin, dos Santos Rosa, & Paulino, 2021). Further addition of λ-carrageenan to this system improved the storage stability of β-Gal, and the immobilized β-Gal could retain over 90% activity after 43 days of storage (Wahba, 2021). In addition to hydrogels, microcapsules can also be used to immobilize β-Gal. The activity retention rate of β-Gal immobilized by liposome microcapsules was 86% at 55 °C, which is much higher than that of free β-Gal (65%) (Rodríguez-Nogales & López, 2006). Overall, encapsulation is an efficient, economical, and feasible immobilization strategy for β-Gal, but it has some drawbacks as well. For instance, the diffusion of the substrate, enzyme, and carrier hinders mass transfer, and the immobilized β-Gal can easily leak out of the encapsulation system during repeated use (Grosova et al., 2008). Moreover, the carrier cannot be reused after exhaustion of the enzyme activity (Ureta et al., 2021).

3.1.2.3. Covalent bonding. In enzyme immobilization by covalent bonding, functional groups unnecessary for the catalytic activity of the enzyme are covalently linked to the carrier (Damin et al., 2021). The stability of immobilized enzyme largely depends on the number of covalent bonds between the enzyme and carrier. Therefore, it is necessary to fully consider the carrier, reaction groups, and immobilization conditions to obtain the maximum number of enzymes covalently linked to the carrier. The carrier contains epoxy groups, which can readily covalently react with amino, thiol, and hydroxyl groups on the enzyme to form covalent bonds (Damin et al., 2021). Choosing appropriate immobilization conditions, including the reaction time, pH, temperature, and inhibitor concentration, is essential for maximizing the number of covalent bonds between the enzyme and carrier (Ureta et al., 2021). β-Gal has been successfully covalently immobilized on inorganic carriers such as graphite (Zhou & Chen, 2001), fiber substances (Zhou, Chen, & Li, 2003), oxides (Di Serio et al., 2003), magnetic materials (Zhang, Gao, & Gao, 2010), and glass microspheres (Gennari et al., 2018). Compared with inorganic carriers, natural biopolymer carriers are safer to be used in the food industry (Elnashar & Yassin, 2009). Chitosan is a natural carrier often used for the immobilization of β-Gal (Elnashar & Yassin, 2009). Chitosan modified with glutaraldehyde can form covalent bonds with β-Gal through the Schiff base reaction. Compared with free β-Gal, β-Gal immobilized in this way has a broader pH and temperature range, and has more stable activity after storage for more than 12 months at 4 °C (Makowski et al., 2007). A comparison of different immobilization technologies (physical adsorption, capture, and covalent bonding) using chitosan carriers demonstrated that covalently bound β-Gal has the highest immobilization efficiency (100%) (Vieira et al., 2013). A study explored the effect of chitosan particle size on the properties of immobilized β-Gal, and found that β-Gal had lower activity in chitosan microparticles than in chitosan nanoparticles. After 50 cycles of use, the chitosan microparticle system showed a β-Gal activity retention rate of 83.20%, which is higher than that of the chitosan nanoparticle system (75.93%) (Klein et al., 2012). A recent study modified chitosan with different substances (glutaraldehyde, epichlorohydrin, and glycidol) and compared the effects on the performance of immobilized β-Gal. Chitosan modified with 0.8% v/v glutaraldehyde (2% w/v) resulted in the highest β-Gal activity and reusability. The immobilized β-Gal activity remained at 100% after 105 days of storage at 4 °C (de Freitas, Hortêncio, de Albuquerque, Rocha, & Gonçalves, 2020). Some studies have found that introducing modified λ-carrageenan into the chitosan system can improve the system's thermal stability. The β-Gal loading rate of a system based on epoxy group-modified λ-carrageenan was three times higher than that based on aldehyde group-modified λ-carrageenan. The loading rate was different because aldehyde groups can react with only one group (-NH_2) on β-Gal, while epoxy groups can react with three groups (-SH, –NH_2, and –OH)

