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LETTER TO THE EDITOR

Letter to the Editor

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Dear Editor,

In the June, 2009 issue of the *Journal of the American Oil Chemists' Society,* Ramli et al. [1] described two steps of a possibly interesting process for the production of a low cloud point palm olein. To put these steps in perspective, I have construed a tentative flow diagram of their entire process shown in Fig. 1 below; the products and process steps discussed in detail in the article concerned [1] have been indicated in bold.

In this process, the starting materials are palm oil and methanol and besides the intended product: palm olein with a low cloud point (indicated as HOPOo in the diagram), the process also produces methyl palmitate, glycerol and palm stearin, all of which are standard products that can be easily disposed of. The palm oil used in the process serves two purposes: it is fractionated to produce a palm olein (POo) and a palm stearin, and it is used as starting material for the production of methyl esters. These methyl esters are then fractionated into a C16-fraction and a C18-fraction, the latter of which is interesterified with the palm olein. This interesterification of **Methyl oleate** and **POo** is discussed in detail in the article [1].

Although the article does not say so in so many words, I would expect the fractionation into C16- and C18 fractions to be by distillation rather than by crystallization since the C18:0 content of the C18-fraction is quite high. I would also expect that in an industrial unit, the FAME collected during the short path distillation of the interesterification reaction mixture would be recycled because this FAME stream has a high oleate content and why have two separate FAME fractionation systems?

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The second process step to be discussed in the article is the dry fractionation of the short path distillation residue (**HOPO**) to yield the low cloud point palm olein as the final product and a stearin (**HOPS**). This stearin is a by-product with non-standard properties so selling it may present difficulties but it can be recycled by mixing it with the palm oil to be transesterified. In that way, full use is made of its enhanced oleic acid content in comparison with palm oil. In my opinion, this constitutes a more attractive method of utilization than selling it at a slightly higher price than normal palm stearin as suggested by the authors.

To facilitate following my subsequent comments, I have copied Table 1 from Ramli et al. below. I have also extended this table with a seventh column headed "Average" and I have inserted a row ("SUM") to indicate that totaling the fatty acid values above does not add up to exactly 100%. In this table, the first column lists the descriptors, values of which are tabulated in subsequent columns. The second column (headed "RBD POo") refers to the refined, bleached and deodorized palm olein used in the interesterification experiments; the third column (headed "Methyl oleate") refers to the methyl oleate used in the interesterification experiments. In these experiments, equal amounts of palm olein (RBD POo) and methyl oleate were mixed and the analytical data of this mixture are given in the fourth column headed "Mixture, Before". This reaction mixture was then interesterified and the analytical data of the reaction product appear in the fifth column headed "Mixture, After" and the sixth column (headed "HOPO") lists the data for the distillation residue that results after short path distillation. Finally, the seventh column (headed "Average"), which I have inserted myself, gives the means of the values of the RBD POo and the methyl oleate.

