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FREEZE-INDUCED FIBRE FORMATION IN PROTEIN EXTRACTS FROM RESIDUES OF MECHANICALLY SEPARATED POULTRY

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Abstract

Coagulated protein obtained by alkali extraction and acid precipitation from the bone-containing residues discarded after mechanical separation of chicken was textured by freezing in semi-infinite cylinders followed by heat-setting in a microwave oven. Macrophotography was used to illustrate textural differences resulting from pH variations (4.5-6.0) in the precipitated protein, and changes in the ambient temperature used for freezing (-5°C to -32°C). Well identified, permanent fibres were formed by the process under all conditions studied. The thickness of the fibres decreased and their radial orientation increased with increasing pH and decreasing ambient temperature of freezing. Cross-linkages between parallel fibres of the main fibre structure were observed primarily as a function of pH, high pH samples showing the highest tendency for formation of these cross-links.

Introduction

Textural modification of proteinaceous materials from animal or plant sources has been gaining importance in contemporary food technology. Macroscopic textural changes based on protein response to heat, chemical, or physical treatments have been exploited for centuries to increase palatability and consumer acceptance of certain foods. Gluten in bread, casein in cheese, or egg white and egg yolk in hard-boiled eggs are common examples of conventional food proteins involved in structure formation. Modern texturization techniques such as spinning or extrusion-cooking were developed relatively recently with the main objective of simulating meat structure in non-meat proteins⁹.

Formation of identifiable texture in proteinaceous materials by freezing has attracted much attention in recent years⁶⁻⁷⁻⁸⁻¹¹⁻¹²⁻¹³⁻¹⁴. However, the technique is not new; in 16th century Japan, freezing preservation of tofu (a soybean protein curd) was found to produce a stable, porous, sponge-like matrix¹⁵. In recent patent literature, the first reference to fibre formation in frozen defatted soybean protein extracts was that by Okumura and Wilkison¹⁴. Boyer and Middendorf¹ and Middendorf *et al.*¹² were awarded the first patents for simulating a meat-like texture in freeze-texturized protein concentrates and slurries. The general process of Middendorf *et al.*¹² is followed in several recent patents⁶⁻⁷⁻⁸⁻¹¹⁻¹³.

While patent literature on freeze-texturization has been growing rapidly, little specific information and explanation of the phenomenon has been forthcoming. It is generally accepted⁵ that the freeze-induced fibre formation is caused by the growth of pure ice crystals in an aqueous system containing dispersed or dissolved proteinaceous material. The growth of the ice crystals forces the proteinaceous material ahead or to the side of the ice crystal boundary, causing a separation and compaction of the protein. Whether any chemical or biochemical reactions in the protein matrix accompany the mechanical effects of the ice crystal growth is not well understood at the present time.

The work reported herein describes the freeze-fibre formation in acid precipitated chicken protein, extracted by alkali from the

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bone-containing residues of mechanical separation ("deboning") of poultry. The aim of this paper is to illustrate the macrostructure of the freeze-texturized chicken protein extracts as recorded by macrophotography. Our objectives were to study the effects of freezing rates (ambient temperature -5°C to -32°C) and pH of the protein extracts (pH 4.5-6.0) on the visual appearance of the freeze-texturized, heat-set protein.

Materials and Methods

Raw material

All freeze-texturization work was carried out with chicken protein extracts obtained from the normally discarded residues resulting from a mechanical separation process. The raw material for the mechanical separation was from chicken backs, necks, and spent layers processed by a Beehive mechanical deboner in a local poultry processing plant. The bone-containing residues were extracted in our laboratory the same day as they were processed. The alkali extraction acid precipitation technique of Jelen *et al.*⁴ was used to produce the chicken protein extracts. Briefly, the method included mixing of the ground bone residues with distilled water at the residue: water ratio of 1:1.25; adjustment to pH 10.5 by NaOH (10% w/w solution) at room temperature (about 22°C); tumbling for 30 min in a Turbula (WAB, Bachofen, Switzerland) mixer; centrifugation at $1750 \times g$ for 15 min to separate the liquid protein extract; acidification of the extract by 6.0 M HCl in a vigorously stirred baffled container; and centrifugation at $2400 \times g$ for 15 min. Protein and total solids of the precipitates (Table 1) were determined by AOAC analytical methods for meat and meat products (micro-kjeldahl and vacuum oven at 80°C for 24 hrs, resp.).

