ELSEVIER

PII: S0308-8146(96)00180-X

Food Chemistry, Vol. 59, No. 1, pp. 95–99, 1997

0308-8146/97 $17.00+0.00

Thermal aggregation of almond protein isolate[1]

S. K. Sathe,* & K. W. C. Sze

Department of Nutrition, Food, and Movement Sciences, Florida State University, Tallahassee, FL 32306-2033, USA

(Received 15 October 1995; accepted 24 February 1996)

Almond protein isolate (API) formed a gel at 90°C in 5 min at a concentration of 4% (w/v) in distilled deionized water adjusted to pH 8.2. The rate of API aggregation was dependent on the composition of the heating medium. Adding NaCl, NaBr, NaI, NaF, or NaSCN (each at 0.25, 0.5, 0.75, and 1 M) to the heating medium decreased the rate of thermal aggregation of API, while addition of 1 M $CaCl_2$ promoted it. Hydrophobic interactions helped stabilize the API against thermal aggregation. Hydrogen bonds, ionic interactions, and disulfide exchange promoted the API thermal aggregation. Contribution of disulfide exchange to the API thermal aggregation was more than that made by the hydrogen and ionic bonds. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The USA is the world's leading almond producer. Almonds contain 16–22% protein and 50–55% lipids, on a dry weight basis. Almonds are a good source of fiber, many minerals, and certain vitamins. Almond lipids are mostly unsaturated (65% monounsaturated and 25% polyunsaturated) and are devoid of cholesterol. The high lipid content of almonds makes them a high energy food ($\approx$600 kcal per 100 g). These nutritious nuts also enjoy worldwide acceptance and are used as a snack or as an ingredient in other food products. Almonds are marketed in different forms to suit the intended end use and include whole natural, in-shell, pieces, diced, flaked, sliced, roasted, roasted and salted, flours, powdered, chopped, blanched slivered, blanched diced, natural splits, almond butter, and almond paste, to name a few (Duxbury, 1989; Anon., 1992). The rich nutty flavor of almond butter (Duxbury, 1989) and almond paste (Anon., 1991) makes these two products versatile and valuable 'value-added' ingredients. The major uses of these and other almond forms are primarily in bakery and confectionery products.

In addition to the rich nutty flavor, the ability to provide unique crunchy, chewy, and smooth textural attributes undoubtedly contributes to the universal appeal of almond and almond products. The textural attributes of almond paste and similar products can be manipulated by controlling the processing parameters (such as the heat input) and/or adding suitable ingredients (such as sugar, sugar syrups, honey, etc.) to produce a range of desirable textures in the final product. Marzipan production is an example of one such manipulation. Most marzipan is made by mixing almond paste with sugar and corn syrup or glucose (Anon., 1991), resulting in a pliable texture. This pliable texture makes it possible to mold marzipan in any desirable shape. Since proteins and lipids account for most of the dry matter in almonds, these two must control the texture of almond and almond products to a major extent.

The protein solubility, distribution, and polypeptide composition of the major US marketing varieties has been recently reported (Sathe, 1993). Almonds typically contain very low (<2%) non-protein nitrogen (Wolf, 1995). Since heat processing denatures the proteins and these denatured proteins produce several desirable textural qualities (such as the crunchiness, crispness, and chewability attributes), we investigated thermal aggregation of almond proteins.

MATERIALS AND METHODS

Materials

Whole almonds (marketing variety Nonpareil) were kindly provided by the Blue Diamond Growers, Sacramento, CA. Tris [tris(hydroxymethyl)aminomethane] was from Bio-Rad Laboratories, Richmond, CA. Ultrapure dithiothreitol (DTT) was from United States Biochemicals, Cleveland, OH. NaBr was from Mallinckrodt, Paris, KY, and was of reagent grade. All other chemicals were from Fisher Scientific, Orlando, FL, and were of reagent (or better) grade.

[1]Paper presented at the Annual Meeting of the Institute of Food Technologists, 3–7 June 1994, Atlanta, GA. Abstract No. S67-1.

*To whom correspondence should be addressed.

