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IMAGE ANALYSIS OF MORPHOLOGICAL CHANGES IN WIENER BATTERS DURING CHOPPING AND COOKING

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Abstract

Histological changes in wiener batters during chopping and cooking have often been illustrated with "representative" fields. The practice of selecting representative fields ignores variation and leads to word descriptions that cannot be correlated with numerical scores for functional or sensory tests. If wieners are regarded as a multi-component system, objectivity can be achieved by selecting many fields for each sample according to a rigid sampling plan. Image analysis quantified parameters of both the fat and protein components. The reduction in size of fat globules during chopping of a commercial formulation, for example, was a function of area and aggregate perimeter of several hundred globules compiled by a computer. There was no relationship between wiener firmness and any feature of the microstructure; but even at a low magnification of 30x, several statistically different factors were exposed during this survey which require further study.

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Introduction

The microstructure of a food is usually studied for one of two reasons. There are basic studies for which the dominant motive is intellectual curiosity, where representative photomicrographs enhance the discussion of results as well as illustrate principal observations. More often, the microstructure is examined to determine how two or more batches of the same foodstuff differ physically as a result of changes in product formulation, production methods or spoilage. In such comparative studies, the use of subjectively selected "representative" microscopic fields may illustrate differences but not quantify them. Knowing that two or more samples are different in some way, a microscopist may tend to select a field that will "represent" only the differences and ignore the similarities. The purpose of this paper is to suggest that multi-component foods be examined according to principles used in mathematical ecology, implying objective sampling plans and statistical analysis to quantify differences. This approach will produce numerical values for structural features which may then be compared to numbers generated by some functional or sensory test.

The subject of this review has been under study for more than twenty years. Hansen (1960) is generally credited as the first to conduct a microscopic examination of finely comminuted meats such a wieners. Making wieners and similar sausage products had long been an industrial art and a commercial success, but fat and water loss during processing was sometimes excessive. Although this problem provided the impetus for Hansen's work, he used low-magnification microscopy to seek an understanding of the basic theory governing the stability of wiener emulsions. He showed that the amount of chopping must be sufficient to form a protein matrix which fully enclosed the dispersed fat globules; that the salt soluble proteins appeared to concentrate at the surface of the fat globules to form a stabilizing membrane; and that the higher temperatures associated with excess chopping might partially denature the protein matrix and permit unprotected fat globules to separate during smoking and cooking. Hansen's only experiment

involving the microstructure of the whole wiener was the one on variable chopping time that emphasized the role of the protein matrix. His demonstration of a protein membrane around the fat globules was obtained experimentally by examining a diluted slurry which had been defatted with xylene, and the essential role of salt soluble proteins was demonstrated in a model system involving meat extracts and pork fat mixed in a laboratory blender.

It was primarily through the use of model systems, rather than further studies of microstructure, that the role played by salt soluble proteins in the formation of stabilized comminuted meat systems was to be studied more thoroughly. Swift, Saffle and their co-workers were among those who categorized meat proteins on the basis of their emulsifying capacity in model systems (Swift et al. 1961; Swift and Sulzbacher 1963; Helmer and Saffle 1963; Saffle and Galbreath 1964; Carpenter and Saffle 1964, 1965; Saffle 1968; Acton and Saffle 1970, 1971). During this period, Borchert et al. (1967) used electron microscopy to show the protein membrane around fat globules and its disruption during cooking; and coincidently pointed to the existence of many fat bodies as small as 0.1 μm with the hope that this would stimulate further work on the nature of meat "emulsions". The significance of the work of Borchert et al. (1967) was that the protein membrane around fat globules could apparently be broken during cooking without causing fat loss.

Work on the problem of fat separation during cooking overshadowed attention to the continuous protein phase described in the microstructure study by Hansen (1960), and there was a semantic difficulty caused by the use of the word "emulsion". For example, Froning et al. (1970) showed that salt soluble proteins were as essential to wieners made from poultry meat as they were to red meats, and by imbedding sections in paraffin they illustrated this with photomicrographs. However, they described meat emulsions simply as "oil in water emulsions with the disperse phase being droplets of the oil and the continuous phase being water". In a similar paper, Hargus et al. (1970) discussed differences in structure between proteins in white and dark turkey meats. These authors seemed willing to interrelate fat and protein in their two-paragraph discussion of a histological survey, but they once again emphasized fat globule size. Angel et al. (1974) did a comparative study of three machines for mechanically deboning poultry meat, using variable chopping times and microstructural differences between raw and cooked products as bases for comparison; but in line with the existing practice, they restricted their analysis of structure to the size and distribution of fat globules.

In the colloquial sense, "emulsion" had been used by sausage makers to imply the dispersion of fat in a protein matrix; whereas, in the physicochemical sense, a true emulsion was defined by Becher (1965) as "a heterogeneous system consisting of at least one immiscible liquid intimately dispersed in another (liquid) in the form of droplets, whose diameters in general exceed 0.1 μm". True emulsions were probably obtained by those such as Saffle (1968) who used oil titration methods to measure the emulsifying capacity of various proteins. Yet van den Oord and Visser (1973) questioned the assumption that wieners were true emulsions and shifted the emphasis from fat alone back to the fat-protein matrix system. Using extensive photomicrographs, they described wiener batters as particles of solid fat rather than droplets of liquid oil, dispersed in a salt-water-protein matrix. After cooking, the fat particles were described as being enclosed in cavities formed by the coagulated, solid, protein-like network. Furthermore, none of the products they studied had a protein membrane that was different from the original fat cell membrane.

It may be impossible to encompass all commercial products within a single theory. Theno and Schmidt (1978), using SEM, found that one brand of frankfurter had a coarse protein matrix which mechanically fixed large fat globules in accordance with the structure proposed by van den Oord and Visser (1973). However, a second brand had a more finely-textured protein matrix and was "emulsion like" while a third had the characteristics of a true emulsion; namely, the fat particles had been reduced to an extremely small size, they did have a proteinaceous coat, and they were bound in place by small protein cross bridges. In a related note, concerning the variability in commercial products, Cassens and Schmidt (1979) were able to statistically support the contention of Angel et al. (1974) that the firmest wieners had the smallest fat globules, but they suggested that firmness was related to the more extensive protein matrix required to surround the larger number of smaller fat globules.

