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COMPOSITION OF BONEFAT

A Thesis Presented to the Chemistry Department of the American University of Beirut in Partial Fulfilment of the Requirements for the Degree of Master of Arts in Chemistry.

by

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A B S T R A C T

The present work is intended to be primarily a study of the fat obtained from "Cattle Bones" of Syrian and Lebanese origin.

The technology of bonefat has been reviewed. Its chemistry has been considered in full details. From the analysis of the crude fat it is seen that the fat proper constitutes around 80% of the total bulk. The water content has been found to be high.

The composition of the insoluble fatty acids obtained from bonefat of local origin is considered to be about as follows: lauric 4.0%, myristic 4.0%, palmitic 20.1%, stearic 16.2%, myristoleic 0.5%, palmitoleic 2.4%, oleic 50.8% and linoleic 2.0%. Following is a brief description of the method adopted:

The fatty material was freed from the calcium soaps and impurities it contains by treatment with hydrochloric acid and subsequent extraction of the fat proper with petroleum ether. The ethereal extract was then filtered and the solvent evaporated on a water bath. The resulting mass was saponified with an alcoholic solution of potassium hydroxide and after removal of the unsaponifiable matter which consists mainly of hydrocarbons and cholesterol the fatty acids were liberated by the addition of an excess of sulfuric acid. These were then separated into "solid" and "liquid" fatty acids by making use of the lead soap-alcohol method. Esterification of each portion came next and the resulting ethyl esters were fractionally distilled under reduced pressure. The saponification and iodine values together with the weight of each fraction were used to calculate the composition of the sample.

A C K N O W L E D G E M E N T

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P R E F A C E

The present work is intended to be primarily a study of the fat obtained from "Cattle Bones" of Syrian and Lebanese origin.

It has been the object of the author to precede the section dealing with the determination of the physical and chemical characteristics of the bonefat by a more or less thorough dicussion of its technology which is supposed to shed some light on its industrial as well as commercial utilization. The composition of the fatty acids making up the sample has been investigated. The numerical results together with a brief description of the procedure adopted have been reserved to the last part of this treatise.

Though extensive work has been done on bonefat in general the literature is scanty with respect to the quantitative and complete examination of this commodity. On the other hand the fatty material dealt with comes from regions where different geographic conditions prevail.

I N T R O D U C T I O N

Among the by-products provided by the meat industry bones are by far the most important inasmuch as they embrace a large number of fields. Being capable of a wide variety of transformations they are used as starting materials for the manufacture of a diversity of useful products which are necessary items to our civilization.

Instead of being exported as they now are, the four thousand tons of bones which are available in Lebanon and Syria each year (1) could be fully exploited thus contributing their part to the modern industrial progress and to the betterment of the economic situation of the country. The unwarranted assumption of considering these valuable raw materials as wastes should be warned against. In this respect it is worthwhile to stress the importance of applied science in fighting against this illogical formulation due most probably to a lack of knowledge.

In a monograph like this it is impossible to consider all the related industries in full detail. Hence bonfat has been selected because of its immediate importance as well as its cheapness which makes it a good and economical source of fatty acids. Attention has been given to its production with due regard paid to the most modern scientific methods in existence. To emphasize throughout the relationship existing between the chemical constitution of this commodity and the almost unlimited number of the

specific economic applications in view it has been required to determine the composition of the fat in terms of the fatty acids it contains besides the usual average values, which are indispensable in the rapid characterization of oils and fats from the technological standpoint.

The lack of well organized abattoirs in Lebanon as well as in Syria coupled with the complete absence of meat packing houses makes it difficult if not impossible to obtain accurate figures concerning the number, kind and nature of domestic land animals serving this industry. However it has been feasible to collect rough estimations from reliable sources (2) of what is known as "cattle bones" in this part of the world. Generally speaking these are distributed as to origin as follows:

1.	Sheep	50%
2.	Ox	25%
3.	Camel and goat	25%

Since our main interest is in the fatty materials extracted from this commodity no attempt was made to isolate and identify fully all the components of these "cattle bones". Nevertheless the fat content was not disregarded and upon our analysis of different samples it has been found to vary within a certain limit i.e. between six and ten per cent giving a mean value of eight per cent. This is true of "old" bones only and does not apply to "green" or "fresh" bones which undoubtedly contain a larger proportion. Lewkowitsch (3) for

example states that "fresh bones from the heads, ribs, shoulder blades, etc. of cattle contain from 12-13% of fat, whilst the large thigh bones ("marrow bones") contain as much as 18 to 20%". According to Smith (4) bones contain something like 15-16% fat.

In this country bone grease is mainly used for leather lubrication and when the price of oils and fats becomes relatively high a large portion of the bone fats are consumed by the soap industry.

It is hoped that this study will promote more interest in this commodity and especially as a source of fatty acids the importance of which cannot be overemphasized. These in turn are prone to undergo a variety of reactions with the net possible production of a large number of substances which are highly valued in all branches of chemistry because of their wide applicability.

THE TECHNOLOGY OF BONEFAT

Before any attempt is made to discuss the technology of bonefat on the basis of existing literature, it is worthwhile to distinguish it from other substances which are more than often undistinguished from it though of marked difference in composition and consistency. At the outset the differentiation between bone oil and bonefat must be stressed. While the latter is defined as the greasy material extracted from bones, the former is the oily material obtained from the bony tissue by a process of destructive distillation and condensation. Neat's foot oil, obtained from the feet and occasionally from the shin-bones of cattle and sheep, is sometimes misnamed bonefat.

Depending upon the age, condition and the state of decomposition of the organic matter in the bones as well as the mode of extraction adopted, the product varies from a white or yellowish substance having a faint odour and containing a trivial amount of impurities and free fatty acids to an ill smelling dark brown fat with a disagreeable taste. The last mentioned variety contains a large amount of free fatty acids besides the presence of an appreciable quantity of calcium soaps formed by the interaction of the mineral lime salts in the bones with the fatty acids. Lewkowitsch (5) for example associates rancidity in the lower grades with the occurrence of calcium butyrate and calcium lactate together with smaller amounts of free butyric acid.

Pretreatment of Bones

Pretreating bones for fat production has an importance that is generally overlooked. Although the pretreatment, if carried out properly, results in a higher yield and a better product, many manufacturers fail to realize its value from the technological standpoint. No matter what the ultimate use of the items derived from bones, the bones should be subjected in advance to a sorting operation which removes all interfereing foreign bodies such as pieces of broken glass, metal scraps, and the like. Iron is easily eliminated by making use of specially designed electromagnets similar to the type described by Riegel (6).

Crushing and screening

Then follows the crushing operation. This reduces the size of the raw material to between 1/4 and 1/2" so that a larger surface area of the substance contained in the inner skeleton is exposed. It is not advisable however, to subject the bones to a finer grinding due to the great losses which sometimes result and consequently decrease the yield of bone grease.

A variety of crushing machines are available to accomplish this purpose. The most widely used machine consists of two heavy crushing rolls with cutters (7). An account with a full description of models which I believe could be used to achieve this end is given by Riegel (8) and Kanowitz (9). Not infrequently use has been made of the swing hammer mills which have almost been completely transplanted by the roll

crushers since the hammer mills are apt to yield a larger amount of excessively fine particles thus rendering them less desirable.

