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Effect of corn oil and amylose on the thermal properties of native soy protein and commercial soy protein isolate☆

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Abstract

Differential scanning colorimetry (DSC) was used to estimate thermal property differences between a commercial soy protein isolate (SPI) and milled defatted soy protein flour (MDF). The measurements were determined in the presence of 15, 20, 25, and 30% corn oil and 2, 4, and 6% amylose. SDS-PAGE showed that the SPI material contains aggregates as a result of the isolation procedures and processing. Upon DSC, this protein isolate showed a 7S protein transition peak at 77 °C and an 11S peak at 170 °C, while the MDF sample had a 7S peak at 69 °C and 11S peak at 177 °C. The MDF sample showed ΔH values 4 times greater than that of the SPI sample. These values reflect the effect of the isolation process on the protein. In the presence of corn oil, the MDF sample showed three transition peaks while the SPI sample displayed only two. The MDF sample demonstrated more interaction with oil than did the SPI sample. The change in the ΔH was reflective of this interaction. The addition of amylose to the SPI sample resulted in the appearance of a third peak. Amylose had a mixed effect on the two proteins; peaks of the same protein reacted differently to amylose level. Increasing the amylose level had the most influence on the third peak of the MDF sample. Amylose influence on the two proteins was attributed to a reduction of the amount of free oil in the system. Published by Elsevier Science Ltd.

Keywords: Soy protein isolate (SPI) and defatted soy flour (MDF) Soy protein; DSC; Onset; Peak; ΔH

1. Introduction

The method of protein isolation and processing will cause physicochemical changes on proteins. Composition of the isolating system and interactions between proteins and other components alter protein functional properties. Conditions under which proteins and other components are emulsified (i.e. temperature, pH, ionic strength, aqueous or fatty medium) play an important role in protein behaviour.

Hagerdal and Martens (1976) reported that, during DSC analysis, the denaturation temperature (peak temperature) of myoglobin decreased with an increase in water content, up to 30%, while the transition heat (ΔH) increased with an increase in water content.

The two major soy protein constituents are 7S and 11S. Both components contain low levels of -helix and consist mainly of β-sheet and random coil (Fukushima 1968; Koshiyama, 1970). 7S is composed of nine subunits with MW 180,000 to 210,000 (Wolf, 1972) while 11S is made of 12 subunits with 309,000 to 363,000 MW. The 7S and 11S globulins are stable at an ionic strength of 0.5 M and pH 7.6, and associate at 0.1 M ionic strength and pH 7.6 (Hermansson, 1978).

Koshiyama (1972) studied the effect of salt on soy protein unfolding and association or aggregation as a result of heat. He concluded that salt may reduce dissociation but does not prevent it; aggregation occurs on heating and is enhanced by salt. Prolonged heating of 11S protein caused aggregation and precipitation of aggregates (Wolf & Tamura, 1969).

Differential scanning calorimetry (DSC) can reveal structural and conformational changes of proteins. Onset, peak temperatures (beginning and peak of denaturation curve), and ΔH (change in denaturation enthalpy) can be determined from the thermograms

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(Donovan, Mapes, Davis, & Garibaldi, 1975). Onset and peak temperatures indicate protein thermostability, while hydrophobic/hydrophilic interactions and compactness of proteins are indicated by ΔH (Ma & Harwalkar, 1991).

Previous works reported that DSC analysis, on aqueous dispersion of soy protein, revealed two peaks. The first is 7S, and the second 11S (Hermansson, 1978). At pH 3.5, a DSC thermogram of a soy protein isolate showed a shoulder at 74 °C that could be due to both 7S and 11S components (Puppo & Anon, 1999). Nyanzi, Maga, and Evans (1995) reported that thermal processing and extrusion had a significant effect on the physical characteristics of soy protein/corn starch blends, as detected by DSC and florescence microscopy. Protein–lipid interactions were demonstrated between bovine α-lactalbumin and lipid bilayers, involving a protein reaction with lipid bilayers which disorganizes acyl groups. This process is influenced by the concentration of protein (Dael & Cauwelaert, 1988).

Sessa (1992) reported that the denaturation temperatures of 7S and 11S in soy flour and in purified form are similar, below 9% moisture content, and differ at higher moisture content. These results were done in crimped (not hermetically sealed) aluminium cover pans. Sessa observed a decrease in ΔH as moisture increased in 11S protein soy flour, while ΔH of 7S increased with moisture.

The objective of this research was to examine possible differences between SPI and native soy proteins. Thermal properties were used as a means of comparison using DSC. Changes due to the isolation process may alter soy protein functional properties, such as foaming, which may limit its uses. Conversely, an isolation process that brings about small changes to the protein structure may also benefit soybean utilization.

2. Materials and methods

2.1. Materials

Protein Technology International, St. Louis, MO, supplied EDI Pro A, a food grade soy protein isolate with 87.3% protein content (Nx 6.25) and 5.3% moisture (this will be called "SPI sample"). A commercial sample of soy flour, purchased from a local store and produced by Hodgson Mill, Effingham, IL, was used for comparison (this will be called MDF). The soy flour sample (4% moisture) was sieved through a 230-mesh screen to enrich protein content. The sieved soy flour was batch defatted 4 times with hexane (1:5 w/v flour: hexane ratio) at room temperature and sieved through a 230-mesh screen and centrifuged (3000 ***g*** for 20 min) after each hexane extraction. After each centrifugation step, the top layer was scraped off with a spatula before the second hexane extraction. The protein content of the MDF sample was 67% (N×6.25). Mazola corn oil was purchased from a local store.

Amylose was isolated from common corn starch using the method of Montgomery and Senti (1958). A starch slurry (20 g/l of water) was added to water at 98 °C while stirring for 11–15 min. The pH of the solution was adjusted to 6.0–6.3. The solution was stirred for 5 min and cooled to room temperature in an ice bath. The cooled solution was centrifuged at 2000×***g***. Amylose was in the supernatant, and amylopectin formed a gel at the bottom of the centrifuge tube. The amylopectin gel was re-dispersed twice in water at 98 °C for 11 min and centrifuged at 2000×***g***. Amylose was recovered from the supernatant and precipitated with butanol. Previous studies, using the same procedure, have shown that some amylose is still present in fractionated amylopectin (L. Grant, unpublished data).

2.2. Methods

2.2.1. Differential scanning calorimetry

Five concentrations of SPI or MDF soy protein and corn oil samples were prepared. Sample weight was corrected for protein content before calculations. Protein (1 g) was mixed well with a spatula in a test tube until homogeneous with 15, 20, 25, or 30% corn oil. The same protein–oil blends were mixed with 2, 4, or 6% amylose. These levels of amylose did not produce any DSC transition peak at the experimental conditions, including 0.2 μW/s sensitivity. The DSC (TA Instrument 2920—dual cell and single cell runs) conditions were set at 5 °C/min from ambient to 250 °C for both SPI and MDF materials. The DSC was calibrated against an indium standard. During each run nitrogen flow rate was 24 cm^3/min. Samples were hermetically double-sealed in coated aluminium pans and calculations were made on corrected soy protein content in the sample. Dry samples were scanned using coated hermetically sealed, uncoated crimped aluminium pans, and high volume stainless steel pans to enable comparison with data reported in the literature.

