Journal of Thermal Analysis and Calorimetry, Vol. 75 (2004) 719–726

DSC AND ELECTROPHORETIC STUDIES ON SOYMILK PROTEIN DENATURATION

H. Zhang[*]*, M. Takenaka and S. Isobe*

Food Processing Lab., National Food Research Institute, Tsukuba Science City, Ibaraki 305-8642, Japan

(Received May 7, 2003; in revised form October 27, 2003)

Abstract

The effects of heat treatment on soymilk protein denaturation were studied by differential scanning calorimetry (DSC) and electrophoresis. Transition behavior of soymilk was studied by DSC. Three endotherms were found in DSC heating curves; the transition observed at around 70°C is attributed to the denaturation of 7S (β-conglycinin) and the transition at around 90°C is to 11S (glycinin). The denaturation temperature increased with the increasing soymilk protein content. The change of electrophoretic patterns after heat treatments indicated that soy proteins were dissociated into subunits, some of which coalesced. When the heating temperature is below their denaturation temperature, the protein fractions cannot completely be denatured even after heat exposure for extended periods of time.

Keywords: denaturation, DSC, electrophoresis, 7S, 11S, soy protein

Introduction

Soymilk and its products have been popular in some Asian countries for centuries. They are generally regarded as nutritious, cholesterol-free, healthy foods, and have considerable potential for greater use in the future. On October 26, 1999, the United States Food and Drug Administration authorized the Soy Protein Health Claim that 25 g of soy protein a day may reduce the risk of heart disease. Since the market is responsive to this assertion, soy foods will penetrate rapidly into western cultures and diets [1, 2].

The most conventional and key processing method of soymilk is heat treatment. Thermal processes applied to soymilk influence the generation and nutritive quality of proteins. The qualities of soy products derived from soymilk, e.g. the texture of tofu and the suitability for lactic acid fermentation, may also be affected by heat treatment. Excess heating will cause undesirable chemical changes, which may lead to the destruction of amino acids and vitamins, browning, and the development of a cooked flavor [3]. Scientific studies on soymilk has scarcely been carried out, and data for optimum processing conditions are deficient [4]. With increasing global ac-

* Author for correspondence: E-mail: zhk@affrc.go.jp

1388–6150/2004/ $ 20.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

ceptance of soymilk and its derived products, research on the effect of thermal processing on the chemical, physical and nutritional properties is imperative.

DSC is a powerful technique to study the thermodynamics of protein stability that can provide a basic understanding of protein denaturation [5–7], and has been applied to various other food systems [8–11]. Electrophoresis is an excellent tool to detect any process that alters either the charge or the conformation of a protein. The objectives of this investigation are to study the thermal denaturation properties of soymilk protein by DSC and electrophoresis, and to provide basic data for the thermal processing of soymilk.

Experimental

Materials

Soybeans of the Proto cultivar (Tsurunoko soybean) used in this study were harvested in 2001 and obtained from Hokkaido.

Soybeans were thoroughly washed and soaked overnight at room temperature in ten times their mass of distilled water. Soaked soybeans were homogenized at 10.000 rpm for 5 min with an ice water bath. Soymilk was obtained from the slurry after centrifugation (1200×g, at 4°C) for 5 min.

Differential scanning calorimetry

The crude protein content of DSC samples was measured using an automatic nitrogen analyzer (LECO FP-528) using the factor of 6.25 to convert nitrogen to protein [12]. Soymilk was heat-treated in glass tubes in a temperature-controlled water/oil bath using water or polyethylene glycol as the heating medium. The heating temperatures and times used covered ranged from 60 to 95°C and from 5 to 120 min. At the end of the heating period, the tubes were immediately transferred to a room-temperature water bath for cooling.

The soymilk sample (0.75 g) was hermetically sealed into an experimental sample vessel of a Micro DSC III microcalorimeter (Setaram Company, France). The same amount of distilled water was used as a reference in a separate vessel. Calorimetric measurements were carried out at a scan rate of 1 K min^{-1} under nitrogen at 1 bar with a rate of 2 L h^{-1} through the range 20 to 105°C. Analyses were conducted in triplicate. Onset temperature (T_{eim}), peak temperature (T_{pm}), and enthalpy of the denaturation endothermic effect (ΔH) were computed from the DSC curves.

