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Molecular encapsulation of linoleic and linolenic acids by amylose using hydrothermal and high-pressure treatments

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ABSTRACT

The health benefits of polyunsaturated fatty acids are well known, however their fragility is a drawback as it leads to their degradation. The objective of this study was to develop innovative assemblies, using the capacity of starch to encapsulate small molecules, for the protection of polyunsaturated fatty acids like linoleic acid and linolenic acid. These assemblies were produced by hydrothermal treatment, and by high-pressure treatment (20 min at 500 MPa at a temperature of 40 °C). They were then analyzed and characterized by X-ray diffraction (XRD), differential scanning calorimetry (DSC), nuclear magnetic resonance (^{13}C CP/MAS NMR) and a heating cell in XRD. The results showed that amylose–ligand complexes were formed during both treatments and two crystalline types V_{6I} and V_{6II} were obtained. The techniques used show excellent complementarities in the determination of important structural features such as crystalline type, helical conformation and nature of the inclusion.

1. Introduction

The primary role of the diet is to provide the body with energy and essential nutrients for its development and protection. However, because of the profound changes in lifestyle in industrialized countries during the 20th century, the composition of the diet has changed dramatically. Studies emanating from statistical institutes and epidemiological research have found an excess of unhealthy saturated fats and sugar and a lack of fruits, vegetables, dairy products and fish in the modern diet. In nutritional terms, this results in an overconsumption of saturated fat and trans fatty acids, an increase in the ratio of n−6 to n−3 fatty acids, a decrease in fiber intake, and a decrease in micronutrient intake (vitamins and minerals). These findings have implications for health and contribute to the development of certain chronic diseases such as cardiovascular diseases and cancers.

An awareness of the consequences of diet on health has led organizations that monitor health to make recommendations about nutrition in order to contribute to improving public health. Research laboratories and food manufacturers have responded to these recommendations by putting on the market food products designed to be beneficial for nutrition and health. Thus, many products enriched with n−6 or n−3 fatty acids, vitamins, minerals, antioxidants, or fibers have been developed, which claim to help combat or prevent various pathologies. However, the addition of some of these constituents in food is not without risk. For example, unsaturated fatty acids are fragile and easily oxidized molecules (Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled, 2009). It is therefore necessary to protect them to ensure their health benefits and avoid the formation of potentially toxic degradation products (Gökmen et al., 2011). To overcome these drawbacks, various encapsulation techniques have been studied and are currently utilized (Barrow, Nolan, & Jin, 2007; Lamprecht, Schäfer, & Lehr, 2001; Serfert, Drusch, & Schwarz, 2009; Xu et al., 2013). These techniques, such as those involving the formation of liposome or cyclodextrin complexes, provide possible solutions for improving storage stability, as well as additional benefits such as an increase in bioavailability and water dispersibility.

Starch is an essential element in human nutrition and an important source of energy in the diet. It also provides very interesting applications for the protection of bioactive molecules. In fact, the ability of amylose, the linear fraction of starch, to interact with certain ligands to form complexes has been known for a long time. These complexes are formed during the gelatinization of starch, as described, for example, by Escher and collaborators (Escher, Nuessli, & Conde-Petit, 2000), or during the subsequent cooling phase in the case of thermal gelatinization (TG) (Kugimiya & Donovan, 1981).

Amylose forms crystalline complexes, known under the generic name of "V-amylose", with a variety of small ligands. Different types of V-amylose, depending on the complexing molecule, can be found in the literature. The best-known and best-described complex is V_{6I} (V_h) amylose, which is obtained with linear alcohols (Brisson, Chanzy, & Winter, 1991; Buléon, Duprat, Booy, & Chanzy, 1984; Le Bail, Bizot, Pontoire, & Buléon, 1995; Whittam et al., 1989) and monoacyl lipids (Godet, Bizot, & Buléon, 1995; Godet, Tran, Delage, & Buléon, 1993). It consists of a six-fold left-handed helix repeating at 0.80 nm, in which

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the complexing agent is included. Three other crystalline types of complex with non-linear alcohols have also been highlighted. These can initially be distinguished by using the constructive amylose helix.

To date, two families of V-amylose complexes have been identified, namely the V_6 and V_8 types, in which "6" and "8" represent the number of D-glucose units per turn. For V_6 types, two trapping modes can be suggested: inclusion V_{6I} (V_h) and induction V_{6II} and V_{6III}, where I, II and III represent the varying volume between helices in the crystalline stacking. For V_{6I} (Brisson et al., 1991), the small molecules can be trapped only in the cavity of the helix (Godet, Tran, Colonna, Buléon, & Pezolet, 1995) while for V_{6II} and V_{6III}, the molecules can also be trapped between the helices (Buléon, Delage, Brisson, & Chanzy, 1990; Helbert & Chanzy, 1994).

The aim of this study is to investigate new methods for obtaining complexes between amylose and polyunsaturated fatty acids using, on the one hand, the traditional hydrothermal treatment and, on the other hand, high-pressure processing with specific parameters. The fatty acids studied in this work are linoleic and linolenic acid, due to their functional properties in health and because they cannot be synthesized in sufficient quantity from their precursors and must therefore be obtained from the diet.

2. Materials and methods

2.1. Materials

Potato amylose (type III), essentially free of amylopectin, was obtained from Sigma-Aldrich CAS 9005-82-7 (France). Polyunsaturated fatty acids (linolenic and linoleic acids) used to form the complexes were obtained from Sigma-Aldrich, CAS 60-33-3 and CAS 463-40-1 respectively, and were stored at a temperature of −20 °C.

