Processing Variables in Peanut Protein Preparation

J. POMINSKI, E. J. LABORDE, V. O. CIRINO, H. L. E. VIX, Southern Regional Research Laboratory,[1] New Orleans, Louisiana

PILOT plant production of peanut protein from hexane-extracted meals for product evaluation has shown the need of detailed technical information on the effects of temperature, time, and water-meal ratio on the peptization and recovery of the protein. The data reported in the literature include the following: the influence of storage (9), pH (3, 4, 8), salts (4), acids (5), and heat, humidity, and length of treatment (6) on the peptizability of the nitrogeneous constituents of the meal; a method for producing light-colored protein (2); the influence of temperature of extract liquor during precipitation of the protein and rate of addition of sulfur dioxide on the settling rate of precipitated peanut protein curds (1). These studies however used room temperatures of peptization, one definite time of extraction, and a single water-meal ratio. Data are reported in this paper which for mathematical and additional product evaluation necessarily included studies on five different temperatures, six water-meal ratios, and four peptization times. Information was also obtained on the effect of grinding the meal before peptization. A general equation for estimating the yield of protein from a meal based on specific analytical data has been developed using some simplifying assumptions. It has practical use for calculating the yield of protein that may be expected for a given meal extracted at different water-meal ratios.

Preparation of Meal

Both unground and ground portions of meal were used in the preparations. The unground meal used was prepared in a pilot-plant continuous counter-current extractor (7) at the Southern Regional Research Laboratory, using commercial hexane. The peanuts had been treated with a 0.5% sodium hydroxide solution to remove objectionable skin color (2), dried at 125°F., and cracked and flaked to a 0.010-inch thickness.

The solvent-extracted meal had the following chemical composition: 6.09% moisture, 8.91% nitrogen, and 3.22% lipids. Dry screen analysis was as follows: 74.1, 43.5, 29.0, 21.5, and 16.8 through U. S. meshes 20, 40, 60, 80, and 100, respectively.

Ground meal was prepared by grinding the unground meal to pass a 60-mesh screen.

General Chemical Processing Conditions

The chemical procedures for all experiments were those used in pilot-plant peanut protein production. Peptization of the meals was accomplished by adjusting to pH 7.5, using sodium hydroxide, and subsequent protein precipitations were made at pH 4.5 with sulfur dioxide as the precipitating agent (1, 4, 5). However distilled water was used to eliminate the effects of inorganic ions (4). No attempt was made to duplicate any spray washing.

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Experimental Procedure and Results

Effect of Time on the Extraction of Nitrogen. In determining percentage of nitrogen extracted, which is equivalent to percentage of protein solubility, 2.5 grams of meal, or multiples of this amount, were mixed with water to give the desired water-meal ratios by weight and then adjusted to pH 7.5 with sodium hydroxide solution. In studying the effect of varying the time on percentage of nitrogen extracted, a wetting time of 7 minutes was required to reach the pH 7.5 and zero starting time. Extraction was carried on with continuous agitation for the desired time interval. After centrifuging and filtering, an aliquot of the filtrate was used for the determination of nitrogen. To obtain percentage of soluble nitrogen, a separate total nitrogen determination was made on the meal and the ratio of peptized nitrogen to total nitrogen was multiplied by 100.

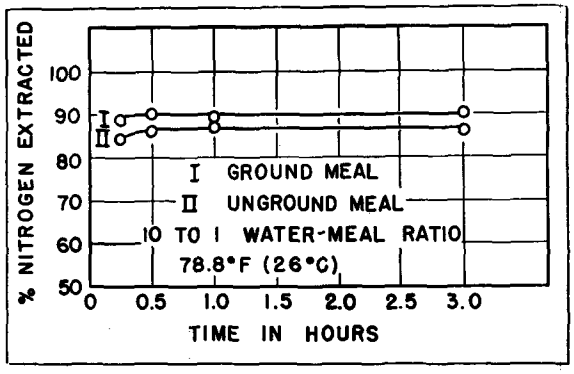

FIG. 1. Effect of time on extraction of nitrogen.

