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Deamidation-induced fragmentation of maize zein, and its linked reduction in fatty acid-binding capacity as well as antioxidative effect

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Zein lost its antioxidative effect in proportion to the degree of deamidation. Changes in molecular weight distribution of the protein fraction by deamidation were examined by means of gel filtration with Sephacry S-200 and SDS-electrophoresis in 12% polyacrylamide gel. The deamidation reaction (mild acid-hydrolysis by 0.05N HCl in 70% ethanol) was accompanied by fragmentation of zein subunits. Concomitantly, the surface hydrophobicity as well as fatty acid-binding capacity decreased with progressed deamidation. There was a close correlation ($r=0.98$) between the antioxidative effect and the fatty acid-binding capacity, but not the surface hydrophobicity. A large part of the antioxidative effect of zein is directly or indirectly attributable to its capacity of burying unsaturated lipid in the inter- or intra-molecular hydrophobic spaces in which the amide groups are intimately involved. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Polyunsaturated lipids easily undergo peroxidation under exposed conditions. Thus, the use of antioxidants is one of the most effective means of protecting food against oxidative deterioration. Food manufacturers, however, may tend to avoid using synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene in for reasons of health. A number of studies are now directed toward natural and safe substances. For example, the application of proteins, peptides and amino acids has been proposed by many investigators (Karel *et al.*, 1975; Kawashima *et al.*, 1979; Laakso, 1984; Yamaguchi, 1989), despite the lack of clear descriptions of the action mechanism. In this connection, plant prolamins such as wheat gliadin (Iwami *et al.*, 1987*a*,*b*, 1988) and maize zein (Wang *et al.*, 1991*a*,*b*; Matsumura *et al.*, 1994) serve as potent antioxidants in powder systems at high humidity. We have recently found that maize zein loses its antioxidative effect in a powder systems proportionally with the progress of deamidation (Chiue *et al.*, 1994). The present paper describes the fragmentation of zein by deamidation, its concomitant diminution in the fatty acid-binding capacity and the loss of antioxidative effect.

MATERIALS AND METHODS

Materials

Wheat, barley, maize, oats, rye, barnyard grass, Job's tears, foxtail millet, buckwheat and sorghum were purchased from a dealer in cereals in Kobe, Japan, 3 years prior to study. Linoleic and palmitic acids were products of Nakalai Tesque Ltd, Kyoto, Japan. These and other chemicals of analytical grade were used without further purification.

Methods

Preparation of prolamins

The above-mentioned cereals were ground into flour with an electric mill in our laboratory and their 'prolamin' fractions were individually prepared by means of extraction with 70% ethanol and exhaustive dialysis against distilled water, lyophilized and pulverized. Maize zein, commercially available, (a product of Nakalai Tesque Ltd) was used as such, for the preparation of deamidated samples, except for antioxidative comparisons of cereal prolamins.

Deamidation of zein
A 1% solution of zein in 70% ethanol and 0.05 N HCl was hermetically sealed in glass tubes, which were placed in a heat block bath at 95°C for designated times. The tubes were in turn withdrawn at stated intervals to obtain zein solutions with diverse degrees of deamidation. Their inner solutions whose aliquots were subjected to ammonia determination (Weatherburn, 1967), were once neutralized with dilute NaOH and freeze-dried after exhaustive dialysis against distilled water. The degree of deamidation was expressed in % as the released amount of ammonia by reference to its latent content in the zein-hydrolyzate with 3 N HCl at 110°C for 3 h (Chiue *et al.*, 1994).

Antioxidation test
An equivolumetric mixture of chloroform and methanol containing linoleic and palmitic (7:2) acids was added with shaking either to various prolamin powders or to diversely deamidated preparations of zein, followed by evaporation to dryness. Then, the content ratio of fatty acids to protein was 1:10 on a dry weight basis. These blendings were divided into small portions and allowed to stand in a chamber controlled at 40°C (humidity; 50–60%). Corn α-starch was used as an 'ineffective' reference instead of protein. A series of samples taken out from the chamber at appropriate intervals were repeatedly extracted with a chloroform–methanol (1:1) mixture. The residue after evaporation was methyl-esterified by the use of a commercial BF_3/methanol solution (Nakarai Tesque Ltd) and subjected to gas chromatography in the following manner: apparatus, Shimadzu GC-7A; column, 0.3 mmϕ×2 m (glass capillary); packing, 10% DEGS on Chromosorb WAW (60–80 mesh); temperature, 180°C (isothermal); carrier gas, nitrogen (40 ml/min); detection, hydrogen-flame ionization. The antioxidative effect was formulated in the proportion of unimpaired 'linoleic acid' for an internal standard 'palmitic acid' without considering extraction and esterification efficiencies (Iwami *et al.*, 1987*a*; Chiue *et al.*, 1994).

