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A differential scanning calorimetric study of β-lactoglobulin and vitamin D_3 complexes

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Abstract In the study, β-lactoglobulin and vitamin D_3 complexes were obtained through spray drying at inlet temperature of 120 or 150 °C. Additionally, complexes with lactose were synthesised. Modulated differential scanning calorimetry (MDSC) was used in order to explain the glass transitional behaviour of spray dried β-lactoglobulin–vitamin D_3 and β-lactoglobulin–vitamin D_3–lactose complexes and the influence of applied spray drying conditions on calorimetric parameters. The glass transition temperatures of the powders in this study ranged from 112.93 to 112.99 °C (T_g onset), from 118.42 to 119.20 °C (T_g midpoint) and from 122.07 to 125.08 °C (T_g endpoint). The present study has shown that the values of glass transition temperatures at $a_w = 0$ did not differ significantly for studied samples obtained in a form of spray-dried powders, despite of various process conditions applied. The different values of heat capacity changes can be related to the various vitamin D_3 content in tested samples.

Keywords β-Lactoglobulin · Vitamin D_3 · Differential scanning calorimetry · Glass transition · Heat capacity

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Introduction

β-Lactoglobulin (β-LG) is a major whey protein possessing nutritional value as well as functional characteristics [1, 2]. Due to its amino-acid sequence and 3-dimensional structure, β-LG has been reported capable of binding a variety of fat-soluble ligands, including vitamin D_3 [3–6]. The fortification of food in vitamin D_3, is usually carried out with some forms of fat as a carrier because of its solubility in fat. As reduced fat products become more popular, the reduction of vitamin D_3 in the diet rendered a nutritional concern [7–9]. The binding properties of β-LG make it a potential ingredient to deliver vitamin D_3 in a form of spray-dried complex with β-LG to fat free food systems. Spray drying is a dehydration technology that converts a suspension or solution into dry powder and belongs to one of the mostly used drying methods in the food industry due to the wide availability of the equipment, a large variety of carriers and good final product stability [10, 11]. Spray-dried powders are economical, compared to other processes such as freeze-drying with advantages of facilitating manipulation, transport, storage and consumption. The major applications in the food industry include the drying of dairy products, fruit and beverages. The spray drying technique has been widely used for drying heat-sensitive foods, pharmaceuticals and micro-encapsulation, due to a solvent's rapid evaporation from the droplet [12]. During the drying process, heat tends to denature the protein and causes the dissociation of the complex, which can result in low retention of vitamins. Certain sugars, such as lactose and trehalose, were found to stabilize whey protein during spray drying [13, 14]. There are many product quality attributes related to the physical state of the ingredients of the dried product. Spray drying results in an amorphous or partially amorphous structure in processed foods, mainly due to insufficient time for

crystallisation within the process [15]. Food powders containing amorphous materials, such as lactose, can undergo physical changes such as sticking, caking, collapse and crystallisation, which impact on quality and functionality of the final product. It is well known that physical changes in food powders are related to the glass transition phenomenon. The most important change in the amorphous state occurs over the glass transition temperature (T_g), which is the temperature at which polymeric materials change from an amorphous solid (glass) to an amorphous rubber. For storage temperature below T_g, the food is expected to be stable; while above T_g, the storage temperature is assumed to control the rate of physical changes [15]. The most widely used technique for studying the glass transition is differential scanning calorimetry (DSC), though several other techniques are available. Modulated differential scanning calorimetry (MDSC) is an enhancement to conventional DSC, which involves the application of a sinusoidal heating or cooling signal to a sample and the subsequent measurement of the reversing and non-reversing components of the heat flow response [16–18]. The technique of MDSC gives the possibility to analyze many aspects of the thermal response of materials, including particularly the glass transition [19]. Quasi-isothermal (QI) modulation at a constant temperature can reduce reversing heat capacity in the melting region. Truly reversible heat capacity can be obtained by increasing the experimental time until a steady state is reached and accurate heat capacities can be calculated by this method [20–22].