Figure 1 shows the effect of time on the extraction of nitrogen from both ground and unground peanut meals, using a 10 to 1 water-meal ratio at 78.8°F. after the meals were brought up to the proper pH for zero starting time. In 15 minutes, for both the ground and unground meals, peptization was practically completed and in 30 minutes it had reached its maximum. Ground meal gave a slightly greater percentage of nitrogen solubility.

Effect of Temperature on the Extraction of Nitrogen. The percentage of nitrogen extracted was determined as described above with the exception that the mixtures of meal and water at pH 7.5 were allowed to extract for 3 hours with occasional rather than continuous shaking. Figure 2 shows the effects of temperature on the extraction of nitrogen from the meals, using the 10 to 1 water-meal ratio. For both the ground and unground meals extraction of nitrogen increased slowly with rising temperature. Though the curves were drawn as straight lines, indications are that they flatten out beyond the upper and lower limits shown.

Effect of Water-Meal Ratio on the Extraction of Nitrogen. The same procedure as used to study the effect of temperature was used to determine the per-

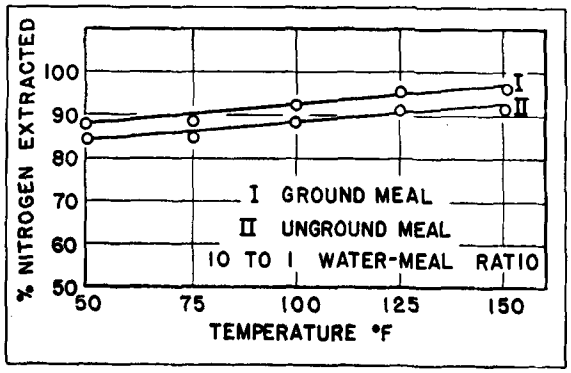

FIG. 2. Effect of temperature on extraction of nitrogen.

centage of nitrogen extracted with various water-meal ratios. Figure 3 shows the effect of water-meal ratio on the extraction of nitrogen at 78.8°F. On increasing the water-meal ratio from 10 to 1 to 40 to 1, the amount of nitrogen extracted from the ground meal increased from 92 to 94% and from 88 to 90% from the unground meal. Thus the peptization of peanut protein may be considered essentially independent of the water-meal ratio.

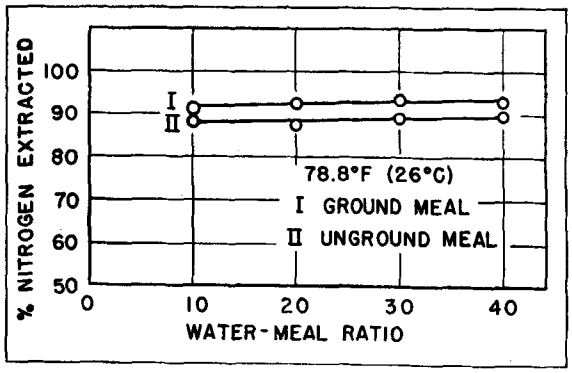

FIG. 3. Effect of water-meal ratio on extraction of nitrogen.

Recovery of Protein and Other Products at Various Water-Meal Ratios. To determine the percentage of protein and spent meal residue recovered, 50-gram portions of meal or multiples of this amount were peptized with continuous agitation for 30 minutes.

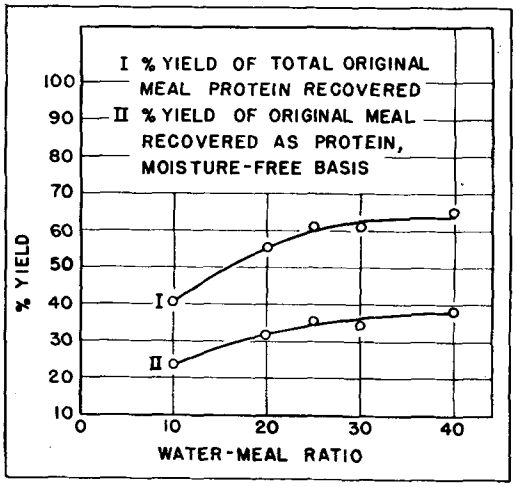

FIG. 4. Recovery of protein at various water-meal ratios.

