THE ISOELECTRIC POINT OF MUSCLE PROTEIN AND ITS RELATIONSHIP TO SOLUBILITY AND DISPERSIBILITY

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Thank you very much. I regret that unfortunately Dr. Hamm couldn't be here, because there is a considerable amount of material I'll give is directly as a result of his work with us at Ohio State. When he agreed to come to the conference, we didn't know it was going to be this late in the year. So, he was out on the West Coast, preparing to leave, and return, and so I agreed perhaps I might pinch hit.

I think you are aware, I want to mention this, I spoke to this group a couple of years ago on the problem of water capacity and its measure. Preceding that even, we reported that a good bit of the properties that we associate with the meat, particularly the tenderness and texture, is the protein's ability to hold water under various circumstances. Dr. Kinsinger has touched on the subject, that has been largely neglected in the field of meat, and it is one of the most profound importance, because there have been a few raw meat eaters, but there are not very many. The changes which take place in proteins during heating, during cooking, or other processes, work rather profound changes on the whole system. And, it was an attempt to try to understand some of these things that we got Dr. Hamm to come to our laboratory to work with us on some of these things, because of his particular work which has already been referred to. It might be of interest to you when he came he was aware that we had used certain methods somewhat different from his, and so he thought perhaps I might expect him to use our methods, rather than his own. I was quick to assure him that if he was right, and that we were right, we would both end up the same place anyway, the method didn't have too much to concern ourselves with if they are on a good foundation. And that proved to be the case. The two methods for cooked meat, perhaps, but it does take a larger sample, and takes a little longer time. But, not to be out done, I might say that Dr. Hamm did a pretty fair job adapting his method to work on cooked meat, and very elegant techniques have been developed. I don't think we should take the time this afternoon to go into the details of the method, but rather, I think it would be well to spend time to show you some of the changes that do take place in fact, or isoelectric points, if you will, on properties of meat. These properties that you are familiar with, that is the cooked meat. The stimulus for this work is the fact that with all due respect to our good friends who are preparing food for the armed forces, it so happens dehydrated meat leaves something to be desired. It is pretty hard to get a nice juicy broiled steak out of some dehydrated meat.

The question of investigating a problem like this, the natural thing I suggest is use shotgun methods of try this, try that, try something else, the reason we began this work some time ago. The observation we made seven or eight years ago that we can take an animal and knock it on the

head and put in sodium chloride in the system before it was dressed out, and have come out with the muscle tissue that was extremely tender, and had 100% ability to hold water, simply by shifting the ionic balance that is taking place in muscle tissue, and make a long story short. The ATP already mentioned various potassium magnesium iron moving on and off the protein surface, is the thing that makes muscle react and contract. It is little wonder the various lines interrupting the normal balance can cause profound changes with the protein.

Now, I hope, I don't think very many of you would be real happy if meat protein were very soluble. Unfortunately, they are not. So, in that sense I am sure the meat packers would be very much concerned about the shrinking in the cans, if it was too soluble. So, this really, I am going to at this point digress off the title here. I think Dr. Hamm would agree, and think it would be better to do so. Let's talk about meat, and worry less about its solubility and dispersibility. Let's see what does take place. This may be a perversion in a little sense, but let's put our minds now back to what is isoelectric point, and the best way I can illustrate that, well, I really almost felt like treating the whole gang this afternoon. I noticed when I was here this afternoon, about the middle of the afternoon, we get thirsty and bored. I thought I would buy some candy. I couldn't find any candy to illustrate what I want to do. Then, I thought I might set you up with the good sour lemonade. I am still a college professor, and didn't feel that I could afford it for this crowd. So, imagine you have a sour lemonade before you, and before you drink, if you take a check on the consistency of the saliva in your mouth, you will find it gets its ropy consistency from a protein. You notice the saliva in your mouth at the present time is rather fluid. However, if you take a good drink of sour lemonade, and notice the saliva, you notice the saliva becomes quite ropy, and that reminds me, perhaps, that the chew of tobacco gets the ropiness from the pH in tobacco. That means it is less soluble. Meat is the same way. All the characteristics are profoundly effected by the isoelectric point.

When we approach the problem of trying to study what makes the various effects of temperature on meat, the effect of freezing per se on meat, and the effect of dehydration per se on meat, they were all approached by studying the isoelectric point in this sense, the buffer capacity, the shift of pH.