Table 1
Composition before freezing of chicken protein coagula obtained by alkali extraction and acid precipitation at various pH levels.

pH	Total Solids (%)	Protein (% of TS)
4.5	14.0	75.2
5.0	12.0	75.4
5.5	9.5	75.4
6.0	8.0	75.3

Freezing

The protein precipitates were tightly packed by hand into sausage casings of 5.4 cm diameter. Cork stoppers of 3.5 cm thickness were placed at both ends of the filled casings to simulate the conditions of heat transfer in a semi-infinite cylinder. The length of the protein-filled cylinders was 15 cm. The cylinders were tied at both ends and hung in a Liquid Carbonic Cryogenic

Freezer. The distances between the individual cylinders were approx. 25 cm. Flow of liquid CO_2 used as the freezant was maintained at a pre-programmed rate giving the desired constant ambient freezing temperature. The gas circulation in the cabinet was maintained by a fan positioned in the vicinity of the CO_2 entrance part. The samples were left in the cabinet until the temperature of the centre of the cylinder, determined by a thermocouple, differed from the ambient temperature by less than 1°C . Correspondingly, the approximate retention times for the four trials were about 5 hrs (-5°C); 3 hrs (-12°C); 2 hrs (-22°C); and 100 min (-32°C). Upon completion of the freezing process, the products were sealed in plastic bags to prevent loss of moisture, and stored at -25°C for two days. The frozen cylinders were heat set by placing them - without the sausage casings - into a Toshiba microwave oven (Model ER-T86BT, 650 W) for 6 min so that the internal temperature of the cylinders reached approximately 85°C . The total solids content of the heat-set samples was determined using again the vacuum-oven method as given above.

Photography

Several thin, 5 mm high circular discs were cut with a sharp razor from the central region of the heat-set cylinders after these cooled to room temperature. The samples with the most consistent flat surface (to facilitate complete focusing) were selected for the photography work. Color pictures were taken under artificial illumination on Kodak Electrochrome 160 ASA Tungsten slide film using a rigidly mounted 35 mm Canon EF camera with a 50 mm, F 3.5 macro lens with a 25 mm extension tube. Higher magnifications were taken using a 24 mm F 2.8 lens which was reverse mounted and combined with the 25 mm extension tube. Maximum depth of focusing was obtained by using apertures between F 16 and F 32. The slides were printed using a positive printing process (Kodak R 1000 chemistry on 2203 paper) to minimize loss of detail. The black-and-white reproductions for this report were produced from the original color prints¹⁰.

Results and Discussion

The overall effect of pH and freezing temperature on the macrostructure of the heat-set, freeze-texturized extracts is shown in Fig. 1. The trend towards finer fibre structure with increasing both pH and the rate of heat removal (i.e. lowering the ambient temperature) is clearly evident. A color change is also noticeable in Fig. 1; the effect - from light pink at pH 6.0 through grey to dark brown at pH 4.5 - is much more pronounced in the colored originals¹⁰. The heat setting caused some shrinkage of the textured material due to the water loss, which was much higher in samples frozen at the low pH and low rate of heat removal (Fig. 2). The differences shown in Fig. 1, Fig. 2, and Table 1 are undoubtedly related to the well known relationships of pH to water holding capacity of meat protein, as well as to the size of the water crystals formed during the freezing process.

