

The cellular distribution of a free cholesterol emulsion indicates an initial uptake by the parenchymal cell and later by the Kupffer cell. Our studies agree with those of Bailey *et al.* (19) that the uptake of cholesterol as well as of various lipids is a general cellular phenomenon and that it is not an exclusive function of the RES (2, 3).

The late rise in Kupffer cell free cholesterol is suggestive of a role of these cells in cholesterol excretion. Although little direct evidence is available, the concept of Kupffer cell participation in the metabolism or biliary excretion of cholesterol has been suggested by Stambul (20). If the excretory function of Kupffer cells in cholesterol metabolism is correct, then the elevated lipid levels in Kupffer cells in the various lipid storage diseases, as well as atherosclerosis may reflect more a failure in metabolism or excretion of cholesterol than of enhanced phagocytosis. Studies on the quantitation of hepatic cell function in cholesterol metabolism are in progress.

Summary and Conclusions

During alimentary lipemia induced in dogs by the feeding of saturated or unsaturated fats no significant alteration occurred in phagocytic function as indicated by colloidal carbon removal and colloidal gold tissue distribution studies. The cellular distribution of intravenously administered triglyceride indicated that most of the injected triglyceride was found in the isolated hepatic parenchymal cell. The distribution of an intravenously administered free cholesterol emulsion indicated initial localization in the parenchymal cell and a later elevation in the Kupffer cell. These findings suggest that both hepatic parenchymal and Kupffer cells participate in the removal of chylom-

iera; the parenchymal cell has the greatest role. The late elevation in cholesterol content of the Kupffer cell is indicative of a metabolic or excretory function of these cells in cholesterol metabolism.

Acknowledgments

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Determination of Water-Dispersible Protein in Soybean Oil Meals and Flours¹

TWILA M. PAULSEN, K. E. HOLT, and R. E. ANDERSON, Archer-Daniels-Midland Company, Minneapolis, Minnesota

INVESTIGATION of the water-soluble or dispersible-protein portion of soya products began in the ADM laboratories in the early 1930's. The original studies were conducted in an effort to find a laboratory method that would measure the comparative fertilization value of various types of soybean oil meals for use in the tobacco industry. In these experiments an adaptation of the A.O.A.C. Nitrogen Activity method (1) was used. This consisted of taking 0.7 g. of soya product, blending with 10 or 15 ml. of water, and allowing to stand 2 hrs. The blend was then filtered through a filter paper, the residue was washed, and a protein analysis was made on the residue. Although this method lacked in accuracy and precision and was highly empirical, it did show that there was a definite correlation between the water-soluble fraction of the

soya protein and the amount of heat treatment given to the meals during processing. J. W. Hayward (2, 3) subsequently noted a correlation between heat treatment and the nutritional value of soybean oil meals. Therefore this method was established as a processing control tool in the ADM laboratories and was used successfully for many years.

Continued research work on soya flours and meals showed a definite correlation between the water solubility or dispersibility of these products and their use in other industrial applications. As a result, terms such as "Water-Soluble Protein," "Protein-Solubility Index," and "Water-Dispersible Protein" began to appear in many customer and product specifications. It was soon found however that the original methods used for determining protein solubility or dispersibility did not have the necessary accuracy or precision

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to permit the close processing control required by these new product uses.

With many processor and consumer laboratories working on the problem, a large number of method variations were proposed and adopted by these laboratories. Each variation gave a slightly different result.

Comparison of Existing Methods

In 1950 ADM laboratories undertook an exhaustive study of the various methods which had been proposed in an effort to determine which method or methods would give the best correlation between the processing control results and the end-product uses. Since so many different types of methods have been used for studying extraction of protein with water, it seems that a brief résumé of the major methods would be in order.

Indirect Methods

ADM Indirect. Blend a 0.7-g. sample of ground soya product with 10–15 ml. of distilled water and soak for 2 hrs., transfer quantitatively with water to a filter and continuously wash with 200 ml. of water, drain and transfer the filter paper and solids to a Kjeldahl flask, and analyze for nitrogen.

ADM Modified Indirect. Extract a 0.7-g. sample of ground soya product with about 45 ml. of water in a 50-ml. centrifuge tube with continuous mechanical agitation for 30 min., centrifuge, decant supernatant, wash once with water for 15 min. with continuous agitation, centrifuge, decant supernatant, transfer residue, using 25 ml. of concentrated H_2SO_4 and a small amount of water, into a Kjeldahl flask for nitrogen analysis.

