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Freeze-dried capsules prepared from emulsions with encapsulated lactase as a potential delivery system to control lactose hydrolysis in milk

Yun Zhang, Qixin Zhong*

Department of Food Science, University of Tennessee, Knoxville, TN 37996, USA

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ABSTRACT

The objective of this work was to study solid/oil/water (S/O/W) emulsions as delivery systems with retained lactase in milk and controlled release during *in vitro* digestion. Spray-dried lactase powder was suspended in anhydrous milk fat/Span® 80 emulsified by sodium caseinate and lecithin (5:1). The S/O/W emulsion had an encapsulation efficiency of 75%, a hydrodynamic diameter of 292 nm, and a zeta potential of −17.37 mV. Cross-linking the dialyzed emulsion with transglutaminase eliminated the detection of free lactase after freeze-drying emulsions and the addition of sodium caseinate further preserved lactase activity. The hydrolysis of lactose in full-fat or skim milk after 3-week storage reduced from > 75% for free lactase to < 15% for encapsulated lactase. The encapsulated lactase was released gradually during the simulated digestions to hydrolyze lactose in milk more efficiently than free lactase. The present findings suggest S/O/W emulsions are potential delivery systems to incorporate lactase in milk products.

1. Introduction

Lactose is the most abundant solute in bovine milk and is hydrolyzed *in vivo* by lactase (β-galactosidase, EC 3.2.1.23) in the intestinal tract as an important source of energy (de Vrese et al., 2001). However, there is a large population of consumers, particularly American Indians and Asians, that are lactose intolerant due to the deficiency of lactase produced *in vivo*, including symptoms of watery stool, bloating and abdominal pain (Swagerty, Walling, & Klein, 2002). This causes the reduced consumption of bovine milk that has high quality proteins. To solve this challenge, lactose is hydrolyzed in milk by addition of lactase, followed by thermal inactivation of lactase before packaging as lactose free milk (Dahlqvist, Mattiasson, & Mosbach, 1973; Scrimshaw & Murray, 1988). In addition to differences in thickness (viscosity) and whiteness between regular and lactose-free milk products, the enzymatic hydrolysis of lactose to D-galactose and D-glucose increases the sweetness, and the extra thermal inactivation step can result in undesirable flavors due to the Maillard reaction (Harju, Kallioinen, & Tossavainen, 2012; Onwulata, Rao, & Vankineni, 1989). Therefore, there is a great need to develop technologies to supply milk to lactose-intolerant consumers.

A potential strategy is to encapsulate lactase for incorporation in milk, if the capsules can retain lactase during storage and release lactase *in vivo*. For this purpose, capsules have to be small enough to eliminate potential sandy texture, which would require a capsule dimension of smaller than 10 μm (Walstra, Walstra, Wouters, & Geurts, 2005), as well as prevention of sedimentation during storage. In addition, the low gastric pH and proteases in the digestive juices can result in losses in lactase activity (Xenos, Kyroudis, Anagnostidis, & Papastathopoulos, 1998). The encapsulated lactase is ideally released mostly in the intestines for hydrolysis of lactose in milk. Lastly, capsules shall be prepared with food grade ingredients, ideally dairy ingredients, for safety and labelling purposes. These practical aspects limit the application of a few encapsulation studies in the literature, for example that based on encapsulation in polylactic acid and pharmaceutical grade cellulose derivative (He, Zhang, & Sheng, 2014) and that based on medium chain triacylglycerols and polyglycerol monostearate (Kwak, Ihm, & Ahn, 2001). As such, much work is needed to provide solutions to the dairy industry.

To prevent the contact with lactose, lactase can potentially be dissolved in the inner droplets of water-in-oil-in-water (W/O/W) emulsions where the oil phase serves as a barrier preventing the contact with the outer continuous aqueous phase. As delivery systems, W/O/W emulsions contain a limited volume of inner water droplets that can reduce the overall ability to dissolve water-soluble compounds (Garti & Bisperink, 1998). Furthermore, the inner W/O interface can be destabilized during storage, strategies are needed to preserve the activity of water-soluble bioactive compounds during processing and storage, and the overall droplet size can easily exceed 10 μm in W/O/W emulsions (Couvreur, Blanco-Prieto, Puisieux, Roques, & Fattal, 1997;

* Corresponding author at: Department of Food Science, The University of Tennessee, 2510 River Drive, Knoxville, TN 37996, USA.
E-mail address: qzhong@utk.edu (Q. Zhong).