Separation of the crushed material into fine dust and rough particles suitable for the subsequent processing is achieved by employing revolving screens (7) which are generally a part of the crushing set-up. For a more elaborate knowledge concerning these, the reader is referred to Kanowitz (10) and Riegel (11).

Washing

Since the presence of dirt, blood, excreta, and other foreign material on the bones at the time of their extraction has a marked effect in depreciating largely the quality of the bonefat it is of the utmost importance to remove these impurities by washing, whether they be extraneous or intrinsic. This washing requires more than ordinary care and great stress must be laid upon this washing as it affects, among other things, not only the quality of the derivatives but also their yield. A thorough washing may be accomplished in any container in which it is possible to provide a large volume of running water (usually warm i.e. 40°-50°C.). Various bone washing machines have been devised. One described by Lewkowitsch (5) seems to be simple in construction and operation as well as efficient.

Bogue (12) advises steeping of the stock in a dilute solution of sulfurous acid in order to obtain a whiter and better product. This treatment has also the advantage of checking rancidification and consequently acts as a preservative. To avoid losses of ossein and fat Smith (13) recommends washing of the uncrushed

bones only.

Extraction of Bonefat

The recovery of bonefat sometimes called bone grease from cattle bones is carried out along two general main lines:-

1. Extraction with water
2. Extraction with organic solvents

Boiling out process

The oldest process of recovering fats from bones consists in boiling out broken (crushed) bones with water in open kettles at atmospheric pressure. The fat separates at the top and is skimmed off from time to time as it accumulates. This mode of extraction is only suitable for batch operations and necessitates the use of fresh bones which are not always commercially available. When the more common old bones are used, the low quality fat obtained the very low yield, and the obnoxious odours evolved in the boiling out method have militated against the spreading of such a method. However it is still used to a certain extent in the large packing houses of the United States and South America for the production of edible bonefat from absolutely fresh bones (14).

Steaming out process

A modification of this boiling out process is treatment of the crushed bones with live steam in an autoclave at a pressure not exceeding three atmospheres for a short period of time. This improvement has the decided advantage of increasing markedly the yield of grease and at the same time leaves both the extracted product and the residue unimpaired. Offensive

odours are completely eliminated and the extraction of the non-fatty materials is reduced to a minimum. Lewkowitsch (3) states that this process makes possible a yield as high as 75% of the fat present in the bones. Smith (15) concludes that this is the best procedure ever used to make bonefat.

Solvent extraction

No matter how old the bones may be and whatever their fat content solvent extraction could be used when other processes fail. The fat obtained by such extraction is dark brown and generally has an offensive penetrating odour. In addition to the considerable amount of free acids the extract may contain calcium lactate, butyrate and other calcium soaps formed by the interaction of free fatty acids and calcium salts in the bone, residue from solvents (16), mineral matter, organic and other impurities.

Solvent extraction is superior however, to the other processes described above in the sense that is is the only available means by which practically the whole fat may be extracted leaving at the same time the bony tissue undamaged (17).

Many of the solvents commonly used in the fat and oil industries are suitable in the extraction of grease from bones. Carbon disulfide, acetone, benzene, carbon tetrachloride, dichloroethylene, trichloroethylene (Westrol), tetrachloroethylene (Westron), special boiling point benzine and extraction naphtas boiling over a wide range have been proposed. Each of these solvents has its merits and demerits. Samples of bonefat extracted with the aid of carbon tetrachloride, for example, were found to be superior to those obtained by using petroleum ether (18). Carbon tetrachloride, however, is not recommended

in conjunction with iron vessels for the extraction because of the facility with which the chloride hydrolyses to produce hydrochloric acid. Instead the chloride is limited to laboratory use. Chlorinated solvents, especially dichloroethylene, have been found to give good results (17). In spite of being non inflammable a fact which largely minimizes fire hazards, the economic factor militates to a much greater degree against their adoption. The solvents employed commercially are almost exclusively petroleum naphthas and in this part of the world the lower boiling portion (60°-90°C.) finds wide acceptance among manufacturers. Were it not for the tediousness involved in removing the naphtha completely from the recovered fat, Scotch shale oil or petroleum naphtha boiling between 110°-120°C. would have been the best if it is required due to certain technicalities to eliminate the preliminary drying or superheating operations. The solvent having a higher boiling point than water, no complications are encountered when dealing with moist bones as the vapours of the solvent carry the water vapour with them when the mixture is heated.

The difficulty with which solvent extraction of bones is accomplished, because of the structural characteristics of the latter which tenaciously retain air and water in their capillaries, led manufacturers to attempt different and varying devices to overcome this obstacle.

Solvent extraction under pressure was in vogue for a certain period of time but the many explosions which resulted made it obsolete as compared to the modern installations for solvent extraction at atmospheric pressure and capable of yielding a

better fat besides leaving the bony tissue unimpaired. Only this latter process will be considered in details. A typical plant which the author had the occasion to see both under construction and actual operation will be fully described. A schematic view is found in fig. 1.

Owing to the drawbacks enumerated above cold solvent extraction is unsuitable for this particular purpose and resort is made to hot extraction processes which introduce the solvent in the form of vapours. A preliminary drying, of which some modifications are described in the literature (19, 20) is preferable although the calcium soaps are generally found in larger proportions in the fat obtained from previously dried bones (21). This preliminary treatment is avoided in to day's plants and instead a provision is made to superheat the S.B.P. benzine to a temperature of 120°-130°C. before its introduction into the extractor.

Description of a Bonefat Extraction Plant Using S.B.P. Benzine from Crushed, Moist (undried) Bones.

In the Beirut bone plant, the solvent extraction unit consists of a battery of four extractors with false bottoms with perforated floors and located just below the discharge doors. These are covered with sack cloths before the crushed bones are put in. aa', bb', cc', dd', are closed steam coils which provide for the heating of the lower compartment. 1, 1', 1", 1'", are live steam pipes. The crushed bones are filled into the extractors and the heated solvent vapours (120°-130°C.) issuing from the superheater H are admitted to the upper part of the lower compartment thru the perforated tubing. The use of the special

boiling point benzine (60°-90°C.) as a solvent and the omission of the drying operation prior to extraction coupled with the difficult and costly recovery of the higher boiling hydrocarbons necessitates the employment of a superheater (22). Extraction proceeds thru the penetration of the solvent vapours into the meal, the condensation of the benzine on the bones and percolation back to the main body of the liquor in A, B, C and D, where the solvent is continually boiled by the closed steam coils. Some solvent vapours distil over with water vapour thru the outlet valve at the top into the condenser W consisting of iron pipes immersed in a large body of running water. From the cooler the mixture of water and special boiling point benzine passes into the separator S which allows the solvent to return to the storage tank (not shown in the figure) while the water is discharged thru the lower outlet. Extraction is judged complete when no more water distils over: seven to eight hours being enough.

