ELSEVIER

PII: S0308-8146(96)00221-X

Food Chemistry, Vol. 58, No. 3, pp. 237–243, 1997

0308-8146/97 $17.00+0.00

The effect of heat- and acid-treatment on the structure of rapeseed albumin (napin)

Y. L. Folawiyo & R. K. Owusu Apenten*

Department of Food Science, University of Leeds, Leeds LS2 9JT, UK

(Received 4 August 1995; accepted 2 May 1996)

The effect of heat and/or acid treatment on rapeseed albumin (napin) structure was monitored, using the hydrophobic fluorescence probe anilinonapthalene-8-sulfonate (ANS), at 20–90°C and pH 1–10. Heat-treatment of napin at $T > 40$°C (20 min) led to an increase in the protein surface hydrophobicity. The mid-point temperature for the irreversible heat-modification of napin at pH 7 (Tm) was 60–67°C depending on NaCl concentration. The Tm value for napin was independent of pH over the range pH 4–8. At pH≤4.0, napin undergoes an acid-induced structural modification leading to a greater change in the apparent surface hydrophobicity compared to the effect of heating alone. These results are discussed in terms of the effect of solvent pH and temperature on the heat-resistance and heat-stability of napin. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Seed albumins exercise well-defined metabolic functions as enzymes, hemagglutinins or proteinase/amylase inhibitors (Derbyshire *et al.*, 1976, Deshpande & Damodaran, 1990). The albumins function as storage proteins at concentrations exceeding 5% of the total soluble seed protein (Derbyshire *et al.*, 1976, Bollini & Chrispeels, 1978; Youle & Huang, 1981, Sauvaire *et al.*, 1984; Prackash & Roa, 1986). Rapeseed albumin (napin) and globulin (cruciferin) constitute about 40 and 50% of the total soluble rapeseed protein, respectively (Murphy *et al.*, 1989). Extraction of defatted rapeseed flour yields about 46% napin and 42% cruciferin (Mahajan & Dua, 1994).

Rapeseed is currently the third most important oilseed crop (Schwenke, 1994). Therefore, a study of the effect of high temperature treatment on the structure of rapeseed protein is timely. Heat-treatment has been shown to alter the proteinase susceptibility and *in-vitro* digestibility of oilseed proteins (Nielsen *et al.*, 1988; Deshpande & Damodaran, 1989). The effect of chemical modification on selected functional properties of rapeseed albumin has also been extensively studied (Nitecka *et al.*, 1986; Schwenke *et al.*, 1989, 1991). However, the effect of high temperature treatment on the structure of napin has yet to be examined in detail.

In this study we investigate irreversible structural change(s) in napin, produced by exposure to elevated temperatures and extremes of pH, using anilinonapthalene-8-sulfonic acid (ANS) as a hydrophobic fluorescence probe (Stryer, 1968; Slavik, 1982; Arakawa *et al.*, 1991; Wicker *et al.*, 1986; Arntfield *et al.*, 1989). ANS fluorescence measurements provide information related to protein surface hydrophobicity (Kato & Nakai, 1980; Akita & Nakai, 1990; Cardamone & Puri, 1992). This study should help to identify heating conditions likely to improve the surface hydrophobicity napin. The effect of acid treatment on napin structure will also shed some light on the structure of napin in the gastric environment.

In this paper, only results obtained with isolated napin, rather than crude rapeseed protein (largely a mixture of napin and cruciferin), will be reported. Folawiyo & Owusu Apenten (1996) have recently presented a study of the effect of pH and ionic strength on the heat resistance of cruciferin. A determination of ANS binding parameters for napin and cruciferin has also been reported (Nyman *et al.*, 1996; Owusu Apenten & Folawiyo, 1996).

MATERIALS AND METHODS

Preparation of rapeseed flour

Rapeseed (*Brassica napus*) was ground into meal using a Horbart Coffee mill and defatted by stirring with 1:4 w/v ratio of hexane (three times) at room temperature. This relatively low temperature defatting procedure is preferable to Soxhlet extraction at about 40–45°C; there

*To whom correspondence should be addressed.

is less likelihood of a heat induced modification of proteins using the former method. During defatting the hull and flour became separated owing to differences in density. The defatted flour was dried in a vacuum oven at room temperature for up to 5 days, powdered using a pestle and mortar and sieved using a 125 μm mesh. The final product was stored in an air-tight container at −28°C. Glycyl-glycine, piperazine hydrochloride and anilinonaphthalene-8-sulphonic acid (ammonium salt), and reagents for sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Sigma, UK. All other reagents were AnalaR grade from Merck Ltd, Lutterworth, UK.