Native polyacrylamide gel electrophoresis (Native-PAGE)

Sodium dodecyl sulfate (SDS) disrupts nearly all noncovalent interactions in native proteins, and reducing reagents (β-mercaptoethanol) break the S–S bonds (disulfide bonds) among protein molecules [13]. In order to investigate the heat treatment effects on the non-covalent interactions and S–S bonds among soy protein subunits, soluble proteins samples were analyzed by Native-PAGE without adding SDS and reducing re-

agent, according to the method of Laemmli [14]. Protein samples for electrophoresis were prepared by diluting each sample in a sample buffer (40% glycerol and 0.02% bromophenol blue) to create 1 mass/mass% final protein concentrations. And also the samples were not boiled before applying. The separating gel was 9% acrylamide and the stacking gel was 2.5% acrylamide. The gels were fixed in 50% MeOH (methanol)/5% AcOH (acetic acid) solution for 45 min, stained with Comassie brilliant Blue (R250) solution for 45 min and destained with a 5% MeOH/7.5% AcOH solution [15].

Results and discussion

Figure 1 illustrates DSC curves of raw soymilk with various protein concentrations. Raw soymilks with crude protein content of 3.6, 5.4 and 7.2% all exhibited three endothermic peaks (peaks A, B and C), while 1.8% soymilk produced only one apparent endothermic peak. The low content of protein fractions in this sample may have influenced this result. It was observed that the onset temperature and peak temperature increased with increasing crude protein content. Peaks appeared sharper and clearer with the increase of endothermic enthalpy.

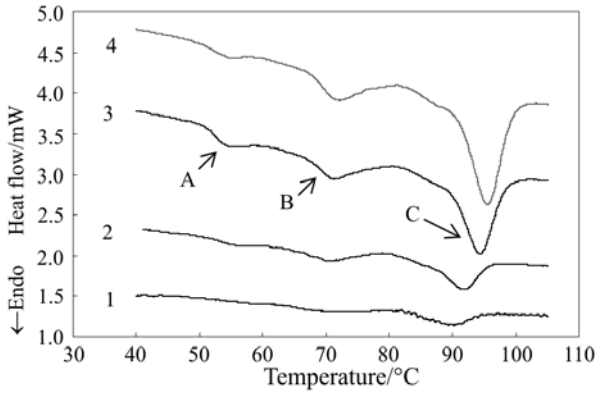

Fig. 1 DSC curves of soymilk with different protein concentration. 1 – 1.8%, 2 – 3.6%, 3 – 5.4%, 4 – 7.2%

Soy proteins in raw soymilk exist in a native state. The transition of proteins from a native to a denatured conformation is accompanied by the rupture of inter- and intra-molecular bonds, and the process must occur concurrently to be discerned by DSC [16–18]. The DSC curve of raw soymilk with a 3.6% protein content was observed to have three endothermic peaks with peak temperatures of 55.69, 70.23 and 91.61°C, and ΔH of 0.0177, 0.0632 and 0.1937 J g^{-1} of solution, respectively (curve 2). Soy proteins in soymilk are composed of two major components, 7S (β-conglycinin) and 11S (glycinin) [19, 20]. From the previous studies on purified soy protein fractions [21], we deduce that peak B and peak C correspond to the denaturation of the two major soy protein fractions 7S and 11S. Peak A corresponds to a minor

protein fraction with low molecular mass. No peaks appeared during the cooling and reheating progress (data not shown here), indicating irreversible denaturation.

