

Comminuted Meat Emulsions—The Capacity of Meats for Emulsifying Fat

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SUMMARY

A method of emulsifying liquefied fat in saline suspensions of meat or meat proteins was used in investigating the factors that influence the capacity of meat to emulsify fat, the characteristics of emulsions, and the relative efficiency of proteins as stabilizers. The capacity of meat, comminuted to an optimum extent, was increased by increasing the proportion of saline phase, increasing the rate of addition of fat, and decreasing the rate of mixing and the temperature (18°C was the lowest investigated). The effects of variables corresponded with concepts of emulsion theory and technology, especially those pertaining to emulsions stabilized by rigid membranes. Emulsions of marked viscosity and stability to heating were prepared. Saline solutions of water-soluble and salt-soluble proteins were also used in preparing emulsions. The salt-soluble proteins were more effective than those extracted by water. The participation of the latter proteins was dependent on the addition of sodium chloride. The efficiency of salt-soluble proteins varied inversely with concentration, and was at a maximum when emulsifying 1.75 ml fat/mg protein. During emulsification, up to 84% of proteins in solution became insoluble. A series of recommendations was advanced pertinent to processing and needed future research.

PRESENT METHODS of manufacturing comminuted sausage, such as frankfurters and bologna, have an empirical basis. These generally perform satisfactorily, but adequate information is lacking for solving processing problems, or improving either products or processing. This especially applies to problems in emulsion curing, the most important as well as the most complex operation in sausage making.

Emulsion curing, as generally conducted, involves 8–10 min of comminution, which converts ice, lean and fat meat, and curing and flavoring agents into batches of batter or meat emulsion. One of the principal problems is binding fat properly. A practice that minimizes this problem, used more in the past than now, consists of limiting the comminution of fatty tissues. This reduces the dispersion of fat and the need for emulsifying it. Recently, however, there has been a trend toward comminuting both lean and fatty materials much more finely. Moreover, the advent of continuous "emulsion curing" is at hand. As a result, comminution will be accomplished not only thoroughly and rapidly, but without the close control possible in batch operations. Efforts to perfect these developments direct increasing attention to the inadequacy of knowledge on the mechanisms of emulsion curing.

A recent investigation of meat emulsions (Hansen, 1960) indicates that fat exists in the emulsions partly

in the form of dispersed fat globules enclosed in matrices formed by protein membranes. A basis for understanding the mechanism involved was provided in 1840, by Ascherson, who observed that "... coagulation in the form of a membrane occurs inevitably and instantaneously when albumin comes in contact with a liquid fat." Subsequently, extensive information has been obtained relative to emulsions in mayonnaise, salad dressings, margarine, and milk, and to means of improving emulsification in producing these foods (Becher, 1957). The need in meat processing is a comparable knowledge of emulsification in emulsion-based meat products. The following experiments were undertaken to obtain information on factors affecting the capacity of meat to stabilize emulsions, the characteristics of meat-stabilized emulsions, and the efficiency of meat proteins as stabilizers.

EXPERIMENTAL

Meat materials consisted of *semimembranosus* and *semitendinosus* bovine muscles removed from carcasses 1–2 hr after slaughter, stored 1–3 days at ca. 3°C, and then trimmed free of fat, and ground. Data presented are averages of duplicate determinations; other observations were confirmed by one or more replications.

Factors affecting the capacity of meat to stabilize emulsions. The results of preliminary experiments indicated that (a) full employment of the capacity of meat to emulsify fat required thorough disintegration and dispersion of tissue in a relatively dilute system, and (b) collapse of an emulsion could serve as an "end point" in determining the capacity of meat in stabilizing emulsions. Six series of experiments were then conducted to determine the effects of varying conditions on the relative capacity of meat to stabilize emulsions, and the character of the emulsions produced. These involved the basic method described, and variations subsequently specified.