Isolation of rapeseed albumin

The approach adopted was essentially as described by Ismond and Welsh (1992) or Leger and Arntfield (1993). Defatted rapeseed flour (10g) was stirred with Tris-HCl buffer (0.05 M, pH 7.0) containing 0.1 M NaCl (200 ml) for 2h at room temperature. The slurry was then centrifuged (15000 rpm or 22700×g, 2h) at 4°C using a Beckman J2-HS centrifuge fitted with a JA-17 fixed angle rotor. The supernatant was dialyzed extensively against several changes of distilled water at 4°C to precipitate globulin and then centrifuged (15000 rpm or 22700×g, 2h). The water-soluble albumin fraction was collected and freeze-dried. The homogeneity of napin preparations and the effect of heat on protein structure were assessed using SDS-PAGE essentially as described previously (Folawiyo & Owusu Apenten, 1996).

ANS fluorescence measurements

Protein samples (0.2 mg/ml) in glycyl-glycyl piperizine buffer were used (10 mM, pH 4.2–10.5; with 0.1–1.0 M sodium chloride. For studies at pH < 4.2 dilute hydrochloric acid (with 0.1–1.0 M NaCl) was used as solvent. Protein samples were heated in test-tubes at 20, 30, 40, 50, 60, 70, 80 or 90°C for 20 min at each temperature and immediately cooled using an ice bath. Thereafter, ANS stock solution (1 mg/ml; 100 μl) was added to each pre-heated protein sample. Fluorescence measurements were made using teflon-stoppered (1 cm) quartz cuvettes The instrument used was a model 203 Perkin–Elmer fluorescence spectrophotometer, fitted with a thermostated twin-cuvette holder. ANS fluorescence measurements were made using a fluorescence excitation and emission wavelength of 360 and 470 nm, respectively.

RESULTS AND DISCUSSION

The addition of ANS to a sample of native rapeseed albumin at pH 7.0 produced a small increase in the ANS fluorescence emission intensity at 20°C (Fig. 1). Pre-heating albumin samples at 90°C for 20 min led to a further increase in the ANS fluorescence. Heat treatment of napin produces an irreversible structural modification associated with an increase in protein surface hydrophobicity (Stryer, 1968; Slavik, 1982; Arakawa *et al.*, 1991).

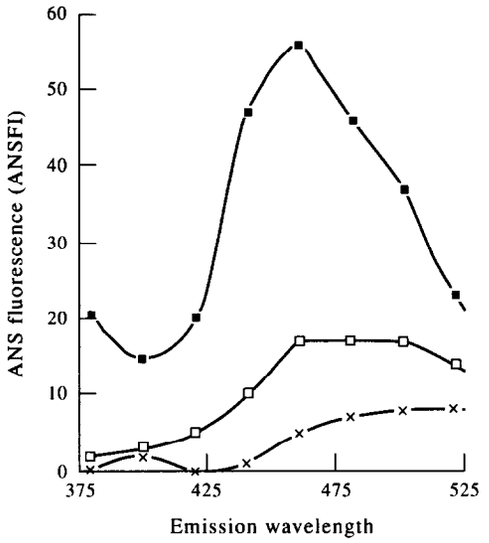

Fig. 1. Fluorescence emission spectrum for 8-anilinonaphthalene sulfonic acid (ANS; X), ANS + napin (□) or ANS + napin pre-heated at 90°C for 20 min, (■).

Figure 2 shows a SDS-PAGE print for napin samples. The SDS-PAGE analysis was performed under non-reducing conditions. The unheated protein gave a single SDS-PAGE band suggesting the napin sample used in this study was homogeneous or consisted of a mixture of proteins with a narrow range of molecular weights (Fig. 2; wells A–C). Rapeseed may contain a group of 4–5 napin with a molecular weight of 12–14.5 kDa (Monsalve *et al.*, 1991*a*,*b*).

Figure 2 shows that heating napin samples at 90°C for 20 min did not produce a change in protein molecular weight at pH≤7. Disulfide linked protein aggregates were not formed. The formation of protein