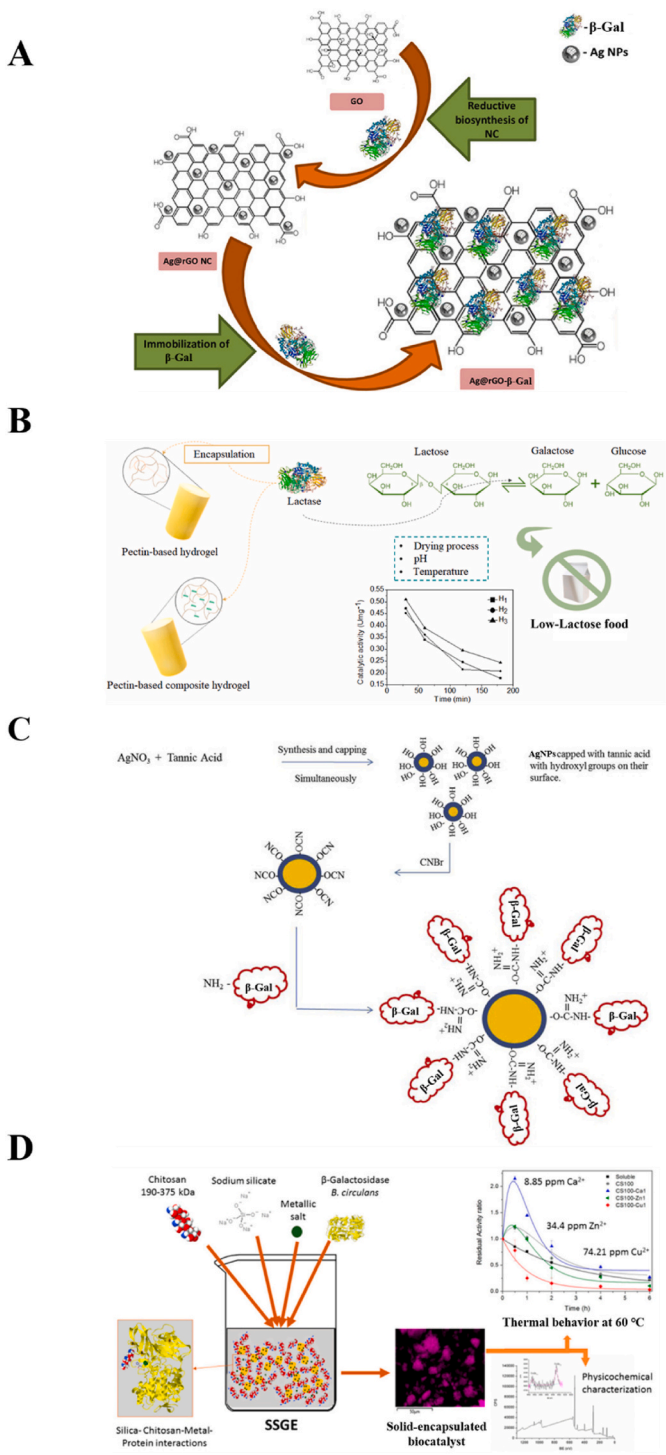

Fig. 3. (A) β-galactosidase immobilization on modified nanosilver-reduced graphene oxide (Ag@rGO) nanocomposites (Shafi et al., 2021). Copyright 2022, Elsevier; (B) Schematic diagram comparing a pectin-based hydrogel and a pectin-based composite hydrogel containing pine fiber to immobilize lactase (Cargnin et al., 2021). Copyright 2022, Elsevier; (C) β-galactosidase immobilization on tannic acid-stabilized silver nanoparticles (AgNPs) (Arsalan et al., 2020). Copyright 2022, Elsevier; (D) β-galactosidase immobilization using the one-pot route, combining the silica sol-gel encapsulation (SSGE) process with a metal chelation strategy by using chitosan and Ca^{2+}, Zn^{2+}, or Cu^{2+} cations (Ospina et al., 2019). Copyright 2022, ACS Publications.

(Elnashar & Hassan, 2014). In recent years, researchers have developed nanomaterials that can provide a larger surface area to covalently immobilize β-Gal (Beniwal, Saini, Kokkiligadda, & Vij, 2018). Silica-based nanocomposites were used to immobilize β-Gal, and the immobilized β-Gal could retain approximately 90% of its activity after 200 h of use (Ricardi et al., 2018). Silver-based nanoparticles have also been used to immobilize β-Gal (Fig. 3C), and the system showed high thermal stability, reusability, and recovery rate (Arsalan, Alam, Farheen Zofair, Ahmad, & Younus, 2020). In general, under covalent immobilization, β-Gal is more tightly linked to the carrier and is not easily dissociated, and β-Gal attached to the carrier surface can readily interact with the substrate. However, covalent bonding is complicated and costly, and the enzyme activity is easily compromised by environmental factors during the immobilization process.

3.1.2.4. Cross-linking. In a crosslinking method for enzyme immobilization, a crosslinking agent is used to form covalent bonds between enzymes. The crosslinking agent has two reactive ends that bind to amino acids in the enzyme (Damin et al., 2021). Crosslinking agents can induce self-cross-linking of the enzyme to form a three-dimensional network (Grosova et al., 2008; Nath et al., 2014). In this case, there is no need for a carrier, avoiding the problem of diffusion and mass transfer limitations. However, enzyme clusters can be formed during the self-crosslinking process, resulting in reduced enzymatic activity (Damin et al., 2021). Many studies have combined crosslinking with other immobilization technologies such as adsorption, encapsulation, and covalent bonding. Adsorption combined with crosslinking was used to immobilize β-Gal on concanavalin A-Celite 545. Compared with free β-Gal, the immobilized β-Gal was more efficient in hydrolyzing lactose in milk (Ansari & Husain, 2012). One study compared the performance of immobilized β-Gal in crosslinked and non-cross-linked systems, whose lactose conversion rate was 77% and 54%, and β-Gal activity retention rate was 86% and 30% after storage for two months, respectively (Haider & Husain, 2009). In another study, β-Gal was covalently immobilized on a chitosan carrier and then crosslinked with metal ions (Fig. 3D). The results showed that the presence of metal ions did not affect the β-Gal encapsulation efficiency but improved its thermal stability (Ospina, Bernal, & Mesa, 2019). The main disadvantage of crosslinking is that it may alter the enzyme structure, leading to the loss of activity by reducing substrate specificity, as well as causing the formation of by-products (Boudrant, Woodley, & Fernandez-Lafuente, 2020).

3.1.2.5. Disadvantages of β-gal immobilization. The main problems for immobilized β-Gal systems are protein adhesion and microbial contamination (Grosova et al., 2008). Therefore, the use of immobilized β-Gal for the industrial production of low-lactose milk requires intermittent cleaning steps, such as washing with general detergents and regular pasteurization (Panesar, Panesar, Singh, Kennedy, & Kumar, 2006). In addition, low-lactose milk is more prone to the Maillard reaction during processing and storage than regular milk, as well as has a higher content of reduced monosaccharides (glucose and galactose), which react more readily with milk proteins than lactose. The Maillard reaction of proteins in milk can cause browning, producing a peculiar smell and reducing its nutritional value (Harju et al., 2012). Moreover, the shelf life of low-lactose milk is shorter than that of regular milk, because the added β-Gal may continue to exert its proteolytic activity in side reactions during storage, which affects the milk's flavor and quality (Bottiroli et al., 2020). Finally, long-term consumption of low-lactose milk can cause health problems related to a lack of calcium, phosphorus, and certain vitamins in the diet, leading to insufficient bone mineralization (Mesa, 2020).

3.2. β-Gal as a dietary supplement

β-Gal can serve as a dietary supplement to relieve the symptoms of lactose intolerance. When ingested with foods containing lactose, it can hydrolyze 70%–80% of the lactose in the food. Since this method does not remove lactose from the food, it will not change the food quality or nutritional status (McClements, 2018). Commercial β-Gal is gaining popularity worldwide, which is formulated as drops, capsules, tablets,