Peroxide value
The hydroperoxide content in the powder model system (peroxide value) was measured colorimetrically according to the ferric thiocyanate method (Iwami *et al.*, 1987*a*) and was conveniently substituted for the L/P ratio in each case.

Molecular weight estimation
Various deamidated preparations of zein were first solubilized using 0.5% SDS in 0.05 M (pH 7.5) Tris-HCl buffer and secondly applied to a Sephacryl S-200 column (2ϕ×100 cm) previously equilibrated with the same Tris buffer. The elution position of protein was monitored by continuous UV measurement. Independently, aliquots of the above zein solutions were electrophoresed in 12% polyacrylamide gel according to the method of Laemmli (1970). The gel after electrophoresis for a prescribed time was stained with 0.2% Coomassie brilliant blue R-250 and thoroughly destained with a 10% solution of acetic acid in 25% methanol. A commercial marker kit from Sigma Chemical Co., St. Louis, U.S.A., was used for the estimation of molecular weights of main protein bands.

Surface hydrophobicity
The surface hydrophobicity was measured using 8-anilinonaphthalenesulfonate (ANS) by a modification of the method of Hayakawa & Nakai (1985). In brief, certain amounts of protein were suspended in 0.05 M (pH 7.5) phosphate buffer at several different concentrations of 0.08–0.3% (w/v); 10 μl of 8 mM ANS in 10% ethanol was added to aliqots (4 ml) so as to obtain the fluorospectrum (excitation at 370 nm) with a Shimadzu RF-535 spectrofluorometer. Subsequently the fluorescent intensity at 480 nm was plotted against protein concentrations and a slope was obtained from the regression line to compare the surface hydrophobicity of each deamidated zein preparation.

Fatty acid-binding capacity
Changes in the binding of fatty acid to zein by deamidation were evaluated according to the method of Tsutsui *et al.* (1986). Specifically, a 1% solution containing linoleic acid and a fluorescent probe '1,6-diphenyl-1,3,5-hexatriene (DPH)' at a 1000: 1 ratio in hexane was poured by portions (1 ml) into test tubes, which were deprived of hexane under a nitrogen stream. To each tube was added 10 ml of a solution containing 0.02–0.12% protein, followed by emulsification at 12 800 rpm for 5 min with a Polytron. The aqueous layer, inclusive of precipitates after centrifugation at 27 000×g for 30 min, was diluted with 20 volumes of the same phosphate buffer and then subjected to fluorescence measurement (exitation, 357 nm; emission, 450 nm) as such or after further dilution in case of not inconsiderable turbidity.

Regression analysis
Data were the means for triplicate assays. A linear regression was obtained in the usual manner (Glanz, 1987) from the relationships between every two variables with various deamidated preparations of zein, i.e. pairs of the antioxidative effect (L/P ratio), the deamidation degree (%), and the fatty acid-binding capacity (DPH hydrophobicity).

RESULTS

Figure 1 compares the antioxidative effects in a powder model system (40°C, RH = 50–60%) of 70% ethanol-soluble proteins from various cereals. The antioxidative effect was evaluated by measuring the ratio of easily oxidizable 'linoleic acid' to stable 'palmitic acid'. Maize