Let's begin now. I'll run through a few slides and you will probably raise more questions than they will answer, but anyway, I hope the time within the next ten minutes are passed you will get some idea of what we are trying to get at. Let's have the first slide. This simply shows you the relationship pH to the ability of the muscle proteins. This is a mixture of proteins, not the muscle is a mixture of proteins. You will see a little later how that affects our results. I might just simply show this as a couple of curves that were obtained, to show the effective pH. We want to show if this was any chain in muscle protein, primarily in the simple process of freezing per se. This is one, the dark curve is the fresh meat and the same meat frozen, and then thawed out, and this is the second pH on the water holding capacity. Notice this isoelectric point, the ability to hold water is the minimum at the isoelectric point. Notice

if we could go down to the saurbraten level, you see the ability of the muscle to hold water. This is raw muscle up to 100%, you can even add water to the muscle where the pH is in the range of 3. Also, if the pH is 7 1/2, you see it will hold better than 100% of its weight, but if the pH is at the isoelectric point, you notice it is 45%.

The next slide will show a similar curve, and this is the thing I want to indicate, sort of plagues you a little bit. This is the same meat, except when heated very modestly here, one that was 20 degrees, and one is about 40 degrees, but the same muscle, and notice the factor that has been produced by that slight amount of heat. Nevertheless, we did get the same isoelectric point even at this low level of heating. Notice that the level of minimum, I mean the minimum hydration here is not becoming less of a heat minimum, but more of a play to it. We will see a little more about that later.

The next slide will show you the effect of dehydration. This is the same meat. This one sample is simply frozen, and fresh dehydrated without storage any length of time. I might say it appears, it has been corrected for any freezing. Notice that we did get somewhat of a shift in the hydration, quite a marked shift in the alkaline range, and quite noticeable in the isoelectric point, simply by dehydrating. We had the feeling perhaps this dehydration, if you would compute, I mean the salting out effect that takes place due to osmotic effect that is, you dehydrate and affect it, the osmotic effect would be comparable to heating the meat to high temperature, and we thought perhaps maybe the adverse effect on hydration was simply similar to that of temperature. That did not prove to be the case. We didn't know what was going on here, but again, show the same technique of protein to hold water. I want to point out to you although the fresh meat had peak hydration, held up to 33% water, the meat that shift from 33 to 22, which is 11% shift in ability to hold water. When I reduce that down from zero up, you see we have actually, although it is only 11% increase in hydration, if you look at it from the other way, it is 50% change, if you look at it from zero point. This is one reason why dehydrated meat is so woody. It is quite fibrous and usually tough.

Next slide, please. Now, let's recollect some things from some years past, this work has been done when Dr. Levitsky was with us, showing the effect of temperature of heating on the ability of meat to hold water. I put this in here to show you that we will end up with information having to do with this very critical range, which is the very range that every meat packer and every good restaurant, and every good cook at home tries to manipulate to give the desirable heating quality that you wish. You notice during the heating temperature here, let's not make too much difference what these three curves are, but you notice the protein's ability to hold water is not any uniformly increasing function with temperature or lack of ability to hold water. You notice the range of 50 degrees there are reactions taking place that help them to hold more water rather than hinder it, so we have an S shaped affair. What is going on? We did do some work to try to find out in terms of water holding capacity, in the terms of weight.

The next slide shows how we approached this thing. If any of you use this pH meter, I simply heat a piece of meat, cook a piece of meat and

find under normal circumstances the beef will increase in pH simply by heating it. If the meat does have the normal pH, which is 5.5, notice the pH here, which is the shift in heat. Notice the delta pH, the net increase in pH, four tenths of a unit. That is quite a remarkable shift. On the change of a protein molecule by the simple expediency of heating it 55 degrees Centigrade, this shows the violent reactions that are taking place when you denature protein under these circumstances. Notice if we adjust the pH of meat by suitable buffers, away from this point here, which is closely isoelectric point of the protein, you recall in the earlier slide you got 100% here. We had pH 3, and pH 7. There is no change in pH on heating when you heat these proteins in the range of pH 3, or pH 7.2. There is a slight shift at 6.8, which would be a real good dark cut. Let's express this in a little different manner, and we will see the next slide, please.

This shows a series of meats which have been studied here, by heating a different temperature. Adjusting the pH at different times. That is composite curve here. That reaches the maximum of pH 5.5, that is essentially pH normal meat. That seems rather a coincidence. We don't know exactly why it is these should have a very sharp maximum at this pH. In other words, what this means, call your attention to it again, is the shift of pH on heating. Why should we get the greatest shift on heating on pH 5.5?

