Food Microstructure: An Integrative Approach

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The integration of information at all levels of organization in a food system - atomic and molecular through macroscopic properties is illustrated with cake batters as an example of a formulated food and bovine muscle as an example of a biologically intact, non-formulated food. Direct examples are given using macroscopic data collected in heat and water transport studies followed by integration of microscopical data for interpretive purposes.

The contribution of starch gelatinization to the characteristics of water loss in batter is related to events such as loss of brieffinge as seen by polarizing microscopy, differential heat input as seen by scanning calorimetry, batter component morphological changes as seen by freeze etch, viscosity differences as characterized by viscometric data, and volume differences of the final baked product. The contribution of myofibrillar protein denaturation to the characteristics of water loss by drip and evaporation is related to events such as changes in sarcomere banding patterns and length as seen by transmission electron microscopy, differential heat input as seen by scanning calorimetry, and by overall muscle sample length shrink.


Introduction

As a food scientist one is intrigued by the number of related food studies that arrive at seemingly dissimilar conclusions. After considerable examination of reliable but conflicting studies, we have concluded that inconsistencies occur when transformations, interactions, and properties at different levels of organization are compared and interpreted as one and the same.

The total system can be evaluated (1) in terms of the overall properties of the product; (2) at the particulate or component level; and (3) at the molecular and atomic level. Therefore, studies of food microstructure, regardless of the type of system being studied, are not entities unto themselves but are simply portions of information needed in an integration of data leading to a fuller understanding of the total system.

As one moves from one level of organization to the next, integration is difficult when a single term such as starch gelatinization or protein denaturation is used to describe a series of broadly based chemical and physical changes. Such problems and the usefulness of integrating information from all levels of organization can be illustrated with batters as an example of a formulated food and with bovine muscle as an example of biologically intact non-formulated food.

Cake batter and its subsequent final crumb structure are often characterized by the effects of different components on starch "gelatinization". Bovine muscle, as meat for human consumption, is often characterized by the effects of different processes on myofibrillar protein "denaturation".

Even though starch "gelatinization" and protein "denaturation" each has a single descriptive word for the transformation in question, many subjective evaluations and objective measurements can be made on products containing starch granules and muscle myofibrils, and can be interpreted at various levels of organization.

Although the molecular structural units are different, the patterns of organization of the macromolecules into a microstructural component are similar. Both can be described as being composed of two or more macromolecular microstructures of multiple repeating smaller molecular weight units such as glucose and amino acids. For example, starches are formed mainly from amylose
and amylopectin macromolecules of differing molecular weight. Muscle myofibrils are formed mainly from actin and myosin molecules of differing amino acid sequences and molecular weights.

The similarities do not end here. Due to the slow rate of solution of many such biopolymers, it is not easy to employ the conventional techniques of solution chemistry. In fact, because of the multilevel nature of these phenomena, data can be collected at each state of the transitions which are representative of the different levels of organization. It is these types of data that can be integrated into a coherent picture of the phenomena. The macroscopic and microscopic properties of volume, size, shape, specific gravity, viscosity, water loss, water holding capacity, elasticity, compression, and shear values, to name a few, are characteristics of the system as a whole; the more microscopic properties of birefringence, X-ray diffraction, X-ray microanalysis, molecular conformation changes and localized temperature changes and phase transitions are more directly related to the microstructural components and individual molecules of the system.

Because these transformations are heat and water dependent, studies in our overall research program have been designed to examine the overall interrelationships among heating profiles, water transport, and physicalchemical properties of foods. The systems are examined at both the macrostructural and microstructural level. Therefore, it is the purpose of this paper to illustrate with data from two of our research areas, cake batters and intact bovine muscle, the information from the various levels of organization which have a better understanding of events that occur under processing conditions or formulation changes and to use this information in theoretical predictive modelling of the process.

Prior to specific illustrations a brief discussion will follow of the general (1) transformations of cake emulsions to a solid porous media, (2) transformations of myofibrillar proteins with shrinkage upon heating, and (3) role of water in such systems.

