Protein-Water Interactions in Muscle Foods

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Part One – Water Retention

Water is the major component in most foods, including muscle foods. Our research interest in water has concentrated on two areas: 1) drip, and the role that salts and pH have on water loss and 2) the interactions of water, protein and fat in comminuted meat products.

Though research has been conducted mainly on fish and poultry, the methods and concepts being proposed may also be applicable to red meats. (The author hopes that a stronger effort will be made to bring together the often overlapping research in the various flesh foods.)

Drip is unsightly in flesh foods. As meat, poultry and fish are prepackaged in central facilities and hence must travel further in their final consumer package, the need to avoid visible drip associated with extended storage required for marketing the product increases. Another important reason for concern with water relationships in flesh food is the economic importance of loss of yield or increase of yield with added water. Both more alkaline pH's and added salts, particularly polyphosphates, increase the water retention of muscle foods.

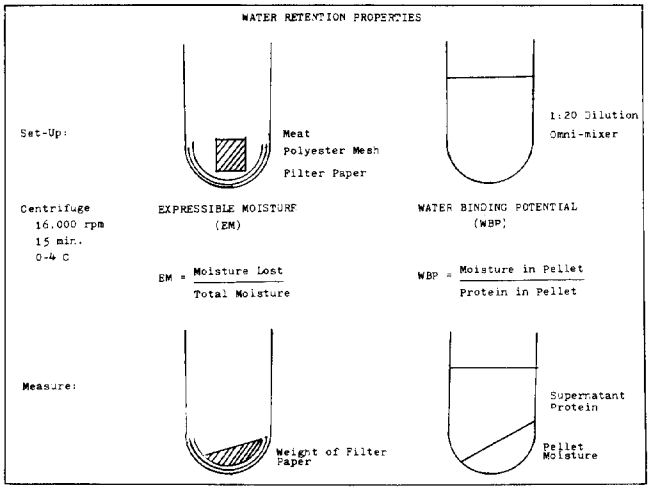
Traditionally, the term "water holding capacity" has been used to describe this functional property. However, a number of different methods are used to measure this property. As we hope to show, two of the major types of water holding capacity methods respond to different properties of flesh. Within each category of measurements, the experimental conditions of each of these measurements may also influence their usefulness as either predictors of the potential of a material for a particular food product or their use as a probe to better understand what is happening in the system. We therefore chose to propose a new set of terms. We call this whole area "water retention properties" studies; and use two different techniques to measure the water retention properties. They are: water binding potential (WBP) and expressible moisture (EM).

Figure 1 attempts to distinguish between the WBP and the EM methodology. For WBP, we take a sample of meat and add 20 parts of the solution to be tested and blended in an Omni-mixer (Sorvall). (We are operating in an environment where there is a great excess of solution.) The property measured, whatever it is, is therefore a reflection of the potential of the system and is not actually a simulation of what might occur under any realistic product conditions. After centrifugation, we remove the supernatant and measure its protein content as a measure of the amount of protein lost from the pellet. This solubility data is also a useful additional bit of information in its own right. Thus, we measure the amount of water bound (held, retained) by the *insoluble* components of the muscle.

The EM methodology has usually been determined by means of a Carver press. In order to make the EM more comparable with WBP, we developed a centrifuge technique which uses conditions similar to that used for WBP. We add the test solution (up to 10% by weight) to the sample and then put the sample in a nylon mesh (to aid in the removal of the muscle). This is then surrounded by layers of filter paper and placed into the centrifuge tube. We then centrifuge the sample and measure the amount of water expressed into the filter paper. The advantage of using this EM technique is that it is then possible to run both EM and WBP at the same time in the same centrifuge – a real plus when making comparisons.

We have been using the Instron in the compression mode for texture measurements and realized that we could simultaneously obtain an expressible moisture measurement by compressing the sample which was on top of a few layers of preweighed filter paper. We then reweigh the filter paper. The amount of moisture expressed using the Instron (EM-I) is much less than with the centrifuge (EM-C), since less force is used. Expressible moisture, as measured by the Instron, gave the best correlation with a series of human raw fish panel evaluations described below.

Figure 1.



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Reciprocal Meat Conference Proceedings, Volume 37, 1984.

These various water retention property measurements have also been used to study the effect of salts, including polyphosphates, on flesh systems. Differentiating between specific ion effects and ionic strength effects with added polyphosphates is difficult as it is difficult to accurately calculate their ionic strength contribution. Thus, generally monovalent and divalent salts were added instead of polyphosphates in order to see if they might be useful as probes. NaI seems to be a particularly appropriate salt for this purpose. Since it is a monovalent salt, it is easier to do comparisons to NaCl.

A few highlights will be described in this review. More details are found in the references at the end of the paper. Figure 2 indicates the pH dependence of the WBP and the EM of trout white muscle. Notice that the changes in the pH 5 to 7 region are much smaller for WBP than in the region above pH 7. For EM a different pH profile is obtained. (The overall direction in both cases is logical. As pH increases in this region, the WBP should go up and the EM down.)