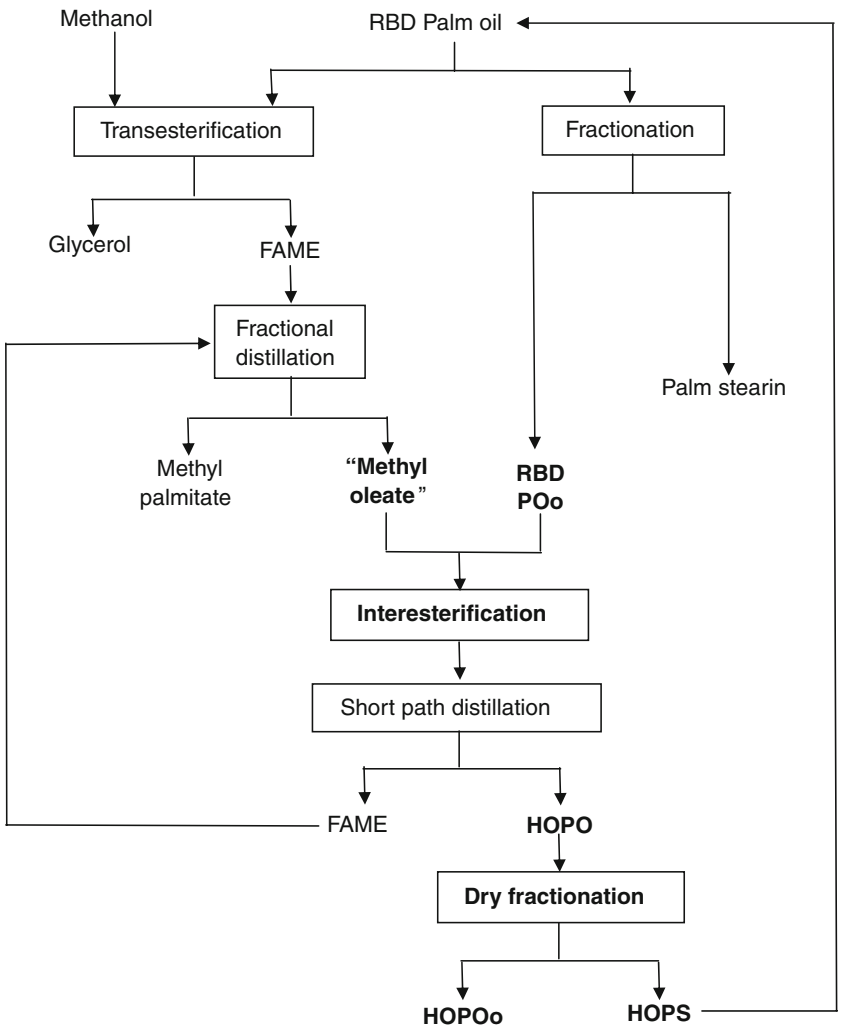

Fig. 1 Tentative flow diagram of complete process

Fatty Acid Compositions

When discussing their results, the authors commented on the fatty acid compositions of the oils before and after 30 min randomization (columns four and five in Table 1) as follows: "The reaction has resulted in an increase of oleic acid content of the high oleic palm oil (HOPO). Palmitic acid had been reduced to nearly half its original content. Stearic acid however had almost double the content in the composition as compared to the initial value." As far as I am aware this is the first time that a substantial change in fatty acid composition as the result of randomization has been reported.

That is the reason why I looked for other possible explanations of the observations listed in Table 1 and one such explanation may be provided by the analytical method used by the authors, which they describe as follows: "Fatty acid methyl esters (FAMEs) were prepared by a rapid method [and here the authors refer to the Malaysian Palm Oil Board (MPOB) test methods]. The oil was transesterified with 0.5 M sodium methoxide. *n*Hexane (0.95 mL) was added to the oil sample in a 2-mL glass vial using a graduated pipette. The mixture was shaken vigorously with a vortex mixer to dissolve the oil. Sodium methoxide (0.05 mL) was then added using a pipette. The vial was well shaken with a vortex mixer for 5 s. After 5 min, the clear upper layer of methyl esters was pipetted off for GC analysis."

This description of the analytical method used by Ramli et al. [1] raises some questions. It does not specify the oil sample size and since sodium methoxide is a powder, a pipette does not appear to be the most appropriate means of dispensing this reagent. I do not understand how the authors arrived at a two-layer system either. A search for articles that also describe the use of this MPOB test method revealed an article [2] by several of the same authors as the article under discussion but it described the same method *verbatim*. Another article [3] explains the FAME

Table 1 Fatty acid compositions of oils before and after chemical interesterification

Fatty acid	RBD POo	Methyl oleate	Mixture		HOPO	Average
			Before	After		
C12:0	0.2	0.0	0.1	0.2	0.2	0.1
C14:0	1.1	0.0	0.3	0.5	0.7	0.6
C16:0	35.7	0.3	8.8	16.4	20.3	18.0
C18:0	3.5	10.0	8.3	7.0	6.1	6.8
C18:1	45.4	71.1	65.2	59.5	56.7	58.3
C18:2	13.1	17.2	16.2	15.2	14.8	15.2
C18:3	0.3	0.4	0.4	0.3	0.3	0.3
SUM	99.3	99.0	99.3	99.1	99.1	99.3
IV	62.7	91.9	84.5	78.7	75.1	77.2
IV conf lim	±0.4	±0.2	±0.4	±0.4	±1.4	–
SFA	40.6	10.3	17.4	24.2	27.4	25.4
MUFA	45.4	71.1	65.2	59.5	56.7	58.3
PUFA	13.4	17.6	16.6	15.5	15.1	15.5

Adapted from [1]

preparations as follows: "Fatty acid methyl esters (FAME) were prepared by dissolving 50 μL oil in 950 μL *n*hexane with sodium methoxide (0.5 mol L^{-1}, 50 μL) and …". Accordingly, it mentions that the sodium methoxide is dissolved and consequently, its solution can be dispensed with a pipette.

