A Rough Determination of Fatty Acid Profile

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Abstract

The determination of fatty acid profiles of fats and oils by standard methods requires expensive and sophisticated pieces of equipment, viz., Thin Layer Chromatography, High Performance/Pressure Liquid Chromatography and Gas Liquid Chromatography. Where such facilities are not available, alternative methods have been devised and employed. Thiocyanogen method is one of such alternatives. However, this method suffers seriously for two main reasons: Thiocyanogen is toxic in nature, and the method itself is not very reliable. At this point, the need for some simpler alternatives cannot be overemphasized. The present paper is one such proposals. The method attempts to manipulate routine tests based on some physicochemical properties of individual fatty acids. Preliminary experimental results were quite encouraging. The method must be validated by some standard methods, for instance, Gas Liquid Chromatography.

Keywords: Hexabromide, Iodine Value, Methyl esters, Saturated, Unsaturated

Introduction

Standard methods for the determination of fatty acid profile in fats and oils entail: (a) conversion of fat or oil in to mixed methyl esters by methanolysis, and (b) analysis and separation by GLC (Sonntag 1979). Sometimes butyl esters are also used. For most general purposes, flame ionization detectors are used for the identification: these detectors can produce sufficiently detailed fatty acid profiles. GLC is combined with mass spectrophotometer where further confirmation is required (Rangana 1986). Other instruments reportedly used for the same are improved versions of TLC and Reversed-Phase-Argentation-HPLC. In particular, the latter is the method of choice for analyzing triglyceride composition and geometric isomers of fatty acids (Sleeter 1985). Because of high cost of the above-mentioned instruments, however they are seldom used on routine basis. Their operation also calls for considerable experience on the part of the analyst.

An alternative method included in the AOCS Methods for the indirect determination of fatty acid profile is the Thiocyanogen Number (Rangana 1986). It was extensively used prior to 1939. With the advent of newer methods, particularly the GLC, this method has become largely obsolete. The main limitations in it were the toxic nature of thiocyanogen and the lack of reliability as confirmed by GLC (Sonntag 1982). For details of Thiocyanogen Number, the reader is referred to AOCS Methods, Vol. 1 (1975).

In this connection, it may be plausible to prescribe determination of PUFAs (polyunsaturated fatty acids) as an alternative to Thiocyanogen technique. Attributing the PUFAs content due alone to C18:2 and C18:3 gives the amounts of linoleic- and linolenic acid respectively in the oil. Oleic acid can then be calculated with the help of Iodine Value, I.V. For rough determination, this method seems to fit the bill. For ω-fatty acids of carbon numbers greater than 18 are present only in minute amounts in normal cooking oils. Table 1 shows the typical fatty acid profile of soybean oil.

Table 1. Typical fatty acid profile of soybean oil

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 14:1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>C 14:0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>C 16:0</td>
<td>7-14</td>
</tr>
<tr>
<td>C 16:1</td>
<td>0.5</td>
</tr>
<tr>
<td>C 18:0</td>
<td>1.4-5.5</td>
</tr>
<tr>
<td>C 18:1</td>
<td>19-30</td>
</tr>
<tr>
<td>C 18:2</td>
<td>44-64</td>
</tr>
<tr>
<td>C 18:3</td>
<td>4-11</td>
</tr>
<tr>
<td>C 20:0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>C 20:1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>C 22:0</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

Source: Pearson (1981)
The detail of the procedure for carrying out PUFAs determination is described in the 13th edition AOAC Methods. However, if the oil has been processed, say hydrogenated, isomerization can take place. Since the method is useful for determining cis isomers only, it will offer little or no advantage at all for hydrogenated products. Besides, since the protocol also mentions a lot of do's and don'ts, for the inexperienced, it cannot be the method of choice.

Effort has been made here to present a crude-but-simple alternative, using entirely different approach. The method does not require sophisticated pieces of equipment. The method is backed by several cogent theoretical arguments.