Methods

Preparation of almond protein isolate (API)
Defatted almond flour was prepared as described earlier (Sathe, 1993). Defatted almond flour was extracted with 20 mM Tris–HCl pH 8.1 buffer (flour:solvent 1:10, w/v) for 1 h with constant magnetic stirring at 4°C. The slurry was filtered through glass wool and the filtrate centrifuged (12 000*g*, 20 min, 4°C). The supernatant was filtered through Whatman No. 4 filter paper (under vacuum, using water aspirator), dialyzed against distilled deionized (DIDI) water (48 h, six changes, 5 litres each) using a dialysis membrane with a molecular weight (MW) cut-off of 6000–8000, and lyophilized. The lyophilized mass was subjected to six bursts (30 s each burst with 30 s rest period between the successive bursts) in an Osterizer blender at a speed setting 'liquefy' to obtain homogeneous powder. The sample was then stored in an air-tight plastic bottle at −20°C until further use.

Aggregation experiments
Typically, aggregation studies were done using autoclavable microcentrifuge plastic tubes (1.5 ml capacity). The API was dissolved in DIDI water (pH of the DIDI water was adjusted to 8.2 with 0.01 N NaOH) with constant magnetic stirring. The solution was centrifuged (12 000*g*, 20 min, 4°C) to remove the insoluble aggregates, and the supernatant was used for aggregation experiments. When required, appropriate additives were dissolved in DIDI water (pH adjusted to 8.2 with 0.01 N NaOH) separately and then added to the protein solution. Typical API concentration used for thermal aggregation studies was 4% (w/v) and the heating temperature was 100°C (boiling water bath). When the heating temperature was variable, a constant-temperature water bath (±0.1°C) was used. In all experiments, at the end of heating period, samples were immediately cooled on ice (4°C) for 30 min, centrifuged at 15 000*g* for 5 min at room temperature (25°C), and the supernatants were analyzed for soluble protein content. Aliquots (0.5 or 1.0 ml) of protein solutions were heated in the autoclavable plastic microcentrifuge tubes. No time corrections were made to account for the time required to reach the desired temperature by the sample.

Analytical
Protein concentration of the appropriate samples was determined by either the micro-Kjeldahl (N×5.18) or the Lowry *et al.* (1951) method. Amount of precipitated protein was calculated by subtracting the amount of protein in the supernatant from the amount of protein in the corresponding sample prior to the commencement of heating.

Three separate protein isolates were used in these studies. Each isolate was run in duplicate. All data points are therefore reported as mean ± SEM of six determinations.

Appropriate data were analyzed for significance using Fisher's LSD (protected test, $P = 0.05$) as described by Ott (1977).

RESULTS AND DISCUSSION

Protein isolate

Typical yield of the API was 18.9 ± 1.9 g per 100 g defatted almond flour ($n = 3$). The isolate had a light cream color and a fluffy appearance. The protein content of the isolate was ≥99% on a dry weight basis (w/w).

Thermal aggregation

Preliminary experiments indicated that the API could form a firm gel at ≥4% (w/v) concentration and ≥90°C in 5 min. For all aggregation studies we therefore used a 4% (w/v) API solution. The choice of heating temperature of 100°C was based on two considerations: (1) it is the most commonly encountered cooking temperature; (2) convenience.

Typically, the API (in DIDI water, pH 8.2) aggregated very quickly (40–50% protein precipitation within 1 min and 80–85% protein precipitation within 2 min) at 100°C. After the first 2 min the amount of protein precipitated did not increase significantly (Fig. 1). Protein gelation is a two-stage process. Protein denaturation occurs during the first stage followed by protein aggregation in the second stage (Flinger & Mangino, 1991). It is therefore apparent that almond