When tripolyphosphate and pyrophosphate are added to flesh foods, the pH changes. Our results suggest that the entire change in WBP of trout muscle could be predicted from the pH changes (Figure 3). Thus, the WBP for these two polyphosphates is accounted for solely by the pH shift. For the two higher polyphosphate polymers studied, and for EM, no such simple relationship with pH was found.

Following the polyphosphate studies, we then turned to various salt studies, generally attempting to study the effect of a series of added cations all added as chlorine salts, and a series of different anions, all added as sodium salts. No attempt was made to be comprehensive in the selection of salts. Figure 4 shows the WBP results as a function of ionic strength. As a first approximation, the different cations gave similar results while the anions gave very different results. Thus, we can refer to WBP as a anion-distinguishing technique, i.e., it is more sensitive to a change of anion than of a cation.

The expressible moisture, on the other hand, is a cation-dependent effect, as can be seen by comparing Figure 4 and Figure 5. Notice that EM, in fact, divides the salts into essentially two classes of behaviors, i.e., those whose EM changes with salt and those that don't. Note that Ca and Mg, two salts of great interest in muscle, are in different classes; the EM of Mg changes with salt concentration, while that of Ca doesn't change.

Thus, we have established that the EM and the WBP

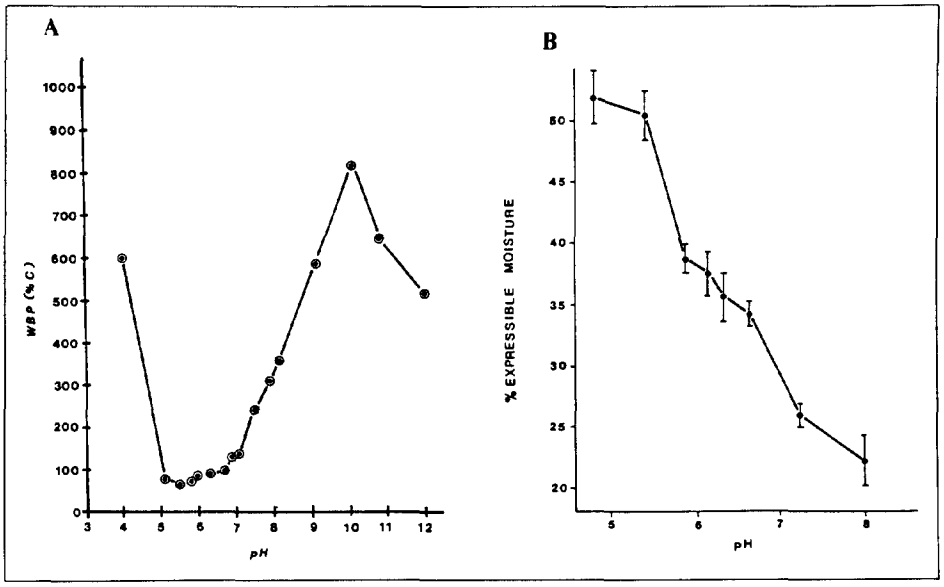

Figure 2.

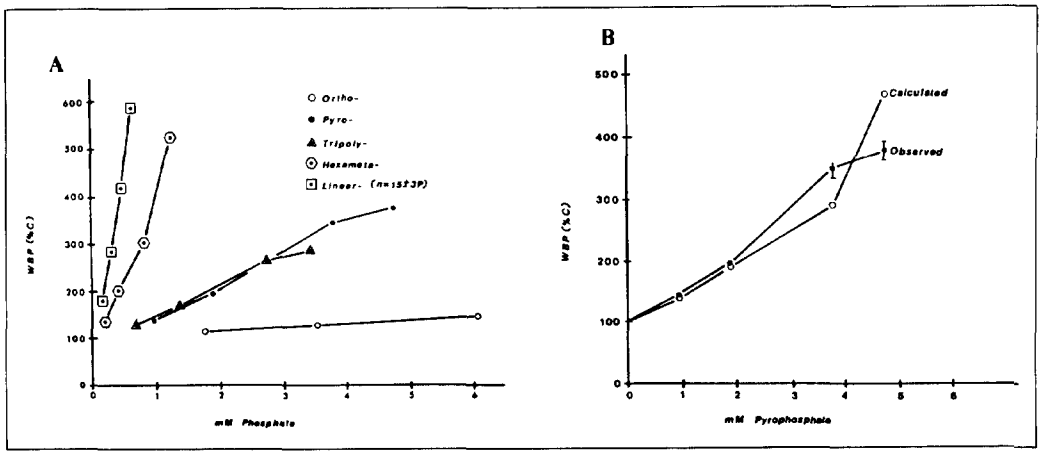

Figure 3.