**Theoretical Considerations**

The proposed method attempts to manipulate the physical and chemical properties of individual fatty acids. The basic assumptions made are:

1. The oil is free from alkyl branching, conjugation, and trace unsaturated entities. The iodine value is due alone to oleic-, linoleic-, and linolenic acid.
2. The oil does not contain saturated fatty acids of carbon numbers greater than 18 and smaller than 16.
3. Hexabromide Test, H.T, can detect linolenic acid down to the level of 1%. The hydrogen bromide and tetrabromide that may may not interfere with the test are neglected.
4. The glycerol moiety of the glyceride molecule and the trace components such as unsaponifiable matter (collectively called Non-FattyAcids, NFA, hereafter) are inert to reactions involving saponification- and iodine value determination.
5. The oil is free from waxes, phosphatides, and lactones that interfere with saponification value determination.
6. The oil is not excessively hydrolyzed.

**Mathematical Derivation**

1. **Iodine Balance**

Using assumptions 1, 2, 4, and the physico-chemical constants of fatty acids shown in Table 2, a simple expression can be obtained:

\[ 9.85x + 181.0y = \frac{10000 \times I.V.}{T} - 273.46z \quad \text{I} \]

Where,

- \( x \): oleic acid (%)
- \( y \): linoleic acid (%)
- \( z \): linolenic acid (%)
- \( T \): true free fatty acid expected (%)
- I.V.: iodine value

The coefficients of \( x \), \( y \), and \( z \) are theoretical iodine values of oleic-, linoleic-, and linolenic acid respectively (from Table 2). The expected true percentage of fatty acids is calculated using the derivation due to Rai (1999).

\[ T = 100 - 0.0226 \times S - P \quad \text{II} \]

where,

- \( S \): saponification value of sample oil
- \( P \): unsaponifiable matter (%)

**Table 2. Some physico-chemical constants of fatty acids**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Mol. Wt.</th>
<th>Saponification value</th>
<th>Iodine value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic, C18:1</td>
<td>282.47</td>
<td>198.64</td>
<td>89.85</td>
</tr>
<tr>
<td>Linoleic, C18:2</td>
<td>280.45</td>
<td>200.06</td>
<td>181.0</td>
</tr>
<tr>
<td>Linolenic, C18:3</td>
<td>278.44</td>
<td>201.51</td>
<td>273.46</td>
</tr>
<tr>
<td>Palmitic, C16:0</td>
<td>256.43</td>
<td>218.81</td>
<td>0.00</td>
</tr>
<tr>
<td>Stearic, C18:0</td>
<td>284.49</td>
<td>197.23</td>
<td>0.00</td>
</tr>
</tbody>
</table>

2. **Total Fatty Acid Balance**

Excluding NFA and using Table 2 again, we have:

\[ x + y + z + \text{Sat.} = 100 \quad \text{III} \]

where,

- \( \text{Sat.} \): saturated fatty acids (%)

Combining equations (I) and (II),

\[ \frac{10000 \times \text{I.V.}}{T} - 273.46z - 89.85(100-z-\text{Sat.}) = 91.15 \quad \text{IV} \]

And thus,

\[ x = 100 - y - z - \text{Sat.} \quad \text{V} \]

**Methodology**

1. **Choice of sample**

Good candidates are: soybean oil, sunflower oil, safflower oil and to some extent cottonseed oil and sesame oil. The crude oil must be free from gummy substances (assumption 5). Samples can be prepared by degumming with acetic anhydride (Norris 1982). To reduce certain unsaponifiable matters such as pigments, bleaching is done with special grades of activated carbon and bleaching earth (Norris 1982). Keeping in view with assumption 6, neutralization is not advised. The sample must be thoroughly dry (Rai 1999).

2. **Iodine Value determination**

Standard Wij's solution can be used for the determination (Sonntag 1982). The weight of the sample to be taken should be according to the formula:
The reagent should be 100 to 150% in excess (Sonntag 1982).

3. Saponification Value

The assumption must be again taken into account here. Accordingly, degumming and bleaching must be carried out with great care (Rai 1999). It cannot be emphasized too often or too strongly that, inspite of its apparent simplicity, the determination of saponification value is a highly skilled piece of analysis, and that it merits the closest attention to detail and care in manipulation. As general precautions may be noted the necessity of avoiding the absorption of CO₂ by the solutions during the test; the desirability of carrying out a blank test with every set of tests; and the ascertaining of the end point of the titration as exactly as is possible (Bolton & Revis 1966).