2.2.2. SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to examine possible aggregate formation during soy protein manufacturing processes. SDS-PAGE was performed according to Laemmli (1970) as described by Khan, Tammiga, and Lukow (1989) with 11.8% acrylamide and 0.1% bis-acrylamide for the separating gel. The stacking gel was prepared with 4.5% acrylamide and 0.1% bis-acrylamide. Samples were analyzed as native or reduced with dithiothreitol (DTT) to test if proteins formed aggregates involving disulfide bonds.

2.3. Statistical analysis

A completely randomized design, with two and three factors ANOVA, was applied using SAS (SAS, 1992). Duncan's new multiple range test, at a level of $\alpha = 0.05$ and 0.01%, was used for means' comparisons following a significant *F*-Test result. Means comparison was made on the combined factors when individual factors were found significant.

3. Results and discussion

DSC thermograms of the two proteins generated three measurable parameters, denaturation enthalpy (ΔH), onset temperature (T_o), and peak temperature (T_p). An SPI protein sample with 6% moisture content and a MDF sample with 4.5% moisture content were each scanned at 0% corn oil level. Consistent with reports in the literature, both the SPI and the MDF samples produced a thermogram with two endothermic peaks (Fig. 1). The first peak of the MDF samples was assigned to 7S protein and the second to the 11S (Hermansson, 1978). With no oil addition, the change in enthalpy (ΔH) of the SPI material gave values of 1.4 J/g and 5.6 J/g for the first and the second peaks, respectively, while the MDF sample gave 5.5 J/g and 29.8 J/g for the first and the second peaks, respectively.

The size and shape of the peaks differed, depending on the type of DSC pans used for scanning. In hermetically double-sealed coated aluminium pans (a special TA, instrument sealer was used), the SPI sample formed its first peak at a higher onset temperature when compared to the first peak of the MDF sample (Fig. 1a, b). The second peak of the SPI sample began at a lower onset temperature than that of the MDF sample, indicating structural differences between the SPI and MDF protein samples, possibly due to the manufacturing isolation process (Fig. 1a,b). Thermograms of the two protein samples scanned in coated crimped aluminium pans (pressure was eliminated as a factor) showed a noticeable difference as compared to the hermetically sealed coated pans and stainless steel high volume pans. In crimped coated aluminium pans, the two protein samples formed two peaks, broader and at different temperatures when compared with hermetically sealed pans scanned under same conditions (Fig. 1c,d). The reason for this difference is that, in crimped pans, proteins start to lose moisture as the temperature increases. The decrease in moisture was reported, by Sessa (1992), to increase the peak temperature and decrease the ΔH of soy proteins. It was also difficult to reproduce the

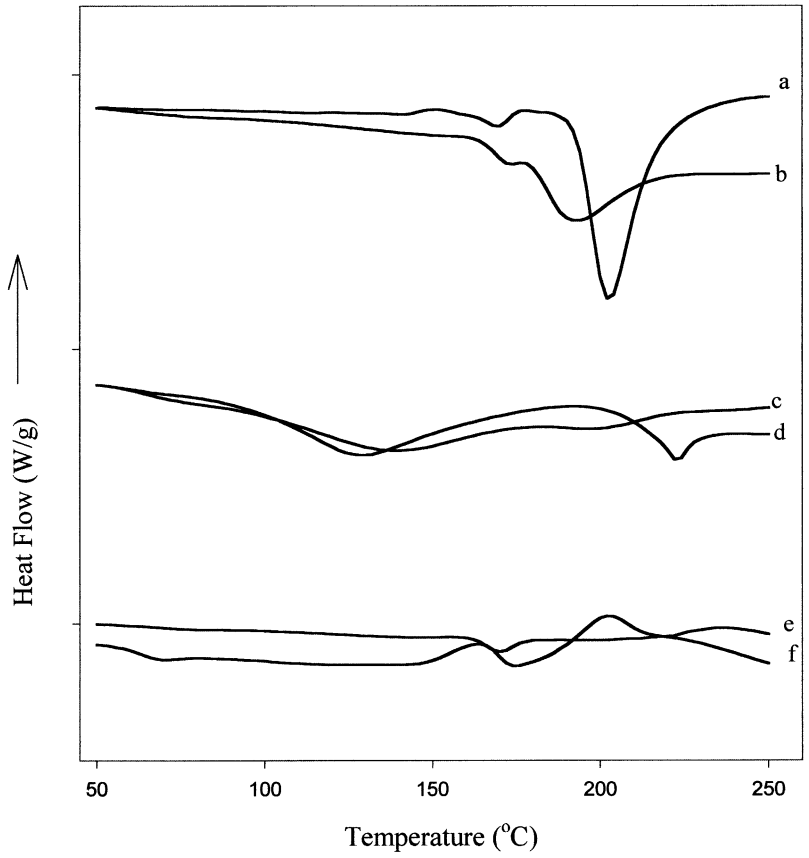

Fig. 1. DSC thermograms of low moisture (4–6%) SPI and MDF soy protein. (a) MDF, coated pan, hermetically sealed; (b) SPI, coated pan, hermetically sealed; (c) SPI, uncoated pan, crimped; (d) MDF, uncoated pan crimped.

runs using crimped pans; as a result, the standard deviation was relatively high.

The scanning also included stainless steel high volume DSC pans. Peaks generated with these pans were different in shape, temperature and ΔH values from those in the other two types of pan (Fig. 1e,f). Consistency of results and low standard deviation made the stainless steel pans a better choice for this study, due to the high temperatures used. Stainless steel pans can maintain higher pressure, generated by the high temperature used in this experiment, than the other two types. Pans were cooled and re-weighed to detect moisture loss during scanning.

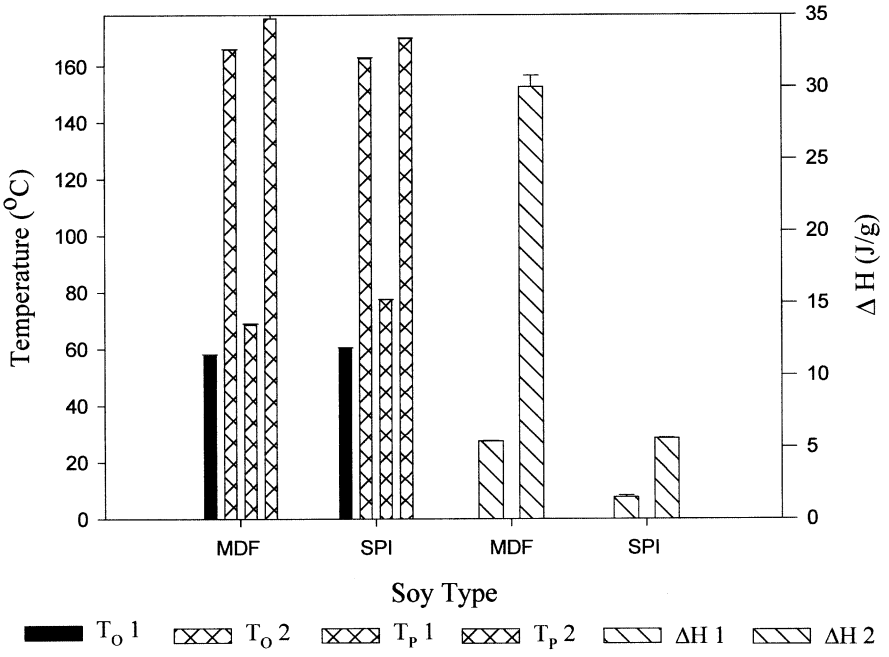

Fig. 2. Thermal properties of MDF and SPI soy proteins with 4–6% moisture content.