The mechanism of cake making can be considered as consisting of three main stages. First, the stage of batter preparation and early part of baking which establishes the macroemulsive characteristics of the system. Second, the intermediate baking stage, where considerable fluid batter flow and chemical transformations occur prior to crumb structure development. Third, the formation of a solid porous crumb structure after component phase changes and interactions have been completed in the early and intermediate stages of batter development. The sequence and specific temperatures of these events are crucial to the quality characteristics of the final product. The general protein changes that the myofilbrils of muscle undergo when heated are best illustrated in Figure 1 from a summary by Lewis (1981). Heating produces a sequence of physical changes in sarcomere banding patterns that are the result of the denaturation of the myofilbrillar proteins in muscle. The ultimate effect is for early myosin denaturation to result in actin thin filaments initially sliding between the myosin filaments and causing some sarcomere shortening, and for later actin denaturation to cause further sarcomere shortening. Sarcomere shortening results in the release by squeezing of large quantities of water as denaturation proceeds (Hung et al., 1978).

The role of water in most food systems, whether intact or formulated, is central to most carbohydrate and protein related chemical transformations. It is of primary importance in heat transfer, as well as participating in chemical transformations such as protein denaturation and starch gelatinization (these two transformations cannot occur without water).

Cake batters

Lean cake formulations (Kissell, 1959) with variations in ingredient ratios and types have been used in many of our studies (Gordon et al., 1979; Hau et al., 1980; Cloke, 1981) as baseline models for more fully-developed batter formulations (see Appendix). The base formula contains water, cake flour, sucrose, shortening, and baking powder (water and sucrose levels result in a 42% sucrose concentration in the batters).

As heat is applied to a batter system, a temperature gradient is established and water is evaporated from the surface simultaneously as reactions take place in the system. If one wishes to study these events dynamically during the heating process, temperature profiles and water loss rate data as a function of time can be collected. We have designed special environmental ovens for this purpose (Godsalve et al., 1977a). The data thus collected are influenced by batter formulation and can be related either directly or indirectly to final cake structure. Typical heat and water transport curves that can be generated in this way for cake batters can be seen in Figure 2.

The following discussion will describe how information from the type of data shown in Figure 2 can be coupled with more microstructurally-related data in order to better understand the role of starch gelatinization in cake structure.

If we divide Figure 2 into parts $T_4$, region $T_0$-$T_1$ is the initial heating up period prior to the surface reaching $100^\circ$C. Between $T_1$ and $T_2$, the slope of the water loss rate curve changes and the centerline temperature of the batter reaches at least $75^\circ$C and may be as high as $95^\circ$C in some cases.

Differential scanning calorimetry (DSC) data of the type found in Figure 3 and Table 1, using model system combinations of the batter components dispersed in 42% sucrose solution, tell us that starch molecules undergo measurable conformational changes in the 74-95°C temperature range, the energy requirement being about 2.6 cal/g starch. Further studies of individual systems such as those that Donovan and others have reported (Donovan, 1977, 1979; Lelièvre, 1974; Eliasson, 1980; Eliasson et al., 1981; Stevens and Elton, 1971; Wada et al., 1979; Wootton and Bamunuarachchi, 1979 a,b; 1980; Larsson, 1980; Kugimiyà et al., 1980, 1981) indicated that this molecular transition is associated with the amylose macromolecular conformational change. Sucrose solution was used in the starch model system since it was the same concentration as that for batters, and has the tendency to delay gelatinization (Bean and Yamazaki, 1978). As one adds different components...
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Figure 1. (a) Diagram showing location of proteins within a single sarcomere of an untreated muscle cell. (b) Diagram, and (c) photograph show maximum changes in structure upon heating.

Figure 2. Typical center position heat curve and overall water transport curve for a 220 g. Kissell cake batter made with hydrogenated vegetable shortening baked at 190°C.

Figure 3. Typical DSC scan for Kissell cake batter. Transition is attributed mainly to starch.

Table 1. Thermal Transitions and Enthalpies of Batter Components Between 40-120°C.

<table>
<thead>
<tr>
<th>System</th>
<th>Transition Temperature Range °C</th>
<th>Enthalpy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat Starch</td>
<td>72-92</td>
<td>2.6 cal/g starch</td>
</tr>
<tr>
<td>Unsaturated Monoglyceride</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Saturated Monoglyceride (SMG)</td>
<td>57-78</td>
<td>58.0 cal/g SMG</td>
</tr>
<tr>
<td>Reheated</td>
<td>63-75</td>
<td>26.0 cal/g SMG</td>
</tr>
<tr>
<td>SMG in H2O</td>
<td>52-69</td>
<td>32.0 cal/g SMG</td>
</tr>
<tr>
<td>Reheated</td>
<td>52-67</td>
<td>18.0 cal/g SMG</td>
</tr>
<tr>
<td>SMG in Oil</td>
<td>58-72</td>
<td>37.0 cal/g SMG</td>
</tr>
<tr>
<td>Reheated</td>
<td>52-62</td>
<td>15.0 cal/g SMG</td>
</tr>
<tr>
<td>SMG + Wheat Starch + Oil</td>
<td>59-72</td>
<td>56.0 cal/g SMG</td>
</tr>
<tr>
<td>Reheated</td>
<td>52-62</td>
<td>15.0 cal/g SMG</td>
</tr>
<tr>
<td>Wheat Starch Batter</td>
<td>74-101</td>
<td>2.6 cal/g starch</td>
</tr>
<tr>
<td>Wheat Flour Batter</td>
<td>74-96</td>
<td>2.5 cal/g starch</td>
</tr>
</tbody>
</table>