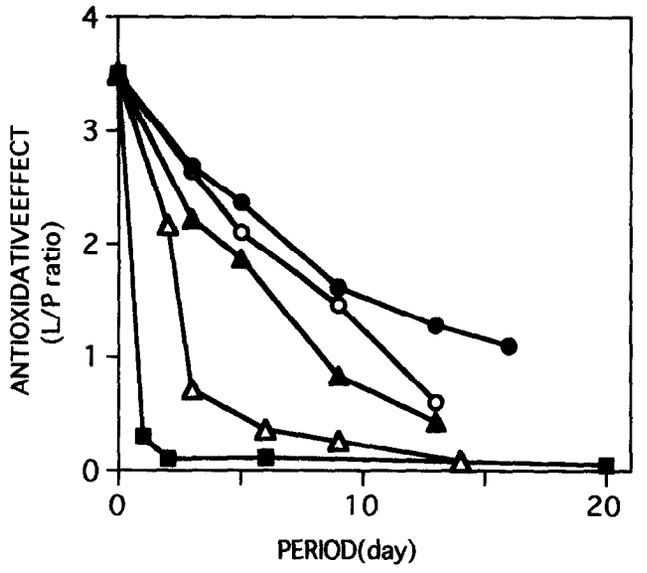

Fig. 1. Comparison of antioxidative effects of 'prolamins' self-made from several cereals. After ground grains were repeatedly extracted with 10 volumes of 70% ethanol, the clarified extract was evaporated to an appropriate volume, and its sticky residue was further dehydrated by lyophilization. Dried powders thus obtained were tested for antioxidation in a powder model system consisting of 'prolamin', linoleic acid and palmitic acid at the weight ratio of 9.1:0.7:0.2. Samples were taken out at stated intervals during storage at 40°C so as to check the impairment of unsaturated lipid. Linoleic and palmitic acids extractable from each sample were gaschromatographically determined following methylesterification. The antioxidative effect was evaluated in terms of the ratio of a variable 'linoleic acid' to an internal standard 'palmitic acid', i.e. L/P ratio. Prolamins herein used are the ones from the following sources: -●-, maize; -○-, wheat; -▲-, oats; -△-, tear grass; and -■-, corn starch for reference.

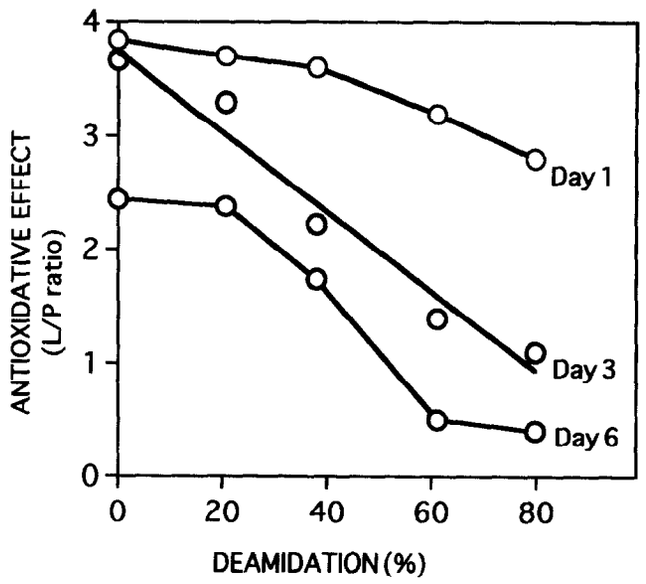

Fig. 2. Relationship between antioxidative effect and deamidation degree with various deamidated preparations from commercially available zein. Zein was heat-treated with 0.05 N70% ethanol in a sealed glass tube.

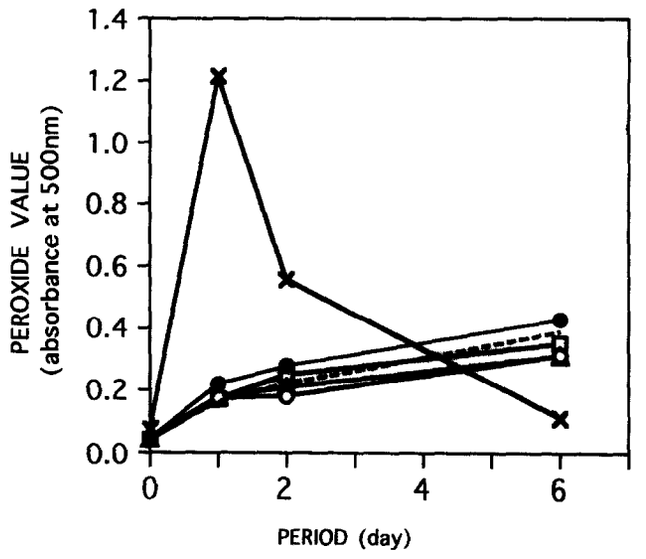

Fig. 3. Changes in antioxidant effect of organic solvent-washed zein. Zein which had been washed with 10 volumes of ether (-○-), ethylacetate (-△-), hexane (-■-), or methanol (-●-) was subjected to the same powder model experiment as mentioned above: –, untreated zein; -X-, corn starch for reference. Samples were withdrawn at stated intervals and their peroxide values were measured according to the ferric thiocyanate method.

zein was the most effective among prolamins tested and the ones from Job's tears, oats and wheat followed in that order. Plant prolamins from barley, buckwheat, rye, foxtail millet and barnyard grass, also were more effective (to one degree or another) than animal proteins such as casein, gelatin and ovalbumin, but were all inferior to maize zein (data not shown). These plant proteins, although belonging to the same classification from the viewpoint of solubility in aqueous alcohol, varied in antioxidation with their origins. For this reason, maize zein of the most antioxidative effect was hereinafter used as a representative of prolamin and its deamidation was adjusted by heat-treatment with 0.05 N HCl in 70% ethanol.