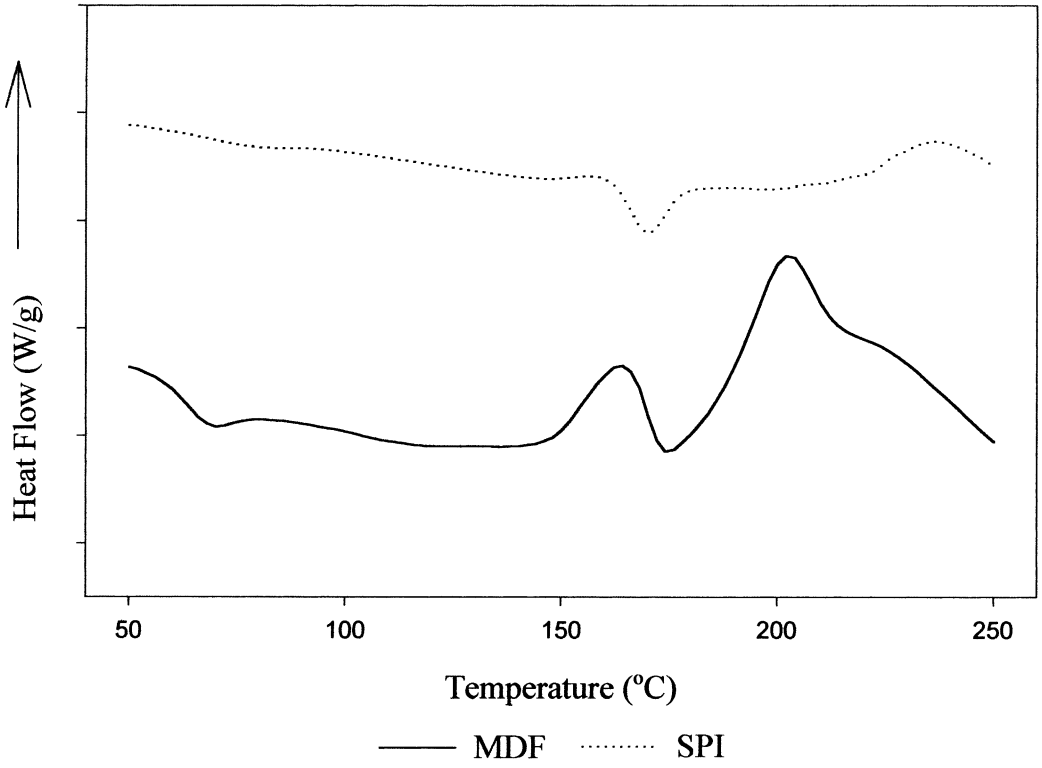

Fig. 3. DSC thermograms of MDF and SPI soy protein with 4–6% moisture content.

Results showed that crimped pans lost up to 35% of their weight, double-sealed lost about 6%, while stainless steel pans lost less than 1%. The stainless steel pans were chosen also for their high volume, which allows larger samples and thus lower error. The stainless steel pans showed the 7S peak (the first peak) at a peak temperature at 69 °C for the MDF sample and 177 °C for the 11S, while the SPI sample showed peak temperatures at 77 °C and 170 °C for 7S and 11S, respectively (Fig. 1). The ΔH values differed more between the samples than did the temperatures.

The differences in the temperatures and ΔH between SPI and MDF samples, scanned with all three types of pans, suggest structural differences between samples due to manufacturing process (Figs. 2 and 3). These structural differences are evident upon SDS-PAGE (Fig. 4), where aggregates are present in the manufactured sample. That could be due to partial protein denaturation, as a result of the isolation procedure, followed by aggregation. The presence of these aggregates is apparent on the gel electrophoresis profile, which shows a peak with 3000 intensity value and zero distance from the top of

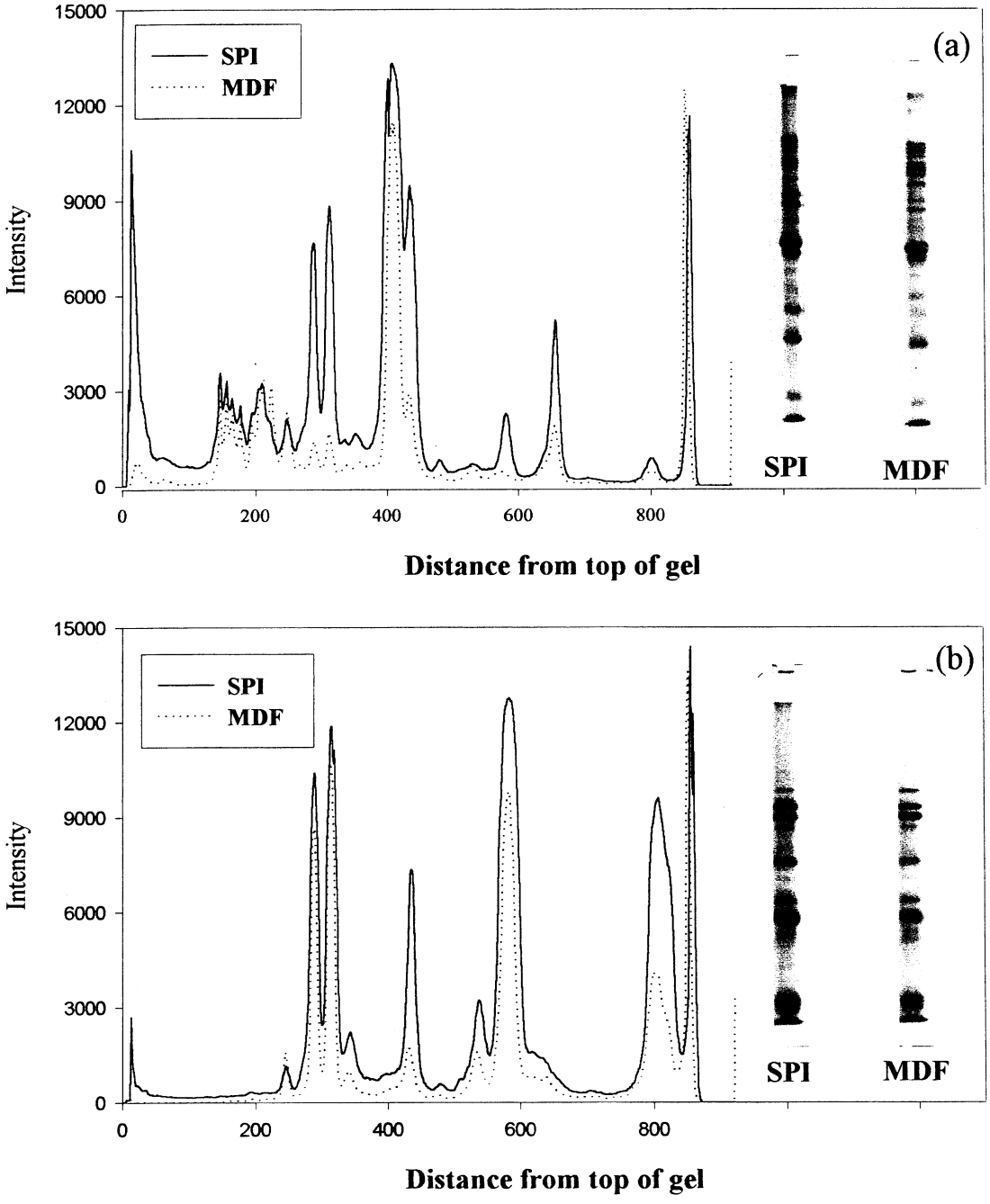

Fig. 4. SDS-PAGE profiles of SPI and MDF soy protein. (a) Reduced (b) Non-Reduced.

