ELSEVIER

PII: S0308-8146(96)00158-6

Food Chemistry, Vol. 58, No. 3, pp. 219–222, 1997

0308-8146/97 $17.00+0.00

Effect of heat treatment on chemically modified proteins of legume seeds

Mirosława Klepacka,* Helena Porzucek & Małgorzata Kluczyńska

Food Technology Faculty, Warsaw Agricultural University (SGGW), ul. Rakowiecka 26/30, 02-528, Warsaw, Poland

(Received 8 July 1994; revised version received 5 January 1996; accepted 5 January 1996)

Legume proteins (unmodified and chemically modified) were subjected to heat treatment (100°C, 60 min). The effects of heating on the protein solubility, the amino nitrogen content and UV spectra were studied. The protein solubility decreased after heat processing. The amino nitrogen content increased after heating of unmodified proteins, indicating polypeptide chain unfolding. But chemical modification resulted in a decrease of this form of nitrogen, suggesting that protein chains were involved. UV spectra indicate exposure of phenylalanine residues in heated unmodified and chemically modified proteins. Acetylation enhanced thermal denaturation and acetylated pea proteins showed an especially high sensitivity to heat. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Thermal behaviour of legume proteins has been discussed by many workers (Hashizume & Watanabe, 1979; Pilosof *et al.*, 1982; Sheard *et al.*, 1986; Bacon *et al.*, 1989; Deshpande & Damodaran, 1989; Carbonaro *et al.*, 1993). Several factors have been shown to have an effect on the denaturation changes of legume proteins. The amount of water associated with the protein can markedly affect the thermal stability and potential application of food proteins (Pilosof *et al.*, 1982; Dutson & Orcutt, 1984). The temperature and heat–moisture conditions are of great importance. Only some properties of bean and cowpea flours affected by heat at different moisture levels have been investigated (Pilosof *et al.*, 1982, 1986; Phillips *et al.*, 1988). However, there is little information on the heat processing of proteins in their natural state or on the effect of chemical modifications on the properties of heat-processed legume proteins.

Therefore, the aim of the present work was to investigate the effect of heat treatment on acylated proteins. Protein solubility, the amino nitrogen content and UV spectra of soluble fractions of proteins were studied to estimate the changes induced by heat treatment.

MATERIALS AND METHODS

Materials

Plant material

Dry seeds of bean, *Phaseolus vulgaris* (var. 'Wenta'), two 'sweet' varieties of lupin, *Lupine albus* (var. 'Hetman' and 'Wat'), and three varieties of pea, *Pisum sativum* ('Koral', 'Poa', 'Ramir'), were obtained from plant breeding stations in Poland.

Sample preparation

The seeds were dehulled, ground, sieved (100 mesh) and held at 5°C until used. The unmodified and acylated (acetylated and succinylated) proteins were obtained by the method previously described (Klepacka & Porzucek, 1994). Flour was dispersed in distilled water (1:10, w/v) and acylated with acetic (acetylation) or succinic (succinylation) anhydride in amounts of 0.2 g or 0.2 ml per 1 g of flour protein, respectively, added over a period of 90 min. During the acylation process the pH was maintained at 7.5–8.0 with the periodic addition of 1 M NaOH. The suspension was centrifuged at 5500*g* for 15 min. Isoelectric precipitation of acylated legume proteins was performed with 2 M HCl at pH 3.5, 3.95 and 3.7 for bean, lupin and pea, respectively. Proteins were recovered by centrifugation at 5500*g* for 20 min at 4°C. The precipitate was re-dispersed in distilled water, adjusted to pH 7.0 and lyophilized. The unmodified proteins were prepared in a similar manner as modified proteins except that no acylating agent was added and the extraction and precipitation were performed at pH 9.2 and 4.5, respectively.

Heat treatment of proteins

Flour and protein samples were packed into glass vials, sealed under nitrogen and heated for 60 min by immersion in a boiling water bath. After heating, the samples were cooled immediately in ice slurries. Such processing was defined as 'heat treatment'. One part of the samples (control) received no heat treatment.

*To whom correspondence should be addressed.

Methods

Protein solubility

The proteins were extracted from unheated and heat-treated samples with 0.5 M NaCl for 60 min. The samples were centrifuged at 10 000*g* for 15 min. After centrifugation, the supernatant was separated. Protein contents (N×6.25) in the supernatants were estimated by the Kjeldahl procedure. Relative decrease of soluble proteins (R_{dsp}) was calculated using the formula:

$$R_{dsp} = \frac{N_{unh} - N_h}{N_{unh}} \times 100\ (\%)$$

where N_{unh} is the nitrogen content in untreated sample supernatant, N_h is the nitrogen content in heat-treated sample supernatant.