The aim of the present study was the thermal analysis of the spray-dried β-LG–vitamin D_3 and β-LG–vitamin D_3–lactose complexes as a part of newly synthesised products' characteristics. Different process parameters were applied to obtain complexes in the form of spray-dried powder in order to explain, whether spray drying conditions influence the calorimetric parameters. The main task was to define the glass transition temperatures and the change in heat capacity of complexes. Understanding the parameters will lead to an explanation of the powders' properties.

Materials and methods

Materials

BioPURE β-LG containing 95% β-LG was provided as powder by Davisco Foods International, Inc. (Le Sueur, Minnesota). The powder was analyzed by high performance liquid chromatography (HPLC) and contained no vitamin D_3. Cholecalciferol (vitamin D_3) and α-lactose monohydrate were purchased from Sigma Chemical Co. (St.Louis, Missouri) and were of the highest analytical quality.

Methods

Solution preparation

400 mL of β-LG (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 homogenous clear solution was formed. Then 0.36 g (0.94 mmol) of cholecalciferol (vitamin D_3) (M = 384 g/mol) dissolved in 800 µL absolute ethanol was added into the solution to obtain 2:1 molar ratio of vitamin D_3 to protein. The solution was incubated at 40 °C for 2 h according to the method described by Kontopidis et al. [5]. Additionally, the complex with lactose was prepared by adding lactose to the protein solution in a weight ratio 5:1. The formulations were kept until the lactose was dissolved and then spray dried.

Powder production

The formulations were spray dried using Anhydro (Denmark) pilot scale spray dryer. The operational conditions of the spray drying were: air inlet temperature: 120 or 150 °C, air outlet temperature: 72–74 °C, rotational speed of the atomizer: 39000 rpm, and flow rate: 15 or 25 mL/min. The powders were collected at the bottom of the dryer's cyclone. The products were kept in vacuum desiccators over $CaCl_2$ at room temperature, in a dry place and in the absence of light. Powders were further dried in a vacuum oven at 50 °C for 24 h before experiments were carried out to remove residual moisture. The processes and analyses were carried out in triplicate.

DSC and MDSC studies

Both the conventional and modulated DSC experiments were performed on a TA Instrument Q200 differential scanning calorimeter (New Castle, USA). The DSC technique was used to obtain heat flow (W g^{-1}) versus temperature curves. MDSC was used to determine the glass transition temperature of β-LG–vitamin D_3 and β–lactoglobulin–vitamin D_3–lactose complexes at water activity $a_w = 0$. The cell was purged with 50 mL min^{-1} dry nitrogen and calibrated for baseline on an empty oven and for temperature using standard pure indium. Specific heat capacity (C_p) was calibrated using a sapphire. An empty sealed aluminium pan was used as a reference in each test.

Complexes powders (10–13 mg) were non-hermetically sealed in aluminium pans (volume 30 µL) and cooled from room temperature to 10 °C at 5 °C/min and equilibrated for 5 min. Samples were scanned from 10 to 170 °C at a heating rate of 5 °C/min [23, 24].

In the case of MDSC, samples were scanned from 10 to 170 °C at a constant heating rate of 2 °C/min with an amplitude of ±1 °C and 60 s period of modulation. Curves were analysed with respect to the total, reversible and non-reversible heat flow [25, 26]. Glass transition was reported with parameters indicating its onset, midpoint and endpoint of a vertical shift in the reversing transition curve. TA Instruments Universal analysis software was used to analyse the glass transition temperature. The measurements were done in three replicates for each sample.

The change in heat capacity, ΔC_p, was determined by taking the heat capacity difference between the solid and liquid baselines at the T_g. The QI runs were done in the modulated mode using an amplitude of ±0.30 °C, a period of 120 s, and stepped incrementally every 3 °C. Measurements were conducted in 5 min intervals. Each measurement lasted 5 min.