At the end of this period the miscella which has collected in the lower space of the extractors is drawn off into the evaporator E thru the valve v. Live steam is blown into the extractor to completely expel all traces of solvent from the bones. All the valves are closed and the top and bottom doors opened to admit of a good circulation of air for drying the residue while still hot.

The plant was run on a semi continuous basis i.e. while one of the extractors was charged or refilled the others together with the evaporator were operated on the condenser.

In the evaporator the larger portion of the solvent is

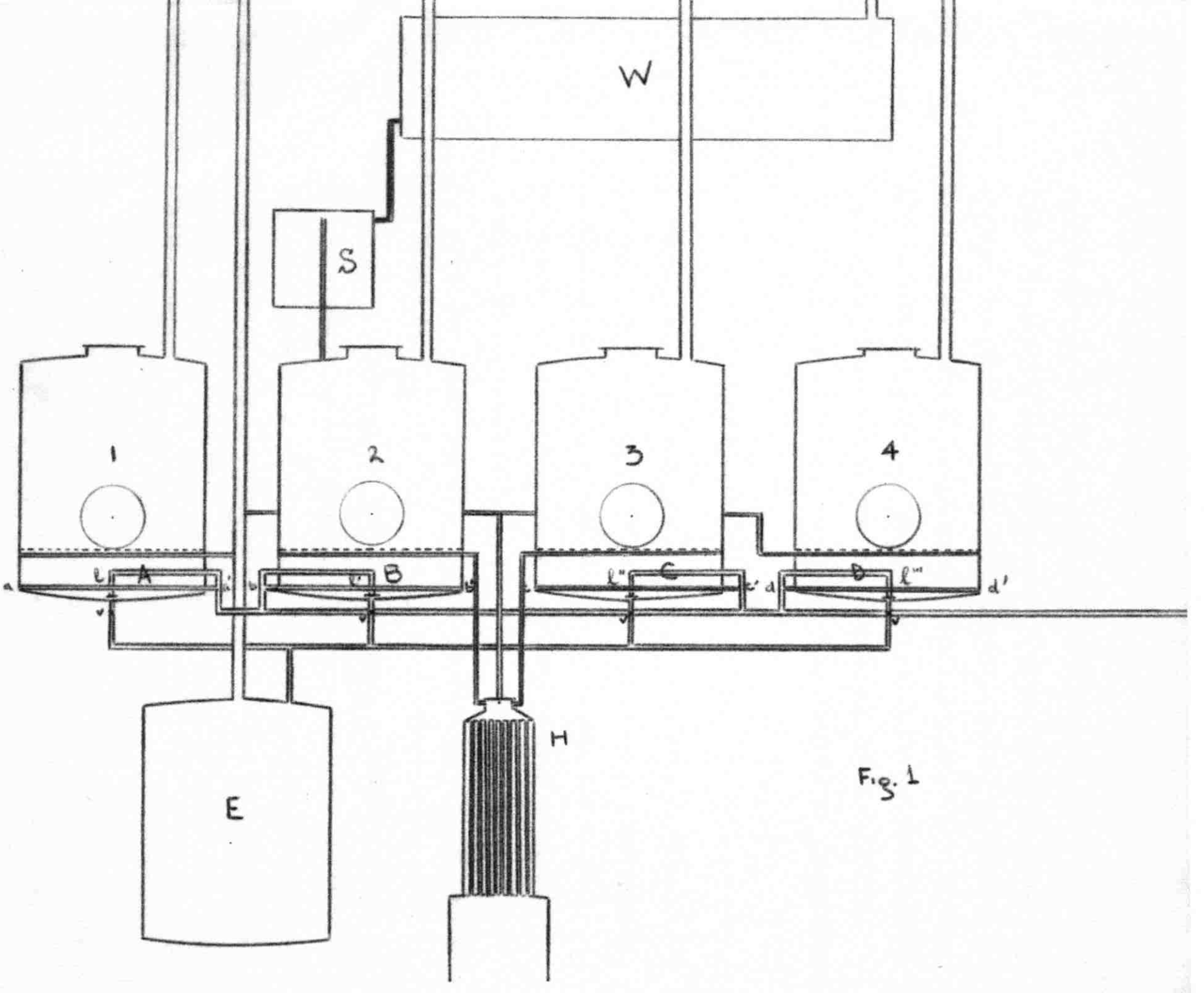


Fig. 1

removed by evaporation at atmospheric or reduced pressure. Then follows the introduction of live steam which is blown into the fat in order to free it from the last traces of benzine. Next all the valves are closed but one and steam under a pressure of twenty pounds per square inch is forced into the still. A pressure is created inside the evaporator and subsequently forces the melted fat out thru the delivery coil to the refinery.

Refining of Bonefat

As it leaves the extraction plant, bonefat is unsuitable for use subsequently as a raw material in manufacturing processes. Mention has been made of its dark brown colour and of its offensive and penetrating smell. Calcium soaps, calcium lactate and calcium butyrate, which are found in varying proportions are objectionable, especially when the fat is intended for soap making or for the production of fatty acids. The presence of hydrocarbons from the solvent, coloring matters extracted from the bony tissue, fine suspension of colloidally dispersed material, coarse suspended matter including mucilage, resinous bodies and albuminoid matter are also objectionable. The considerable amount of free fatty acids it generally contains offers no serious problem.

Since bonefat is at best a technical grease, the use of elaborate and costly processes, as applied to the refining of edible fats and oils, is undoubtedly out of the question. Instead one should look for an economic and reasonably effective way of purification. Several methods of bleaching

and deodorization of bone grease are described in the literature. In 1910 Volland (23) obtained a patent covering the use of barium peroxide at a high temperature for decolorizing and deodorizing bonefat. Wolff (24) treats the fatty material with hydrochloric acid to desintegrate lime soaps which he claims to be responsible for causing emulsions and poor yield in the cleavage process. This is followed by the addition of aluminum sulfate which acts as a purifying agent. Bolis (25) describes in detail the treatment of bonefat with sulfuric acid and states that, besides improving the colour as well as the odour, it composes the calcium soaps. Moreover the process is advantageous because of the "improved results and subsequent bleaching" even when no calcium soaps are present. For the removal of the odour due to putrefaction, he recommends the use of benzoyl peroxide in addition to sulfuric acid. He warns against the use of barium peroxide even in small proportion because of the possible formation of barium soaps. Gunther (26) advises treating bone grease with a 30-60% solution of hydrogen peroxide at 50°-55°C. in lead lined tanks. However this is a very delicate operation and great care should be exercised not to expose the material to too high a temperature. Residual colored solvents are objectionable and consequently should be eliminated previously. A preliminary purification with sulfuric acid is required (in order to decompose lime soaps). Eckart (27) opposes using hydrogen peroxide as the peroxide is likely to oxidize albuminous matter in the grease thus preventing complete clarification and decolorization. He favours the use of adsorptive agents such as activated carbon or fuller's earth.