Figure 2 illustrates the plots of changes in the ratio of linoleic acid to palmitic acid (L/P ratio) on day 1, 3 or 6 against deamidation degrees of maze zein. On days 1 and 6, both curves were nonlinear because of the disorder of low-deamidated zein (day 1) and/or the high-deamidated one (day 6). The curve on day 3 was almost linear, which provided a straight line with correlation coefficient of $r = -0.95$ ($p < 0.05$). This implies that the deamidation degree of maize zein is intimately involved in the disappearance of its antioxidative effect.

Deamidated maize zein may have been deprived of concomitantly occurring antioxidative substances. In practice, tocopherols had been listed as potent candidates for such substances (Wang *et al.*, 1991*a*). Figure 3 examines to what extent linoleic acid becomes labile to peroxide formation by washing deamidated preparations with various organic solvents. As a result, neither ether nor ethylacetate nor hexane took out antioxidative

concomitants, if any. Tocopherols seemed not to be affirmatively correlated with the decreased antioxidative effect of maize zein by deamidation (Chiue *et al.*, 1994). Nothing but them has been established in regard to presumed antioxidants of low-molecular weight. In view of this status quo, our attention was directed to physicochemical properties of zein itself.

Incidentally we had noted a gradual increase in the UV absorption for TCA-soluble fractions in the process of 'deamidation'. Therefore, it appeared highly possible that zein undergoes fragmentation rather than polymerization even by such mild acid hydrolysis. Figure 4 depicts the elution profiles by Sephacryl S-200 gel filtration of maize zein at various deamidation degrees. The gel filtration pattern of non-deamidated zein was in agreement with that by Abe *et al.* (1981), who had reported that three major peaks corresponded to polymer, dimer and monomer, respectively, in order of elution. Their peak components with molecular weight of more than 21 000 dalton were observed to change into smaller sizes (< 20 000) as the deamidation reaction proceeded. At half the deamidation, the ratio of the monomer (21 000–25 000) to its derived fragments (< 20 000) became fifty–fifty and at above 70% deamidation, zein was mostly fragmented into molecular sizes of less than 20 000. This series of deamidated preparations were furthermore subjected to SDS-PAGE. The analytical results are given in Fig. 5. The main bands corresponding to two monomers faded away as the deamidation reaction proceeded. Then a new broad band appeared further ahead of it. These changes in electrophoresis faithfully reflected those in gel filtration.

Differences in hydrophobicity among various deamidated preparations of zein were assessed by the use of ANS as a fluorescent probe. Figure 6 correlates shifts in fluorescence with the degrees of deamidation. The emission spectrum was found to shift to the side of a somewhat long wavelength with increasing deamidation, as shown in Fig. 6(a). There was a distinction in this respect between the merely deamidated preparation and the once-lyophilized one. On the other hand, no distinction was observed between them for the surface hydrophobicity which was expressed as the fluorescent intensity under the routine assay condition. The surface