Basic method. Fifty g of ground meat were placed in a 1-pt. jar (diam 7.3 cm), 200 ml of cold 1M NaCl (0–5°C) were added, and the mixture was comminuted 2 min in a Servall Omni-mixer (blade span 5.7 cm) at ca. 13,000 rpm (trade names are mentioned only for identification, implying no endorsement). During the operation, the jar and contents were cooled by immersion in an ice bath. Twelve and five-tenths grams of the resulting slurry were placed in another jar, 37.5 ml of cold 1M NaCl (0–3°C) were added, and the two were mixed for a few seconds in the Omni-mixer at ca. 1000 rpm. Fifty ml of melted lard (30°C) were then added from a graduated cylinder. High-speed cutting-mixing (ca. 13,000 rpm) was begun. Immediately thereafter, melted lard was added at a rate of about 0.8 ml per sec from a graduated separatory funnel through Tygon tubing into the stirred mixture. Emulsion formed, persisted, and finally collapsed, the transition being marked by a gradual increase, followed by a sudden decrease, in viscosity. Addition of fat was immediately terminated on observation of the abrupt transition. Occasionally, near the "end point," rigid emulsions formed that resisted mixing, although the above conditions were chosen to

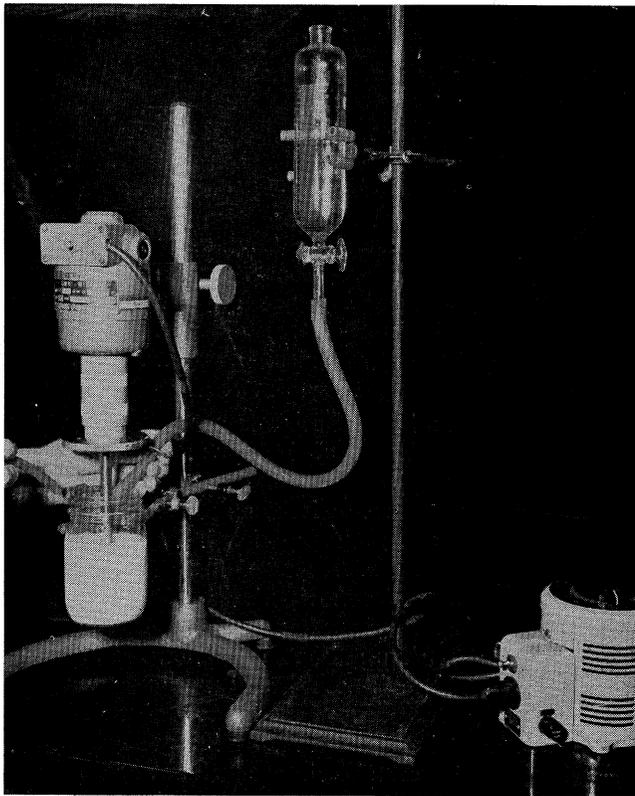


Fig. 1. Arrangement of apparatus for emulsification.

minimize this. In these instances it was necessary to assist the flow of mixture manually into the blades of the Omni-mixer, using a short section of tubing. The volume of fat added, 50 ml plus the additional fat withdrawn from the separatory funnel, just exceeded the emulsifying capacity of the meat sample under the conditions of the method, and is reported as emulsifying capacity, ml fat emulsified/2.5 g meat. Fig. 1 shows the apparatus used.

Comminution of meat in 1M NaCl. Mixtures, consisting of 50-g portions of a sample of ground beef and 200 ml cold 1M NaCl, were converted into slurries by comminution for 1, 2, 3, or 4 min. Emulsions were then prepared from them as described in the basic method.

Dilution of meat slurry. Samples of meat slurry, prepared from a sample designated A, were used in preparing emulsions by the basic method, or by variations in which 12.5 or 87.5 ml 1M NaCl, rather than 37.5 ml, were used to dilute 12.5 g of slurry. In addition to determining emulsifying capacity, observations were made of the general character of emulsions.

Emulsification at different rates of mixing. Samples of meat slurry were used to prepare emulsions by following the basic method, except that emulsions were also prepared with the Omni-mixer operated at ca. 9,200 and 6,500 as well as at 13,000 rpm. These results were obtained with a meat sample designated B.

Addition of fat at different rates. Fat was emulsified with a series of portions of a meat slurry using the basic method, except that the rates of addition of fat were 0.48, 0.57, 0.77, or 1.05 ml per sec.

Temperature. The capacity for fat emulsification of a series of 12.5-g samples from a meat slurry was determined by the basic method, except that maximum temperatures attained during emulsification were varied to include 18.0, 21.5, 32.0, 36.5, 44.5, and 46.0°C. This was accomplished by varying the temperature of the fat used in preparing the emulsions and that of the water bath in which the jars were immersed. Cottonseed oil was used as fat in measurements at 18.0 and 21.5°C.