He succeeded in obtaining a high quality bonefat and the method he worked out is fully described in the reference. In 1939 Urbain and Jensen (28) patented the use of hydrogen peroxide with a nitrite for bleaching. Erdheim (29) found that bleaching could be carried out satisfactorily with mixtures of highly active fuller's earth and decolorizing carbon. In an attempt to reproduce these results the author found that mixtures of fuller's earth, activated carbon (provided by the Pittsburgh Coke and Chemical Co.) and bone black were less effective than animal charcoal alone. Phelps and Black (30) succeeded in deodorizing animal fats by treatment with steam at 200°-250°C. and a vacuum of at least twenty seven inches after the addition of 0.001 - 1% of gum guaiac, citric acid or mixtures thereof. The resulting product showed a marked resistance to rancidity and reversion. However and as far as the author is aware this kind of fat has not been bleached successfully. Only improvement is obtained, the extent varying with the methods and substances used. One of particular importance and which has been adopted by manufacturers in general is chemical bleaching with combined oxygen, employing in this case potassium or sodium dichromate and an acid (sulfuric or hydrochloric acids). A full account of the procedure usually followed is given by Smith (31): to the melted fat is added a solution of dichromate, followed by the acid. All the bulk is agitated to bring about intimate mixing and then allowed to stand after which the chromic chloride solution is drawn off and the fat washed with water until free from acids.

A preliminary treatment with sulfuric acid especially if the grease is intended for hydrolysis by the Twitchell process is advisable (32). A description of this pretreatment will be found in Smith's (33).

Since the refined bonefat has a certain tendency to revert to its original colour as well as odour, attempts have been made to use antioxidants. Some of these have been studied and discussed by Vas (34).

U S E S O F B O N E F A T

The stearine and candle industries absorb large amounts of bonefat. This is hydrolyzed usually by the sulfuric acid or clave process and the resultant fatty acids purified by subjecting them to distillation under reduced pressure (14).

Monopol and Tetrapol soaps are made by saponifying bonefat in the presence of a "sulphonated oil" (usually sulphonated castor oil) the proportion of which varies from about half to an equal amount of the fat used (34a).

Lubricating greases for coarse bearings contain a high percentage of bonefat or soaps derived from it (34b).

It is a major component of the "stuffing" greases (34c) used in conjuction with degras for the treatment of leather.

It is more than often incorporated to the oil used in soap manufacture in an attempt to improve the lathering quality and at the same time decrease the price of the resulting mass. The persistence of the obnoxious odour, however, has prevented it from gaining ground in the high grade soaps.

METHODS FOR PHYSICAL AND CHEMICAL ANALYSIS

The structural complexity of the natural fatty materials coupled with the impracticability of their rapid resolution into their ultimate individual components has lead to the development of specially elaborate technological methods of analysis specific numbers (though fluctuation is apt to occur within very narrow limits) or "analytical constants" which are very helpful in the assessment or identification of a particular fat or oil have accumulated during the last fifty years. Not only do these chemical criteria give us average approximation of the composition of a given sample but they also enable us to judge of the quality of same. Consequently their importance is fundamental to the analyst engaged in industries related to fats and oils in general.

Lewkowitsch (35) subdivides these "analytical constants" into two main classes:

1. The "Characteristics" which depend solely on the specific nature of the fat and oil.

2. The "Variables" which give an idea of the quality of a given oil or fat.

The "Acetyl Value" belongs to both categories and circumstances will determine whether it is a characteristic or a variable.

Component Fatty Acids

Different procedures together with their modifications are available for the identification and quantitative estimation of the fatty acids contained in an oil or fat. Although the process is a difficult one and the manipulative technique cumbersome, none of these methods have lead to the quantitative isolation in a pure form of an individual component of a complex mixture. Despite this fact, the conventional methods provide a means of separating complex fractions into simpler ones, the composition of which may be ascertained with reasonable certainty.

Before a description is made of the method selected and its experimental application, it has been judged advantageous to devote some lines to a brief discussion of the applicability and limitation governing the laboratory operations so far devised to accomplish such a purpose. No observation of the chronological order has been made in referring to them. Instead the sequence has been reversed and the most modern methods are considered first.

Chromatographic Separation

The recent advances in chromatography has made possible its application to the isolation and determination of the individual components of a mixture of fatty acids. Since the resolution is carried out at room temperature it has the merit specially when dealing with highly unsaturated fractions of leaving the product unaltered.

A variety of solvents have been proposed but benzene and petroleum naphtha were found to be most effective. Among the

suggested adsorbents alumina and silica gel are the most widely used.

Although some work related to this particular subject has already been done and the experimental results proved to be fruitful the field is still virgin and much improvement is to be desired.

Special attention is called to references 36 - 43.

Distillation Methods

Distillation of a fatty acid mixture particularly after conversion to esters of monohydric alcohols (usually ethyl or methyl) is the most common laboratory operation used for determining the individual components making up a natural fat or oil. Various types of stills have been described in the literature (44). None of the available fractionating columns has actually effected complete separation of a particular constituent. Instead and depending largely upon the relative complexity and percentage composition of the original mixture they yield intermediate fractions boiling over a definite range of temperatures. By a knowledge of a very few "analytical constants" and by making certain assumptions which have been experimentally established it is possible to calculate the composition of each resulting fraction and consequently of the whole sample.

Solubility Methods of Separation

As its name implies this procedure depends upon the relative solubility of the individual fatty acids, their salts or bromo derivatives in different solvents or in the same solvent at different temperatures. Owing to the mutual solubility effects exerted by one component of the mixture on the solubility of the

other components (45) a sharp line of demarcation cannot be drawn.

Low temperature Crystallization

Speaking of the preparation of large quantities of saturated and unsaturated acids, Markley (46) claims that low temperature crystallization is simpler, more rapid and more economical than any other method.

It depends upon the relative solubility of the fatty acids or their esters in solvents at different temperatures (very low).

An investigation made by Hartsuch (47) shows that this method is superior to either the lead soap-alcohol or the barium soap-benzene method which will be mentioned later in effecting the separation of saturated from unsaturated fatty acids.

This process has found wide application when the mixture intended for separation contains a relatively large amount of highly unsaturated acids thus eliminating the possibility of isomerization or other chemical changes in the final product.

References 48 - 53 are of special interest.

Lead Soap Method

The lead soap method which originated in 1828 (54) is still the most widely used for the separation of saturated from unsaturated fatty acids. It is based upon the differential solubility of the lead soaps in ether or alcohol (55).

The Barium Soap Method

The barium soap-benzene method has been applied by some investigators (56 - 57) to the isolation or purification of a

particular fatty acid in a mixture. Though originally proposed as a means for separating saturated from unsaturated acids (58) it has been largely superseded by the Twitchell modification (55) which kept gaining ground since its introduction.

The Magnesium Soap Method

No sharp and effective separation of a mixture into unsaturated and saturated fatty acids has ever been obtained by this procedure. It has been widely applied however for the purification of specific fatty acids (59 - 61) and in this respect it gives quite reasonable results as a qualitative means of examination.

The Lithium Soap and Bromination Methods (48, 62-74)

The lithium soap-acetone method and the same is true of the bromo derivatives method give satisfactory results when it is intended to separate polyethelenic from monoethylenic and saturated acids. Though their importance as analytical tools of examination cannot be overemphasized we should admit that they are of great merit from the preparative standpoint.