Renewed emphasis on the function of proteins was most important to those who wished to replace some of the meat with comparatively less expensive vegetable and milk proteins while retaining maximum nutritional value and satisfactory functional and sensory properties. Randall et al. (1976) found that the use of extenders such as textured soy protein, powdered soy protein, soy isolate, gluten and egg white, as well as tripe and cooked beef, decreased firmness and increased water loss during cooking in proportion to the rate of substitution. However, the fat loss was not severely affected until the replacement rate exceeded 40% of the meat protein. Referring to the microstructure study of Cassens et al. (1975) for support, they surmised that the texture of the replacement protein governed the texture of the finished product. They concluded that fat and water binding are not unique factors contributing to textural properties, and that a property of the protein such as thermal coagulability exerted a greater effect. Smith et al. (1973) concluded that finely-divided protein solids can play a role in emulsion stabilization based more on their physical state than on their solubility. Comer (1979) showed decisively that conventional measurements such as the emulsifying capacity of proteins did not correlate with

either emulsion stability or texture in wiener formulations. On the contrary, various fillers which had favourable effects upon homogenate stability had negative effects upon product texture. He stressed the observation that water binding and gelation characteristics of the proteins probably account for the comparative softness of wieners containing vegetable protein.

A previous report from this laboratory (Kempton et al. 1982) showed that the inclusion of nonmeat proteins in wiener formulations caused major distortions in the microstructure of the batters which were clearly visible at a magnification of 30x under a light microscope, but only in some fields. Therefore, a large number of photographs was obtained for each product by a rigid, objective sampling plan. The degree of distortion in each photograph was measured by pattern analysis and the results were subjected to computer analysis by a statistical package known as the "two-within-four randomization test". The results were expressed as the number of slide preparations on which the ingredients were "clumped" rather than having a random pattern. Emphasis was placed on the unreliability of "representative" fields, which would have been more unreliable at higher magnifications. This present study pursued the concept that the microscopic evaluation of wieners should be based on an objective evaluation of many fields selected by following a rigid sampling plan. Image analysis, coupled to statistical methods which are comparatively simple and readily available, was applied to a survey of the changes that occurred during the chopping and cooking of various industrial wiener formulations. The program used measured the total area and perimeter of both the fat and protein phases because current theories of wiener structure involve the formation of a continuous protein matrix of sufficient perimeter to surround the discontinuous globules of fat. This demonstration of image analysis stresses the difference between the type of information obtainable by quantitative analysis and subjective word descriptions of representative fields.

Materials and Methods

Materials

The "commercial" batters were prepared and supplied by a major producer of comminuted meats and represented a typical least-cost formulation. The precise ratio of ingredients was not divulged. A 100-lb lot referred to only as a "final blend" was placed in a Kramer-Grebe silent cutter. Grab samples of several pounds each were removed by the sausage maker after 10, 20, 30, 40 and 50 revolutions. A portion of each sample was

Table 1. Characteristics of commercial products

Designation	Revolutions in cutter	Yield[1] (% volume)	Fat loss[1] (% volume)	Firmness: (kg)
Coarse	10	90.55	0.99	1.99
-	20	91.08	0.52	1.95
Medium	30	92.55	0.22	2.19
-	40	92.44	0.17	1.94
Fine	50	92.53	0.18	1.96

[1] All values are means of 10 determinations

Table 2. Characteristics of experimental products

Sample No	Designation	Revolutions in cutter	Ingredients (Protein Source)	Total Shrink (% weight)	Texture (kg) HSD_{90}=0.22[3]
1	Medium	150	ALL MEAT	10.98	3.00
2	Coarse	75	MEAT + EXTENDER NO. 1	12.15	1.85
3	Medium	150	"	12.04	1.89
4	Fine	225	"	11.77	1.93
5	Medium	150	MEAT + EXTENDER NO. 2	11.29	1.96
6	Medium	150	MEAT EXTENDER NO. 3	11.05	1.93
7	Fine	225[1]	MEAT + EXTENDER NO. 1	10.71	1.58
8	Fine	225[2]	"	10.52	1.50

[1] Extender added after 150 revolutions
[2] Extender and binder added after 150 revolutions
[3] Honest Significant Difference based on Tukey's test (90% level of probability) = 0.22.

frozen at -18°C and retained as the raw product, and the remainder was sent to D. Raymond, Food Research Institute, Ottawa, Ont., where cook stability was determined by the method developed by Ranken (1973) as modified by Randall et al. (1976). Firmness of the cooked product was measured using the method described by Voisey and Randall (1977) for the Universal Food Rheometer (UFR). Pertinent performance data are summarized in Table 1. Finished wieners were frozen, and returned for microstructure analysis.

The "experimental" batters containing significant amounts of nonmeat protein were supplied by a second industrial concern. Batters were prepared in a silent cutter. Wieners representing each batter were prepared and evaluated as described above. However, Table 2 was constructed from data supplied by the manufacturer. Complete formulations and variables in preparation were made available (Kempton, unpublished), but Table 2 contains only information required for this paper. The involvement of different industrial and governmental laboratories, and the transport of large samples, made it logistically impossible to immerse samples in liquid nitrogen. Preliminary studies showed that the structural damage caused by freezing to -18°C did not obscure the ability of image analysis to quantify similarities and differences among the various products.

Sample Preparation and Microscopy

Fig. 1 is an abbreviated illustration of the procedure used to obtain microscopic fields suitable for statistically valid analysis. For additional information see Kempton et al. (1982). Five sample units were taken at random from the 2-kg frozen sample of raw product and trimmed to 2 cm x 2 cm x 1 cm rectangular prisms, while maintaining the temperature at -18°C. For cooked products, the sample units were 5 wieners made from the batter, frozen for transport, and kept frozen at -18°C. Slices 20 µm thick were made with a Cryo Cut Cryostat (Model 840, American Optical (A/O), Buffalo, N.Y.) set at -25°C. Three slices were taken from each unit by selecting the first slice, discarding the next nine, selecting the eleventh, discarding the next nine and finally selecting the twenty-first slice.

The slices were fixed on slides by immersion in a 1:1 (v/v) solution of saturated mercuric chloride and absolute ethanol for a minimum of 6 h. The fixed slices were stained for 20 min in a working solution of Oil Red O according to Disbrey and Rack (1970). The slides were washed in running water for 10 min, counterstained in a 0.5% solution of Light Green (C.I. No. 42095), then washed again in running water for 10 min. Fat retains the red stain and the protein component is stained green.