the gel after disulfide bond reduction (Fig. 4). Reduction of disulfide bonds with DTT did not help the SPI sample penetrate the stacking gel but it stayed at zero distance from the top of the gel. This indicates that physicochemical bonds other than disulfide bonds held the SPI aggregate together. SDS-PAGE profiles of the reduced protein samples showed clear differences in electrophoretic band intensities; the absence of some bands signified structural differences between the two samples (Fig. 4). The thermogram of the SPI sample showed a wider (higher difference between onset and peak temperatures) profile, indicative of a less cooperative denaturation process, more heterogeneous than for the MDF sample (Fig. 3). The manufacturing process effect on soy proteins was also reflected by denaturation energy (ΔH) values: native proteins (MDF) required 4 times the energy to denature the 7S and 5 times the energy to denature 11S as compared to the SPI sample (Figs. 2 and 3).

The thermal property differences between the SPI and MDF protein samples remain apparent in the presence of corn oil (Figs. 5 and 6). An increased level of corn oil tended to increase the onset and peak temperatures of both samples, but this was more noticeable with the SPI sample, indicating protein surface stability (Fig. 5). A possible explanation for this stability could be a relatively hydrophobic protein surface, which would be more stable in the hydrophobic oil environment, thus showing higher denaturation temperatures. The 11S protein (second peak) showed more interaction with oil than 7S, possibly due to its higher denaturation temperature and MW. The higher denaturation temperature increases the mobility of oil molecules and thus facilitates interaction with 11S. This interaction is reflected by the T_o, T_p, and ΔH shifts (Figs. 5 and 6). The aggregation of the SPI sample may help in burying the hydrophobic groups and limit oil–protein interactions on the surface. The increase in oil concentration caused an increase in the ΔH of the 11S

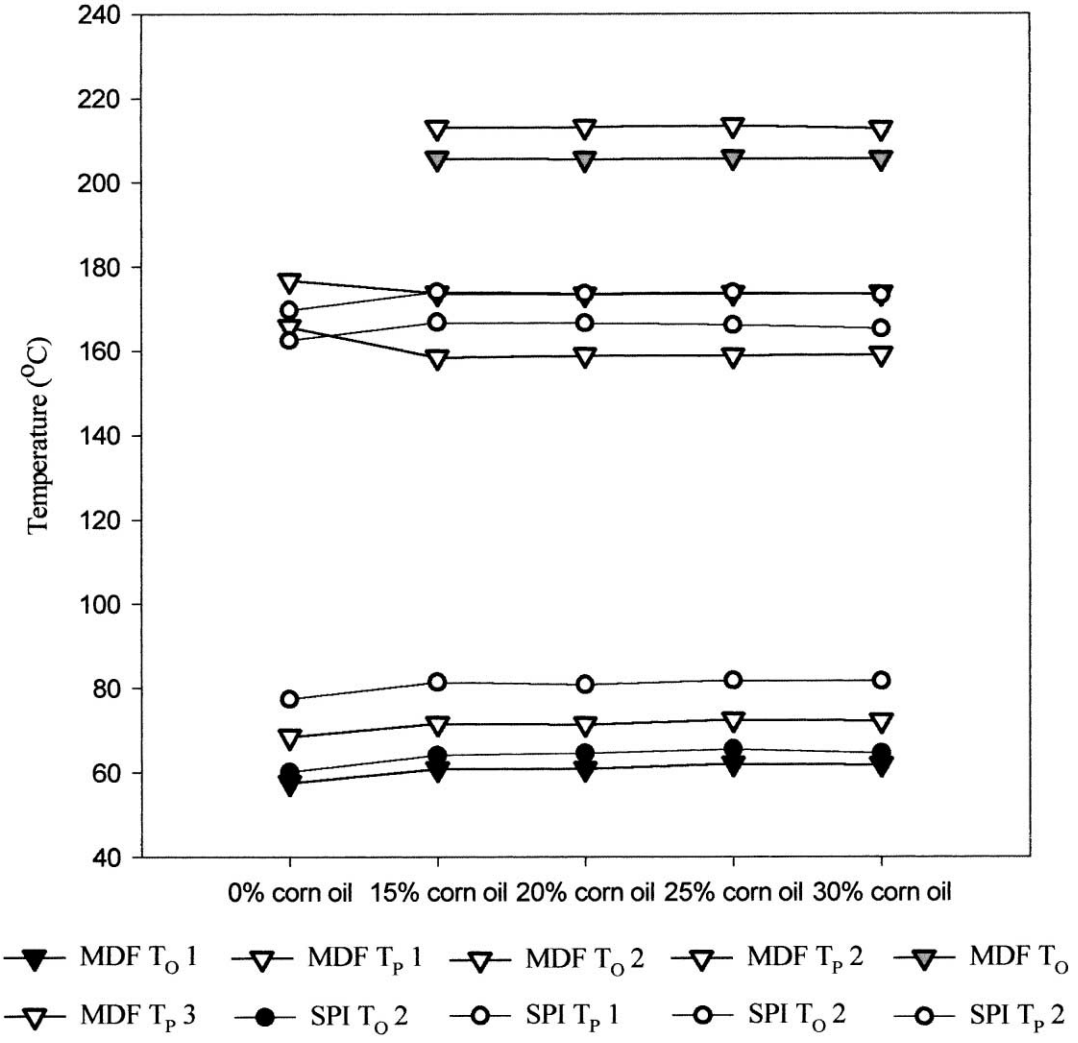

Fig. 5. Effect of corn oil concentration on the onset and peak temperatures of MDF and SPI.