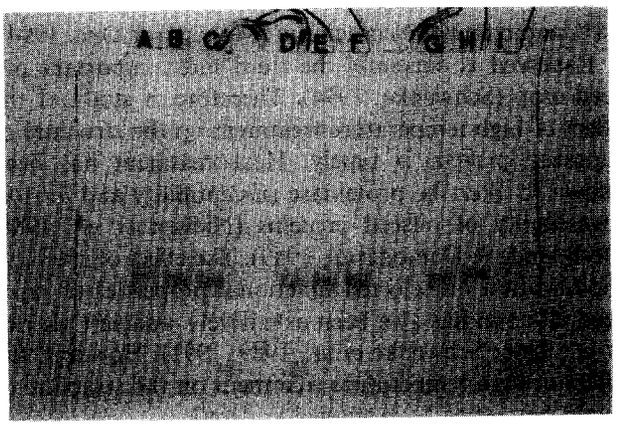

Fig. 2. A photograph of an SDS-PAGE gel for napin analyzed using a an 8–15% gradient gel. Protein samples were pre-treated for 20 min at **20°C** (A = pH 4.4, B = pH 7.0 and C pH 10), **50°C** (D = pH 4.4, E = pH 7.0 and F = pH 10) or **90°C** (G = pH 4.4, H = pH 7.0 and I = pH 10).

aggregates via non-covalent interactions would not be detected by SDS-PAGE. At pH 10 heating at 90°C produced a reduction in the SDS-PAGE band intensity for the native protein, suggesting that disulfide-linked aggregates were formed (well I; Fig. 2).

It is essential to use a homogeneous protein (e.g. isolated napin) sample in conjunction with fluorescence monitoring. Rapeseed storage protein consists of cruciferin and napin in almost equal amounts. Cruciferin is an aggregate of six different protein subunits whilst napin consists of one polypeptide subunit. Crude rapeseed protein contains seven or more polypeptides, cruciferin and napin, as well as other protein constituents. Fluorescence measurements on crude rapeseed protein could not be correlated with the heat modification of individual protein components.

Monitoring heat induced irreversible structural change in napin

Figure 3 shows the irreversible heat modification of napin structure as detected from changes in ANS fluorescence. The extent of irreversible structural modification (Fm) is expressed as a fraction of the maximum ANS fluorescence change at pH 2 and 90°C;

$$\mathrm{Fm} = \mathrm{F470(obsv)} - \mathrm{F470(min)}/\mathrm{F470(max)} - \mathrm{F470(min)} \quad (1)$$

where F470(min) is the ANS fluorescence for rapeseed albumin at pH 7.0 (20°C) and F470 (max) is the maximum recorded fluorescence at pH 2.0/90°C. Since fluorescence measurements were made at room temperature Fig. 3 shows that rapeseed albumin undergoes an irreversible heat-induced structural change after preheating at temperatures above 40°C. Rapeseed albumin (0.3 mg/ml in a salt free medium, pH 8.0) exhibits heat induced gelation, as monitored by turbidity changes, at or above 40°C (Schwenke *et al.*, 1991).

Increasing NaCl concentration had little or no effect on the heat-resistance of napin (Fig. 3). With glycyl-glycine buffer (10 mM, pH 7) containing 0.1–1.0 M NaCl as solvent, the mid-point temperature (Tm) for the irreversible structural modification of napin was 60–67°C. In contrast, previous results show that the Tm for cruciferin increases linearly with NaCl concentration according to the relation, Tm(°C) = 14.7 [NaCl] + 71 at pH 7.0 (Folawiyo & Owusu Apenten, 1996). The Tm range for the irreversible heat-modification of napin (60–67°C) compares with values of 71–95°C obtained for cruciferin under identical conditions. Therefore, rapeseed albumin is less heat resistant than rapeseed globulin. This conclusion has been confirmed by gel permeation chromatography of heated rapeseed proteins (Folawiyo & Owusu Apenten, unpublished).

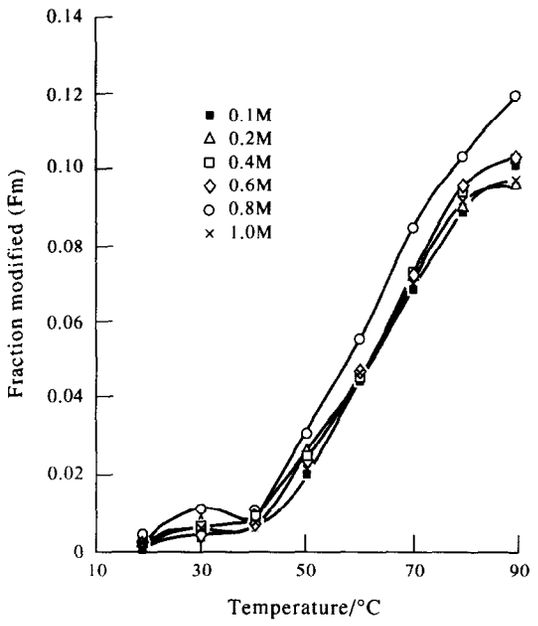

Fig. 3. The effect of sodium chloride concentration on the fraction of heat-modified rapeseed albumin (Fm). The NaCl concentration given in the graph. Protein samples heated for 20 min in 10 mM glycyl-glycine buffer (pH 7; 0.1–1.0 M NaCl).

