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T. Beveridge

L. Jones

M. A. Tung

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STRANDED STRUCTURE DEVELOPMENT IN THERMALLY PRODUCED WHEY PROTEIN CONCENTRATE GEL

T. Beveridge*[1], L. Jones[2] and M. A. Tung[2]

[1]School of Food Science
Macdonald Campus of McGill University
Ste. Anne de Bellevue, Quebec, Canada
H9X 1C0
[2]Department of Food Science
University of British Columbia
Vancouver, British Columbia, Canada

*Present Address:
Agriculture Canada
Research Station
Summerland, British Columbia
V0H 1Z0, CANADA

Abstract

Scanning electron micrographs of thermally induced whey protein concentrate gels were taken. Sample preparation was accomplished by glutaraldehyde fixation, osmium tetroxide post-fixation and critical point dehydration. Stranded or beaded gel structures were observed on the external surface of a gas bubble, suggesting that a "string-of-beads" gel microstructure may result from bubble formation during thermal treatment.

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Direct inquiries to T. Beveridge.
Telephone number: 604-494-7711.

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Introduction

Scanning electron microscopy (SEM) has become a standard technique for the examination of the microstructure of thermally formed protein gels (Kalab and Harwalkar, 1973; Hermansson, 1979; Yasui et. al., 1979). Generally, in critical point dried preparations, these gels have been observed to consist of a network of globular particles of aggregated protein, apparently adhering together. Occasionally, during the SEM examination of egg albumen and whey protein concentrate gels, areas were encountered which appeared as a "string-of-beads" entanglement of strands. Indeed, the appearance of bead strands is not uncommon in the literature (Hermansson, 1979; Yasui et. al., 1979) and it is possible that this forms the normal gel matrix. However, considerable variability in gel microstructure has been observed, and this paper offers a possible explanation for some of this variation.

Methods

Gels (22.8% w/w solids, room temperature) were formed from a commercial whey protein concentrate (WPC: Dairyland Products Inc., Savage, Minnesota, USA) containing 30.5% protein (N x 6.38), 9.5% moisture, 6.0% ash and $<$ 0.5% fat, as determined by standard methods (AOAC, 1975). Heating in a water bath for 30 min. at 90°C in screw-capped test tubes gelled the solutions. WPC gels were scalpel cut to approximately 2 x 2 x 4 mm pieces and fixed overnight in 4% glutaraldehyde in 0.07 M phosphate buffer, pH 7.0, followed by phosphate buffer rinse (0.07 M, pH 7.0, 3 x 10 min.). Osmium tetroxide fixation (1% OsO_4 in 0.07 M phosphate, pH 7, 3 x 10 min.) was followed by ethanol dehydration (50, 70, 80% in distilled deionized water, 5 min. each; 90% 2 x 10 min., 100%, 3 x 10 min.), then transferred to amylacetate (25, 50, 75% in ethanol, 10 min. each; 100%, 1 hr.). Critical point drying using liquid CO_2 completed the process. The dried pieces were fractured and mounted on aluminium stubs with epoxy glue and coated with gold-palladium alloy in a sputter coating device (Technics Inc., 7950 Cluny Court, Springfield, Virginia, USA). A Cambridge Stereoscan scanning

electron microscope operated at 20 kV was used to examine the gels.

Results and Discussion

Figure 1 shows a gas bubble which has been cut through with the scalpel during the initial specimen preparation. The view is on the cut edge of the piece and not the fractured surface and extensive scalpel damage on the cut edge around the gas bubble is obvious. Figure 2 is a magnified image of the inside surface of the gas bubble. The stranded, beaded or filamentous appearance of the gel formed at the bubble surface is evident. The features of figure 2 may be compared to those of figure 3, an image obtained from a fractured surface of gel, free of apparent bubbles. It is clear that the movement and stretching of developing coagulum in the region of a forming and expanding bubble combined with the surface tension forces at the interface can markedly influence gel ultrastructure.

The source of the gas responsible for bubble formation in the gels is unclear, but it may arise from gas occluded during solution preparation, evolution of dissolved gas during heating or steam generation. Also unclear is how numerous the bubbles are and to what extent they develop during gel formation because the WPC solutions are themselves very cloudy, almost opaque. Observations of the formation of thermally induced protein gels of 5-6% egg albumen at pH 9 suggest that bubbles could be quite numerous and extensively developed. In opalescent albumen systems, many small bubbles could be seen forming throughout the gel during heating over 30 min. Larger bubbles formed at the test tube wall and all bubbles disappeared on cooling. The possible physical or chemical effects of gas evolving within thermally gelling protein systems have not been discussed by other workers in this field. Certainly the extent of sulphydryl oxidation in heated protein solutions depends upon the amount and nature of the gas present (Beveridge and Arntfield, 1979). The evolution of gas in the developing structural matrix could also substantially influence the perceived rheological properties of the gels.

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Discussion with Reviewers

D. N. Holcomb: Why were such large (2 x 2 x 4 mm) specimens used? Are the authors sure that the fixatives penetrated throughout the specimens?