Amino nitrogen determination

The thermal changes of proteins were examined by monitoring amino nitrogen in the presence of *o*-phthaldialdehyde (OPA) by a spectrophotometric procedure (Church *et al.*, 1983; Ma *et al.*, 1990). To determine amino nitrogen, sample solutions containing 100–200 μg of soluble proteins were mixed with 0.15 ml of 20% sodium dodecyl sulphate (SDS) and 2.85 ml of OPA reagent (OPA and mercaptoethanol, but without SDS). The OPA reagent was prepared daily. The solutions were mixed and incubated for 2 min at ambient temperature and the absorbances were measured at 340 nm with the Beckman 24 spectrophotometer. Amino nitrogen was calculated from a standard curve prepared with glycine (Lie, 1973) and expressed as μg amino nitrogen per 1 mg soluble protein.

UV spectrophotometry

Ultraviolet spectra of unheated and heated samples (0.04% protein) were recorded on the Beckman 24 spectrophotometer in the 250–300 nm region. Data for UV spectra were expressed as wavelengths of absorption maxima (λ_{max}).

RESULTS AND DISCUSSION

Data on the protein, salt-soluble nitrogen, amino groups and moisture contents are summarized in Table 1. As previously reported (Porzucek *et al.*, 1991; Klepacka & Porzucek, 1994), samples of bean and pea flour contained 25–28% and lupin flour 34–41% protein on a dry basis; the protein contents were 76–86% in unmodified samples and 65–82% (dry weight basis) in acylated samples.

The differences in solubilities between control and heated to 100°C samples, expressed as relative decreases of soluble proteins (R_{dsp}), are shown in Table 2. Heating caused part of the proteins to be rendered insoluble,

Table 1. Protein, salt-soluble nitrogen, amino nitrogen and moisture contents of flour (F), unmodified (UP), acetylated (AC) and succinylated (SP) protein samples

Sample	Protein (N×6.25% dry weight)	Salt-soluble nitrogen (% total N)	Amino nitrogen (μg g^{-1} protein)	Moisture (%)
Bean				
'Wenta'				
F	28.5 (0.69)	84.2 (0.42)	12.3 (0.10)	10.9(0.05)
UP	76.0 (0.38)	79.5 (1.66)	6.8 (0.06)	6.8 (0.06)
AP	65.5 (0.77)	94.9 (1.34)	1.0 (0.05)	9.0 (0.08)
SP	71.7 (0.35)	71.0 (0.65)	5.2 (0.01)	3.2 (0.07)
Lupin				
'Hetman'				
F	41.5 (0.34)	81.2 (0.39)	7.8 (0.12)	9.6 (0.07)
UP	80.6 (0.58)	65.3 (0.35)	4.6 (0.09)	4.9 (0.02)
AP	75.4 (0.40)	88.0 (0.84)	0.8 (0.01)	4.3 (0.11)
SP	79.6 (0.80)	73.9 (0.75)	3.1 (0.04)	3.3 (0.06)
'Wat'				
F	33.8 (0.17)	82.3 (0.58)	8.9 (0.09)	7.9 (0.04)
UP	83.6 (0.48)	75.3 (0.78)	4.1 (0.02)	4.2 (0.13)
AP	75.3 (0.66)	95.4 (1.27)	1.1 (0.01)	8.0 (0.15)
SP	66.7 (0.72)	62.0 (0.69)	3.8 (0.01)	2.9 (0.03)
Pea				
'Koral'				
F	26.4 (0.89)	83.0 (0.91)	17.3 (0.17)	9.9 (0.10)
UP	84.7 (0.38)	53.8 (0.54)	4.7 (0.06)	5.8 (0.03)
AP	82.3 (0.49)	92.5 (1.13)	1.9 (0.01)	3.8 (0.15)
SP	77.4 (0.48)	94.5 (0.60)	3.0 (0.05)	4.0 (0.05)
'Poa'				
F	26.2 (0.89)	74.6 (0.39)	15.8 (0.12)	10.5(0.06)
UP	85.6 (0.03)	55.9 (0.56)	6.2 (0.01)	5.9 (0.17)
AP	78.9 (0.84)	96.9 (1.25)	0.9 (0.01)	7.8 (0.12)
SP	75.2 (0.64)	86.9 (0.90)	2.8 (0.02)	3.7 (0.10)
'Ramir'				
F	24.9 (0.80)	82.5 (1.18)	12.6 (0.12)	11.4 (0.05)
UP	84.8 (0.13)	59.2 (0.59)	6.5 (0.04)	5.1 (0.12)
AP	71.8 (0.62)	90.4 (0.56)	1.0 (0.01)	2.8 (0.01)
SP	76.1 (0.66)	96.7 (1.01)	1.3 (0.01)	3.6 (0.04)