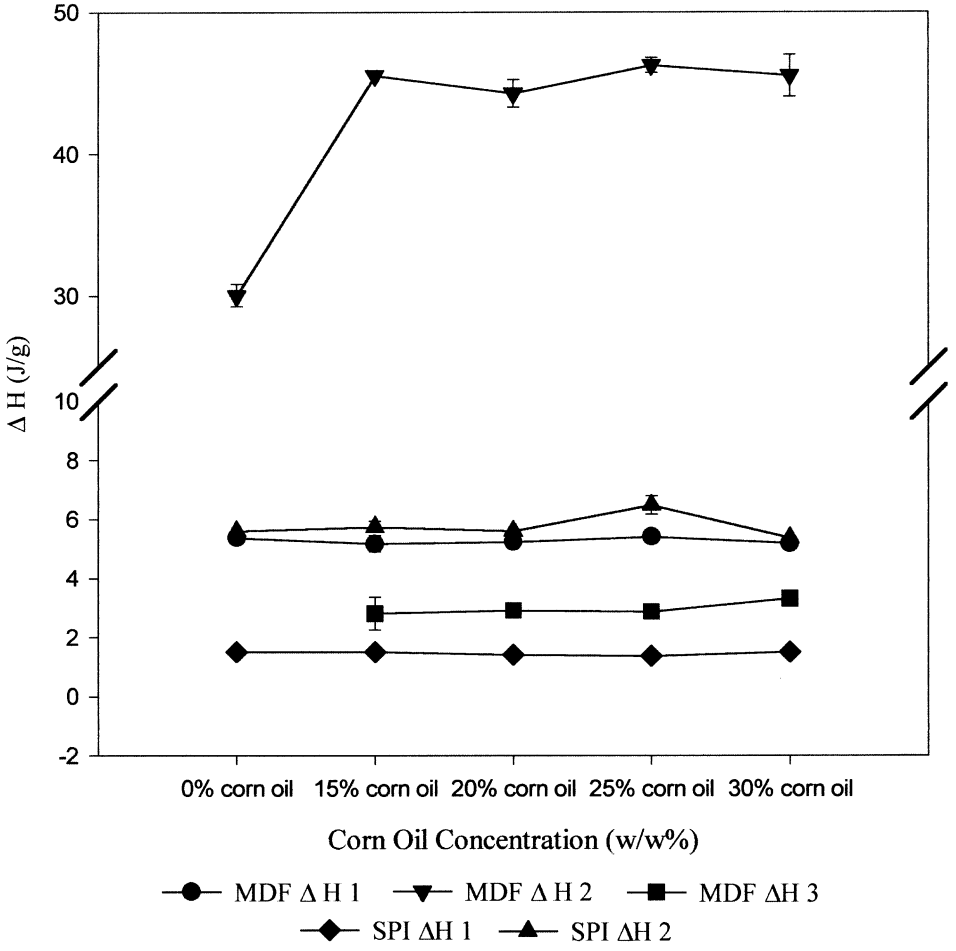

Fig. 6. Effect of corn oil concentration on the ΔH of MDF and SPI.

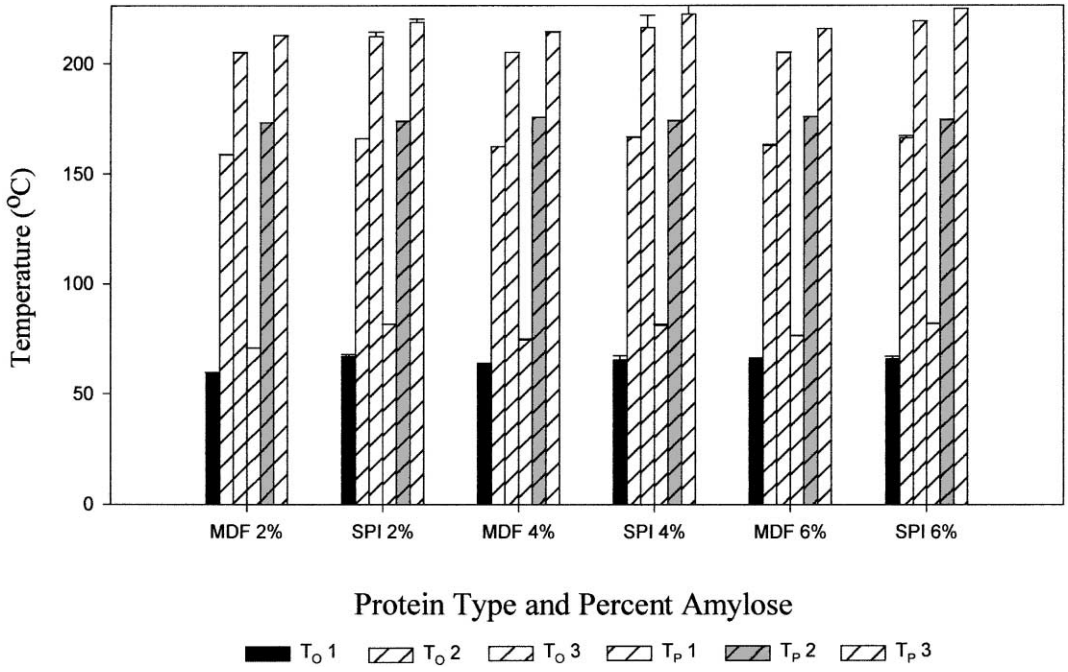

Fig. 7. Effect of 15% corn oil and 2, 4, and 6% amylose on the onset and peak temperatures of MDF and SPI.

peak of the MDF sample, indicating interaction, while it increased the ΔH of the SPI sample only slightly. Overall, the onset and peak temperatures of 7S in both samples were increased as a function of oil increase, while the oil increase had no significant effect on 11S. The effect of corn oil on the onset and peak temperatures of 7S in both samples was similar: addition of 15% oil significantly increased the temperatures, while further oil increase had little effect. The SPI sample had a higher peak temperature than the MDF (Fig. 5). This indicates that manufacturing isolation of soy protein facilitated greater 7S aggregation than 11S, and thus increased the peak temperature in the presence of oil. Conversely, the 11S protein shifted more upon oil addition, where the onset and peak of 11S of the MDF were decreased, and that of the SPI sample was increased upon oil addition (Fig. 5). The two protein samples showed significant ΔH_2, T_{o2} and T_{p2} differences between protein types at $\alpha = 0.05$ and 0.01%. The ΔH values of the two protein samples are significantly different across protein type ($\alpha = 0.05$ and 0.01), where the first and second peaks of the MDF sample corresponded to four and five times,

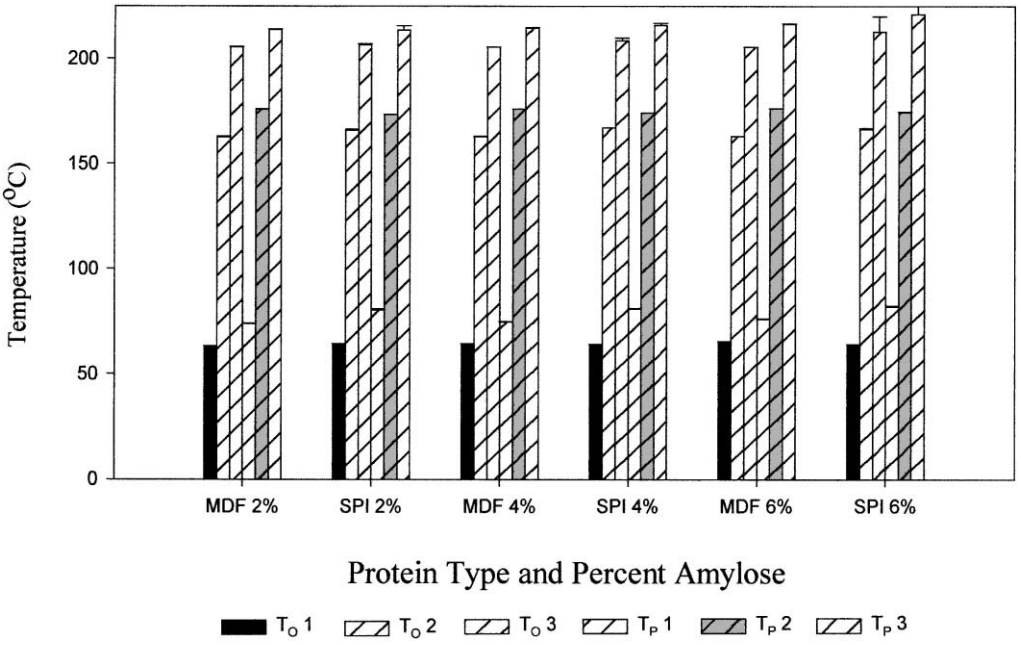

Fig. 8. Effect of 20% corn oil and 2, 4, and 6% amylose on the onset and peak temperatures of MDF and SPI.