FREEZE-INDUCED FIBRE FORMATION IN PROTEIN EXTRACTS

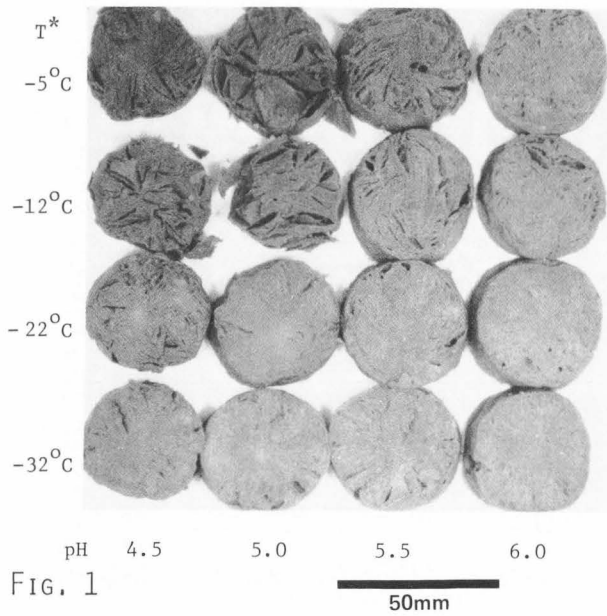


FIG. 1

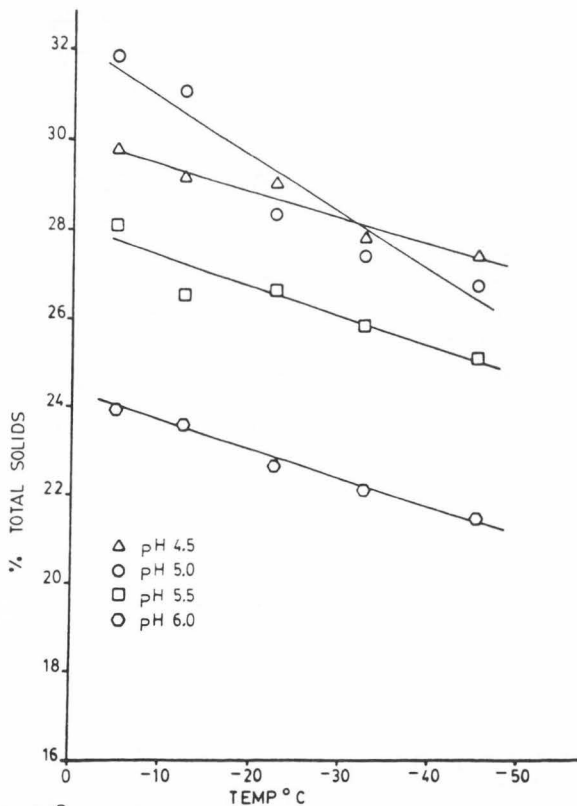


FIG. 2

Fig. 1. Effect of freezing temperature (T^*) and pH of chicken protein extracts on the freeze-induced fibre structure.

Fig. 2. Effect of freezing temperature and precipitation pH on total solids of the heat-set, freeze-texturized protein.

Higher magnifications of the freeze-texturized heated samples, showing the most striking changes in texture, are shown in Fig. 3 to 7. Fig. 3 shows a sample frozen at pH 4.5 and -5°C . The fibres (or, more specifically, the fibre planes) are thick, easily identifiable, and

