

Meat Batters: Why It is Not an Emulsion

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The concept of what a meat emulsion is has been changing. Recent studies suggest that the structural elements of muscle (i.e., the highly organized myofibrillar, sarcoplasmic reticulum system, etc.) in meat batters may be more important than the behavior of proteins at the oil/water interface. In this presentation, I would like to review some of the research by our group that has been done to develop methods to study this problem and some of the more interesting results of this work.

Our interest in meat batters was stimulated initially by the work of Tsai et al. (1972). They studied individual muscle proteins using the classical emulsion capacity test; i.e., they measured the amount of oil that had to be added to a fixed quantity of aqueous solution until the emulsion either broke or inverted. Their results suggested that the emulsion capacity was essentially independent of which protein was used. Unfortunately, we found this a difficult idea to accept, given the vastly different structure of the various proteins that were studied – ranging from the water soluble, supposedly non-participatory, globular sarcoplasmic proteins to the uniquely-structured myosin with its coiled-coil tail and two heads. In fact, the hyperbolic function of ml oil emulsified/g protein versus mg protein that they obtained suggested that emulsion capacity measures a constant amount of oil emulsified with an ever-changing denominator of protein content (Galluzzo and Regenstein, 1978a). We showed that the amount of oil emulsified remained pretty constant but that changes in the amount of soluble protein (with and without the addition of ATP to identical samples) would change the apparent emulsion capacity values (Galluzzo and Regenstein, 1978a). On the other hand, if the initial protein concentrations (soluble plus suspended material) were used, the various samples gave the same emulsion capacity. The small differences in the amount of oil emulsified with different proteins and protein concentrations may be due to the effect of viscosity on the efficiency of the equipment, rather than to protein differences. For example, actomyosin is much more viscous than the sarcoplasmic proteins and therefore the EC test might not reach an endpoint until slightly more oil had been added. Other reports in the literature had already suggested that the technique had a number of limitations due to problems with controlling various experimental variables.

Thus, at best, the test is a complicated way to measure solubility!

The emulsion capacity test is used in the meat industry as part of the calculations leading to the bind value, i.e., a factor that supposedly reflects the solubility and the emulsion capacity of various meat components which can then be used in obtaining least-cost formulations for producing comminuted meats. The bind value is based on the emulsion capacity of the soluble protein fraction; no measure of batter/emulsion stability is incorporated into the measurements. The numbers are tabulated by meat cut (with no consideration of batch-to-batch variation). The formulator then specifies the desired total bind value for the final product, and the least-cost software insures that the formulation meets this standard. After some investigation, I learned that, for most meat products, this constraint was rarely invoked by the computer in formulating a product. Apparently constraints such as the regulatory requirements for such formulations controlled the formulation selected. However, at least one company is trying to produce a true "least-cost" product and they have found that the computer-generated formulations lead to a surprisingly large percentage of product failures.

We therefore set out to develop a test system that would allow us to accomplish two objectives. The first was to have a system which would be convenient and easy to run so that it might actually be usable in a plant. The second requirement would be that it would provide us with some insights into the emulsion (batter) process in meat products so that we might test some of the hypotheses that had developed along with the technology. Here we will focus on how timed cream layer formation (formerly called "timed emulsification") and the subsequent cream layer stability measurement (formerly called "emulsion stability") have allowed us to tackle such questions as the importance of protein solubility in meat emulsions and the possible role of muscle structure in comminuted meat products.

Timed cream layer formation (TCLF) uses a fixed oil-to-water (protein solution) ratio (Galluzzo and Regenstein, 1978a). The ratio we selected was 6 ml oil and 3 ml water. The original test conditions were optimized using myosin. The ratio of oil to water is greater than that used in real meat products where the legal requirement is usually a maximum of 30% fat.

