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INFLUENCE OF ENZYMATIC HYDROLYSIS ON FUNCTIONALITY OF PLANT PROTEINS DSC studies

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Abstract

DSC was used to study the extent of denaturation of hemisphaericin and mexicain hydrolysates from corn gluten, soybean and sunflower meals. It was observed that the defatted meals studied exhibited only one broad peak transition. The data obtained demonstrated that the partial protein denaturation found with hemisphaericin or mexicain is correlated to modifications of functional properties. The two enzymes display different modes of action, according to the protein source.

Keywords: DSC, enzymatic hydrolysis, functional properties, hemisphaericin, mexicain

Introduction

One of the most efficient means of improving the functional properties of proteins is to submit them to enzymatic hydrolysis. Control of the molecular size of hydrolysates is essential if optimum and reproducible changes in functional properties are to be achieved. This can be accomplished by using highly specific proteases [1]. The functionality of protein hydrolysates is influenced by the degree of hydrolysis (DH), the enzyme specificity, the physical and chemical natures of the intact parent protein and the hydrolysis conditions. Partially and extensively hydrolysed proteins acquire properties that affect the utilization of the final product.

In oilseed processing, during oil extraction, the proteins remaining in the meal usually exhibit minimal functional properties that are essential in food formulation. These protein sources may have an excellent amino acid balance and all prerequisites for nutritionally superior proteins, but may not have an impact on human nutrition unless they have the functional properties necessary for their incorporation into food systems.

Likewise, it is essential to develop a better understanding of the structure – property relationship of proteins. Thus, further research is needed in order to understand the relationship between DH and hydrolysate functionality. Denaturation of proteins changes their viscosity, solubility and emulsifying properties. At present, attempts are being made to use DSC to gain information on the protein functionality and the effects of the processing conditions, both related directly to food systems.

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Since thermal analysis is an excellent tool for the selection of processing conditions to maximize the functionality of vegetable proteins [2] in specific applications, we have used DSC to compare the extent of denaturation reached by hemisphaericin and mexicain, proteinases from the Mexican plants *Bromelia hemisphaerica* [3] and *Pileus mexicanus* [4, 5], hydrolysates from corn, sunflower and soybean meals, produced at two different values of DH.

Experimental

Materials

The defatted soybean type I and corn gluten (from corn endosperm) meals used were commercial products (Sigma Chemical Co.). The protein contents were 44.5 and 56.9% (N×6.25), respectively. Defatted sunflower meal was obtained by the Sodini and Canella [6] procedure. The protein content was 54.6% (N×6.25).

Proteinases

Refined hemisphaericin and mexicain preparations were obtained by a pilot plant procedure that includes the following operations: extraction of juice from *Bromelia hemisphaerica* fruits, or homogenization of *Pileus mexicanus* latex; centrifugal clarification, ultrafiltration and spray drying (mexicain) of freeze drying (hemisphaericin). The proteolytic activities obtained were 1.06 U/mg for mexicain and 0.87 U/mg for hemisphaericin.

Assay of proteolytic activity

Hemisphaericin and mexicain activities were assayed by using casein as substrate [7, 8]. The reaction mixture contained 1.9 ml of 1% casein in 0.020 *M* phosphate buffer *pH* 7.6 and 0.1 ml of enzyme dissolved in the same buffer. The mixture was incubated at 35°C for 10 min. The precipitate was removed by centrifugation and the absorbance of the supernatant was determined at 280 nm. One unit of proteolytic activity was defined as the quantity producing an absorbance difference of 1.0 between the blank and sample.

Functional properties

Protein solubility was determined by the Kabirullah and Willis procedure [9], while water absorption and oil absorption were measured by the centrifugation method of Lin *et al.* [10] as modified by Paredes-Lopéz and Ordórica-Falomir [11]. Emulsifying activity was determined by the Pearce and Kinsella procedure [12].

Differential scanning calorimetry

Differential scanning calorimetry studies were performed with a Shimadzu DSC-50 instrument. Protein samples from corn gluten, soybean and sunflower de-

fatted meals were placed in DSC hermetic pans with a good contact between the sample and the capsule bottom ensured. An empty hermetic pan was used as reference. All the samples were scanned at 5°C min^{-1} over the range 10–120°C. The endotherm areas and enthalpies of transitions were measured with the thermal analysis software TA-50WSI.

Results and discussion

DSC curves

In general, it was observed that the sunflower, corn gluten and soybean defatted meals studied exhibited only one broad peak. In this broad transition, overlapping of the thermal transition of starch gelatinization that shifted to higher temperatures in the presence of proteins could have occurred, as reported by Mahmoud [13] for tapioca starch in combination with casein and whey protein hydrolysates. Sorgentini *et al.* [14] observed the disappearance of 7 S protein when soybean isolates were treated at 80°C. Wright and Boulter [15] studied the DSC curves of several legume meals, including soybean, fababean, cowpea, dry bean and three lupin species. They observed that 7 S peaks were present in all meals, but the 11 S transition was absent in some species. Equally, in some flours they observed a starch gelatinization peak, but in soybean and lupin meals these peaks were absent. In this context, our results are similar.