such as emulsifiers to the batter system, DSC data have the potential of indicating emulsifier transformations along with the changes that take place in the starch molecule. For example, a saturated monoglyceride (SMG) dispersed singly or in aqueous or oil media has a transformation beginning at about 52°C and requires 15-38 cal/g emulsifier depending on whether it is run alone or in oil or in aqueous media. Since emulsifiers can bind with other components in a system, as well as undergo phase transitions on their own, the potential exists for differences in starch transformation energy requirements to develop. In a study by Cloke (1981), the total energy requirement for the initial starch transformation, which is associated with the loss of birefringence, remained the same upon the addition of SMG. Therefore, wheat starch and wheat flour batters with added SMG continue to show the same energy requirements as the starch.
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transformation shown in Table 1 (2.5-2.6 cal/g starch).

On a microscopic level, representative freeze fracture micrographs of batter samples at various stages of the heating process are shown in Figure 4 and are similar to data presented in an earlier publication (Hsieh, et al., 1981). More specifically, in Figure 4a and 4b one can compare starch granules in 42% sucrose before and after heating to 93°C. Although there are some differences in morphology, only minimal in swelling can be observed. Although size differences inherently exist in wheat starch, Figure 4b shows morphological changes in starch heated to 93°C which is evidenced by ridges about individual starch granules (arrows).

From the data in Figure 2, for the region defined by T2-T3, one sees that water loss rates are constant. Reasoning from temperature data and freeze-etch studies, during this period water being evaporated is not the surface as well as being retained in the batter components. Internal temperatures are between 93-102°C, and the freeze-etch data, as seen in the micrograph Figure 4c, shows that the starch granules in batters sampled at 102°C appear significantly more swollen than those sampled at 93°C. Furthermore, this integrated information helps to interpret apparently conflicting amylograph viscosity data. For example, the temperature at which loss of birefringence occurs more nearly coincides with the temperature of molecular transformations as seen by DSC. However, the maximum amylograph viscosity occurs at the higher temperatures at which maximum granule swelling occurs, as seen by freeze-etch in Figure 4c. It takes place after the molecular conformational changes as seen by DSC are completed. Since this sequence of transformations occurs in batters, we see that at T3 (of Figure 2) when the temperature at the center is about 102°C, water loss must occur from a batter which is no longer fluid. Starch granules have ceased to visibly swell, and water moves to the surface in an increasing rate of water loss through the now developed porous media. Once the boiling point of the batter has been reached until a constant rate of water loss commences, a dehydration front from edge to middle is established at the point of "doneness" at T4.

On an even more macrostructural level, photographs such as those seen in Figure 5 can be taken of the cakes at the end of the baking period. Evaluations can be made which are representative of the system as a whole on such physical properties as cake volume, final cake weight, and cake volume at 4% SMG addition. However, at 7.5% SMG addition, the volume increased but with more open structure and tunneling. The increase in volume has the general trend of decreasing specific gravity. From DSC data, it is deduced that early starch transformations which occur in the second stage of cake baking, are not affected, yet the later changes as observed by freeze-etch involve less granule swelling and fuller matrix development.

SEM data such as that found in Figure 6 by Hsu, et al. (1980) can play a significant role in characterizing component interactions and their role in localized areas of the system; for example, the state or character of fat (whether droplets or pools), matrix development and starch granule swelling and their role in the final crumb structure development. In Figure 6a cake crumb is seen which contains hydrogenated vegetable shortening in the formulation, and the matrix appears developed about swollen starch granules with fat areas (arrows) appearing globular throughout the matrix. However, in Figure 6b, cake crumb is seen which contains vegetable oil in the formulation, and the matrix appears more compact and smooth with large fat pools (arrow) dispersed throughout the matrix. The manner in which fat is distributed in cake crumb can help one understand the stability of the batter as well as other things.