Statistical analysis

Each measurement was triplicate. The data were reported as the means ± standard deviation. Two-way ANOVA was conduced using Statgraphics Plus for Windows program, version 4.1 (Statistical Graphics Corporation, Warrenton, VA, USA). Differences were considered to be significant at a p value of 0.05, according to Tukey's Multiple Range Test.

Results and discussion

In the study, the binding properties of β-LG were used in order to obtain β-LG–vitamin D_3 complexes. Previous studies and data from fluorescence spectroscopy have shown that β-LG is capable of binding two moles of vitamin D_3 per mole [27]. Since β-LG is produced as a spray-dried powder, it is possible to obtain vitamin-fortified β-LG as a powder form for its convenience use in food applications and for its shelf stability. In order to produce such complexes with high vitamin yield, it is important to protect β-LG from denaturation and the complexes from dissociation during the drying process. Sugars were found to stabilize the conformation of β-LG when drying whey protein [13, 14]. Thus, additionally, β-LG–vitamin D_3–lactose complexes were synthesised in a form of spray-dried powders. It is thought that sugar will protect the complex from destruction by stabilizing protein and the vitamin will be retained more in the dried powder. Figure 1 shows the data obtained from DSC measurement of β-LG, β-LG–vitamin D_3 and β-LG–vitamin D_3–lactose complexes. For clarity, only three model curves are presented in Fig. 1. For every sample, an endothermic peak is observed in the vicinity of 87.74, 96.99 and 97.62 °C, respectively. Binding of vitamin D_3 to β-LG increased the stability of the protein and its resistance to denaturation and moved the curves of complex towards higher temperatures. The data from DSC indicated that the binding of the ligand shifted the denaturation temperature of β-LG of +9.25 °C. This observation is in agreement with Puyol et al. [28], who showed that addition of palmitic acid and retinol increased the denaturation temperature of β-LG of 8 and 4 °C, respectively. It can also be seen that incorporation of lactose into β-LG–vitamin D_3 complex shifted the transition temperature to higher value, similarly as the results obtained by De Wit [13]. Additionally, in the case of β-LG–vitamin D_3–lactose complex a dehydration endotherm of lactose with a peak maximum of 144.5 °C is observed.

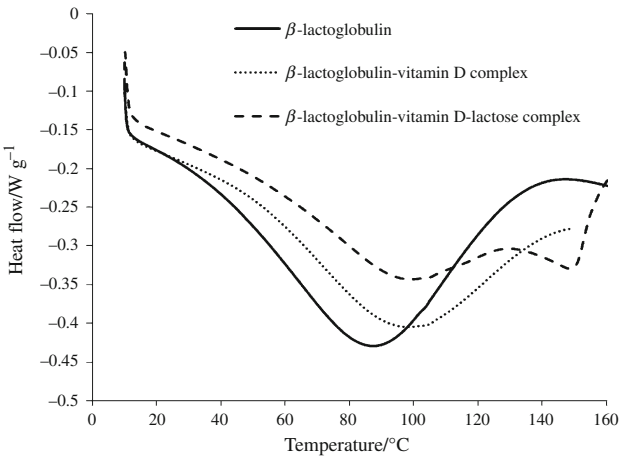

Fig. 1 Curves of β-LG, β-LG–vitamin D_3 and β-LG–vitamin D_3–lactose complexes obtained by spray drying (inlet temperature: 120 °C, flow rate: 15 mL/min)

It has been proved that the stability of food containing sugars is often related to the properties of their amorphous phases. Lactose and proteins in foods are often in an amorphous state and may undergo time-dependent changes [29, 30]. Lactose is the main component affecting stickiness in powders. During the drying of sugar-rich products, they may either remain as syrup or stick on the drier chamber wall, what usually lead to lower product yields and operating problems. Knowledge of the T_g can be used to evaluate causes of stickiness problems, especially in the production of amorphous powders. The glass transition temperature has been proven to be an effective indicator for food quality changes during storage [15, 31]. In the study, different process parameters were chosen to obtain complexes in the form of spray-dried powders in order to explain, whether spray drying conditions influence the calorimetric parameters. Experimental values of the glass transition temperatures and specific heat change through the glass transition zone for the tested samples are presented in Table 1. The glass transition is reported with parameters indicating its onset, midpoint and endpoint, so