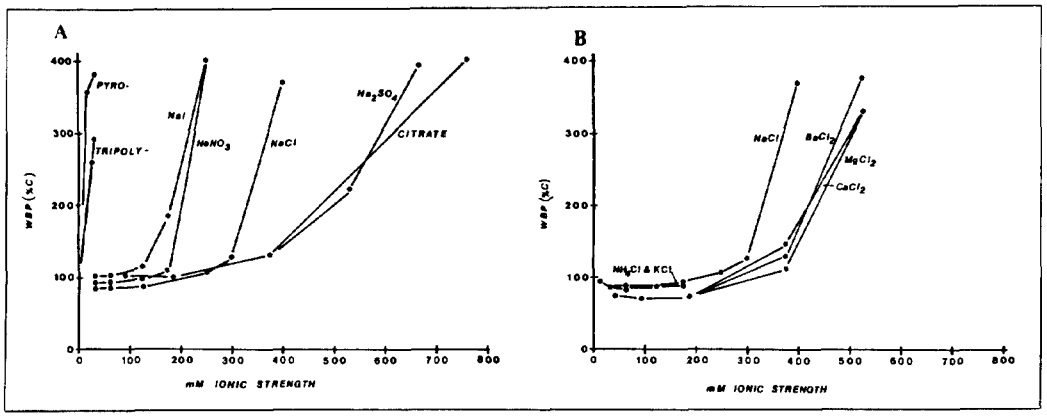

Figure 4.

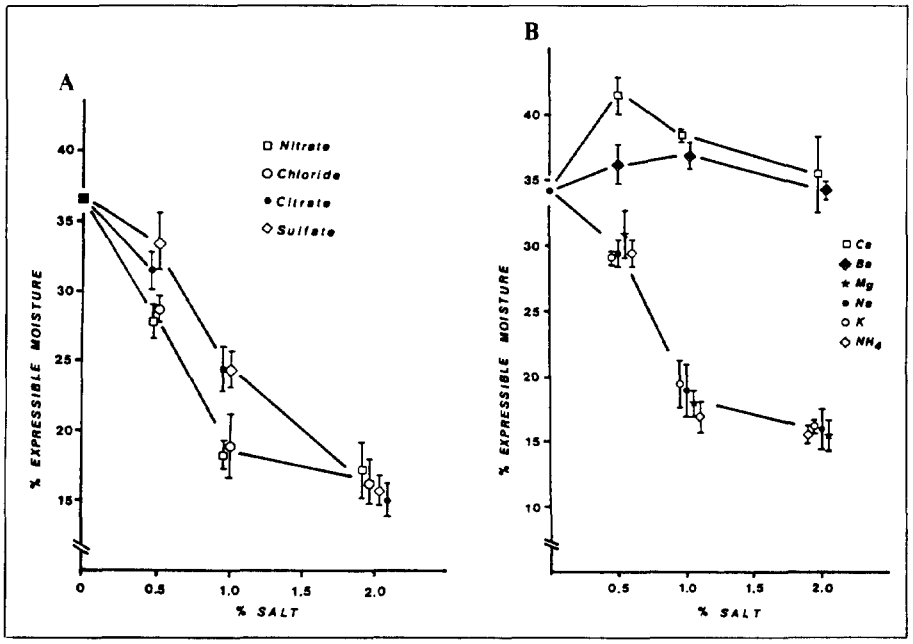

Figure 5.

techniques, at least in one case, measure different water retention properties. Does either type of measurement have any practical value? This question must, unfortunately, be approached on a case-by-case basis, both with respect to the material and to the question being asked. For example, we have one result which clearly indicates the correlation of EM-C to other properties, one for EM-I, and some preliminary data that suggests the correlation of WBP of a NaI solution with another property of interest.

In the first case, a semi-trained panel was used to evaluate the binding (cohesiveness) of cooked codfish loaves. Figure 6 indicates the cooked binding scores, relaxation time, EM of the raw starting material and the WBP for various cations. Figure 7 is the same four graphs for the anions. These results confirm the difference between WBP and EM for very different fish species (i.e., trout, a fatty freshwater fish and cod, a low fat marine fish). These results show that EM of the raw material correlates with the binding of the cooked fish loaves.

In the second case, the change in textured (springiness) of frozen cod muscle was measured on samples stored at two different storage temperatures for both fillet and frame mince. (A frame is the "backbone" of the fish after the fillets are removed. Mince in this case refers to the material obtained from a meat-bone separator with 5 mm holes.) The EM-I results most closely parallel the texture score for the raw samples (Figure 8) while EM in the centrifuge was not as sensitive to sample differences; all samples gave almost identical results. WBP results are not shown as they were very different from the texture results.

Finally in comparative studies with various frame minces; for cod (a gadoid that develops a "cottony, spongy" texture during frozen storage which is supposedly unique to this group of species) the WBP of the sample containing added NaI changed rapidly during frozen storage while the WBP changes with NaI were much slower for flounder, and showed almost no change for fresh water perch. However, this work is still too preliminary to show any results or to draw any firm conclusion.

