DETECTION OF ALPHA-PALMITO-DISTEARINE IN PRESENCE OF BETA-PALMITO-DISTEARINE

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By F. J. F. MUSCHTER AND G. VISSER

In the literature on oils and fats (Holde, Marcusson, Gruen, Benedikt-Ulzer, etc.) methods are given for differentiating lard (from which alpha-palmito-distearine is crystallized) in presence of tallows (the associated component of which is the beta isomer); among others there is the method of Boemer, which is known as the Boemer melting-point-difference method. On account of lack of space we refer those interested to the literature, but for clarity we note that the value Sg + 2d, in the case of hog fat is more than 71, while in mixtures of lard with tallow, for example, beef fat in a minimum amount of 10 per cent of tallow, this value becomes smaller than 71.

Ad. Gruen, in his recent work, "Analysis of the Fats and Waxes," has assumed a very skeptical attitude toward the two-sided application of this Boemer value; on the ground that Boemer values above 71 do not on the whole give any guarantee of the purity of lard (ibid, page 360). The trade buys and sells on Boemer values. If these are above 71 the goods pass for pure lard, and, if not, the lot is declined (justly so, in this case) as adulterated with substances that are not lard, and among these tallow comes first into consideration.

It is precisely on account of the fact that Boemer values above 71 tell nothing, that an incentive is given to the use of methods like that in the distinguished work: "Food Inspection and Analysis," by E. Leach and A. E. Winton. This method consists chiefly in determining the iodine number, refraction, and saponification number of the sample, and tests are also made for vegetable constituents, train-oil, hardened oils; and in addition a microscopic investigation is made as to the presence of any tallow, which may be in the mixture (quantities of 0.5 — 1 per cent can now be detected), and whereby a possible adulteration with beef, goat or sheep fat may be brought to light.

This involves the microscopic determination of alpha or beta palmito-distearine, a method of differentiation which in fact is so sensitive that in this place we wish to go into the matter somewhat further.

After various attempts with the method as given in the literature it soon transpired that it must be discarded as much too troublesome; the differentiation of the two crystal forms, without some better means, is almost impossible, so that, somewhat regretfully, attempts were made to find better methods.

We tried to make the two isomers each separately visible in presence of the other, which might, for example, be done in the following manner:

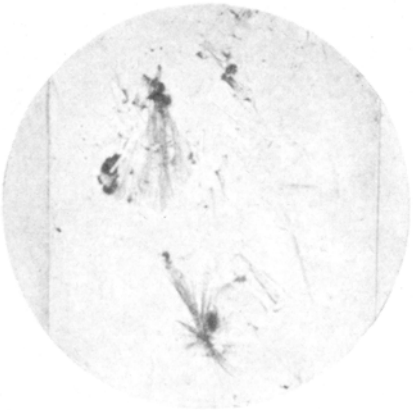

Fig. 1

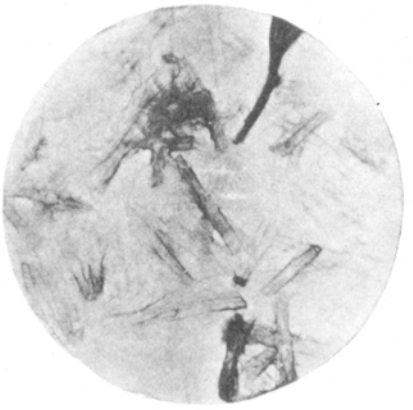

Fig. 2

Fig. 3

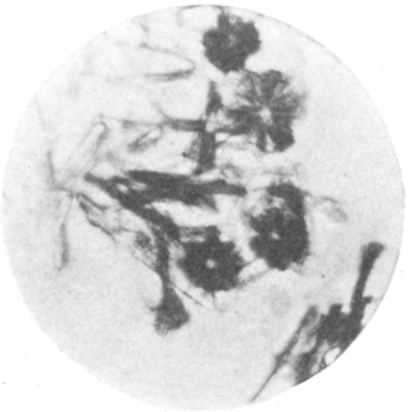

Fig. 4

Fig. 5

Fig. 6

One of the isomers may be camouflaged by placing it in a liquid medium of the same index of refraction, and thus, if the index of refraction of the other isomer is different, only one of the isomers will be visible.

While it was seen, *a posteriori,* that this method must be discarded, we will here add certain particulars regarding the determination of the refractive index—among others the corrections—chiefly for the reason that in the customary American method this determination is prescribed.

