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Stabilization of alpha-lipoic acid by complex formation with octenylsuccinylated high amylose starch

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ABSTRACT

The thermal and oxidative stability of alpha-lipoic acid (ALA) in aqueous dispersions containing beta-cyclodextrin (CD), native high amylose (HA) and octenylsuccinylated high amylose (OS) starches (0.1% ALA and 1.0% CD or starch solids) were compared. Both native and modified starches increased the stability of ALA against thermal degradation and oxidation at higher degrees than CD. The OS was more effective in stabilizing ALA than HA. The ALA loss in the dispersions occurred mainly in the supernatant, suggesting that the complex formation of ALA with amylose played a key role in the stabilization. In an *in vitro* digestion test, the release of ALA from OS dispersion was less than that of HA dispersion, indicating that ALA complexed with OS amylose was most stable against digestion. The octenylsuccinylated high amylose starch was an effective protecting agent for ALA in aqueous media, as well as a delivery carrier for ALA in digestive tract.

1. Introduction

Alpha-lipoic acid (1,2-dithiolane-3-pentanoic acid; ALA), a medium chain (C_8) fatty acid with an intramolecular disulfide bond, serves as a cofactor in the mitochondrial energy metabolism in prokaryotic and eukaryotic cells (Packer, Witt, & Tritschler, 1995). It contains a cyclic disulfide, which has an ability to create a low redox potential and thus provides a strong antioxidant activity. There has been a growing interest in the therapeutic application of ALA to prevent various diseases associated with oxidative stress (Holmquist et al., 2007; Smith, Shenvi, Widlansky, Suh, & Hagen, 2004). Currently, ALA is used as a dietary supplement (Shay, Moreau, Smith, Smith, & Hagen, 2009) and a functional cosmeceutical to retard the adverse effects from aging (Beitner, 2003). However, the utilization of ALA, due to its poor water solubility and the instability against oxidation and thermal process, has been hampered. The distorted five membered ring of dithiolane may allow ALA to polymerize, especially during the thermal process at a temperature above its melting range (48–50 °C). The polymerization and oxidative degradation of ALA result in the loss of its bioactivity and formation of unpleasant sulfurous odor. The elimination rate of ALA with a biological half-life of less than 30 min (Teichert, Hermann, Ruus, & Preiss, 2003) hinders its clinical application. Therefore, it needed a strategy to enhance its stability and bioavailability of ALA.

Entrapment within structural cavities provided by polymeric carriers is an approach widely practiced to stabilize unstable ingredients. In addition to stabilizing effect, it may control the release or delivery of entrapped ingredients, improving its bioavailability in human (Dima, Dima, & Iordăchescu, 2015).

Water solubility of a bioactive substance is another factor determining its biological half-life and bioavailability (Lobenberg, Vieira, & Amidon, 2000). Reducing particle size of an insoluble substance improves its dispersion rate and storage stability, which often enhances bioavailability. Accordingly, encapsulation to nano-sized particles has been suggested as an effective technique to enhance water dispersibility and bioavailability of various non-polar bioactive substances (Rabinow, 2004). Nano-sized encapsulation based on self-assembly of carrier substances such as cyclodextrin and amylose has been suggested as one of the effective processes in preparing aqueous dispersions of hydrophobic substances (Augustin & Hemar, 2009; Kim & Lim, 2009; Kim, Seo, & Lim, 2013).

Starch has been extensively studied in its use as a carrier vehicle for a variety of bioactive substances (Zuidam & Nedović, 2009) because it is cost-effective and safe. Amylose, an essentially linear starch chains, has an ability to form a stable complex with polyunsaturated fatty acids serving as a protective vehicle against oxidation and a carrier for delivery (Lalush, Bar, Zakaria, Eichler, & Shimoni, 2005; Yang, Gu, & Zhang, 2009). It allows hydrophobic guest molecules to incorporate in its core by forming a signal helix, so called V-crystalline complex. Alternatively, modified starches have been tested as carrier materials to control the release of bioactive agents or flavor ingredients. Notably, a substitution with amphiphilic

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octenylsuccinyl groups on starch molecule endows the starch with an improved miscibility and emulsifying ability for hydrophobic components. This modification could enhance the dispersion rate of hydrophobic bioactive agents, and retention of flavors (Baranauskiene, Bylaite, Zukauskaite, & Venskutonis, 2007; Liang, Shoemaker, Yang, Zhong, & Huang, 2013; Paramera, Konteles, & Karathanos, 2011). Furthermore, hydrolyzed octenylsuccinylated starches are commercially available as the substitutes of gum Arabic to encapsulate various food ingredients (Bhosale & Singhal, 2006). In a previous study, it was found that an octenylsuccinylated high amylose starch improved the dispersion ability and storage stability of ALA in aqueous media through the V-type amylose complex formation. These effects were higher than that provided by unmodified high amylose starch (Li & Lim, 2016).

In the present study, the feasibility and efficacy of high amylose starches, both in native and in octenylsuccinylated states, were tested as protective agents for ALA against thermal degradation and oxidation, in comparison with those of beta-cyclodextrin. Additionally, the release of ALA from the amylose complex during an *in vitro* digestion was investigated using simulated digestive tracts.