Figures in parentheses are standard deviations of samples.

which was attributed to protein denaturation. The thermal denaturation of proteins has traditionally been associated with loss of solubility (Pilosof *et al.*, 1982; Sheard *et al.*, 1986; Phillips *et al.*, 1988; Carbonaro *et al.*, 1993). The results for R_{dsp} showed that sensitivities to heat treatment were different and dependent on the type of sample. The unmodified and modified proteins of bean and pea were far more sensitive to heat than were the flour proteins. A similar phenomenon was observed by Pilosof *et al.* (1982). The marked changes were seen in all heated acetylated proteins. An especially significant increase in R_{dsp} was noted for acetylated pea proteins. This indicates that acetylated proteins were more sensitive to heat than were succinylated and unmodified proteins. Based on these results, it can be deduced that pea proteins were more affected by heat treatment than were bean and lupin proteins. This suggests that genetic variations may affect the solubility of

Table 2. Effect of temperature on the relative decrease of protein solubility (R_{dsp}) in salt solution (0.5 M NaCl)

Sample	R_{dsp} (%)			
	F	UP	AP	SP
Bean				
'Wenta'	16.7	24.4	55.4	18.2
Lupin				
'Hetman'	21.7	23.2	54.9	19.5
'Wat'	21.0	19.5	47.4	21.9
Pea				
'Koral'	27.8	38.2	80.8	27.7
'Poa'	25.6	36.3	76.7	32.9
'Ramir'	20.9	41.2	77.8	39.8

heated proteins. Comparing the behaviour of proteins after heat treatment, we found that the R_{dsp} values of succinylated proteins were lower than those of acetylated and unmodified proteins. This suggest that the thermal denaturation of proteins can be prevented by net charge, as was shown by Lakkis & Villota (1992) and Ma & Holme (1982).

The results obtained by Pilosof *et al.* (1982) indicated that a greater reduction of nitrogen solubility occurred when the samples were heated at higher moisture content. It has been suggested (Sheard *et al.*, 1986; Phillips *et al.*, 1988) that water is the decisive factor in the denaturation, not the protein content. In the present study, the flour moisture contents ranged from 8% to 11% and from 3% to 9% for the protein samples. This level of moisture presumably has a negligible influence on the protein denaturation process. Such an effect can be observed when the water content is much higher (i.e. >20%) (Sheard *et al.*, 1986; Phillips *et al.*, 1988). The effect of heating on proteins also depends on other components and carbohydrate–protein interactions (Back *et al.*, 1979; Sheard *et al.*, 1986; Yamasaki & Ikebe, 1992). Sugars protect proteins against heat denaturation due to their effect on hydrophobic interactions. We observed (data not shown) that the sugar content decreased in all unmodified and modified proteins (Porzucek *et al.*, 1993).

The extent of the protein changes upon heat treatment was investigated by OPA spectrophotometric assay. Amino nitrogen increased after heating of flour and unmodified proteins, but decreased in acylated proteins (Fig. 1). Heating of flour and unmodified proteins probably resulted in unfolding of polypeptide chains, but in the case of chemically modified proteins the chains were involved.

The changes in thermally denaturated proteins remaining soluble in salt solution (0.5 M NaCl) were observed by UV spectroscopy. The results, expressed as wavelengths of the absorption maxima (λ_{max}), are shown in Table 3. Heating of flour caused a shift in λ_{max} to longer wavelengths (red shift). On the other hand, the heating of unmodified and chemically modified proteins

