Histological Techniques to Study Emulsion Microstructure

Glenn R. Schmidt*, Leader
James C. Acton, Cooperator
Frederick K. Ray, Recorder

Introduction

Finely comminuted meat products are a combination of an oil water emulsion and a protein gel. Reports differ as to which mechanism is most important in determining the overall properties of the meat product. Light, transmission electron, and scanning electron microscopy have been used to study the microstructure of finely comminuted meat products (Schmidt, 1984). Light microscopy and histochemistry have been used to characterize the fat droplet sizes and the protein distribution within the gel matrix in sausages (Froning et al., 1970; Cassens et al., 1975; 1977; Jones and Mandigo, 1983; Hermansson and Olsson, 1982). These workers utilized histochemical stains to differentiate connective tissue protein from other proteins in the matrix. In addition, Hermansson and Olsson (1982) utilized differential interference contrast lighting to elucidate the structure at the interface of the lipid and proteins within the comminuted meat product. The effects of including nonmeat proteins on finely comminuted meat product microstructure have been studied by Cassens et al. (1975) and Kempton et al. (1982). Studies with uncooked wiener batters containing substantial amounts of nonmeat protein showed that nonmeat protein prevented the formation of typical fat and protein matrix that is characteristic of all meat batters, but only in some microscopic fields of some slices.

Hansen (1960) reported one of the earliest works on the use of transmission electron microscopy to study the emulsion formation in finely comminuted sausages. Later, Borchert (1967) also used transmission electron microscopy to study meat emulsions. The work of Theno and Schmidt (1978) utilized scanning electron microscopy and light microscopy to study the microstructure of three commercial frankfurters. Although these earlier studies did show differences in product microstructure, they did not utilize a treatment of the materials to study a cause and effect between ingredients, preparative techniques and cooking with product microstructure. More recent workers, (Carroll and Lee, 1981; Hermansson, 1980; Jones and Mandigo, 1982; Lee et al., 1981; Swasdee et al., 1982) have utilized specific ingredients and preparation techniques to study the effects of processing conditions on microstructure and functional properties of comminuted meat. Specialized studies by Ray et al. (1979, 1981) used techniques to study the microstructure of liver sausage and attempted to identify fat and protein components in meat emulsions, using scanning electron microscopy and light microscopy of sectioned liver sausage.

*G.R. Schmidt, Department of Animal Sciences, Colorado State University, Fort Collins, Colorado 80523

J.C. Acton, Clemson University, Clemson, S.C. 29631

F.K. Ray, Oklahoma State University, Stillwater, OK 74078

Reciprocal Meat Conference Proceedings, Volume 38, 1985.

A Comparison of Methodologies

Results of a preliminary study to determine the effect of ionic strength and pH on product yield of a comminuted beef product are presented. Light, transmission, scanning electron and freeze fracture microscopy were utilized to illustrate the microstructures of a comminuted beef product as affected by pH and ionic strength.

Product Manufacture

Commercially produced coarse ground beef trimmings were obtained from a packing plant and aged in chubs for 25 days. Product was prepared which included beef trimmings, 75.1%; ice, 22.5%; corn syrup solids, 2%; white pepper, 0.3%; nutmeg, 0.06%; sodium erythorbate, 0.05%; sodium nitrite, 0.015%. Three salt concentrations were utilized, as shown in Table 1. All ingredients were mixed and chopped in a 35 liter Meissner vacuum-bowl chopper (Model VE, RMF Steel, Kansas City, Missouri) at a blade speed of 4,000 rpm to a final temperature of 13° C. Each treatment batter was extruded with an EZ Pak water-powered stuffer into 303 × 406 cans. Cans were heated in a water bath to 70° C internal temperature as monitored with thermocouples and a recording potentiometer. The heated product was cooled under a water shower (5° C) and stored at 6° C.

Cook losses were measured on canned product 14 to 18 hours after cooking. Overall cook loss equaled the percentage of weight of the drained liquid divided by the sum of the solid product and the drained liquid weight. Calculated ionic strengths, cooked product pH and cook loss of the three treatments are shown in Table 1.

Table 1. The Effect of Salt Level and the Addition of Sodium Tripolyphosphate (STP) on Ionic Strength, Cooked pH and Cook Yield of a Comminuted Beef Product.