Stability and rigidity of emulsions. Emulsions were prepared by the basic method except that the procedure was terminated when emulsions attained maximum viscosity. These were used

in limited tests of properties related to stability. Included were heating emulsion 30 min at 75°C in a large test tube, storing 7 days at ambient laboratory temperatures, and centrifuging at 1300×G. A small quantity of viscous emulsion was diluted with 1M NaCl, placed on a slide, and stained with a 1:10 dilution of Lillie-Mayer's hematoxylin solution (Lillie, 1954). A photomicrograph of the stained emulsion was prepared, and the average diameter of fat globules was calculated.

Emulsions prepared with meat protein extracts. Extracts of *longissimus dorsi* muscles were prepared as follows: One hundred grams of ground tissue were homogenized in the Omni-mixer with 100 ml of water at approx 5°C. The slurry was centrifuged 30 min at ca. 3000 rpm in a Servall Model SP centrifuge, and the resulting supernatant layer was decanted. The residue was successively re-extracted in this manner with 75-, 50-, and 50-ml portions of water. The extracts were combined and then clarified by an additional centrifugation.

The extracted residue was comminuted 1 min at 13,000 rpm with 200 ml 1M NaCl at 3–5°C. The resulting mixture was stored overnight at 2°C. Two hundred ml of additional 1M NaCl were added and thoroughly mixed, and the diluted mixture was again centrifuged as described. The supernatant was decanted and diluted with an equal volume of 1M NaCl. Finally, it was centrifuged 15 min at 15,000 rpm in a Servall Model SS-3 centrifuge to remove heavy matter.

Emulsifier efficiency. Fifty-ml portions of each of the two extracts and dilutions of these, were used as the aqueous phase in preparing emulsions by the basic method. Solid NaCl was added to the aqueous extracts to adjust them to 1M NaCl before their use. Initially this was done for the practical reason that salt is present in all emulsion-cured meat products. Subsequently, it was found that water-soluble proteins had no marked capacity as stabilizers in the absence of salt. The broken emulsions were centrifuged to clarify the saline layer. Aliquots of the saline layers were withdrawn for analysis. Total N and nonprotein nitrogen were determined in both the original and the recovered extracts. Nitrogen determinations were made by the micro-Kjeldahl method (Ma and Zuazaga, 1942). Non-protein was determined on fractions soluble in 5% trichloroacetic acid (Hiller and Van Slyke, 1922). Protein changes were calculated from the data obtained. Sedimentation data of components of the original and the recovered extracts from an additional, similar experiment were obtained with the ultracentrifuge. The amounts of proteins in the latter solution were: original saline protein solution, 3.81 mg/ml; after emulsification 0.59 mg/ml; original water-soluble protein solution, 10.65 mg/ml; after emulsification, 3.56 mg/ml.

Membranes formed by water-soluble and salt-soluble proteins. Emulsions of relatively low viscosity were prepared by emulsifying only 75-ml portions of fat with 50 ml of the extract of water-soluble proteins (made to 1M NaCl by addition of crystalline salt) or with 50 ml of the extract of salt-soluble proteins. After mixing for 10 sec at 13,000 rpm, the emulsions were diluted with 5 volumes of water. Slides were dipped into the preparation, and one side of the slides was wiped clean. Several drops of a 1:10 dilution of Lillie-Mayer's hematoxylin stock solution were added (Lillie, 1954), and photomicrographs were prepared of the stained emulsions.

RESULTS AND DISCUSSION

Factors affecting the capacity of meat to stabilize emulsions. Table 1 shows the results of varying the time of comminution of meat in 1M NaCl in the prepa-

Table 1. Effect of differences in time of comminution of meat slurry.^a

Minutes at 13,000 rpm	Emulsifying capacity/2.5 g tissue
	ml
1	137
2	148
3	133
4	131

^a 50 g tissue in 200 ml 1M NaCl.

ration of slurries. The data indicate that capacity to emulsify fat was influenced somewhat by varying comminution. Emulsification of fat was maximum with the slurry prepared by comminuting 2 min. Comminution for only 1 min apparently failed to disintegrate tissue sufficiently. The fact that samples comminuted 3 or 4 min exhibited a lower capacity than those comminuted 2 min suggests that some protein denaturation occurred during comminution, its effect becoming apparent only when optimal (2 min) comminution was exceeded.