ADM Centrifuged and Filtered Indirect. Blend a 0.7-g. sample of finely ground soya product with 10–15 ml. of distilled water in a 50-ml. centrifuge tube and soak for 2 hrs., dilute with 35 ml. of water, stir, and centrifuge. Filter supernatant, and wash filter paper with water several times. Wash solids in centrifuge tube with about 35 ml. of water, stir well, and centrifuge again. Filter supernatant, using the same filter paper as above, and wash with water again. Quantitatively transfer the solids from the centrifuge tube with water to the same filter paper, allow to drain, and transfer filter paper and solids to a Kjeldahl flask for nitrogen analysis.

Direct Methods

Tentative Method of the Soy Flour Association, Revision of December 10, 1946 (4). Extract a 10-g. sample of 100-mesh soy product with 200 ml. of distilled water at 30°C. for 2 hrs. with continuous mechanical agitation, transfer to a 250-ml. graduate, make to volume, centrifuge, and analyze an aliquot of the filtrate for nitrogen.

Quartermaster Food and Container Institute Method (5). Extract an 8.0-g. sample of 100-mesh soy product with 100 ml. of distilled water in a centrifuge bottle, at 80°C., for 30 min. with continuous agitation, centrifuge, and analyze an aliquot of the supernatant for nitrogen.

U. S. Regional Soybean Laboratory Method (6, 7, 8). Shake a weighed amount, usually 2.5 g., of fat-free meal with 100 ml. of distilled water at room temperature for 30 min. on a mechanical shaker, and centrifuge. Use an aliquot of the filtrate for nitrogen analysis. Shake the residue again with another portion

of water for an equal length of time, and analyze the liquid as before. Repeat this process as many times as is considered necessary (usually three).

Waring Blendor Direct Method. Extract a 21.0-g. sample of soy flakes, meal, or flour in 300 ml. of distilled water, using a Waring Blendor at full speed for 10 min., centrifuge, and determine the nitrogen on a suitable aliquot of the supernatant liquid.

Table I summarizes the chief differences in methods described above.

The objectives of this study were to develop a method of analysis for determining "Water-Dispersible Protein of Fat-Free Soya Products" that had greater precision and accuracy, required a minimum of time per analysis, employed a sufficient sample to insure uniformity, minimized the rise in temperature during the extraction, minimized the effect of this increase in temperature on the results, and maintained the use of relatively simple equipment that could be readily standardized.

More than a thousand analyses were made in this comparative study. Results on three typical samples are shown in Figure 1.

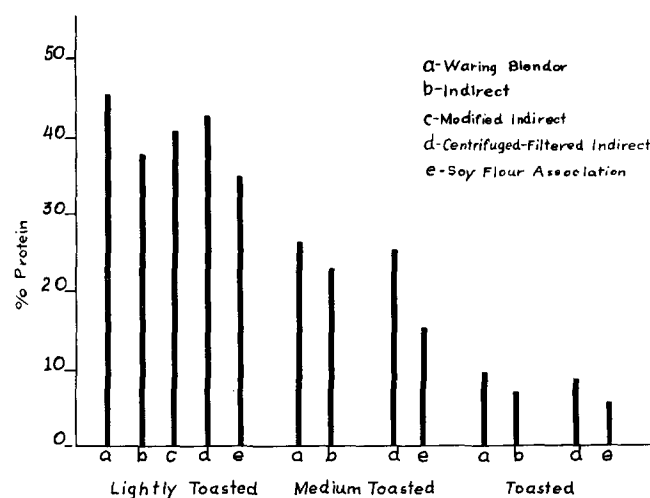


FIG. 1. Comparison of water-soluble protein methods.

The NRRL method, like the ADM Centrifuged-Filtered method, includes multiple extractions of a small sample so both were eliminated as a possible method for product control. The Quartermaster Food and Container Institute method did not seem realistic for our purpose because the extraction was carried out at 80°C. The ADM Indirect and ADM Modified Indirect methods were eliminated because of the small sample size, time factor per analysis, and lack of precision.

The Waring Blendor method afforded improvement in all of the objectives except the temperature factor and standardization of equipment. At the conclusion of this study the Waring Blendor method was adopted for processing and product control in the ADM laboratories and plants.

Terminology

A variety of terms have been used by laboratory and sales personnel to describe or characterize the protein extracted by these general analytical procedures. In an effort to avoid confusion for the reader, the terminology used in this paper is defined.