The solids were removed by centrifuging in a bottle-type centrifuge at a force approximately 1,000 times gravity for 10 minutes and dried at 221°F. The percentage of moisture retained in the spent pulp residues were comparable to those obtained on a pilot-plant scale. Protein was precipitated from clarified peptized liquors at pH 4.5 and was separated by centrifugation at approximately 1,000 times gravity for 10 minutes and dried at 120°F.

These experiments were carried on at room temperature (from 78.8° to 86°F.). Figure 4 shows the effect of water-meal ratio on percentage of protein recovered, based on both the total protein in the unground meal and the total unground meal. The yield of protein increases as the water-meal ratio increases, levelling off at a ratio of about 25 to 1. The yield of protein is largely affected by the amount of peptized solution retained in the residue. The lower the water-meal ratio, the greater the proportion of peptized liquor retained in the residue (see Figure 6).

Grinding of the meal increased the protein yield due to increased peptization and less retention of liquid in the residue. At a 25 to 1 water-meal ratio, ground meal yielded a protein recovery of 42.9% of the original meal on a moisture free basis, equivalent to a recovery of 73.5% of the total protein, as compared to 35.3 and 61.1%, respectively, for unground meal.

In an extraction in which the unground peanut meal was peptized successively three times at a water-meal ratio of 15 to 1, the protein product was 42.1% by weight of the original meal (moisture free), equivalent to 71.3% of the total protein in the meal.

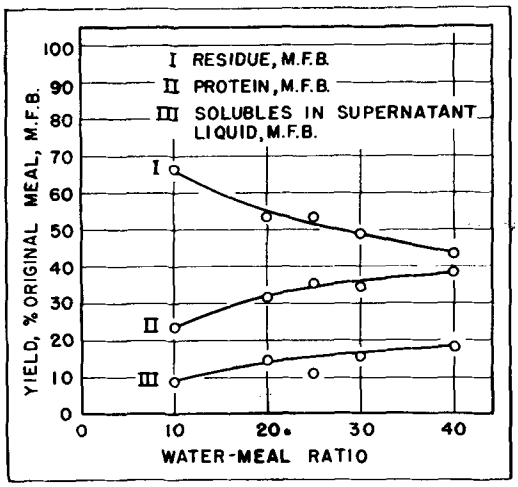

FIG. 5. Yield of products from original meal for various water-meal ratios (moisture-free basis).

Figure 5 shows the effect of water-meal ratio on the yield of products obtained on a moisture-free basis from the unground meal. Actual determinations were made for percentage of spent meal residues and protein products. The percentage of solubles in the supernatant liquid was obtained by difference. The weights of sulfur dioxide and sodium hydroxide used were not considered in the balance since they amounted to only about 1% of the original peanut meal. With increase in water-meal ratio the total amount of residue decreased, and the total amount of solubles in the supernatant liquid increased. These

results were due to the decrease in soluble materials retained by the spent pulp. At a 25 to 1 water-meal ratio, ground meal gave 41.7% residue and 15.4% solubles as compared to 53.5 and 11.0%, respectively, for unground meal. The triple extraction using a water-meal ratio of 15 to 1 yielded 38.9% residue and 19.0% solubles. For unground meal repeated extractions gave the greatest yield of protein with the least yield of residue.

TABLE I

Analyses of Products from Preparation of Protein at Various Water-Meal Ratios from Unground Meal

Water-Meal Ratio	Residue		Protein
	Moisture	Nitrogen M.F.B.	Nitrogen M.F.B.
	%	%	%
10	89.2	7.29	16.35
20	91.8	6.18	16.69
25	91.6	5.68	16.42
30	92.1	5.45	16.78
40	93.3	5.07	16.18
25 (Ground)	87.5	4.01	16.28
Triple Extraction	93.2	3.58	16.36

Table I shows analyses of spent pulps and proteins produced from the meals at various water-meal ratios. For the residue the nitrogen content decreased and the amount of moisture held increased with increase of water-meal ratio. At a 25 to 1 water-meal ratio the ground meal residue retained less moisture than the unground meal residue. The nitrogen content of the protein showed no significant changes.