Table 2 shows the data obtained by varying the dilution of the slurry containing the meat. As the total volume of saline solution increased, the increasingly diluted 2.5-g meat content was capable of emulsifying an increasing amount of fat. One factor accounting for this result is the high efficiency typical

Table 2. Effect on emulsifying capacity of diluting emulsion and varying rate of mixing.

Meat sample	Total volume 1M NaCl/ 2.5 g tissue	Rate of mixing	Emulsifying capacity	Description of emulsion
	ml	rpm	ml	
A	22.5	13,000	82	Semi-solid
	47.5	13,000	127	Viscous, mixable
	100.0	13,000	179	Moderately viscous
B	47.5	13,000	142	Viscous, mixable
	47.5	9,200	168	Slightly viscous
	47.5	6,500	185	Grainy suspension

of salt-soluble proteins at low concentrations, as shown later. The dilution also affected viscosity. This would be expected on the basis of the recognized influence on viscosity of volume concentrations (Becher, 1957). Because of the marked viscosity, but general responsiveness to mixing, of emulsions prepared from 12.5 g meat slurry diluted with 37.5 ml 1M NaCl, "end points," or the breakdown of emulsions, were most readily detected using these proportions.

Table 2 also shows the results obtained by emulsification with different rates of mixing and stirring. The data indicate that varying this rate markedly affected both the amount of fat emulsified and the character of the emulsions produced. An increase in viscosity with increasing rate of shear has been shown to be associated with a decreased particle size in emulsion systems (Becher, 1957). The breaking points of the viscous, but mixable, emulsions prepared at 13,000 rpm were readily detectable.

Figure 2 shows the results obtained from adding fat at different rates during emulsification, plotted to show the relation between the amount of fat emulsified and rate of addition. The relationship was linear ($r = 0.995 \pm .004$, $P < .001$), indicating that the amount of fat emulsified increased as the rate of fat addition increased. An explanation of this result can be advanced by assuming that the rate at which protein membranes form approaches "instantaneous" and that vigorous mixing-stirring more than assured adequate dispersion of fat and intermixing of components. Under these circumstances, rate of emulsification would not be a limiting factor. In this case, increasing the rate of addition of fat would more fully utilize the potentially rapid rate of emulsification

FAT EMULSIFIED WITH CHANGE IN RATE OF ADDITION

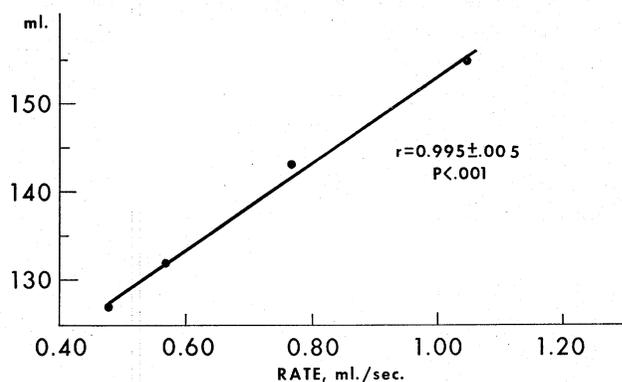


Fig. 2. Influence of rate of addition on amount of fat emulsified.

while progressively tending to reduce damage to protective membranes produced by unnecessarily extended mixing.

Fig. 3 shows the data obtained in investigating emulsification at different temperatures, plotted to show the amounts of fat emulsified by the samples as a function of maximum temperature. The amount of fat emulsified was inversely and linearly related to the maximum temperature attained during emulsification ($r = 0.995 \pm .003$, $P < .001$). In view of evidence of the stability of emulsions at 75°C, presented later in this paper, heating at temperatures in the range 18–46°C should not cause emulsions to break down. Rather, anticipating an effect associated directly with the process of emulsification appears warranted. Factors possibly contributing to this unresolved "temperature effect" include (a) a chain of events leading from increased temperature to decreased surface tension of the fat, increased dispersion and surface area, and, consequently, an increased need for stabilizing membranes; (b) denaturation of protein prior to formation of protective membranes; and (c) altered formation of membranes.

