Errors in the Acid and Saponification Values of Fatty Acids

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Attention is directed to the variation in ester value of fatty acids depending upon method of isolation. The formation of sterol esters increases the ester value and produces errors in the acid and saponification value of fatty acids. The increase of ester value with elapsed time is discussed. The effect of ester value variation on the results obtained in soap analysis is described, and a possible basis of explanation for the anomalous results frequently obtained with the Goldschmidt method is presented.

THE NUMERICAL DIFFERENCE between the acid and saponification values of a fatty acid is traditionally defined as the ester value. Browne (1) has described the effect of the ester value on the determination of the chemical constants of fatty acids, particularly the acetyl value, when hydroxy acids are present.

Hydroxy acids which contain the hydroxyl group in the 4- or 5-position exhibit such a marked tendency towards lactone formation that the free acid is frequently extremely difficult to obtain (2). For example, 4-hydroxy-valeric acid, after separation from its salts or esters, passes immediately into a lactone.

Of the 17 theoretically possible hydroxystearic acids, 10 have been synthesized. Browne (1) postulated a 4-hydroxy stearic acid and employed it in his paper as an example. This concept has since been shown to be erroneous, but the mathematical aspects of the example are still valid.

It can be calculated that if half of a sample of pure hydroxy-stearic acid were to undergo lactonization the acid value would be depressed from 186.85 to 96.31 and the saponification value increased from 186.85 to 192.62. The original ester value of zero would then become 192.62 − 96.31 or 96.31 mg. KOH per g. of fatty acid. The theoretical basis for this calculation is the fact that stearo-lactone does not titrate with KOH, but does undergo saponification to potassium hydroxy-stearate.

Lewkowitsch (3) has ascribed increasing divergence between the acid and saponification values of aging oil samples to the slow formation of hydroxylated fatty acids which subsequently undergo loss of water with the formation of lactones.

In addition to postulating the presence of lactones as accounting for the ester value, there is the real possibility that sterol esters may also increase the ester value. The presence of sterols in soap is widespread. In this laboratory a series of 14 different bar soaps were found by digitonin precipitation to contain an average of 0.33% sterol calculated as cholesterol. Assumption of the view that lactones and sterols account for the greater portion of the ester value leads to some interesting deductions pertaining to soap analysis.

If the unsaponifiable matter (consisting principally of sterols) and unsaponified compounds are removed from a soap solution by extraction with petroleum ether and the soap subsequently split with mineral acid to liberate the fatty acids, the latter still yield an ester value. The ester value is less than that of the same soap split with acid, but not extracted with petroleum ether prior to splitting, and is due possibly to hydroxy acids (which are insoluble in petroleum ether in the form of their alkali salts) undergoing lactonization.

When a soap is split with mineral acid and cleared in acid solution by boiling or by standing on a steam bath for an extended period of time, as is the usual procedure, conditions are favorable for the slow formation of sterol esters in addition to the rapid lactonization of any hydroxy acids that may be present. The ester value is appreciably greater than in the case in which the soap is freed of sterols by prior extraction with petroleum ether.

An experiment to illustrate this point consists in adding approximately 0.5% by weight of cholesterol to the fatty acids liberated from soap prior to proceeding with the clearing operation. The ester value of these fatty acids is invariably greater than that of the same soap fatty acids to which no cholesterol is added.

The difference in the magnitude of the ester value, depending as it does upon the treatment of the fatty acids, probably explains the very puzzling results frequently obtained by use of the Goldschmidt (4) procedure in the analysis of soap. In this procedure the fatty acids are extracted from a soap by ethyl ether subsequent to liberation of the fatty acids by mineral acid. The fatty acids are then titrated in ether solution with KOH solution previously standardized against a sample of fatty acids from the same soap prepared in greater amount for the determination of titer and iodine value. The merits of the procedure consist in avoidance of the loss of volatile fatty acids, since it is not necessary to dry them to constant weight, and the direct standardization of the alkali against identical fatty acids by weight instead of a primary standard such as acid potassium phthalate. In practice the soap content of the sample calculated from the titration is invariably greater by a substantial amount than anticipated. This results from the relatively higher ester content of titer fatty acids which leads to a decrease in acidity. On a weight basis of the titer fatty acids, the normality (in mg. of fatty acid per ml. of alkali) of the alkali appears greater than the actual strength.

It has been noted in this laboratory for many years that ester values of fatty acids split from soap by acidification show a slow increase with elapse of time. Table I presents some typical results that demonstrate this phenomenon. The increase results from a simultaneous slow decrease of the acid value and a relatively more rapid increase of the saponification value. Since lactones are formed almost immediately, the increase

TABLE I

Increase in the Ester Value of Fatty Acids with Time at Room Temperature

Individual soap sample		Constants of fatty acids (mg. KOH/gram)					
		Immediately after splitting			1 Week after splitting		
No.	Stock composition	Acid value	Sap. value	Ester value	Acid value	Sap. value	Ester value
1	Tallow	203.4	206.0	2.6	201.6	209.0	7.4
2	Tallow	202.7	206.7	4.0	199.0	209.4	10.4
3	Tallow	196.5	202.9	6.4	195.7	205.4	9.7
4	Tallow	199.0	203.6	4.6	197.8	205.5	7.7
5	Tallow	198.6	204.7	6.1	197.4	209.3	2.1
6	Coconut	271.0	271.4	0.4	269.9	272.0	11.9
7	Grease	203.5	204.2	0.7	202.7	205.7	3.0

is presumed to be due principally to the slow esterification of sterols. The increase in ester value is noticeable within 24 hr. of liberating the fatty acids and the ester value continues to increase slowly for a period of a month or two before leveling off.