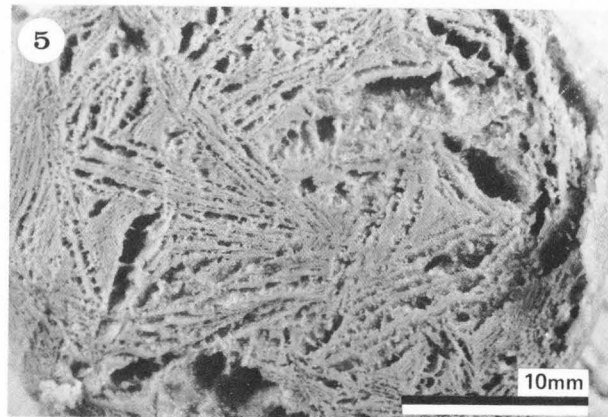
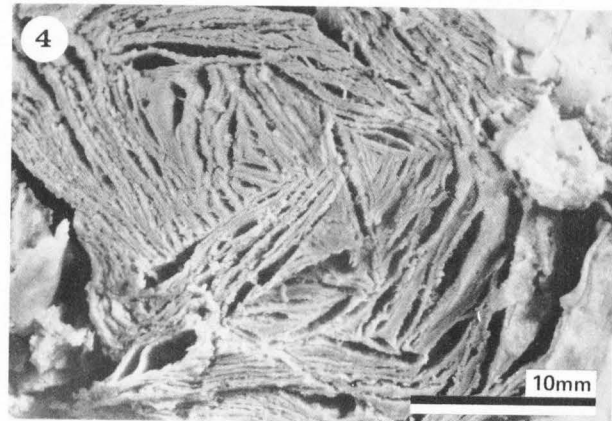
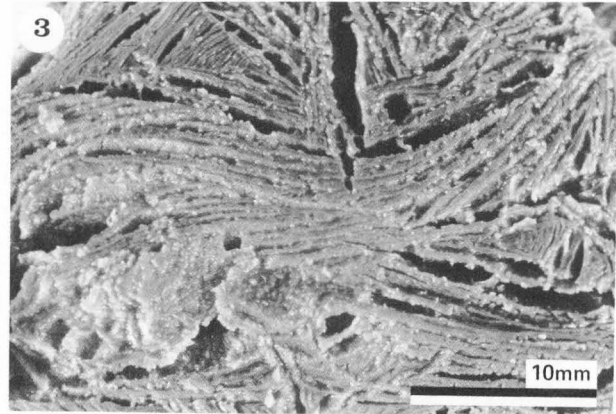


Fig. 3. Fibre formation in a protein precipitate frozen at -5°C , pH 4.5.

Fig. 4. Protein precipitate frozen at -12°C , pH 5.5.

Fig. 5. Protein precipitate frozen at -12°C , pH 6.0.

relatively randomly oriented. Large voids are apparent with little visible cross-linking. This allowed easy flaking of the fibres. The large, randomly oriented fibres are typical of the low pH, high ambient temperature freezing.

Increasing the freezing rate by decreasing the ambient temperature to -12°C , while simultaneously increasing the pH to 5.5 (Fig. 4) or 6 (Fig. 5) produced a noticeable decrease in the fibre size and increase in fibre cross-linking. This dense, sponge-like structure was especially characteristic of all pH 6.0 samples examined. Decreasing the freezing temperature to -22°C (Fig. 6) or -32°C (Fig. 7) further decreased the fibre thickness. In addition, a clearly noticeable pattern of radial fibre orientation was produced in all samples regardless of pH.

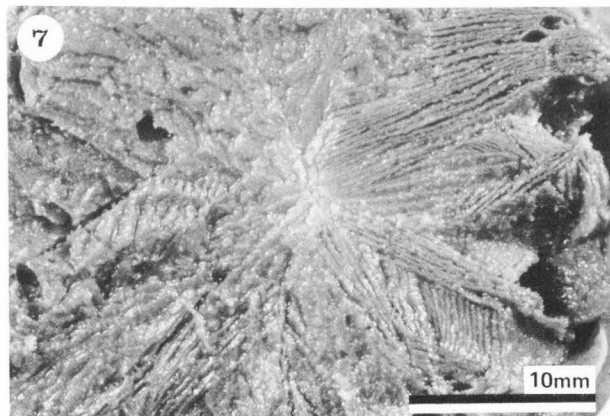
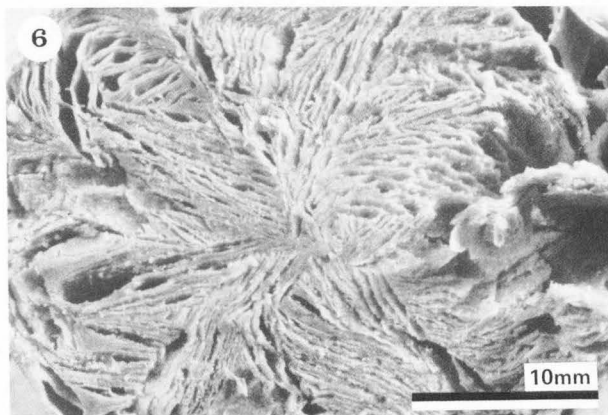


Fig. 6. Protein precipitate frozen at -22°C , pH 5.5.

