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

formation, you try to keep it moving because, if it sits around before it is stuffed into casings or linked, then you will have greater opportunity for breakdown of that product during thermal processing. This is separate from preblending and before the fine-chopping step.

Schmidt: Along that line, I believe there's been research done where they have looked at pumping batter along pipe lines. The further along the pipeline, the more coalescence you get of lipid. The lipid droplets even in the raw material got bigger the further it was pumped.

Acton: Is that temperature-related or is it structure-related also? I'm going back to the matrix structure that hasn't really set yet.

Schmidt: I think it's mainly structure-related because I don't think there was much temperature differential. It was a three or four-inch pipeline and they were pumping a long distance.

Benedict: One of your early slides showed staining for collagen, probably Weikert's stain. Have you done any other stains similar to that, which would show any of the other proteins or something for the fat?

Schmidt: That was a slide from a paper by A.M. Hermansson. We haven't been successful in getting any level of staining like they have in their lab in Gothenberg.

Benedict: This would seem to indicate if there was any association between a particular protein of the meat and any portion of the fat like phospholipids.

Schmidt: An area ready for additional research is protein-specific staining and localization by light microscopy. Cassens and group have done a lot of work in that area and also this group in Sweden. We are simply not that good at that. It takes people who are very good in histochemistry to do that; perhaps there is someone here who could enlighten us more than I could. It is a viable area for research. If you are going to work on tissue that's been cooked and heat-denatured for any time, your histochemical stains don't work the way they did back before it was heat-denatured. That's the problem.

Jim Guenther: How do you measure the particulate size where the protein was concerned? There has been a lot of work done on trying to numerically analyze fat droplet size. I'm not sure one can do it. There are all types of particle-size analyzers available now that you could integrate into computers and readout. I'm still not sure that it tells you a true answer.

Schmidt: I agree it's a very nice academic exercise like mental gymnastics and if you have a few weeks to wait, but . . .

Guenther: You don't get a lot of answers.

Schmidt: No, and everyone says to pick a typical micrograph; well, typical is the best one you've got. It would be very difficult to view these pictures the way they show up. I think transmission electron microscopy is our best evidence for protein aggregation where large aggregates and big capillaries are dominant at low salt and low pH. When you have high salt and adequate pH, you form a very fine three-dimensional matrix. It's very clear in the transmission electron micrographs and when you look at samples that went through the other procedure and you get supporting evidence, then I almost believe it. I'm not sure going through and taking a ruler on the photograph and measuring them and averaging 5000 from each one makes you a whole lot smarter than looking at several pictures.

Regenstein: Do you have any extensive work on what those capillary structures actually are in a muscle-structure sense? What are we looking at? What's aggregating and what's making capillaries?

Schmidt: I don't know and that's a very good question. Again, if one could do labeling of proteins; have this label hold during the cooking process and then be able to see what you've labeled and where it is in the matrix, that would be a very nice experiment.

Acton: Since all of these have to do with finely comminuted systems, why is it that the system is so efficient that you never even show a myofibril unit anywhere in the magnifications? Are the choppers really that good?

Schmidt: The choppers are razor-sharp and we have a very good piece of equipment. We have the Meissner 35 liter bowl chopper we run at 4000 rpm's and we chop to 15° C. We just get a very good chop even with a small load in that chopper. That's a real top-notch little chopper.

Acton: My concern is that I have never seen a particle here and there and I just can't believe that every one of them are destroyed.

Schmidt: You certainly would see them in light micrographs if they were there. This material has been aged three weeks in the chub so it's pretty liable to fragmentation index. In fact, if you open that chub in three weeks, you get a pretty good load of volatiles. But when the pH is adjusted to the right level, that material is as functional as fresh beef. But I suspect that it might be more liable to getting knocked apart by the chopper.

Regenstein: Why are you using such old meat? What would happen if you went to something like a kosher-type hot dog with a fairly coarse chop. Beermann did some work where they looked at kosher versus an Oscar Mayer and you get the extreme difference and you can really still see the myofibrils.