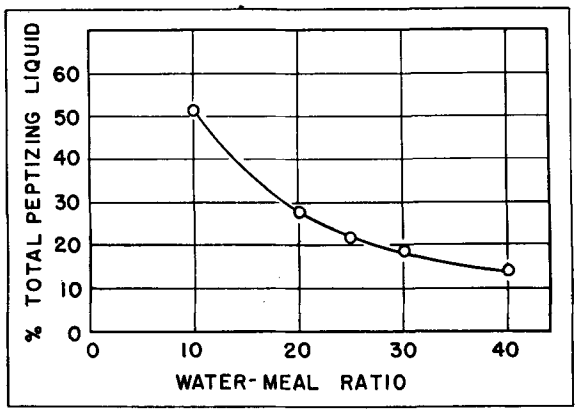

FIG. 6. Effect of water-meal ratio on percentage of total peptizing liquid remaining in residue.

Figure 6 shows the effect of water-meal ratio on the percentage of total peptizing liquid remaining in the residue from the unground meal. This percentage times the water-meal ratio is practically a constant and may be represented by the equilateral hyperbolic equation $XY = 541$, where X is the water-meal ratio and Y is the percentage of the peptizing liquid remaining. At low water-meal ratios excessive percentages of peptizing liquid containing soluble protein are retained in the residue. The values shown would vary with meals of other particle size.

The residue from a 25 to 1 water-meal ratio, using ground meal, contained only 11.1% of the original peptized liquid as compared to 21.9% for unground meal at the same water-meal ratio. The centrifuging operation showed that a decrease in particle size reduced the amount of liquid retained.

The ratio by weight of peptizing liquor in the residue to the original unground meal used is shown below:

Water-Meal Ratio	Retained Water in Residue / Original Meal
10	5.1
20	5.6
25	5.5
30	5.7
40	5.7

This ratio is practically constant and represented by the average value (V_S in the formula) of 5.5. This ratio would vary with particle size of meal and method of dewatering. From these data it is evident that wetting of the meal with peptizing liquid requires 5.5 times as much liquid as meal by weight.

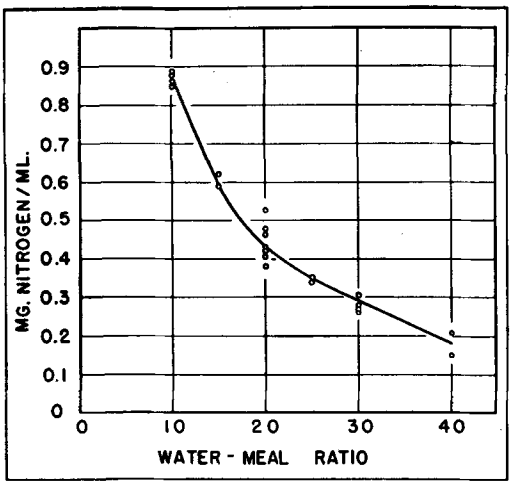

FIG. 7. Effect of water-meal ratio on nitrogen in solution at pH 4.5.

Figure 7 shows the effect of water-meal ratio on nitrogen in solution at pH 4.5 after precipitation of the protein. The amount of nitrogen remaining in solution per ml. times the water-meal ratio is approximately a constant and may be represented by the equilateral hyperbolic equation $XY = .0085$, where X is the water-meal ratio and Y is grams of nitrogen per ml. in the supernatant liquid. This value 0.0085 is also equal to the constant C_1 in the derived equation (7) which follows later. Analysis for non-protein nitrogen, 5.9% of the total meal nitrogen, was too low to account for this phenomenon.