Since the denaturation temperature of soymilk protein can be altered by the protein concentration, soymilk with a 3.6% protein was used as the sample in the following heat treatment experiments. Figure 2 shows DSC curves of soymilk after being heated at different temperatures for 5 min. It was observed that after heating at 60°C for 5 min (No. 2), peak A disappeared while peaks B and C decreased. This suggests that the soy protein fractions with a denaturation temperature of 55.69°C had been completely denatured during the heat treatment. Peaks A and B disappeared in the sample heated at 70°C for 5 min (No. 3). This result indicated that the 7S fraction was also completely denatured during the heating process. As the temperature increased, peak C became smaller, with all the peaks disappearing when soymilk was heated at 95°C for 5 min (No. 6). This result confirmed that the denaturation temperature of the 11S fraction was approximately 90°C. The absence of endothermic peaks in the DSC curves indicated soy protein fractions had been denatured before DSC analysis. It is evident from the above results that denaturation temperatures were an important parameter for heat treatment.

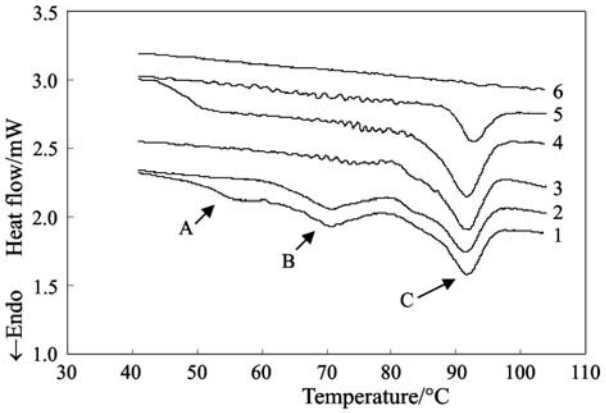

Fig. 2 DSC curves of soymilk after heated at different temperatures for 5 min.
1 – control, 2 – 60°C, 3 – 70°C, 4 – 80°C, 5 – 90°C, 6 – 95°C

Native-PAGE was applied without adding SDS, reducing reagents or heat. Native-PAGE patterns differ from SDS-PAGE patterns, with 7S and 11S bands appearing indistinguishable. Under these conditions, electrophoresis would provide information regarding: 1) relative charge for molecules having the same size and shape or 2) the relative size of molecules with the same charge. Consequently, the native-PAGE patterns can detect the effects of heating on the change of soy protein subunits without the interference from SDS, reducing reagents and sample heating. Native-PAGE patterns (Fig. 3) characterized the change of soy protein molecules after being heated at different temperatures for 5 min. Compared to the control sample, band A appeared after being treated at 60 to 90°C, and disappeared at 95°C. When heated to 90°C or higher, new bands (C and D) appeared, and band F disappeared. It

was observed that increasing the heating temperature gradually eliminated band F. This denoted that this subunit can be gradually dissociated with the increase of treatment temperature. The occurrence of band change, including new band generation and old band disappearance suggests that the soy protein fractions dissociated and associated under different heating temperatures.

The previous analysis results of raw soymilk samples concluded that the denaturation temperature of the 7S protein in soy milk is 70°C and that of 11S protein in soy milk is 90°C. In order to find the influence of heat treatment duration on different protein fractions, soymilk was heated to 70 and 90°C, and then analyzed. Figure 4 corresponds to DSC curves of soymilk after being heated to 70°C for different durations. After the soymilk was heated at 70°C for 5 min, peaks A and B disappeared (No. 1). This indicated that part of the fractions (e.g., 7S) had been completely denatured. Prolonging heating time to 120 min did not significantly change the ΔH of peak C. The ΔH value is actually a net value from a combination of endothermic reactions, such as the disruption of hydrogen bonds and exothermic processes, includ-

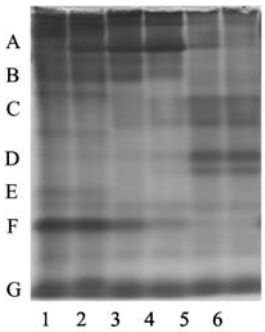

Fig. 3 Native-PAGE patterns of soy protein in soymilk after heated at different temperatures for 5 min. 1 – control, 2 – 60°C, 3 – 70°C, 4 – 80°C, 5 – 90°C, 6 – 95°C