Yet another article [4] referring to the same official method also used 50 mg fat, 950 μL hexane and 50 μL of 1 M sodium methoxide and it also mentions that water (1 mL) was added. It is a *verbatim* copy of the method described in [5]. However, none of the articles [1–5] mentions in which solvent the sodium methoxide was dissolved; I presume that it was methanol since this compound is an essential reagent in FAME synthesis by transesterification.

According to the analytical method used, the sample, which consists of triglyceride oil and FAME, is dissolved in hexane, an interesterification catalyst is added to start the conversion of the triglyceride oil to FAME and this conversion is subsequently interrupted/terminated by addition of water. At that point in time, the triglyceride oil conversion need not be complete so that the hexane will not only contain the FAME that was already present in the sample and the FAME formed by the conversion of the triglyceride oil but also the residual triglyceride oil. Injecting an aliquot of this hexane solution onto a GLC column causes the FAME to elute but not the oil. Accordingly, incomplete transesterification of the triglyceride oil present in the sample will cause the FAME originating from this oil to be under-represented in the FAME mixture being eluted and measured.

Incomplete transesterification of the triglycerides present in the sample consisting of FAME and triglyceride oil is therefore a possible explanation of the observation that the fatty acid composition before randomization differs from the fatty acid composition after randomization. Moreover, this possible explanation is supported by the fact that the analytical results in the fourth column of Table 1, which refer to the mixture of palm olein and methyl oleate before interesterification, are much closer to the values reported for the methyl oleate itself because of incomplete transesterification of the palm olein present in the sample.

In theory, complete randomization of the reaction mixture causes the triglyceride oil and the FAME present in the interesterified mixture to have identical fatty acid compositions and indeed the values reported for the interesterified mixture (column five) are much closer to the mean values listed in the last column. However, they still deviate significantly from these mean values and are still slightly biased towards the methyl oleate. This can be tentatively explained by assuming that the interesterification between the palm olein and the methyl oleate has not reached equilibrium.

This assumption is further supported by the observation that the fatty acid composition of the interesterified triglyceride oil (HOPO, sixth column in Table 1) is biased towards that of the palm olein used as starting material. Take the C16:0 content for instance. In the palm olein (second column), this equals 35.7%. For the interesterified reaction product (fifth column), 16.4% is reported whereas for the HOPO, Table 1 lists 20.3%. Comparing these with the mean value of 18.0% indicates incomplete transesterification in two ways. The HOPO analysis does not suffer from incomplete transesterification during FAME preparation, so we can assume the value of 20.3 to be quite likely. It is larger than the mean value and closer to the palm olein, which indicates incomplete randomization. This incomplete randomization also causes the FAME present in the reaction mixture to be biased towards the methyl oleate and given the analytical bias towards the FAME present in the sample, a low value of the C16:0 content is only to be expected.

The authors do not discuss the possibility that their interesterification had not reached full randomization. It is not even clear whether or not they asked themselves that question. This is surprising, since the HOPO is an intermediate product serving as starting material for a subsequent dry fractionation and thus affects the composition and properties of the fractionation products.

Iodine Values

In Table 1, Ramli et al. [1] also list the iodine values (IV) for the various products and as expected, palm olein has a

lower IV than methyl oleate. Table 1 also shows that the IV of the HOPO short path distillation residue (75.1) is slightly lower than the average (77.2) listed in the last column. This can be tentatively explained by again assuming that the randomization was incomplete. However, I have not managed to arrive at an explanation for the reported observation that on randomization, the IV drops from 84.5 to 78.7. In fact, I have difficulty in believing that the same values would be observed when the measurements were to have been repeated which is what I would have done myself when carrying out this research.

When copying Table 1, I did not include all the ± values after each analytical result. I only copied them for the iodine values in a separate row. In the notes underneath Table 1 the authors mention that their values are means of three determinations and that the ± indicates the standard deviation. However, they do not say what they mean by 'determinations'. Do they mean just the measurement or the entire determination including sample preparation? In this context, I found it interesting to note that in Table 1, the standard deviations relating to fatty acid content vary between ±0.0 and ±1.8 whereas in Table 3, they vary between ±0.0 and ±0.1. In Table 5 they vary between ±0.0 and ±1.8 but in Table 6, the variation is again much smaller and between ±0.0 and ±0.1. The text provides no explanation of these differences. Moreover, I would have expected the standard deviations themselves to be normally distributed and they are certainly not. This is something I can only explain by assuming the data to include several rogue values.