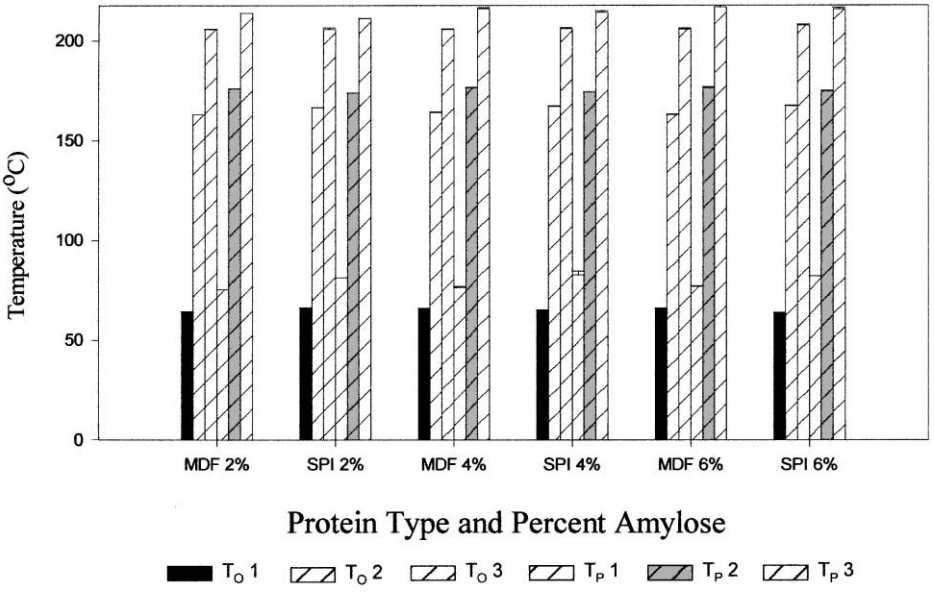

Fig. 9. Effect of 25% corn oil and 2, 4, and 6% amylose on the onset and peak temperatures of MDF and SPI.

respectively, more denaturation energy than those of the SPI sample (Figs. 5 and 6).

Upon adding corn oil to the MDF sample, a third peak at a higher temperature and lower H than for 7S and 11S emerged. This third peak was not present in the SPI sample, possibly due to aggregation with 7S or 11S during isolation. Addition of corn oil to the MDF sample seemed to distinguish this protein from 7S and 11S. The higher ΔH value indicates that the third peak could have a more hydrophobic surface; thus it was affected more by addition of corn oil than were 7S and 11S proteins (Fig. 6). The increase in corn oil content facilitated the increase in ΔH of the 11S (ΔH_2) and ΔH_3 significantly ($\alpha = 0.05$ and 0.01%), while ΔH_1 showed a significant difference only between protein types. The interaction between corn oil and SPI sample was noticeable as oil levels changed: 15% oil yielded the highest ΔH_2 increase for the MDF sample. The presence of 20% oil, however, showed the lowest ΔH_2 value (Fig. 6). The 11S protein of the SPI sample

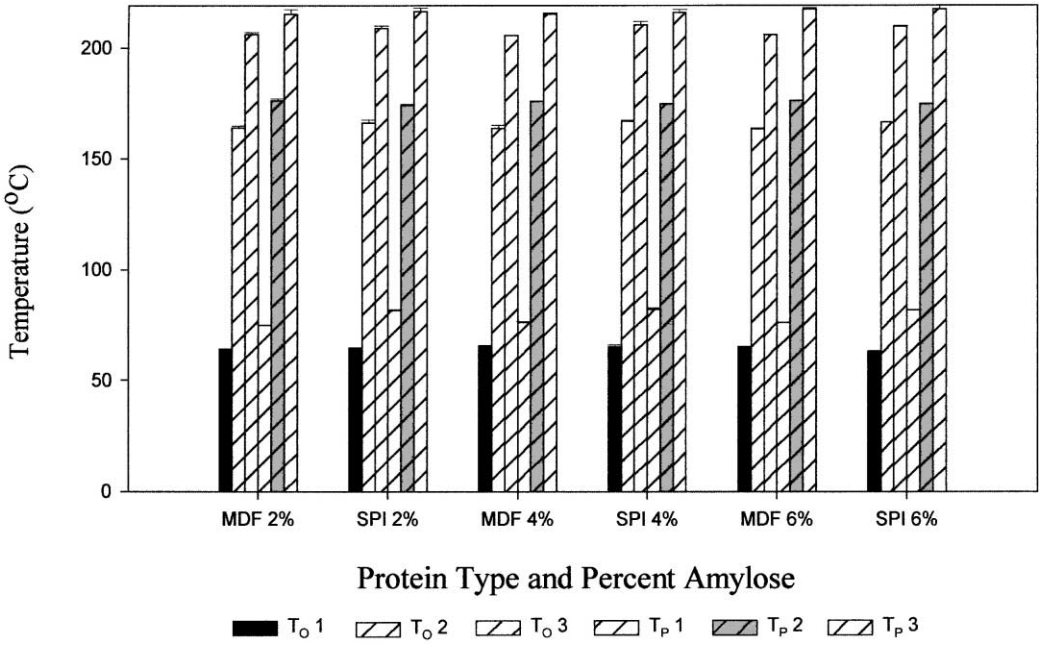

Fig. 10. Effect of 30% corn oil and 2, 4, and 6% amylose on the onset and peak temperatures of MDF and SPI.