The stained slices were mounted under a Nikon Aphophot microscope (Nippon Kogaku KK, Japan) equipped with a 35 mm Nikon M-35 FA camera. Each slice was sampled by photography of as many as four adjacent fields on each of two linear transects (called Planes on Fig. 1). This plan potentially yields 120 objectively selected

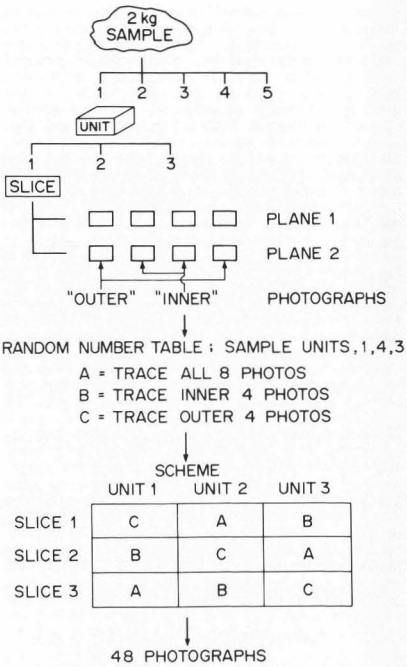

Fig. 1. Sampling procedure from which as many as 120 photographs could be obtained from each sample (5 units x 3 slices x 8 microscope fields), reduced to 48 photographs by random selection. See Kempton et al. (1982) for complete details.

photographs of each sample. This plan was reduced to 48 randomly selected photographs by the procedure detailed on Fig. 1. After experience with the degree of homogeneity found, the sampling was reduced to as few as 20 photographs during the course of this study.

Component Analysis

In order to analyze each component in the photographs, it was necessary to trace each separately onto a transparency sheet using a black, felt-tipped pen. Each tracing was analyzed using an Image Analyzer (Quantimet 720, Imanco, Monsey, N.Y.). The program, written in Basic Language, inputs area and perimeter for each feature and accumulates area and perimeter to give aggregate totals.

The data were analyzed by a two-way fixed effects analysis of variance using BMDP7D. The

factors themselves were PREPARATION [RAW, COOKED] and GRIND [COARSE, MEDIUM, FINE]. No post-hoc tests were performed.

Results and Discussion

Chopping and Cooking of an All Meat Commercial Formulation

Experiments involving variable chopping regimes have been done several times with wieners of varying composition. However, the first part of this study differs from previous work in that changes in both the fat and protein phases were described in numerical terms, the amount of void or open space was measured, and quantitative measurements of the interaction between the amount of chopping and changes during cooking are also novel. Kempton et al. (1982) explained why many microscopic fields of each sample must be selected by an objective sampling plan such as Fig. 1 if the results of histological studies of wieners are to be presented as statistically valid numbers. In this present paper, image analysis was used instead of pattern analysis because the major expected change was that the fat component would be dispersed into more but smaller particles as chopping continued. Wiener batters were tested for fat loss and yield during cooking, and UFR measurements of firmness were made on the finished products (Table 1) so that the potential for comparing morphological changes and functional characteristics would at least exist.

Ingredients of a commercial formulation chopped for 10 revolutions in a silent cutter produced a batter which the sausage maker described as "coarse"; with batters chopped for 30 and 50 revolutions described as "medium" and "fine" respectively. The performance data given in Table 1 show that even the "coarse" batter produced a wiener of acceptable firmness, and the fat loss from this batter during cooking, while highest of the three, was not considered excessive. Fig. 2 may be of interest as a reference, because as far as is known this is the first publication of a color plate in which changes in the fat and protein during chopping and after cooking have been shown using a differential stain. These photomicrographs are representative in the statistical sense because they were assembled to illustrate mean values that had been ascertained by image analysis of 36 microscopic fields of each treatment. They are presented here to discuss how appearances may be deceiving when any one photograph is selected to represent a product. Subjectively, the fat globules in the coarse batter (Fig. 2a) could be described as large amorphous masses that became more regular and ovoid as chopping progressed, as well as becoming smaller (Fig. 2b, 2c). Similarly, the protein in the coarse batter could be described subjectively as thick strands retaining some evidence of tissue structure which was dispersed into a thinner, more uniform matrix during chopping. The point is that these simple and expected changes were not substantiated by visual inspection of all the photomicrographs obtained by the random sampling plan outlined in Fig. 1. When all 108 photographs of these batters were shuffled like a deck of cards, they could not then be divided accurately into three piles corresponding to the amount of chopping.

On the other hand, all 216 photographs could be easily and accurately separated into two piles corresponding to raw batters and cooked wieners. This separation was based primarily on the darker hues of both the fat and protein phases after cooking, and secondarily on the impression that there was less material and more open space, generally, in the photographs of the cooked product. The subjective observation of greatest theoretical importance was that most of the fat "globules" in the raw batters appeared to be closely surrounded by a coating of protein; while after cooking (Fig. 2d, 2e, 2f) the fat could be described better as irregularly shaped "particles" which appeared to be retracted from their protein coating although most remained trapped in crevices within a protein matrix. This subjective analysis of Fig. 2 agreed more with the theory proposed by van den Oord and Visser (1973) than with the emulsion theory. The description of fat in raw batters as amorphous masses surrounded by protein corresponds to the concept of a dispersion of solid fat, and the enclosure of fat in cavities formed by the coagulation of protein during cooking suggested a physical network rather than an emulsion. However, as it was with photographs of the raw batters, the 108 photographs of the cooked wieners could not be subdivided visually into three piles corresponding to the amount of chopping. Therefore, the program of image analysis was broadened to determine whether there were quantitative differences between a raw batter and its corresponding cooked product in either the fat, protein or open space components and to determine whether there were subtle changes during chopping which could not be discerned by visual analysis of the randomly selected microscopic fields.