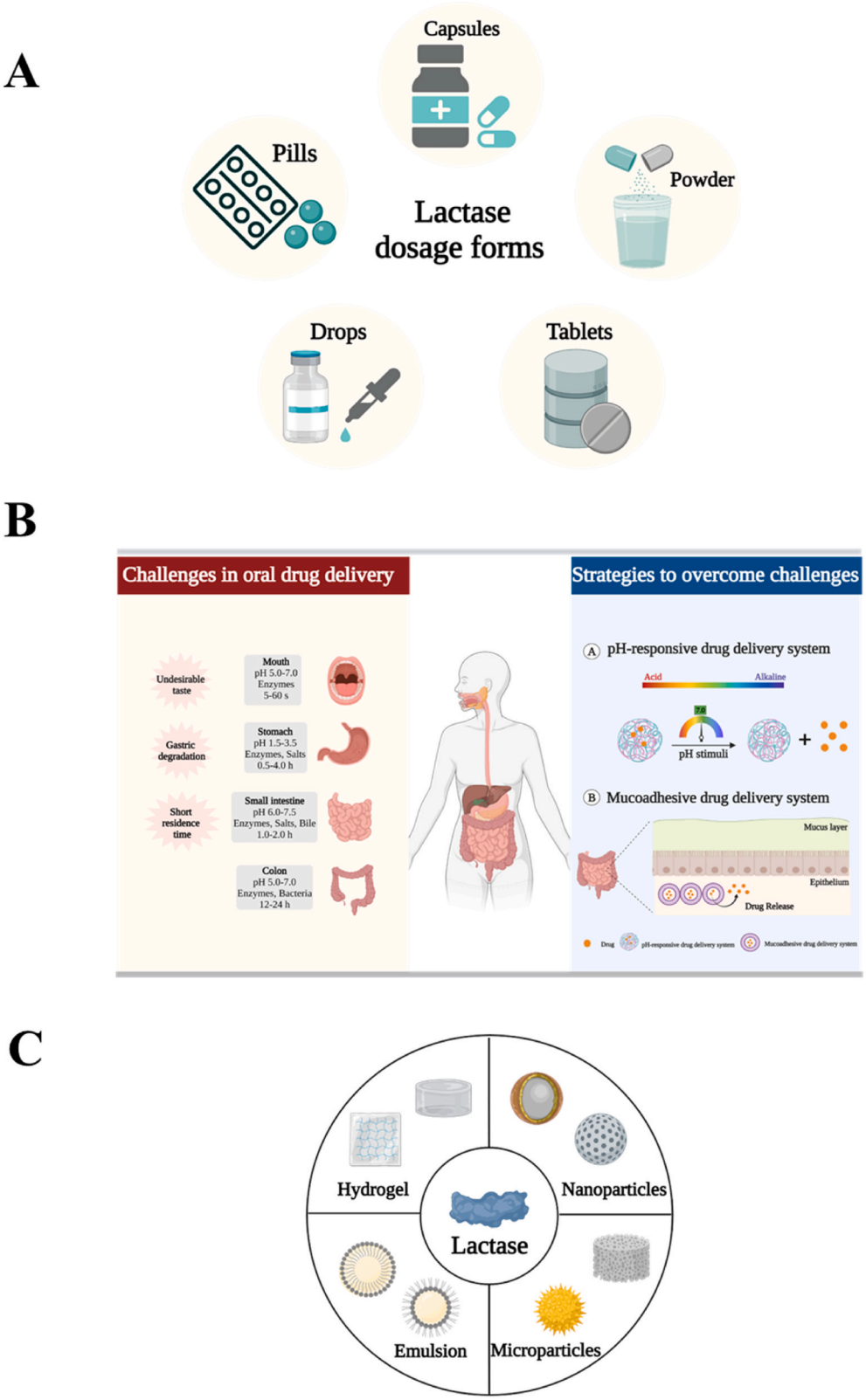

Fig. 4. (A) Lactase dosage forms; (B) Challenges faced by oral drug delivery systems and strategies to overcome them; (C) Oral delivery systems that can potentially be used to encapsulate and protect lactase.

pills, and powders (Fig. 4A). Oral lactase preparations are easy to use and have a long shelf-life, which are particularly suitable for lactose-intolerant individuals with the need of long-term β-Gal intake (Raeisi Estabragh et al., 2021). They also have unique advantages for doctors and industry, such as flexible administration time, no need for specialized equipment or well-trained professionals, and low production costs (Liu et al., 2017).

3.2.1. Challenges faced by oral β-gal preparations

Oral β-Gal preparations must maintain sufficient activity to hydrolyze lactose in the small intestine. However, they are faced with harsh environments during processing, transport, storage, and ingestion, including freeze-drying, temperature changes, acidic pH values in the gastrointestinal tract, and the action of digestive enzymes. In its native form, these environmental attributes will change the three-dimensional structure of β-Gal, thereby considerably reducing its activity and therapeutic effect (Dan et al., 2020; Liu et al., 2017; Perry & McClements, 2020; Raeisi Estabragh et al., 2021).

3.2.1.1. Product stability. Oral β-Gal preparations are exposed to different temperature, light, oxygen, humidity, and mechanical stress conditions during manufacturing, storage, and transport (Kuchay, 2020). These environmental factors can denature, aggregate, or hydrolyze β-Gal, thereby reducing its activity. Therefore, it is necessary to design matrix and processing operations for oral β-Gal to ensure its integrity and avoid the negative impacts of environmental factors on its activity. β-Gal can be encapsulated in various oral delivery systems, which can change the microenvironment to make β-Gal more stable (McClements, 2018; Mesa, 2020; Perry & McClements, 2020).

3.2.1.2. Gastrointestinal stability. Oral β-Gal preparations must pass through the gastrointestinal tract to hydrolyze lactose in the small intestine. Several special features of the gastrointestinal tract may negatively affect β-Gal activity. Therefore, it is essential to understand the physical and chemical properties of the human gastrointestinal tract for better design of the oral delivery systems for β-Gal. Fig. 4B depicts the microenvironment of different parts of the human gastrointestinal tract. Oral cavity (pH of 5.0–7.0) is the first environment. The saliva in the mouth contains amylase and lipase, which break down starch and fat. In the oral cavity, the ingested substance is moderately sheared by chewing, with a residence time of 5–60 s before entering the esophagus. In general, very little protein is degraded in the oral cavity and esophagus (Xiong et al., 2020). The ingested substance then passes through the esophagus to reach the stomach, a highly acidic environment (pH 1.5–3.5) containing a variety of digestive enzymes (protease, amylase, and lipase). Direct exposure of β-Gal to gastric juice can destroy its activity. Therefore, the gastric environment is the main challenge faced by oral β-Gal preparations. The residence time in the stomach is generally 0.5–4.0 h, during which the substance will be digested into a thick semi-fluid form called chyme. Some simple chemical digestion may also occur in the stomach. After passing through the antrum and pylorus, the chyme enters the small intestine (pH 6.0–7.5), where the chyme is further digested into large molecules (proteins, fats, and polysaccharides) (Schubert, 2014). The pancreas and gallbladder provide enzymes to the small intestine, such as pancreatic alpha-amylase, protease, lipase, and bile salts, to facilitate digestion and absorption (Xiong et al., 2020). The residence time in the small intestine is about 1–2 h. Most of the ingested substances are degraded and absorbed after passing through the stomach and small intestine, but some substances, such as dietary fiber, are not thoroughly digested by this point. These substances will enter the colon (pH 5.0–7.0), where they are broken down by microorganisms (Xiong et al., 2020). Since the pH changes throughout the gastrointestinal tract, the oral delivery carrier for β-Gal needs to be pH-responsive, maintaining integrity in the acidic gastric environment to avoid β-Gal release, and decomposing slowly to release β-Gal in the small intestine with near-neutral pH.

