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Microencapsulation technology for lipase added infant formula to improve gastrointestinal digestion: a review

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ABSTRACT

The role of lipase in milk lipid digestion is critical since fat is the common source of infant nutrition. Human milk, the gold standard of infant nutrition, contains bile salt-stimulated lipase, which plays an important role in infant weight gain. In achieving the goal of infant formula to mimic human milk, researchers are concerned to add lipase in infant formula which is called LAIF (lipase added infant formula). The lipase is called bile salt stimulated lipase (BSSL). Since lipase is heat labile, microencapsulation is needed as an approach to stabilize lipase addition in infant formula. This article reviews the possibility of microencapsulation to optimize lipase addition. One type of encapsulate is alginate beads from algae, which can be used since it is commonly used in commercial powder infant formula. Milk proteins also an option since the proteins contains high nutritional values and categorized as safe materials. In the legume group, pea protein could be the prospective option since the material has good solubility in water, stability at high temperatures and good foaming capacity. Microencapsulation technology can be used to extend the shelf life and improve the stability of lipase in infant formulas.

Keywords: alginate beads; bile-salt-stimulated-lipase; milk protein; pea protein

INTRODUCTION

Human milk is the golden standard for infant feeding, while some groups of mothers cannot breastfeed their babies due to various reasons (Donovan et al., 2016; Sari et al., 2021). Infant formula is one of the alternatives for infant nutrition, as researchers and pediatricians try to improve the quality of infant formula by mimicking the contents of human milk. The development of infant formulas is progressing for both term and preterm infants (Estorninos et al., 2022)(Moreira-Monteagudo et al., 2022). Due to the special case of preterm infants who cannot digest their food optimally than the term ones, as well as the higher risk and difficulties for the mother to breastfeed, researchers need more improvement in preterm infant formula (Moreira-Monteagudo et al., 2022).

One of the main concerns in preterm infant formula

development is how to optimize digestion to gain more weight (Id et al., 2020). Fat is the main composition of human milk, which serves as the main source of energy (Delplanque et al., 2015). Fat digestion is considered as the most important part of infant weight gain, especially for the preterm infants. Previous studies have shown that the digestive system of breastfed infants is more efficient, while those who consume formula are excreted in feces (He et al., 2020). This paper reviewed the history of bile salt stimulated lipase (BSSL) invention and supplementation requirement, microencapsulation technology, and the possible microencapsulate source including alginate beads, milk protein, and pea protein.

BILE SALT STIMULATED LIPASE INVENTION IN INFANT FORMULA AND ADDING REQUIREMENTS

Newborn babies have an immature digestive tract, including the pancreatic organ, which produces bile salt stimulated lipase that helps digest fat. Human milk is supplemented with bile salt stimulated lipase (BSSL) in human milk which was found as a proof of better digestion of breastfeeding baby. The invention of BSSL enzyme in infant formula began in 1974 when Hernell studied two lipases in human milk (Hernell & Olivecrona, 1974). Recently, human milk bile salt stimulated lipase was found to have different pattern in each stage (Sari et al., 2023).

On July 31, 1990, a United States patent was published on dietary compositions and methods using bile salt-activated lipase, detailing the source and proportion of BSSL added and the formula base milk (Wang et al., 1990). Wang showed that the most effective dosage of BSSL addition is 1:200 (lipase:fat), which means 1 mg of BSSL in 200 mg of infant formula fat content. The above patent explained various methods for adding BSSL from the different sources, such as from pancreatic BSSL, milk BSSL from other species, genetic engineering, or the most popular one is recombinant human bile salt stimulated lipase (rhBSSL). Once the amino acid sequences of these enzymes or their functional fragments have been identified, synthetic BSSL or its active fragments can be artificially constructed. It will be appreciated that these various technologies can produce enzymes that are functionally similar but not structurally identical. The invention of rhBSSL has progressed to a 3 phase clinical trial conducted by Casper in 2016. This research found that there was no significant weight gain in premature infants from these samples (Casper et al., 2016).