It was not possible to perform image analysis directly on the colored photographs by using filters to block one color while measuring the complimentary color, which would appear black. There were two technical problems which prevented this preferred approach. First, the colors were not uniform and no single filter would block out all of the fat, which varied in hue between pale pink and deep red. Similarly, the color of the protein varied from blue-green to black. The second problem was that there were areas in every photograph where fat and protein were overlayed, and red and green together blocked all transmitted light and produced a black photographic image in those areas. Both problems are related to the fact that a slice has depth. This difficulty could probably be reduced by obtaining thinner slices, but it will be inherent in images obtained by transmitted light through any histological preparation. Bergeron and Durand (1977) overcame the problem of conveying histological information obtained from colored photographs with word descriptions by tracing the structures they wished to discuss. Tracing each component in the wiener separately

produced a series of three images for each photograph as illustrated by Fig. 3. Diagrams of the empty spaces (Fig. 3c, 3f, 3i), which are either air pockets or water, were used to ensure that the sum of the three components equalled the total area of the photograph. The tracing process introduced subjective decisions in areas of the colored photographs where fat and protein were overlayed. Furthermore, it is unlikely that all of the very small holes in the protein matrix, and all the very small fat particles particularly evident in Fig. 2c, were accurately portrayed in Fig. 3g. However, Fig. 3a, 3d, 3g illustrate the dispersion of fat, and Fig. 3b, 3e, 3h portray the formation of a "honeycomb" protein matrix more clearly than Fig. 2. Admission of this difficulty is one reason why the procedure was evaluated first on an all meat formulation, about which there is a large body of histological knowledge and predictable results were expected. Computer printouts of data obtained from image analysis of the chopping and cooking of an all meat wiener formulation are given as Fig. 4, Fig. 5, Fig. 6 and Fig. 7.

No a priori assumptions were made regarding the degree of central tendency that would emerge from analysis of tracings of 36 photomicrographs or what type of distribution around the mean could be expected. A relatively large range was expected from cursory analysis of the many fields selected by the objective sampling plan. For example, one photograph of the coarse raw batter was about 80% blank space. In spite of the range, the results approximated a normal distribution around mean values. Based on mean values, the area occupied by protein did not change at all during chopping (Fig. 4) and an apparent upward trend in the area occupied by fat during chopping was not statistically significant (Fig. 5). Since the total area occupied by fat or protein was not expected to change during chopping, these consistent mean values, each derived from 36 photographs, indicated that this technique probably quantified histological changes with greater accuracy than could be achieved by any assessment of single representative fields.

The average size of the fat globules was expected to change during chopping, by all previous reports and by visual assessment of the photographs. This could not be adequately illustrated by dividing the area occupied by fat by the number of fat globules to give a numerical "average". Kempton (unpublished) produced histograms showing that chopping caused an increase in the number of smaller fat globules; but the numerical average size was weighted in the direction of one or two large globules that accounted for as much as 50% of the fat, even in a finely chopped batter. However, image analysis showed that the aggregate perimeter of the fat phase in the raw batters increased from 1000 mm in the coarse batter to 1500 mm in the fine batter: an increase of 50% (Fig. 6.) Since the area remained statistically constant, this increase in perimeter became an indirect numerical measurement of the decreasing size of the fat globules. This increase in the fat perimeter may be a direct measurement of the increase in protein perimeter required to enclose the fat. Angel et al. (1974) and Cassens and Schmidt (1979) concluded that firmness was directly related to the size of the fat particles. That was true in their studies of various finished products, but if universally applied it would mean that continued chopping should increase firmness. In this study, UFR measurements of firmness (Table 1) did not correlate with the decreasing size of the fat globules. The decrease in size of the fat globules was the only structural change that was directly correlated with the amount of chopping; but contrary to other reports, this was of no practical significance as a criterion for performance.

All other statistically different observations showed that the medium batter, and the wieners made from it, were distinct in several ways. For example, and to complete the discussion of changes caused by chopping, Fig. 7 shows that the perimeter of the protein phase increased between the coarse and medium chopping regimes, but fell back as chopping continued to the "fine" stage. After cooking, the area occupied by protein was greater in the medium-ground product, but unchanged (at 2700 mm^2) in the other two (Fig. 4). Fat loss during cooking, expressed as area (Fig. 5), was expected to correlate with the actual loss of fat shown in Table 1 but it did not. Although both extreme batters lost fat, according to the morphological data there was no loss of fat when the medium batter was cooked. Since the area occupied by fat and protein distinguished the medium batter but did not change in any consistent pattern during cooking, the question of whether the fat shrank or the protein expanded (Fig. 2) was resolved. The two phases simply drew apart. Fig. 7 singles out the medium batter again as the only one to show a loss in the aggregate perimeter of the protein phase during cooking.

Further discussion of the unique nature of the "medium" chopping regime, including interactions among structural parameters, would not be productive at this time. Empirically, the medium batter was expected to produce the firmest wiener, with the least amount of fat loss and total shrink during cooking. This was not borne out by measurements conducted by Agriculture Canada and given in Table 1. Therefore, there is no performance criterion to which the observed structural differences associated with the medium batter can be compared.

Chopping and Cooking of Experimental Formulations Containing Vegetable Proteins.

Wieners made with significant amounts of nonmeat protein were known to be fundamentally different, histologically, than comparable all meat products. Pattern analysis showing clumping was one way of measuring changes visible at low magnification (Kempton et al. 1982). Image analysis exposed further differences. Fig. 8 includes an all meat control, but otherwise it is analagous to the way changes in the area of the protein were displayed in Fig. 4. The area of

