

Thermal properties of β -lactoglobulin – vitamin D₃ complexes obtained by different technological processes

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The properties of β -lactoglobulin make it a potential ingredient to deliver vitamin D₃ in a form of dried complexes with β -lactoglobulin to fat-free food systems. Two different drying methods were employed to obtain β -lactoglobulin – vitamin D₃ complexes in a form of powder. Powdered complexes were tested by differential scanning calorimetry. The addition of lactose or trehalose to complexes influenced the course of DSC curves. The thermal properties of study complexes were not dependent on the technological drying processes (freeze- or spray-drying).

Key words: β -lactoglobulin, vitamin D₃, differential scanning calorimetry.

Introduction

The functional properties of milk whey proteins may be changed by heat treatment, what is essential for their application as food ingredients. During heating, intramolecular and intermolecular changes and reactions can occur, which are generally denoted as denaturation and aggregation [1, 2]. The major whey protein is β -lactoglobulin (β -Lg), which constitutes >50 % of total whey proteins in bovine milk. β -Lg is a globular protein (molecular mass 18.3 kDa and radius 2 nm), which contains two intramolecular disulfide bonds and one thiol group [3, 4]. Due to its amino acid sequence and 3-dimensional structure, β -Lg has been reported as capable of binding a variety of fat-soluble ligands, including vitamin D₃ [5–8]. The binding properties of β -Lg make it a potential ingredient to deliver vitamin D₃ in a form of complexes with β -Lg to fat free-food systems [9]. The studies have showed [10] that the major binding site is inside the hydrophobic pocket with its carboxyl group of amino acid residues. It is the central cavity, the calyx that provides the ligand-binding site.

Because of its thermally unstable and molten-globule nature, β -Lg has been studied extensively for its physical and biochemical properties in the past 40 years. Although the biological functions of the protein still remain elusive, some essential functions of β -Lg, such as cholesterol lowering, immune system modulation, transport of retinol, fatty acid, and vitamin D, and prevention of oxidative stress, have been reported [11]. Spray-drying and freeze-drying are the two methods widely used for drying heat-sensitive foods, pharmaceuticals and micro-encapsulation [12]. Spray drying is a dehydration technology that converts a suspension or solution into dry powder and belongs to the drying methods most widely used in food industry due to the wide availability of the equipment, a large variety of carriers and a good final product stability [13, 14]. Freeze-drying is a process in which water is frozen, followed by its removal from the sample, initially

by sublimation (primary drying) and then by desorption (secondary drying). In this process, the moisture content of the product is reduced to such a low level that it does not support biological growth or chemical reactions [15].

Differential scanning calorimetry (DSC) is an experimental technique to measure the heat energy uptake taking place in a sample during a controlled increase (or decrease) in temperature [16]. DSC is an established analytical method that enables characterizing the thermal properties of powder materials [17, 18]. The use of thermal analytical techniques and the information obtained are useful in controlling quality changes in food during processing and storage [18, 19].

The aim of the study was to evaluate the effect of drying methods on the thermal properties of β -lactoglobulin – vitamin D₃ complexes. Two different drying methods were employed to obtain β -lactoglobulin – vitamin D₃ complexes in the form of powder. It is of great importance to protect the protein from denaturation during the drying process. Sugars, such as lactose and trehalose, were found to stabilize the conformation of β -lactoglobulin. So, additionally complexes with lactose and trehalose were synthesized.

Materials and methods

BioPURE β -lactoglobulin containing 95 % β -lactoglobulin was provided as a powder by Davisco Foods International, Inc. (Le Sueur, Minnesota). Cholecalciferol (vitamin D₃) and α -lactose monohydrate were purchased from Sigma Chemical Co. (St. Louis, Missouri) and were of the highest analytical quality. Trehalose was purchased from Hortimex Ltd Co (Konin, Poland).

400 ml of 2 % β -lactoglobulin (M = 18 400 g/mol) solution was prepared by gently adding distilled water into 8.6 g (0.47 mmol) of the protein while stirring slowly to avoid heavy foaming. The mixture was kept at room temperature until a homogeneous clear solution was formed. Then 0.36 g (0.94 mmol) of cholecalciferol

(vitamin D₃) (M = 384 g/mol) dissolved in 800 µl absolute ethanol was added into the solution to obtain a 2 : 1 molar ratio of vitamin D₃ to protein. The solution was incubated at 40 °C for 2 hours. Additionally, complexes with lactose and trehalose were prepared by adding sugars to the protein solution in a weight ratio 5 : 1. The obtained products were dried using two different drying processes: spray- and freeze-drying.