General Formula for Protein Recovery

The data show that for practical purposes nitrogen solubility is independent of the water-meal ratio, that water-meal ratio times the amount of nitrogen in the supernatant liquid gives a constant, and that the ratio by weight of peptizing liquid in spent pulp to original meal at all water-meal ratios is a constant for the method of dewatering used. With this information a general equation can be derived, showing the percentage yield of meal nitrogen or meal protein in the crude protein products as a function of the water-meal ratios. The formula is derived, using 1 gram of meal as a basis so that weight and volume units may be interchangeable since milliliters of water are used. All weight units are expressed as grams and volume units as milliliters.

Specifically, the following units are used:

- P = percentage of original meal nitrogen or percentage of original protein recovered in protein product.
- S = fractional solubility of meal nitrogen or protein.
- N_o = weight of nitrogen in original meal, grams.
- N_1 = weight of nitrogen recovered in protein product, grams.
- V_T = total volume of liquid used in peptization, milliliters. (Since 1 gram of meal is used, this represents the water-meal ratio.)
- P_T = weight of protein per milliliter in peptized solution, grams.
- V_S = volume of liquid in residue. This is a constant at all water-meal ratios for the type dewatering used, milliliters.
- V_D = volume of supernatant liquid after protein precipitation, milliliters.
- P_D = weight in nitrogen per milliliter in supernatant liquid, grams.

Now, nitrogen recovery = total soluble nitrogen available − (soluble nitrogen in spent meal + nitrogen in supernatant liquid) giving the following equation:

$$N_1 = V_T P_T - (V_S P_T + V_D P_D) \quad (1)$$

and percentage of recoverable protein or nitrogen may be expressed as follows:

$$P = \frac{N_1}{N_o} \times 100 \quad (2)$$

Total peptized nitrogen is a constant for any water-meal ratio;

$$V_T P_T = C \text{ or } P_T = \frac{C}{V_T} \quad (3)$$

Total volume of peptizing solvent is equal to liquid in spent pulp plus supernatant liquid. (The amount held by the precipitated and centrifuged wet protein is negligible and varies approximately from 1 to 2.5% for water-meal ratios from 40 to 1 to 10 to 1.)

$$V_T = V_S + V_D \text{ or } V_D = V_T - V_S \quad (4)$$

The water-meal ratio or volume times the nitrogen per unit-volume in the supernatant liquid is a constant;

$$V_T P_D = C_1 \text{ or } P_D = \frac{C_1}{V_T} \quad (5)$$

Substituting (3), (4), and (5), in (1)

$$N_1 = (C - C_1)\left(1 - \frac{V_S}{V_T}\right) \quad (6)$$

Substitute (6) in (2) to obtain percentage of recoverable protein or nitrogen.

$$P = \frac{N_1}{N_o} \times 100 = \frac{(C - C_1)\left(1 - \frac{V_S}{V_T}\right) \times 100}{N_o}$$

$$P = \frac{100}{N_o}(C - C_1)\left(1 - \frac{V_S}{V_T}\right) \quad (7)$$

C, C_1, N_o, and V_S will be constants.

Using the following experimental data reported in this paper, C may be calculated:

$$N_o = 0.089 \text{ g.}; \; S = 0.89; \; V_S = 5.5.$$

In deriving the equation (7), the expression $C = V_T P_T$ was used. This is equal to the total soluble nitrogen so that the following equation may also be used: $C = N_o S$. From which $C = 0.089 \times 0.89 = 0.0792$ g.

$C_1 = V_T P_D$ which is equal to 0.0085 as previously shown.