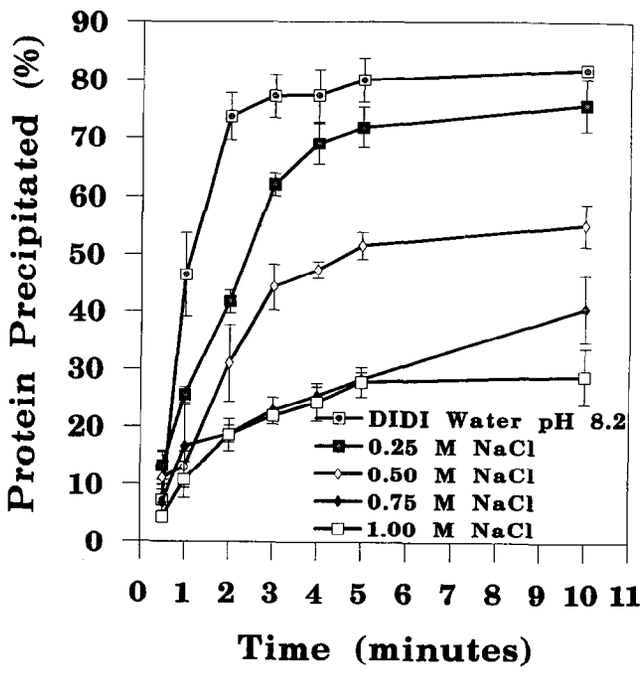

Fig. 1. Effect of NaCl concentration on API thermal aggregation.

proteins can denature and aggregate rapidly. The majority of almond proteins (≥95%) are water-soluble proteins and a single oligomeric protein dominates the total protein composition. This oligomeric protein is primarily composed of two kinds of polypeptides with estimated MW of 38 000–41 000 and 20 000–22 000 (Sathe, 1993). Ultracentrifugation studies of almond proteins suggest that this oligomeric protein has sedimentation characteristics similar to the soybean 11S protein (W. J. Wolf & S. K. Sathe, unpubl. data). The rapid thermal aggregation of almond proteins at 100°C observed in this study is similar to the one reported for the thermal aggregation of soybean 11S protein by Wolf and Tamura (1969). The gelation temperature of 90°C for the API is identical to the one reported for the soybean 11S globulin (Peng *et al.*, 1984).

Adding NaCl to the heating medium caused a significant decrease in the rate of API aggregation and this decrease in rate was dependent on NaCl concentration (Fig. 1). These results are consistent with similar findings by several investigators on soybean 11S protein (Wolf & Tamura, 1969; Hashizume & Watanabe, 1979; Shimada & Matsushita, 1980; Kinsella *et al.*, 1985; Yamauchi *et al.*, 1991). Salts are known to disrupt ionic attraction between protein molecules, break hydrogen bonds, and indirectly promote hydrophobic interactions (Nandi & Robinson, 1972). The progressive decrease in thermal aggregation rate with increasing NaCl concentration in the heating medium suggests that the hydrophobic interactions stabilize the API. The decrease in thermal aggregation rate with increasing NaCl concentration also suggests that the ionic and hydrogen bonds are important in API thermal aggregation. This thermal aggregation rate decrease was not only dependent on the salt concentration but also on the type of anion present in the heating medium (Fig. 2). Generally, as the salt concentration increased, the rate of thermal aggregation decreased, regardless of the anion type and the heating period (for both up to 5 and 10 min heating periods). The effect of anion type did not follow the typical lyotropic series $SCN^- > F^- > I^- > Br^- > Cl^-$ as suggested by Hatefi and Hanstein (1969), which has been explained on the basis of reduction in the free energy required to transfer polar groups to water (von Hipple & Wong, 1964). The 'non-ideal' effects of the anions observed in the present investigation may arise from at least two major factors: (1) since we did not use a buffer as a heating medium the pH of the heating medium could be different depending on the anion type and the salt concentration used; (2) it has been reported that the soybean protein solubility decreases with added salt to a minimum and then increases to a constant value when salt concentration is approximately 1 M (Shen, 1976, 1981). The API may have behavior similar to that of the soybean proteins in this regard. We therefore also evaluated the effect of heating time on thermal aggregation of API at a constant salt concentration of 1 M (Fig. 3). The data again show that the effect of anion did not follow the lyotropic series regardless of the heating time. $CaCl_2$ (1 M) promoted the thermal aggregation (Fig. 3) more than any of the sodium salts tested. These aggregation results (that the $CaCl_2$ promotes thermal aggregation) are consistent with the literature reports (Peng *et al.*, 1984; Yamauchi *et al.*, 1991; Kohyama *et al.*, 1995). Results of API thermal aggregation in the presence of salts clearly demonstrate that the hydrogen and ionic bonds are important contributors to the aggregation mechanism.