At low salt concentration (e.g. [NaCl] < 0.5 M) the stabilization of cruciferin by salt proceeds via the shielding of unfavourable electrostatic interactions in the native protein. At high salt concentration (e.g. [NaCl] = 1–4.5 M) protein stabilization results from a preferential hydration effect. The degree of stabilisation produced by various salts depends on their position in the Hoffmeister series (Arntfield *et al.*, 1986). The above data imply that, unlike cruciferin, napin is not subject to electrostatic destabilization in low ionic strength media.

The effect of pH on napin was also examined at pH 1–10 at 20–90°C (Fig. 4). Heating rapeseed albumin to 90°C at pH 7.0 apparently results in two possible effects: (a) the irreversible structural change corresponding to ≤10% of the maximum shown in Fig. 4; or (b) the irreversible thermal modification of ≤10% of the total population of protein molecules (see below). For a homogeneous protein isolate option (b) can be dismissed because it is unlikely that 90% of rapeseed albumin molecules are resistant to heat modification at 90°C (see Figs 1 and 2 and Schwenke *et al.*, 1991). That is, high temperature treatment of napin is associated with a relatively minor irreversible structural change in native structure (Fig. 4). For comparison, acid treatment produced a far greater change in the apparent hydrophobicity as monitored from F470 readings. It is most likely that napin undergoes unfolding at low pH according to the relation:

$$N \leftrightarrow U_{(\mathrm{pH1-4\&T<40°C})} \quad (2)$$

where N = the native conformation for napin and U = the acid unfolded state. The effect of acid on napin

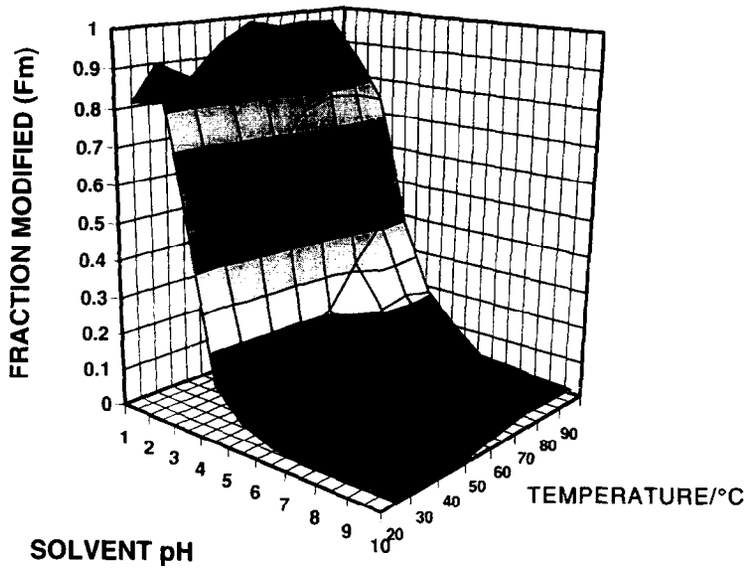

Fig. 4. A three-dimensional plot showing the fraction of denatured rapeseed albumin as a function of solvent pH and pre-heating temperature. Fm was calculated from eqn 1 (see text for details).

is reversible. Neutralising an acidified sample of napin reverses the ANS fluorescence increase observed at low solvent pH (data not shown).

The heat-resistance of napin, as indicated by the value of Tm, was constant over the range pH 4–8 (Fig. 5). There was a reduction in the heat-resistance at pH < 4 and pH > 8. For cruciferin at pH 1–10, Tm was found to be a simple linear function of solvent pH according to the relation: Tm (°C) = 4.16 pH + 41 (Folawiyo & Owusu Apenten, 1996).

The modification of globular protein structure by high temperature treatment can be discussed with reference to the 2-stage model for protein irreversible deactivation (Owusu *et al.*, 1992; Owusu & Berthalon, 1993; Owusu Apenten & Berthalon, 1994; and refs therein). Upon heating to temperatures greater than 40°C, the native conformation for napin (N) begins to unfold leading to the heat-unfolded (U^*) state. The $N \leftrightarrow U^*$ reaction is followed by the formation of an irreversibly modified form of napin (I) with increased surface hydrophobicity compared to the N-state. The preceding reactions take place at high temperature. Any U^*-state remaining, when the temperature is reduced to room temperature, either refolds to the N-state or proceeds to form more of the I-state(s) which are detected by the F470 readings.