EXPERIMENTAL WORK

In this section we deal with the analysis of bonefat as it reaches the refinery.

The specimen analysed was obtained from the solvent extraction unit of the Beirut bone plant similar to that described in figure 1. Sampling has been carefully carried out by the author in the factory and the secured mass could be safely considered to be a representative sample of the bulk. The bones used average about 50% sheep, 25% ox, and 25% camel and goat, by weight.

In the laboratory the crude fat was melted on the water bath in a large porcelain dish after which it was subjected to a vigorous stirring while cooling it down slowly in order to distribute the water and the present impurities thru the whole body. Settling down of the heavy particles to the bottom of the dish is thus eliminated and consequently a uniform and homogeneous product results.

All the available modifications of determining a particular characteristic are too numerous and time consuming to be considered separately as applied to the assessment of the fatty material in "bonefat". Instead adherence has been made to a refinement which in the mind of the writer is the best with respect to reliability, validity and simplicity.

Preparation of the Fatty Matter for Examination

Before any attempt is made to determine the physical and chemical characteristics of the fat proper, the fatty material must be freed from the impurities and foreign bodies it

contains. This separation has been fully described by Shukoff and Schestakoff (75) and is carried out in the following manner which, as will be observed, also gives proportions.

Fatty Matter 83.42%

Five drops of concentrated hydrochloric acid were added to 9.5062 grams of the crude fat contained in a 125 cc. Erlenmeyer flask. The mixture was warmed on a water bath for an hour and agitated from time to time whereby the lime soaps are decomposed. Forty cc. of petroleum ether (fraction boiling below 70°C.) were added to the content of the flask and the whole agitated and left overnight for a complete extraction of the fatty material. Next the solution was poured off carefully through a tared quantitative filter paper into a second 125 cc. Erlenmeyer flask, the weight of which is known, making sure not to transfer the acid drops collecting at the bottom of the container. The flask and the filter paper were washed respectively several times with petroleum ether and the washings received in the second flask. The solvent was distilled off on a steam bath and the fat dried at 105°C. until the weight remained constant. 7.9306 grams were obtained giving a percentage of 83.42% of fatty matter.

Impurities (Organic and Inorganic) 6.588%

The acid solution and the dirt from the first flask were washed on the tared filter paper and the washings received in a container which has been previously dried, cooled and weighed. The filter paper and its content were dried at 105°-110°C. to constant weight. This represents the water insoluble impurities and has been found to amount to 0.5030 grams or 5.29% of the crude fat.

The wash water undoubtedly contains the acid with the lime and other soluble substances. Heating and drying at 105°-110°C. leaves behind the water soluble impurities. This has been found to yield 0.1234 grams which represents 1.298%.

Moisture 9.98%

Since lime soaps tenaciously retain water, as has been previously discovered, a direct determination will inevitably give too low a result. Consequently this was obtained by difference and showed a value of 9.98%.

Ash 1.797%

This was arrived at by incinerating a fresh portion of the sample (6.2365 gr.) whereby the calcium soaps are converted into calcium carbonate. Care should be exercised to restrict the temperature of calcination, so as not to overheat the resulting mass from fear of decomposing it into calcium oxide and carbon dioxide. The weight of the residue was found to be 0.1121 grams representing 1.797% of the fat (crude basis).

By dissolving the residue in a known volume of standard hydrochloric acid solution and back titrating with a standard solution of sodium hydroxide a percentage of 1.768 in terms of calcium carbonate was obtained.

Analyses and Tests

The fat proper, having been freed from the foreign substances and impurities it contains, is now ready for examination.

A logical step would be to consider first the physical characteristics of the fatty material in the light of those tests which besides being relatively simple from the manipulative point of view furnish more decisive results than could be obtained by more complicated procedures.

Specific gravity (76) 0.8957 at 40°/25°C.

The specific gravity of the bonefat in question has been ascertained by means of the Sprengel's pycnometer and was found to be 0.8957 at 40°/25°C.

Melting point 38°C.

The method of the Association of Official Agricultural Chemists (77) which is a modification of the original procedure proposed by Wiley (78) was adopted. The temperature at which the disc of fat rounded up (thus becoming a sphere) was taken as the melting point, showing a value of 38°C.

Refractive index 1.4592 at 40°C.

This was determined at 40°C. and showed a value of 1.4592. An Abbe refractometer was used.

Solidifying point 15°C.

The melted fat was cooled down slowly in a water bath until it solidified which occurred at 15°C.

Saponification value (Koettstorfer Number) 201.55

This is defined as the number of milligrams of potassium

hydroxide required to completely saponify one gram of fat or oil. The sample gave a value of 201.55.

Saponification equivalent 278.4

It is defined as the number of grams of oil that is completely saponified by 56.11 grams of potassium hydroxide. The value for the submitted specimen was found to be 278.4.

Since we are interested in the technical application as well as the complete analysis of bonefat with respect to fatty acids both values have been quoted. The former gives us directly the amount of alkali which is required to saponify a charge of soap and the amount of glycerol which is supposed to yield for example while the latter throws some light on the composition of the sample as it gives immediately the mean molecular weight of the glycerides and fatty acids contained herein.

For a full account of the manipulative details and the methods used in preparing the reagents reference is made to Griffin's monograph (79).

The alcohol was purified by working along the suggestions presented by Malfatti (80) and which have been adopted by the Association of Official Agricultural Chemists (81).

Iodine value 52.65

This "analytical constant" is one of the most valuable characteristics as it tells at once the group to which the fat belongs and consequently is of great help in the identification of an unknown sample.

It is an average measure of the total unsaturation present. The Iodine Value represents the number of grams of iodine absorbed by hundred grams of fat or oil.

The determination has been carried out by following the procedure proposed by Hanus which has been adopted by the Association of Official Agricultural Chemists (82) and fully described by Jamieson (83).

The value for the sample is 52.659, thus placing it in the non-drying oil category.

Reichert-Meissl Value (84) 1.46

The Reichert-Meissl Number is an indication of the proportion of soluble volatile fatty acids i.e. those which can be separated from the saponified fat after subsequent acidification by distillation with steam. It is defined as the number of ccs of 0.1 Normal alkali solution required to neutralize the soluble volatile fatty acids from five grams of the sample. The value for this particular bonefat is 1.46 or 0.26% as butyric acid.

Hehner Value (Insoluble Fatty Acids) (85,86) 97.

This is the percentage of insoluble fatty acids obtained from a fat or oil. Usually it includes the percent quantity of the unsaponifiable matter present in the sample. Bonefat of local origin showed a value of 97.

Soluble Fatty Acids (85) 0.28%

The soluble portion of the fatty acids was determined in conjunction with the Hehner value and was found to represent 0.28% of the total fatty matter.

Acid Value (87) 94.72

The acid value is a measure of the quantity of free fatty acids and is defined as the number of milligrams of potassium hydroxide required to neutralize the fatty acids found in one gram of the fat or oil.

This value is of a primordial importance when dealing with fats and oils intended for lubricating and pharmaceutical purposes besides being used as a criterion of edibility. As to the fat in question it gave a value of 94.72, in terms of milligrams of potassium hydroxide or 47.60 as percentage of oleic acid.