Since BSSL is a heat-labile lipase, the condition of BSSL addition should be reviewed to optimize the effectiveness during infant formula processing, which usually uses thermal process for long-term use. Feeding strategy and formula production technology can be used for improving the result of BSSL added infant formula. The enzymes are either of prokaryotic or eukaryotic origin, isolated from fermentation broth or tissue, or expressed from recombinant gene sequences. The enzyme is provided in the form of an additive to the formula prior to feeding the infant or at the time of feeding. In the preferred form, the enzymes are provided in a matrix with an enteric coating that releases the enzyme in the upper portion of the intestine (Firm & Karlf, 2007). The enzyme should be added at a maximum ratio of 1:200 (BSSL: fat in infant formula) to optimize the function, even in 1:1000 still work (Wang et al., 1990).

MICROENCAPSULATION IN INFANT FORMULA

Microencapsulation is the process by which active ingredients (core materials) are packaged within a secondary material. The size of particles formed by encapsulation can be classified as macro (>5000 microns), micro (1.0-5000 microns), and nano (<1.0 micron). Capsules smaller than 1.0 micrometer are often referred to as nanocapsules (Choudhury et al., 2021; Trojanowska et al., 2017). The global microencapsulation will reach 13.70% to 19.35 billion dollars by 2025 (Yang et al., 2020). Microencapsulation is a common step to preserve the perishable materials and improve the digestibility, for example, in tuna oil could improve the DHA absorption in infant formula (Fard et al., 2020).

The method for encapsulation has been found in various methods including lyophilization, spray drying, extrusion, coacervation, complexation, and supercritical anti-solvent drying (Zabot et al., 2022). The specialty of infant formula composition that should be considered during encapsulation is the material composition that is safe as infant formula composition and the suitable process to preserve other and core material, beyond the function of encapsules to protect core material during digestion to reach its final destination. Since the function of BSSL is to improve the digestibility of fat, it should be released in the intestine after mixing with bile salt as a stimulator. It means that the encapsulated candidates should be robust in gastric condition to preserve the BSSL during gastrointestinal digestion. This review will focus on the encapsulation based on the alginate beads, milk protein and pea protein as the possible

encapsulates for lipase in infant formula.

ALGINATE BEADS ENCAPSULATES

Alginate is a product derived from algae that can be used to encapsulate lipase. Algae is also used in commercial powdered infant formula (PIF) as an encapsulation of decohexanoic acid (DHA). Alginate is a linear polysaccharide consisting of 1-4 linked β-(D)-glucoronic and α-(L)-mannuronic (M) acids derived from bacterial or brown algae sources. Alginate is commercially available and, due to its lack of toxicity and generally recognized as safe (GRAS) status, is well suited for use as an encapsulator in infant formula compositions. Research by (Zhang et al., 2016) showed that buffer-loaded hydrogel beads (microgels) can be prepared from food-grade ingredients using simple processing steps. It was also shown that these beads can be used to optimize the stability of lipase encapsulation under in vitro gastric conditions, but can be released smoothly under small intestinal conditions (Zhang et al., 2016). The research described the preparation of hydrogel beads in detail by using alginate solution (dissolved alginic acid in phosphate buffer and continuously stirred at 600C, then reduced at 350C with continuous stirring until completely dissolved). The alginate solution was then mixed with lipase to obtain the specific concentration and combined with or without Mg(OH)2 0.15%. The mixture was stirred continuously and injected into calcium chloride (CaCl2) 10% solution using a commercial encapsulation device with 120 µm vibrating nozzle to prepare the hydrogel beads. The encapsulation device was operated under fixed conditions: frequency 800 Hz, electrode 800 V, pressure 500 mbar. The formed beads were kept in Ca2+ solution for 30 min at ambient temperature to promote alginate cross-linking and bead hardening. The research also revealed the influence of encapsulation on the ability of lipase to perform lipid digestion, which was studied using an automated titration (pH-stat) method. Lipase-loaded beads, with or without co-encapsulated Mg(OH)2, were passed through the oral and gastric phases and then incubated with emulsified lipids. Meanwhile, the final result of free fatty acid (FFA) showed that single encapsulation of lipase using alginate beads was insufficient for protection in low gastric pH. Co-encapsulation of alginate beads with Mg(OH)2 was reported as more effective to protect the lipase activity as the core material. For infant formula application, further research might be needed in supplementing or replacing Mg(OH)2 in standardized

requirement, which is safe for newborn and infant consumption. Alginate beads were also used to control the lipase digestibility of emulsified lipids (Li et al., 2011). It showed that alginate had ability to protect under different pH in gastrointestinal track. Encapsulation of lipase was also found to be optimized by the presence of alginate beads and combined by chitosan as co-encapsulation (Pereira et al., 2018). Research by Pereira showed that the combination of chitosan can optimize the encapsulation of lipase from *Yerrowia lipolitica* in combination with lyophilization technology, as well as extend the shelf life at room temperature. However, the potential for application in infant formula needs to be further investigated.