FAT EMULSIFIED WITH CHANGE OF TEMPERATURE

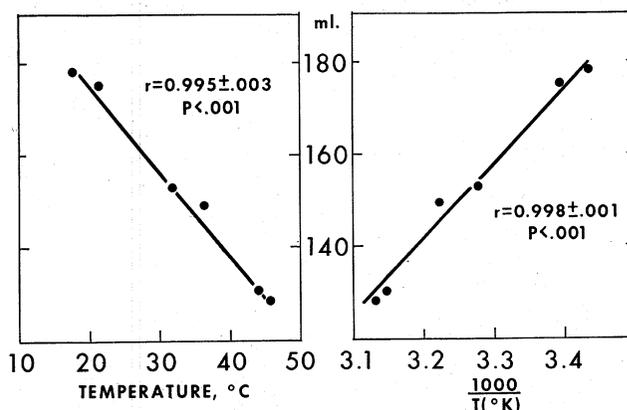


Fig. 3. Influence of maximum temperature attained on amount of fat emulsified.

Data obtained on the stability and rigidity of meat emulsions indicate that fat was well bound in them. Storage for a week at ambient laboratory temperatures produced no observable evidence of breakdown. Centrifugation at 1300×G separated some water but no fat. Heating 30 min at 75°C produced no separation of fat. Fig. 4 is a photograph of a sample so heated and then cooled to 30°C. The high viscosity of uncooked emulsion was converted into the rigidity of the column of cooked emulsion. The fat globules shown in Fig. 5 had an average diameter of 18 μ . The exterior surfaces of fat globules outlined by stained protein membranes had a non-uniform polyhedral form. According to Becher (1957), such forms result from deformation of the spherical globules usually obtained at a high concentration of disperse phase.

The rigid character of the membranes pictured in Fig. 5 is indicated by the manner in which the polyhedral form of stabilized globules was retained after dilution. This is evidence that the films formed by the meat proteins behaved like those described by Dean (1948), who stated that "some proteins are sorbed and denatured at oil-water interfaces, and the denatured film has considerable rigidity." He further stated that "an emulsion stabilized by rigid films may be very stable so long as it is left undisturbed. It may have a long 'shelf life,' but since rigid films are brittle, such emulsions may have a very poor 'service life.'" Consequently, owing to their rigidity, the susceptibility of meat protein films to mechanical damage can be predicted. In sausage manufacture,

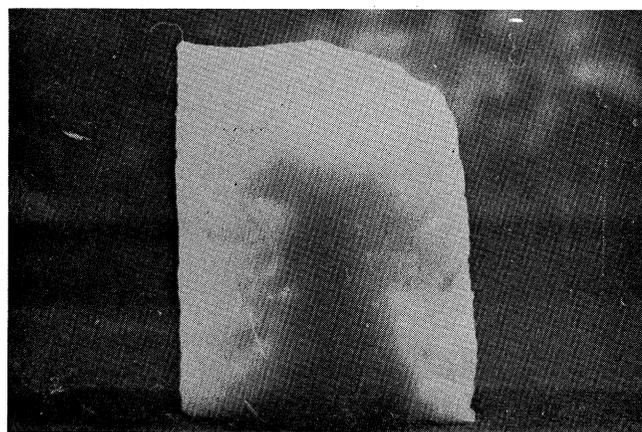


Fig. 4. Emulsion at 30°C after heating 30 min at 75°C.

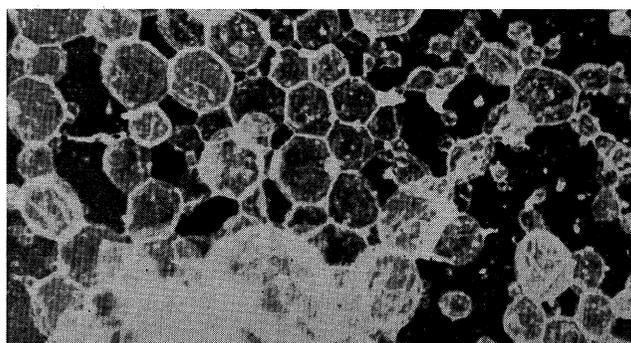


Fig. 5. Fat globule membranes formed in emulsion prepared from meat (800×).

poor "service life" would be reflected in the inability of emulsions to withstand reworking or extended comminution, as in overchopping.