Schmidt: As long as you have the appropriate ionic strength and pH, I think the results would be very similar. This was simply an example we took for this paper from work that is being reported by Andrew Clarke in the grad students' poster session. We thought that we'd lose functionality upon storage of the meat. But as long as we adjust the pH and the ionic strength back to a certain level, this aged meat is as functional as fresh meat – which was a total surprise to us. But if you have a lot of lactic acid bacteria growing on aged meat and you don't adjust the pH up with phosphates, you really lose functionality and that's what this is showing. If you don't have lactic acid bacteria growing, but you have some other types that don't drop the pH, it will remain functional.

Regenstein: You are adjusting the pH with what?

Schmidt: Sodium tripolyphosphate.

Regenstein: How do you separate what is affected, by just adjusting the pH or the phosphate?

Schmidt: Then you have to do another experiment which again gets difficult. Graham Trout had a technique where he could adjust the pH with hydrochloric acid or sodium hydroxide, add the phosphate and come out with a given ionic strength and pH. That was on a coarse ground beef roll product and one would have to repeat that on a finer chopped item. We're using some of the thinking from the project to extrapolate here to give the reason why we see what we see.

I think we are right. You can do the other experiment, but I think you'll find it's right.

Guenther: I wonder what the optimal pH and ionic strength is?

Schmidt: It sure looks like about 0.4 ionic strength pH 6.0. If you drop the pH in that batter down to about 5.6, you have problems.

Regenstein: Is that species-specific in any way?

Schmidt: It doesn't appear to be.

Regenstein: You are just working mainly with beef?

Schmidt: Yes, we work mainly with beef in Colorado.

Dale Rice: On those emulsion pictures, how would you say other ingredients fit into that structure, such as milk protein, soy protein and other things of that sort?

Schmidt: When you start including nonmeat ingredients, there are other interactions that take place. With milk proteins and soy proteins, you can do a prechop and indeed you do emulsify fat. If you don't prechop, and you have milk and soy proteins, they do indeed bind water within the capillaries within the particle of the nonmeat ingredients. Somehow they have a high water-binding capacity which makes it necessary for the meat to bind less water itself. There is a positive influence of these ingredients on water binding and fat emulsifying, depending on the level used and how they are applied.

Rice: So in the photographs, would you picture a particle of soy protein in there and maybe hydrated in the middle of that capillary matrix?

Schmidt: Hermansson actually has some pictures of that in some of her papers.

Rice: And the same with milk proteins and things of that sort?

Schmidt: Yes, as long as there is a structure for it to bind water. Bucheim of Germany is an expert on microstructure and functionality of those proteins.

Darrel Cornish: What effect does vacuum have on the emulsion microstructure?

Schmidt: Vacuum takes the air out of the batter. It makes a nice looking product, but I'm not sure a vacuum does a lot more than take air out of material. It gives nice color stability, helps the shelf life, makes a more dense product and more uniform density for linking, but I'm not sure it does much more.

Rice: If that's the case, how come vacuuming seems to work with tumbling when we are actually extracting protein? Is there a different mechanism that tumbling works under?

Schmidt: If you tumble large chunks and don't have a vacuum, you stir up the proteins and create a foam. Foam is a lot worse glue than a continuous liquid medium.

Marcos: In the sausages that you prepared, the formulation is 20% fat, 63% water and 12% protein. Do you have a new proposition to increase water in those kind of sausages? According to regulations, we shouldn't put more than 10% water in the final product and you have 15%.

Schmidt: Yes, we are aware of that. Our final composition wasn't too different than the American Meat Institute petition for the low-fat frankfurter. As long as you have 12% protein in the product, you can drop the fat to the level you want. So that is the hot dog of the future. But again, it's just a model system. There is a desire in this country to make a frankfurter or bologna with somewhat elevated protein and to allow the calories to be reduced by reducing the fat and using a higher level of water. That's what this does.

Acton: I have a question that relates to definition or terminology. On one set of literature you use the term "fine structure." I also see the terms "macroscopic, microscopic and ultrastructure." Is ultrastructure and microstructure one and the same? How are we to read the literature, look at magnifications and resolution capability?

Schmidt: In my vocabulary, microstructure and ultrastructure are the same.

Bob Miles: In the light micrograph, I have a question about spots where you didn't see any fat. Is it possible that it was just air? I didn't see any air vacuoles or anything.