Finally, returning to Figure 2, the dehydration of the cake continues past the "doneness" range at T4 and is of interest in terms of defining the release of water from the developed structure and was not evaluated in this study. The discussion of the heating profiles and water loss curves to this point has been in general terms. The specifics of the transitions characteristically change with alterations in formulation. The interpretation of these changes is facilitated by the use of the composite information generated from DSC data, SEM and freeze-etch micrographs, and other specified specific properties of the system. Furthermore, it was from the heat and water loss rate data analysis in conjunction with such composite information that inconsistencies in starch gelatinization data were rationalized as representing a sequence of events in a whole series of starch-related transformations. For example, the loss of birefringence coincides with the observed DSC transformations, but observable swelling of the granule with significant changes in viscosity occur past this point in the thermal process. Freeze-etch and SEM data support this interpretation.

This integrative approach has been applied to the use of emulsifiers in batter, Cloke, et al. (1981); several papers will be published later.

Bovine Muscle

As heat is applied to frozen intact aged bovine muscle, temperature gradients are established and water transport takes place. Several studies from our laboratories have looked at the transport properties of batters and porcine muscles as they are heated in controlled environment ovens that can be operated using electrical resistance (conventional) heating or hybrid combinations of conventional-forced hot air and microwave heating (Godsalve et al., 1977a,b; Hung et al, 1978; Cloke et al., 1981; Wei et al., 1981). A muscle cut into g. cylinders and heated in the conventional environmental oven at 177°C (see

*Scanning Electron Microscopy

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Figure 4. Freeze-etch micrographs of batter at different stages of heating. Starch (S). a. unheated; b. heated to 93°C (arrow is swollen granule ridge); c. heated to 102°C (arrow points to typical boundary between two starch granules, and the hilum is depicted by H.)

Figure 5. Typical cross-sections of baked cakes containing (a) 0%, (b) 4.0% and (c) 7.5% of saturated monoglycerides.

Figure 6. SEM micrographs of cake crumb which have been vacuum desicator dried and coated with Au-Pd. Formulation containing (a) hydrogenated shortening, and (b) oil. Light areas are matrix containing significant amounts of lipid. Arrows in (a) point to swollen starch granules with fat areas. In (b) arrow points to large fat pool.

Figure 7. Typical water loss and drip rate for aged bovine ST muscle cut into 600 g. cylinders and heated in conventional environmental oven at 177°C.
Appendix for details of method). A general description of the type of supportive data obtained at different levels of structural organization will be integrated into the curve interpretations as related to the mechanisms of water loss by evaporation and drip. An understanding of the mechanisms of water loss is important not only in predictive modelling but also, from the practical standpoint, in defining optimizing conditions that will minimize muscle shrink and water loss. For example, recommendations for the coupling of conventional and microwave heating of meat for fast and efficient cooking can be based on such data.

We will illustrate this approach by considering the role of myofibrillar proteins in water loss and shrink mechanisms in heated bovine muscle. Therefore, it is not an exhaustive discussion of the muscle system with all the protein and components present in the entire muscle.

Figure 7 can be divided into seven regions defined by lines T0-T7. Table 2 summarizes other types of data obtained in these studies that can be integrated into the interpretation of the sarcomeres. Specifically, we will focus on surface and internal temperatures, drip losses, evaporative losses, and shrinkage as measured at the sarcomere level and for the total sample.

In region T0-T1, the rate of water loss rises slowly as the sample thaws and the surface temperature rises to 100°C. Thaw drip (where water and protein have been determined to be present) becomes significant and there is a significant rise in water loss rate until the surface of the muscle reaches 100°C at T2. At this time, water loss rate is constant and drip loss rate is minimal. Once the surface temperature rises above 100°C to 130°C (T3), the rate of water loss begins to steeply increase. This is also the point at which significant protein denaturation begins and much of the sample reaches 62°C. However, drip rate (the drip was found to be only water) also increases significantly until water loss and drip rate are maximized at T4 to a second constant rate period. At this time, the internal temperature range is 62°C and surface temperature range is 100°C. The ratio of sample length to original length (1/10) is 0.82. This second constant rate period continues until T5 when internal temperatures range 87-100°C and 138°C at the surface with a 1/10 of 0.82. By T5, the sample 1/10 has become 0.78 and all portions of the sample are at least 100°C with a surface temperature of 160°C. At essentially total dryness, T7, the sample 1/10 is 0.65.