3.2.2. Requirements for an oral β-gal delivery system

An oral delivery system must protect β-Gal activity and allow its release in the small intestine. Moreover, this system must be manufactured using food-grade processing operations and ingredients. It must also be sufficiently stable to meet the needs of commercial applications with a low cost (Perry & McClements, 2020). The following sections will highlight the essential factors that should be considered when designing and preparing a β-Gal oral delivery system.

3.2.2.1. Ingredient selection. Compared with that in other industries, the choice of ingredients in the food industry is much more restricted. Additionally, many consumers prefer products made from natural ingredients such as proteins, polysaccharides, phospholipids, and lipids from plants, meat, eggs, or milk. Furthermore, some consumers have special dietary requirements, such as kosher and vegetarian restrictions, which also limits the selection of food ingredients (Perry & McClements, 2020). The choice of ingredients will affect the functional properties of an oral delivery system. These properties will determine the location of the gastrointestinal tract in which they are digested and release β-Gal. Finally, considerations should also be given to the cost, shelf life, ease of use, and stability of the ingredients (McClements, 2018).

3.2.2.2. Stability. An oral delivery system should be stable and able to resist the influence of external factors, which can be ensured by appropriate composition and structure (McClements, 2018). To improve the bioactivity of orally ingested β-Gal, the delivery system needs to release β-Gal at an appropriate time and place in a targeted and sustained manner. Therefore, the oral delivery systems are designed to be pH-responsive and mucoadhesive (Fig. 4B). The pH-responsive delivery system can remain stable under acidic conditions and be disintegrated under neutral or alkaline conditions to achieve targeted release of β-Gal (Asgari, Pourjavadi, Licht, Boisen, & Ajalloueian, 2020). The mucoadhesive nature of the delivery system allows it to stick to the mucous layer of the small intestine, which can extend its residence time and allow sustained release of β-Gal, thereby improving the effectiveness of β-Gal (Malhaire, Gimel, Roger, Benoît, & Lagarce, 2016). Such delivery systems contain mucoadhesive polymers such as chitosan, cellulose derivatives, guar gum, xanthan gum, and alginate [56]. The interactions between these polymers and the mucous membrane (containing mucins) are mainly based on non-covalent bonds. Some polymers can also form covalent bonds. The carrier polymers have a high molecular weight, which can delay the release of β-Gal owing to steric hindrance (Swaminathan & Ehrhardt, 2012). The system should also be amenable to continuous industrial production at an appropriate scale and cost (McClements, 2018).

3.2.3. Different types of oral delivery system for β-gal

Various oral delivery systems with different structural designs have been developed for β-Gal, including emulsions, hydrogels, nanoparticles, and microparticles (Fig. 4C). The following sections will summarize the advantages and disadvantages of these delivery systems.

3.2.3.1. Emulsions. An emulsion is composed of two immiscible liquids (usually oil and water), with one dispersing in tiny droplets within the other (McClements, 2004). According to the relative positions of the oil and water phases, emulsions can be classified as oil-in-water (O/W) or water-in-oil (W/O) emulsions. Previous research has used O/W emulsions to prepare pH-responsive microparticles with large pores for efficient loading of β-Gal. The pores are closed under acidic conditions but opened under neutral conditions, by which the system protects β-Gal in the gastric environment and releases it in the intestinal environment. In simulated intestinal fluid (SIF), the residual activity of β-Gal in this system is about 15 times greater than that of commercial preparations

(Kumar, Montemagno, & Choi, 2017). However, this system requires a complicated manufacturing process, making it unsuitable for industrial large-scale production. Moreover, the preparation process involves an organic solvent not allowed for use in the food industry.

Compared with O/W emulsions, W/O emulsions are more suitable for encapsulating β-Gal owing to their internal hydrophilicity. However, this system is only suitable for encapsulating β-Gal after addition of a continuous lipid phase, such as salad oil, butter, spreads, or oil-filled capsules (McClements, 2004). This limitation can be overcome by further homogenizing the W/O emulsion with an aqueous phase containing a hydrophilic emulsifier to form a water-in-oil-in-water (W/O/W) emulsion (McClements, 2004). One study encapsulated β-Gal in a W/O/W emulsion prepared from gelatin, gum arabic, and potassium ions (Fig. S1), with a β-Gal encapsulation rate of 98.67%. This system improved the thermostability and storage stability of β-Gal. The system remained stable in simulated gastric fluid (SGF), and β-Gal was not released. In SIF, the system was demulsified, and β-Gal was released; the residual activity of β-Gal was 83% (Souza, Comunian, et al., 2019). The main disadvantages of W/O/W emulsion systems are that they are expensive and laborious to manufacture, requiring two homogenization steps and two emulsifiers (Jiménez-Colmenero, 2013). In another study, β-Gal was encapsulated in a solid-in-oil-in-water (S/O/W) emulsion, with an encapsulation rate of 76%. During pasteurization and storage, the emulsion remained evenly dispersed in milk and maintained β-Gal activity for three weeks. The S/O/W-encapsulated β-Gal was gradually released during the simulated digestion process, and could more effectively hydrolyze the lactose in milk than free β-Gal (Zhang & Zhong, 2017, 2018). However, the study did not perform sensory evaluation on the milk containing the S/O/W emulsion and investigated the system *in vivo*.

3.2.3.2. Hydrogels. Hydrogels are crosslinked hydrophilic polymer networks, and are biodegradable, biocompatible, and non-toxic. Thus, they are widely used in food, biology, pharmaceutical, and medical fields. The overall structural characteristics of hydrogels can be adjusted according to the need. A crosslinked hydrogel network structure was shown to protect β-Gal from a harsh environment. The characteristics of the crosslinking agent determine the pore size of the gel network, which affects the loading and diffusion of the enzyme (Liu et al., 2017). A silica gel composite material was successfully used to encapsulate β-Gal, protecting β-Gal from adverse external conditions (extreme pH and high temperature) (Nichele et al., 2011). Moreover, ionic hydrogels are widely used to encapsulate other biologically active proteins (Traffano-Schiffo et al., 2017).