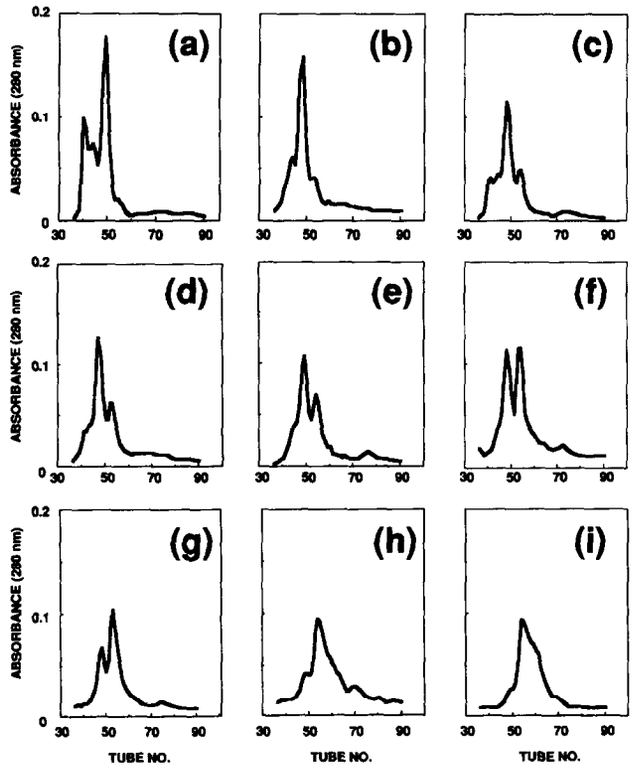

Fig. 4. Variation by deamidation in molecular weight distribution of zein subunits on Sephacryl S-200 column. Deamidated zein preparations used for gel filtration are as follows (in deamidation degree): (a) 0%; (b) 10.2%; (c) 20.6%; (d) 29.8%; (e) 38.6%; (f) 49.9%; (g) 61.1%; (h) 71.2%; and (i) 79.8%.

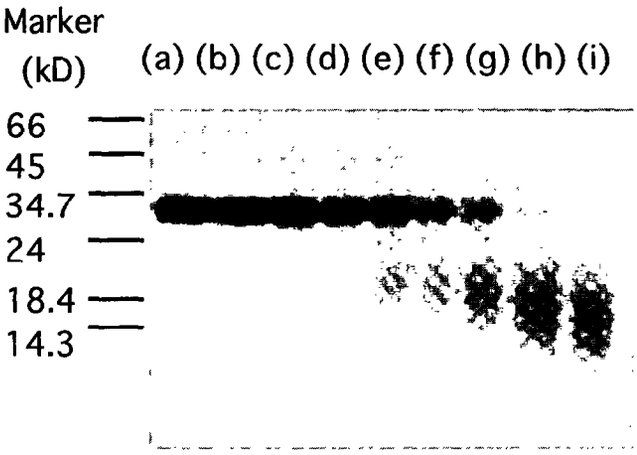

Fig. 5. Variation by deamidation in molecular weight distribution of zein subunits in SDS-polyacrylamide gel. Deamidated zein preparations used for SDS-electrophoresis are the same as (a)–(i) in Fig. 4. For molecular weight estimation the following marker proteins (in kDa) were used: bovine serum albumin, 66; ovalubumin, 45; pepsin 34.7; trypsinogen, 24; β-lactoglobulin, 18.4; lysozyme, 14.3.

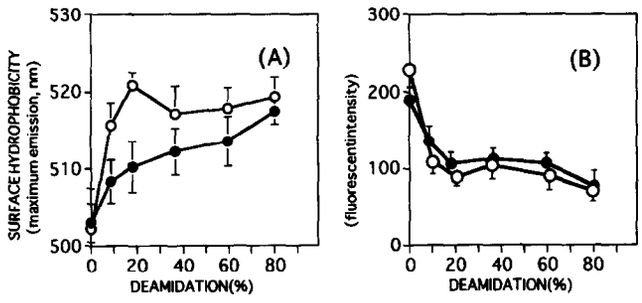

Fig. 6. Deamidation-related changes in surface hydrophobicity of zein using anilinonaphthalenesulfonate as a fluorescent probe. (A) Shift of the maximum emission to longer wavelength regions and distinction between freeze-dried (-●-) and non-treated (-○-) zein preparations of the same deamidation degree; *, significantly different at $p < 0.05$ by the student's t-test; (B) irregular decrease in fluorescent intensity (excitation, 370 nm) with progress of deamidation; there was no significant difference between samples with and without freeze-drying.

hydrophobicity was considerably lost at the stage of 10% deamidation, but invariably maintained in the range of 20–60% deamidation.

Another fluorescent probe 'DPH' was used in order to assess the fatty acid-binding capacity instead of the ANS hydrophobicity. The amount of fatty acid bound to protein was evaluated by fluorescence measurement of the protein–fatty acid-probe mixture. Figure 7 lists the analytical data obtained with various deamidated preparations of zein, in which the fluorescent intensity is tentatively plotted against the degree of deamidation. A good relationship ($r = -0.95, p < 0.01$) was valid between these parameters. Accordingly, changes in the 'DPH hydrophobicity' by deamidation were somewhat at variance with the ones in the ANS hydrophobicity. Based on this observation, the antioxidative effects of zein preparations at different deamidation degrees (Fig. 2; on day 3) were plotted against their fatty acid-binding capacities (Fig. 7). As a natural consequence it followed that a high coefficient of $r = 0.98$ was obtained from the plot, its regression line being drawn in Fig. 8. The antioxidative effect of deamidated zein in our powder model system was rather faithfully reflected in the fatty acid-binding capacity, that is, DPH hydrophobicity.