These techniques collectively have, however, begun to give us some new tools and some new insights into the relationship of water with the proteins of meat *in situ*.

Figure 6.

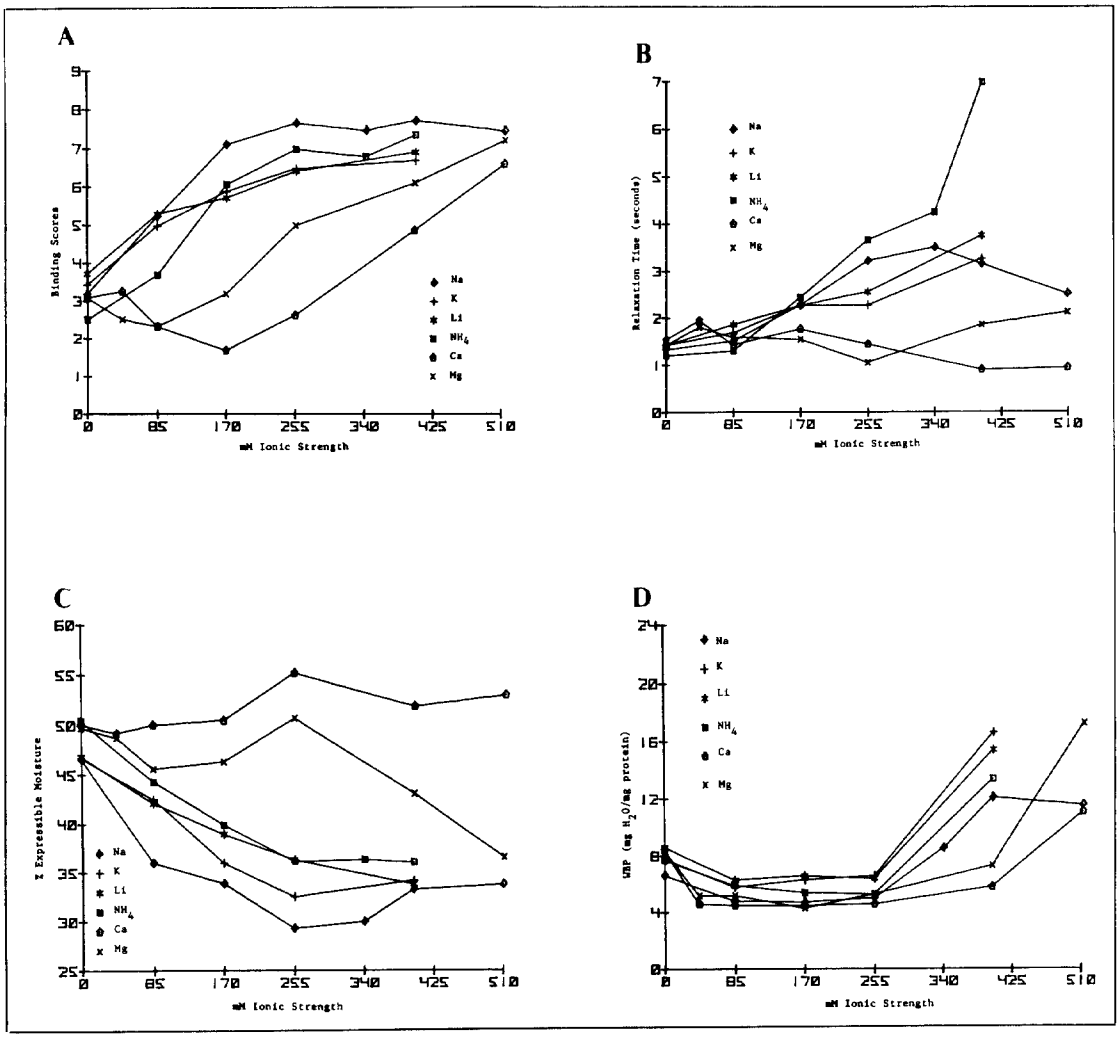

Figure 7.