TABLE I
Summary of Five Water-Soluble or Dispersible-Protein Methods

Method identification	Sample size (g.)	Water per analysis (ml.)	Type of mixing	Extraction time (min.)	Separation
Indirect ^a					
ADM indirect.....	0.7	ca. 200	Soak	120 ^c	# 4 Whatman
ADM modified indirect.....	0.7	ca. 45	Mechanical	45	Centrifuge 2,700 r.p.m., 10 min.
ADM centrifuged and filtered indirect.....	0.7	ca. 45	Soak and hand stirred	120 ^c	Centrifuge 2,700 r.p.m., 10 min., # 40 Whatman
Direct ^b					
Soy Flour Association direct.....	10	200	Mechanical medium rate	30	Centrifuge 1,500 r.p.m., 15 min.
Q.F. and C.I.	8	100	Mechanical medium rate	30	Centrifuge 1,800 r.p.m., 10 min.
Regional Soybean Laboratory.....	2.5	100	Mechanical shaker	30	Centrifuge 3,000 r.p.m., 6 min.
Waring Blender.....	21	300	Waring Blender	10	Centrifuge 2,700 r.p.m., 10 min.

^a Residue analyzed for nitrogen. ^b Filtrate analyzed for nitrogen. ^c Dependent on filtration time.

Water-Soluble Protein. The first methods which were developed and used for this analysis employed hand-stirring or mild mechanical blending of the soya product and water with the extracted protein separated by filtration. The protein extracted in this manner was defined as water-soluble protein; this term however has been indiscriminately applied to all methods employing this general extraction procedure.

PSI (Protein Solubility Index). This term expresses the ratio of water-soluble protein to total protein and is calculated as follows:

$$\frac{\text{Water-Soluble Protein} \times 100}{\text{Total Protein}} = \text{PSI (Protein Solubility Index)}$$

While the use of the term PSI has become common in the soya industry, the value cannot be calculated unless the total protein is known. Expressing results as PSI introduces the additional errors encountered in running the total protein analysis, thereby affecting the accuracy and precision of the water-soluble protein method.

Water-Dispersible Protein. Methods which employ high-speed Blenders to extract the soya product, followed by centrifugal separation, extract more protein than earlier single extraction-methods that used mild agitation. The protein extracted in this manner cannot be classified as water-soluble since proteins are defined as a class of compounds which may be dispersed in water as colloids. Concomitant with the colloidal protein there may be a fraction, which we have called suspensoids, that is difficult to remove in the centrifugal separation. The latter is especially encountered in the high-extractability range of soya products. The term water-dispersible protein is used to characterize the protein extracted by methods which employ single extraction, using high speed Blenders.

PDI (Protein-Dispersible Index). Since the soya industry is accustomed to expressing the extracted protein as PSI, a counterpart of this term, PDI, can be used if desired. As pointed out above, the accuracy and precision of the method are reduced when results are expressed in this manner. Therefore all of the results and calculations in this paper are expressed as water-dispersible protein.

Method Development

In 1958 ADM laboratories resumed the study of methods of analysis for Water Dispersible Protein in an effort to improve or eliminate the two major inadequacies of the Waring Blender method, *i.e.*, temperature factor and readily standardized equipment.

While the blending and grinding action of the Waring Blender provides a very rapid determination, excessive heat is developed during the blending operation because of the placement of the motor directly

beneath the cup. This is particularly true when the bearings on the Blender become worn or when one sample follows another in quick succession. These conditions produce erratic temperatures in the blend, thereby seriously affecting the precision of the method. Figure 2 shows the relationship between the blending time, the rise in temperature, and the protein dispersibility on a typical sample run by the Waring Blender method.

In an effort to minimize the temperature rise during blending and still maintain the principle of the Waring Blender, a Hamilton Beach Drinkmaster #30 was adapted for use with Waring Blender blades and cups. This modified Blender is shown in Figure 3. The relationship between blending time, temperature rise, and protein dispersibility when using the Modified Hamilton Beach Blender is shown in Figure 4. It will be noted that the temperature did not rise as rapidly as with the conventional Waring Blender (Figure 2),

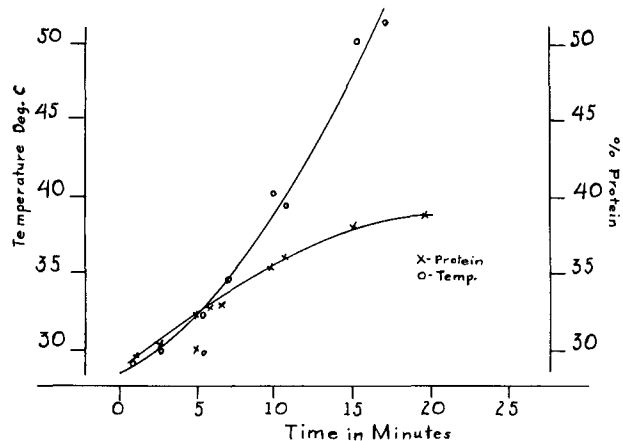


Fig. 2. Time-temperature relationship, using Waring Blender.

and the rate of temperature rise tended to level off after 10 min. of blending.