Over an extended time period of 4 mo., the acid value of a sample of tallow fatty acids decreased from an initial 205.2 to 203.2 while the saponification value increased from 205.6 to 207.3. The ester value originally was 0.4 and increased to 4.1.

In all work of this nature, involving as it does small differences between acid and saponification values, it is necessary to operate with great care. In this laboratory it has been found possible by rigorous adherence to prescribed experimental conditions to run replicate saponifications that do not differ by more than ± 0.2 saponification value units (5).

The preceding discussion has shown that the acid and saponification values of commercial fatty acids, as commonly determined, can only be approximations. Fortunately the variations in the acid and saponification value are limited in magnitude and do not seriously interfere in the normal course of analytical work. For example, in calculating the anhydrous soap content of a soap containing 72.05% of fatty acids, a negligible difference of 0.05% in the anhydrous soap content results depending upon whether acid value (222.1) or saponification value (224.2) is employed in the calculation.

On the other hand, it does make quite a difference whether the acid value or saponification value is employed in determining the composition of two component fatty acid mixtures. Thus a table of acid values constructed for a mixture of coconut oil (saponification value 266.1) and tallow (saponification value 205.0) gives a result for coconut oil content of 28% versus 31% depending upon which value is used. It is more realistic to employ the acid value (222.1) even though a corrected acid value, if it were practical to correct it, would fall somewhere between 222.1 and 224.2. It would not, except in a minority of cases, constitute an advantage to utilize instrumental methods of analysis in determining the exact nature of the compounds which determine the magnitude of the ester value. However, unless such a study is made, it is improper to apply such corrections to the acid and saponification value, as have been proposed from time to time (1,6). Fortunately for the analytical chemist the advent of vapor phase chromatography provides a far more accurate technique for the determination of the composition of mixed fatty acids than the use of the acid and saponification value.

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Fatty Acid Composition of *Choanephora Cucurbitarum*[1]

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Crude lipid extracts were prepared from *Choanephora cucurbitarum* which had been grown on a glucose-glutamic acid medium for periods up to 12 days. Methanolysis of the extracts and gas chromatography of the esters revealed that palmitate accumulated continuously at a rate 7-fold that of palmitoleate. Oleate accumulation followed a different pattern at a rate nearly 6-fold that of stearate. The esters included linoleate and four others which were not identified.

MANY FUNGI, particularly when grown under conditions of high carbohydrate and low nitrogen supply, synthesize large amounts of lipid (1,2, 3,4,5). *Choanephora cucurbitarum*, a carotene-producing phycomycete (6) which attacks squash blossoms and fruits, can produce as much as 50% of the mycelial dry weight as lipid (2). This report is concerned with the fatty acid composition of this lipid and some effects of culture conditions and age of fungus on the proportions of the acids.

Materials and Methods

Stock cultures of *Choanephora cucurbitarum* NRRL-A-6097 (+) and NRRL-A-6098 (−) were maintained on glucose-yeast agar slants. Transplants were made once a month.

An inoculum was prepared by subculturing from the stock cultures into flasks containing aliquots of a solution composed of 10 g. glucose and 3 g. yeast extract in a liter of water. After 3 days at 28C the cultures were combined in a sterilized Waring Blendor and macerated for 10–20 sec. Unless otherwise stated a 1-ml. sample of the homogenate from the combined + and − cultures was used to inoculate each 100 ml. of the basal medium.

The basal medium contained glucose, 20 g.; L-glutamic acid, 2 g.; KH_2PO_4, 1 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; thiamine, 100 mg.; Fe^{+++}, 0.2 mg.; ZN^{++}, 0.2 mg.; Mn^{++}, 0.1 mg.; Ca^{++}, 10.0 mg.; and water to 1 liter. The pH was adjusted to pH 6.0 before autoclaving.

Cultures were incubated in air at 28C in continuous darkness on a reciprocating-platform shaker operating with 4-in. strokes 86 times a min. Trays were rotated daily.

Two or more cultures were harvested at the desired intervals (7). After extraction of the cultures with 50–100 ml. of hexane-acetone (10:7) in a Virtis homogenizer, they were re-extracted at least twice with 50–100 ml. of acetone, and the homogenate filtered.

The combined hexane-acetone extracts were washed with 50 ml. each of water, methanol-water (9:1), and water in that order. Following passage of the hexane solution through an anhydrous sodium sulfate column the solvent was removed in vacuo and the crude lipid weighed on an analytical balance.

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