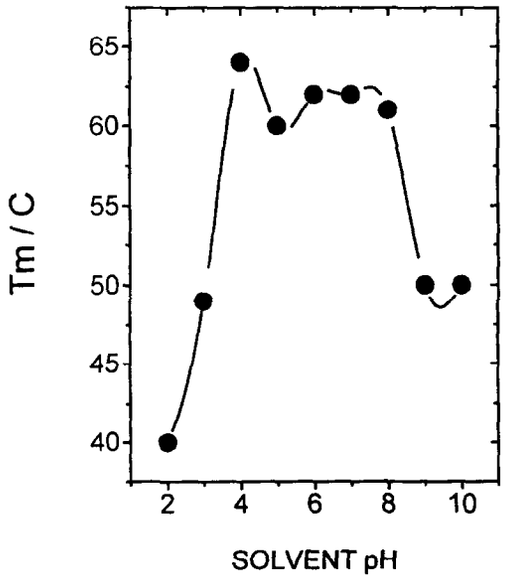

Fig. 5. The effect of solvent pH on the heat resistance of rapeseed albumin (napin). Tm is the temperature for 50% heat modification of napin.

$$N_{(\text{pH6-10and}T<40^\circ\text{C})} \leftrightarrow U^*_{(T>40^\circ\text{C})} \rightarrow I \qquad (3)$$

The overall reaction for irreversible thermal modification of napin can be summarized using eqn 4:

$$N \rightarrow I \qquad (4)$$

It is not at all easy to measure only the irreversible ($U^* \rightarrow I$) component for the $N \rightarrow I$ transition. We have previously achieved some success by pre-unfolding proteins at low temperature using guanidine hydrochloride, urea (Owusu & Berthalon, 1993; Owusu Apenten & Berthalon, 1994), or extremes of pH (see Fig. 4) before heat-treatment. Most 'pre-unfolding' agents, however, interfere or eliminate certain $U^* \rightarrow I$ transitions (Ahern & Klibanov, 1988; Owusu & Berthalon, 1993; Owusu Apenten & Berthalon, 1994).

A completely reversible heat-induced (N/U^*) transition could not account for the fluorescence change produced by high temperature treatment (Fig. 1, 3 and 4). Therefore, it can be concluded that high temperature treatment leads to an irreversible change in the structure of napin.

At moderate temperatures (e.g. below 100°C) the *I*-state (eqns 3 and 4 is an incorrectly refolded protein and/or an aggregated protein (Ahern & Klibanov, 1988). Protein aggregation, usually mediated by sulfur/di-sulfide exchange reactions at high pH, is detectable by SDS-PAGE (Fig. 2, Well I). Non-covalently bonded aggregates, or incorrectly refolded proteins, would not show up in SDS-PAGE analysis (Fig. 2, Wells A–H).

Both acid- and heat-treatment cause a partial unfolding of globular proteins (Hirose, 1993). In contrast, strong denaturants like guanidine hydrochloride and urea normally cause complete unfolding. The heat- and acid-unfolded states of napin are probably similar in structure. However, the heat-unfolding process was not observable because fluorimetric analysis was performed at room temperature on pre-cooled samples (see below).

The relatively modest increase in the protein surface hydrophobicity produced by high temperature treatment is therefore due to the formation of an *I*-state having non-polar amino residues removed from solvent contact. This would account for the reduction in F470 values for a heat-treated napin (pH 7.0) *relative to the F470 values of the acid unfolded state*. Exposure of napin to low pH solvents (pH < 4.0) led to a (10-fold) larger increase in protein surface hydrophobicity and/or solvent (and ANS) accessibility to nonpolar amino residues previously buried in the native protein (Fig. 4).

Monitoring of napin heat unfolding

So far, the present study involved preheating napin at 20–90°C for 20 min, cooling the protein samples in an ice bath followed by spectrofluorimetric measurements at room temperature. This 'heat-cool-assay' experimental design is a common one for the evaluation of enzyme/protein heat-resistance. The method is, however, only capable of detecting heat-induced, irreversible, structural changes.

There is some sympathy for a view that irreversible heat change (eqn 4) provides information about protein heat-*resistance* and not about protein heat-*stability*. Accordingly the term heat-stability is considered synonymous with protein conformational stability determined by monitoring protein unfolding at *high temperature*. Protein unfolding transitions can be reversible or apparently irreversible. Strategies for eliminating certain $U^* \rightarrow I$ reactions during measurements of protein unfolding include the use of, a low protein concentration, an appropriate choice of buffer and solvent pH or the presence of a reducing agent such as dithiothreitol.

Protein stability (as defined here) can be described in terms of the temperature necessary to produce unfolding of 50% of protein molecules (Tm*). Depending on the detailed experimental design, the value for Tm* may or may not equal the Tm for irreversible heat modification.