Treatment	*Salt level, %*	*[I]*	*Cooked pH*	*Cook loss, %*
A	0.58	.10	5.35	56
B	2.82	.48	5.61	35
C	2.82 plus 0.37 STP	.55	6.16	9

Microscopic Analysis Procedures

Scanning Electron Microscopy

A 2.54 cm core was removed from the central portion of each canned treatment. From each core, 10 to 12 3mm cubes were removed; frozen in a bath of Freon 12 suspended over liquid N2; fractured; and further prepared for scanning electron microscopy (SEM). Samples were fixed for one hour in 3% glutaraldehyde, 0.1 molar phosphate buffer, pH 7.2; washed twice in 0.1 molar phosphate buffer; then postfixed for 14 hours in 1% osmium tetroxide, 0.1 molar phosphate buffer, pH 7.2 at 4° C. Five to six samples were dehydrated in acetone; critical point dried in a Polaran critical point dryer; sputter coated with gold in a Hummer unit; affixed to a disc with colloidal graphite and examined on a Hitachi HHS 2R SEM.

Transmission Electron Microscopy

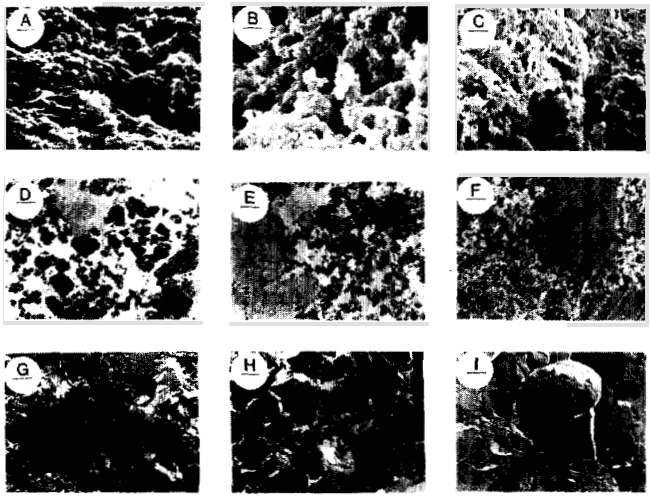
The remaining five to six samples, after postfixing in osmium tetroxide, were prepared for thin section transmission electron microscopy. Samples were dehydrated in acetone, embedded in Spurr's plastic and sectioned on a Reichert Om-U2 ultramicrotome. Sections were picked up on grids, stained with uranyl acetate and lead acetate and viewed on a Philips 200 transmission electron microscope.

Freeze Fracture

Samples (4 × 4 × 2 mm) were removed from the cores for freeze fracture and were surface frozen on a slammer unit and immediately immersed in liquid N2. The samples were fractured in a Balzer 301 unit at −105° C and coated with carbon-platinum to form a replica. The tissue was digested from the replicas with chlorine bleach for 4 hours, the replicas were rinsed with distilled water and then soaked in a chromsulfuric acid bath for 2 hours. The replicas were examined with a Philips 400 T operated at 80 kv.

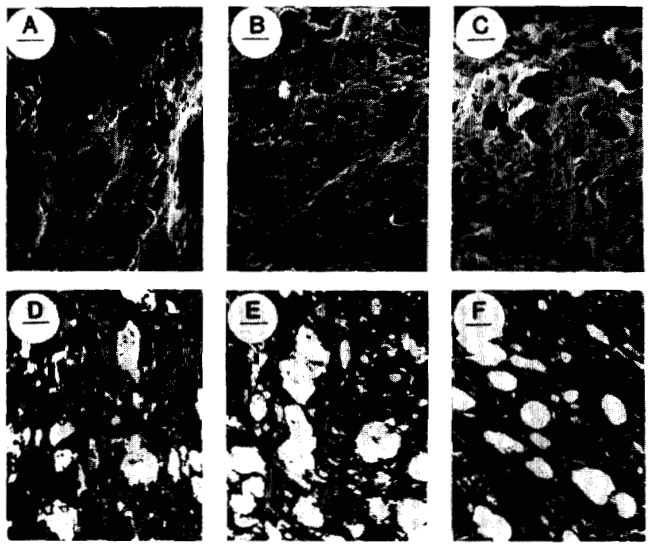
Frozen samples were sectioned at −20° C on a microtome, allowed to dry covered with a saffron stain, rinsed with water, covered with a coverslip and photographed through a Zeiss light microscope. Micrographs from the four techniques utilized are shown in Figures 1 and 2.