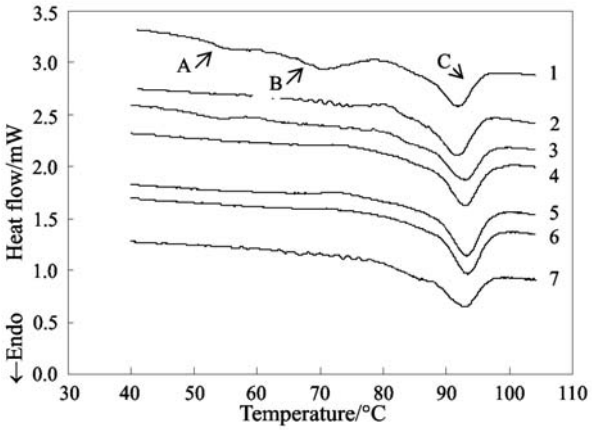

Fig. 4 DSC curves of soymilk after processed at 70°C for different durations. 1 – control, 2 – 5 min, 3 – 10 min, 4 – 20 min, 5 – 30 min, 6 – 35 min, 7 – 120 min

ing protein aggregation and the breakup of hydrophobic interactions [22, 23]. This indicated that the 11S fraction was not wholly denatured. When the heating temperature is below the denaturation temperature (90°C), even prolonged heating times (120 min) cannot denature the 11S fraction completely.

Figure 5 presents native-PAGE patterns of soy protein in soymilk (heated at 70°C for variable durations). Compared to the control sample, a new band (A) appeared and some existing bands disappeared after heated. With the prolongation of heating time, band E gradually became weaker and eventually disappeared. This phenomenon was similar to that of band F in Fig. 3. This result indicated that this subunit was sensitive to both heat treatment temperature and time. Lanes 4, 5, 6 and 7 appeared alike, and were coincident with their corresponding DSC curves. These outcomes indicated that different soy protein fractions had different denaturation temperatures, and that suitable heating temperature should be ensured.

Figure 6 depicts the DSC curves of soymilk after being heated to 90°C for various times. After heated at 90°C for 5 min, fractions with low denaturation tempera-

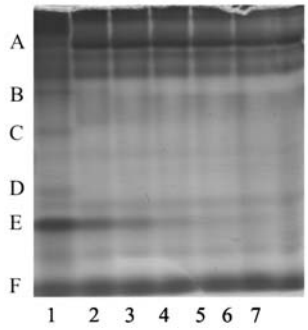

Fig. 5 Native-PAGE patterns of soy protein in soymilk of after heated at 70° C for different durations. 1 – control, 2 – 5 min., 3 – 10 min, 4 – 20 min, 5 – 30 min, 6 – 35 min, 7 – 120 min

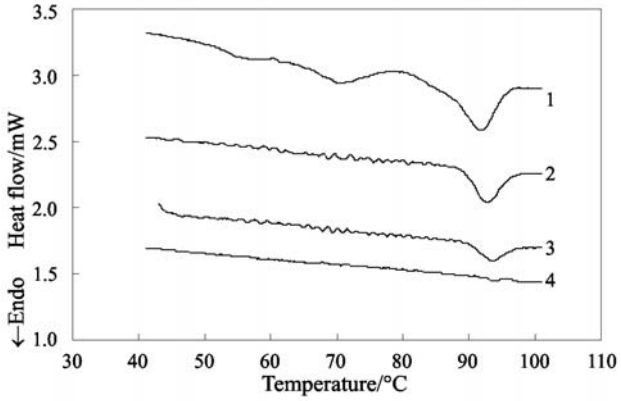

Fig. 6 DSC curves of soymilk after processed at 90°C for different durations. 1 – control, 2 – 5 min, 3 – 10 min, 4 – 15 min

tures (e.g. 7S) had been completely denatured and fractions with higher denaturation temperatures (e.g. 11S) had been partially denatured. No peaks appeared on the DSC curves when the heating time was extended to 15 min. After heat treatment, the soy protein molecules changed from a native state to a denatured state, dissociation into subunits. The rupture of hydrophobic bonds was followed by irreversible denaturation and aggregation through the exposed hydrophobic regions. Endothermic peaks could not be detected by DSC analysis.