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Vasiljevic, Parojcic, Primorac, & Vuleta, 2006). Instead of water droplets, water-soluble compounds in the solid form can be included in oil droplets to form solid-in-oil-in-water (S/O/W) emulsions, which has been studied for superoxide dismutase and horseradish peroxidase in droplets emulsified by poly(D,L-lactic-*co*-glycolic acid) and/or poly(D,L-lactic acid) (Morita, Sakamura, Horikiri, Suzuki, & Yoshino, 2000). To relate to food applications, spray-dried glutamine powder (Zhang & Zhong, 2015) and probiotic bacteria (Zhang, Lin, & Zhong, 2015) have been encapsulated in S/O/W emulsions with anhydrous milk fat (MF) being the oil phase and dairy proteins – whey protein isolate or sodium caseinate (NaCas) being the emulsifier. Based on our earlier studies, we hypothesize spray-dried lactase powder can be encapsulated in S/O/W emulsions to retain lactase during storage and release lactase after the outer protein interfacial layer is digested by proteases after ingestion. To facilitate incorporation of encapsulated lactase in milk, the S/O/W emulsions are to be dehydrated, and we hypothesize treating the interfacial film by enzymatic cross-linking using microbial transglutaminase (mTGase) can improve the retention of lactase after dehydration using freeze-drying. Cross-linking caseins on emulsion droplets is well-established (Macierzanka et al., 2011), but has not been studied for the impact on retaining encapsulated water-soluble compounds after dehydration.

The first objective of the present study was to study the possibility of encapsulating spray-dried lactase powder in S/O/W emulsions that can retain lactase after freeze-drying and maintain dispersed after hydration in milk and subsequent refrigerated storage. The second objective was to characterize the hydrolysis of lactose in milk incorporated with freeze-dried capsules during refrigerated storage and simulated gastric and intestinal digestions.

2. Materials and methods

2.1. Materials

Commercial grade lactase (Maxilact® LGX5000F, in liquid form) and mTGase (Activa TG-TI, in powdered form) were purchased from DSM Food Specialties (San Diego, CA, USA) and Ajinomoto Food Ingredients, LLC (Chicago, IL, USA), respectively, and were used directly without further purification. Span® 80 and Glucose (HK) Assay Kit were procured from Sigma-Aldrich Corp. (St. Luis, MO, USA). Soy lecithin (laboratory grade) was ordered from Fisher Scientific (Pittsburgh, PA, USA). NaCas (93% protein, dry basis) was a commercial preparation from American Casein Co. (Burlington, NJ, USA). Anhydrous MF was a kind gift of Land O'Lakes, Inc. (Arden Hills, MN, USA). Horizon® organic skim and full fat pasteurized milk (Broomfield, CO, USA) were bought from a grocery store. Pepsin (product P7000, lot #SLBR2349 v, ≥250 U/mg solid), bile extract (product B8631, lot #SLBM3454), trypsin (product T0303, lot # SLG6471v, 13,000–20,000 BAEE U/mg), and pancreatin (product P1750, lot #SLBM4075v, 4×USP specifications) were purchased from Sigma-Aldrich Corp. (St. Luis, MO, USA). Other reagents were products of Fisher Scientific.

