Food Hydrocolloids 24 (2010) 347–357

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Structural characterization of amylose-long chain fatty acid complexes produced *via* the acidification method

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ARTICLE INFO

Article history:
Received 13 August 2009
Accepted 30 October 2009

Keywords:
Amylose inclusion complexes
Lamellar model
Encapsulation
Small angle X-ray scattering (SAXS)
Unsaturated fatty acids

ABSTRACT

Amylose molecular inclusion complexes, or V-amylose, have been studied as a possible nano-sized delivery system for unsaturated fatty acids. This study aimed to study three different structural levels of V-amylose produced via an acidification method. Molecular attributes were studied using XRD, DSC and ^{13}C CP/MAS NMR, nanostructures using SAXS and AFM, and the microscopic level by SEM and AFM. ^{13}C labeled fatty acids revealed head groups were entrapped in both COO- and COOH forms. SAXS data, showed that conjugated linoleic acid yield particles with the highest values for parameters like average crystalline lamellar thickness ($\varphi = 0.46$) and characteristic particle dimension ($R_g = 1011$). AFM revealed surface roughness increases from 7.72 ± 4.34 nm to 11.54 ± 6.05 nm during the formation of V-amylose. The insights described contribute to the understanding of V-amylose structure and help establish a model for V-amylose structure which may prospectively be used in the fabrication of a novel delivery system.

1. Introduction

Consumption of poly-unsaturated fatty acids (PUFA) has been enthusiastically advocated due to their essential role in human health. Studies have shown PUFA support improved performance of the immune system, reduce blood pressure, decrease the chance for heart attacks and the recurrence rate of certain cancers (Shahidi & Miraliakbari, 2004, 2005). Familiar to the public as omega 3 and omega 6 fatty acids, PUFA include 2 or 3 methylenes interrupted by double bonds which render them susceptible to heat, light and oxidation. PUFA cannot be synthesized by the human body and should be provided with the diet however the daily uptake is, on average, lower than the recommended amount. Enrichment of food products with PUFA is a challenging technological task due to their tendency to degrade and autoxidize at high rates during production, storage, and passage in the digestive system. Various encapsulation platforms have been suggested to be suitable for the controlled delivery of lipophilic nutraceuticals such as PUFA and omega 3 rich oils (Barrow, Nolan, & Jin, 2007; Lalush, Bar, Zakaria, Eichler, & Shimoni, 2005; McClements, Decker, & Weiss, 2007; Semo, Kesselman, Danino, & Livney, 2007). This study focused on amylose-based molecular inclusion complexes or V-amylose as a prospective controlled delivery system for PUFA, as suggested in previous work (Lalush et al., 2005; Lesmes, Barchechath, & Shimoni, 2008; Lesmes, Cohen, Shener, & Shimoni, 2009; Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled, 2009).

The molecular organization of amylose complexes with various fatty acids has been studied extensively and complex formation has been shown to be affected by parameters such as complexation temperature and lipid structure (monoglyceride or free fatty acid), with increased fatty acid (FA) chain length and decreased unsaturation increasing V-amylose thermal stability (Biliaderis & Galloway, 1989; Godet, Buleon, Tran, & Colonna, 1993; Godet, Bizot, & Buleon, 1995; Tufvesson, Wahlgren, & Eliasson, 2003b). Also, two main crystalline polymorphic forms have been identified. Type I is considered to be amorphous, while the semi-crystalline type II displays three peaks at Bragg angles of 7.4°, 13.1° and 19.8° in its X-ray diffraction pattern (Biliaderis & Galloway 1989; Lesmes et al., 2009). However, the nanostructure of V-amylose has been explored to a lesser extent compared to its molecular structure. Transmission electron microscopy (TEM) micrographs of amylose fatty acid complexes revealed uniaxial layout of amylose molecules, which were locally interrupted by amorphous segments with a thickness of no more than 4.6 nm (Godet, Bouchet, Colonna, Gallant, & Buleon, 1996). Other studies suggest amylose–alcohol complexes have

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doi:10.1016/j.foodhyd.2009.10.015

a lamellae folding length of about 10 nm (Biliaderis & Galloway 1989; Jovanovich & Maria, 1999). Recently AFM work has shown that V-amylose also exhibits an aggregative nature, with aggregates being comprised of small spherulites of ~50–100 nm, lamellae of a few microns in length and ~10 nm of thickness and some other ill-defined structures (Lalush et al., 2005; Lesmes et al., 2009).

Understanding the structure and physicochemical properties of V-amylose is believed to be important for the future design and fabrication of such systems (Lesmes & McClements, 2009; Mcclements, Decker, & Park, 2009; McClements, Decker, Park, & Weiss, 2008). Accordingly, we have already investigated the effect of guest chemistry on the structure of V-amylose inclusion complexes produced via dilution of dimethylsulfoxide (Zabar et al., 2009). To the best of our knowledge, this was the first study to combine molecular level investigations with nanostructure and microscopic characterization. The effect of fatty acid unsaturation on V-amylose structure has been shown to span throughout the different structural strata studied. In the current study, we broaden our previous research toward a different production method more suitable for food applications. Thus, amylose complexes with fully saturated 18:0 stearic acid (SA), 18:2 linoleic acid (LA) and naturally occurring 18:2 conjugated linoleic acid isomer mixture (CLA) were produced using a previously described acidification method. X-ray diffraction (XRD), ^{13}C solid sate CP/MAS NMR and differential scanning calorimetry (DSC) were used to study the molecular organization and thermal properties of the amylose complexes produced while Small angle X-ray scattering (SAXS), scanning electron microscopy (SEM) and atomic force microscopy (AFM) probed the nano and micro structures of the complexes. The effect of the production method as well as the effects of the various guest molecules on the structural characteristics is discussed.