The rate of thermal aggregation of API was initially (first minute of heating) promoted by a reducing agent (20 mM DTT). The amount of protein precipitated during the first minute of heating in DIDI (pH 8.2) water was 46.42% and in the presence of 20 mM DTT it was 52.75%. With increasing heating time, the rate of

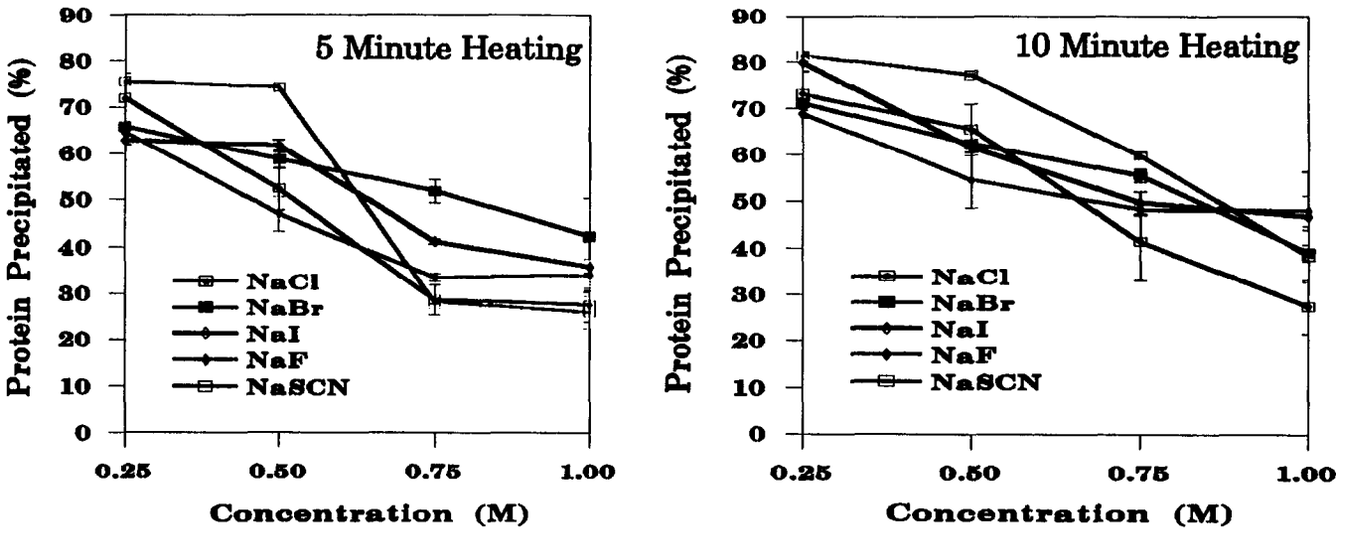

Fig. 2. Effects of concentration, anion type, and the length of heating time on API thermal aggregation.