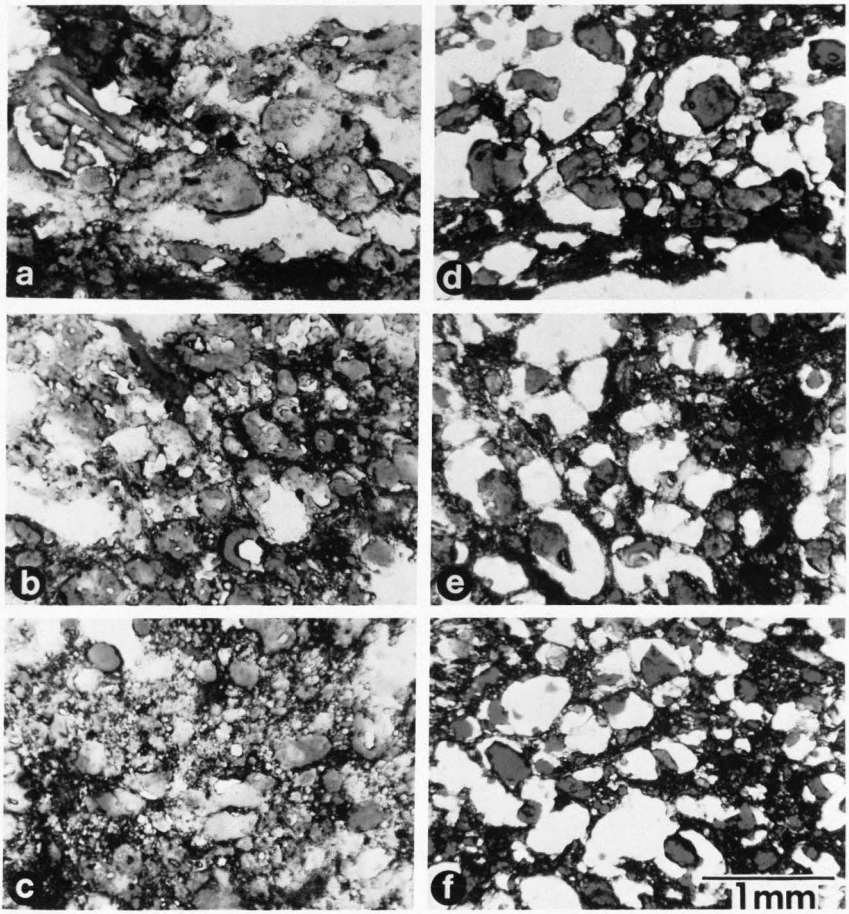

Fig. 2 Photos a-c: Representative fields of commercial wiener batter chopped to coarse, medium and fine stages respectively. Fat is stained red, protein is green. Photos d-f: Representative fields of commercial cooked wieners produced from batters chopped to coarse, medium and fine stages respectively. Fat appears to be trapped in protein matrix.

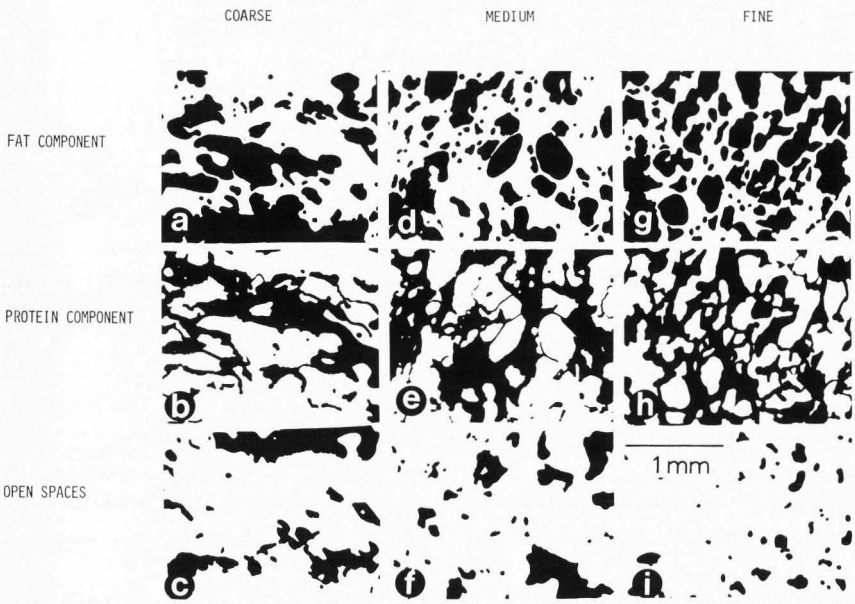

Fig. 3 Typical tracings of each component in commercial wiener batters, showing changes during chopping. These tracings were subjected to image analysis.

the photomicrographs occupied by protein did not remain constant during chopping the way it did with all meat batters. Compared to the specific control for this experiment, the coarsely chopped amended batter appeared to have more protein, the medium batter had the same amount as the control (3300 mm^2) and the finely chopped batter had less. A loss of protein area occurred during cooking, except for the finely shopped amended batter which having lost the most during chopping posted a gain during cooking. The large shrinkage in the protein component of the all meat product (Sample 1) from 3300 to 2000 mm^2, was totally unexpected. This may be a measure of the variation encountered in comparative studies of wieners made by different companies. Fig 9 is introduced to show that this was not an isolated occurrence. Various formulations were identical when chopped to the medium stage, and the apparent shrinking of the protein during excessive chopping could not be avoided by adding the vegetable component later in the chopping process. Changes in the area of protein in photomicrographs indicate a change in the density of the protein, not in the amount of protein present. Varying degrees of hydration would be one possible explanation for the changes noted. Whatever the explanation may be, these changes and differences were not related to wiener firmness.

Had this study been performed solely to relate microstructure to wiener firmness, there would have to be one feature emerge that would separate the control wieners that had a texture-firmness value of 3.0 kg (Table 2) from all wieners containing some vegetable protein which had UFR values in the relatively narrow range of 1.58-1.96 kg. Although this study was not successful in this respect, Fig.10 may further illustrate the wrong conclusion that could be drawn from visual assessment of photomicrographs while it serves to show what image analysis actually measured. These tracings represent mean values and there is a striking visual difference between the typical "filagree" or "honeycomb" appearance of the protein phase in the all meat control (Fig. 10a) and the severely fragmented protein in the finely ground batter that contained a proportion of vegetable protein (Fig. 10d). If there were only these two samples, it would be subjectively concluded that fragmentation is typical of nonmeat products and is the cause of the difference in texture. Image analysis again showed that, contrary to appearances,

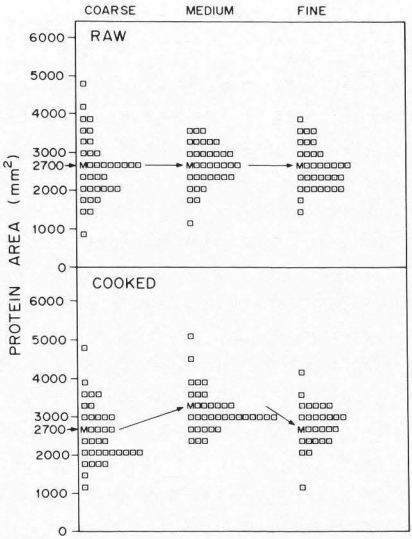

Fig. 4 Mean values and variation in area of photomicrographs occupied by protein, as affected by chopping and cooking a commercial wiener formulation.