An attempt was made also to determine the acidity of the original sample i.e. including the impurities and foreign substances. The result given in terms milligrams of potassium hydroxide amounts to 67.47 or 33.9% oleic acid.

Unsaponifiable Matter 1.81%

All substances which are not saponified by alkali but are soluble in ether and petroleum ether are classified as unsaponifiable matter. Generally they are made up of sterols with add-mixtures of small quantities of other alcohols and hydrocarbons. Though their nature has not been fully ascertained, resinous bodies and colouring matters have been reported to be present in certain portions of unsaponifiable matter (88).

The modified Kerr-Sorber method described by Jamieson (89) was used. It is based upon a ready solubility of the unsaponifiable matter in ether in contradistinction to the practical insolubility of the soap in this solvent.

The bonefat dealt with contains 1.81% unsaponifiable matter.

For a discussion of its composition the reader is deferred to a later section of this monograph.

Acetyl Number 14.68

The Acetyl Value indicates the number of milligrams of potassium hydroxide required for the neutralization of the

acetic acid obtained by the hydrolysis (or saponification) of one gram of the acetylated product.

The determination has been carried out by making use of the procedure proposed by Andre - Cook a modification of which is found in Jamieson's (90). The method depends upon the fact that hydroxy acids and alcohols, when heated with acetic anhydride undergo a change consisting in the substitution of the acetyl radical for the hydroxyl. The figure obtained is 14.68.

The Thiocyanogen Value 50.9%

This "analytical constant" is of special interest when it is desired to descriminate between oleic, linoleic and linolenic acids. It presupposes a knowledge of the iodine value. The test is a very delicate one and unless attention is paid to conditions such as the concentration of thiocyanogen reagent and the excess of it used, the time allowed for the reaction, and the temperature of the reacting substances, it is liable to give misleading results.

The procedure followed is that of the Committee on the Analysis of Commercial Fats and Oils of the American Chemical Society which was first proposed by Kaufmann (91-96) in 1925. It is based upon the fact that while oleic acid reacts quantitatively with thiocyanogen only one double bond of the linoleic acid and two in the case of the linolenic acid are involved in the reaction.

The figure obtained is 50.9%. This will be taken up again in calculating the composition of the fatty acids.

Separation and Fractionation of the Fatty Acids

Some of the most important laboratory operations for use in the analysis of fatty acid mixtures are discussed in an earlier section. It is desirable to state here the method as applied to local bonefat.

Saponification

Saponification was effected by means of an alcoholic solution of potassium hydroxide. 400 grams of the sample were mixed with 2000 grams of absolute alcohol containing 135 grams of potassium hydroxide and the whole boiled vigorously under a good reflux condenser for three hous (97). At the end of the operation the alcohol was partially distilled and the residue diluted with a large volume of water. Removal of the alcohol was continued until most of it passed over with the distillate.

Removal of the Unsaponifiable Matter

This was accomplished by using a continuous extractor similar to that described by Hilditch (98). Petroleum ether (boiling below 70°C.) was substituted for ethyl ether.

Recovery of Fatty Acids from the Saponification Medium

After removal of the unsaponifiable matter the fatty acids were liberated by the addition of an excess of sulfuric acid i.e. until acid to congo red. The whole bulk was left to stand overnight after which it was filtered to separate the insoluble fatty acids floating at the top of the aqueous medium. This was followed by a thorough washing being careful to receive the wash water into the filtrate

which was subsequently extracted with petroleum ether to remove the water soluble fatty acids. Evaporation of the solvent and short periods of drying until constant weight came next. As to the portion making up the longer chain acids it was dried at 105°C. and then subjected to a preliminary separation into saturated or "solid" and unsaturated or "liquid" acids.

Separation of Saturated from Unsaturated Acids

For analytical purposes the estimation of the relative percentages of "solid" and "liquid" acids was arrived at by making use of the lead soap-alcohol method proposed by Twitchell (55).

Since our ultimate purpose is the determination of the individual constituents by fractional distillation of the mono-esters a preliminary separation of the mixture into saturated and unsaturated fatty acids was considered desirable. Owing to the bulkiness of the sample dealt with it was found convenient to carry out the operation in the light of the suggestions presented by Hilditch (99).

300 grams of the fatty acid mixture were dissolved in 1500 cc. of 95% ethanol. Likewise an alcoholic solution of lead acetate was prepared by dissolving 210 grams of the salt in 1500 cc. of 95% ethyl alcohol containing 25 grams of glacial acetic acid. Both solutions were heated to boiling following which they were slowly mixed with constant stirring. After cooling to room temperature the resulting mixture was stored overnight in the icebox and subsequently filtered through a 20 cms. Buchner funnel into a four liter suction flask. Recrystallization of the

precipitated lead soaps from the same volume of solvent under the same conditions of temperature was followed by a thorough washing with 95% ethyl alcohol.

The precipitated lead soaps were suspended in a large volume of water and concentrated hydrochloric acid was added until the aqueous layer was distinctly acid to congo red. The whole batch was heated on a water bath thus causing the fatty acids to float on the top and consequently rendering their separation easier upon cooling. They were then transferred to a separatory funnel, the lead chloride and the aqueous solution extracted with petroleum ether and the extracts added to the funnel containing the main bulk of acids. A thorough washing with water eliminated the mineral acids while the addition of anhydrous sodium sulfate assisted in the removal of the last traces of moisture. The dried petroleum ether extract was then filtered using a Buchner funnel and a suction flask which have been previously heated in the oven at 110°C. and cooled. Next the solvent was evaporated on the water bath and the resulting saturated or "solid" acids dried at 102°C. Before they were subjected to esterification, their saponification and iodine values were determined and were found to be 203.3 and 10.3, respectively.

As to the portion containing the soluble lead soaps, it was heated on a water bath, after addition of water, to remove the excess alcohol. Concentrated hydrochloric acid was added next and the liberated fatty acids were extracted with petroleum ether, washed until free from mineral acids and dehydrated with anhydrous sodium sulfate. The extract was filtered thru a Buchner funnel and the filtrate received in a previously dried suction flask. The solvent was then evaporated and the resulting "liquid" fatty acids were dried in the oven for short periods until the weight remained constant. The saponification and iodine values obtained are 209.1 and 83.4 respectively.

Calculations

Weight of the "solid" fatty acid fraction	1.8013 g.
Weight of the "liquid" fatty acid fraction	2.4748 g.
Total weight of the fatty acids	4.2760 g.

or expressed in terms of percentage:

"Solid" fatty acids	42.12%
"Liquid" fatty acids	57.88%

From the knowledge of the iodine value of the "solid" fatty acid fraction it is possible to calculate the relative amount of unsaturated acids it contains. Assuming that the only unsaturated component is oleic acid, we can correct for the above value in the following manner (100) :

$\frac{10.3 \times 100}{90} = 11.44\%$ = percentage of unsaturated acid in "solid" acid fraction.

$\frac{1.8012 \times 11.44}{100} = 0.2061$ g. unsaturated acid in the "solid" acid fraction.