Table 1 Experimental glass transition temperatures and specific heat change values through glass transition zone of β-LG–vitamin D_3–lactose complexes obtained by spray drying

	T_g onset/°C	T_g midpoint/°C	T_g endpoint/°C	ΔCp/J g^{-1} $°C^{-1}$
A 1	112.93 ± 1.087[a]	118.68 ± 0.879[a]	122.07 ± 1.008[a]	0.392 ± 0.077[a,b]
A 2	112.97 ± 1.113[a]	118.42 ± 1.276[a]	122.27 ± 1.070[a]	0.304 ± 0.059[a]
A 3	112.93 ± 1.248[a]	118.53 ± 0.912[a]	122.07 ± 1.114[b]	0.406 ± 0.091[a]
A 4	112.99 ± 0.962[a]	119.20 ± 1.184[a]	125.08 ± 1.915[b]	0.308 ± 0.046[a]

Values represent means ± standard deviations. Different letters indicate that the samples are considered significantly different at the 5% level ($p < 0.05$)

A 1 β-LG–vitamin D_3–lactose complex, spray dried; inlet temp.: 120 °C, flow rate: 15 mL/min; *A 2* β-LG–vitamin D_3–lactose complex, spray dried; inlet temp.: 120 °C, flow rate: 25 mL/min; *A 3* β-LG–vitamin D_3–lactose complex, spray dried; inlet temp.: 150 °C, flow rate: 15 mL/min; *A 4* β-LG–vitamin D_3–lactose complex, spray dried; inlet temp.: 150 °C, flow rate: 25 mL/min

the width of the transition is clear. No glass transition was detected at $a_w = 0$ in the case of β-LG–vitamin D_3 complexes in applied conditions.

In the case of β-LG–vitamin D_3–lactose complexes a single glass transition was observed. The onset glass transition temperature of the powders in this study ranged from 112.93 °C ± 1.087 to 112.99 °C ± 0.962, the midpoint T_g from 118.42 °C ± 1.276 to 119.20 °C ± 1.184 and the endpoint T_g from 122.07 °C ± 1.008 to 125.08 °C ± 1.915. The results has shown the lactose impact on product features. The present study has shown that the values of glass transition temperatures at $a_w = 0$ did not differ significantly for studied samples obtained in a form of spray-dried powders, despite of various process conditions. The results confirmed the statement that the glass transition temperatures of foods are mainly dependent on the moisture content and chemical composition of the material [25]. Due to the importance of lactose in the food industry, an understanding of its impact on phase transitions can be very useful in predicting shelf life and stability during storage under varying temperature, moisture content and time. The glass transition temperatures of synthesised complexes were measured for powders conditioned at $a_w = 0$. Therefore, in the future, the glass transition temperature should be obtained for a wide range of water activities.

QI operation mode was used to reach highest precision for the measurement of heat capacity. There was no significant differences observed in the shape of QIDSC curves, thus, for clarity, the model curve of β-LG–vitamin D_3–lactose complex (spray dried: inlet temperature: 120 °C; rate flow: 15 mL/min) is presented in Fig. 2.

The heat capacity changes of β-LG–vitamin D_3–lactose complexes during glass transition range from 0.304 J g^{-1} $°C^{-1}$ ± 0.059 in the case of powder spray dried at 120 °C and rate flow of 25 mL/min to 0.406 J g^{-1} $°C^{-1}$ ± 0.091 in the case of powder spray dried at 150 °C and rate flow of 15 mL/min. It is well recognized the heat capacity of an object is determined by both its composition and mass. The evidence of HPLC previous studies (not published so far) indicated the higher vitamin D_3 quantity in β-LG–vitamin D_3–lactose complexes obtained by spray drying at rate flow of 15 mL/min and inlet temperature of 120 °C or 150 °C. More association of vitamin to the protein can explain higher heat capacity changes in samples spray dried at rate flow of 15 mL/min.