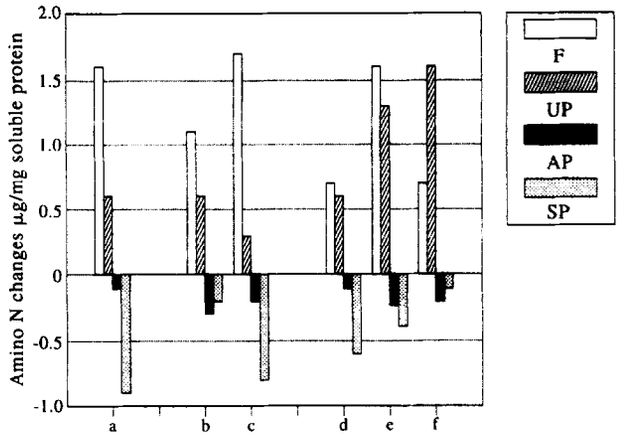

Fig. 1. Changes of amino nitrogen content after heat treatment of bean, lupin and pea proteins. F, flour proteins; UP, unmodified proteins; AP, acylated proteins; SP, succinylated proteins. Bean: a, var. 'Wenta'. Lupin: b, var. 'Hetman'; c, var. 'Wat'. Pea: d, var. 'Poa'; e, var. 'Koral'; f, var. 'Ramir'.

Table 3. Changes in the UV absorption maxima (λ_{max}) of unheated and heated legume proteins

Sample	λ_{max} (nm)			
	F	UP	AP	SP
Bean				
'Wenta'				
Control	269	275	268	275
Heated	274	268	260	269
Lupin				
'Hetman'				
Control	274	270	268	274
Heated	276	265	262	268
'Wat'				
Control	273	270	265	274
Heated	275	269	260	265
Pea				
'Koral'				
Control	265	265	265	269
Heated	274	260	260	267
'Poa'				
Control	269	264	265	274
Heated	276	259	260	269
'Ramir'				
Control	265	265	260	268
Heated	274	260	259	264

For abbreviations, see Table 1.

shifted λ_{max} to shorter wavelengths (blue shift). These results have been used to obtain information about the state of aromatic residues in proteins. The peaks between 250 and 270 nm are due to phenylalanine, while those between 270 and 300 nm are contributed predominantly by the tyrosine and tryptophan residues (Ma & Harwalkar, 1988; Yamagishi *et al.*, 1982, 1983). The present data show that denaturation by heat treatment exposed the aromatic residues and this may be attributed to various protein fractions. It is known that

globulins are the predominant fraction of legume flours (Bhatty, 1982; Deshpande & Nielsen, 1987; Gueguen & Barbot, 1988). Yamagishi *et al.*, (1981, 1982) reported differences in the states of aromatic amino acid residues in heated soybean 7S and 11S globulins. Electrophoretic studies (data not shown) confirmed that flour proteins consisted of 7S and 11S globulins, while 7S globulin was the main component of unmodified and modified proteins. All heated unmodified and chemically modified proteins showed the denaturation blue shift and this could be connected with predomination of phenylalanine residues, while heating of flour proteins gave a red shift caused by the exposure of tyrosine residues.

In conclusion, the results of our study indicate that acylated proteins were affected by heat treatment differently from flour and unmodified proteins. The trends of heat denaturation were similar for both applied chemical modifications. However, acetylated proteins were more sensitive to heat than succinylated proteins, probably because of prevention of thermal protein denaturation by the negative charges of succinylated proteins. Comparing the behaviour of the legume proteins investigated upon heating, a higher susceptibility of pea proteins than bean and lupin proteins to heat processing was found.

REFERENCES

Back, J. F., Oakenfull, D. & Smith, M. B. (1979). Increased thermal stability of proteins in the presence of sugars and polyols. *Biochemistry*, **18**, 5191–5196.

Bacon, J. R., Noel, T. R. & Wright, D. J. (1989). Studies on the thermal behaviour of pea (*Pisum sativum*) vicilin. *J. Sci. Food Agric.*, **49**, 335–345.

Bhatty, R. S. (1982). Albumin proteins of eight grain legume species: electrophoretic patterns and amino acid composition. *J. Agric. Food Chem.*, **30**, 620–622.

Carbonaro, M., Vecchini, P. & Carnovale, E. (1993). Protein solubility of raw and cooked beans (*Phaseolus vulgaris*): role of the basic residues. *J. Agric. Food Chem.*, **41**, 1169–1175.

Church, F. C., Swaisgood, H. E., Porter, D. H. & Catignani, G. L. (1983). Spectrophotometric assay using *o*-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. *J. Dairy Sci.*, **66**, 1219–1227.

Deshpande, S. S. & Damodaran, S. (1989). Heat-induced conformational changes in phaseolin and its relation to proteolysis. *Biochim. Biophys. Acta*, **998**, 179–188.