4. Hexabromide Test

The test is generally used for the detection of linseed oil in other non-linolenic oils. It can be carried out using the method outlined by Rangana (1986). The test is also used for semi-quantitative determination of fish or marine oils in vegetable oils (Sonntag 1982). The visible precipitate observed in the test is due to the formation of hexabromide from linolenic acid. Some experience is needed to be able to detect the 1% level of linolenic acid. If a large amount of precipitate is observed, the sample may be serially diluted in a non-linolenic oil, e.g., coconut oil, and the test performed again. The final dilution should just fail to answer the test. The linolenic acid content in the original sample can then be back-calculated.

5. Unsaponifiable matter

Unsaponifiable matter includes, inter alia, hydrocarbons, higher alcohols, and sterols. Some of the unsaponifiable matters are removed during the degumming and bleaching process. The rest can be determined by any routine method, for instance the one described by Bolton and Revis (1966).

6. Saturated fatty acids

The dominant saturated fatty acids in a typical cooking oil are stearic and palmitic acid. Several methods (or their variations) are available for the determination of saturated fatty acids, for instance the one described in the 11th edition AOAC Methods. Bolton and Revis (1966) have mentioned a method based on oxidation of unsaturated fatty acids by potassium permanganate. The method described by Sonntag (1982), called modified Twitchell method, appears more appropriate. The method is based on the insolubility of lead salts of saturated fatty acids and solubility of unsaturated fatty acids in 95% ethanol. The method is not applicable to fats of the lauric group. It is also not applicable to oils with fatty acids of very large molecular weights, e.g., rapeseed- or mustard oil. In practice, the fat or oil is saponified, and the potassium salts are acidulated and treated with lead acetate in alcohol. After 2 hours at 15℃, the insoluble lead salts are removed by filtering, dried, and weighed. Isooleic acid may be estimated by attributing the entire iodine value of the solid acid due to it. The percentage of pure (isooleic acid-free) saturated fatty acid can be calculated by:

\[
\text{Sat.} = \frac{10000 \times B \times 89.85 \times I}{T \times A \times 89.85} \quad \ldots \text{VI}
\]

Where,

- A: weight of oil sample taken for the determination of lead salt (usually 20 g)
- B: weight of impure saturated fatty acid obtained after drying (in grams)
- I: iodine value of the impure saturated fatty acid
- T: free fatty acid expected (%)

Computation

Since the quality of result is very much dependent on how well the above-mentioned tests have been performed, it naturally calls for great attention during lab analysis. During computation, as a rule of thumb, if a negative value is obtained for any of x or y, the lab analysis is faulty: the reverse, however, is not always true.

Result

An experiment was carried out using soybean oil sample. Below are given details of the findings:

Sample: fully degummed and bleached soybean oil (not refined)

- Weight of sample taken for lead salt preparation, A = 20 g
- Weight of impure fatty acid (dry), B = 2.8 g
- Iodine value of impure fatty acid, I = 6.8
- Saponification value of oil, S = 192.01
- Iodine value of oil, I.V. = 128.3
- Unsaponifiable matter, P = 1.18%

Hexabromide test: 2%, using 1% sensitivity level, or \( z \) (linolenic content) = 2%

Calculation

Using equation II,

\[
T = (100 - 0.0226 \times 192.01 - 1.18)
= 94.48
\]
Using equation VI,
\[
\text{Sat.(%)} = \frac{10000 \times 2.8 \times (89.85-6.8)}{94.48 \times 20 \times 89.85} = 13.7
\]

Using equation IV,
\[
\frac{10000 \times 128.3}{94.48} - \frac{273.46 \times 2 \times 89.85(100-2-13.7)}{91.15} = 59.88\%
\]

Using equation V,
\[
x = (100 - 59.88 - 2 - 13.7)\%
= 24.42\%
\]

Hence,
- Oleic acid = 24.42%
- Linoleic acid = 59.88%
- Linolenic acid = 2%
- Saturated = 13.7%

**Discussion**

The method described herein is only a rough and ready method: it is relatively crude in that too many assumptions have been made and so may not be applicable to oils with fatty acid profiles that greatly differ from those of normal cooking oils. Presence of fatty acids with carbon numbers lower than 16 and higher than 18 has been considered negligible, which is again not true. The result obtained above is the actual finding of one of my tests. Although the profile of the major fatty acids (above) seems to fall neatly within the range (Table 1), its validity must be checked by GLC. The present work should therefore be taken with some reservations. The major saturated fatty acids in oils thus far considered are palmitic- and stearic acid. By further manipulation, it is thus possible to break down % Sat. in to % palmitic and % stearic acid. For this, saponification value of the fatty acids prepared from the lead salts has to be determined first. With the help of Table 1 and the test results, following equations can be derived:

Issoleic acid + palmitic acid + stearic acid
= total impure fatty acids … … VII

S_p \times \text{isooleic acid} + S_p \times \text{palmitic acid} + S_s \times \text{stearic acid}
= S_{sat} \times \text{impure fatty acids} … … VIII

where,
Si, S_p, and S_s are the theoretical saponification values of isooleic-, palmitic-, and stearic acid respectively;
S_{sat} = \text{saponification value of impure saturated fatty acids.}

Solving Equations VII and VIII will give the percentages of palmitic- and stearic acid.

The present method utilizes four variables at a time. As such, the greater the number of component fatty acids taken in to account (in terms of entirety that is), the more reliable the result will be. Also, more will be the number of equations and, of course, more complex the entire calculation. Normally, with a limited number of major fatty acids, the equations can be conveniently solved.

**Conclusion**

It is evident from the above-mentioned, despite the complexity in the derivation of the final working equations, the actual test to be carried out are quite simple. The method can be (if validated) of considerable value for both the hydrogenation industry and the testing body. It must be mentioned here, the technique is not advocated if GLC facilities are available. The value of this technique for the testing body lies in the fact that selectivity of hydrogenation can be quickly and simply detected. Selective hydrogenation refers to hydrogenation of linolenic acid in particular. The hydrogenation should proceed in the sequential order: C18.3→C18.2→C18.1 → C18.0. Hydrogenation leads to solidification of oil because of the saturation mechanism. In actual practice, most hydrogenation plants carry out, unwittingly though, trans isomerization of oleic acid. Trans oleic acid (isooleic acid) has a melting point above 40°C while the natural cis form has a melting point below 16°C. It is unfortunate that the chemists involved in the hydrogenation plant (in Nepal) take isomerization (and therefore solidification) as selective hydrogenation. The method described here can be fruitfully used as a guide as to whether the hydrogenation has been carried out selectively. To ascertain it, one has only to compare the iodine value of the saturated fatty acids (prepared by lead salt method) of the original oil with that of the hydrogenated oil. If the iodine value of the saturated fatty acid (impure) from the hydrogenated oil is significantly higher than that from the original oil, it is a sure indication that trans isomerization has taken place. What is more, a bit of manipulation can give the percentage of isooleic acid also.

Another important finding of this work is that attempting to characterize any given oil in terms of iodine value and saponification value only can sometimes be utterly misleading. In fact, unless one of the component unsaturated fatty acids, e.g., linolenic acid, is stipulated in terms of maximum or minimum limit, the possible number of combinations of fatty
acids for exhibiting the same iodine- and saponification values can be unlimited. As proof, we can examine Table 3. The combination shown in the table was generated using iterative program in a hand-held programmable computer fx 4000 P.

Table 3. Feasible solutions at I.V. = 123.8, Sap.V. = 192.01, Sat. = 13.70

<table>
<thead>
<tr>
<th>Manipulated values</th>
<th>z (linolenic acid)%</th>
<th>x (oleic acid)%</th>
<th>y (linoleic acid)%</th>
<th>Saturated %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22.39</td>
<td>63.91</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23.40</td>
<td>61.90</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>27.46</td>
<td>53.84</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>32.53</td>
<td>43.77</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>37.60</td>
<td>33.70</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>42.68</td>
<td>23.62</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>47.75</td>
<td>13.55</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>52.82</td>
<td>3.48</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>31.727</td>
<td>54.57</td>
<td>0.00</td>
<td>13.70</td>
<td></td>
</tr>
</tbody>
</table>

We can also see from the table above a remarkable fact: the linolenic acid content can vary freely between 0 and 31.70% without least affecting the iodine value (128.30) and the saponification value (192.01). Although it is evident that the balance of iodine and saponification value is achieved by the rearrangement of the component oleic- and linoleic acid, it is also clear that linseed oil, if carefully mixed with other oils, can easily pass as soybean oil were only the saponification- and iodine values to be used to characterize soybean oil. This work therefore places due emphasis on the routine Hexabromide Test for approximating linolenic content as well.

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References