While the Modified Hamilton Beach Blender eliminated a definite source of error, the method still lacked the desired precision for close product control. Therefore it was decided to investigate all probable sources of variation in the method that could be specified and controlled. The following were listed for study: blending time, temperature rise, blending speed, type of blade, pH of blend, centrifuge time, centrifuge speed, sample size, and sample grind. Two other probable sources of variation which were not made a part of this study are the protein determination and sampling procedure.

Temperature Rise and Blending Time. The effect of blending time on temperature rise and protein an-

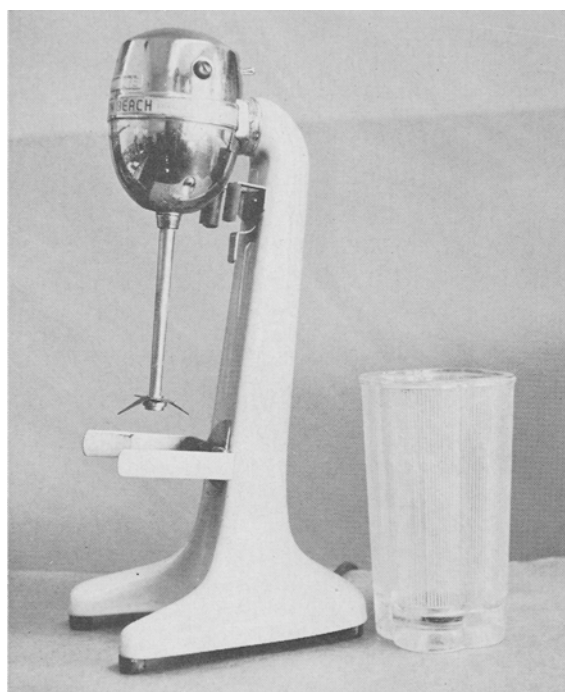


FIG. 3. Modified Hamilton Beach Drinkmaster #30.

alysis is shown in Figure 4. It will be noted that, after a rapid initial temperature rise, the rise in temperature was directly proportional to time. Also the increase in dispersible protein was proportional to time. Since there was no levelling off in the dispersed protein, no exact or maximum results could be obtained. Therefore an arbitrary mixing time was selected. To keep the effect of temperature rise at a minimum, a 10-min. blending time was chosen. There was approximately a 12°C. rise in temperature when the initial temperature of the blend was 25°C. Experimental work also showed that the initial temperature was an important factor and should be specified as a part of the method.

Blending Speed. The effect of the blending speed was determined by connecting a variable transformer into the line and removing the top cap of the Hamilton Beach Drinkmaster so that the r.p.m. of the motor could be measured with a tachometer. Then 300 ml. of water were placed in the blending cup and the

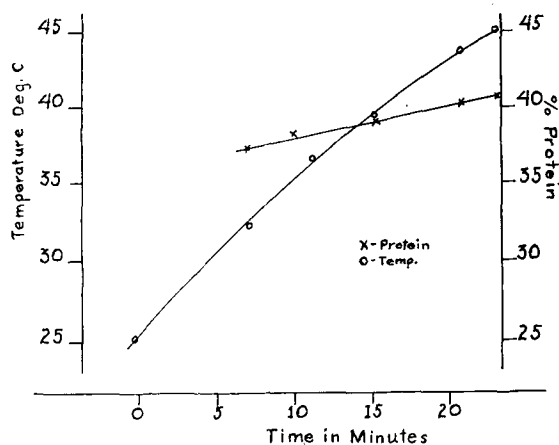


FIG. 4. Time-temperature relationship, using modified Hamilton Beach Blender.

transformer was adjusted to give the blending speed desired. Figure 5 shows results obtained on samples blended for 10 min. at speeds varying from 4,000 to 9,000 r.p.m. It was found that the maximum protein dispersibility was reached at 8,000 r.p.m. Since the maximum speed of the Blenders is approximately 9,000 r.p.m. under load, it was decided to standardize the speed at 8,500 r.p.m.

pH of the Blend. Soybean meals and flours have a natural buffered pH of 6.6 to 6.8. To find the effect

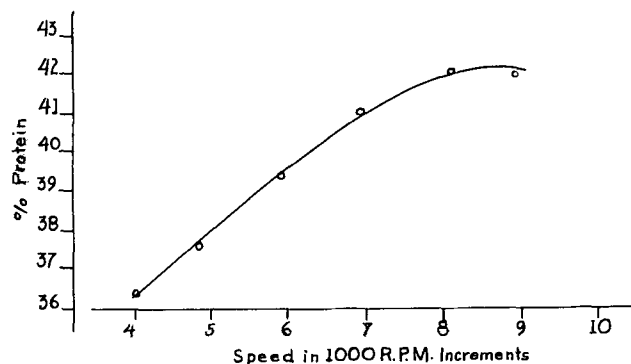


FIG. 5. Effect of blending speed.

of the change of pH on dispersibility of the protein, alkali and acid were added to give pH's which ranged between 5.0 and 8.0. It was found that a change in pH had a very marked effect on the dispersibility of the protein as shown in Figure 6. However there appeared to be no advantage to the changing of the pH of the blend when neutral distilled water was used for the determination.