Thus, $C - C_1 = 0.0707$. Substituting these data in (7)

$$P = 100 \times \frac{0.0707}{0.089}\left(1 - \frac{V_S}{V_T}\right) = 79.4\left(1 - \frac{V_S}{V_T}\right)$$

$$P = 79.4\left(1 - \frac{5.5}{V_T}\right)$$

$$P = 79.4 - \frac{437}{V_T}.$$

TABLE II

Actual and Calculated Percentage Yields of Original Meal Protein or Nitrogen

V_T Water-Meal Ratio	Calculated Yield N	Actual Yield N
	%	%
10	35.7	40.9
20	57.6	55.7
25	61.9	61.1
30	65.0	61.0
40	68.5	65.2

A comparison in Table II of the calculated and actual yields for the unground meal shows a fairly good correlation, with the yield for the 10 to water-meal ratio having the greatest discrepancy. Since the constants will vary from meal to meal for a given material, N_o, S, V_S, V_T, and P_D should be experimentally determined for some water-meal ratio such as 20 or 25 to 1, and from this information the protein recovery can be calculated for any water-meal ratio. Obviously in a true countercurrent extraction V_T would equal infinity and the maximum possible yield would be $P = 79.4$. Thus V_S is not needed in determining the maximum yield available in a given meal in a countercurrent system. The general formula has practical application in large-scale work, and its use has proved valuable in pilot-plant work, results of which will be reported in a subsequent paper.

Summary

A general equation was derived with which the percentage yield of protein may be calculated for a solvent-extracted peanut meal at various water-meal ratios.

Results of investigations showed that nitrogen solubility for ground and unground meal increased slowly with temperature but was little affected by the water-meal ratio and that peptization might be considered complete in 30 minutes.

For unground meal the yield of protein increased with increase in water-meal ratio. Low water-meal ratios left excessive amounts of peptized protein in the spent pulp. The ratio by weight of peptizing liquid in spent pulp to original meal at all water-meal ratios was practically a constant. Repeated peptizations increased the yield, indicating the desirability of a countercurrent system of peptization. Grinding of meal resulted in increased peptization and increased protein yields.

Acknowledgment

The authors wish to express their appreciation to Kenneth M. Decossas for drawing the graphs shown in this paper.

REFERENCES

1. Arthur, J. C. Jr., Crovetto, A. J., Molaison, L. J., Guilbeau, W. F., and Altschul, A. M., J. Am. Oil Chem. Soc., *25*, 398-400 (1948).
2. Burnett, R. S., Chem. Eng. News, *24*, 478-480 (1946).
3. Burnett, R. S., and Fontaine, T. D., Ind. Eng. Chem., *36*, 284-288 (1944).
4. Fontaine, T. D., and Burnett, R. S., Ind. Eng. Chem., *36*, 164-167 (1944).
5. Fontaine, T. D., Irving, G. W. Jr., and Markley, K. S., Ind. Eng. Chem., *38*, 658-662 (1946).
6. Fontaine, T. D., Samuels, Carolyn, and Irving, G. W. Jr., Ind. Eng. Chem., *36*, 625-627 (1944).
7. Gastrock, E. A., and D'Aquin, E. L., Oil Mill Gaz., *53* (4), 13-21 (1948).
8. Hoffpauir, C. L., and Guthrie, J. D., J. Biol. Chem., *178*, 207-212 (1949).
9. Pickett, T. A., J. Am. Chem. Soc., *70*, 3516-3517 (1948).

[Received June 4, 1951]

The Hydrogen Value—Refractivity Relationship of Unsaturated Fatty Acids of Natural Origin

F. A. VANDENHEUVEL [1] and E. H. FARMER [2]

IN a previous paper (2) the authors have shown that a relationship exists between the refractive indices and the unsaturation value (iodine or hydrogenation value) of unsaturated fatty esters derived from cod liver oil, haddock liver oil, and sardine oil. When the refractive indices were plotted against the hydrogenation values, a straight line D_1H (Fig. 1) was obtained; this line remained unbroken until a point H, corresponding with the C_{22} hexaene acid, was reached, after which it continued in a new direction. The curve was directly applicable to pure individual esters as well as to unresolved fractions unless the latter contained components belonging to the different branches of the curve. It was stated that "whatever the precise significance of the hydrogen value/refractivity curve, the latter appears to offer direct and trustworthy empirical indication as to whether a product is an original component or only a secondary one."

Since the publication of this paper, some pure methyl esters of unsaturated fatty acids have been derived from natural materials of widely different origins. The experimental conditions under which they were prepared offer a good guarantee as to the original structure being conserved. Their constitution is well established, and they cover a wide range of unsaturation.