2.2. Thermal treatment and storage of samples

Amylose dispersions (300 mg) were prepared with distilled and deoxygenated water (20 mL) in a 50-mL Sovirel bottle. The mixtures were stirred for 15 min with nitrogen bubbling to prevent any further oxidation. After that, the mixtures were heated for 45 min at 165 °C using a silicon oil bath, under magnetic stirring at 200 rpm.

In parallel polyunsaturated fatty acid solutions were prepared with distilled and deoxygenated water (2 mL) and 100 μL of linolenic or linoleic fatty acid in a sealed flask and stirred for 10 min with nitrogen bubbling to prevent oxidation.

After 45 min at 165 °C, amylose mixtures were placed in the silicon oil bath and heated at 90 °C for 15 min under magnetic stirring of 200 rpm, then transferred to the bottles containing fatty acid solutions (to minimize the possible loss of fatty acid) and put back in the water bath for 15 min at 90 °C, under magnetic stirring at 300 rpm.

After cooling, the mixtures were stored at room temperature for 48 h and then centrifuged (Jouan GT 4 22) for 30 min at 20 °C and 3500 *g*. After the phase separation, the solid phase was recovered, weighed into a tare box and placed under an atmosphere saturated with NaCl (water activity, $a_w = 0.75$ at room temperature) until equilibration of the mass (about 72 h). After that, the samples were ready for μDSC and X-ray diffraction analyses.

2.3. High-pressure treatment and storage of samples

The amylose dispersions were prepared in the same quantity (300 mg of amylose and 20 mL of water), stirred for 15 min with nitrogen bubbling to prevent any further oxidation and then heated for 45 min at 165 °C using a silicon oil bath, under magnetic stirring at 200 rpm. After the dispersions had cooled down to a temperature of approximately 40 °C, they were vacuum-packaged with the addition of 100 μL of linoleic or linolenic acid in plastic bags (low density polyethylene) and pressurized at 500 MPa for 20 min in a high pressure vessel (ACB Pressure Systems, Nantes, France) at a temperature of 40 °C. The temperature was maintained by temperature-controlled water circulating in a jacket placed around the vessel. The temperature increase due to the adiabatic heating effect was lower than in the case of strict adiabatic conditions for water due to the heat exchange between the vessel and the pressurization fluid. The low concentration of amylose meant that the adiabatic heating of the suspension could be assumed to be very close to that of pure water. Experimental measurements of the temperature rise of the amylose suspension were carried out using a K-type thermocouple. The effective temperature rise at the end of the pressure rise to 500 MPa was +4 °C starting from 20 °C and +9 °C starting from 40 °C. The temperature was re-equilibrated within a few minutes of the end of compression to the set point temperatures of 20 °C and 40 °C. The come-up time for the pressure rise was 3 min and the pressure release was almost instantaneous. After high-pressure processing, some of the samples were stored at room temperature for 24 h and the water content was adjusted by desorption until equilibrium with saturated NaCl solution (water activity, $a_w = 0.75$) before X-ray measurements were carried out.

2.4. High-pressure low-temperature microscopic cell prototype (HP LT MC)

The high-pressure microscopic cell (HP LT MC 1114/06, Unipress, Warsaw, Poland) used in this study is made of beryllium copper. The distance between the sapphire windows allows introduction of a sample of 0.2 mm thickness and maximum diameter of 10 mm. The high-pressure microscopic cell is linked to a high-pressure manual pump (609 29 00 PU, Top Industrie, Vaux le Pénil, France).

In the bottom of the cell body, a sapphire window is placed, co-operating with the objective of an inverted microscope (Eclipse TS 100, Nikon, Champigny, France). In the upper part of the pressure cell (closing screw), an identical sapphire window is bonded, co-operating with the condenser of the microscope. The pressure cell was used to investigate the behavior of linoleic and linolenic acids under different pressure and temperature conditions to determine the parameters under which polyunsaturated fatty acids are transformed into crystals. The samples were placed in the microscopic cell with the micropipette and examined. The sample was isolated from the pressurization fluid (water) with an O-ring gasket (0.5 mm ring diameter), which was pressed against two sapphire windows (gap of 0.3 mm between the two windows). The pictures were taken using a digital camera (Sony DFW-SX910). A computer with image analysis software (Visilog 6.2, Noesis, Courtaboeuf, France) was coupled to the camera for evaluation and archiving of the recorded pictures.

2.5. Microcalorimetry

Thermograms were recorded by a micro-differential scanning calorimeter, SETARAM™ MicroDSC7 (SETARAM instrumentation, Caluire, France). Stainless steel pans were used and a pan containing deionized water was taken as the reference. For the amylose samples after the hydrothermal processing and weight equilibration, 50 mg of sample was placed in the pan and 500 μL of deionized water was added. The pans were then sealed and placed in a thermally isolated detector. The sample and the reference were subjected to heating and cooling controlled by the computer. The chosen kinetics of the reaction were 1 °C/min from 10 °C to 120 °C followed by a cooling of 1 °C/min to 10 °C. A second rise in temperature was performed at 1 °C/min to 120 °C in order to determine whether the observed thermal transitions were reversible.

2.6. X-ray diffraction

Fifty milligrams of sample, equilibrated at $a_w = 0.75$, was sealed in a copper ring between two adhesive tape sheets to prevent any change in water content. The sample was examined by wide-angle X-ray scattering. Measurements were performed using a D8 Discover