DISCUSSION

Prolamin [i.e. gliadin (wheat), zein (maize) or hordein (barley)], is a peculiar protein (soluble in aqueous alcohol) characterised by its high glutamine content. The occurrence of abundant amide groups in the prolamin must cause its insolubility in water as well as its antioxidative effect in powder model systems. It is known, however, that the amide bond is readily cleaved on exposure to elevated temperature, extreme pH, altered ion-strength, or certain enzymes (Wu *et al.*, 1976; Kato *et al.*, 1987, 1988; Hamada & Marshall, 1989; Shin, 1991; Vaintraub *et al.*, 1992). Because of this fragility, the deamidation reaction was carried out by heating zein for a short time in the presence of 0.05 N HCl and 70% ethanol. In this way, some control of deamidation was achieved after several trials.

It has been previously demonstrated that the antioxidative effect of prolamin, e.g. gliadin or zein, is much affected by humidity (Iwami *et al.*, 1987*a*,*b*, 1988; Wang *et al.*, 1991*a*,*b*). At moderate humidity, zein was superior in antioxidation to gliadin and was far more effective than other food proteins. A similar effectiveness was also observed in this experiment (Fig. 1). It was the view of Wang *et al.* (1991*a*) that such an effect was largely mediated by tocopherols remaining in zein throughout its preparation process. On the other hand, we have found that there is no direct relationship between changes in the tocopherol content and in the antioxidative effect by varying deamidation of zein (Chiue *et al.*, 1994). In this experiment, zein was washed with ether, ethylacetate and hexane, but a statistically significant decrease in the antioxidative effect was not reproduced (Fig. 3). As regards natural antioxidants, flavonoids and/or phenolic compounds are considered to remain in soy flour as well as its derivatives and to function as antioxidants against the oxidation of concomitant polyunsaturated lipids (Hammerschmidt & Pratt, 1978; Pratt & Birac, 1979). In fact, soy protein isolate is effectively deprived of these components by washing with water-saturated buthanol but not with organic solvents alone. Analogously, there remains a possibility

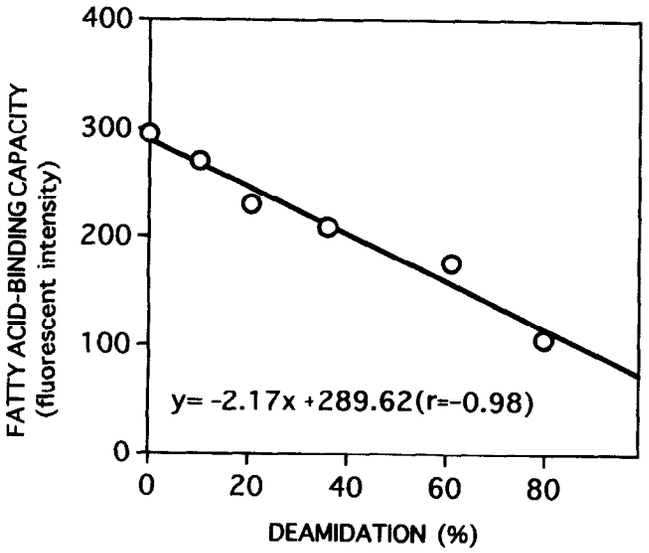

Fig. 7. Plot of fatty acid-binding capacity against deamidation degree with various deamidated zein preparations. Fatty acid-binding capacity (DPH hydrophobicity) was measured in the manner above-mentioned and expressed as the fluorescent intensity (excitation, 357 nm; emission, 450 nm).