Fig. 7. Protein precipitate frozen at -32°C , pH 5.5.

The effect of the freezing rate on the fibre orientation in the semi-infinite cylinder configuration can be illustrated by the schematic representation shown in Fig. 8. Initially, at low rates of heat removal, a few randomly nucleated ice crystals appear in the surface regions of the protein cylinder. These crystals can grow in various directions, producing the wedge-shaped areas of fibre "bundles" (Fig. 3 to 5). As the

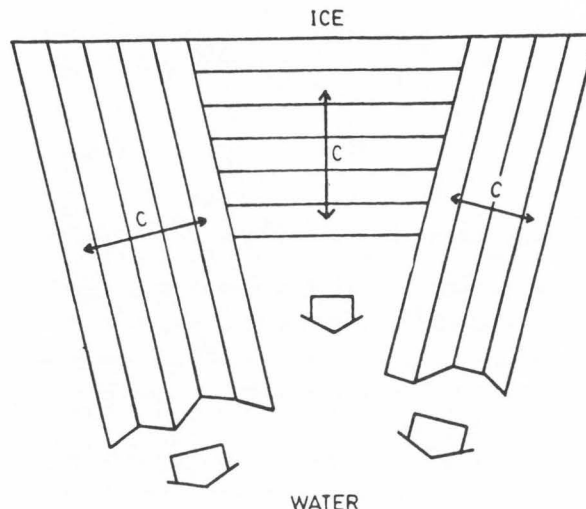


Fig. 8. Schematic representation of ice crystal growth (from de Quervain, 1975). (Reference 2)

rate of energy removal is increased (Fig. 6 and 7) the amount of fibres normal to the surface (i.e. giving an impression of radial orientation) is greatly increased. This demonstrates the rapid production and growth of ice crystal nuclei from the surface inwards. According to Hallett³ ice crystal growth occurs most rapidly in a planar direction, defined as the *a* axis, while growth "across" the planes (the *c* axis) is much slower. Crystals with the *a* axis perpendicular to the surface of the cylinder nucleate and grow much more rapidly at low freezing temperatures; this rapid growth inwards suppresses any crystals growing with their *a* axis parallel to the surface. The faster the rate of energy removal, the more numerous the nuclei, and the finer the fibre structure.

The increased water holding capacity of the protein at higher pH, characteristic of most meat proteins, is undoubtedly responsible for the finer structure of the high pH samples. It is probably also related to the sponge-like appearance of these high pH samples (Fig. 9) which is caused by the numerous cross-linking fibres. These cross-links appear to have formed in the interstitial areas between the branches of the ice-crystals, running perpendicular to the large fibres. During the acidification step to precipitate the proteins from the alkaline extracts, it was noted that at pH 4.5 and 5.0 the protein formed small, easily recognizable floccules. At pH 6.0, the precipitate was quite homogeneous with little flocculation occurring. It appears that the flocculated protein is not capable of forming the cross-linked structure upon its freeze-concentration into the voids between the ice crystal planes (Fig. 10). This may be due to the non-homogeneous nature of the precipitate or the more unfolded state of the protein at the higher pH. Alternatively, the explanation of this phenomenon may be related to differences in the ice structure as effected by the pH variations. At higher pH, the ice