Interpreting these results requires at least an integration of data obtained by TEM* (Figure 8) for raw and heated muscle and by DSC for myofibrillar proteins. Special focus will be put on the fibrinous structures: actin and myosin filaments. Although there are many studies on myofibrillar and other muscle protein structures in heated samples (Paul, 1963; Gilles, 1969; Schaller and Poulte, 1971; Jones et al., 1977; Hearne et al., 1978; Dahlin et al., 1976; Hegarty and Allen, 1975; Bouton et al., 1976; Davey and Gill, 1976; Hamm and Deatherage, 1960; Locker and Daines, 1974; Leander et al., 1980; Manashi et al., 1976) we will discuss here TEM myofibrillar data for the same type of muscles used in the water transport experiments and subjected to the same heat treatment (Hung, 1980). Details of the TEM preparation procedures can be found in Hung (1980) and are summarized in the Appendix of this paper. Raw samples (T0) as seen in Figure 8a, had average sarcomere of 2.5 μm and samples heated below 75°C (T1-T4) as seen in Figure 8b, had a bimodal distribution of sarcomeres with lengths at about 2.50 and about 2.05 μm. When heated samples reached at least 75°C (T4-T6 as seen in Figure 8c, the sarcomere lengths were bimodal with averages at 2.05 or 1.55 μm. At total dryness (T7), the sarcomeres averaged 1.55 μm. The sarcomere shortening results and TEM micrographs are consistent with those presented by Leander et al. (1980). The variations are due to differences in experimental procedure. They also noted several visual changes in myofibrillar structure, but observed that sarcomere shortening occurred in two narrow temperature ranges. Also, their microscopy is a more in-depth presentation of bovine ST muscle changes upon heating and can be consulted for further detail than is presented in this overview paper.

In order to better understand the reasons for the relatively discrete sarcomere length differences, we looked at the differential thermal analysis work of Karmas and DiMarco (1970), Wright et al. (1977), and Appel and Löfqvist (1978). The following information is based on muscle, post rigor, shows significant differential heat input beginning at about 49°C and ending at about 90°C. Sometimes two peaks are observed, one at about 66°C and the other at about 82°C (Karmas and DiMarco, 1970). It is beyond the intent of this review to discuss the instrumental design factors that result in peak position not being constant and, therefore, temperatures of peak height results are not constant. For this reason, only an approximation of peak maxima are utilized in this discussion. For other types of muscle, Appel and Löfqvist (1978) give three endothermic peaks at about 62 (I), 69 (II), and 83°C (III). Also, Wright et al. (1977) using model system studies found three characteristic thermal peaks: actin-related for I, sarcoplasmic protein-related for II, and actin-related for III. These three thermal peaks are also found by Quinn et al. (1980) for bovine muscle and are irreversible endothermic transitions. More detailed model system studies are in progress by other workers such as that reported by Swenson and Ritchie (1980), in order to better understand the whole muscle protein denaturation.

These results, nevertheless, lead to more complete interpretation of the TEM results in which three relatively discrete sarcomere lengths were found for post rigor aged bovine ST muscle at subsequent stages of heating. The 2.5 μm sarcomere would be related to thermally undenatured myofibrils with banding patterns similar to aged post rigor bovine muscle (Figure 8a).

Based on the differential thermal data, the 2.05 μm sarcomere would be related to the denaturation of myosin which results in the A band region (Figure 8b) becoming more densely packed and first sarcomere shortening. In fact, significant amounts of the muscle have reached 62°C by T3 and the sarcomere length range is 2.05-2.5 μm.

*Transmission Electron Microscopy
Figure 8. TEM of raw and heated aged bovine ST muscle. a. raw sample. Z disc (Z), A is placed on part of A band region, I on part of I band region; arrows point to M line (M), N line (N) and H zone (H); b. 65-70°C; c. 75-90°C.