Where, from eqn 4, protein unfolding ($N \leftrightarrow U^*$) is the rate-limiting step for irreversible deactivation, values for Tm and Tm* will be nearly identical. Under such circumstances, the $U^* \rightarrow I$ reaction (eqn 3) will have little or no impact on the observed thermal transition profile (Figs 3 and 4). In situations where protein unfolding occurs quickly, or at a lower temperature compared to the $U^* \rightarrow I$ reaction, the value for Tm will be expected to be considerably higher than the unfolding temperature Tm* (Owusu *et al.*, 1992; Owusu & Berthalon, 1993; Owusu Apenten & Berthalon, 1994).

In order to examine the possible relationship between Tm (for irreversible heat-deactivation) and Tm* (for heat unfolding), F470 readings were also recorded for napin samples at high temperature (Fig. 6a). Figure 6a also shows the percent of heat denatured protein (i.e.

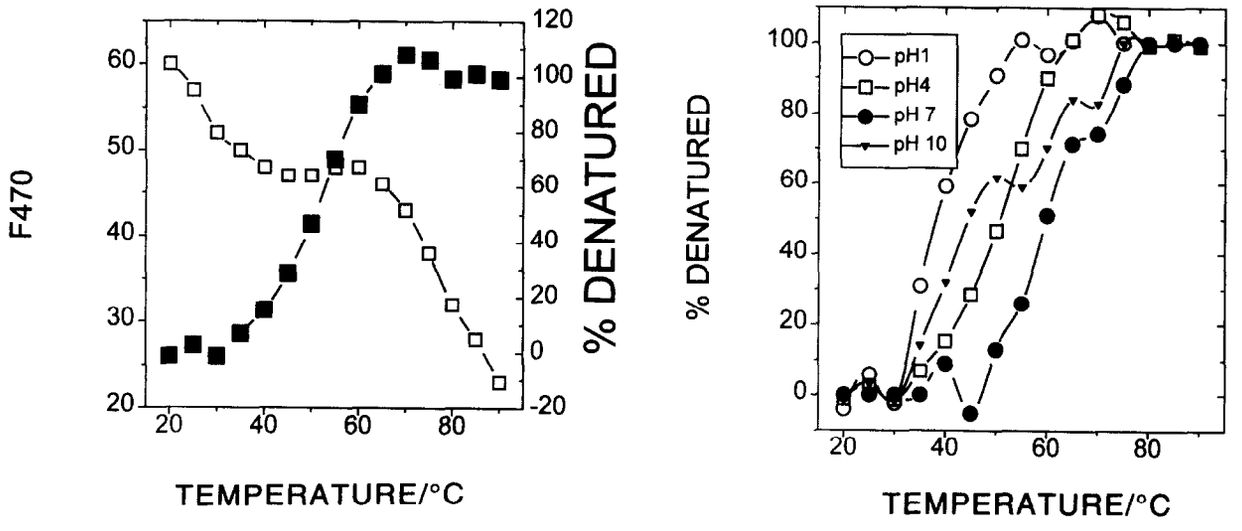

Fig. 6. Determination of rapeseed albumin (napin) heat-unfolding temperature (Tm*) at pH 4 using ANS fluorescence measurements (F470). (A: left). (□) F470 values, (■) % denatured protein. (B: right). Determination of rapeseed albumin (napin) heat unfolding temperature (Tm*). Results for napin heat unfolding at (○) pH 1, (□) pH 4, (●) pH 7 and (▼) pH 10.

Fm×100; cf. eqn 1) at different temperatures. The procedure for analysing protein thermal-unfolding curves using fluorescence monitoring has been presented in detail elsewhere (Owusu Apenten, 1995; Amiza & Owusu Apenten, 1996).

Figure 6(b) shows a summary of results for the heat-unfolding of napin at pH 1, 4, 7 and 10. The unfolding temperature (Tm*) for napin was estimated from Fig. 6 as 39°C at pH 1, 51°C at pH 4, 63°C at pH 7 or 45°C at pH 10. From the 'heat-cool-assay' method, the corresponding Tm estimates (Fig. 5) were 64°C at pH 4, 62°C at pH 7 and 52°C at pH 10. At pH 1 no irreversible structural change was observed at $T \leq 90$°C (Fig. 4).