Centrifuge Time and Speed. Since the high-speed blending and grinding operation used in this method may produce colloids, suspensoids, and suspended solids, the time and speed of centrifuging may be critical for some samples.

Table II shows the effect of speed while Table III shows the effect of time on the dispersible protein analysis. As a result of these experiments, centrifuging for 10 min. at 2,700 r.p.m. was chosen since this

TABLE II
Effect of Centrifuge Speed on Water-Dispersible Protein
Using One Sample of Soyflour. Time 10 Min.

Run	R.P.M. Centrifuge				
	1,200	2,000	2,500	3,000	3,500
#1.....	43.5	43.5	43.0	42.5	42.5
#2.....	43.0	42.5	42.5	41.0	41.0
#3.....	44.5	43.5	43.0	42.5	42.0
Av.....	43.5	43.0	43.0	42.0	42.0

appeared to be the minimum time and speed that would give the most complete separation of nondispersible protein and suspended solids from the supernatant liquid.

Type of Blade. During the course of the experimentation it was noted that standard Waring Blender blades became very dull after a few determinations, thus affecting the grinding action and the water-dispersible protein results. Springsteel blades were substituted for the standard blades with the result that these blades could be used for long periods of time without any apparent effect on the sharpness of the blade. Also the springsteel blades tended to give

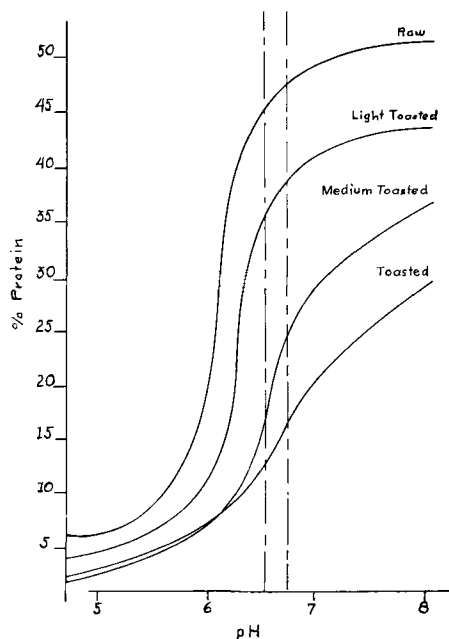


FIG. 6. Effect of pH on protein dispersibility.

slightly higher results than the standard blades on most samples.

Sample Size. For simplicity of calculation 20-g. samples were used in the initial experimental work on the method. To determine if this were the optimum sample size, a series of experiments was run by using

TABLE III
Effect of Centrifuging Time at 2,700 r.p.m. on
Water-Dispersible Protein Analysis

Centrifuge time	Sample 1	Sample 2	Sample 3	Sample 4
10 Min., unfiltered.....	38.5	29.5	42.0	24.0
10 Min., filtered.....	29.5	29.5	41.5	24.5
30 Min., unfiltered.....	38.5	29.5	41.0	24.0

samples up to 30 g. Slightly lower results were obtained when larger samples were used. Thus a 20-g. sample was selected as the optimum size.

Sample Grind. The Waring Blendor cup and blades provide a very efficient wet grinder, making it unnecessary to pregrind the sample prior to running the determination. A series of samples was pre-ground on a Weber hammer mill to 100 mesh. The results are shown in Table IV. It will be noted that the preground samples gave lower results and poorer precision than the unground samples. The reason for these lower erratic results was not determined.

Based on the results obtained in the above experiments, the method shown in detail below was written, and a collaborative experiment was set up within the ADM laboratory organization to determine the accuracy and precision of the method.

TABLE IV
Effect of Sample Grind on Water-Dispersible Protein
(Each value is average of 24 determinations)

Type of sample	Unground sample	Ground sample
44% Meal.....	14.0	13.6
Grits #1.....	16.9	14.7
Grits #2.....	14.3	12.2
Standard deviation.....	0.85	1.34

Method

Apparatus

Hamilton Beach Drinkmaster #30. Modified to accommodate Waring Blendor blade and cup (Figures 3, 8, 9 and Note 1).

Blade Assembly. Cenco-Pinto blades. Central Scientific Company, #17251-L55. Use two blades, one horizontal, and one with tips pointing down with the cutting edge in the direction of rotation.

Waring Blendor Cup. 1-qt. capacity with bottom sealed with #3 stopper.