Between T3 and T4 the bulk of the sample ranges between 1.55-2.05 μm. Most of the sarcomeres measure 2.05 μm (shortened with A band density increasing) and are 82% of the original sarcomere length. It is not surprising that the muscle at time T4 has reached 85% of its original length. It is therefore speculated that at this point the myosin denaturation mainly contributed to this initial sample shrink. It is about in this region that the sample is used as cooked meat muscle. Furthermore, at T4 the temperatures reach the range at which actin denaturation begins and significant water is squeezed to the muscle surface and appears as drip and evaporative losses. Thus, the surface temperature returns to 100°C as a wet bulb temperature predominates. The actin denaturation causes further sarcomere shortening with less defined I band region (Figure 8c) and breaks at the Z disc. Since sarcomere shortening is not maximized nor complete after some of the sample reaches actin denaturation temperatures (above about 83°C), some sarcomeres are 1.55 μm and some remain at 2.05 μm due to the stretcher-like effect of intact muscle and surface crust. This distribution is maintained until a state of dehydration is reached throughout the sample, at which time the sample 1/10 ratio is 0.65. This interpretation is supported by the fact that sarcomeres that are 1.55 μm are 62% of the original length. It is interesting to note that it seems that actin denaturation is just beginning in dry cooked beef roast at doneness and that the myosin denaturation is more nearly complete. It is intriguing to speculate that control of this second stage of myofibrillar denaturation (i.e., actin) is necessary, for example, in microwave-conventional heating of bovine muscle. In fact, in a recent publication by Wei et al. (1981), the 1/10 ratios for conventionally heated, microwave heated, and combined conventionally-microwave heated bovine muscle at doneness indicated that control of crust formation and actin-related sarcomere shortening (1.55 μm) is critical in muscle shrink and water loss. These events do not necessarily predict tenderness. Carroll and Jones (1979) and Hegarty and Allen (1975), as well as others have recognized that connective tissue such as collagen, method of cookery, origin of muscle, age of animal, all contribute to quality at doneness and that the relative shortening of the sarcomere (depending on how they are restrained) is only a portion of the story. More studies are necessary to further evaluate the system.
This example is by no means an exhaustive discussion of the physicochemical events that take place in heating of bovine muscle, but merely a small example of how one integrates information from all levels of molecular and structural development in order to understand some of the events that we observe in nature.

**Future Projections**

Ultrastructure studies are playing a primary role in defining the problems that can be investigated by new as well as existing instrumentation. However, complex sequences of events which occur during the processing and heating of anisotropic systems are often defined by single word terms, such as protein denaturation and starch gelatinization. This contributes to the direct comparison of experimental results which in fact represent different stages in the sequence of events. The next decade should be an especially exciting one to food scientists because of all of the information coming together in proper sequence as methods of analysis are perfected and quantified.

Formulated foods are becoming more prevalent as food sources and it is imperative to understand the exact cause and effect of chemical and structural changes. For example, chlorination of cake flour and possible alternate sources of treatment thickeners, gelatin and similar flour functionality are important. Soy substitutions in meat emulsions as well as batter and dough systems pose special problems in terms of functionality, safety, and sensory characteristics.

Advanced instrumentation which will gain more use in the near future in studies of food microstructure includes surface tensiometers, thermal conductivity probes for localized thermal conductivity measurements as reactions proceed, and low angle x-ray diffractometers of polymer systems such as protein and carbohydrate based gels. X-rays can now be produced for routine application from high velocity synchrotrons which can emit monochromatic X-ray radiation ranging from 1 kev to 1 Kev for high resolution X-ray patterns. Differential scanning calorimeters will continue to play a primary role in the study of the thermal properties of foods, not only in heating but in freezing studies. The bridging of basic knowledge on the interfacial properties of microemulsions to food macroemulsive systems will result in a better understanding and control of processes of formulated and restructured foods. Rheological data with various types of viscometers and textural measuring devices on Instron instruments will be better standardized for various systems.

Central to all this is light, polarizing and electron microscopy which will continue to remain central to understanding the resultant physical structures of foods and the relationships of those structures to the sequence of chemical events that foods undergo upon freezing, heating and processing.

It will be crucial to standardize measurement procedures across laboratories as new methodologies and instrumentation leave the realm of research and are applied more widely to industrial use. A prime example of this need can be seen in the area of microwave processing of foods commercially and in home preparation. The problem which arises in the standardization procedures, is that the food systems are not completely understood for conventional heating, let alone for microwave heating.

**Appendix**

**Cake Formula and Preparation**

The formula given in Table 3, was based on the lean research formula of Kissell (1959). The levels of sucrose and water were adjusted, in preliminary trials, for the characteristics of the flour being used. Hydrogenated vegetable shortening was used in the preliminary standardization experiments. Criteria for the accepted standard cake were a slightly rounded top, and an even and moderately open crumb structure. All ingredients, except the emulsifiers, were of household types as described in Table 3.

Two shortenings served as controls: hydrogenated vegetable shortening and corn oil. Emulsifiers were added to corn oil at different % levels of the oil by weight, and the weight of oil reduced accordingly.