Calcium alginate has also been successfully used to encapsulate β-Gal. In this system, guar gum was used as an excipient to enhance β-Gal stability during freeze drying and vacuum drying (Fig. 5A) (Traffano-Schiffo, Castro-Giraldez, et al., 2017). In another study, β-Gal was encapsulated in a gel formed by κ-carrageenan and potassium ions, with an encapsulation rate of 63% (Zhang et al., 2016). The efficiency of β-Gal release in SIF was two times greater than that of commercial tablets (Silva, Trevisan, & Garcia, 2020); however, the system could not prevent β-Gal inactivation in SGF. Addition of an antacid (magnesium hydroxide) to the system (Fig. 5B) to maintain the gel microenvironment at a neutral pH in SGF could prevent the loss of β-Gal activity (Zhang et al., 2017). A recent study compared the protective effects of gels formed by crosslinking carboxymethyl tuckahoe polysaccharide (CMP) with different metal ions (Fig. 5C). The hydrogel formed by CMP and aluminum ions was pH-responsive and could encapsulate β-Gal, which could maintain 72% of its activity after 24 h in a simulated digestion system (Deng, Pei, et al., 2020). However, ionic hydrogel formulations have many problems, including uncontrollable swelling, fragility, and chemical instability, which can lead to β-Gal leakage. To overcome these limitations, the hydrogel system can be coated. In one study, researchers used chocolate to coat β-Gal-loaded agarose, achieving sustained release of β-Gal in simulated digestive fluid (Nussinovitch et al., 2012). ε-Polylysine was successfully used to coat β-Gal-loaded alginate via electrostatic adsorption (Fig. 5D), which significantly increased the residual activity of β-Gal in SGF (94.6%) and SIF (76.0%) (Wang et al., 2018). Compared with cationic chitosan (Facin et al., 2015), ε-polylysine is more soluble in water and less sticky; therefore, it allows β-Gal to better retain its activity and has a higher encapsulation efficiency. In a recent study, a gastrointestinal synthetic epithelial lining system was developed to orally deliver β-Gal (Fig. 5E). This system contained a dopamine monomer and a small amount of hydrogen peroxide. When it encounters catalase in the small intestine, the hydrogen peroxide is quickly decomposed into oxygen by catalase, which will oxidize the dopamine monomer to polydopamine, forming a thin and robust polydopamine coating on the small intestine surface. Polydopamine has properties similar to those of glue, and can tightly adhere to the intestinal wall. This system was shown to increase lactose digestion in lactose-intolerant sows by 20 times (Li et al., 2020). No noticeable side effects were observed in pigs, but further preclinical safety studies are needed in other large animal models (such as dogs and non-human primates), as well as human clinical trials.

3.2.3.3. Nanoparticles. Nanoparticles of 1–1000 nm have been used for the oral delivery of biologically active proteins. For the loading of β-Gal, nanoparticles have been made from inorganic materials such as silica nanospheres and various polymers. Nanocapsules prepared with polylactic acid and hydroxypropyl methylcellulose phthalate (HP55) have been successfully used to encapsulate β-Gal, with an encapsulation rate of 90%. Compared with free β-Gal (61.7%), encapsulated β-Gal had a higher rate of lactose hydrolysis in milk (100%) (He et al., 2014). However, the polymers used to make these nanocapsules cannot be used in the food industry. Nanoparticles (NPs) prepared with β-chitosan were used to encapsulate β-Gal, with a loading rate of 68.32% (Fig. 6A) (Zhang et al., 2018). The addition of cellulose nanocrystals (CNCs) to these NPs enhanced the protection on β-Gal in an acidic environment. After 2 h in a pH 4.5 solution, the residual activity of β-Gal in CNC-containing NPs was 81.23%, while that in NPs without CNC was only 30% (Deng, Zhu, et al., 2020). However, the study did not perform *in vitro* simulated digestion experiments with this system. A recent study used NPs formed by zein and low-methoxy beet pectin to encapsulate β-Gal (Fig. 6B), achieving an encapsulation efficiency of 93.0%. The β-Gal-loaded NPs were added to milk, and less than 40% of the lactose in the milk was hydrolyzed after three weeks. After *in vitro* simulated digestion, the β-Gal was released, and 100% of the milk lactose was hydrolyzed (Dong & Zhong, 2019). The advantages of nanocarriers include reducing the enzymatic degradation of proteins in the gastrointestinal tract and achieving targeted delivery (Date, Hanes, & Ensign, 2016). At present, research on nanocarriers for use in oral lactase preparations is still preliminary, and there is a lack of *in vivo* studies. Nanocarriers are also faced with challenges of long-term stability and industrial scalability.

3.2.3.4. Microparticles. Microparticles range from 1 to 1000 μm in size and have been studied for their application in the oral delivery of β-Gal (Homayun & Choi, 2020). For instance, lipid-coated mesoporous silica particles were used to encapsulate β-Gal, achieving an encapsulation efficiency of 35% (Pavel et al., 2018). However, solid lipid microparticles prepared from tri-myristic acid glyceride could achieve over 95% of β-Gal encapsulation efficiency (Fig. 7A). The system remained stable in SGF, and the activity residual rate of β-Gal was 70% in SIF (Bertoni et al., 2018). However, these microparticles are composed of lipids, and therefore are difficult to be stored for a long time.

One study used hydroxypropyl methylcellulose phthalate to coat lactase-loaded microcapsules, which did not affect the physical, chemical, or sensory properties of the system (Ahn et al., 2019). In a recent study, hollow microcapsules were extracted from the spores of