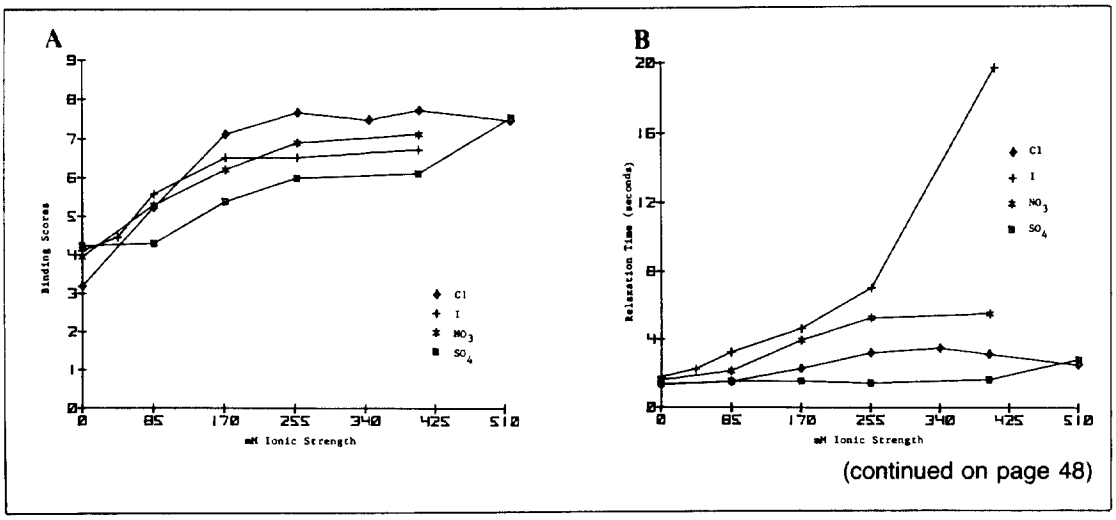

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Figure 7. (continued from page 47)

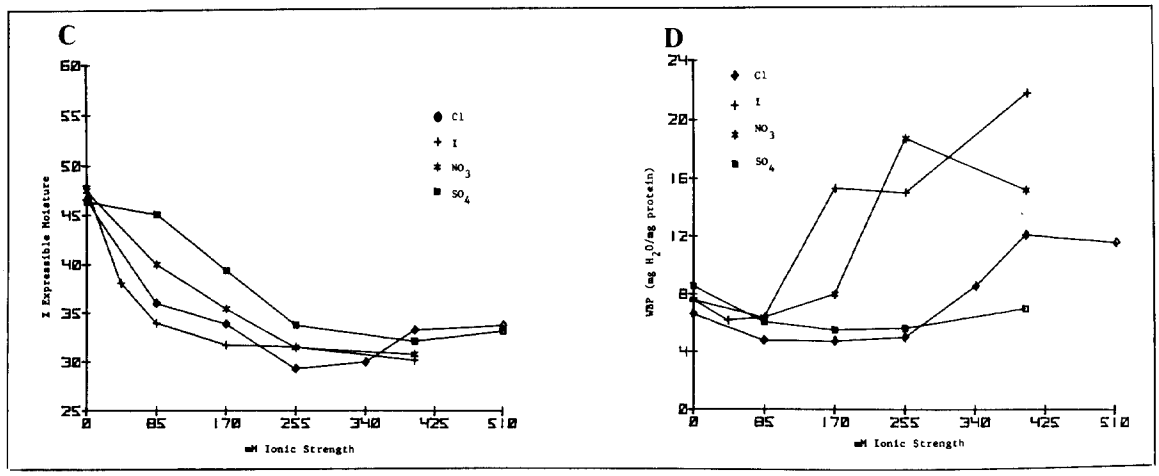
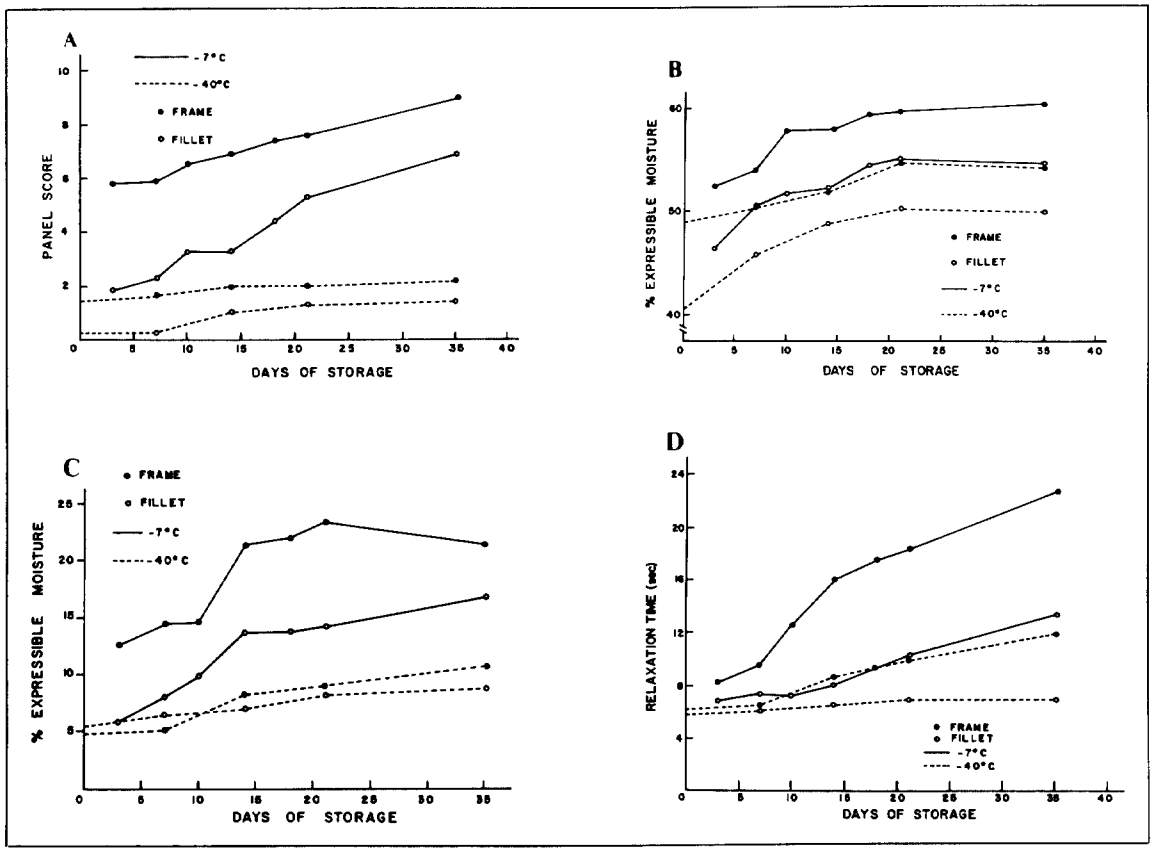