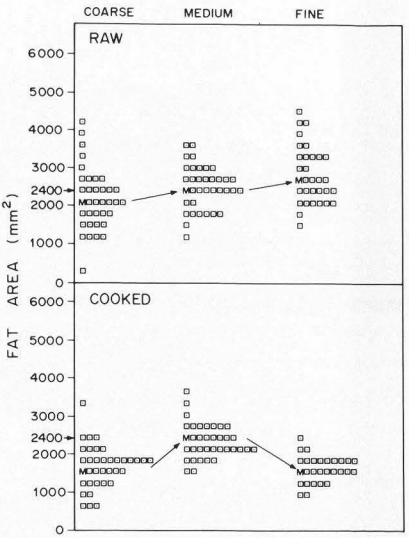

Fig. 5 Mean values and variation in area of photomicrographs occupied by fat, as affected by chopping and cooking a commercial wiener formulation.

the number of "features" (in this instance bits of protein) was not statistically different and the "average" size of the protein features was nearly the same. That is because the apparent "honeycomb" picture shown as Fig. 10a contained a number of very small features. As it was with the fat phase in the all meat products, the apparent differences in the protein strands could be measured only by the interaction of protein area (Fig. 8) and protein perimeters shown in Fig. 11. The coarse batter that had the largest protein area also had the smallest protein perimeter of the four batters. In other words, the large protein mass which occupied 4200 mm^2, as quantified in Fig. 8 and illustrated in Fig. 10b, had a perimeter of only 1650 mm. The finely ground batter that had only 57% as much protein area (2400 mm^2) had as much perimeter as the coarse batter (1800 mm). This high ratio of perimeter to area was the true measure of the smaller size of the protein features in the fine batter shown in Fig. 10c. The "control" batter (Sample 1, Fig. 10a) and the medium batter (Sample 3, Fig. 10e) were similar in both area and perimeter (Fig. 11). Thus what was seen pictorially was caused by chopping, but it was apparently not related to firmness. The two that looked most alike (the control and the medium batter) produced wieners that were widely different in texture. Two that looked quite different (the coarse and medium batters) produced wieners that were texturally similar.

Any emphasis on the fat component at the expense of the protein component seemed unwarranted because it was the protein that was substituted. In this study, the behaviour of the fat phase was like a mirror image of the changes in the protein phase (Fig. 12 and Fig. 13). During chopping, the area occupied by fat was initially less in the coarse batter than in the all meat control and then it expanded in the fine batter (while the protein first expanded and then shrank). After cooking, the only anomaly was the very large contraction of the fat in the finely ground product, from 2400 mm^2 to 1200 mm^2 (mean values).

This finely chopped product was unique. It appeared to be composed of protein fragments (shown in Fig. 10d) enclosed in a continuous fat phase rather than the conventional view of a wiener (Fig. 2) as fat particles dispersed in the continuous protein phase. In spite of a total inversion of the accepted model this product did not lose more fat during cooking, as measured by total shrink, than the other batters. Although no conclusion should be drawn from this single

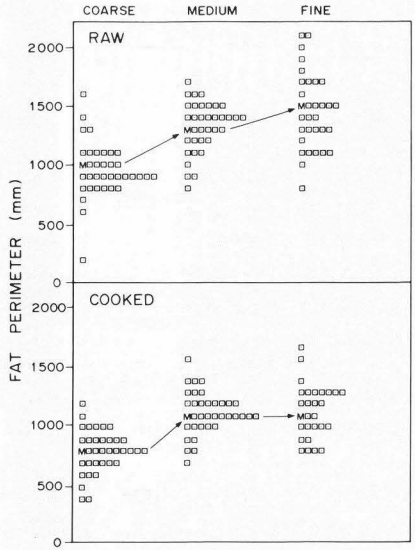

Fig. 6 Mean values and variation in total perimeter of the fat in photomicrographs, as affected by chopping and cooking a commercial wiener formulation.

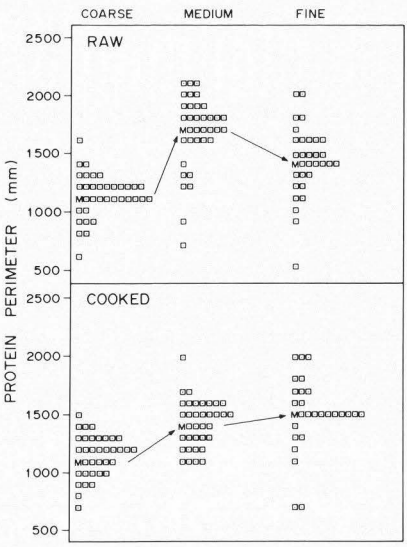

Fig. 7 Mean values and variation in total perimeter of the protein in photomicrographs, as affected by chopping and cooking a commercial wiener formulation.

example, the good cook stability of this visually peculiar product tends to support the school of thought espoused by Randall et al. (1976) and Comer (1979), who placed more emphasis on the properties of the protein than on the fat emulsions.

Summary

Although the microstructure of wieners has been studied for more than twenty years, more discussion seems to have centered on the use of model systems than on studies of raw batters or cooked wieners. It was Hansen's 1960 work with model systems, rather than his study of product microstructure, that helped Saffle (1968) and others achieve an understanding of the cook stability problem, which in turn led to the development of computer controlled least-cost formulations; and it was Hansen's model that has been challenged by others such as Comer (1979).

Studies in this laboratory were commissioned to provide a visual dimension to empirical experiments conducted by industry on wieners containing nonmeat protein. A relationship between microstructure and wiener texture was an implicit goal rather than an explicit requirement. The first decision which had to be made concerned the selection of an appropriate magnification. In the first phase (Kempton et al. 1982) a major distortion in wiener microstructure caused by the presence of nonmeat protein was observed at 30x. Unless this crude "clumping" of ingredients could be overcome, there was no practical reason to examine similar amended products under higher magnification. For comparative studies, it is suggested that the lowest magnification capable of detecting differences in structure should be used; and that the significance of these differences be reported in the statistical sense, which requires an objective sampling plan. However, pattern analysis described the problem but offered no solution. Higher magnifications obtainable by electron microscopy could be used to conduct a basic study of this aberration, which could ignore those portions of the wiener not affected.