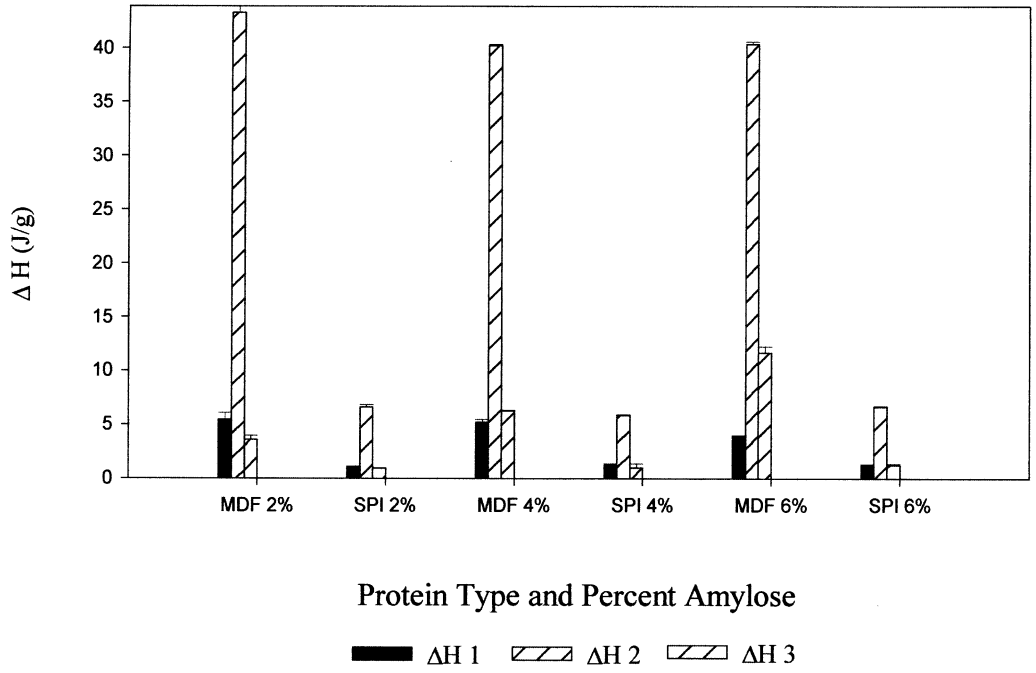

Fig. 11. Effect of 15% corn oil and 2, 4, and 6% amylose on the ΔH of MDF and SPI.

showed a mixed change with increased oil content. The 25% oil content displayed the highest value while 30% showed the lowest value. The decrease in ΔH_2 of the SPI sample, in the presence of 30% corn oil, could be due to disaggregation of aggregates as oil content increased. The presence of oil may disrupt interactions between 7S and 11S during isolation, as Wolf (1970) suggested. Possible hydrophobic interactions between corn oil and 7S or 11S could take place, which help their disaggregation and thus lower ΔH values. Although the two proteins showed significantly different ΔH_1values, the oil level within the MDF sample did not show a significant difference (Fig. 6).

Amylose addition generated the third peak for the SPI sample that was detected by oil addition, as in the MDF sample. The introduction of amylose, at different levels, altered the thermal behaviour of the protein–oil mixture. Amylose seemed to have an opposite effect on the

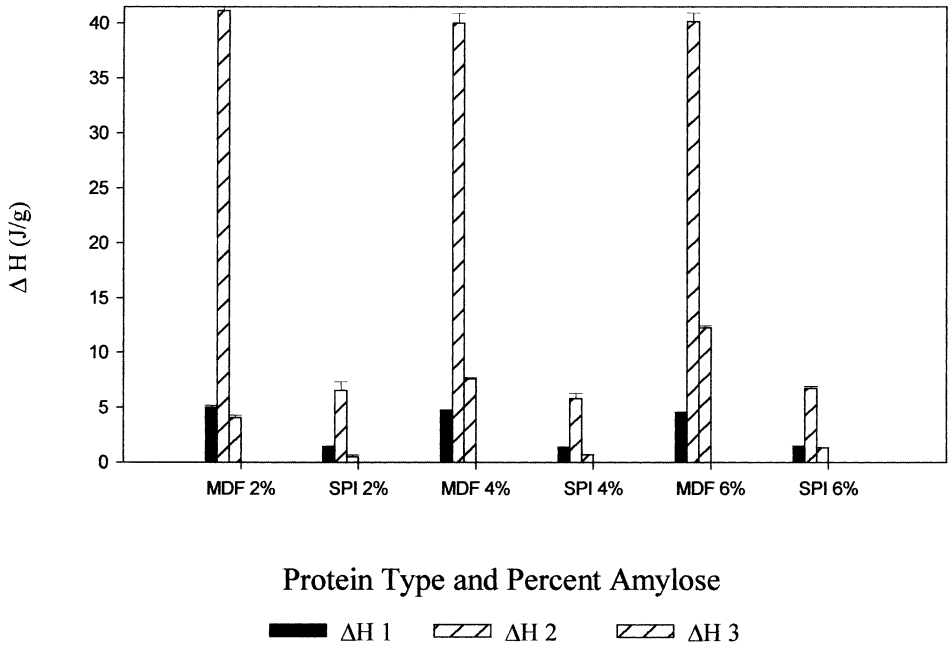

Fig. 12. Effect of 20% corn oil and 2, 4, and 6% amylose on the ΔH of MDF and SPI.

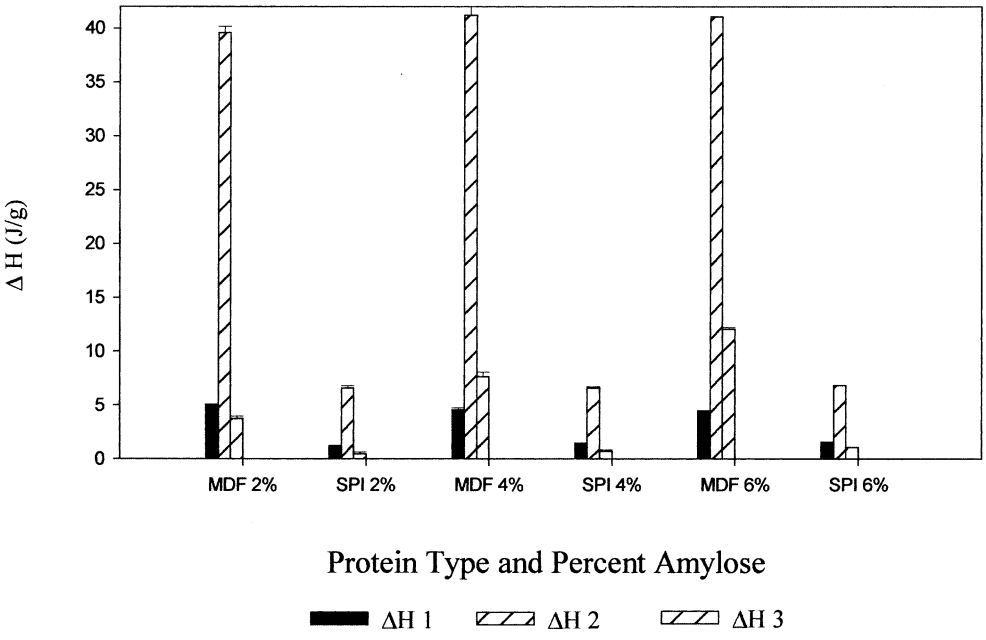

Fig. 13. Effect of 25% corn oil and 2, 4, and 6% amylose on the ΔH of MDF and SPI.

protein samples as compared to oil. That is apparent in Figs. 7–14, where oil addition to MDF sample increased ΔH while amylose added to the same sample, in the presence of oil, decreased ΔH. Oil seemed to protect the protein, but it is also known to form a complex with amylose's helical structure (Carl Hosney, 1990). The formation of an oil–amylose complex appears to take away oil and reduce protein protection. A change in the thermal properties of the SPI protein, indicative of interactions, was more apparent for SPI than the MDF protein. Exposure of groups, during manufacturing, to the protein surface could explain this interaction. The statistical data demonstrated that amylose level did significantly affect some thermal parameters of the protein samples. Since the statistical analyses demonstrated that protein type, oil level, and amylose level have significant effects on ΔH_1, and ΔH_3, means comparison was done only on the combined effect of the two.