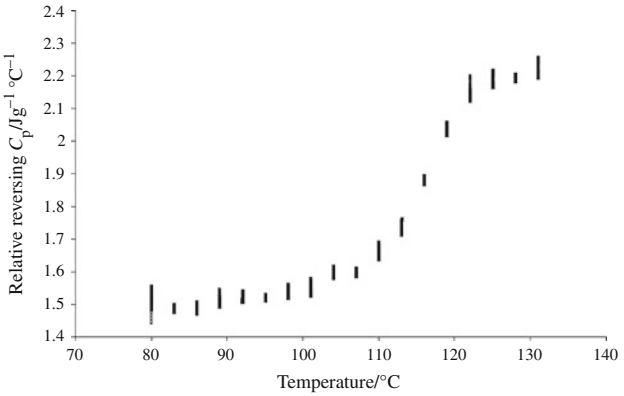

Fig. 2 QI modulated heat capacity curve for β-LG–vitamin D_3–lactose complex (spray dried: inlet temperature: 120 °C; rate flow: 15 mL/min)

Conclusions

β-LG has been reported capable of binding a variety of fat-soluble ligands, including vitamin D_3. The importance of the binding property is that it is possible to deliver vitamin D_3 using β-LG as a carrier without the presence of the fat in which it normally associates. In the study β-LG–vitamin D_3 and β-LG–vitamin D_3–lactose complexes were obtained in form of spray-dried powders. The differential scanning calorimetric analysis of synthesised complexes has shown that the values of glass transition temperatures at $a_w = 0$ did not differ significantly, despite of various process conditions applied. The various values of heat capacity changes can be related to the different vitamin D_3 content

in tested samples. Further research is needed to define the glass transition temperatures for a wide range of water activities, which will enable the powders' shelf life stability to be evaluated using a state diagram.