Glassware. 300-ml. volumetric flask, 15-ml. pipette, 600-ml. beaker.

Centrifuge. International Type SB size 1, 2,700 r.p.m., with 50-ml. tubes or any equivalent, capable of delivering 1,400 relative centrifugal force at the tip.

Balance. 0.1 g. accuracy, important.

Timer. Interval, alarm.

Variable Transformer.

Standard Kjeldahl Equipment. See A.O.C.S. Method Ac-4-41 (9).

Tachometer. Range to 10,000 r.p.m.

Voltmeter. (Use optional.)

Reagents

Distilled Water. Neutral.

Standard Reagents as used for protein determination. See A.O.C.S. Method Ac-4-41 (9).

Preparation of Sample

No preparation necessary; use sample as received.

Standardization of Blendor

Measure 300 ml. of distilled water into the Blendor cup, and place in position on the mixer.

Remove chrome cap, which covers the top of the drive shaft. Using the proper tip, place tachometer in position on the rotating shaft.

With the switch in high position, gradually increase the transformer setting until the shaft shows 8,500 r.p.m. on the tachometer.

Note voltmeter reading and transformer setting, and use for blending of sample.

Standardization of machine should be done before each series of tests to eliminate errors on account of fluctuation in line voltage.

Procedure

Weigh 20 ± 0.1 g. of fat-free soya product.

Fill a 300-ml. volumetric flask with distilled water at $25 \pm 1^\circ\text{C}$. Pour about 50 ml. of the water into the Blendor cup. (Water-dispersible protein is related to temperature so the Blendor cup should be at room temperature.) Transfer the weighed sample quantitatively to the Blendor cup. Stir with a spatula to form a paste. Add remainder of water in increments, with stirring, to form a smooth slurry. Use last of water to rinse spatula and Blendor cup walls. Place cup in position for blending.

Turn Blendor on with switch in high position, and gradually adjust the variable transformer to the point indicated by the water standard at 8,500 r.p.m. Blend at this speed for 10 min.

Remove the Blendor cup, and pour the slurry into a 600-ml. beaker. After the slurry has separated, decant or pipette a portion into a 50-ml. centrifuge tube, and centrifuge 10 min. at 2,700 r.p.m.

Pipette 15 ml. of supernatant liquid into a Kjeldahl flask, and determine protein by using A.O.C.S. Method Ac-4-41 (9) (15 ml. = 1.0-g. sample).

Calculation of Results

Percentage of protein obtained in procedure = Water-Dispersible Protein.

% Water-Dispersible Protein $\times 100/\%$ Total Protein = Protein-Dispersible Index (PDI).

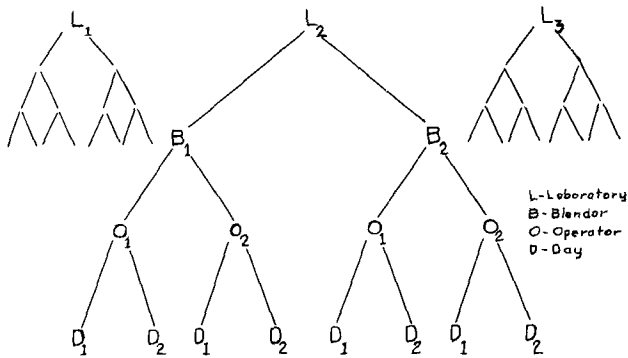


FIG. 7. A.O.C.S. experimental design.

Note 1. Adaptation of Hamilton Beach Drinkmaster #30 for use with a 1-qt. capacity Waring Blendor Cup.

Remove the top and bottom brackets that come with the machine.

Attach the top bracket, shown in the adapter assembly drawing (Figure 9), with the switch bar in the slotted hole. This locks the switch in the "on" position.

Attach the bottom bracket (Figure 9), and adjust to accommodate the cup height before tightening the bolt.

Remove the blades and baffle washer from the mixer shaft, and substitute two of the three Cenco-Pinto blades, using the proper assembly (screw, blade holder, and washer, Figure 8).

Loosen the two bolts on the motor mount, and insert shims to centralize the shaft in the glass cup. Approximately 1 mm. on each side is necessary for this adjustment.

The tips of the top bracket may be covered with rubber tubing as a cushion for the glass cup.

Note 2. The method as written carries the A.D.M. Designation of Ce-4a-59.

Discussion of Results

In setting up the collaborative study, the A.O.C.S. nested statistical design (9) was used as shown in

Figure 7. The A.O.C.S. design tests four variables, that is, laboratories, operators, days, and duplicates. The Modified Hamilton Beach Blendor is an integral part of this method and probably has more effect on the precision than any other factor. Therefore it was decided to eliminate the duplicate analysis and add the Blendor as a fourth variable. Three ADM laboratories were set up with two Modified Hamilton Beach Blendors in each laboratory, making a total of six different Blendors for this study. Five samples were used and a total of eight determinations were made in each laboratory on each sample. The results of these determinations are shown in Table V.