The second decision was to give equal emphasis to the fat and protein phases. The most significant change in wiener technology in recent years has been the replacement of some of the meat proteins by vegetable, milk, egg and single-celled protein. It would seem logical to place more emphasis on the protein matrix than had been done in the past. No doubt existed regarding the

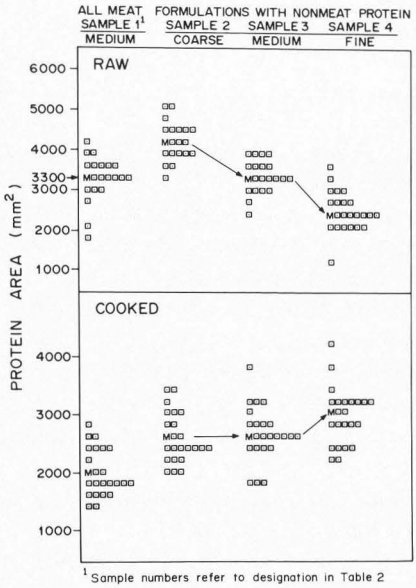

Fig. 8 Mean values and variation in area of photomicrographs occupied by protein, as affected by chopping and cooking experimental wiener formulations.

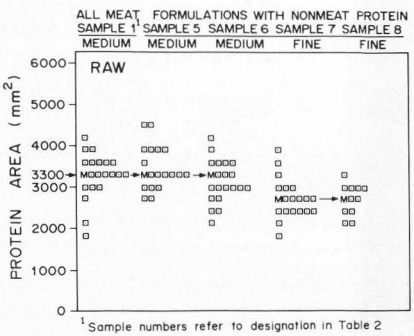

Fig. 9 Similarities in the area of photomicrographs occupied by protein in various experimental wiener formulations chopped the same amount. Samples 1,5 and 6 were "medium" batters. Sample 7 and 8 were "fine" batters with nonmeat protein added late in the chopping regime.

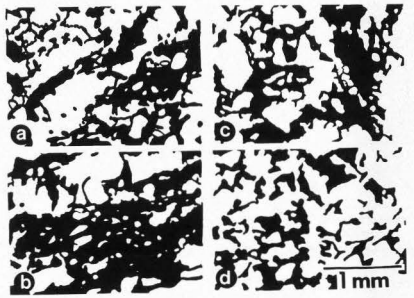

Fig. 10 Photo a: Protein component of all meat control (Sample 1). Photo b: Protein component of coarsely-chopped wiener batter containing non-meat protein (Sample 2), showing thick strands occupying large area of photograph. Photo c: Protein component of medium chopped Sample 3: not visibly different from all meat Photo a. Photo d: Protein component of finely chopped wiener batter containing nonmeat protein (Sample 4). Matrix appears fragmented.

need for salt-soluble protein to impart cook stability, but the size of the fat droplets, or globules, became common currency in studies on microstructure of wieners, from Froning et al. (1970) to Carroll and Lee (1981). Yet the problem with wieners containing nonmeat proteins is not stability, but firmness (Table 2). Comer (1979) dealt with both properties simultaneously as they are affected by proteins. Cassens and Schmidt (1979), while being careful not to claim a cause and effect relationship, did show that texture was related to the size of the fat entities. Therefore, both phases were given equal attention by using image analysis.

The nature of open spaces or voids has been addressed by Carroll and Lee (1981). They are either air spaces or trapped water droplets, which one, cannot be determined after the specimen has been prepared for microscopic study. Many are large enough to be considered as features of the macrostructure and can be seen by the unaided eye when a wiener is sliced. In microstructure, at 30x they can fill some fields of view completely and they will be present in fields selected objectively. They, too, can be quantified by image analysis.

Image analysis showed several differences which were not obvious from visual assessment. In both experiments, there were greater differences between the behaviour of proteins than between the fat distribution patterns. However, image analysis failed to separate the amended products from their all meat control on the key factor of firmness.

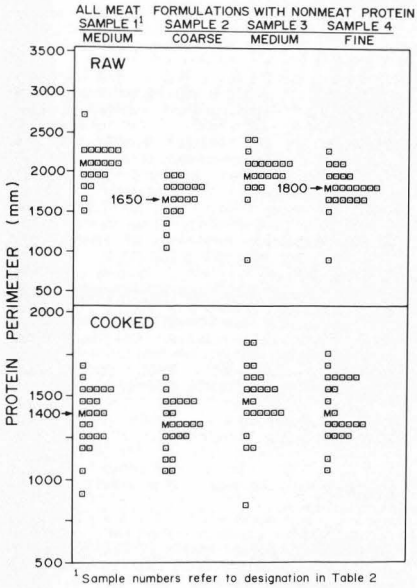

Fig. 11 Mean values and variation in total perimeter of the protein and photomicrographs, as affected by chopping and cooking experimental wiener formulations.

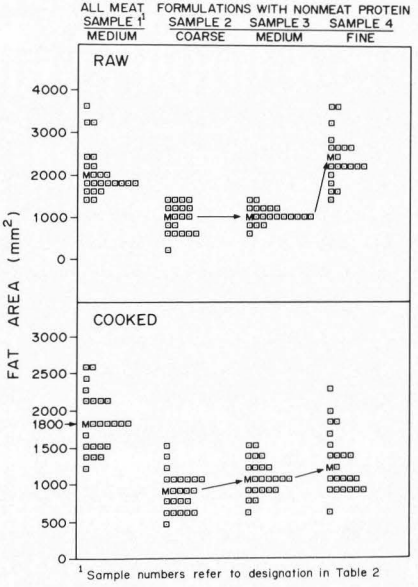

Fig. 12 Mean values and variation in area of photomicrographs occupied by fat, as affected by chopping and cooking experimental wiener formulations.

The loss of firmness in products containing vegetable proteins may depend on the physical and molecular structure of the protein, and thus the mechanism may be beyond the capacity of any microscopic study. It is suggested that this be tested by further study of the morphology of the protein phase under more controlled conditions. Slices of raw batters and cooked wieners fixed on microscope slides can be defatted by a 20% solution of chloroform in ethanol without distorting the protein matrix (Kempton, unpublished). This eliminates the need to trace the protein in a multicomponent system; and it is now possible to project the image directly onto a screen without taking photographs. Chopping should be done in a Mince Master, which is used in industry more widely than a silent cutter; but the chopping time variable could be excluded. The samples should be immersed in liquid nitrogen to eliminate the distortion caused by freezing. The point is, that image analysis is objective rather than subjective and if differences among samples that vary in firmness can be detected at 30x the use of higher magnifications should be temporarily shelved. However, if the results of the proposed study confirm the similarity observed in this preliminary survey, the magnification could be increased to the limit of the light microscope using the same program of image analysis providing the number of fields analyzed was increased sufficiently to establish reliable mean values and describe the distribution about the means.