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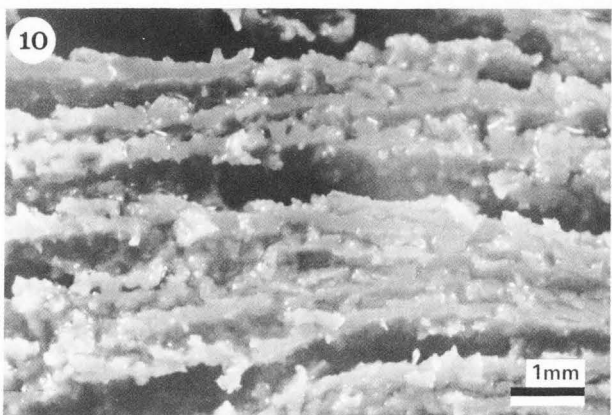
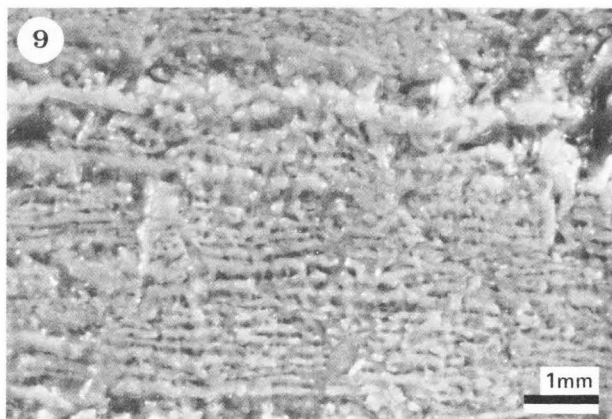


Fig. 9. Illustration of the sponge-like structure of freeze-textured chicken protein frozen at -32°C , pH 6.0.

Fig. 10. Illustration of the incohesive structure of freeze-textured chicken protein frozen at -5°C , pH 4.5

structure may have changed due to the lower NaCl content and/or differences in water binding to the meat protein.

Conclusions

Our intention in this study was to demonstrate the texture forming effects of freezing in an aqueous protein concentrate. The major observed effects of the temperature of freezing and pH of the protein material on the heat-set structure formed by the freezing process are readily explainable from current knowledge of protein-water interactions as effected by pH, and from the theory of ice crystal formation. However, the formation of cross-linked, sponge-like structures observed primarily in the high pH samples appears to be a more complicated phenomenon which may need further elucidation.

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Discussion with Reviewers

D.S. Reid: Have the authors made any measurement of the rate of propagation of the ice front during freezing under various conditions or have they calculated this quantity?
Authors: Yes, this was determined experimentally from time-temperature profiles measured by four thermocouples equally spaced along the radius¹⁰. For each temperature studied, the frozen boundary movement progressed at approximately constant rate (lower temperatures showing faster rates) in

the outer half of the radius. In the inner half of the radius, the rates of the frozen boundary movement increased. A detailed account of these measurements will be published (in preparation). Similar effects were described by Meryman¹⁹ for a 7% starch solution frozen in cylindrical forms.

D.S. Reid: Rohatji PK, Brush EJ, Jain SM, Adams CM (Materials Sci. and Eng. 13, 1974, 3-18) showed a relationship between freezing rates and the dimensions of ice columns in dendritic freezing of salt solutions. Have the authors looked for similar relationships between their observed freeze-induced structures and freezing conditions?

Authors: We did not measure the structure of the ice crystals *per se*; rather, the object of our study was the structure developed in the frozen-thawed protein. Obviously, the ice crystal structure should have an effect on the textural phenomena as illustrated in this paper. As one of the quantitative correlations between the freezing rates and the textural phenomena observed, we adopted a measure of "fibre density". This was determined as a number of fibres crossing an arbitrary 1 cm long line drawn perpendicular to the main bundles of fibres. The correlation, as shown in Table 2 below, confirms what was shown qualitatively in this paper. Higher pH and lower ambient freezing temperature resulted in higher fibre density, i.e. more fibres crossing the 1 cm line. The details of this work will be published later.