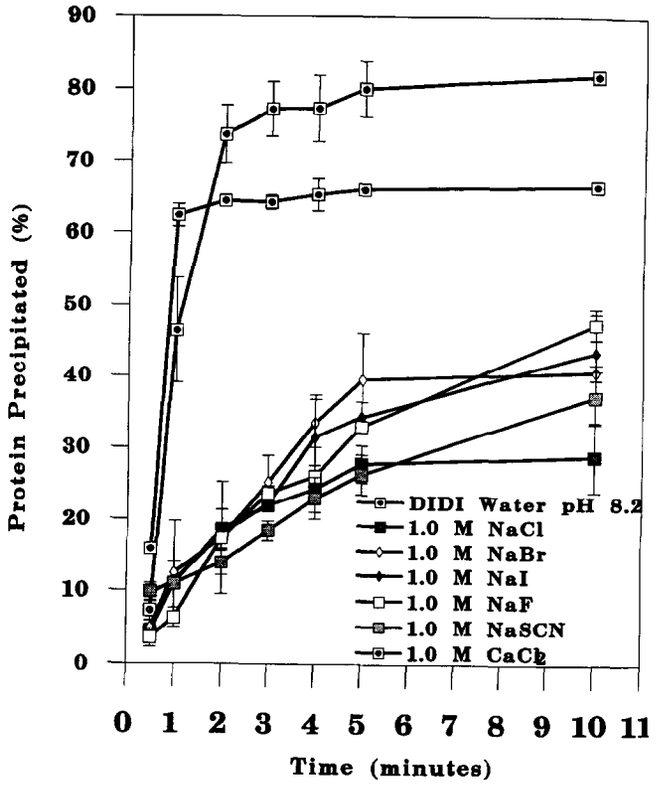

Fig. 3. Effect of anion type at fixed salt concentration (1 M) on API thermal aggregation.

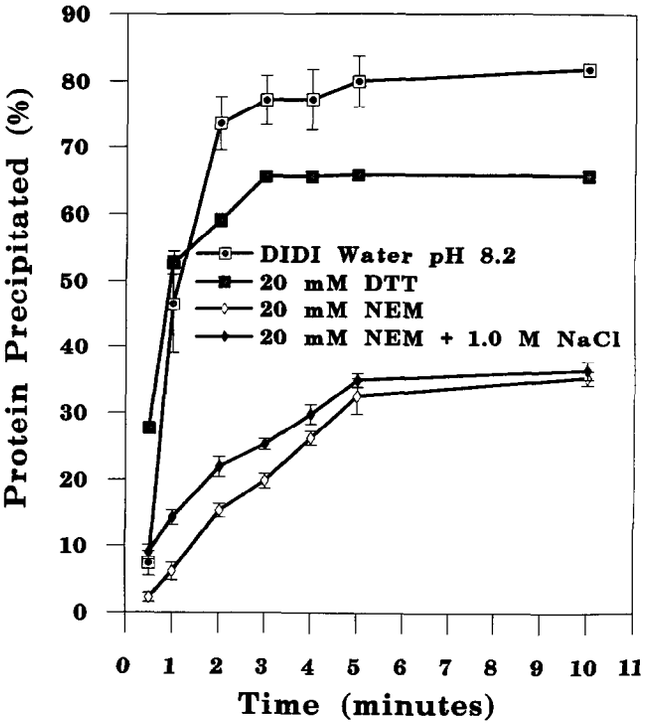

Fig. 4. Effects of reducing agent dithiothreitol (DTT) and sulfhydryl blocking agent *N*-ethylmaleimide (NEM) on API thermal aggregation.

thermal aggregation in the presence of 20 mM DTT was lower, however, compared to that in the DIDI water (pH 8.2). This suggested that the disulfide bonds in the API may help stabilize the proteins against heat denaturation only during initial heating ($\leq$1 min). The disulfide bonds of the native proteins therefore did not appear to promote the thermal aggregation under experimental conditions. Adding a sulfhydryl blocking agent (20 mM *N*-ethylmaleimide) to the heating medium decreased the thermal aggregation rate significantly (Fig. 4). These data indicate that the sulfhydryl exchange was important in the formation of thermally induced aggregates. Similar results have been reported for the soybean 11S globulin (Yamagishi *et al.*, 1981, 1982, 1983; Mori *et al.*, 1986) and soybean 7S basic globulin (Sathe *et al.*, 1989). Adding 1.0 M NaCl to the heating medium in the presence of sulfhydryl blocking agent (20 mM *N*-ethylmaleimide) increased the thermal aggregation rate by a small amount (Fig. 4). This suggests that the contribution by the hydrogen and ionic bonds to the API thermal aggregation may be much smaller than the one made by the sulfhydryl exchange.

ACKNOWLEDGEMENT

We gratefully acknowledge the generous gift of Nonpareil almonds from Dr Sam Cunningham of the Blue Diamond Growers, Sacramento, CA.

REFERENCES

Anonymous (1991). *Tree Nut News*. Blue Diamond Growers, Sacramento, CA.

Anonymous (1992). *Tree Nut News*. Blue Diamond Growers, Sacramento, CA

Duxbury, D. D. (1989). Almond butter versatility, flavor, and nutrition. *Food Process.*, **50**, 28.