Figure 8.

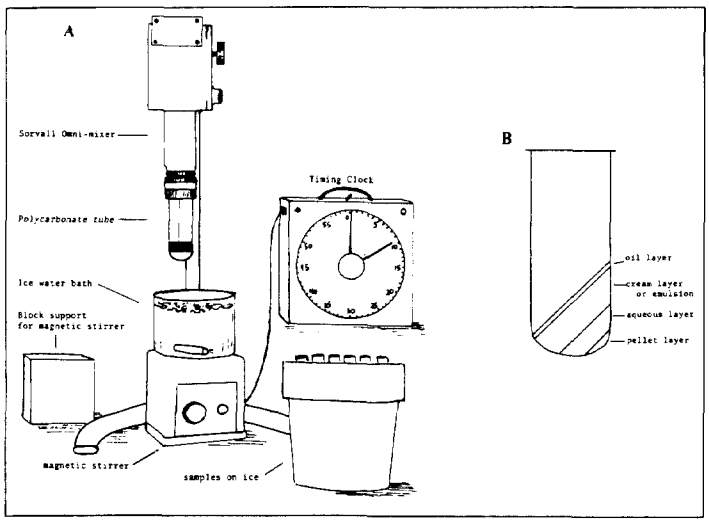


Part Two – Meat Batters

The traditional emulsion capacity measurements seemed to be questionable, both theoretically and in actual practice. Therefore, we felt it might not be appropriate to obtain results at an extreme endpoint (i.e., emulsion failure), where slight changes in viscosity, energy transfer etc., may be more important than the inherent properties of the meat. Also, using this technique the individual muscle proteins did not show any differences, despite the large differences in the proteins' size and shape (Tsai et al., 1972).

We thus undertook the development of a new technique, timed emulsification. We begin by mixing 6 ml of oil with 3 ml of an aqueous protein solution/suspension. This mixture is then blended in an Omni-mixer for a series of different time periods (between 15 sec and 5 min). After centrifugation of each differently timed sample, a cream layer (and/or an oil layer) and an aqueous phase can be observed (Figure 9).

Figure 9.



Sometimes due to insoluble materials, a pellet phase is also observed. The disappearance of protein from the aqueous phase can be measured, for example, by the Lowry method. (It is easier to measure aqueous disappearance of protein than the increase in the protein in the cream/fat phase.) Figure 10 shows the difference between the timed emulsification (aqueous protein lost) of actin and myosin. Myosin is a good emulsifier (high disappearance), while actin is not. The quality of the resulting cream layers also supports this concept.

The remaining aqueous phase can also be analyzed by SDS gel electrophoresis and the relative disappearance of specific proteins from this phase can be detected. In natural actomyosin, actin is removed along with the myosin under conditions where these two proteins are associated as an actomyosin complex, while in the presence of ATP or pyrophosphate, conditions that dissociate actomyosin, the actin is left behind in the aqueous phase (Figure 11). Thus, with timed emulsification, the participation or lack of participation in emulsion formation of different proteins in the complex mixture can be analyzed. For example, the differences between actin and myosin can be shown.

We have also used timed emulsification to examine the role of protein solubility. Solubility is an important water-protein property. This property is usually considered in the meat emulsion literature to be an important prerequisite for protein interactions at the oil/water interface. To study the role of solubility in meat emulsions, we chose to investigate the timed emulsification of exhaustively washed muscle. Exhaustively washed muscle is prepared by washing muscle twice with 0.05 M NaCl and then four times with 0.6 M NaCl using an Omni-mixer at a higher speed setting than was used for the timed emulsification treatments. The final material, now presumably devoid of soluble protein, should be a poor emulsifier. Experimentally, the material (a suspension) disappeared rapidly from both the aqueous and pellet phases into the cream layer. These results can be more easily explained if we assume that a protein-water matrix was formed rather than a classical emulsion.