Table 2
Fibre Density in Freeze-Texturized, Heat-Set Chicken Protein Extracts.

pH	No. of fibres crossing an arbitrary 1 cm long line in samples frozen at different temperatures			
	-5°C	-12°C	-22°C	-32°C
4.5	10	12	18	21
5.0	10	11	21	24
5.5	13	11	23	26
6.0	12	16	30	34

D.S. Reid: On the subject of freezing conditions, the authors quote "ambient freezing temperatures" of -5°C to -30°C. Since at the higher temperatures used, especially -5°C, ice formation will not be complete, a significant ice growth could take place after the transfer of the cylinder to -25°C storage. This two-stage freezing process could produce modified freezing. If a cylinder frozen at -5°C is immediately cooked, is the protein fibre structure similar to that obtained if cooking occurs after a period of -25°C storage?

Authors: For logistical reasons related to the experimental design, it was necessary to keep all frozen samples in storage before the heat-setting. We did not attempt to heat-set any samples prior

to the frozen storage at -25°C. It is recognized that storage at -25°C after freezing to -5°C will diminish the amount of unfrozen water, and thus will cause additional water to freeze out. However, as our photographs show, the additional freezing is unlikely to change substantially the structural patterns of the protein fibres which were set by the initial freezing conditions. (If it did, then in fact our Fig. 3, 4 and 10 would not look the way they do). The absolute dimensions of the individual fibres could perhaps be subject to change although the comparison of the individual samples (Fig. 1) still shows rather substantial differences related to the initial freezing conditions. A possibility can be considered that a secondary dendritic growth of ice crystals might occur in the samples frozen at -5°C as a result of the two stage freezing. This, in turn, might be related to the occurrence of some of the cross-linkages observed. However, Fig. 3, 4 and 10 (illustrating samples frozen at -5°C or -12°C) do not show any cross-linking, and thus make such a conjecture dubious. The reason why -25°C was used for storage of all samples, was to minimize any protein denaturation which is known to occur in frozen storage of meat proteins at relatively high ambient temperatures¹⁷⁻¹⁸. As certain properties of the freeze-textured samples were to be measured (e.g. water-holding capacity, strength of the textural patterns, retort stability), it was deemed desirable to minimize any likelihood of protein denaturation occurring in the frozen materials.

P.B. Addis: Have the authors considered that explanation for the changes in color as a function of pH shown in Fig. 1 could be provided by autoxidation reactions?

Authors: The pH-induced color variations in the freeze-textured extracts were recorded as an incidental - although consistently occurring - observation. This color change was also apparent in the fresh extracts before the freeze - texturization and heat - setting. We have not determined the exact cause of these color changes, although it is believed that the primary explanation would be related to the pH-related chemistry of myoglobin.

P.B. Addis: It is known (Owen JE and Lawrie RA, J. Food Technol. 10, 1975, 169-180) that oxidation in meat occurs more rapidly at lower pH than at higher pH. Was any evidence obtained of phospholipid oxidation (warmed-over flavor) in the studies conducted by the authors?

Authors: Flavor aspects were not part of this study. In related studies, similarly prepared (but not freeze-textured) chicken protein extracts were used in luncheon meat-type products without any adverse effects on the flavor.

D.F. Wood: Have the authors determined whether or not the extracted protein has any functionality in a comminuted meat system? Before heat-setting and after heat-setting?

Authors: As mentioned above, certain functionality tests were carried out with the freeze-textured samples. In other studies, the fresh extracts were used as an ingredient in luncheon meats. All these tests indicated that the extracted protein

appears to have functional properties similar to the mechanically separated protein paste. However, a thorough study of functional properties of the protein extracts before and after the freeze texturization appears necessary and is being planned.

D.F. Wood: Have the authors tried looking at the protein matrix using TEM or SEM, especially to better investigate the structure of the cross-linkages observed in the high-pH samples?

Authors: No. This is another area requiring future work which is now in progress.

Om Johari: Can you please provide a reference to AOAC analytical methods you used?

Authors: Please see ref. 16.

Om Johari: Please provide the titles of patents in refs. 1,6,7,8,11,12,13 and 14.

Authors: Please see below.

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7. British Patent #1,537,173 (1978) Textured Fibrous Protein Product.

8. U.S. Patent #4,001,459 (1977) Fibrous Protein Materials.

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