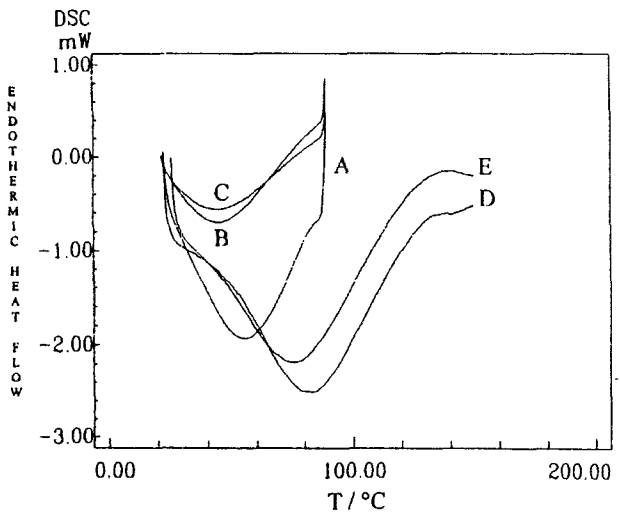

Fig. 1 DSC curves of corn gluten meal treated with proteinases. A, untreated, B, hemisphaericin for 1 h, C, hemisphaericin for 3 h, D, mexicain for 1 h, E, mexicain for 3 h

Under these conditions, it can be deduced that the soybean, corn and sunflower meals assayed were composed of proteins in a much more highly denatured state. Nevertheless, as has been claimed by other researchers [16–19], these proteins have

no functional properties, but are materials susceptible to improvement with proteolytic enzymes.

Figure 1 shows DSC curves of corn gluten meal and hydrolysates obtained with hemisphaericin and mexicain. The following characteristics were observed: hemisphaericin hydrolysates after reaction times of 1 and 3 h exhibited an endotherm with T_{max} shifted to lower temperatures. In both cases, the areas (Table 1) were diminished appreciably by the proteolytic effect. On the other hand, mexicain hydrolysates show transitions with T_{max} shifted to higher temperatures and the peak obtained is broader than that in the intact protein profile. In general, the endotherm areas diminished more on hemisphaericin treatment than on mexicain treatment, which is indicative of action differences between the proteinases. However, this partial degree of protein denaturation corresponds to improvements in solubility, water absorption capacity, oil absorption capacity and emulsifying activity index (Table 2). The results of corn gluten meal protein enzymatic denaturation suggest that the protein hydrophobic zones, initially inaccessible, were exposed by the proteolytic treatment, improving their functionality.

Table 1 Data obtained from DSC curves

Meal	T_{onset}/°C	T_{endset}/°C	T_{max}/°C	ΔH/J g^{-1}
Corn gluten (endosperm)				
untreated	21.9	94.9	54.7	188.5
w/hemisphaericin 1 h	20.1	90.6	44.1	158.7
w/hemisphaericin 3 h	21.3	90.5	44.2	127.1
w/mexicain 1 h	40.0	125.3	83.4	148.3
w/mexicain 3 h	33.0	123.9	75.6	146.7
Soybean				
untreated	25.2	134.4	75.8	227.6
w/hemisphaericin 1 h	40.0	128.9	92.4	143.4
w/hemisphaericin 3 h	45.5	124.0	83.6	106.5
w/mexicain 3 h	35.5	121.6	75.6	142.7
Sunflower				
untreated	33.6	139.3	85.1	229.2
w/hemisphaericin 1 h	26.2	140.7	81.3	223.7
w/hemisphaericin 3 h	24.9	134.0	80.7	194.0
w/mexicain 3 h	34.5	154.3	84.9	180.0

The DSC curves of untreated defatted soybean meal revealed one major broad endothermic peak (Fig. 2), with T_{max} at 75.8°C (Table 1). T_{max} of this broad peak

shifted to higher temperature (92.4°C) on hemisphaericin treatment for 1 h, and presented an area reduction of 36.58% (Table 1). When the hydrolysis duration reached 3 h, the T_{max} peak had shifted to 83.60°C and the area had diminished by 53% (Table 1) with respect to that for the intact soybean meal proteins. This result could be ascribed to enzymatic action on soybean proteins. On the other hand, on mexicain protein treatment for 3 h there was no modification of T_{max}, although it was observed that the peak area diminished by 37% (Table 1). These changes cor-