Deshpande, S. S. & Nielsen, S. S. (1987). Nitrogenous constituents of selected grain legumes. *J. Food Sci.*, **52**, 1321–1325.

Dutson, T. R. & Orcutt, M. W. (1984). Chemical changes in proteins produced by thermal processing. *J. Chem. Educ.*, **61**, 303–308.

Gueguen, J. & Barbot, J. (1988). Quantitative and qualitative variability of pea (*Pisum sativum* L.) protein composition. *J. Sci. Food Agric.*, **42**, 209–224.

Hashizume, K. & Watanabe, T. (1979). Influence of heating on conformational changes of soybean proteins. *Agric. Biol. Chem.*, **43**, 683–690.

Klepacka, M. & Porzucek, H. (1994). Some properties of chemically modified bean, lupin and pea proteins. *Pol. J. Food Nutr. Sci.*, **3**(44), 45–56.

Lakkis, J. & Villota, R. (1992). Effect of acylation on substructural properties of proteins: a study using fluorescence and circular dichroism. *J. Agric. Food Chem.*, **40**, 553–560.

Lie, S. (1973). The EBC–ninhydrin method for determination of free alpha amino nitrogen. *J. Inst. Brew.*, **79**, 37–41.

Ma, C.-Y. & Harwalkar, V. R. (1988). Study of thermal denaturation of oat globulin by ultraviolet and fluorescence spectrophotometry. *J. Agric. Food Chem.*, **36**, 155–160.

Ma, C.-Y. & Holme, J. (1982). Effect of chemical modifications on some physicochemical properties and heat coagulation of egg albumin. *J. Food Sci.*, **47**, 1454–1459.

Ma, C.-Y., Harwalkar, V. R. & Paquet, A. (1990). Physicochemical properties of alkali-treated oat globulin. *J. Agric. Food Chem.*, **38**, 1707–1711.

Phillips, R. D., Chinnan, M. S., Branch, A. L., Miller, J. & McWatters, K. H. (1988). Effects of pretreatment on functional and nutritional properties of cowpea meal. *J. Food Sci.*, **53**, 805–809.

Pilosof, A. M. R., Bartholomai, G. B., Chirife, J. & Boquet, R. (1982). Effect of heat treatment on sorption isotherms and solubility of flour and protein isolates from bean *Phaseolus valugaris*. *J. Food Sci.*, **47**, 1288–1290.

Pilosof, A. M. R., Boquet, R. & Bartholomai, G. B. (1986). Effect of heat–moisture treatment of field bean (*Phaseolus vulgaris*) flour and protein isolate on water uptake. *Cereal Chem.*, **63**, 456–458.

Porzucek, H., Larsson-Raznikiewicz, M. & Klepacka, M. *In vitro* protein digestibility of flours and protein isolates from seeds of some leguminous plants. *Swed. J. Agric. Res.*, **21**, 49–53.

Porzucek, H., Paprocka, M. & Klepacka, M. (1993). Effect of heat treatment on oligosaccharide content in some legume protein sample. Proceedings of XXIV Session of Food Technology and Chemistry Committee of Polish Science Academy, Wrocław, pp. 189–193, in Polish.

Sheard, P. R., Fellows, A., Ledward, D. A. & Mitchell, J. R. (1986). Macromolecular changes associated with the heat treatment of soya isolate. *J. Food Technol.*, **21**, 55–60.

Yamagishi, T., Yamauchi, F. & Shibasaki, K. (1981). State of aromatic amino acid residues in soybean 11S globulin heated in the presence of *N*-ethylmaleimide by derivative spectrophotometry. *Agric. Biol. Chem.*, **45**, 459–467.

Yamagishi, T., Ebina, F. & Yamauchi, F. (1982). Analysis of the state of aromatic amino acid residues in heated soybean 7S globulin by absorption derivative spectrophotometry and spectrofluorimetry. *Agric. Biol. Chem.*, **46**, 2441–2448.

Yamagishi, T., Miyakawa, A., Noda, N. & Yamauchi, F. (1983). Isolation and electrophoretic analysis of heat-induced products of mixed soybean 7S and 11S globulins. *Agric. Biol. Chem.*, **47**, 1229–1237.

Yamasaki, N. & Ikebe, K. (1992). A new amylose derivative for the preparation of protein–carbohydrate conjugates. *Biosci. Biotechnol. Biochem.*, **56**, 2091–2092.