Now, let's take another look and do a similar study here of the fact that adjusting the pH of meat that you have through a range of 3 to 8, and heat these samples to determine their water holding capacity at various temperatures. Here is the normal temperature here, 20 degrees, and you will notice down here, the lower pH values, we had a little bit difference even than we have at the 45. What I want to point out here is two things, as the temperature of heating increases, or cooking, whatever you want to call it, increases, notice this peak or this minimum value. We get two things, the minimum drifts over toward the alkaline side. That shows us the isoelectric point is changing over toward the alkaline side. In other words, there are several explanations of this, and I won't have the anticlimax and try to show you what it is. But, there is another thing that this thing shows, that the raw meat, the composite of the isoelectric point, all the proteins in meat are pretty well centered around pH 5. However, as we heat the meat, well, now that this minimum is now broad, notice the difference of 15 degrees here, and how broad this becomes at 60 degrees and reflecting back a little on the S shaped curve I showed you, that came from some years back. Notice going from 60 to 80 doesn't produce much difference from 45 to 60. This is the real change that takes place when you smoke, when you cook meat, make it into sausage, and so forth. Now, you see each individual protein is deserting itself. Each protein behaves differently on changing pH for heating. There are some that do not change pH with isoelectric point. Still others the pH and isoelectric point is reacting remarkably on the heating. This area broadens out. Consequently we really do not get an increase in water holding capacity on cooked meat until we get over here to three, the broad end of things, and that would be pretty sour for our German friends.

Now, let's take a look at the next slide. This is a curve, and again, I am not going to take time to go into methods here. I don't think Dr. Hamm would either. Here is a curve that shows the rate of change of pH

with respect to pH, the rate of change from the acid principle of the protein, with respect to pH. This curve is developed from the study of what we call buffer capacity. Relative terms, but it has to be repeated over a wide pH range. This shows you two pieces of meat which have been used, the only difference is in freezing here, but the change of pH, the acid groups in the protein indicates that we receive them shown here negative, and means the acid groups or buffer capacity ability to withstand the increment of basis decreased, is dropping as we go up, the pH scale.

The next slide. Let me show you before we change, you know the order here, shifting in units. It is a very minimum or change on the alkaline side of the isoelectric point.

The next curve will show it will have to be used in the different change to show comparable things on this alkaline side.

Nex slide, please. Here is the next slide showing the change that takes place, change of acid groups which take place with pH, and alkaline side. Now, we have had to multiply our scale by tenford to get a good reproducable difference. Here two different meats that you can see. After all, muscle is fairly uniform, and they expect it to be so. Well, the inference now is that we have approached this study of the ability to hold water, and study this with respect to isoelectric point, the protein. Now, can we translate these curves of buffer capacity, curves, into actual figures of what might be going on? Our first speaker indicated to you proteins are polymer and occasionally had free carboxyl groups, and free amino groups, free basic group, and unattached. It is in these groups where these are of various kinds attach themselves to protein rather profoundly affecting the protein to hold water. It is impossible to determine the whole number of free ionized carboxyl group, or free ionized groups. This is by methods that I won't go into details of, but I think that we can show you the net result as to what is happening when we heat a piece of meat, and where we get the change in pH that we get.

The next slide will show that. As we heat meat, and you will recall now try to keep in the back of your mind the S shape curve and critical temperature of 50 to 60 degrees centimeters. You notice as we heat meat the number of acid groups or basic groups for ten grams of protein, this is composite of protein and muscle. You get a change in acid group if you go to 20, to 30, to 40, but notice the rapid drop in free carboxyl groups as we heat the protein through this very critical range of 40 to 60 degrees. Quite a precipitous drop, and actually, it is practically complete. There will be little change beyond this point. Notice, however, the basic groups show a little shift, but not any consequence. It is total number of basic groups.

Now, the fact there are more acid groups than basic groups tells us why the isoelectric point was on the acid side of the problem. The fact that the carboxic (?) groups decrease tells us why, or explains in part, and a good part, why we cook meat, we have the protein, or the isoelectric point shifts toward the alkaline side, and total pH of the meat shifts toward the alkaline side. If you are interested in meat shrinkage, I am not selling the most water with your product, you want to get close to the alkaline and

still palatable as possible. That also holds for tender meat too. Get the product so it will still hold water, the product will be satisfactory and tender. I think probably I have taken more time than I should have. I thought you might want to see a brief glimpse of what Dr. Hamm has been doing. I think he made a real contribution to our knowledge of beef. Thank you very much.

(Applause)

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MR. PEARSON: Thank you, Dr. Deatherage. I appreciate you bringing us up to that recent work that you have been doing in Ohio with Dr. Hamm.

The next paper is entitled Changes Occurring During Rigor Mortis and Subsequent Ripening of Muscle Tissues, given by E. J. Briskey, University of Wisconsin. Ernie.

MR. E. J. BRISKEY: Mr. Chairman, Members of the Conference, and Guests:

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