Figure 10.

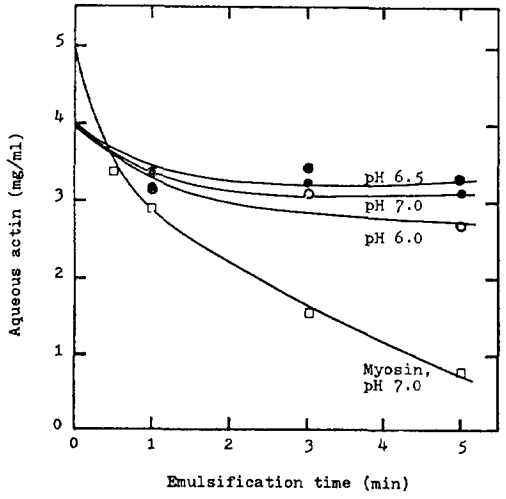
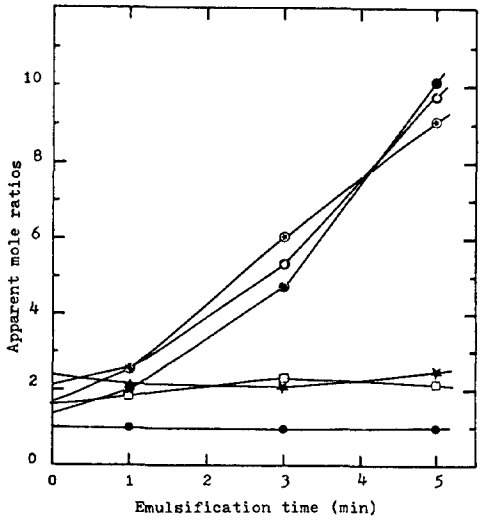


Figure 11.



The original timed emulsification experiments were done at cold temperatures, (i.e., the samples were kept in an ice-water bath or in an ice bucket at all times.) It is also important to investigate the heat stability of this system. For this purpose we needed to develop an appropriate stability measurement. The weight loss of the cream layer when placed between filter paper and stored for up to 4 days appeared to be an experimentally appropriate measurement. We could then show that the cream layer was relatively heat stable (up to 75°C), when heated after blending in the Omni-mixer or after formation of the cream layer. On the other hand, if the sample was heated before mixing (i.e., muscle in aqueous suspension), the stability at higher temperatures was not as good (Figure 12). This suggests that the matrix might be heat set in place, but if the proteins are heated before blending, the proteins can no longer form the matrix. From these types of experiments we infer that the properties at the fat/protein-water interface may be less important in meats then previously thought. (Would it be appropriate to say that comminuted products are simply a meat loaf with trapped oil?) A concern we have not yet addressed is how gelation fits into all of this.

The interaction of water and protein is extremely important to meat systems. More information is clearly needed. Further progress in this area of research will require improvements in both methodology and understanding.

As a final thought for those of you who are more practically concerned with meats: Is it possible for some of the techniques discussed in this paper to be used for developing a better data base for use in least-cost formulations of comminuted meat products?

Figure 12.

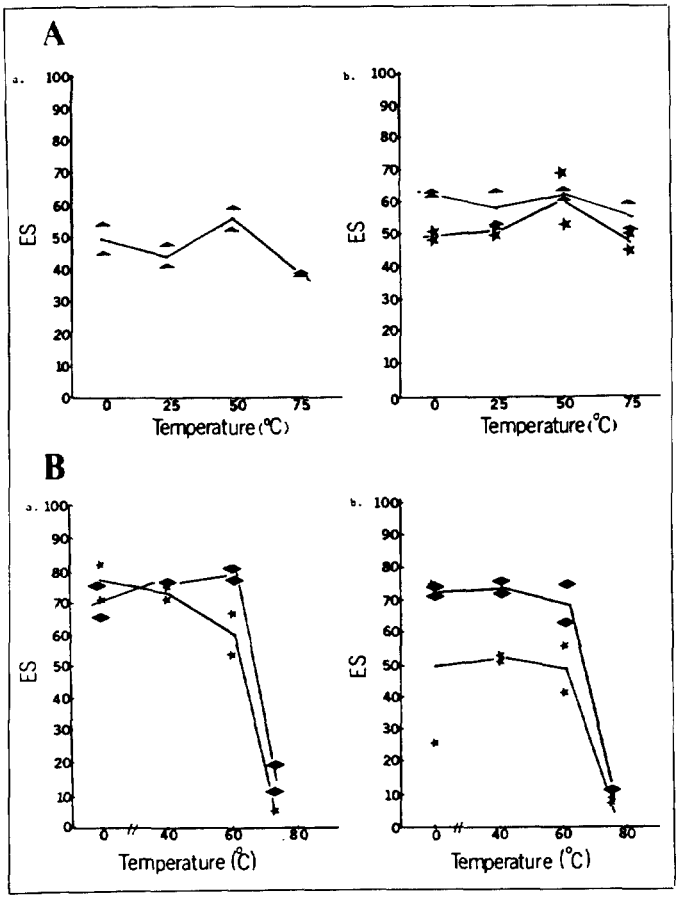


Figure 13.