It is the purpose of this paper to show that the refractive indices published for these products as well as their unsaturation values also verify the line D_1H of the graph shown in Figure 1 (l.c.).

This line is defined by the co-ordinates of the two points D_1 and H:

$$D_1\begin{cases} n_D^{20}=1.4589 \\ H.V.\times 100=120 \end{cases} \qquad H\begin{cases} n_D^{20}=1.4930 \\ H.V.\times 100=355 \end{cases}$$

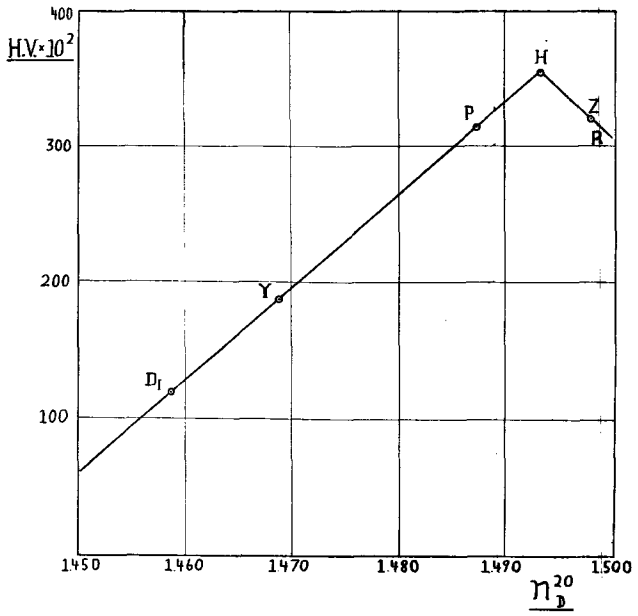

FIG. 1.

From the equation of this line the following empirical expression can be derived:

$$n_D^{20} = 1.44163 + H.V. \times 0.0145 \qquad (I)$$

In this expression H.V. represents the hydrogenation value (mg. of hydrogen necessary to saturate 1 g. of compound). The iodine value is linked to the hydrogenation value by the following simple relationship:

$$H.V. = I.V. \frac{1.008}{126.93} = I.V. \times 0.79414 \times 10^{-2} \qquad (II)$$

In Table I are listed a series of methyl esters, their origin, and method of isolation.

[1] Fisheries Research Board of Canada, Halifax, Nova Scotia.
[2] British Rubber Producers' Research Association,, Welvyn Garden City, England.

TABLE I

Source and Method of Isolation of Unsaturated Methyl Esters

Sample No.	Name — Methyl:	Source and Method of Isolation	Reference
1	Oleate (9-octadecenoate)	From olive oil by the fractional crystallization method.	(6)
2	Petroselinate (7-octadecenoate)	From coriander seed oil by fractional crystallization.	(1)
3	Linoleate (9,12-octadecadienoate)	From tobacco seed oil by chromatographic separation on silicic acid.	(7)
3a	Linoleate (9,12-octadecadienoate)	From cotton seed oil through debromination followed by molecular distillation.	(9)
3b	Linoleate (9,12-octadecadienoate)	From cottonseed oil through the same process as 3a.	(6)
3c	Linoleate (9,12-octadecadienoate)	From cottonseed oil by chromatographic separation on alumina.	(8)
4	Linolenate (9,12,15-octadecatrienoate)	From Linseed oil by chromatography on silicic acid.	(7)
4a	Linolenate (9,12,15-octadecatrienoate)	From Linseed oil by debromination followed by molecular distillation.	(9)
4b	Linolenate (9,12,15-octadecatrienoate)	From Linseed oil by the same method as 4a.	(6)
5	Arachidonate (6, 10, 14, 18-eicosatetraenoate)	From beef suprarenal gland lipids by fractional crystallization.	(4)
6	Docosahexaenoate	From cod liver oil, haddock liver oil, and sardine oil, by molecular distillation.	(3)