A modified two-stage method of mixing was used. Flour and baking powder were sifted together, and the shortening and sucrose solution were added. The batter was mixed for 3.5 min with a mixer (Kitchen-Aid, Model K45). The distilled water was added and the batter was mixed for an additional 3 min. Two-hundred-twenty grams of batter were weighed into an aluminum pan (15.2 cm diam x 3.2 cm deep). All cakes were baked for 25 min in the controlled environment oven. Data reported are based on a minimum of three replications of each emulsifier type and concentration.

**Cake Water Loss and Temperature Measurements**

Water loss and internal temperatures were measured during baking in the controlled environment oven. Details of the design and operation of the oven as well as method of calculation of water loss rates were given by Godsalve et al. (1977b). Briefly the purpose of the oven is to provide a uniform and controlled environment for cooking experiments. It consists of a cooking chamber, heated by electrical resistance, and a piping network that circulates air through the oven at a controlled rate. The temperature, air flow rate, and humidity are known at all times. For the experiments reported for the cake batters, air flow rate was 10.1 m³/hr, and the temperature was 190 ± 1°C, with experiments starting at ambient humidity. Continuous humidity determinations of the air before and after it passes through the oven chamber are made from wet bulb-dry bulb thermometers positioned in the piping network. From these measurements, water loss rates are calculated, as described by Godsalve et al. (1977b).

Temperatures were monitored by thermocouples placed at four locations in the cake: center, 2.5 cm, 5.1 cm, and 6.9 cm radially from the center of the pan, which had a total radius of 7.6 cm. Each thermocouple was positioned 7 mm above the pan base. One of the interchangeable doors of the oven was fitted with a frame to hold the cake pan and thermocouples. Thermocouple wires were introduced into the oven chamber through a bulkhead fitting in the oven door. Details of the assembly are shown by Gordon et al. (1979).
Commercial bovine semitendinosus muscles were obtained from USDA Choice carcasses after a 5-day postmortem aging period at 4°C. These muscles were cut into cylindrically-shaped samples with fibers parallel to the axis of the cylinder. Sample size was 600g. The dimensions (length x diameter) of these samples were 19.1 cm x 6.35 cm. A small hole (about 3.5 mm diameter) through the axis of every sample was drilled for insertion of a glass rod that suspends the muscle sample in the air. Muscle samples were kept frozen throughout the procedure of sample preparation. Unless otherwise stated, the samples were cooked from frozen state. 600g frozen samples, with axes vertical, were cooked in the controlled environment oven at 177°C. One sample was cooked in each run. The rate of air flow through the oven was set at 13.7 m³/hr in all runs. The weights of the samples, the sizes of the drip droplets, the number of drip droplets formed per minute and the lengths of the samples were measured throughout the experiments. From these data, the weight loss, the rate of weight loss, the rate of drip in g/min (assuming density of drip is 1 g/cm³), the rate of evaporation (difference between the rate of total loss and the rate of drip) and the weight ratio of water to dry solid in meat can be calculated. The approximation that the density of the drip is 1 g/cm³ is based on the assumptions that under the cooking conditions used, the majority of the drip is water, and that the densities of protein and lipids are not making a major contribution. Temperature ranges were obtained by monitoring temperatures continuously using copper-constantin thermocouples placed surface, center, 1/3 and 2/3 positions from the center.

**Muscle TEM Preparation**

TEM data were obtained from the retail bought semitendinosus (ST) bovine muscle, raw or heated as described above. Samples were placed in 2% glutaraldehyde - 0.1 M cacodylate buffer (pH 6.9), followed by 0.1 M cacodylate washes, then placed in 1% Os04 - 0.1 M cacodylate. They were subsequently washed with 0.1 M cacodylate buffer, then serially dehydrated in acetone, after which they were embedded in Epon 812 and polymerized. All data were recorded on a Philips 300 TEM operated at 60 kV or a Hitachi H-10 EM operated at 70 kV.


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have argued in the text of the paper, we propose that a sequence of transitions occurs over the temperature range encompassing the various definitions of gelatinization. Certain of these would be reversible in a thermodynamic sense; others would not be of this type. It is the sorting process that is one of the themes of this paper.

M.A. Christman: Where and how is the temperature measured for the temperature curve in Figure 2?
Authors: These measurements are for the center position and were made with a copper-constantan thermocouple. We typically monitor temperature at least two other positions. Details of the apparatus are given by Gordon et al. (1979).