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References

1. Bordin G, Cordeiro Raposo F, De la Calle B, Rodriguez AR. Identification and quantification of major bovine milk proteins by liquid chromatography. J Chromatogr A. 2001;928:63–76.
2. Chatterton DEW, Smithers G, Roupas P, Brodkorb A. Bioactivity of b-lactoglobulin and a-lactalbumin—technological implications for processing. Int Dairy J. 2006;16(11):1229–40.
3. Brownlow S, Cabral JHM, Cooper R, Flower DR, Yewdall SJ, Polikarpov I, North AC, Sawyer L. Bovine β-lactoglobulin at 1.8 Å resolution—still an enigmatic lipocalin. Structure. 1997;5(4):481–95.
4. Papiz MZ, Sawyer L, Eliopoulos EE, North AC, Findlay JB, Sivaprasadarao R, Jones TA, Newcomer ME, Kraulis PJ. The structure of beta-lactoglobulin and its similarity to plasma retinol-binding protein. Nature. 1986;324(6095):383–5.
5. Kontopidis G, Holt C, Sawyer L. Invited review: β-lactoglobulin: binding properties, structure, and function. J Dairy Sci. 2004;87:785–96.
6. Sawyer L, Kontopidis G. Review: the core lipocalin, bovine β-lactoglobulin. Biochim Biophys Acta. 2000;1482:136–48.
7. Janssen H, Samson MM, Verhaar HJJ. Vitamin D deficiency, muscle function, and falls in elderly people. Am J Clin Nutr. 2002;75:611–5.
8. Weaver CM, Fleet JC. Vitamin D requirements: current and future. Am J Clin Nutr. 2004;80(suppl 6):1735S–9S.
9. Harris SS, Soteriades E, Stina Coolidge JA, Mudgal S, Dawson-Hugues B. Vitamin D insufficiency and hyperparathyroidism in a low income, multiracial, elderly population. J Clin Endocrinol Metab. 2001;85:4125–30.
10. Mermelstein N. Spray drying. Food Technol. 2001;55(4):92–5.
11. Reineccius GA. Flavor encapsulation. Food Rev Int. 1989;5(1):146–76.
12. Re MI. Microencapsulation by spray drying. Dry Tech. 1998;16(6):1195–236.
13. De Wit JN. Structure and functional behavior of whey proteins. Neth Milk Dairy J. 1981;35:47–64.
14. Murray BS, Liang HJ. Evidence for conformational stabilization of β-lactoglobulin when dried with trehalose. Langmuir. 2000;16:6061–3.
15. Bhandari BR, Howes T. Implication of glass transition for the drying and stability of dried foods. J Food Eng. 1999;40:71–9.
16. Gill PS, Sauerbrunn SR, Reading M. Modulated differential scanning calorimetry. J Therm Anal Calorim. 1993;40:931–9.
17. Reading M, Elliott D, Hill VL. MDSC, a new approach to the calorimetric investigation of physical and chemical transitions. J Therm Anal. 1993;40:949–55.
18. Reading M. Modulated differential scanning calorimetry—a new way forward in materials characterisation. Trends Polym Sci. 1983;1:248–53.
19. Zeng JL, Yu SB, Cao Z, Yang DW, Sun LX, Znahg L, Zhang XF. Synthesize, crystal structure, heat capacities and thermodynamic properties of a potential enantioselective catalyst. J Therm Anal Calorim. 2011;105:961–8.
20. Boller A, Jin Y, Wunderlich B. Heat capacity measurement by modulated DSC at constant temperature. J Therm Anal. 1994;42:307–30.
21. Judovits L, Gupta R. Detection of obscured glass transition by QiDSC. J Therm Anal Calorim. 2011;106:299–303.
22. Shanks RA, Gunaratne LMWK. Comparison of reversible melting behaviour of poly(3-hydroxybutyrate) using quasi-isothermal and other modulated temperature differential scanning calorimetry techniques. J Therm Anal Calorim. 2011;104:1117–24.
23. Khalloufi S, Ratti C. Mathematical model for prediction of glass transition temperature of fruit powders. Food Sci. 2000;65:842–8.
24. Telis VRN, Martı′nez-Navarrete N. Collapse and color changes in grapefruit juice powder as affected by water activity, glass transition, and addition of carbohydrate polymers. Food Biophys. 2009;4:83–93.
25. Jakubczyk E, Ostrowska-Ligęza E, Gondek E. Moisture sorption characteristics and glass transition temperature of apple puree powder. Int J Food Sci Technol. 2010;45:2515–23.
26. Rahman M, Al-Marhubi I, Al-Mahrouqi A. Measurement of glass transition temperature by mechanical (DMTA), thermal (DSC and MDSC), water diffusion and density methods: a comparison study. Chem Phys Lett. 2007;440:372–7.
27. Wang QW, Allen JC, Swaisgood HE. Binding of vitamin D and cholesterol to β-lactoglobulin. J Dairy Sci. 1997;80:1054–9.
28. Puyol P, Perez MD, Peiro JM. Effect of binding of retinol and palmitic acid to bovine β-lactoglobulin on its resistance to thermal denaturation. J Dairy Sci. 1994;77:1494–502.
29. Roos YH, Karel M. Phase transitions of mixtures of amorphous polysaccharides and sugars. Biotechnol Prog. 1991;7:49–53.
30. Slade L, Levine H. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. Crit Rev Food Sci Nutr. 1991;30(2–3):115–360.
31. Le Meste M, Champion D, Roudaut G, Blond G, Simatos D. Glass transition and food technology: a critical appraisal. J Food Sci. 2002;67(7):2444–58.