Complexes B-1, B-2 and B-3 were prepared by spray-drying, and complexes B-4, B-5 and B-6 were prepared by freeze-drying. Lactose was added to powders B-2 and B-5. Powders B-3 and B-6 contained trehalose. The process of spray-drying was conducted in a laboratory spray-dryer (Anhydro, Denmark). The operational conditions of the spray drying were as follows: air inlet temperature 120 °C, air outlet temperature 72–74 °C, rotational speed of the atomizer 39000 rpm, and flow rate 51.4 ml/min as typical of liquids of such viscosity. The powders were collected at the bottom of the dryer's cyclone. For samples obtained by freeze-drying, the process was carried out in an ALPHA1-4 LDC-1m Christ laboratory scale freeze dryer at constant parameters: pressure 63 Pa, time 24 h, and the heating shelf temperature of the freeze-dryer 30 °C.

Powders were further dried in a vacuum oven at 50 °C for 24 h before experiments to remove residual moisture. The products were kept in vacuum desiccators over CaCl₂ at room temperature, in a dry place and in the absence of light.

Powder complexes were tested with a differential scanning calorimeter (DSC, TA Instruments Q 200). The cell was purged with 50 mL/min dry nitrogen and calibrated for baseline on an empty oven and for temperature using standard pure indium. An empty sealed aluminium pan was used as a reference in each test. Powders (5–8 mg) were non-hermetically sealed in aluminium pans. A sample was heated from 10 to 170 °C with the heating rate 5 °C/min. The DSC technique was used to obtain curves of heat flow (W/g) versus temperature curves. All analyses were accomplished in triplicate [20, 21].

Results and discussion

β -Lg has been reported capable of binding a variety of fat-soluble ligands, including vitamin D₃. The importance of the binding property is that it is possible to deliver vitamin D₃ using β -Lg as a carrier without the presence of fat with which it normally associates. In the study, β -Lg –

vitamin D₃, β -Lg – vitamin D₃ – lactose and β -Lg – vitamin D₃ – trehalose complexes were obtained in the form of powders [9]. Certain sugars, such as lactose and trehalose, were found to limit the conformational changes and stabilize whey protein during drying [22, 23]. The temperature of spray drying and the stresses imposed by freeze drying could easily degrade or decompose the protein during the process. The low temperature of freeze drying does not guarantee protein stability because many proteins experience cold denaturation or denaturation at interfaces [24, 25]. Saccharides are able to form a glassy state of a very high viscosity and low mobility and restrict the mobility of protein as well as its unfolding [26]. Sugars such as lactose and trehalose remain in the amorphous phase with the protein and bind to the protein instead of water during drying, this preventing the stability problems.

The composition of the synthesised complexes is presented in Table 1.

Fig. 1 shows the DSC curves obtained for the studied samples in the form of spray-dried powders: β -lactoglobulin – vitamin D₃ complex (B-1) and complexes with addition of lactose (B-2) or trehalose (B-3). DSC curves for all powders were characterized by mild courses. The first endothermic peak was observed in the B-2 complex curve at a temperature of 66.7 °C and for B-3 at a temperature of 62.3 °C. Complexes B-2 and B-3 contained lactose or trehalose and β -lactoglobulin. Under the influence of thermal processes, reactions between sugars and free amino group and amino acids, peptides or proteins can occur and result in the newly formed compounds [27]. The samples were exposed to heat during the test. The endothermic peaks were probably the result of new compounds, such as pyrroline and melanoidin synthesis. Characteristic peaks generated by phase transitions running within the studied samples of complexes during heating were observed.

In the case of sample B-1, an endothermic peak at a temperature of 107.4 °C was observed. Similar phase transitions were characteristic of samples B-2 and B-3, but the temperatures of the endothermic peaks were lower than for B-1 (respectively 93.9 °C for B-2 and 98.2 °C for B-3). The temperatures of endothermic peaks for B-1, B-2 and B-3 were the result of binding vitamin D₃ to β -lactoglobulin [28].