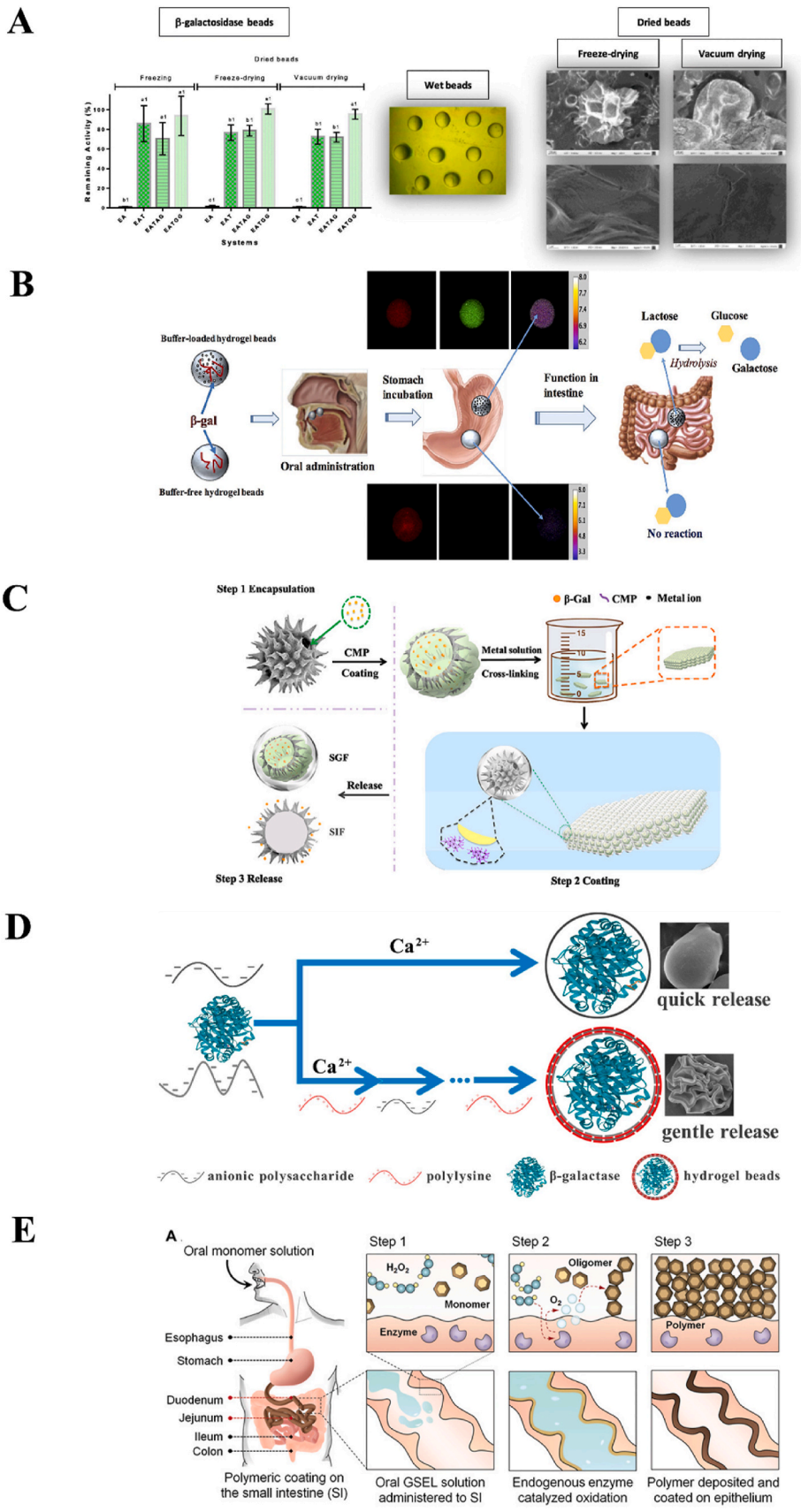

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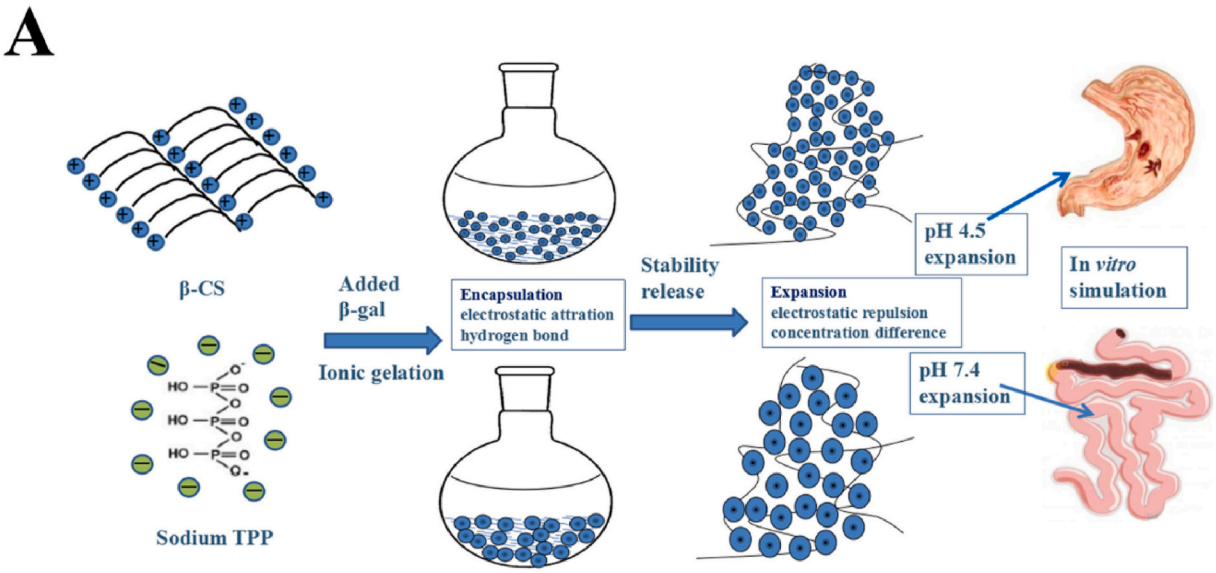
Fig. 5. (A) Effects of trehalose, gum arabic, and guar gums on the preservation of β-galactosidase activity in freeze-dried and vacuum-dried Ca (II)-alginate beads (Traffano-Schiffo, Castro-Giraldez, et al., 2017). Copyright 2022, Elsevier; (B) β-galactosidase (β-gal) encapsulation in hydrogel beads with self-regulating internal pH to retain enzyme activity after exposure to gastric conditions (Zhang et al., 2017). Copyright 2022, Elsevier; (C) Development of a new oral vehicle for β-Gal delivery based on a hydrogel formed by crosslinking carboxymethyl tuckahoe polysaccharide (CMP) and metal ions, including the encapsulation, coating, and release processes (Deng, Pei, et al., 2020); (D) A novel oral β-Gal delivery system based on sodium alginate/κ-carrageenan binary polysaccharide gel beads with and without ε-polylysine coating (Wang et al., 2018). Copyright 2022, Elsevier; (E) Gastrointestinal synthetic epithelial lining (GSEL) technology. Orally administered dopamine monomers in a GSEL solution rapidly oxidize in the presence of hydrogen peroxide (H2O2) via endogenous catalase activity and form a polydopamine coating on the small intestinal epithelial surface. Specific small intestine coating and targeting are achieved because of the uneven distribution of catalase along the digestive tract (i.e., high catalase expression in the small intestine) (Li et al., 2020) Copyright 2022, Science.



