A Method of Testing Pure Lard[1]

Differentiating Lard and Lard Compounds, Particularly Those Containing Hydrogenated Vegetable Oils

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THE commonly accepted method of determining the presence of mixtures of vegetable oils in animal fats is by determining the presence of phytosterol in the unsaponifiable matter. This is done by crystallizing the sterols from the unsaponifiable matter, examining them microscopically, preparing the acetates of the sterols, purifying them by recrystallization from alcohol, and determining the melting points. The procedures outlined in the Methods of the Association of Official Agricultural Chemists are satisfactory. The preparation of the unsaponifiable matter by either the alcohol extraction method or the digitonin method is satisfactory, but the microscopical examination does not give very dependable results, particularly when there are mixtures of cholesterol and phytosterol present.

On acetylization, however, pretty definite results are generally given by determining the melting points of the various crops of crystals of sterol acetates. After the second or third recrystallization, the melting points should approach definitely the melting points of the pure acetate, if only one of the sterols is present, but if the phytosterol acetate is present in the cholesterol acetate, the melting points should show a gradual increase in value with each successive recrystallization.

Further indications are given by concentrating the mother liquors of each crop of crystals, and then obtaining further deposits from these liquors. Theoretically, if only one kind of sterol is present, such as cholesterol, the crystals from the mother liquor should melt at the same point as the preceding crop from the same liquor. In practice these may be contaminated with impurities from the mother liquor and need recrystallization. After such purification, valuable indications may be obtained by the above procedure, as the acetates of the sterols do not have the same solubilities in alcohol, and the least soluble crystallizes first. If only one acetate is present, the second crop from the mother liquor will be of the same kind of acetate as the first.

Table No. 1 gives the results of some tests made on samples which were all vegetable, and were of known origin.

TABLE No. 1

Melting Points of the acetates from:	1st crys.	2nd crys.	2nd crop 1st mother liquor	2nd crop 2nd mother liquor	2nd crop from 80% alc.
Sample No. 1	120	126.5	—	—	121.0
" " 2	125	125.4	*122.	—	119.0
" " 3	121.4	125.6	*119.2	—	—
" " 4	123.2	124.8	—	122.6	—
" " 5	127.4	128.9	—	127.	—

*Not Purified

On a sample containing 20% tallow and 80% cottonseed oil, these results were obtained:

TABLE No. 2

Melting Points of the acetates from: 1st crys.	2nd crys.	1st mother liquor
120	122.6	114.8
121.5	124.0	—

A sample of lard gave the following results:

TABLE No. 3

Melting Points of the acetates from: 1st crys.	2nd crys.	2nd crys. 2nd mother liquor	3rd crop
111.0	113.8	108.0	113.8

On this sample there is no evidence of the presence of any vegetable oil.

[1] Presented at the Third Fall Meeting of The American Oil Chemists' Society.

Quicker Methods Desirable

THESE tests show the results that may be obtained by the use of the sterol acetate test, but the method is long and tedious, as large quantities of oil must be saponified and extracted to get enough of the unsaponified matter for further tests. Then this unsaponifiable matter must be resaponified and re-extracted to insure the removal of all saponifiable oil.

For the above reasons, the use of other means of determining this question of admixture of vegetable oils in lard, particularly of determining the presence of hydrogenated vegetable oils in pure hog lard, seemed very desirable, at least for confirmatory evidence.

A sample of lard, whose purity was questioned, showed the following analysis:

Free Fatty Acids (Oleic)	1.29%
Iodine Value (Wijs)	63.3
Titre	39.8° C
Iodine Value (Wijs) of total fatty acids	64.7
Melting Point (capillary tube method)	43.3° C
Unsaponifiable Matter (FAC)	0.62%
Halphen Test (cottonseed oil)	Negative
Villavechia Test (sesame oil)	Negative

The following results were obtained by another laboratory on another sample of the same parcel of lard:

Melting Point	46.5° C
Iodine Value (Hanus)	60.7
Saponification Value	191.4
Unsaponifiable Matter	0.39%
Solid Fatty Acids	30.7%
Iodine Value (Hanus) of the liquid fatty acids	89.2
Iodine Value (Hanus) of the total fatty acids	62.1
Melting Point of the total fatty acids	41.6° C

Although no evidence of the presence of any fat, except lard, was indicated by the above analyses, further evidence was necessary to prove its purity.

In 1913-14, Bomer published the results of a splendid piece of research on the constituents of hog lard, tallow and other fats, and showed that among the constituents of lard was alpha-palmitodistearin, which has a melting point of 68.5°C, and whose fatty acids show a melting point of 63.3°c. He also showed that a glyceride of the same empirical formula was found in tallow, but that it was betapalmitodistearin, with a melting point of 63.3°C, and that the acids from the glyceride melted at 63.2°C.