MILK PROTEIN BASE ENCAPSULATES

Milk proteins are widely available, inexpensive, recognized as GRAS raw materials with high nutritional value. From a regulatory perspective, their use as encapsulating materials in infant formulas is not an issue as the majority of formulas, especially those containing cow's milk proteins. These proteins posse structural and physicochemical properties that make them natural vehicles for bioactive. These properties include high gelling properties, surface and self-assembly properties, small molecule and ion binding capabilities, and excellent buffering capacity against the harsh acidic environment of gastric hydrochloric acid. Milk protein encapsulation also worked in combination with starch (Eraso & Aníbal, 2014).

The method using rennet-induced gelation of milk protein and emulsification to encapsulate the bioactive component is usually used for probiotics. Meanwhile, it has possible option to be applied for lipase. As it can be used in infant formula, andget pass trough the gastric juice. Meanwhile, the proportion should be further investigated to modify it until the design suitable for release under intestine. Usually, milk protein encapsulation is used for enteric release. The milk protein encapsulation is suitable for both spray drying and lyophilization technology (Encina et al., 2016).

The encapsulation of the bioactive component using casein and denatured whey showed the efficacy of both combinations compared to the single component alone, achieving an encapsulation rate in 97%. Casein is a milk protein often used as a water-soluble matrix to protect the bioactive component during gastric transit. Casein is considered as an abundant,

cheap and easy to modify and useful as robust material as wall microencapsulate (Acuña-Avila et al., 2021).

The combination of casein and pectin was used by Oliveira et al. to form coacervates, which were then spray dried. This resulted in an impressive increase in the activity of the bioactive components at very low pH, which means stronger encapsulation under gastric conditions (Oliveira et al., 2007). Milk protein has also been used as a material to preserve the probiotics during storage (El-salm & El-Shibiny, 2015).

PEA PROTEIN ENCAPSULATION

Plant based protein is also an alternative for encapsulation of bioactive material that can be used in non-diary product, as groups of infants cannot consume the milk. In infant formula, pea protein may be suitable for encapsulating soy-based dairy products or other groups of specially designed infant formulas (Kent & Doherty, 2014).

Dry field peas and other grain legumes, commonly referred to as pulses, are the edible seeds of the pods of legume plants and are widespread throughout the world. Peas are widely used as a source of starch, fiber and protein due to the economics associated with fractionation, extensive growth and the simple dehulling process that removes the protective, fiber-rich outer seed coat. Pea protein isolate (PPI) has a number of beneficial functional properties such as good solubility in water, stability at high temperatures, good foaming capacity and high oil-in-water emulsifying power, and good shear and retort stability (Donsì et al., 2010). Protein could be the great option as it has also been reported to improve the delivery of conjugated linoleic acid and stabilize during shelf life (Costa et al., 2015).

The research conducted on the combination between pea protein and alginate showed the improvement of cell viability in the intestinal microbiota when compared to the alginate alone. Encapsulation can also be carried out in Bacillus adolescentis strain by pea protein and alginate. Pea protein helped to protect the component during gastric challenge and shelf-life study and rapidly degraded at the destination of bioactive designed released under gastrointestinal condition (Khan et al., 2013). Pea protein is gaining popularity due to its nutritional and functional properties (Bajaj et al., 2017).

CONCLUSION

The availability of BSSL in infant formula can be of great help in improving fat digestion, especially for low birth weight and preterm infants. However, the production and feeding strategy should be

selected since the recent phase 3 clinical trial did not show significant improvement in infant weight. Encapsulation might be one of the most possible option to improve the effective BSSL addition. Alginate, milk protein and pea protein were the three encapsulating materials that could be investigated in the future research to protect BSSL as core material and can be applied in commercial infant formula for longer shelf life. As the current trend in infant formula improvement is increasing, encapsulation of BSSL could be a good option to optimize lipid digestion.

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