The sample is blended for various time periods in an Omnimixer (Dupont Sorvall, Norwalk, CT) at an intermediate blade speed. (The same equipment can be used at higher settings to wash materials in the same solvent prior to testing so that the additional solubilization of material during the actual TCLF experiment is minimized.) Sharp blades are essential for good results. An ice-water bath is used to

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maintain temperature. Some heating still occurs during the extensive blending, initially up to 5 min, now more usually a maximum of 3 min. The Omnimixer uses centrifuge tubes that can then go directly into the centrifuge without requiring a quantitative transfer of viscous emulsions. The sample is then creamed in the centrifuge running at about $30,000 \times G$ (16,000 rpm in the Sorvall RC2B centrifuge with the SS-34 rotor). The various fractions can then be analyzed using a variety of techniques.

The major measurement is the loss of protein (usually by Lowry) from the aqueous layer. If any pellet phase remains, its protein content can also be measured. The actual composition of the proteins remaining in the aqueous (or pellet) phase can be determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Working with both natural actomyosin (i.e., soluble thick and thin filaments) or with glycerinated myofibrils (i.e., the insoluble components of muscle tissue), we could show that the presence of ATP (or a food analog polyphosphate such as pyrophosphate) leads to the selective removal of myosin to the cream layer or the selective retention of actin in the aqueous phase (Galluzzo and Regenstein, 1978b,c). Thus the myosin is the important "functional" component in "actomyosin" fat stabilization. This was the first example of where our newer methodology detected effects that were not obtained from the emulsion capacity test.

By measuring the amount (weight or height) of the cream layer formed, we could determine the relative amount of oil incorporated into the cream layer. Only in a few marginal cases (e.g., with very low protein concentrations) were we **unable** to incorporate all of the oil. Furthermore, the cream layer formed during the centrifugation step has sufficient mechanical strength that it could be removed from the centrifuge tube and used to measure the cream layer stability.

The cream layer stability (Gaska and Regenstein, 1982a,b; Perchonok and Regenstein, 1986a,b) is measured by placing the cream layer in the center and on top of three to four layers of filter paper (in a petri dish) topped with a polyester mesh (to allow easier handling of the cream layer). A similar series of materials is placed on top of the cream layer and the petri dish covered. The entire sample is placed in the cooler (0° to 1°C). Weight loss of the pellet is measured every day for four days. The filter paper is replaced each day. Thus, the stability measurement is made at a little greater than one-atmosphere pressure: the filter paper provides some capillary action and the top layer of the filter paper also provides a small amount of extra pressure.

The timed cream layer formation test has three different stages where heat may be applied (Perchonok and Regenstein, 1986a,b). The first is while the proteins are still in the aqueous solution. The second is when the samples have been blended but not creamed. And the third is when the sample has been creamed. To a first approximation, these three points would mimic the three major steps of the meat sausage/batter preparation procedures. That is, first one chops the meat with ice and water (heating the protein in aqueous solution). Second, one adds the fat and chops to a slightly higher temperature (heating the protein/oil blend after the Omnimixer step but before centrifugation to form a cream layer). And, third, one cooks the batter (heating the cream layer).

One of the issues in dispute by meat scientists is whether the final maximum chopping temperature is determined by the behavior of the fat or the protein. Based on the differences in melting point temperature between the fat thermal transitions (below 20°C) from various meat sources and the maximum chopping temperatures for different meats, e.g., beef versus chicken, liquification of fat during chopping seems to be detrimental to product formation. If the muscle proteins were emulsifying the fat at this stage, as classical theory supposes, liquification at this stage should not be a problem. On the other hand, our results on the protein phase (i.e., very little changes in the timed cream layer formation or the cream layer stability between 0°C and 30°C), suggest that changes in the protein at low temperature are unimportant. However, the formation of an aqueous phase protein matrix (the alternative hypothesis that we are proposing) might not occur rapidly enough during chopping to prevent oil coalescence. These large oil droplets may then be large enough that they interfere with matrix formation and are thus never stabilized.