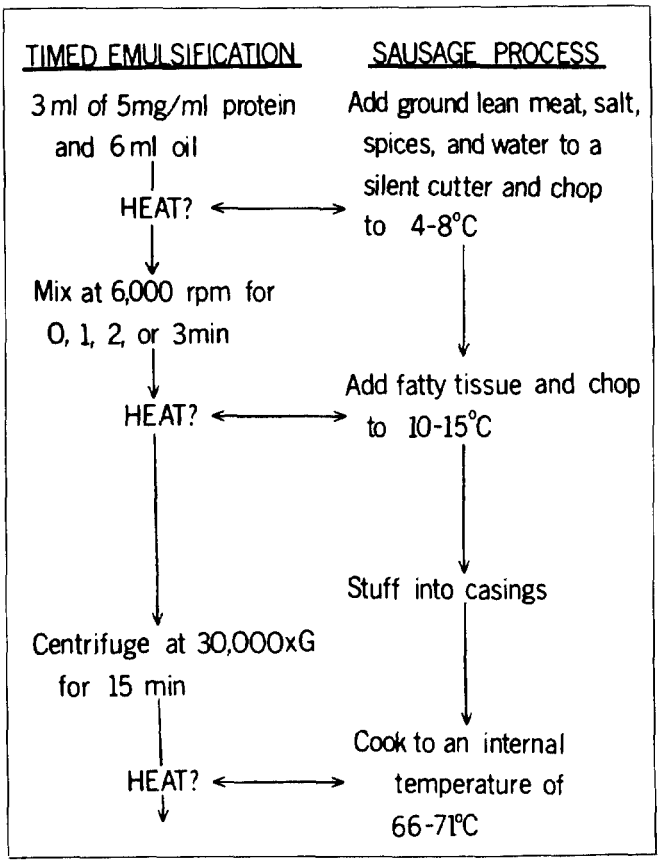


Figure 13. Comparison of timed emulsification and the sausage making process.

Acknowledgement

I would like to thank all of the technicians and graduate students who have participated in this research for their help. They are listed in the papers in the reference section. I would also like to thank my colleagues for their help and discussions. Besides those listed in these papers, I would specifically like to thank Dr. Wilding at Unilever.

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Discussion

Trout: There is one comment I do have, Joe. You are talking about developing methodology for determining functional properties of different types of meats. Why isn't it better to use a miniature version of the product you are making, rather than using a method which is pretty well removed from a meat or fish product and then correlating the results to what happens in a real product? Why not use a small-scale version of the product you are interested in?

Regenstein: I guess that's one way to approach it and certainly in the short term it is a fair approach. Part of the reason for our approach is that the basic thrust of our work is to try and understand what is going on in these systems. We were really interested in the mechanism. We found, for example, that working with myosin and actin, which is the obvious way to start from a biochemist's or biophysicist's point of view, wasn't getting us anywhere. In this binding study on the cod fish loaves, we were using a cooked product, which has gone through formulation, etc. We thought if we could take the raw materials and get, hopefully, a universal number such as emulsion capacity, then it can be used in computer formulation. We could just as easily have made hot dogs and come up with a hot dog value! People are looking for a number that characterizes an ingredient, which they can use for computer formulation rather than by analysing samples. The question of which method you use, I think, depends on the success of the results. In this case, at least, if you are making cod fish meatloaf you can use a simple raw analysis.

Trout: The point I'm getting at is that you seem to be a little too far removed from the actual product and your correlation with product performance is weak. Therefore, you can't really predict accurately what's going to happen in products using these methods.

Regenstein: It's surprising how well we did, and how far the industry has taken emulsion capacity (which I think is a terrible method) and used it successfully in least-cost formulating. I think that's far more removed from a real hot dog than our timed emulsification which is still far removed from a hot dog. But I think we can get useful numbers.

Trout: It doesn't necessarily mean you should take a bad example of emulsion capacity and work from there.

Cassens: Joe, I think the point that was made in our paper was that we were surprised to find there was little difference in an emulsifying capacity between these very different proteins. As I recall, we cast some suspicion at the time on continued use of emulsifying capacity.

Regenstein: I wasn't going to identify it as your paper but since you have, in all fairness, I think you and other people regarded it as an upsetting result. But the fact is, in least-cost formulations they're still using numbers based on this concept and it does leave one a little uneasy. We do feel, that we don't know how useful these tests will be. It is certainly not our main thrust. I threw it out as a challenge to the industry people to see whether it does have some value to them. We can argue these points and I hope people will reciprocate later.