Flinger, K. L. & Mangino, M. E. (1991). Relationship of composition to protein functionality. In *Interactions of Food Proteins*, eds N. Parris & R. Barford. American Chemical Society, Washington, DC, pp. 1–12.

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

Hatefi, Y. & Hanstein, W. G. (1969). Solubilization of particulate proteins and nonelectrolytes by chaotropic agents. *Proc. Natl. Acad. Sci. USA*, **62**, 1129.

Kinsella, J. E., Damodaran, S. & German, B. (1985). Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. In *New Protein Foods*, Vol. 5, eds A. Altschul & H. Wilke. Academic Press, New York, pp. 107–179.

Kohyama, K., Murata, M., Tani, F., Sano, F. & Doi, E. (1995). Effects of protein composition on gelation of mixtures containing soybean 7S and 11S globulin. *Biosci. Biotechnol. Biochem.*, **59**, 240.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**, 265.

Mori, T., Nakamura, T. & Utsumi, S. (1986). Behavior of intermolecular bond formation in the late stage of heat-induced gelation of glycinin. *J. Agric. Food Chem.*, **34**, 33.

Nandi, P. K. & Robinson, D. R. (1972). The effects of salts on the free energies of nonpolar groups in model peptides. *J. Am. Chem. Soc.*, **94**, 1308.

Peng, I. C., Quass, D. W., Dayton, W. R. & Allen, C. E. (1984). The physicochemical properties of soybean 11S globulin—a review. *Cereal Chem.*, **61**, 480.

Ott, L. (1977). *An Introduction to Statistical Methods and Analysis*. Duxbury Press, Belmont, CA.

Sathe, S. K. (1993). Solubilization, electrophoretic characterization, and *in vitro* digestibility of almond (*Prunus amygdalus*) proteins. *J. Food Biochem.*, **16**, 249.

Sathe, S. K., Mason, A. C. & Weaver, C. M. (1989). Thermal aggregation of soybean (*Glycine max* L.) sulfur-rich protein. *J. Food Sci.*, **54**, 319.

Shen, J. L. (1976). Soy protein solubility: the effect of experimental conditions on the solubility of soy protein isolates. *Cereal Chem.*, **53**, 902.

Shen, J. L. (1981). Solubility and viscosity. In *Protein Functionality in Foods*, ed. J. P. Cherry. ACS Symposium Series 147. American Chemical Society, Washington, DC, pp. 89–109.

Shimada, K. & Matsushita, S. (1980). Gel formation of soybean 7S and 11S proteins. *Agric. Biol. Chem.*, **44**, 637.

von Hipple, P. H. & Wong, K. Y. (1964). Neutral salts: the generality of their effects on the stability of macromolecular conformation. *Science*, **144**, 577.

Yamagishi, T., Yamauchi, F. & Shibasaki, F. (1981). Electrophoretical and differential thermal analysis of soybean 11S globulin heated in the presence of *N*-ethylmaleimide. *Agric. Biol. Chem.*, **45**, 1661.

Yamagishi, T., Yamauchi, F. & Shibasaki, K. (1982). Effect of sulfhydryl and disulfide compounds on the formation and quality of thermal aggregates of soybean 11S globulin. *J. Sci. Food Agric.*, **33**, 1092.

Yamagishi, T., Takahashi, N. & Yamauchi, F. (1983). Spectroscopic studies on the aggregates and the dissociates induced by heating soybean 11S globulin in the presence of *N*-ethylmaleimide. *Agric. Biol. Chem.*, **47**, 2475.

Yamauchi, F., Yamagishi, T. & Iwabuchi, S. (1991). Molecular understanding of heat-induced phenomena of soybean protein. *Food Rev. Int.*, **7**, 283.

Wolf, W. J. (1995). Gel electrophoresis and amino acid analysis of the nonprotein nitrogen fractions of defatted soybean and almond meals. *Cereal Chem.*, **72**, 115.

Wolf, W. J. & Tamura, T. (1969). Heat denaturation of soybean 11S protein. *Cereal Chem.*, **46**, 331.