Table 1. The composition and drying process applied for complexes B1-B6

Sample	B-1	B-2	B-3	B-4	B-5	B-6
Ingredients						
β -lactoglobulin	+	+	+	+	+	+
Vitamin D ₃	+	+	+	+	+	+
Lactose	-	+	-	-	+	-
Trehalose	-	-	+	-	-	+
Drying process applied	Spray-drying	Spray-drying	Spray-drying	Freeze-drying	Freeze-drying	Freeze-drying

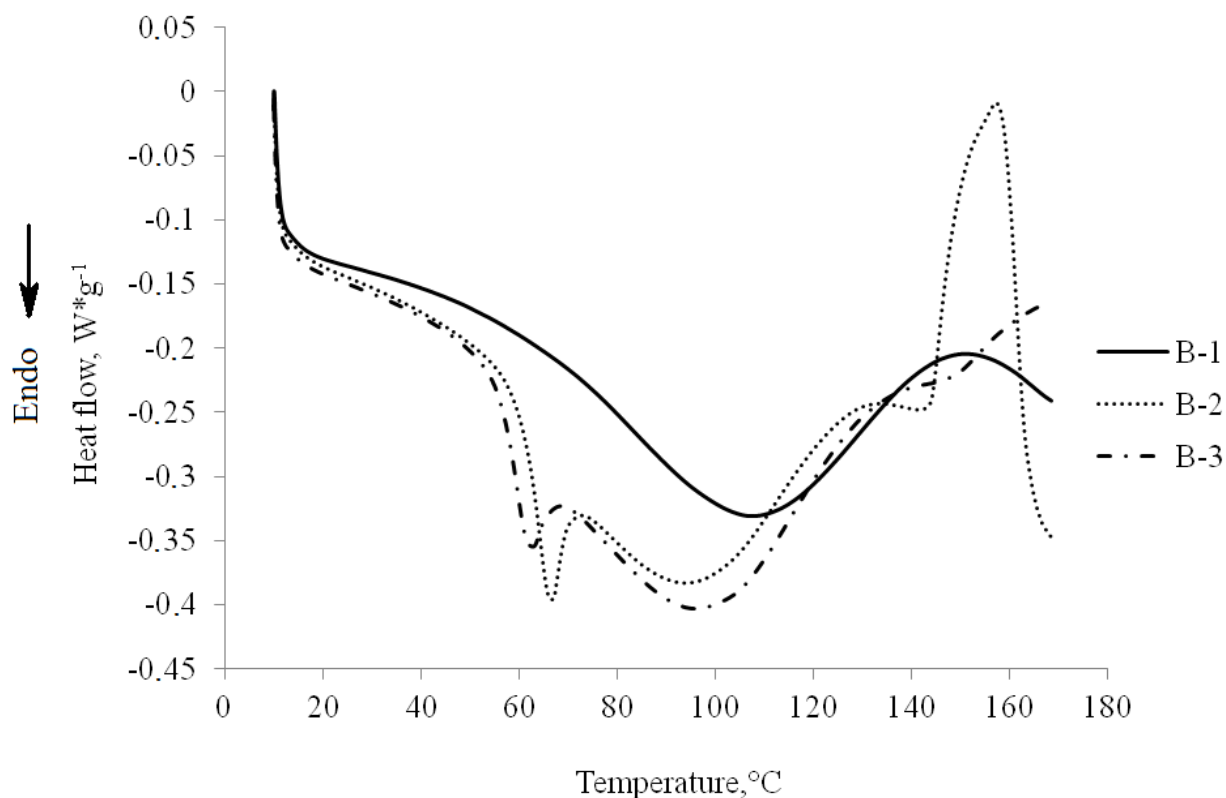


Fig. 1. DSC curves of β -lactoglobulin – vitamin D₃ complexes obtained by spray-drying method

The observed course of endothermic phase transitions was similar to the DSC diagrams of polymers. The DSC curve of sample B-2 was characterized by a distinct exothermic peak at a temperature of 157.9 °C. Lactose, which was present in product B-2, could occur in an amorphous or crystalline form or in their mixture. The observed exothermic peak that represented phase transition

was characteristic of amorphous lactose. Gombas et al. [29] studied the temperatures of phase transitions of both amorphous and crystalline lactose.

Fig. 2 shows the DSC curves obtained for the studied samples: β -lactoglobulin – vitamin D₃ complex (B-4) and complexes with lactose (B-5) or trehalose (B-6), obtained by the freeze-drying method.