$\frac{(1.8012 - 0.2061)}{4.2760} \times 100 = 37.3\%$ of the total fatty acids of the saturated type contained in the "solid" fatty acid fraction.

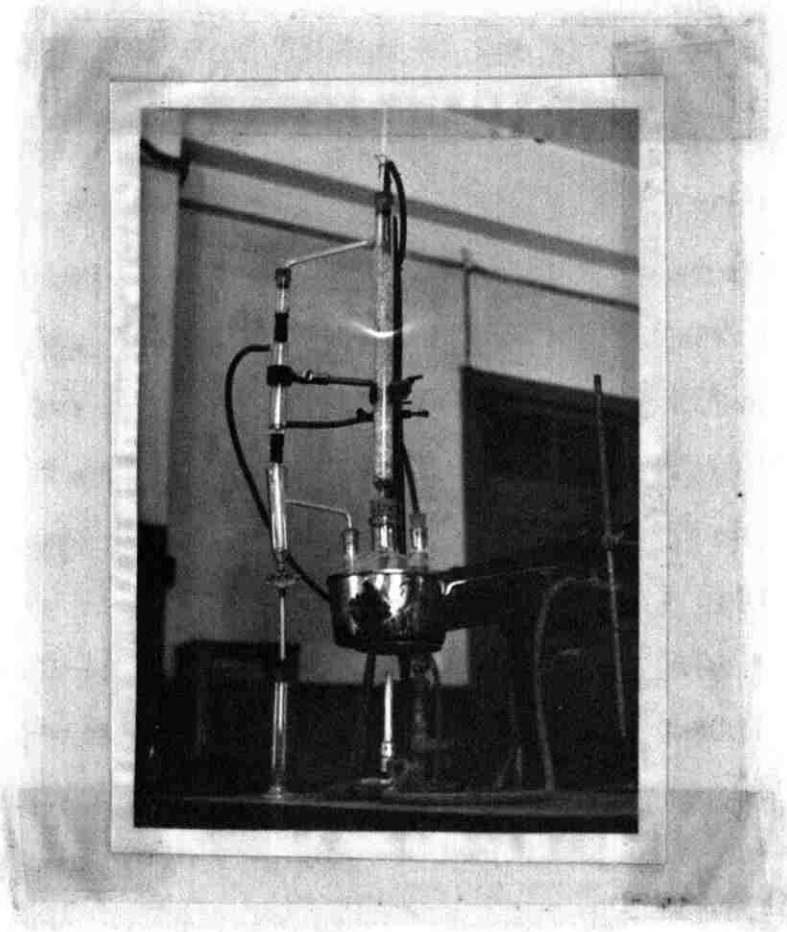
The percentage of oleic acid contained in the "solid" fatty acid fraction is therefore 4.82% of the total fatty acid fraction.

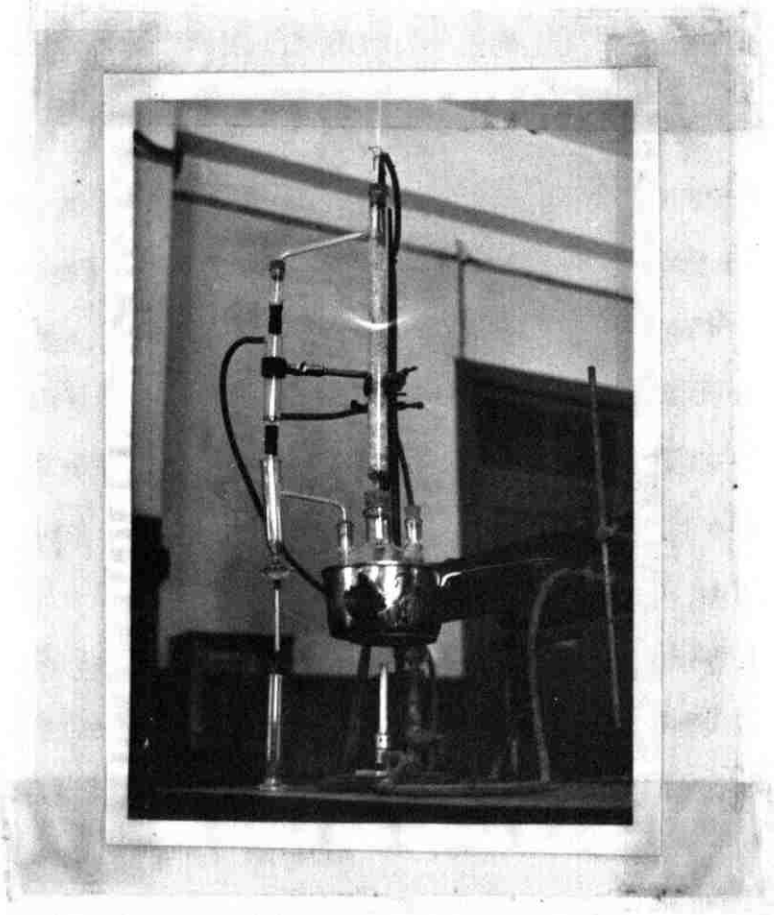
Esterification

The direct alcoholysis (101) of the sample would have eliminated several time consuming steps. This has not been possible, however, due to the presence of an appreciable amount of unsaponifiable matter which otherwise will tend to remain in the esterified mixture and consequently introduces some errors in the calculation of the composition of the fat.

Workers in that field have suggested many procedures (102-5) which, despite the unity of the principle underlying each of them, are quite dissimilar with respect to manipulative details.

Ethyl alcohol was used and the esterification of each of the fractions resulting from the lead soap separation was carried out in the apparatus shown in the accompanying photograph. The fatty acids, the corresponding alcohol and the benzene in the molar proportions of 0.5, 0.75 and 0.65, respectively, were charged in the three necked flask. 1 cc. of concentrated sulfuric acid was added to the mixture and the whole heated slowly in an oil bath. The water formed during the reaction was removed azeotropically, a provision being made to return the mixture of alcohol and benzene to the reaction flask. This kind of equipment has the advantage of eliminating the use of an undue excess of alcohol and sulfuric acid. Besides it makes possible practically complete esterification in 4-5 hours. At the end of the operation, the excess alcohol and benzene was





removed by heating at a relatively low temperature under the vacuum of a water pump. The residue was poured in a large volume of water and the esters extracted with petroelum ether. The ethereal extract was then washed with a dilute solution of sodium carbonate and water respectively. After a preliminary drying with anhydrous sodium sulphate the solvent was removed and the esterified portions subjected to distillation.

Vacuum Fractional Distillation of the Mixed Fatty Acids Ester Fractions.

Fractionation of the esters was achieved in a still consisting of a 500 cc. flask provided with a side arm for filling and discharging purposes and upon which was sealed a column 48 cm. long and 3 cm. internal diameter. Broken glass tubings were used as the packing medium. The top was fitted with a rubber stopper carrying a thermometer and a small water reflux condenser. The rubber stopper was protected from the solvent action of the hot vapours by a disk of cork stuck to its lower part. At a distance of 3.5 cm. from the top and just facing the bulb of the thermometer a side arm extends for 11 cm. This is followed by a 32.5 cm. air condenser which in turn is connected to a rotating receiver holding 12 small test tubes.