2.2. Enzymatic activity assay

The activity of lactase was quantified with a literature method (Jasewicz & Wasserman, 1961) with some modification. The substrate solution was prepared with lactose at 5.0% w/v in 0.01 M phosphate-buffered saline (PBS) at pH 7.0. An aqueous sample with lactase was adjusted to pH 7.0 using 1.0 M HCl or NaOH and was mixed with the substrate solution at a volume ratio of 1:2. After incubation in a shaking water bath at 37 °C for 5 min (New Brunswick Scientific Co., Edison, NJ, USA), the mixture was heated in a boiling water bath for 5 min to deactivate the enzyme, followed by cooling in an ice/water bath immediately. The glucose (HK) assay kit was used to quantify the amount of glucose produced after hydrolysis. The molar amount of lactose hydrolyzed was then used to estimate the activity of lactase with one unit (U) representing the ability of an enzyme sample to hydrolyze 1.0 μmol of lactose in one min at the test conditions.

Table 1
Summary of properties of commercial lactase preparation based on spray dried powder.*

Property	Protein content (mg/g)	Activity (U/g)	Specific activity (U/mg protein)
Value	643.76 ± 11.93	10876 ± 22	16.44 ± 0.37

* Numbers are mean ± standard deviation (n = 3).

Table 2
Efficiency of encapsulating (EE) lactase in S/O/W emulsions prepared with an O:W phase volume ratio of 1:6 or 1:8, and the arithmetic mean diameter ($d_{1,0}$) and zeta-potential of corresponding emulsion droplets at pH 7.0.*

O:W phase volume ratio	$d_{1,0}$ (nm)	Zeta potential (mV)	EE (%)
1:6	420.13 ± 8.93^{a}	-14.63 ± 1.53^{b}	70.26 ± 4.43^{b}
1:8	291.90 ± 1.78^{b}	-17.37 ± 1.08^{a}	74.70 ± 0.42^{a}

* Numbers are mean ± standard deviation (n = 3). Different superscript letters in the same column represent difference in the mean ($P < 0.05$).

Table 3
The amount of free and total lactase activity in suspensions hydrated with freeze-dried powder.#

Treatment before freeze drying	Lactase activity (U/g powder)*	
	Free	Total
None	20.68 ± 14.63^{d}	341.98 ± 58.50^{c}
mTGase	N/A	604.98 ± 36.56^{a}
mTGase + 5% NaCas	N/A	573.09 ± 30.47^{b}

Freeze-dried powder was prepared from emulsions that were dialyzed, added with mTGase to cross-link proteins on oil droplets, and freeze-dried with or without adding 5% w/v sodium caseinate (NaCas). Tween® 20 was added to the suspension to release encapsulated lactase to obtain total lactase activity in the powder.

* Numbers are mean ± standard deviation (n = 3). Different superscript letters in the same column represent difference in the mean ($P < 0.05$).

2.3. Preparation of spray-dried lactase powder and characterization of properties

The liquid form lactase preparation was spray-dried (model B290, BÜCHI Corporation, Flawil, St. Gallen, Switzerland) at a pump rate of 15%. The inlet temperature was 105 °C, and the outlet temperature was 56 °C. The powder was collected and stored at a −20 °C freezer before further use.

The protein content of the spray-dried powder was determined using the bicinchoninic acid (BCA) method (Walker, 2009). Reagent A (Bio-world, Dublin, OH, USA) and Reagent B (prepared by dissolving 0.4 g $CuSO_4 \cdot 5H_2O$ in 10.0 mL of deionized water) were mixed at a volume ratio of 50:1 to prepare a working reagent. After mixing a lactase sample with the working reagent and incubation at 60 °C for 30 min, samples were cooled to room temperature, and absorbance at 562 nm (Evolution 201, Thermo Scientific, Waltham, MA, USA) was measured to determine protein content based on a standard curve created using standard solutions of bovine serum albumin.

Spray-dried lactase powder was dissolved at 40.0 mg/mL in PBS. To characterize the thermal stability of lactase, the lactase solution was heated in a water bath at 72 °C for 0.5, 1, 5, 10, 20 and 30 min, followed by immediate cooling in an ice/water bath. The activity of lactase before and after heating was measured using the above method. The deactivation data were fit to the first-order kinetics to estimate the percentage of lactase loss after heating at 72 °C for 15 s, which are thermal pasteurization conditions of Grade A milk (FDA, 2011).