2. Materials and methods

2.1. Materials

High amylose maize starch (HA, 70% amylose) was obtained from Ingredion Incorporated (Bridgewater, NJ, USA). Alpha-lipoic acid (ALA, > 98%), beta-cyclodextrin (β-CD), 2-octen-1-ylsuccinic acid anhydride (OSA), pepsin (P7125, from porcine gastric mucosa), pancreatin (P7545, from porcine pancreas), bile extract (B8631, from porcine), amyloglucosidase (A9913, from *Aspergillus niger*) and glucose assay kit (GAGO-20) were all purchased from Sigma-Aldrich Company (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Preparation of ALA dispersions

High amylose maize starch was modified using 2-octen-1-ylsuccinic acid anhydride (3% based on the dry starch solids) following the previously reported method (Li & Lim, 2016). The octenylsuccinylated high amylose starch (OS, 600 mg, dry weight) was dispersed in a NaOH solution (1.0 M, 3 mL) to gelatinize the starch, and then distilled water (54 mL) was added to the starch solution. The starch solution was neutralized by adding 0.1 M HCl solution followed by adjusting the total volume to 60 mL with distilled water. Then the starch solution was autoclaved for 20 min at 121 °C to complete gelatinization subsequently after nitrogen flushing. The pre-dissolved ALA (60 mg in 0.3 mL absolute ethanol) was added to the starch solution after being cooled to 70 °C. The mixture was incubated at 70 °C for 3 h while stirring, and then was allowed to cool to room temperature with continuous stirring (12 h). The ALA dispersions were also prepared under the identical procedure using either native high amylose starch (HA) or β-cyclodextrin (CD) for comparison. The dispersions were then treated by ultrasonication (VCX 500, Sonics & Materials Inc., CT) for 3 min at 40% amplitude with on-off cycles of 3 s each in an ice bath. To prevent ALA from photo-oxidizing, exposure to light was minimized during the dispersion process by covering the glass container with aluminum foil.

2.3. Quantification of ALA

The ALA content in various dispersions was measured using a capillary gas chromatography. The samples were firstly hydrolyzed with methanolic NaOH (0.5 M, 5 mL) in a flat-bottom flask equipped with a condenser for 10 min in a boiling water bath. Then they were methylated with boron trifluoride-methanol solution (14% v/v, 5 mL), and were extracted with hexane (5 mL). The methylated samples were then analyzed with a Hewlett Packard 5890 gas chromatography equipped with an FID and an Omegawax capillary column 320 (30 m × 0.32 mm × 0.25 μm film thickness). The column temperature was initially kept at 140 °C for 3 min, then was gradually increased to 200 °C at a rate of 10 °C/min and maintained for 3 min, and finally was increased to 230 °C at a rate of 5 °C/min and was maintained for 4 min. Both injector and detector temperatures were equally 280 °C. Then ALA content was quantified using methyl heptadecanoate as an internal standard. The percentage ALA retention was defined as the remaining ALA content in the whole dispersion or supernatant or precipitate divided by the ALA content initially added during the preparation of the dispersion (×100).

2.4. Stability of ALA dispersion

The stability of ALA in the dispersions containing CD or starches was tested after different treatments including thermolysis, accelerated oxidation and UV irradiation.

The thermal treatment was carried out by heating the ALA dispersions at 100 °C for 50 min in a water bath after being flushed with nitrogen with continuous stirring (500 rpm). The ALA content in whole dispersions as well as that in supernatant and precipitates obtained by centrifuging the dispersions (20,000 × *g*, for 20 min at 4 °C) were determined.

The accelerated oxidation was performed by adding hydrogen peroxide solution (0.1 M, 5 mL) to the ALA dispersions (50 mL) and incubating the mixture while stirring (500 rpm) for 2 h. The UV irradiation was conducted by exposing the ALA dispersions to UV light (G4T5E UV-B, Sankyo Denki, Tokyo, Japan) while stirring (500 rpm) for 24 h. The distance between the bottom of the glass vial and the UV lamp was kept at 10 cm.

The percent loss of ALA was calculated by measuring the ALA contents before and after the treatments.

2.5. In vitro digestion

The *in vitro* digestion of the ALA dispersions was performed under simulated sequential gastric and intestinal conditions (Englyst, Kingman, Hudson, & Cummings, 1996; Lalush et al., 2005). Simulated gastric fluid (SGF) composed of pepsin dissolved in 0.1 M HCl (4 mg/mL, pH 2.0), and simulated small intestinal fluid (SIF) composed of pancreatin (4.4 mg/mL) and bile extract (2 mg/mL) dissolved in a potassium phosphate buffer (20 mM, pH 7.0) were used. Additionally, amyloglucosidase was added into the SIF as the brush border enzyme in human intestine. The ALA dispersions (50 mL) were mixed with 10 mL basal saline (140 mM NaCl, 5 Mm KCl, and 150 μM butylated hydroxytoluene) to mimic the natural perfusion under normal digestion, and then the pH of the mixture was adjusted to 2.0 by adding 0.1 M HCl. An aliquot (7.5 mL) of the SGF was then added to the dispersion and the mixture was incubated at 37 °C for 1 h. After neutralization by adding $NaHCO_3$ solution (0.2 M), an aliquot of the SIF (15 mL) was added to the dispersion and the mixture was incubated at 37 °C for 12 h. To induce churning, the digestion was performed in a shaking water bath (170 rpm) with eight glass balls (10 mm diameter) added to the dispersions. At time intervals during the simulated digestion, aliquots (5 mL each) were taken from the dispersions for the ALA analysis. Meanwhile, another aliquot (5 mL) was taken, mixed thoroughly with absolute ethanol (10 mL), and the mixture was centrifuged for 20 min (20,000 × *g*, 4 °C). The ALA retention in both supernatant and precipitate based on the ALA initially added were analyzed.

2.6. Statistical analysis

Experiments were carried out at least in triplicate, and data were presented as the averages and standard deviations. Statistical analysis was performed by the one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS17.0, IBM Corp., New York, USA).