Emulsions prepared with meat protein extracts. Table 3 shows data obtained in preparing emulsions stabilized with protein extracts, including the results of analyses and calculations made relative to the role of water-soluble and salt-soluble proteins. The results indicate that both water- and salt-soluble proteins were capable of stabilizing emulsions. The efficiency of water-soluble proteins, in terms of ml of fat emulsified per mg protein, did not vary to any appreciable extent with changing concentration, in contrast to the increasing efficiency of the salt-soluble proteins on dilution. The efficiency of the salt-soluble proteins tended to vary inversely (and curvilinearly) with respect to both the amounts of protein removed from solution and the original content of protein. Because of this tendency, results obtained with the basic method can be only relative estimates of emulsifying capacity. The data also show that the percentage of proteins removed from solution varied from 59.5 to 75.0% for water-soluble proteins, and from 79.8 to 84.1% for salt-soluble proteins. Hence, a relatively large part of the potential emulsifying capacity of the proteins was used in all cases, assuming that removal from solution signified use in emulsification. The relatively complete recovery of non-protein substances from the solutions used in emulsification indicates that these were not involved directly.

Fig. 6 shows the patterns obtained by ultracentrifugal analysis of extracts, before and after use in emulsification. These indicate that an appreciable

Table 3. Effects of varying type and amount of proteins on emulsification.

Protein group	Experiment No.	Changes in nitrogenous substances										Fat emulsified	
		Original solution			After emulsification			Differences		Protein removed from solution (N×6.25)		Volume	Emulsifier efficiency ^a
		Total N	NPN	Protein N	Total N	NPN	Protein N	NPN	Protein N	Total	% of original		
		mg	mg	mg	mg	mg	mg	mg	mg	mg	%	ml	ml/mg
Water soluble	1	173.0	68.0	105.0	111.0	68.5	42.5	0.5	62.5	390.6	59.5	176	0.45
	2	121.0	47.5	73.5	71.5	48.0	23.5	0.5	50.0	312.5	68.0	132	0.42
	3	69.0	27.0	42.0	37.0	26.5	10.5	-0.5	31.5	196.9	75.0	94	0.48
1M NaCl soluble	1	86.7	4.8	81.9	22.8	6.5	16.3	1.7	65.6	410.0	80.7	248	0.61
	2	60.7	3.4	57.3	17.6	6.0	11.6	2.6	45.7	285.6	79.8	220	0.77
	3	34.6	1.9	32.7	10.2	5.0	5.2	3.1	27.5	171.9	84.1	194	1.12
	4	17.3	1.0	16.3	5.6	2.5	3.1	1.5	13.2	82.5	80.9	144	1.75

^a Fat emulsified, ml/mg protein removed from solution.

amount of salt-soluble proteins and a smaller amount of water-soluble proteins were withdrawn from their respective solutions during emulsification. The patterns differed only quantitatively, except that a very small heavy fraction was completely removed from solution during emulsification. Calculations based on the chemical data showed that 84.5% of salt-soluble proteins and 66.6% of water-soluble proteins were removed from solution during emulsification. These, as well as the results in Table 3, indicate that salt-soluble proteins were utilized more completely than water-soluble proteins. Possible variations in effectiveness with varying ionic strength, or pH, and the effectiveness of the two classes of proteins when acting simultaneously, remain to be investigated.

FAT EMULSIFICATION

Effect on Protein Solutions

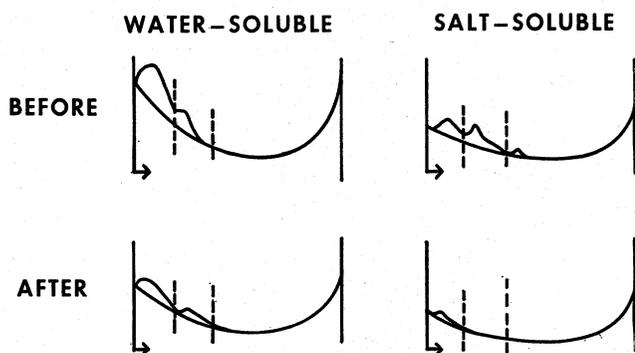


Fig. 6. Sedimentation of salt-soluble and water-soluble proteins in solutions before and after emulsification.