The only temperature effects that we have seen were with myosin, NAM (natural actomyosin), or exhaustively washed muscle (see below) when the muscle was heated to above 40°C in the aqueous solution before any emulsification. The denaturing temperature in the presence of structurally intact muscle (i.e., exhaustively washed muscle which is presumed to still have myofibrils, including thin and thick filaments) was higher (60° to 75°C) compared to NAM (40° to 60°C), which has approximately the same proteins but in solution. Exhaustively washed muscle is prepared by first washing the muscle tissue two times at low salt (0.15 M NaCl, which is used to remove the "sarcolemmal" proteins) and then four times at high salt (0.6 M, which is used to remove the soluble "myofibrillar" proteins such as NAM). The Omnimixer speed is much higher than that used for the timed cream layer formation studies. Preliminary evidence from SDS-PAGE suggests that not all of the actomyosin is removed from the material. However, that which remains after exhaustive washing is probably still a part of the myofibrillar structure (insoluble) and is very likely in rigor linkage. (Are there two populations of actomyosin, soluble and insoluble?)

Current theories suggest that protein solubility is an important property in emulsion formation. The hypothesis is that proteins migrate to the interface between the aqueous and oil phases and then undergo conformational changes leading to the hydrophobic groups entering the oil phase while the hydrophilic regions remain in the aqueous phase. Using this model, exhaustively washed muscle should not form a good emulsion. The results, however, showed a stable cream layer was formed with insoluble protein, with and without heating up to 75°C , which is higher than the temperature used for meat processing.

The highly varied, essentially "globular" sarcoplasmic proteins, by the classical model, ought to be good emulsifiers if surface denaturation at interfaces were important. Other globular proteins, such as BSA, are believed to be able to unfold easily at these interfaces in order to minimize their free energy. Unfortunately, both our results and the classical meat processing wisdom suggest that these proteins are in fact poor participants in meat batter systems, possibly because they are not able to form a gel structure and/or they

interfere with the gel (matrix) structure of myofibrillar proteins (Gaska and Regenstein, 1982a).

By using lower amounts of protein to determine the minimum amount of protein needed for forming relatively stable cream layers, we showed that the amount of protein needed for stable cream layers of exhaustively washed muscle was only twice that of myosin (Gerwig Huber and Regenstein, 1988). (The cream layer stability at higher protein concentrations was about 10% less for exhaustively washed muscle [70%] than for myosin.)

We also measured droplet sizes. The greatest error in the droplet surface area measurements would be due to any smaller droplets we might miss in the pictures. (These would have a great deal of surface area in comparison to the larger droplets.) Calculations of initial protein concentration divided by droplet surface area showed that we had less than the 2 to 3 mg of protein per mm of interface surface area that is required to give a protein monolayer to emulsified droplets in emulsions (Graham and Phillips, 1979a,b), strongly suggesting that the stable cream layers formed by myosin did not depend on surface droplet coating. Both myosin, an aggregated, coiled-coil alpha helical protein for most of its length, and insoluble exhaustively washed muscle were not likely to be easily denatured at the oil/water interface. (Unfortunately we have not been able to recover any protein participating in the cream layer in order to test whether it is denatured or not.) We thus believe that these experiments force us to look for new explanations of the meat batter process and to take into account the role of structure.

Our current hypothesis is that the meat proteins set up a structure in the aqueous phase, i.e., a gel matrix based on the myofibrillar proteins. This matrix is different from that of gelatin since it is known that gelatin interferes with the formation of good hot dog-like products.

Food additives in processed meats may then function in one or more of three ways: 1) to supplement the formation of the protein matrix in the aqueous phase by directly participating in the myofibrillar matrix, 2) to improve water and fat retention by binding the water or fat and/or 3) to supplement the activity of the aqueous phase muscle proteins by acting as surface active material at the oil/water interface, i.e., to act as classical emulsifiers. The ability of proteins, either from meat or an additive, to aggregate in the appropriate way to form the aqueous matrix may then be more important than their solubility (Toro-Vazquez and Regenstein, 1988).