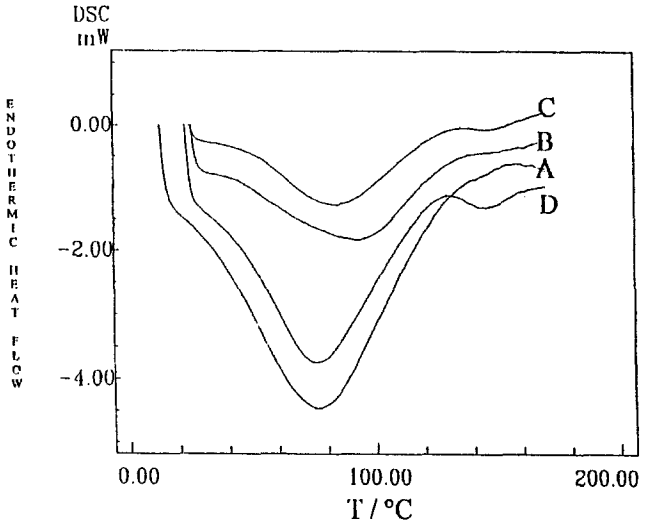

Fig. 2 DSC curves of soybean meal treated with proteinases. A, untreated, B, hemisphaericin for 1 h, C, hemisphaericin for 3 h, D, mexicain for 3 h

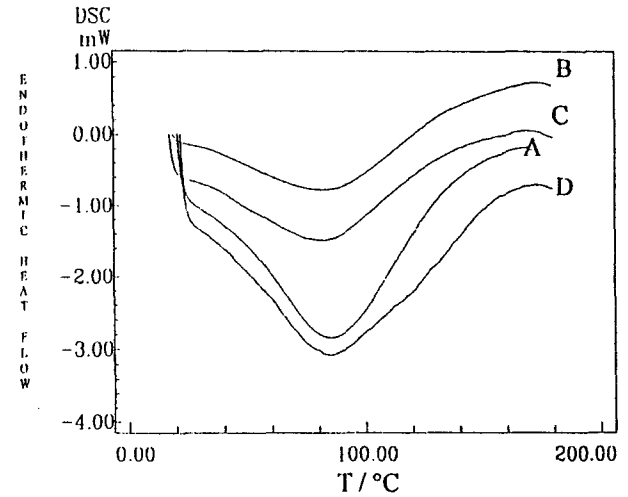

Fig. 3 DSC curves sunflower meal treated with proteinases. A, untreated, B, hemisphaericin for 1 h, C, hemisphaericin for 3 h, D, mexicain for 3 h

respond to an improvement in oil absorption capacity and decreases in solubility, emulsifying activity index and water absorption capacity.

Untreated sunflower meal proteins gave a DSC curve (Fig. 3) with a T_{max} peak at 85.10°C. T_{max} shifted slightly to lower temperature on hemisphaericin digestion for 1 and 3 h (81.3 and 80.7°C, respectively). The peak areas diminished by 15.34% for the 3 h hydrolysate on both enzymatic treatments (Table 1). On mexi-

Table 2 Functional properties of hemisphaericin and mexicain hydrolysates from corn gluten, soybean and sunflower meals

Meal	Solubility (at *pH* 7)/ %	Water absorption capacity (g/g protein)	Oil absorption capacity (g/g protein)	Emulsifying activity index ($m^2 g^{-1}$ protein)
		Hemisphaericin		
Corn gluten				
untreated	42	3.36	2.45	4.69
1 h treatment	64	3.88	6.88	5.39
3 h treatment	64	4.23	7.52	4.99
Soybean				
untreated	95	4.74	3.2	11.33
1 h treatment	63	6.31	8.52	7.52
3 h treatment	55	5.46	9.21	7.49
Sunflower				
untreated	55	5.4	5.65	6.17
1 h treatment	81	4.78	7.18	4.11
3 h treatment	84	4.44	7.36	4.24
		Mexicain		
Corn gluten				
untreated	42	3.36	2.45	4.69
1 h treatment	75	3.0	5.48	8.03
3 h treatment	82	3.13	7.4	9.19
Soybean				
untreated	95	4.74	3.2	11.33
1 h treatment	96	4.45	5.28	6.33
3 h treatment	51	3.32	7.21	6.68
Sunflower				
untreated	55	5.4	5.65	6.17
1 h treatment	30	5.43	7.61	6.62
3 h treatment	47	5.19	7.93	7.13

cain treatment for 3 h, T_{max} and the peak area did not change appreciably, which would suggest the lack of enzyme action. However, with respect to functional properties, solubility and oil absorption capacity, improvements were obtained with hemisphaericin hydrolysates from sunflower meal. On the other hand, mexicain improved the emulsifying activity index and oil absorption capacity, but diminished the solubility (Table 2).

The data indicate that partial protein denaturation, with hemisphaericin or mexicain, is correlated to modifications of functional properties. The two enzymes act differently, according to the protein source.

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