As media for the determination of the refractive index of the crystals we have chosen ethereal oils of different refractive indices, which, by mixing in various proportions, would give media with refractive indices like those of the isomers. On determining these indices by microscopic methods it immediately became apparent that the indices of the two isomers here considered lie very close together.

Due to the fact that the composition of the medium under observation was subject to change by partial evaporation during the operations, the exact determination of the indices was very troublesome. After various experiments we are inclined to ascribe to the alpha isomer a somewhat greater index than that of the beta isomer, namedly n_{15}^{D} 1.518 — 1.5186 for the alpha, and n_{15}^{D} 1.5180 — 1.5182 for the beta compound.

In the melted condition (at 70° C.) the indices are practically equal and differ at most by one unit in the fourth decimal place, namely: For the alpha isomer n_{70}^{D} 1.4423 — 1.4424; for the beta compound n_{70}^{D} 1.4424.

The alpha compound we obtained from leaf lard, the beta compound from beef fat, both by crystallization as is more particularly described at the end of this paper.

We determined the indices in the liquid phase, with the help of an Abbe refractometer.

As a correction factor for one degree centigrade, 0.0004 has been assumed for neutral fat. If passage into another phase should not require any other value for the correction factor, then in recalculating n_{70}^{D} into n_{15}^{D} there must be added to the value of n_{70}^{D} 55 $\times$ 0.0004 or 0.0220, so that the value of n_{15}^{D} will be found to be: 1.442 + 0.0220, or 1.4644.

In reality n_{15}^{D} is 1.5183, so that it follows at once that the correction factor holds only for the liquid phase.

In connection herewith we call attention to the fact that the determination of the index of refraction of lard or of beef fat at a temperature of 40° C., where two phases are present, may not be accurate. The reading at this temperature (below the melting-point) is in fact very unsatisfactory, since the line of division between the two fields is not sharp, and in consequence there can be no talk of an accurate adjustment of the cross-hair on this line.

It is thus desirable to give the indices at a temperature which lies somewhat above the melting-point, because here there is only one phase present, and by means of a correction-factor it can then be recalculated to the index at the temperature of the melting-point, or higher.

It has also repeatedly seemed to us to be advisable to determine the index at constant or slowly rising temperatures, not at decreasing temperatures when operating much above the melting-point. Under the last named circumstance the refraction does not increase with the fall in temperature (as would be expected), but remains stationary, so that application of a correction leads to totally misleading results. It is remarkable that the index does not remain stationary at progressive (rising) temperatures. No single case of this kind has been observed. Whether the opposite phenomenon has any connection with the known condition of over-melting is still an open question.

For completeness, we will note that the value of the refractive index for one degree of higher temperature becomes 0.0004 lower, and conversely becomes 0.0004 higher for one degree of lower temperature than is read off on the refractometer.

Since these optical methods have not supplied us with a method for easily differentiating the isomers, we have made use of a means employed in bacteriology, namely, coloring by means of organic dyes.

That an improvement would also result in the taking of microphotographs was to be expected. The coloring of the crystals facilitates the distinguishing of the crystal form, chiefly by showing the particular isomer in perspective. For this purpose a very small portion of the mixture of glycerides (first crystallization direct from ether, after which the crystals from the first crystallization are recrystallized from ether—absolute alcohol 50:1) is obtained, placed upon an object glass, and stirred with one or two drops of a Romanowsky-Giemsa solution (azure-eosin, as it occurs in the trade, is diluted for this purpose with six times its amount of absolute alcohol).

The crystals become colored after 4 to 6 minutes—that is, they become covered with the dye particles, and can now be studied under the microscope.

One begins with a small magnification (100 to 180 fold) and a search is made for typical growth-forms such as are pictured in the accompanying micro-photographs. These growth forms (crystal habit) are characteristic of each isomer.

After this, the magnification is increased to 400 or 500, this time to determine the form of the rhombohedrons or the cylinders. If necessary, the magnification is increased to 900, but in this case it must be borne in mind that no immersion is to be used, because immersion liquids often

wash off the dye with which the crystals are covered (not impregnated). During these observations the following is kept in mind: The alpha isomer occurs mostly in flat rhombohedra, which can easily be distinguished at a magnification of 100; they appear as bodies on the surfaces of which are seen evenly spaced parallel ribs, while the ends of the crystals are sharply cut off. These bodies may appear singly, crossed, in regular star forms, in rosettes and also (though seldom) in fan shapes.