Authors: The samples were of convenient size for handling. Adequate fixative penetration was not a problem since a uniform yellow color was noted on the cross-section of freshly trimmed, glutaraldehyde fixed specimens and a uniform black-gray color was seen on fractured surfaces of osmium post-fixed specimens. Protein gels of this type seem quite porous and allow rapid fixative penetration.

M. Kalab: Was the whey protein concentrate degassed (e.g., by lowering the pressure) and did this treatment affect the incidence of the gas bubbles?

Authors: The solutions of whey protein concentrate were not degassed. The observations reported here were made during studies relating rheological, chemical and ultrastructural properties of protein gels (Beveridge et. al., J. Agric. Fd. Chem., in press) and were not pursued further. Since it is probable that dissolved gases are a major source of gas for bubble formation, it is likely degassing will affect gas bubble incidence.

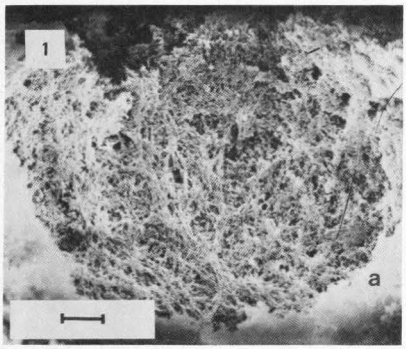
M. Kalab: What happened to the protein matrix at the test tube wall where gas bubbles were evident at 90°C but vanished after cooling? Were there any signs of the string-of-beads structure where the bubbles had been or was such a structure observed only around permanent bubbles? What was the consistency of the whey protein concentrate at 90°C before cooling? Was it already a gel (holding the shape of the container) or a highly viscous liquid which actually settled on cooling? (This question is closely associated with the previous one because it is aimed at finding out whether the beaded structures originated at 90°C or during cooling.)

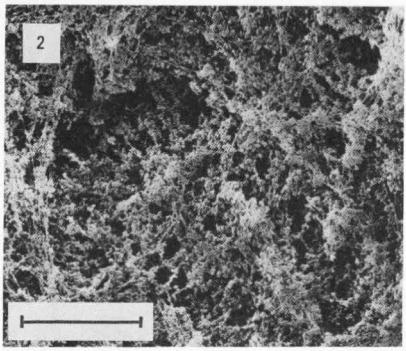
Authors: We cannot answer the first questions since the specimens were scalpel cut from the central portion of the thermally produced gel, specifically to avoid "artifacts" generated by possible interactions between the test tube wall and the gelling protein. At the time of preparation, the possibility of bubbles producing structure within the gel was not considered. The gelled whey protein concentrate at 90°C is sufficiently stiff to hold the container shape on inversion, however, the possibility of flow

(bubble disappearance) on cooling cannot be eliminated. It seems likely that the structures observed here originate at 90°C and are fixed on cooling. Obtaining definitive answers will require experiments designed to study the effects of bubble formation on ultrastructure modification rather than the simple observation reported here.

D.N. Holcomb: Figure 1 has scratches or some similar defect. Perhaps the authors have an unblemished copy or the Figure could be trimmed to elimate the "scratches".
Authors: The defects are "scratches" on the negatives. Since the number of pictures available are limited and cropping this one would eliminate the edge of the bubble, the decision was taken to go "warts and all" rather than lose the image of the bubble.

M. Kalab: The incidence of the permanent gas bubbles in the gel would be easy to demonstrate by freezing the fixed and dehydrated (in absolute ethanol) samples, fracturing them, melting in absolute ethanol, CPD, and SEM. Would you please show a micrograph of a characteristic fracture and indicate the average bubble diameter?
Authors: The resources to do this are not available. Care should be taken when examining a fractured surface for gas bubbles. Since it is likely that the fracture would pass through the bubble, this could occur at any level of the bubble, and with an irregular edge. It may not be obvious that the structure imaged was once on the surface of a bubble.





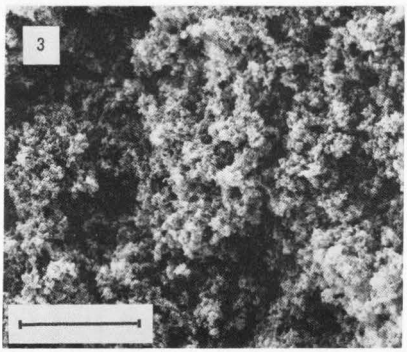


Fig. 1. Scanning electron micrograph of structure on the surface of a gas bubble formed in heat-induced whey protein concentrate gel. a: cut edge of bubble damaged by scalpel. Bar represents 50 microns.
Fig. 2. Detail of the central area of Fig. 1 showing stringed microstructure. Bar represents 50 microns.
Fig. 3. Microstructure of whey protein concentrate gel on fractured surface, remote from gas bubbles. Bar represent 50 microns. Note the magnification of Figs. 2 and 3 are the same.