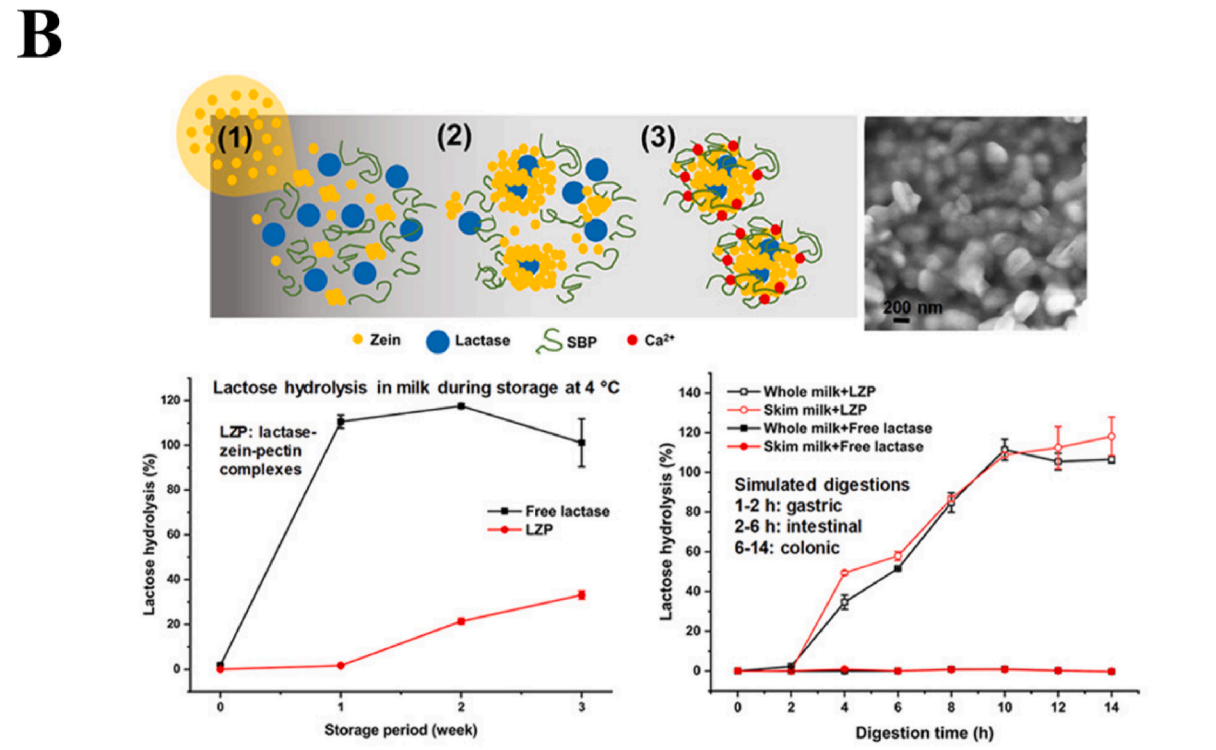


Fig. 6. (A) Use of β-galactosidase (β-gal)- loaded β-chitosan (β-CS) nanoparticles for *in vitro* digestion (Zhang et al., 2018). Copyright 2022, Elsevier; (B) Use of zein and low-methoxy beet pectin (SBP) to form composite nanoparticles to encapsulate lactase in milk (Dong & Zhong, 2019) Copyright 2022, ACS Publications.

Lycopodium plants (Fig. 7B), which were used to encapsulate β-Gal with a loading rate of 79.40% (Deng, Pei, et al., 2019). The researchers then screened various sources of plant-based microcapsules and finally promoted the β-Gal loading rate (82.75%) using microcapsules extracted from sunflower pollen (Deng, Wang, et al., 2020). Non-covalent and covalent methods were used to coat the β-Gal-loaded plant microcapsules. The results showed that covalent coating contributed to better targeted and sustained β-Gal release under simulated gastrointestinal