These fans are always simple, that is, they consist of crystals which, beginning at a point, grow radially in definite directions out into space. Double fans (two simple fans, with their pointed sides placed opposite each other) often occur in the beta isomer. Here the two halves mostly differ in dimensions, and are therefore often unsymmetrical.

These fans or sectors, on their circular outer boundary, have projecting crystal ends which in the alpha form are sharply cut off; in the beta isomer they are pointed or rounded off. In a mixture of both isomers, observation of the outer ends of these clusters will disclose both sharply cut off and rounded ends; in such a case it is difficult to decide whether a fan is formed principally of the one or the other isomer; coloring the complex intensifies the perspective and one generally succeeds in making the desired identification. In general, it is the form of the outer end of the crystal (aside from the formation) that is decisive, and if the same isomer is found in *different* parts of the preparation, a conclusion can be drawn as to the presence of the corresponding species of fat.

The beta crystals occur in cylinder-like needles, the outer ends of which appear round or pointed, and if the needle can be observed in such a position that the light from a polarizer can pass through it, a small bright circle (ellipse) may be seen. One should be careful, however, not to confuse small fat globules (consisting of liquid fat) with

Fig. 7

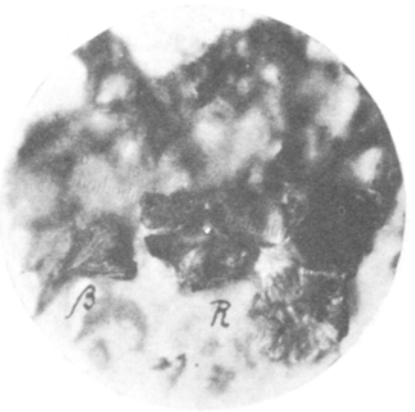

Fig. 8

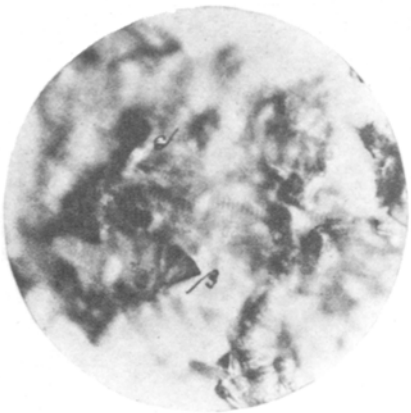

Fig. 9

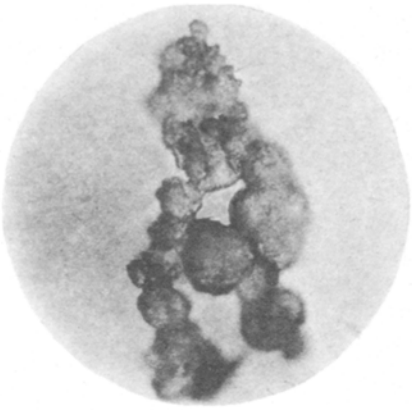

Fig. 10

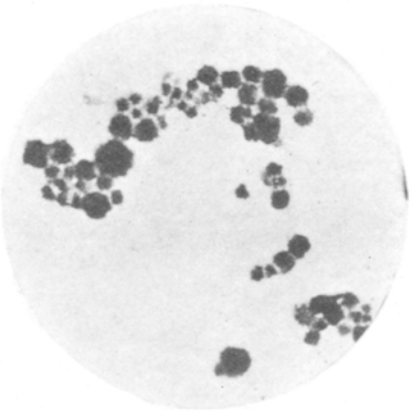

Fig. 11

these terminations of the needles; an unsatisfactory crystallization results in the undesirable presence of more over-saturated components in the preparation, which evaporation of the fat-solvent leaves behind as small globules. This phenomenon will occur if the crystals are not washed.

The beta crystals occur also in bent form, whereas the alpha isomer is exclusively rectilinear. The fans formed from the alpha isomer are also formed by joining together their obtuse angled ends, so that from rectilinear crystals, what on superficial observation has the appearance of a bent needle may be formed.

As illustrations we have inserted eleven of the photographs of the colored photographs, which, we hope, will contribute to a clearer idea of the crystal forms which are to be differentiated. It should not be forgotten, however, that when working with the microscope and making use of the stronger objectives or oculars, only one surface can be brought

sharply into the focus, so that details of the whole crystal or its growth-habit cannot be shown in a single microphotograph.