The column was lagged with asbestos and then covered with several pieces of wool cloth in an attempt to reduce heat losses as much as possible.

The flask was almost completely immersed in an oil bath the temperature of which could be kept relatively constant by using a Bunsen burner.

The results of the fractional distillation together with the saponification and iodine values are tabulated in the following two pages:

Fractional Distillation Data of Ethyl Esters of the "Solid" Fatty Acids

Weight of the Ester Charged in the Still 106.34

Sample No.	Temp. °C.	Pressure mm / Hg	Weight g.	% of total	Sap. No.	Iod. No.	Estimated Acid Components % of total: Myristic %	Palmitic %	Stearic %	Oleic %
1	147-65	7	5.22	4.97	201.7	0.7	1.05	3.9	---	0.02
2	165-70	7	5.55	5.32	199.8	0.73	0.67	4.6	---	0.05
3	170-72	6	6.06	5.87	195.83	0.94		4.66	1.14	0.07
4	172	6	5.56	5.32	193.8	1.17		4.3	0.94	0.08
5	172	5	5.73	5.48	192.9	1.28		4.15	1.24	0.09
6	172	5	5.71	5.49	192.8	1.28		4.1	1.3	0.09
7	173	5	5.24	5.03	191.	1.56		3.83	1.1	0.1
8	173	5	5.72	5.43	191.	1.7		3.7	1.62	0.11
9	173	5	6.03	5.74	190.9	1.9		3.7	1.91	0.13
10	173	5	5.66	5.4	187.	2.02		3.53	1.74	0.13
11	174	5	5.62	5.38	186.5	2.9		2.12	3.06	0.2
12	174-75	5	6.3	6.11	184.13	7.78		2.	3.54	0.57
13	175-78	4	6.	5.71	183.25	13.62		1.15	3.6	0.96
14	178-82	4	6.34	6.08	182.8	20.6		1.05	3.5	1.53
15	182-84	4	6.37	6.06	182.6	21.8		0.96	3.5	1.6
16	184-dropping	4	6.08	5.77	180.7	26.88		0.3	3.6	1.9
residue			11.33	10.81	181.4	19.04		1.	7.31	2.5
total			104.52	100.			1.72	49.05	39.10	10.13
Per cent. Acids.							0.7	20.15	16.24	5.03

Fractional Distillation Data of Ethyl Esters of the "Liquid" Fatty Acids

Weight of the Ester Charged

in the Still 116.50

Sample No.	Temp. °C.	Pressure mm / Hg	Weight g.	% of total	Sap. No.	Iod. No.	Estimated Acid Components % of total: Lauric %	Myristic %	Myristoleic %	Palmitoleic %	Oleic %	Linoleic %
1	114-48	9	5.77	5.01	307.58	15.3	4.18	---	0.17	0.66		
2	148-55	9	4.9	4.26	232.6	28.52	2.96	---	0.26	1.04		
3	155-75	9	5.32	4.63	213.58	29.27		3.15	0.26	1.22		
4	175-80	8	4.75	4.12	212.35	33.5		2.6	0.18	1.34		
5	180	5	5.87	5.11	180.73	84.85					4.92	0.19
6	180-82	4	5.12	4.45	180.73	84.98					4.28	0.17
7	182	4	6.6	5.74	180.7	85.89					5.45	0.29
8	182	4	6.43	5.6	180.7	86.25					5.3	0.3
9	182	4	6.42	5.6	180.7	86.42					5.3	0.3
10	182	4	6.56	5.7	180.7	87.3					5.32	0.38
11	182	4	5.86	5.1	180.7	87.4					4.75	0.35
12	182	4	6.82	5.94	181.05	88.07					5.5	0.44
13			44.51	38.74	180.7	83.9					37.74	1.
total			114.93	100.00			7.14	5.75	0.87	4.26	78.56	3.42
per cent. Acids.							3.95	3.24	0.49	2.42	45.8	1.98

The composition of the insoluble fatty acids obtained from bonefat of local origin is therefore considered to be about as follows:

Lauric acid	4.0 %
Myristic acid	4.0
Palmitic acid	20.1
Stearic acid	16.2
Myristoleic acid	0.5
Palmitoleic acid	2.4
Oleic acid	50.8 (by difference)
Linoleic acid	2.0
Total	100.0

The absence of linolenic acid was ascertained by making use of the hexabromide test, with negative result.

The percentage of linoleic acid has also been calculated from the iodine and the thiocyanogen values, and the result agrees pretty well, within experimental error, with the figure obtained from fractional distillation of the ethyl esters. The calculations involved are shown below:

	Iod. No.	SCN Value
x Linoleic acid	181.0	96.3
y Monounsaturated acids	same in both cases	
z Saturated acids	0	0

$$x.181 + y.N = 100x52.65$$
$$\underline{x.96.3 + y.N = 100x50.97}$$
$$x.84.7 + 0 = 168$$
$$x = 1.98\%$$

N being the iodine or thiocyanogen number of the mono-unsaturated acids.

Unsaponifiable Matter

Upon boiling the mass of unsaponifiable matter with acetic anhydride a turbid solution resulted with a clear layer floating on the top. Cooling precipitated down an appreciable amount of crystals. These two phenomena indicate the presence of both hydrocarbons and cholesterol or (and) phytosterol (106). Udranszky reaction (107) confirmed the presence of cholesterol.

SUMMARY AND CONCLUSIONS

The technology of bonefat has been reviewed. Its chemistry has been considered in full details. From the analysis of the crude fat it is seen that the fatty matter (fat proper) constitutes around 80% of the total bulk. The water content has been found to be high. The physical and chemical characteristics, determined for this particular bonefat, show its approximate relative composition in terms of fatty acids. The iodine value for example tells us that it contains an appreciable amount of unsaturated fatty acids while the thiocyanogen number informs us of the presence of the diunsaturated acid (linoleic). The saponification value, on the other hand, shows that the major components are fatty acids lying between C_{16}-C_{18}. From a knowledge of the Reichert-Meissl number it is found that butyric acid or rather soluble volatile fatty acids are present in relatively minute quantities. Free fatty acids as determined from the acid value constitute a large percentage of the bulk.

The fractional distillation of the ethyl esters shows that the composition of the fatty acids making up the fat lies between C_{12}-C_{18}. The relatively larger amount of unsaturated fatty acids as found by the lead soap-alcohol method is confirmed. The amount of linoleic acid obtained from distillation agrees very well with that calculated from the iodine and thiocyanogen values. Linolenic acid is undoubtedly not present. This has been ascertained by the absence of a hexabromide.

The qualitative tests run on the unsaponifiable matter indicates the presence of hydrocarbons and cholesterol.

As to further work it would be desirable to refractionate in order the different fractions obtained. Also it would be desirable to isolate if possible the component or components which impart the unpleasant smell to the fat. It has been noted already that the odour remained in the unsaponifiable matter after its extraction from the saponification medium. But since no complete qualitative and quantitative tests were run on it no conclusions concerning it could be safely drawn.

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