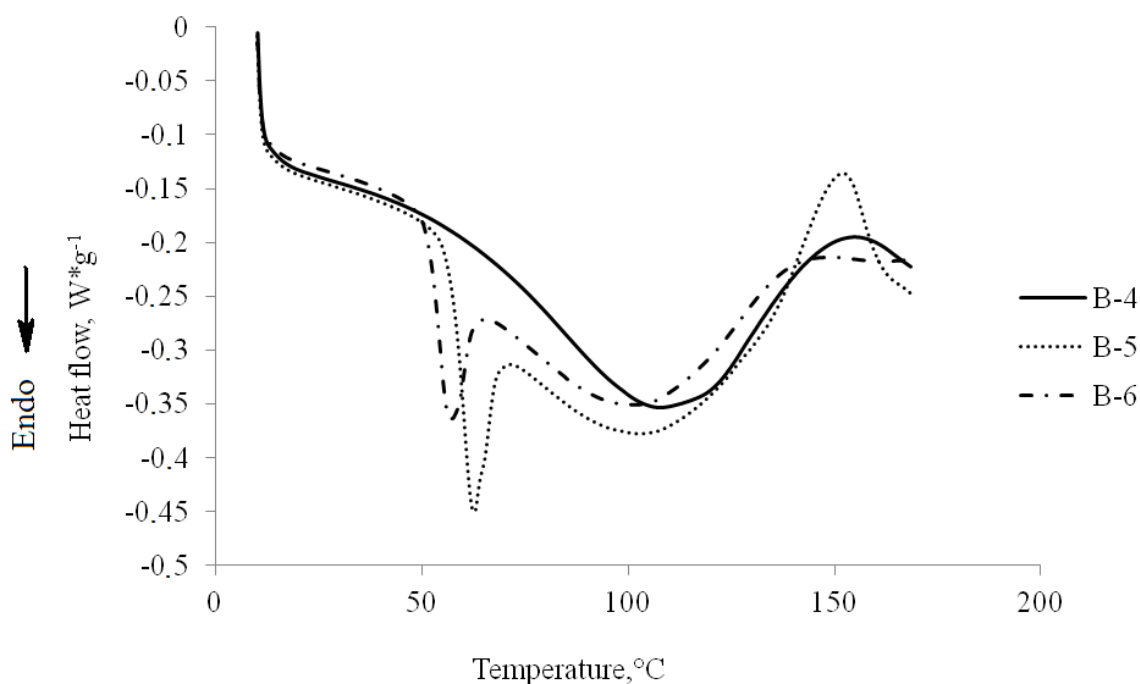


Fig. 2. DSC curves of β -lactoglobulin – vitamin D₃ complexes obtained by freeze-drying method

The shape of DSC curves for freeze-drying complexes was similar to those obtained for spray-drying powders. Two distinct endothermic peaks, at a temperature of 62.9 °C (B-5) and 56.7 °C (B-6), were observed. The range of temperatures in the case of the first endothermic phase transitions was similar in both groups of complexes (spray- and freeze-dried). Gentle endothermic peaks were characteristic of DSC curves in complexes B-4, B-5, and B-6. The phase transitions indicating protein aggregation were observed in the case of all curves. Phase transitions at temperatures of 107.5, 101.5 and 100.7 °C were present in the DSC curve. The exothermic peak at a temperature of 152.6 °C of powder B-5 was characteristic of amorphous lactose, which was an ingredient of the B-5 complex. A more intensive peak of amorphous lactose was observed for the complex obtained by the spray drying-method.

Conclusions

1. The addition of lactose or trehalose to complexes influenced the shape of DSC curves.
2. The thermal properties of the study complexes were not dependent on the technological process (freeze-drying or spray-drying).
3. The temperature of endothermic, gentle peaks indicates the formation of β -lactoglobulin – vitamin D₃ complexes.
4. The observed exothermic peaks confirmed the presence of lactose in an amorphous form.
5. DSC is the method that could be successfully used for thermal characteristics of newly obtained products.

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SKIRTINGAIS TECHNOLOGINIAIS BŪDAIS GAUTŲ β -LAKTOGLOBULINO IR VITAMINO D₃ KOMPLEKSŲ ŠILUMINĖS SAVYBĖS

S a n t r a u k a

Vitaminas D₃ į maistą, neturintį riebalų, įterpiamas naudojant sausus β -laktoglobulino ir vitamino D₃ kompleksus. Sausi β -laktoglobulino ir vitamino D₃ kompleksų milteliai buvo ruošiami taikant du skirtingus džiovavimo būdus – purkštuvinį džiovinimą ir sublimaciją. Kompleksai tirti diferencinės skenuojamosios kalorimetrijos metodu. Nustatyta laktozės ir trehalozės priedų įtaka DSK kreivėms. Tirtų kompleksų šiluminės savybės nepriklausė nuo džiovavimo būdo.