We will now pass to the descriptions of the microphotographs, which were taken with the help of a pointed light and chromate filter (saturated aqueous potassium chromate solution) on Agfa-chromo-isolar plates.

We see in Fig. 1 a picture of uncolored alpha crystals. Only the fan forms are clearly visible, the flat rhombohedra do not appear to their best advantage. How this condition may be improved is shown by Fig. 2, where the flat bodies with their sharply cut off ends are clearly visible. Fig. 3 shows a collection of beta crystals here one sees the double form, mostly consisting of a large and a small fan, which show a fine rectilinear rayed structure. This rayed structure is caused by the radial positions of the needles, which, however, do not all lie in one plane.

The pretty rosette forms are found in Fig. 4, while Fig. 5 is a greatly enlarged picture of an alpha fan. It is only necessary to look at the end-points to be certain of the identification of the alpha isomer. We here see that what is stated for example in Gruen's work (previously cited) regarding the beta isomer on page 359, that the "horse-tail-like" form indicates the presence of beef fat, is not necessarily true, since the same form is also possible with the alpha isomer. By stronger magnification a fan which is at first regarded as a beta fan may really turn out to be an alpha fan.

The series, Figs. 6, 7, 8, 9, shows pictures of forms which owe their origins to mixtures of beef and hog fats.

In all the figures of this series one clearly sees the beta fans, while in Fig. 7 a deceptive alpha fan occurs; deceptive in that, on first sight, it has the appearance of a beta fan, but which on closer examination is found to be classifiable among the alphas.

In Fig. 8, a beta fan occurs along with an alpha rosette.

If crystallization is effected from a mixture of ether (4 parts) and of the stronger objectives of oculars, only one surface can be brought absolute alcohol (1 part), the fan forms seldom occur, and when they do occur, they cannot be distinguished. The fan forms occur practically only on crystallization from ether (a little absolute alcohol is not disturbing).

The differentiation of the beta isomer by crystallization from ether and alcohol requires long practice.

We note that here and there evenly spaced cylindrical forms occur, the ends of which are cleanly rounded in contrast to almost similar appearing alpha forms with their sharply cut off ends, while the ribs of the rhombohedrons give the impression of evenly spaced staves, which might easily be confused with the cylinders of the beta isomer.

Finally, we will make a few remarks on the method of crystallizing.

To obtain well-formed crystals, crystallize twice, the first time from ether (one part fat, 2 parts ether), the next from ether and about 2 per cent of absolute alcohol (one part fat and 5 to 6 parts ether alcohol) in order to avoid malformations as in Fig. 10, where the various crystal forms are covered with lower melting fractions. Further, the crytallization should be done rapidly, that is to say, with vigorous agitation and at as low a temperature as possible (10° to 15° C.), otherwise not clearly definable groupings will be formed, such as may be seen in Fig. 11.

After each crystallization wash once with 1 to 2 cc. of the solvent. A small suction filter can be used to advantage.

We can recommend to the reader to make one trial with the Giemsa-Romanowsky dye (azur-eosin, as used in bacteriology).

The method of working is very simple: A very small quantity of the crystals is placed upon a preparation glass. After this one or two drops of the dilute dye solution are carefully dropped on; the crystals are carefully stirred and thinly spread out with a thin rod, one or two mm. in diameter, the end of which has been rounded by fusion; after 4 to 6 minutes the preparation is examined under the microscope. The preparation thus obtained keeps well without cover glass or fixation and can be kept standing on end in preparation-beakers without fear of the crystals falling off.

It is self-evident that the coloration of crystals, where the perspective is heightened, opens new paths for fundamental crystallographic investigations in other fields than the one here considered. In conclusion, we will gladly furnish further information or particulars to those interested in the matter.

Summary

The testing of hog fat for the presence of tallows according to the Boemer melting-point-difference method gives, in a case where Sg + 2d lies above 71, no certain indication of a possible adulteration, so that reliance cannot be placed on this method in this connection.

Only a competent microscopic examination, in which the isomers alpha and (in presence of) beta palmito-distearine are identified, does this test give certain proof of a possible adulteration with tallow. For the purpose of simplification, the crystals may be colored with the Romanowsky-Giemsa dye whereby the certainty of recognition of these isomers is greatly increased.

Micro-photographs, which have been taken after previous coloration on Agfa-chromo-isolar plates (with yellow filter) show a perceptible improvement of the picture, especially as regards the background.

Preparations of these colored substances can be kept in an upright position on the object glass without special fixation.