Based upon this research, in which he examined fats from all parts of the animals, including hogs, cattle, etc., and all kinds of mixtures of these different kinds of fats, Bomer devised a method which is called the Bomer Test, in which the solid glycerides of the fat are crystallized from ether solution, endeavoring to eliminate all glycerides of lower melting point than the palmitodistearins. The glycerides are subsequently saponified, and the fatty acids separated from them. After determining the melting points of the glycerides and the fatty acids from them, a value is obtained for the Bomer number, which is obtained by subtracting the melting point of the fatty acids from that of the glycerides; doubling that difference, and adding it to the melting point of the glycerides. If this value is less than 71, the hog fat is considered adulterated with tallow. This is due to the fact that the betapalmitodistearin of beef fat melts at 63.3, the same as the melting point of the fatty acids from it, and, therefore, there would be none or only a very small difference between the melting points of the glycerides and the fatty acids. Lards containing considerable quantities of beef or mutton fat give Bomer numbers from 66 to 68, although there are mixtures which closely approach the minimum of 71. Bomer also tested the effects of various vegetable oils on this test, and found that they do not interfere with its efficacy. He does not give any data on the question of detecting the presence of hydrogenated fats, although he does mention that they would act like tallows.

Results on Compounds

QUITE independently, we applied this method to the above sample of lard, and also six samples of American lard compounds, three of which were made wholly from cottonseed oil, and the other three of which were well known standard brands of commercial vegetable oil products, with results as follows:

TABLE No. 4

Samples	A	B	C	D	E	F	LARD
Melting Point Glycerides	58.5	65.3	64.4	63.8	58.7	60.3	64.9
Melting Point of Fatty Acids	70	64.5	64.3	63.5	55.4	56.9	60.2
Bomer Number	—	67.2	64.6	64.4	65.3	67.1	74.3

As will be seen, the lard easily passed the requirements of the Bomer test for pure hog fat, but all the hydrogenated vegetable oil compounds failed. Of these lard compounds, C and D were mixtures of hydrogenated and unhydrogenated oils, and E and F were all

hydrogenated products. The composition of A and B were not definitely known, but apparently were also all hydrogenated. These samples gave the following results when tested further:

TABLE No. 5

	A	B	C	D	E	F
Free Fatty Acids	0.07%	0.07%	0.06%	0.06%	0.18%	0.12%
Iodine Value	48.4	69.1	98.6	98.4	71.1	67.2
Iodine Value of the Crys. Glycerides	42.3	—	5.2	6.7	9.9	12.8
Melting Point (approx.)	—	—	43.5	45.0	—	—
Solid Acids	—	—	35.0	34.0	—	—
Iso-oleic Acids	—	—	2.5	3.0	14.38	8.53
Halphen Test	Trace	Neg.	Pos.	Pos.	Neg.	Neg.

Thus by the use of this test, it was proved conclusively that the sample of lard was pure lard, not containing any fats of vegetable origin, and this result could be obtained in a much shorter time, and with as much, if not more certainty, than could be given by the phytosterol acetate test.

Also this test demonstrates that the hydrogenated fats act as tallows under its conditions.

The method for this test is as follows:

"Dissolve 50 grams of the clear dry filtered fat in 50 cc of ether, cool to 15°C for one hour with occasional agitation. Filter off the crystals on a Buchner funnel with suction, pressing the crystals dry. Redissolve in 50 cc of ether again, and recrystallize as before. If these second crystals do not melt above 61°C, the crystallization is repeated a third time.

A portion of these crystals is saponified with caustic potash and alcohol, dissolved in 100 cc of water, acidified with dilute hydrochloric acid, and extracted in a separatory funnel with 50 cc of ether. This ether extract is washed twice with 10 cc of water, filtered into a dry beaker, evaporated to dryness, and heated in an oven at 105°C for 30 minutes.

The melting points of the glycerides and fatty acids are now determined in capillary tubes, placing one of each on each side of the thermometer, and heating in a water bath. Subtract the melting point of the fatty acids from that of the glycerides, double this difference and add it to the melting point of the glycerides. This Bomer number should not be less than 71, but may run as high as 78 for leaf lard.

The following precautions must be observed: The glyceride crystals must not be melted prior to the determination of the melting point.

The fatty acids can be melted into the capillary tubes, however, but must be kept in a desiccator until the melting point is determined, as they will pick up ammonia very rapidly, forming soaps, and increasing in melting point considerably.

If the fat is rather liquid, the quantity of fat can be increased on the first crystallization, the time can be extended to two hours, the temperature can be lowered to 5°C, anhydrous acetone may be used, or a mixture of 3-4 parts of ether and 1 part of alcohol can be used, in order to get a sufficiently large first crop of crystals. Subsequent recrystallizations must be from ether."

Bibliography

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The Baltimore office of the Spencer Kellogg & Sons Sales Corporation, which is in charge of Alfred Day, has been moved to a new location on the eighth floor of the American building, where more space is available.

The Greek tobacco control office is endeavoring to promote the manufacture of the expressed oil from tobacco seed. The oil is said to be edible as well as useful as a soapstock, and the oil meal can be used as a cattle feed.

Correction

ON page 27 of *Oil & Fat Industries for* November, in the article entitled "Proposed Thiosulphate Number for Olive Oil" by Wallace H. Dickhart, the fourth column in the table of *"Experimental Data"* should be headed "Index of Refraction at 40° C." instead of "Valenta Index" as originally printed. The footnote exponent [1] in the first column of the same table should have appeared one line lower, i.e., against the figures 5.28, instead of against 4.78, as printed.—*Ed.*