Values for Tm and Tm* were virtually the same at pH 7 (Tm* = 63°C and Tm = 62°C). At solvent pH values away from pH 7, Tm was greater than Tm*. The differences were: 7°C at pH 10, 14°C at pH 4 and > 50°C at pH 1. Therefore, a 'heat-cool-assay' is capable of providing information about the heat unfolding stability of napin at pH 7 (Fig. 6). There was increasing disparity between the indices of heat-resistance (Tm) and heat-unfolding stability (Tm*) at solvent pH values removed from neutrality. Such results can be explained in terms of greater pH-dependence of the $N \leftrightarrow U^*$ reaction as compared to the $U^* \rightarrow I$ reaction(s) for irreversible heat-deactivation of napin.

At pH 7 where Tm* ≅ Tm then the $N \leftrightarrow U^*$ reaction is the limiting step for the irreversible heat-modification of napin. These results agree with those obtained for cruciferin. At pH 7 the Tm for cruciferin (determined by 'heat-cool-assay' fluorescence study) agreed with the temperature for 50% protein unfolding measured using a differential scanning calorimeter (Folawiyo & Owusu Apenten, 1996).

The main findings of this study can be summarized as follows. Napin undergoes acid induced unfolding under conditions similar to those prevailing within the stomach. Heating samples of napin to 90°C produced an irreversible but relatively moderate change in the surface hydrophobicity. Napin is less heat resistant than cruciferin. The heat stability of napin is also less affected by ionic strength and solvent pH.

Heat-processing operations usually involve either a 'constant' application of heat or the use of a well defined *transient* temperature-time profile. The majority of experiments described above (Figs 1–5) provide information about the heat resistance of napin. Protein heat-resistance is arguably a more important concept than protein stability when discussing thermal processes involving a transient heat profile.

REFERENCES

Ahern, T. J. & Klibanov, A. M. (1988). Analysis of processes causing thermal inactivation of enzymes. *Meth. Biochem. Analyt.*, **33**, 91–127.

Akita, E. M. & Nakai, S. (1990). Lipophilization of β-lactoglobulin: Effect of hydrophobicity, conformation and surface functional properties. *J. Fd Sci.*, **55**, 711–717.

Amiza, M. A. & Owusu Apenten, R. K. (1996). Urea and heat unfolding of cold-adapted Atlantic cod (*Gadus morhua*) trypsin and bovine trypsin. *J. Sci. Fd. Agric.*, **70**, 1–10.

Arakawa, T., Kita, Y. & Narhi, L. O. (1991). Protein-ligand interactions as a method to study surface properties of protein. *Meth Biochem. Analyt.*, **35**, 87–125.

Arntfield, S. D., Murray, E. D. & Ismond, M. A. H. (1986). Effect of salt on the thermal stability of storage proteins from fababean (*Vice faba*). *J. Fd Sci.*, **51**, 371–377.

Arntfield, S. D., Murray, E. D., Ismond, M. A. H. & Bernalsky, A. M. (1989). Role of the thermal denaturation-aggregation relationship in determining the rheological properties of heat induced networks for ovalbumin and vicilin. *J. Fd Sci.*, **54**, 1624–1631.

Bollini, R. & Chrispeels, M. J. (1978). Characterization of subcellular localization of vicilin and phytohemogglutinin, two major reserve protein in *Phaeolus vulgaris*. *Planta*, **142**, 291–298.

Cardamone, M. & Puri, K. (1992). Spectrofluorimeteic assessment of the surface hydrophobicity of proteins. *Biochem. J.*, **282**, 589–593.

Derbyshire, E., Wright, D. J. & Bouller, D. (1976). Leguminin and vicilin, storage proteins of legume seeds. *Phytochem.*, **15**, 3–24.

Deshpande, S. S. & Damodaran, S. (1990). Food legumes: chemistry and technology. *Advances Cereal Chem.*, **10**, 147–241.

Deshpande, S. S. & Damodaran, S. (1989). Structure-digestibility relationship of legume 7-S proteins. *J. Fd Sci.*, **54**, 108–113.

Folawiyo, Y. L. & Owusu Apenten, R. K. (1996). Effect of pH and ionic strength on the heat stability of rapeseed 12S globulin (Cruciferin) by the ANS fluorescence method. *J. Sci. Fd Agric.*, **70**, 241–246.

Hirose, M. (1993). Molten globule state of food proteins. *Trends Fd Sci.*, **4**, 48–51.

Ismond, M. A. H. & Welsh, W. D. (1992). Application of new methodology to canola protein isolation. *Food Chem.*, **45**, 125–127.

Leger, L. W. & Arntfield, S. D. (1993). Thermal gelation of the 12S canola globulin. *J. Am. Oil Chem. Soc.*, **79**, 853–861.

Kato, N. & Nakai, S. (1980). Hydrophobicity determined by a fluorescent probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta.*, **624**, 13–20.