How do we measure the structural properties of proteins (both meat and non-meat) in meat batters? Two tests may be relevant. The first is the measurement of the textural properties of cooked batters using the General Foods Texture Profile Analysis on the Instron or similar equipment to characterize the actual textural properties of: 1) an experimental system such as our cream layers, 2) a model meat batter product such as that developed by Whiting and Miller (1984) or 3) an actual meat-batter product. Comparisons between the three types of products should permit critical testing of various hypotheses.

The second test may be derived from studies of the water retention properties of muscle/meat systems. Water-holding capacity measurements in the literature often are of two distinct types: either 1) some force is used to remove the "free" water, which we now refer to as an "expressible

moisture" measurement or 2) the ability of a sample's non-soluble component to incorporate additional water, which we now refer to as a "water-binding potential" measurement (Jauregui et al., 1981; Regenstein et al., 1984). An examination of the pH profile and the effect of various salt ions on these two types of measurements has established that, although they both may be called "water-holding capacity," they are very different measurements (Regenstein et al., 1984). The latter is in reality a measure of the swelling capacity of muscle under various conditions. Offer and Trinick (1983) showed that muscle tissue in the presence of salts will show an increase in the thin to thick filament distance due to swelling of the sarcomere, presumably by adsorbing additional moisture as sarcomere length does not change. Of particular interest is the effect of NaI on this system. This salt, at about 0.25 M, will cause a significant swelling of muscle, but only after the resolution of rigor has begun (Weinberg et al., 1984; Lillford et al., 1983). At this NaI concentration, the myofibrillar proteins are not solubilized. Thus, it becomes possible under these conditions to separate swelling effects from solubilization effects. Preliminary results using this salt in the timed cream layer formation system with whole muscle (unwashed) suggest that the behavior of 0.25 M NaI samples is very similar to that of 0.6

Table 1. Major Points Arguing Against Meat Batters Being an Emulsion.

1. In the emulsion capacity (EC) measurements, different muscle proteins function the same way. This is probably an artifact of the method. EC does not measure protein participating at an interface, but rather the amount of soluble protein. Therefore the test does not contribute anything to the argument one way or the other.
2. Exhaustively washed muscle (no soluble protein) can incorporate a great excess of oil. The cream layer formed is both cold and heat stable. It is unlikely that this tissue will denature at the oil/water interface.
3. The concentration dependence of exhaustively washed muscle and of myosin for the cream layer stability measurements follow similar shaped curves, suggesting that the mechanism is not significantly different for myosin. The amount of exhaustively washed muscle needed to reach maximum cream layer stability is only about twice that of myosin.
4. Sarcoplasmic proteins, which should easily denature and would be expected to stabilize an oil/water interface, are poor participants in the timed cream layer formation test.
5. The amount of protein needed to cover the droplets of oil is less for myosin than that needed for other more easily denatured proteins. The methods used to make these measurements would most likely miss smaller droplets – which have a greater area; thus, the error is in the other direction.
6. The amount of protein left behind in the aqueous phase is a constant ratio of the amount of protein used. This protein has also been demonstrated to be active in stabilizing oil. If a monolayer coverage was formed at some stage, the equilibrium should shift and give a different ratio of coverage.

M NaCl samples (where both swelling and protein solubility occur) supporting the concept that swelling (structural changes) may be more important than solubility (Shin and Regenstein, 1988). Allen Foegeding (personal communication) has asked the important question, "Can we make a hot dog with 0.25 M NaI?"

In summary, the use of these newer methods to examine meat batters has led to a reevaluation of the mechanisms involved in meat batter products. The results suggest a major role for the aqueous phase protein matrix compared to the traditional emphasis on reactions at the interface. The tests are simple enough to run that they may also prove practical in determining the functional parameters for use in least-cost formulations on individual lots of meat.

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