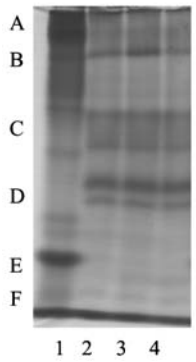

Fig. 7 Native-PAGE patterns of soy protein in soy milk of after heated at 90° C for different durations. 1 – control, 2 – 5 min, 3 – 10 min, 4 – 15 min

Figure 7 presents native-PAGE patterns of soy protein in soymilk after heat treatment at 90°C for different durations. The pattern changed significantly compared to that of the control sample. It was evident that some bands (E) disappeared and some new bands appeared (C and D). Lanes 2, 3 and 4 were similar with those of heat treated samples for a long time durations. This indicated that most of the protein fractions had been changed to denatured states after heat treatment for 5 min. This was also consistent with the DSC results (Fig. 6, curve 2) which exhibited a small peak with ΔH 0.0748 J g^{-1} of solution.

Conclusions

DSC and electrophoresis were applied to analyze denaturation states of protein fractions in soymilk after heat treatments. Thermal studies of soy proteins using DSC revealed thermal transitions occurring at 70°C for 7S and 90°C for 11S. The denaturation temperature increased with the increase of protein content. After heat treatment, soy proteins were dissociated into subunits, some of which might coalesced. The protein fractions cannot be denatured completely even after heat treatment for long durations when the heating temperature is below their denaturation temperature.

* * *

The authors are indebted to the Japan Society for the Promotion of Science (JSPS) for financial support.

References

1 D. Fukushima, Food Sci. Technol. Res., 7 (2001) 8.
2 A. M. Hermansson, J. Texture Stud., 9 (1978) 33.
3 K. C. Kwok and K. Niranjan, Int. J. Food Sci. Tech., 30 (1995) 263.
4 J. de dios Alvarado, J. Food Proc. Eng., 14 (1991) 189.
5 K. A. Thorarinsdottir, S. Arason, M. Geirsdottir, S. G. Bogason and K. Kristbergsson, Food Chem., 77 (2002) 377.
6 B. I. Kurganov, A. E. Lyubarev, J. M. Sanchez-Ruiz and V. L. Shnyrov, Biophys. Chem., 69 (1997) 125.
7 S. F. Wang and D. M. Smith, Poultry Sci. Rev., 5 (1994) 145.
8 P. Relkin, Thermochim. Acta, 246 (1994) 371.
9 J. W. Donovan and R. A. Beardslee, J. Biol. Chem., 250 (1975) 1966.
10 M. Lu, B. Wang, Zh. Li, Y. Fei, L. Wei and Sh. Gao, J. Therm. Anal. Cal., 67 (2002) 689.
11 A. Raemy, J. Therm. Anal. Cal., 71 (2003) 273.
12 AACC, proved Methods of the AACC 10th edition, method 44 (2000) 110.
13 J. M. Berg, J. L. Tymoczko and L. Stryer, Biochemistry, W. H. Freeman and Company, New York, 2001, p. 84.
14 U. K. Laemmli, Nature, 227 (1970) 680.
15 V. Neuhoff, N. Arold, D. Taube and N. Ehrhardt, Electrophoresis, 9 (1988) 255.
16 C. Y. Ma and V. R. Harwalkar, Adv. Food and Nutr. Res., 35 (1991) 317.
17 E. A. S. Rosa and A. S. Rodrigues, HortScience, 36 (2001) 56.
18 M. M. Falcão-Rodrigues, M. Moldão-Martins and M. L. Beirão-da-Costa, Eur. Food Res. Technol., 215 (2002) 317.
19 F. Yamauchi, T. Yamagishi and S. Iwabuchi, Food Rev. Int., 7 (1991) 283.
20 S. Utsumi, A. B. Gidamis, J. Kanamori, I. J. Kang and M. Kito, J. Agric. Food Chem., 41 (1993) 687.
21 N. Kitabatake, M. Tahara and E. Doi, Agri. Biol. Chem., 54 (1990) 2205.
22 W. M. Jackson and J. F. Brandts, Biochemistry, 9 (1970) 2294.
23 S. D. Arntfiel and E. D. Murray, Can. Inst. Food Sci. Technol. J., 14 (1981) 289.