Mahajan, A. & Dua, S. (1994). Physicochemical properties of rapeseed (*Brassica compestris* Var. Toria) seed proteins: viscosity and ionizable groups. *J. Agric. Food Chem.*, **42**, 1411–1414.

Monsalve, R. I., Lopezotin, C., Villalba, M. & Rodriguez, R. (1991*a*). A new distinct group of 2-S albumins from rapeseed. Amino acid sequence of 2 low molecular weight napins. *Febs Lett.*, **295**, 207–210.

Monsalve, R. I., Villalba, M. , Lopezotin, C. & Rodriguez, R. (1991*b*). Analysis of the small chain of the 2-S albumin, napin NIII from rapeseed. *Biochim. Biophys. Acta*, **1078**, 265–272.

Murphy, D. J., Cummins, L. & Kang, A. S. (1989). Synthesis of the major oil-body membrane proteins in developing rapeseed (*Brassica napus*) embryos: Integration with storage-lipid and storage-protein synthesis and implications for the mechanism of oil-body formation. *Biochem. J.*, **258**, 285–293.

Nielsen, S., Deshpande, S. S., Hermodson, M. A. & Scott, M. P. (1988). Comparative digestibility of legume storage proteins. *J. Agric. Fd Chem.*, **36**, 896–902.

Nitecka, E., Raab, B. & Schwenke, K. D. (1986). Chemical modification of proteins. 12. Effect of succinylation on some physico-chemical and functional properties of the albumin fraction from rapeseed (*Brassica napus* L). *Nahrung-Food*, **30**, 975–985.

Nyman, R. Folawiyo, Y. L. & Owusu Apenten, R. K. (1996). The effect of heat treatment on anilinonapthalene-8-sufonate binding to rapeseed albumin (napin) *J. Sci. Fd Agric.* (submitted March 1996).

Owusu, R. K., Makhzoum, A. & Knapp, J. S. (1992). Heat inactivation of lipase from psychrotroph Pseudomonas fluorescence P38: Activation parameters and enzyme stability at low or ultra-high temperatures. *Food Chem.*, **44**, 261–268.

Owusu, R. K. & Berthalon, N. (1993). A test for the two-stage thermoinactivation model for chymotrypsin. *Food Chem.*, **48**, 231–235.

Owusu Apenten, R. K. & Berthalon, N. (1994). Determination of enzyme global thermostability from equilibrium and kinetic analysis of heat inactivation. *Food Chem.*, **51**, 15–20.

Owusu Apenten, R. K. (1995). Thermodynamic parameters for 3-state thermal denaturation of human and bovine a-lactalbumin. *Themochimica Acta*, **262**, 1–12.

Owusu Apenten, R. K. & Folawiyo, Y. L. (1996). The effect of pH on rapeseed globulin (cruciferin) binding to anilinonapthalene-8-sufonate. *J. Food Biochem.* (submitted).

Prackash, V. & Roa, M. S. N. (1986). Physicochemical properties of oilseed proteins. *CRC Crit. Rev. Biochem.*, **20**, 265–363.

Sauvaire, Y., Baccou, J.-C. F. & Kobrehal, K. (1984). Solubilisation and characterisation of fenugreek seed proteins. *J. Agric. Fd Chem.*, **33**, 41–47.

Schwenke, K. D. (1994). Rapeseed protein. In: *New and Developing Sources of Food Proteins*, ed. B. J. F. Hudson. Chapman and Hall, pp. 281–306.

Schwenke, K. d., Raab, B., Pahtz, W., Sirwer, D. & Hak, K. Y. (1989). Modification of the low molecular weight basic albumin fraction from rapeseed (*Brassica napus* L) by acetylation Chemical and physicochemical aspects. *J. Fd Biochem.*, **13**, 321–334.

Schwenke, K. D., Kim, Y. H., Kroll, J., Lange, E. & Mieth, G. (1991). Modification of the low molecular weight basic albumin fraction from rapeseed (*Brassica napus* L) by acetylation. 2. Selected functional properties. *Die Nahrung*, **35**, 293–301.

Slavik, J. (1992). Anilinonapthalene-8-sulfonate as a probe for membrane composition and function. *Biochim. Biophys. Acta*, **694**, 1–25.

Stryer, L. (1968). Fluorescence spectroscopy of proteins. *Science*, **162**, 529–533.

Wicker, L., Lanier, T. C., Hamann, D. D. & Akahane, T. (1986). Thermal transitions in myosin-ANS fluorescence and gel rigidity. *J. food Science*, **51**, 1540–1543, 1562.

Youle, R. L. & Huang, H. C. A. (1981). Occurrence of low MW and high cysteine containing albumin storage proteins in oilseeds of diverse species. *Am. J. Bot.*, **68**, 44–48.