The results of Table V were analyzed statistically and can be summarized as follows:

Standard deviation due to machines.....	0.31
Standard deviation due to operators.....	0.23
Standard deviation due to days.....	0.47
Standard deviation due to labs.	0.60
Standard deviation within a lab. (O + B + D).....	0.60
Standard deviation between labs. (O + B + D + L).....	0.85

Calculating Student's T from data at a 95% confidence limit, the following precision was established.

Two single determinations run within a laboratory should not differ by more than 1.4%.

Two determinations run in two different laboratories should not differ by more than 2.0%.

This method is highly empirical with a large number of potential sources of error. The results shown however indicate extremely good precision can be obtained by this method if proper attention is paid to equipment and techniques. In actual practice this method has enabled the ADM plants to maintain close control over products produced. The correlation between the results obtained and the end-product uses has been excellent.

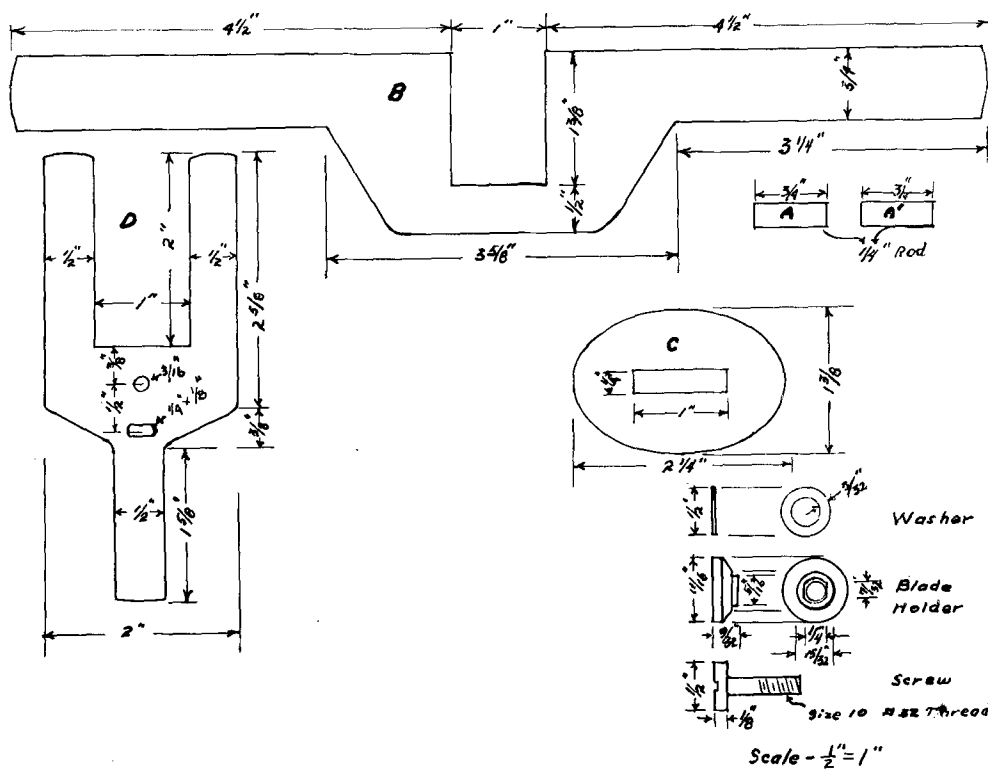


FIG. 8. Hamilton Beach adapter unit. 16-Gage stainless steel type 302.