Oates et al. (1987) and Sessa (1992) reported that lower moisture content increased the denaturation temperature

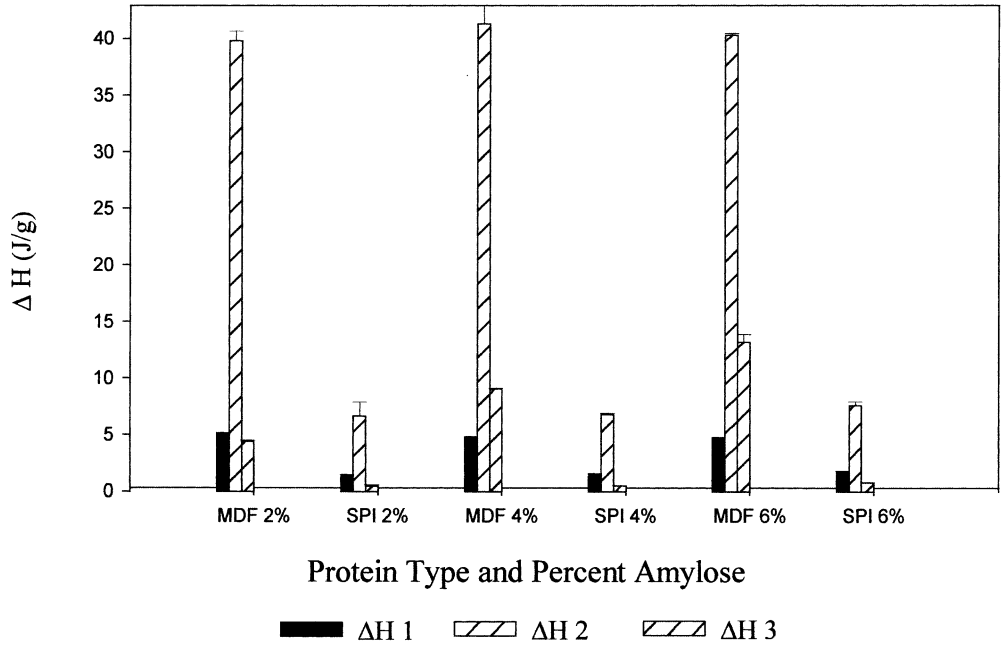

Fig. 14. Effect of 30% corn oil and 2, 4, and 6% amylose on the ΔH of MDF and SPI.

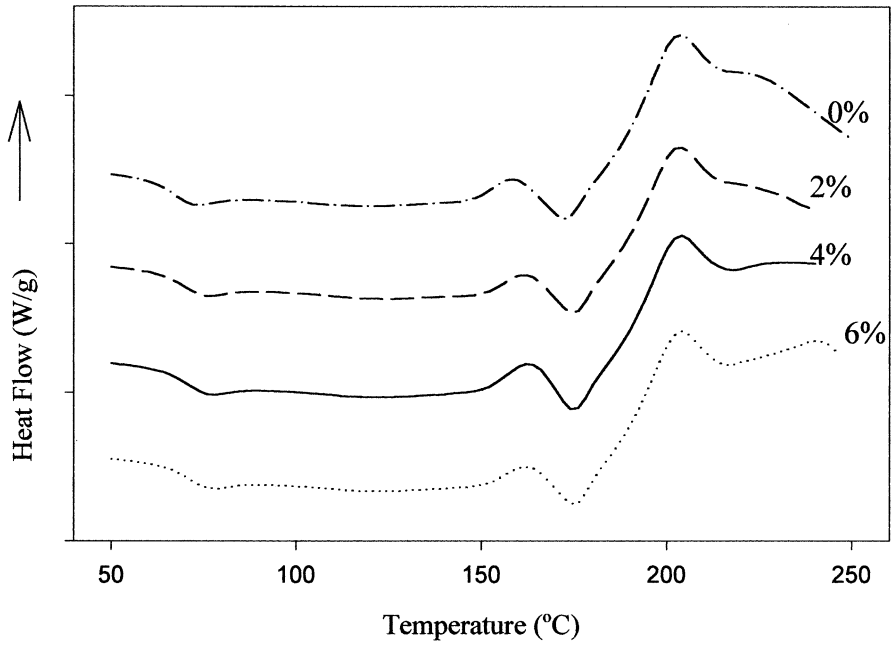

Fig. 15. DSC thermograms of MDF with 25% corn oil and 2, 4, and 6% amylose.

of soy protein while ΔH increased with moisture up to 31% water content. Low moisture increases protein thermal stability in a relationship connected with insufficient available water for protein hydration; incomplete protein unfolding results, which in turn decreases H and increases the onset temperature. In general, 15% oil content slightly increased the onset temperature of both protein samples with the increase on amylose content from 2 to 4 and 6% (Fig. 7). Amylose addition differentiated the first two peaks and the third peak of the MDF sample, where T_o and T_p of the first two peaks increased with amylose addition while the third peak stayed the same. This may indicate that the third peak, generated upon oil addition to the MDF sample, was less reactive with amylose. The effect of amylose on onset and peak temperatures of 7S and 11S of the MDF sample is similar to that of moisture: low moisture content increased onset and peak temperatures. Amylose addition seemed to have little influence on 7S and 11S of the SPI protein, in which onset and the peak temperatures remained unchanged (Figs. 7–10).

The increase in amylose content significantly reduced ΔH of the 7S protein as more oil was added to the MDF sample (Fig. 11). This phenomenon could mean that the first peak (7S) of the MDF material became less stable due to amylose–oil interaction (amylose helix–oil complex), which left the protein less protected, thus lowering ΔH. The 11S protein of the MDF sample showed results similar to the 7S only at 15 and 20% oil contents, while the 25 and 30% oil resulted in a ΔH increase (Figs. 11–14). This indicates that the amount of oil that interacted with amylose is limited, and the remaining oil helped to protect the protein and thus increased the ΔH at 25 and 30%. The third peak, however, showed ΔH increases at all oil and amylose levels (Fig. 15). This indicates that this peak, generated upon oil addition, needs a small amount of oil for it to stabilize and stay distinct and separate from the other two peaks. The effect of amylose on the third peak was similar to that of the 11S protein, in which 25 and 30% oil both significantly increased ΔH (Fig. 6).

Conversely, the SPI sample showed no change of ΔH values at 15 and 20% oil levels of the 7S and 15% oil level of the 11S peaks at 2, 4 and 6% amylose. All remaining oil levels showed significant increases on ΔH of all three peaks. The third peak of the SPI sample showed a similar effect upon amylose addition to that of the MDF sample, but the third peak of the later sample had higher ΔH values, indicating organization and compactness of the native protein. This supports the speculation of the effect of manufacturing during isolation on soy proteins. In general, cooperative denaturation of either protein was not noticed. That was evident from the homogeneous difference between the onset and peak temperatures and the wide shape of the DSC thermogram (Fig. 3). A small difference between onset and peak temperatures of any protein is indicative of cooperative denaturation.

More samples from other soy protein manufacturers are needed to complete this study to confirm the results reported in this paper. Hydrophobicity testing results of the samples used in this study will be reported separately. The new system consisted of 50% amylopectin and 50% soy protein mixture at different ionic strengths, moisture level, and pH. This should give a more complete picture of protein behaviour in hydrophilic (phosphate buffer) and hydrophobic (corn oil) environments, which reflect some current uses of soy protein isolates.

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