Figure 1



Scanning electron micrographs, A, B and C; transmission electron micrographs of thin sections D, E and F; and carbon-platinum replicas of a freeze fracture surface, G, H and I of a comminuted beef product. A, D and G are of product with 0.58% NaCl; B, E and H are of product with 2.28% NaCl; C, F and I are of product with 2.82% NaCl and 0.37% STP.
Bar = 1um.

Figure 2



Scanning electron micrographs, A, B and C and light micrographs, D, E and F of a comminuted beef product. A and D of product with 0.58% NaCl; B and E are of product with 2.28% NaCl; C and F are of product with 2.82% NaCl and 0.37% STP.
Bar = 100um.

Results and Discussion

Considerable effort has been spent on characterizing protein gels by scanning and transmission electron microscopic techniques (Hermansson and Buchheim, 1981; Hermansson, 1982; 1983; Hermansson and Lucisano, 1982). When finely comminuted raw meat suspensions are heated, there may be a partial disruption of the gel network and the gel will become more aggregated (Hermansson, 1982). With this change comes an increase in moisture loss during heating, since water is easily lost through larger capillaries in the structure. Gels with larger capillaries have a

lower water-binding capacity (Labuza and Lewicki, 1978; Labuza and Busk, 1979).

It is clear in micrographs A, D and G, at the 0.58% salt level, that larger aggregates and larger capillary areas are formed than in micrographs B, E and H, at the 2.82% salt level (Figure 1). In addition, the protein matrix is extremely fine in micrographs C, F, and I at the 2.82% salt + 0.37% STP level. As the matrix structure and capillary size become smaller, there is a great increase in water-binding capacity, as seen by the decline in cook loss from 56% to 35% to 9% (Table 1, Figure 1).

Davis and Gordon (1984) reported that use of several types of microscopic methods is necessary to guard against misleading artifacts and confirm true structures. In this study, use of several techniques gave much greater confidence in verifying microstructural appearance resulting from different ionic strength and pH treatments. Different levels of organization, as studied by varied magnifications of gels, must be evaluated to fully understand gel structure (Davis and Gordon, 1984).

Micrographs shown in Figure 2 compare scanning electron micrographs with light micrographs. At this low magnification (approximately 100X), it is difficult to see clear differences in the protein matrix structure. In micrographs A and D, the fat droplets are somewhat larger and irregular in shape compared to the micrographs from treatments at higher ionic strength levels. More coagulation of proteins seems to be present at the lower ionic strength treatments; however, this is not as clear as when observed at a higher magnification (Figure 1). All micrographs in Figure 1 utilize a magnification of approximately 10,000X. The greater abundance of tiny droplets in micrographs C and F (Figure 2) agrees with the observations at higher magnification (Figure 1). However, it would be very difficult to conclude from low magnification (100X) the necessary understanding of product microstructure. It is evident that one must choose the correct magnification to study appropriate and important levels of microstructure.

The light micrographs shown in D, E and F (Figure 2) require very careful methodology to prevent the loss of lipids. Previous studies by Hermansson and Olsson (1982) and Cassens et al. (1977) had excellent color micrographs of protein and lipid stained material. This requires careful histochemical and photographic technique and this detail is often not visible in black and white reproductions.

Summary

Reviewed literature and results of this work reveal several factors of importance in microstructural study of processed muscle foods. In order to study cause and effect relationships in muscle foods, the researcher must have control of 1) ingredients, 2) method of preparation and 3) accessibility to several analytical techniques to verify microstructural variation. Selection of proper magnification levels is also important. The work reported herein supports the basic concept of water-binding ability in processed meats being controlled by protein dissolution, due to appropriate ionic strength and pH (Trout and Schmidt, 1984a, 1984b). The resulting protein aggregation and capillary formation function to bind water (Hermansson, 1982; Labuza and Lewicki, 1978) and therefore control product cook yield and texture.

References

Borchert, L.L.; Greaser, M.L.; Bard, J.C.; Cassens, R.G.; Briskey, E.J. 1967. Electron microscopy of a meat emulsion. J. Food Sci. 32:419.

Carroll, R.J.; Lee, C.M. 1981. Meat emulsions – Fine structure relationships and stability. Scanning Electron Microsc. 1981. III:447.