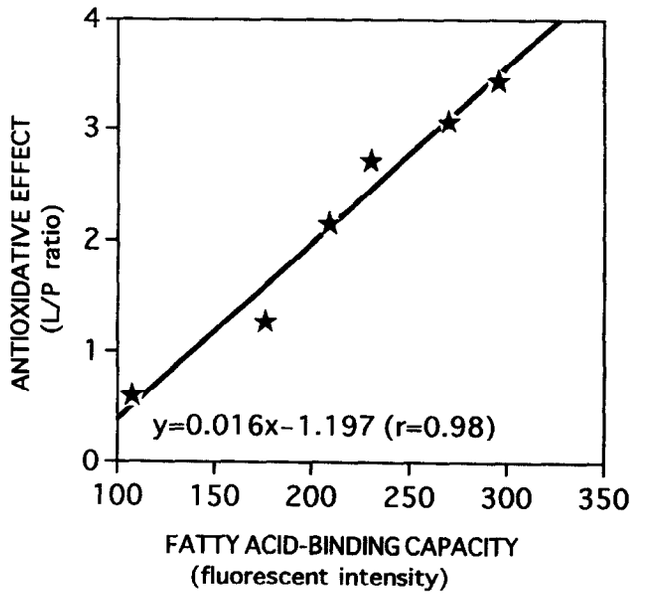

Fig. 8. Plot of antioxidative effect against fatty acid-binding capacity. The data on day 3 in Fig. 2 were used as representatives of the antioxidative effects of deamidated zein preparations, because of favourable linearity and plotted against their corresponding fatty acid-binding capacities (DPH hydrophobicities).

that similar components may have to be mixed in zein. Even so, it seems more likely that these components are more or less extractable with aqueous alcohol and therefore are mostly excluded from the mother liquor during dialysis. In any case, the isolation and identification of concomitant non-proteinaceous substrates will be required to justify the validity of such an assumption.

A recent interesting explanation for the antioxidative effect of intact zein is that it arises from the protein structure inclusive of β- and γ-type subunits at any given content. Matsumura *et al.* (1994) have referred to a close correlation between the antioxidative effect against docosahexaenoate of several zein preparations from different maize varieties and the existence of β- and γ-subunits. These subunits are abundant in sulfur amino acids, which may serve as free-radical trapping agents. Besides the reactivity of these and other amino acids, it is feasible that the conformation of zein subunits is responsible for the antioxidative effect, probably *via* lipid embedment in hydrophobic crevices of the zein particles. It seems likely that glutaminyl or asparginyl amide-groups take part in such a spatial arrangement of zein molecules. Deamidation, i.e. ammonia release from amide groups, breaks the hydrogen or hydrophobic bond in gluten and thereby rearranges its molecular weight distribution without serious cleavage of the peptide bond (Wu *et al.*, 1976; Matsudomi *et al.*, 1981, 1982; Ma *et al.*, 1986). A progressive increase in the surface hydrophobicity is then observed below 30% deamidation. This is explained by deamidation-caused exposure of buried hydrophobic groups. The surface hydrophobicity of deamidated zein, was presumably not increased because the emission spectra shifted to longer wavelengths (Fig. 6a), but rather reduced to one-half its initial value at 20% deamidation (Fig. 6b). A gap in the maximum emission between deamidated zein preparations with and without lyophilization may be accounted for by dehydration-caused structural changes. Zein is much more hydrophobic than other cereal prolamins and even soluble in a polar lipid-directed solvent (chloroform–methanol). This high hydrophobicity is why zein had to be solubilized by 70% ethanol containing 0.05 N HCl prior to the deamidation reaction (gliadin is soluble in aqueous acid solution), while unmodified zein and its deamidated preparations were pretreated with 0.5% SDS for solubilization prior to gel filtration and electrophoresis.

A by-product (so-called 'gluten-meal') of corn-starch manufacturing can be relied on for the supply of zein sources, but it is not effectively used for food materials. The main reason is that the protein is, not only poor in its nutritive value (low in tryptophan and lysine), but also cumbersome for food processing because of its gummy cohesion in a hydrated state. Deamidation, or partial cleavage of the peptide bond, leads to a pronounced change in the functional properties of zein. As the fragmentation reaction proceeds, however, a favourable feature, represented by antioxidation, vanishes. Additionally, the possibility cannot be excluded that a crude zein preparation contains physiologically active substances which benefit rather than damage health.

REFERENCES

Abe, M., Arai, S. & Kato, H. (1981). Electrophoretical analysis of zein and isolation of its components. *Agric. Biol. Chem.*, **45**, 1467–1472.

Chiue, H., Iwami, K., Kusano, T. & Ibuki, F. (1994). Decreased antioxidative activity of maize zein in response to deamidation rate. *Biosci. Biotech. Biochem.*, **58**, 198–199.

Glanz, S. A. (1987). How to test for trends. In *Primer of Biostatistics*, 2nd edn. McGraw-Hill, New York, 191–244.

Hamada, J. S. & Marshall, W. E. (1989). Preparation and functional properties of enzymatically deamidated soy proteins. *J. Fd Sci.*, **54**, 598–601.

Hammerschmidt, P. A. & Pratt, D. E. (1978). Phenolic antioxidants of dried soybeans. *J. Food Sci.*, **43**, 556–559.

Hayakawa, S. & Nakai, S. (1985). Relationship of hydrophobicity and net charge to the solubility of milk and soy proteins. *J. Fd Sci.*, **50**, 486–491.