Schmidt: When you put that stain on and rinse it off, there is a very good chance that you lose lipids. Also we find that while we have very low water-binding capacity and you section the tissue on a freeze microtome, that tissue is very brittle. I think you lose some lipid just in sectioning. In this material, I don't think we have many air vacuoles at all. Wherever there is an open space, I think it is lost material.

Acton: Would you comment on making your microstructure analyses on raw material as a predictive effort for what you would see in the cooked product which you showed here?

Schmidt: I have a very difficult time extrapolating from raw materials to cooked materials. If you're making meat mayonnaise, that's okay, but if you want to make a frankfurter and cook it, then I think looking at the cooked material is of greatest importance. I suspect that with raw material you could have a batter that is very high in gelatin and some other things that would look very nice on your microscope. When you heat it, it's all going to change. Obviously, in this case, when we put the material in a can it all looked very similar. But when it was heated, some of the material lost half of its weight as exudate and some lost 10% of its weight. So obviously, looking at the raw material should be coupled with looking at the cooked material.

John Carpenter: How did you decide those round objects were bacteria?

Schmidt: They seemed to fit the pictures that we see in books. We've seen them now in a number of studies. In this study, where you have finely chopped material, they are probably dispersed. In other studies where we've had a different kind of material, we see them in clusters, which really made us think strongly that they were bacteria.

Carpenter: Wouldn't it be possible to stain them and positively identify them whether they were or not? I wouldn't exactly trust what someone else has published in a book either because they might have thought they looked like bacteria too.

Schmidt: In the scanning electron micrograph, you can't really stain them. I would say the best would be to grow a culture; put them on some tissue or on a plate; go through the scanning electron microscope procedure and compare a culture.

Carpenter: At that magnification, microorganisms have distinctive characteristics that distinguish themselves among each other as well and they ought to show up somewhat differently than just round or oval objects.

Schmidt: I'm happy with the criticism, but I don't know what else they'd be.

Jim Price: I would agree with the observation that John makes about microorganisms. I don't think there is very conclusive evidence that those are microorganisms. The other question is in your work where you described the last series of slides on light microscopy and wet cover slip. It seems to me that looking at those pictures, the last few, light microscopy, that maybe it's just my bias, or my experience, I could see just as much with those as I could with some of the more sophisticated techniques. Do you have any comment on that?

Schmidt: At that magnification, I think you see the large aggregates of protein and the clustering of the lipid. But you don't really see the matrix structure to the aggregate of the protein. With the scanning and transmission electron microscopy, I think you get some idea of how the proteins are aggregating or not aggregating in that space between the large lipid droplets. I think freeze fracture may be taking you a step further and give you maybe a better indication of the exact protein arrangement within those aggregates. We haven't done that here.

Price: Just one more question. It may or may not be related to that. I am interested in trying to differentiate in either one of those techniques – collagen and muscle protein. Can you do that with that technique?

Schmidt: I would like to ask for some help on this, but I think labeling with isotopes may be a technique to find various proteins once they've entered the structure. I would ask Dr. Cassens if he has any comments along this line.

Bob Cassens: In regard to the comment, you can differentially stain proteins. I've never been terribly happy with the results because I think you have to know what you are looking for. The cues are not at all distinct. You could go to the trouble of labeling with an isotope or with an antibody, a fluorescent antibody or whatever, but then you are into the situation of isolating proteins, putting them back together and then making a sausage from that. So it's a ways from reality. Have you noticed tiny fat particles on top of the section, as opposed to actually imbedded in the section. This is one method we use for making some judgement of the extent of artifact, possible artifact, or the extent of damage during sectioning. By focusing carefully, you can see whether the fat is sitting on top of the section or if it's actually imbedded in the section.

Schmidt: We haven't noticed that. One thing we did notice is that the tissue that has a high water-binding and fat-binding capacity is a lot easier to section than tissue that has low water-binding capacity. If you section it, and it shatters, then you don't get a section.

Kevin Jones: On the transmission slides, I noticed that there was a lot of streaking on the fat globules like knife marks. Is that right?

Schmidt: That is right, they were knife marks.

Jones: I guess my speculation would be that with knife marks like that, you are introducing a fair amount of artifact, perhaps sufficient to destroy such things as a membrane surrounding a lipid droplet.