Acknowledgements

This work was performed under contract with Agriculture Canada (Contract Serial OSU78-00130). The basic language program for the image analysis was developed by J. Terry, Department of Materials Chemistry, Ontario Research Foundation. Dr. G.W. Bennett, Statistical Consultant in the Dept. of Statistics, University of Waterloo, performed all statistical evaluations. This work is dedicated to Dr. John Quinn (deceased) of Agriculture Canada who provided inspiration and encouragement.

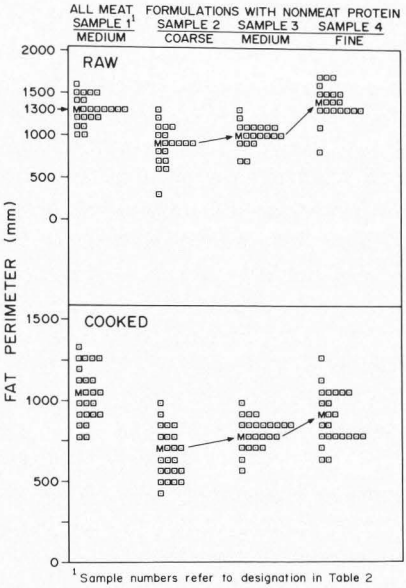

Fig. 13 Mean values and variation in total perimeter of the fat in photomicrographs, as affected by chopping and cooking experimental wiener formulations.

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Discussion with Reviewers

Reviewer I: Although this paper is unique in introducing image analysis to food microstructure, there are serious flaws. One is the selection of samples with little or no control over significant variables such as using samples prepared with different sources of meat protein. Another is the failure to avoid the distortion that results from storage at -18°C by freezing in liquid nitrogen and sectioning immediately.

Reviewer II: A very large number of products of different compositions and chopping protocols are being considered. Is this a realistic number of variables to be analysed within the limitation of sample number, etc. of this paper?

Authors: Theno and Schmidt (1978) and Cassens and Schmidt (1979) showed that theories relating wiener microstructure to performance characteristics must encompass the variation that exists among commercial brands, and Comer (1979) showed that the model developed by Hansen (1960) did not explain the performance of wieners containing nonmeat protein. Our introductory survey was intended to introduce image analysis to the study of food microstructure, and it was purposely designed to include as many variables as are likely to be encountered in further studies. There are probably some other variables in commercial wieners which are proprietary, such as variation in least-cost formulations. When all the principal variables have been identified, it may become possible to use multiple regression to develop a predictive mathematical model that will relate wiener microstructure to texture, and image analysis (or preferably image processing) is a method of obtaining the numerical values necessary to construct such a model. The primary purpose of this paper was to demonstrate the type of information that can be obtained from image analysis, and the text has been amended to explain why freezing samples in liquid nitrogen was not possible. An example of the distortion caused by freezing to -18°C was that the protein matrix surrounding some fat globules was sometimes cracked and occasionally discontinuous. However, the key parameters of total area and total perimeter of the fat and protein features were not seriously affected. Cracks in the protein matrix might have been serious if the emulsion theory had been the target of this study; but Borchert et al. (1967) had already shown that such cracks in the protein "membrane" occurred during cooking without causing fat loss.

E.W. Ross: Occasionally, bimodal histograms are seen, as in the cooked, all meat sample in Fig.8. Can a visual interpretation be given for such cases?

Authors: The direct answer to your question is, no it cannot. By the time we had progressed to the study of products containing some vegetable proteins, the number of photographs subjected to image analysis had been reduced to 24 because of the relative homogeneity of the samples. Additional photographs may have re-established a more normal distribution about the mean values, but the practical difference in all meat samples in Fig. 8 is the decrease in the meat protein area, from 3300 mm^2 in the raw product to 2000 mm^2 in the cooked product. The significant difference between means seemed more important than distribution about mean values. Fig. 9 also contains bimodal and skewed histograms but the mean values (denoted by M) were identical within treatments. We would have been very concerned if the difference between medium and fine chopping in Fig. 9 had been obscured by variation in the shape of the histogram; but once again the consistency of mean values within a set seemed to be more important than the distribution of values about the means.

E.W. Ross: Since areas and perimeters were obtained for each feature in each drawing, it might have been possible to calculate for each feature the quantity: $\Sigma = 1\text{-}4\pi \cdot (\text{area})/(\text{perimeter})^2$ which is a measure of the "out of roundness" of the feature.

Authors: We are aware of the potential for shape measurements such as rectangularity, circularity and invariant moments, but not of the comparatively simple equation you have given us. We did not measure shape because we feared that this was a parameter that was affected by the constraints that let us to examine samples frozen to -18°C (which did not affect size measurements by area and perimeter) instead of using samples frozen in liquid nitrogen.

E.W. Ross: In Table 2, the HSD is given for texture, but not in Table 1. Why?

Authors: The data in the Tables were supplied by our industrial and governmental colleagues. The methods for determining texture and the methods used to express statistical differences are not standard across the meat industry.

R.J. Carroll: You may wish to explain the difference between image analysis and pattern analysis for readers not knowledgeable in this area.

Authors: This is a difficult question to answer briefly. We recommend that those not familiar with these terms read Castleman KR (1979) Digital Image Processing, Prentice-Hall Inc., Englewood Cliffs, N.J. The term "image analysis" was used in the title of this paper to imply the use of a high-quality image digitizer as distinguished from image processing which requires a high-

quality image display device as well (Castleman, 1979, page 13). Pattern analysis, used in the text of this paper, usually refers to a specific statistical test such as the two-within-four randomization test used by Kempton et al. (1982) to analyze spatial patterns in wiener batters. "Pattern recognition" is the accepted term for both aspects of this field of study. Cassens et al. (1975) recognized that the ingredients of wieners containing soy flour had a different distribution pattern than wieners containing only meat proteins, when viewed under a light microscope. Essentially, Kempton et al. (1982) digitized these patterns, meaning that the difference in patterns observed through the microscope could be expressed numerically. Castleman (1979) devoted two chapters (pages 299-346) to "statistical pattern recognition as applied to digital images," which he considered as little more than an introduction to the subject. Directions are given on how to select the "features" (fat and protein in our system) and how to measure size (from which we selected area and perimeter). To answer the question as briefly as possible, "pattern analysis" involves comparisons among photographs and "image analysis" determines what is in the pictures. Both procedures express microscopic observations in numerical terms.