Cassens, R.G.; Terrell, R.N.; Couch, C. 1975. The effect of textured soy flour particles on the microscopic morphology of frankfurters. J. Food Sci. 40:1097.

Cassens, R.G.; Schmidt, R.; Terrell, R.; Borchert, L.L. 1977. Microscopic structure of commercial sausages. Research Report R2878. Ag. Bulletin Building, 1535 Observatory Drive, Madison, WI 53706.

Davis, E.A.; Gordon, J. 1984. Microstructural analyses of gelling systems. Food Technol. 38(5):99.

Froning, G.W.; Andersen, J.; Mebus, C.A. 1970. Histological characteristics of turkey meat emulsions. Poultry Sci. 49:497.

Hansen, L.J. 1960. Emulsion formation in finely comminuted sausage. Food Technol. 14:565.

Hermansson, A.-M. 1980. Effect of processing conditions on microstructure and functional properties of comminuted meat batters. Proceedings of European Meeting of Meat Research Workers, p. 134, Colorado Springs, CO.

Hermansson, A.-M. 1982. Gel characteristics – Structure as related to texture and water binding of blood plasma gels. J. Food Sci. 47:1965.

Hermansson, A.-M. 1983. Protein functionality and its relation to food microstructure. Qual. Plant Foods Hum. Nutr. 32:369.

Hermansson, A.-M.; Buchheim, W. 1981. Characterization of protein gels by scanning and transmission electron microscopy. A methodology study of soy protein gels. J. Coll. Int. Sci. 81(2):519.

Hermansson, A.-M.; Lucisano, M. 1982. Gel characteristics – Waterbinding properties of blood plasma gels and methodological aspects of waterbinding of gel systems. J. Food Sci. 47:1955.

Hermansson, A.-M.; Olsson, E. 1982. Studies av livsmedelsstrukturer med ljusmikroskopi. Sartryck ur Livsmedelsteknik 24(7):336.

Jones, K.W.; Mandigo, R.W. 1982. Effects of chopping temperature on the microstructure of meat emulsions. J. Food Sci. 47:1930.

Kempton, A.G.; Polonenko, D.R.; Bissonnette, J. 1982. A statistical evaluation of histological changes caused by nonmeat proteins in wiener batters. Can. Inst. Food Sci. Technol. J. 15(2):85.

Labuza, T.P.; Lewicki, P.P. 1978. Measurement of gel waterbinding capacity by capillary suction potential. J. Food Sci. 43:1264.

Labuza, T.P.; Busk, G.C. 1979. An analysis of the water binding in gels. J. Food Sci. 44:1379.

Lee, C.M.; Carroll, R.J.; Abdollahi, A. 1981. A microscopical study of the structure of meat emulsions and its relationship to thermal stability. J. Food Sci. 46:1789.

Ray, F.K.; Miller, B.G.; Van Sickle, D.C.; Aberle, E.D.; Forrest, J.C.; Judge, M.D. 1979. Identification of fat and protein components in meat emulsions using SEM and light microscopy. Scanning Electron Microscopy III:473.

Ray, F.K.; Miller, B.G.; VanSickle, D.C.; Aberle, E.D.; Forrest, J.C.; Judge, M.D. 1981. Microstructure of liver sausage. J. Food Sci. 46:694.

Schmidt, G.R. 1984. Processing effects on meat product microstructure. Food Microstructure 3:33.

Siegel, D.G.; Tuley, W.B.; Schmidt, G.R. 1979a. Microstructure of isolated soy protein in combination ham. J. Food Sci. 44:1272.

Siegel, D.G.; Church, K.E.; Schmidt, G.R. 1979b. Gel structure of nonmeat proteins as related to their ability to bind meat pieces. J. Food Sci. 44:1276.
Swasdee, R.L.; Terrell, R.N.; Dutson, T.R.; Lewis, R.E. 1982. Ultrastructural changes during chopping and cooking of a frankfurter batter. J. Food Sci. 47:1011.
Theno, D.M.; Schmidt, G.R. 1978. Microstructural comparisons of three commercial frankfurters. J. Food Sci. 43:845.
Trout, G.R.; Schmidt, G.R. 1984a. The effect of phosphate type and concentration, salt level and method of preparation on binding in restructured beef rolls. J. Food Sci. 49:687.
Trout, G.R.; Schmidt, G.R. 1984b. The effect of processing temperature and effective salt concentration on the water binding ability of frankfurt type sausages, made with and without added fat. Proc. Eur. Meeting Meat Res. Workers, Bristol, UK.