Fig. 7 and 8 are photomicrographs prepared from the stained emulsions stabilized by the water-soluble and salt-soluble groups of proteins. Examination indicates that the membranes formed by salt-soluble proteins consisted of thicker layers than those formed by water-soluble proteins. Hansen (1960) failed to obtain evidence of membranes formed from water-extracted proteins; unlike the present case, however, salt was not added to the emulsion mixture in his work. As noted in the foregoing, the action of salt appears to enhance the tendency of the water-soluble proteins to stabilize emulsions. This may be explained by hypothesizing that salt altered these proteins, possibly by unfolding their globular structure, to an extent that affected their tendency to form membranes. Under the conditions investigated, even with the addition of salt, the capacity of water-soluble proteins was lower than that of salt-soluble proteins.

CONCLUSIONS

The method employed affords relative estimates of the capacity of meats or meat proteins to stabilize emulsions of liquefied fat. Pending investigation of the emulsification of fats containing various proportions of liquid and solids, the principles governing the

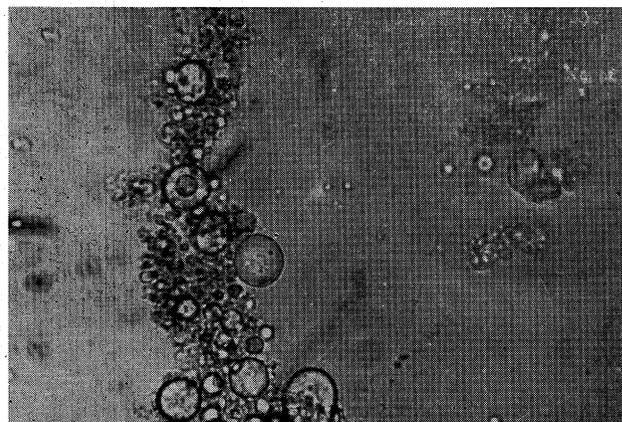


Fig. 7. Fat globule membrane formation in emulsion prepared from water-soluble proteins (800X).

emulsification of these and liquid fat may be assumed to be generally similar. The method merits consideration for determining the relative capacity for emulsion stabilization of the meats used in sausage formulas. For this purpose, a supplementary, or even an alternative, method, based on content of soluble proteins, also appears promising provided a basis is developed to adjust for the unequal capacities of water- and salt-soluble proteins.

The ability of meat to stabilize emulsions was at a maximum with optimal comminution of lean tissue, followed by diluting the external (saline) phase, mixing at low speed, adding fat rapidly, and operating at low temperature (18°C was the lowest investigated). Based on the importance accorded to these findings, the following four facts appear to have been established with regard to the production of meat emulsions. Obtaining a very thorough, but not excessive, disintegration of lean tissue offers a measurable advantage. Meat acts most efficiently when maximally dispersed in water. Coordinating sufficiently rapid mixing with rapid addition of fat employs emulsifying capacity most effectively. Exposure of emulsions to shearing forces should be kept at a minimum once emulsions are stabilized.

Recommendation of an optimum temperature must be deferred. The results indicate that such temperature is 18°C or lower. Investigations must be extended

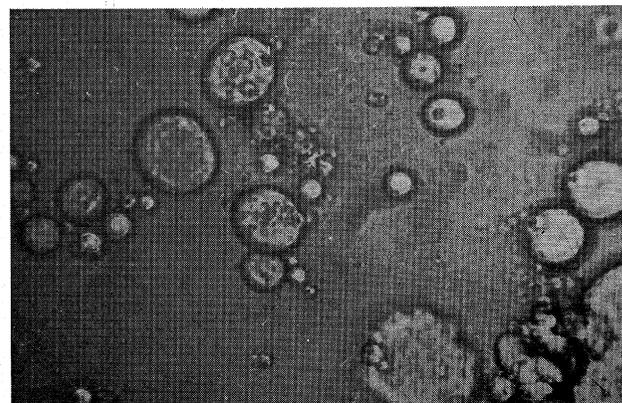


Fig. 8. Fat globule membrane formation in emulsion prepared from salt-soluble proteins (2400X).

to include the lower range of temperatures at which fats exist in varying degrees of solidification.

The results direct attention to differences in the effectiveness of water-soluble and salt-soluble proteins. The evidence indicates that full use of these in the emulsification process will require more information on means of securing maximum activity from both the water-soluble and salt-soluble groups.

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