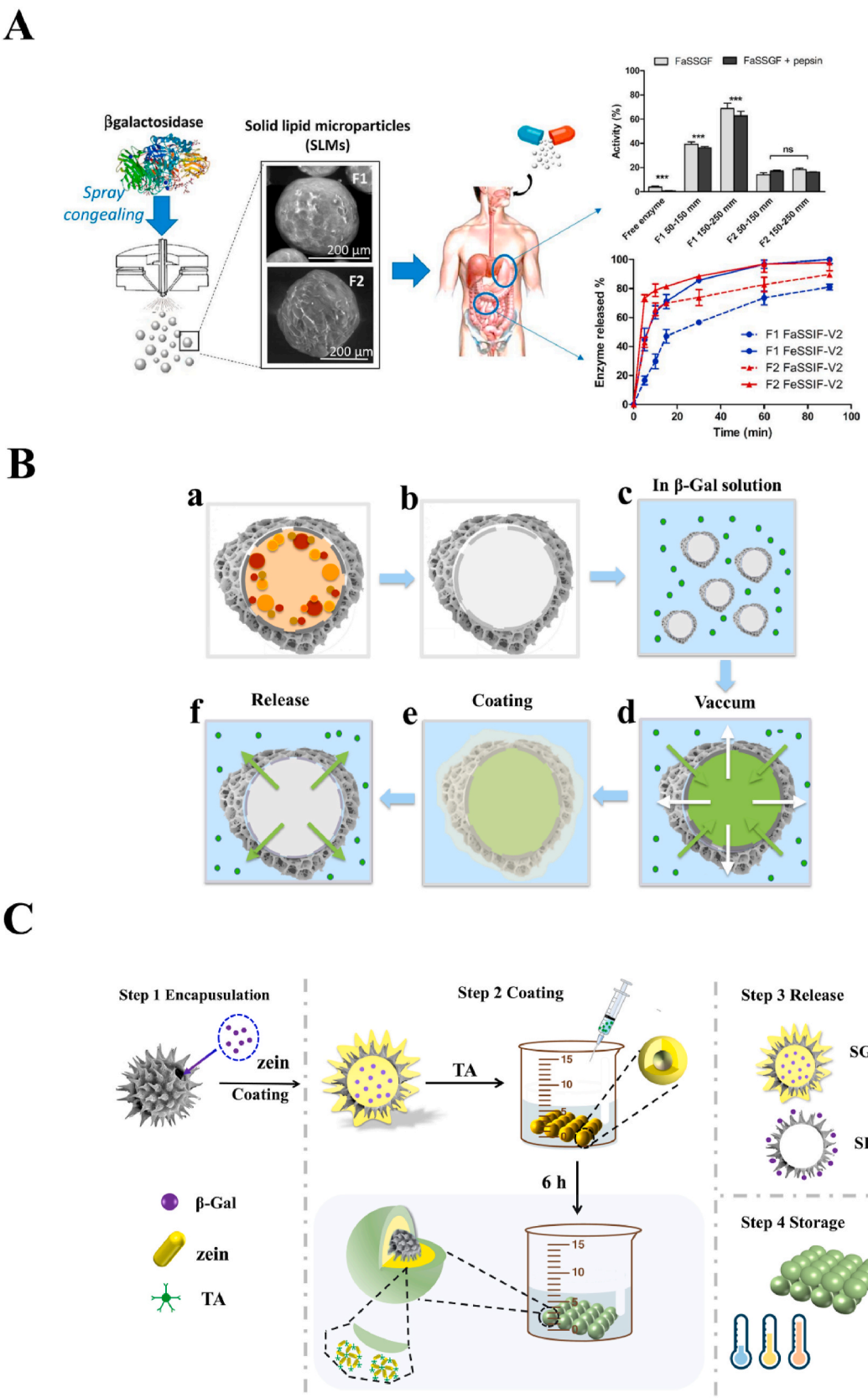


Fig. 7. (A) An oral lactase delivery system based on solid lipid microparticles prepared by spray coagulation of glyceryl trimyristate (Bertoni et al., 2018) Copyright 2022, Elsevier; (B) *Lycopodium. clavatum* sporopollenin exine capsules (SECs) and processing techniques for β-galactosidase (β-Gal) encapsulation. (a) Natural spores, (b) spores converted into empty shells, (c) and (d) encapsulation of β-Gal, (e) carboxymethylpachymaran (CMP) coating, and (f) β-Gal release under simulated gastrointestinal conditions (SGCs) (Deng, Pei, et al., 2019); (C) Development of a new oral vehicle for β-Gal delivery based on the interaction among SECs, zein, and tannic acid (TA), including the processes of encapsulation, coating, release, and storage (Deng, Wang, et al., 2021).

conditions than non-covalent coating (Fig. 7C). The residual activity of β-Gal in SIF exceeded 70%. In addition, after storage at 25 °C for 28 days, the residual β-Gal activity in this system exceeded 90% (Deng, Wang, et al., 2021). However, there have been no *in vivo* oral efficacy measurement and sensory evaluation on this system. In summary, microparticles have great potential in the food industry for the oral delivery of β-Gal, but further studies are needed to determine their effects on the human body and whether they can be economically produced.

3.2.3.5. Liquid β-gal. Liquid β-Gal is the most suitable for babies with difficulty in swallowing or chewing. It has been demonstrated that children under 6 years have difficulty in swallowing solid formulations, such as capsules, tablets, and pills (Schirm, Tobi, De Vries, Choonara, & De Jong-van den Berg, 2003). Liquid β-Gal preparations contain excipients that can protect β-Gal activity during processing and storage. Common excipients such as benzyl alcohol, glycerol, propylene glycol, and dextran can be used safely in adults but are toxic to children (Breitkreutz & Boos, 2007). Some commercial liquid β-Gal preparations contain almost 50% glycerol because it can stabilize β-Gal under heat treatment. However, excessive glycerol intake can cause nausea and dizziness in children. Therefore, the potential pediatric toxicity of excipients must be considered (Dan et al., 2020). Researchers have been devoted to developing a biocompatible, effective, and safe liquid β-Gal excipient for children (Deng, Li, et al., 2019; Mesa, 2020). Recently, some researchers have found that the oligosaccharides isomalto-oligosaccharide (IMO), xylo-oligosaccharide (XOS), and konjac-oligosaccharide can significantly increase β-Gal thermostability (Fig. S2). The circular dichroism, fluorescence, and Fourier transform infrared spectroscopy results have shown that these oligosaccharides can stabilize the secondary and tertiary structure of β-Gal under thermal conditions through hydrogen bond interactions. Under heat treatment, the residual activity of β-Gal with the optimal composition (30% IMO, w/v and 40% XOS, w/v) was 82.1%, which is significantly higher than that of native β-Gal (20%) (Deng, Li, et al., 2019).

3.2.3.6. Problems faced by oral delivery systems. At present, most relevant studies have been focused on the stability of oral β-Gal delivery systems in different pH environments. However, their stability is also affected by other factors in the human gastrointestinal tract, such as the concentrations of ions and enzymes. Ions can cause the aggregation or precipitation of some delivery systems through ionic interactions, and enzymes can degrade the delivery systems based on starch, protein, or lipids. Therefore, it is necessary to investigate how ions and enzymes influence the stability of oral delivery systems. Recent research has also focused on the *in vitro* release of β-Gal in oral delivery systems. Because multiple complex factors may affect their stability and bioactivity, *in vivo* studies are needed to prove the efficacy of these oral delivery systems. Moreover, the safety and nontoxic nature of the oral delivery systems also need further confirmation. *In vivo* experiments will be valuable to test the toxicity and side effects. In addition, the storage stability of oral β-Gal delivery systems has not been systematically studied. A combination of *in vivo* and *in vitro* experiments would facilitate the commercialization of food-grade oral β-Gal delivery systems.

4. Conclusion

Here, we comprehensively review the application of β-Gal in alleviating lactose intolerance. Traditionally, β-Gal was immobilized to remove lactose from food, which can continuously produce lactose-free products at an industrial scale, but this method has negative impacts on the flavor and nutritional value of dairy products. Furthermore, long-term absence of lactose in the diet can induce various diseases in humans. New methods to utilize β-Gal as a dietary supplement, which do not change the dietary structure, are more amenable to human health. Oral lactase preparations must be properly formulated because β-Gal is prone to denaturation, hydrolysis, and aggregation during processing, transport, and storage and in the human gastrointestinal tract. Decline of β-Gal activity will significantly reduce the effectiveness of these preparations. Different oral delivery systems for β-Gal have been extensively studied, including emulsions, hydrogels, nanoparticles, and microparticles. These systems can protect β-Gal activity in different phases. Future studies should clarify the *in vivo* stability and toxicity of these oral lactase preparations to promote their commercial application. Further development of oral lactase preparations has great potential, and this treatment method has become the first choice for individuals with lactose intolerance. This review may promote the development of advanced oral β-Gal delivery systems to relieve lactose intolerance.

Author statement

Ziyu Deng: Investigation, Analysis, and Writing- Original draft preparation. Qianchun Deng: Visualization and Review. Bin Li: Writing - Review & Editing. Jing Li: Conceptualization. Sangyong Jung: Conceptualization, Writing - Review & Editing. Nam-Joon Cho: Writing - Review & Editing, Supervision. Hongshan Liang: Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no competing financial interest.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2023.104244.

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