D.F. Lewis: What role does protein play in cake structure and can this be related to the DSC data in Table 1?
Authors: Successful cakes cannot be made without gluten proteins for structure even though the primary function of cake flour is attributed to starch. Gluten's role is to participate in amorphous matrix development between starch granules. However, not in the ordered or layered sense of bread making which is a more limited water system. DSC thermograms by Eliasson and Hegg (Eliasson A-C, and Hegg F-O. Thermal stability of wheat gluten. Cereal Chem. 57, 1980; 436-437) show four peaks for extracted wheat gluten between 30°-115°C. The first and fourth peaks are attributed to contamination from wheat starch while the middle two are attributed to gluten. The enthalpies of the wheat gluten are below 0.06 cal/g gluten. Usually large enthalpies are associated with protein denaturation (of the order of 4 to 6 cal/g protein). These results mean that protein is denatured at room temperature, isotropic and lacking order, or so highly ordered that it is extremely stable to heat. The thermograms that Eliasson and Hegg observed could also be the contribution of complexed gluten with other components during extraction. Therefore, the importance of gluten in baked products may be prior to baking and not during the baking process. Thus, the DSC data in Table 1 is primarily due to starch with the gluten contribution not being measurable in this temperature range.

D.L. Lewis: Why do you use the term "doneness" instead of "when the product is fully baked"?
Authors: The term "doneness" is certainly an ambiguous term especially for some systems such as muscle. However, we often study the water emission patterns prior or past the point when the consumer would consider a product cooked or baked. In that sense, "fully baked" can be equally ambiguous and certainly should be related to certain objective criteria such as temperature patterns. The term "doneness" for purposes of discussion is used as an approximate anchor in time during a long sequence of events as a sample is being baked or heated to some level of dryness.

W.A. Christman: How can one differentiate in freeze-fracture micrographs between fat and starch particles? They are both round and in the same size range.
Authors: In preliminary studies we prepared specimens in which only one of the batter components was present in order to become familiar with the
morphology of each component.

D.F. Lewis: Have you tried thin sectioning any batters to complement your freeze-etching results and give information on extraction of amylose from the starch grains into the sugar matrix?
Authors: No, we have not due to the limitations of sample preparations in these emulsive systems prior to loss of fluidity.

R.J. Carroll: What was the pH of the muscle used in the TEM studies? Why was the pH 6.9 selected for fixation?
Authors: The pH of the muscle was not checked at the time of this study and a pH of 6.9 was selected based on earlier work with homogenized myofibrils and/or fixation methods. In recent time, it has become evident to us that future work might benefit from the use of buffers closer to the final pH of the muscle samples.

D.F. Lewis: To what extent do you think that the interactions between proteins in meat will affect the transition temperatures produced by the DSC compared with those obtained from protein extracts?
Authors: This is difficult to say since we have not done this in our studies. However, for years myofibrils have been extracted for EM work in neutral buffers and sarcomere banding patterns have been observed. This indicates that at most, incomplete protein myofibrillar denaturation takes place depending on the extracting medium, and possibly none. Some soluble proteins could denature upon extraction. In fact, the study by Appel and Lofqvist shows distinct thermograms extracted myosin and actin similar to those found in intact muscle.

S.H. Cohen: What were the distributions and standard deviations of sarcomere lengths at the various T levels?
Authors: The data reported in Table 2 are based on a detailed study of the distribution of sarcomere lengths at various temperatures (Hung, 1980). All of the sarcomeres shown for one fiber on a micrograph were measured, and the average value was used in the distribution. The number of sarcomeres that could be measured on a micrograph ranged from four to ten. The distributions were based on approximately 30 average sarcomere lengths for each treatment. The mean and its standard error for the uncooked samples were 2.6 and 0.063, respectively. For the 70-80°C range, the values were 1.7 and 0.042; and for the 65-70°C range, 1.90 and 0.090. We examined the distribution of average sarcomere lengths within the various temperature ranges, but found it was instructive also to examine the distributions of specific sarcomere lengths across temperatures. The usefulness of the latter distribution is illustrated by the observation that no lengths less than 1.75 were found in the uncooked sample and no lengths greater than 2.25 were found in the 70-80°C range. In the 65-70°C range, two distributions appear to be present, one with a range of 2.05-2.35 and another with a range 2.35-2.89. It was the observation of frequency distributions of these various types that lead us to the conclusion that in the intermediate temperature ranges a bimodal distribution is present due to the sequential transformations during cooking.

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