Iwami, K., Hattori, M. & Ibuki, F. (1987). Prominent antioxidant effect of wheat gliadin on linoleate peroxidation in powder model system at high water activity. *J. Agric. Food Chem.*, **35**, 628–631.

Iwami, K., Hattori, M., Nakatani, S. & Ibuki, F. (1987). Spray-dried gliadin powders inclusive of linoleic acid (microcapsules); their preservability, digestibility and application to bread making. *Agric. Biol. Chem.*, **51**, 3301–3307.

Iwami, K., Hattori, M., Yasumi, T. & Ibuki, F. (1988). Stability of gliadin-encapsulated unsaturated fatty acid against autoxidation. *J. Agric. Fd Chem.*, **36**, 160–164.

Karel, M., Schaich, K. & Roy, R. B. (1975). Interaction of peroxidizing methyl linoleate with some proteins and amino acids. *J. Agric. Fd Chem.*, **23**, 159–163.

Kato, A., Tanaka, A., Lee, Y., Matsudomi, N. & Kobayashi, K. (1987). Deamidation of food proteins by protease in alkaline pH. *J. Agric. Fd Chem.*, **35**, 224–227.

Kato, A., Shibata, M., Yamaoka, H. & Kobayashi, K. (1988). Deamidation of lysozyme during the storage of egg white. *Agric. Biol. Chem.*, **52**, 1973–1978.

Kawashima, K., Itoh, H. & Chibata, I. (1979). Synergistic ternary antioxidant compositions comprising tocopherol, partially hydrolyzate of gelatin and organic acid. *Agric. Biol. Chem.*, **43**, 827–831.

Laakso, S. (1984). Inhibition of lipid peroxidation by casein. Evidence of molecular encapsulation of 1,4-pentadiene fatty acids. *Biochim. Biophys. Acta*, **792**, 11–15.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bactriophage T4. *Nature*, **227**, 680–685.

Ma, C-Y., Ooman, B. D. & Holme, J. (1986). Effect of deamidation and succinylation on some physicochemical and baking properties of gluten. *J. Fd Sci.*, **51**, 99–103.

Matsudomi, N., Kaneko, S., Kato, A. & Kobayashi, K. (1981). Functional properties of deamidated gluten (in Japanese). *Nippon Nogeikagaku Kaishi.*, **55**, 983–989.

Matsudomi, N., Kato, A. & Kobayashi, K. (1982). Conformation and surface properties of deamidated gluten. *Agric. Biol. Chem.*, **46**, 1583–1586.

Matsumura, Y., Andonova, P. P., Hayashi, Y., Murakami, H. & Mori, T. (1994). Antioxidant activities of zeins from different maize varieties against docosahexaenoic acid ethylester. *Cereal Chem.*, **71**, 428–433.

Pratt, D. E. & Birac, P. M. (1979). Source of antioxidant activity of soybean and soy products. *J. Fd Sci.*, **44**, 1720–1722.

Shin, F. F. (1991). Effect of anions on the deamidation of soy protein. *J. Fd Sci.*, **56**, 452–454.

Tsutsui, T., Li-Chan, E. & Nakai, S. (1986). A simple fluorometric method for fat-binding capacity as an index of hydrophobicity of proteins. *J. Fd Sci.*, **51**, 1268–1272.

Vaintraub, I. A., Kotoba, L. V. & Shaha, R. (1992). Protein deamidase from germinating wheat grains. *FEBS Lett.*, **302**, 169–171.

Wang, J. Y., Fujimoto, K., Miyazawa, T. & Endo, Y. (1991). Antioxidative mechanism of maize zein in powder model systems against methyllinoleate. Effect of water activity and coexistence of antioxidants. *J. Agric. Fd Chem.*, **39**, 351–355.

Wang, J. Y., Miyazawa, T. & Fujimoto, K. (1991). Inhibition of methyllinoleate peroxidation by maize zein in powder model system at high water activity. *Agric. Biol. Chem.*, **55**, 1531–1536.

Weatherburn, M. M. (1967). Phenol-hydrochloride reaction for determination of ammonia. *Analyt. Chem.*, **39**, 971–974.

Wu, C. H., Nakai, S. & Powrie, W. D. (1976). Preparation of acid-solubilized gluten. *J. Agric. Fd Chem.*, **24**, 504–510.

Yamaguchi, N. (1989). Antioxidative effects of peptides (in Japanese). *New Fd Indust.*, **31**, 18–22.