TABLE V
Protein Results on Water-Dispersible Collaborative Study

	Type of Product				
	44% S.B.O.M.	Medium toasted flakes	Toasted grits	Light toasted flakes	Light toasted flour
Laboratory 1					
O1, B1, D1	15.0	18.4	14.3	39.7	39.7
D2	14.5	18.5	14.3	39.5	39.9
B2, D1	14.6	17.6	13.3	39.3	39.5
D2	14.0	17.1	13.5	39.2	39.5
O2, B1, D1	14.7	18.7	14.5	40.4	40.6
D2	15.0	18.2	14.8	39.7	40.0
B2, D1	14.2	18.2	13.9	39.9	40.1
D2	14.6	17.3	14.2	39.8	40.1
Laboratory 2					
O1, B1, D1	13.2	15.9	13.5	39.4	39.9
D2	13.7	16.1	13.4	38.8	39.0
B2, D1	12.8	14.6	12.8	39.0	39.4
D2	13.2	15.8	13.9	40.1	41.1
O2, B1, D1	13.6	15.4	15.2	39.4	39.5
D2	12.8	17.4	14.2	40.2	39.7
B2, D1	13.8	15.9	13.5	39.2	41.0
D2	13.3	16.0	14.0	40.7	41.0
Laboratory 3					
O1, B1, D1	14.1	16.9	14.1	39.7	39.4
D2	14.2	17.4	15.3	39.4	40.1
B2, D1	14.2	16.2	15.3	40.3	40.3
D2	13.7	15.9	14.5	39.1	39.9
O2, B1, D1	14.7	17.2	15.2	39.5	39.2
D2	14.4	16.8	15.0	39.5	39.7
B2, D1	14.2	16.7	14.8	38.8	37.8
D2	14.0	16.5	14.3	39.2	39.3
Average	14.0	16.9	14.2	39.6	39.8
Std. deviation	0.74	1.25	0.75	0.59	0.79

O—Operators, B—Blenders, D—Days. See experimental design, Figure 7.

Summary

A method is presented for determination of Water-Dispersible Protein, using a Hamilton Beach Drink-master #30 modified to accommodate Waring Blender blades and cups. The probable sources of error in the method are discussed, and the precision of the method

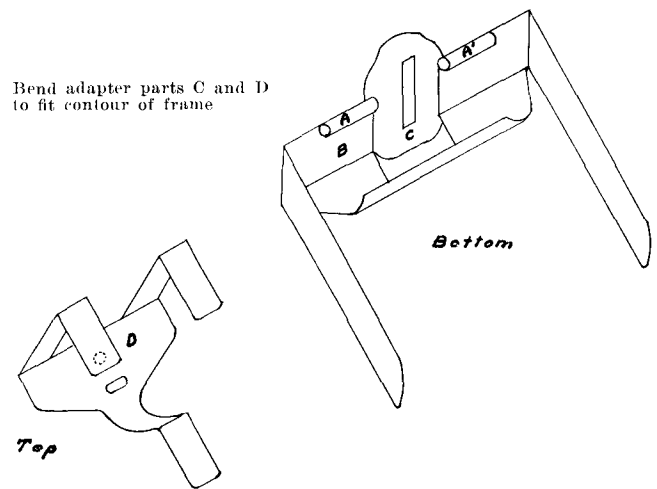


FIG. 9. Adapter assembly.

is shown, based on the results obtained in three laboratories using six different Modified Hamilton Beach Blenders.

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Surface-Active Properties of Sodium Salts of Sulfated Fatty Acid Monoglycerides

A. K. BISWAS and B. K. MUKHERJI, Department of Applied Chemistry, University College of Science and Technology, Calcutta, India

STUDIES ON THE SULFATION of fatty acid-glycerol esters (1) were started as far back as 1909, but the utility of the products as surface-active agents was not realized until sulfated monoglycerides of coconut oil fatty acids (R-COO-CH₂CHOHCH₂OSO₃Na) were put in the market under the trade name, Arctic Syntex-M,L, etc. However very little published information has appeared on the fundamental relations between constitution and properties. The present investigation on the comparative surface-active properties of the sodium salts of monoglyceride sulfates of C₁₂-, C₁₄-, C₁₆-, and C₁₈-saturated, oleic, and linoleic acids was undertaken with a view to elucidating any possible influence of the molecular weight and unsaturation of the fatty acids on the surface-active properties of the products of sulfation of the monoglycerides.

Raw Materials and Reagents Used

Fatty Acids. Lauric and myristic acids were separately crystallized from 25% solutions of the com-

mercial products in acetone at -25°C. and -20°C., respectively. Four such crystallizations yielded the following products: lauric acid having neutralization equivalent (N.E.) 201.0, iodine value (I.V.) less than 0.1, m.p. 44.1°C., literature value 44.2°C., and myristic acid having N.E. 228.1, I.V. less than 0.1, m.p. 54.2°C., literature value 54.4°C. A 20% solution of palmitic acid in acetone was cooled to 5°C. to remove insoluble stearic acid. The filtrate was chilled to -12°C. and kept at that temperature for 2 hrs. Repeated crystallization gave palmitic acid having N. E. 257.2, I.V. nil, m.p. 62.7°C., literature value 62.9°C.

Three successive crystallizations of stearic acid from 10% solution in acetone at -5°C. for 3 hrs. gave a sufficiently pure stearic acid having N.E. 284.2, I.V. nil, m.p. 69.5°C., literature value 69.6°C.

Successive crystallizations of 7.5% of oleic acid solution in acetone at -25°C. and -35°C. precipitated crops of palmitic and oleic acid. Three recrystallizations of the oleic acid from acetone yielded a product