Discussion

Joe Regenstein: In the initial uncooked product, did you see if you can predict what will happen after it is cooked?

Glenn Schmidt: That's a good question. No, we did not. I'm not sure that what you would see would tell you a lot about what is going to happen. I would be interested if anyone would chime in on that. I shy away from looking at raw material and trying to predict what's going to happen to it. You can make excellent meat mayonnaise that doesn't make a real good hot dog. Most products are cooked.

Regenstein: You point out that when the protein has not aggregated tightly, then water is bound and fat will stay also. The fat will not leave until the water leaves. Why is that?

Schmidt: I think that fat, being a semi-solid, cannot migrate to the surface whereas water, unless it is in very tiny capillaries, will. So it takes the water moving to the surface of the product before the fat can make it.

Regenstein: Do you have any evidence for it?

Schmidt: We can think of no examples in any of the work that we've done where you lose fat and not water. But we do find that the ionic strength in the aqueous phase is a critical factor in binding both. As you add more fat to the product, if you keep the percent salt constant in the product, you increase the ionic strength in the aqueous phase. This is something Warren Tauber used to talk about a lot and I think he was right. So as we go to lower salt products, if we concurrently go to lower fat products, we are compounding the ionic strength effect. Anyone agree or disagree with that?

Regenstein: What about setting up the experiment so that the aqueous phase is at constant salt with different amounts of fat?

Schmidt: We have done that in the poster that's reported here; if you are at optimal ionic strength and pH, you bind both.

Regenstein: But if you're not, can you show that you lose the water? As you go away from there, you would expect to lose the water first.

Schmidt: That is our belief.

Regenstein: But you don't have data from those experiments then?

Schmidt: No, not here at the moment.

Jim Acton: I'd like to offer a very simple explanation and then you can comment on it relative to the release of water before fat release. First of all, it's in a capillary and irrespective of the relationship of microstructure, it is a continuous phase or flow. The fat has to either coalesce or make its movement and it's in a discontinuous state. That's why you will always find water released. The earlier problems were always with fat release or fat stability, but you always found water in every case that you found fat instability. In some cases, there was a relationship between the amount of water released and fat. If it was a high fat release, you had a very high moisture release at the same time.

Allen Foegeding: We've done some work along that line looking at transitions in water release, fat release and texture development. When a student did a bad chop, we did not get a good emulsion and we would lose fat and water as low as 30° C, when you see fat melting. If we had a good emulsion, you wouldn't see anything until around 50° C. Once you started setting up your texture, that's when you started being able to lose some type of moisture.

Schmidt: What's a good chop?

Foegeding: A good chop is what the student does after he has been working at it for about a month. When they started goofing up at the beginning, they were overloading a small bowl chopper so it wasn't finely comminuted enough. That was the only time I ever saw any fat released at the low temperature.

Schmidt: This is one thing I'm very concerned about in doing research in this area. We happen to have a chopper where we know the knife speed, the bowl speed and the number of counts and all these materials are subjected to the same mechanical energy. If you don't have that, I think it is difficult to do a controlled treatment.

Foegeding: That's true along with vacuum along with everything else.

Schmidt: Well, I don't think you use vacuum on this.

Foegeding: These batches were quite repeatable; but as far as the transitions were concerned, it appeared that you could not set up a structure that would hold the fat, due to the mechanical action. When done properly it could set up a structure.

Schmidt: How would you design an experiment to check mechanical action? A.M. Hermansson reported in 1980 at the European meeting at Colorado Springs that she looked at different pieces of equipment and that you can get a different microstructure based on equipment. If ionic strength and pH are constant across treatment, I question whether you'd see differences in cookout even with different equipment.

Foegeding: I think that would be a good experiment.

Acton: Glenn, this is along the same line. You asked how an experiment can be designed to show mechanical action. We do this all the time to compare the function of one piece of equipment manufactured by manufacturer A versus B versus C. Basically, we evaluate it on a performance basis in the plant. The question is in whether structure is affected or not from a functionality standpoint, whether it damaged a product, particularly in finely chopped systems. The story in industry is "when you get it moving, keep it moving." If you stop it and hold it for any length of time and run it through another pumping machine into the linkers or casings, many times you will have problems, because in essence you have already begun to set up structure. Once you get into batter