2. Experimental

2.1. Materials

2.1.1. Potato amylose (Av. DP 900)

Potato Amylose (Av. DP 900) is essentially free of amylopectin was purchased from Sigma Co., Israel (A0512) and used as received.

2.1.2. Long chain fatty acids

The complexation experiments were conducted using three different fatty acids of various degrees of unsaturation. Fully saturated 18:0 (18 carbons and 0 double bonds) stearic acid (SA) (Sigma S-4751), 18:2 cis-9,cis-12-octadecadienoic acid or linoleic acid (LA) (Sigma L-1376); and 18:2 mixture of cis-and trans-9,11 and −10,12-octadecadienoic acids or conjugated linoleic acid (CLA) (a mixture of cis-and trans-9,11 and −10,12-octadecadienoic acids. Linoleic acid <1% – (Sigma O-5507); all of at least 99% purity. Additionally, molecular level studies aimed at determining the positioning of fatty acid in V-amylose used uniformly labeled $^{13}C_{18}$-stearic acid (605 581) and uniformly labeled $^{13}C_{18}$-linoleic acid (605 735), both with at least 99% isotope enrichment were used. Complexes Produced with these fatty acids were used in solid state ^{13}C CP/MAS NMR experiments.

2.1.3. Other reagents

Potassium Hydroxide (KOH), Hydrochloric Acid (HCl), and all other reagents were analytical grade chemicals.

3. Methods

3.1. Formation of V-amylose molecular inclusion complexes

Production of V-amylose complexes via acidification of an alkali solution mixture of amylose and guest fatty acid dilution was carried out based on a method previously described (Eliasson & Krog, 1985; Karkalas, Ma, Morrison, & Pethrick, 1995; Lalush et al., 2005). 600 mg of amylose were dissolved in 40 mL of preheated (90 °C) 0.1 M KOH then cooled to crystallization temperature of 30 °C, 60 °C or 90 °C. Similarly, an alkali fatty acid (FA) solution (60 mL, 1 mg/ml, 0.1 M KOH) preheated to 90 °C, was cooled to the same crystallization temperature of 30 °C, 60 °C or 90 °C and then mixed together with amylose solution. The solution mixture was titrated under gentle stirring to a final pH of 4.7 using 2 M HCl solution. The resulting suspension was incubated at a constant temperature for 24 h under gentle stirring. At the end of incubation phase, the suspension was cooled to 25 °C.

3.1.1. Separation of the complexes

Separation of the V-amylose from the suspensions was done by centrifugation (2000 g, 20 min). The wet pellet was washed using 50% ethanol/water mixture (v/v) and centrifuged as before. This step was repeated three times to remove residues of uncomplexed FA, and to obtain salt-free complexes, before the resulting pellet was transferred to petri dishes, freeze dried and pulverized into a fine powder.

3.2. Molecular level investigations

Investigation of the molecular level characteristics of the amylose-FA complexes powders produced was studied through X-ray diffraction (XRD), ^{13}C solid state CP/MAS NMR (ssNMR) and differential scanning calorimetry (DSC). These methods have already been successfully and extensively used by others to verify formation and study V-amylose inclusion complexes (Biais, Le Bail, Robert, Pontoire, & Buleon, 2006; Bulpin, Welsh, & Morris, 1982; Godet, Bizot, et al., 1995; Godet et al., 1993; Godet, Tran, Colonna, Buleon, & Pezolet, 1995; Jouquand, Ducruet, & Le Bail, 2006; Kawada & Marchessault, 2004; Lalush et al., 2005; Le Bail, Rondeau, & Buleon, 2005; Tozuka et al., 2006).

The formation of a V type amylose-FA complex was verified by measuring the X-ray diffraction of powders produced from the suspensions. These XRD measurements were carried out on a Philips PW 3020 powder diffractometer equipped with a graphite crystal monochromator (Philips, The Netherlands). The operating conditions were $CuK_\alpha 1$ radiation ($\lambda = 0.154$ nm), voltage 40 kV and current 40 mA. Approximately 200 mg of sample powders were loaded onto a poly(methyl methacrylate) plate and scanned over the angular range of 2θ from 5° to 35° with step size 0.02°. Counting time was 4 s per step.

The thermal properties of the amylose-FA complexes were studied by DSC. These were obtained from the heating curves of obtained for 7 mg of powdered sample suspended using 21 mg of double distilled water placed in a sealed stainless steel DSC pan (Perkin–Elmer stainless steel pressure-tolerant pans). These curves were determined using a Perkin Elmer DSC- 7 system (The Perkin Elmer corp., Norwalk Conn, USA). The system was first calibrated using Indium and then samples were measured against a 20 mg pure water reference pan. Samples were scanned from 25 °C to 150 °C with a 5 °C/min ramping. The transition temperatures and enthalpies were calculated using the Pyris thermal analysis system version 3.72 of Perkin Elmer LLC.

Further molecular level characterization of the amylose-FA complexes was achieved through ^{13}C CP/MAS solid state NMR spectroscopy measured by a 300 MHz chemagnetics-Infinity spectrometer operating at 75.45 MHz. Lyophilized powder samples were packed into 7.5 mm zirconia rotors and spun at 5000 Hz at the Magic Angle. The ^{13}C spectra were obtained by direct excitation of the ^{13}C nuclei and by cross polarization achieved via ^{1}H nuclei adjacent to the resonating carbons.