Schmidt: I doubt that. We've looked at those at different mags and you see the knife marks and they run completely across the picture. I don't think you lose that membrane. But we just haven't seen that, especially a lipid droplet of that kind and size.

Jones: That was my next question. What was the size on that – one micron on your bar?

Schmidt: Yes. Those lipid droplets that are 100 microns in diameter would reach completely across the screen at that magnification.

Jones: Would you speculate that they are coalesced fat?

Schmidt: The big droplets that you see at the low magnification are coalesced fat. In a lot of products, that's the way fat is. It's just coalesced fat, a large deposit – 100-200 microns across.

Jones: Where on your freeze fracturing are you running into problems in resolution in technique? What are the secrets there?

Schmidt: The way we actually did it is a little different from the initial diagram. We used a device called a slammer device and you mount the tissue on a specimen holder and everything happens kind of fast, but you have a super-cooled metal bar that comes up and the sample slams on this cold metal bar. It freezes the surface and then it all drops in the liquid nitrogen. That surface is very rapidly frozen. You put that in the Balzer apparatus and cleave a new surface, then coat it and make the replica. If that is all done properly, one should then be able to take that replica and get all the tissue off the surface, which we were not that good at. Continuing to go up in magnification, you should be able to get to a very fine resolution to look at individual globular proteins within that fine matrix. I don't want to say what the ultimate resolution is. There has been some very nice work done on actin gels where you see the individual G-actins where you're up to a 100,000X magnification.

Jones: I did have another question that is related to that area. I've seen a lot of freeze-fractures of milk and of these where you have beautiful surfaces to work with. But when you get into these deep crevices, it seems like it would be easy to either destroy your replica or break it or not get all the tissue out. I was wondering if you had some techniques or what can be done to get a clean replica?

Schmidt: I suspect several laboratories now are taking pure protein gels and deep-etching them and looking at how the proteins are arranged to make that gel. There's room for some good work there. Freeze-fracture technique is not real cheap. The apparatus costs a lot and one run of that apparatus costs us $40 or $50.

Acton: Would you comment about the effects of pH, ionic strength, etc., on structure, the mechanism of that structure formation?

Schmidt: These results were actually a bit of a surprise to us because we were thinking that enzymatic action would cleave the protein and you would lose functionality. However, at the same time, Graham Trout has done a series of experiments where he explains that a lot of water-binding capacity and the resulting fat-binding capacity is dependent on a certain ionic strength and a pH of about 6. These experiments have fairly well convinced me that, even if the tissue is to the point that it is well-aged and has been subjected to a lot of bacterial growth, if the pH is adjusted back to 6 and you have the appropriate ionic strength, you get as much functionality as you had with fresh material. So, for sausage processors, this becomes critical that if you are using aged trimmings, providing you adjust the pH and ionic

strength properly, you can get a high-yielding product with a strong binding capacity. If you use aged trimmings with only salt in batter and not phosphate, some aged trimmings, with the right bacterial growth, would show a pH below the critical point. You could get a very low-yielding product. So enzymatic action does not seem to have as much importance as we thought when we started this experiment. The pH of the product is the critical thing.

Acton: I have one additional question that is related to that. Would you compare prerigor and postrigor tissue functionality? Would you speculate on some ultrastructure differences there?

Schmidt: I think it wold be an interesting experiment to take relatively pure myosin and subject it to different levels of enzymatic cleavage; then subject these different levels to different ionic strengths and pH, and look at the structure and water-binding capacity of the resulting gels. That's a lot of work. The Japanese workers have done some of this work and their final paper agreed with us that the strongest gel was that gel made of intact myosin. In a system such as this, even aging it for a few weeks, you still have more than enough intact myosin heavy chains that you don't get into any problems. But this is working in the total meat system and when you go to isolated system, you don't know what you've done to all the components. So I'm just speculating.

Acton: Does that speculation go for prerigor meat versus postrigor tissue?

Schmidt: I think the literature would tell us that, with prerigor meat, you simply have more readily available protein. You can get the protein extracted at a lower ionic strength. What ionic strength of prerigor meat is critical in getting maximum water-binding capacity would be another good experiment. Can you get maximum water-binding capacity with prerigor meat at a lower salt level than you can with postrigor meat? That may be one of the better approaches for producing low-salt, low-fat products.