Another matter I do not understand is why they have calculated a standard deviation for each value? As far as I am aware, the standard deviation of a measured value depends primarily on the analytical method and the skill of the laboratory analyst rather than on the value itself.

Perhaps it would have been more appropriate if the authors had determined the standard deviation of their iodine value determination or its variance. They could have used the data available to arrive at a combined variance V, according to:

$$V = \frac{\sum (x_1 - \bar{x}_1)^2 + \sum (x_2 - \bar{x}_2)^2 + \ldots + \sum (x_k - \bar{x}_k)^2}{N - k}$$

The procedure is to calculate the sum of the squared deviations from the sample mean for each sample that has been analysed several times, add these for all samples and divide the sum by $(N - k)$ where N is the total number of observations and k the number of samples. Doing this would have avoided statements that the standard deviation of the IV of the HOPO = 1.4 whereas that of the methyl oleate equals only 0.2. Doing this might even have gone some way in explaining the reported decrease in iodine value as a result of interesterification.

Tricaylglycerol Compositions

The anomalies in the paper by Ramli et al. [1] are not limited to their Table 1. In their Table 2, they report the "Major triacylglycerols (TAGs) composition of oils before and after chemical interesterification." So they list triacylglycerols such as OLL, OLO, PLO etc. However, they do not inform the reader that OLL really stands for OL_2 and thus includes LLO and LOL. The reader has to find this out for himself. I did this by determining the total of all TAGs listed. For the palm olein (RBD POo) the total comes to 85.7% and accordingly, there is little room left for isomers.

The palm olein is then diluted with methyl oleate, which should not affect the TAG composition, but nevertheless, vast changes are reported. As is only to be expected, interesterification causes changes in the TAG composition but subsequent removal of the FAME apparently also leads to highly significant increases in all TAGs listed. It turns out that the authors who state that their data refer to the "composition of oils", actually express the TAG content as a percentage of the sample, and this sample may contain FAME or may not. So adding FAME apparently decreases the OLL content of the oil.

Because the fatty acid composition of the HOPO is known, this can be used to calculate the triglyceride composition of the randomized product and comparing this calculated composition with the measurements should indicate whether randomization has been achieved. I attempted such a comparison but did not get a clear answer. Perhaps the fact that the fatty acid compositions reported do not add to 100% is partially (?) responsible for this failure.

I now want to discuss what is Fig. 1 in the article being discussed and which has the legend: "Fig. 1 Effect of reaction time on oleic content and formation of free fatty acid (FFA) and diacylglycerol (DAG) in the interesterified oil". To add to the confusion, the authors talk in the text about "the oleic content" and in the figure, the ordinate legend reads "C18:1 composition"; in my opinion, these terms only make sense when both are replaced by "oleic acid content". Since interesterification is random, I would expect the oleic acid content to change monotonically; however, according to Fig. 1 in Ramli et al., this content rises in the early stages of the reaction, reaches a maximum and then diminishes.

The same figure also shows what happened to the FFA composition and, in the text, it is mentioned that the FFA formation increased; presumably the authors mean FFA-content in both instances. But why would any FFA be formed in a basically anhydrous system? When venturing an explanation for the decrease in DAG content, the authors mention hydrolysis of diacylglycerol since "at this stage, the

DAG started to hydrolyze to form FFA and MAG." This is conjecture since no analytical data for MAG content have been provided and moreover, the conjecture does not make sense since the increase in FFA content is far less than the equivalent decrease in DAG content. According to Fig. 1, the DAG content starts at 5.3%, increases to 13.1% after 60 min and then decreases to 10.3%. An decrease in DAG content of (13.1 − 10.3=) almost 2% corresponds to an FFA increase of just under 1% but the FFA composition [*sic*] that starts at an estimated 0.045% increases after 60 min only to about 0.07% or by less than 0.03%. This leads me to reject the conjecture presented by the authors.

Discussion

The work described by Ramli et al. [1] aims at producing a low cloud point cooking oil based on palm oil. Accordingly, the oil should have a negligible content of trisaturated triglycerides and a low content of disaturated triglycerides. This can be attained by fractionation since that process removes high melting triglycerides. Interesterification on the other hand, randomises the triglycerides and generates high melting triglycerides from low melting, monosaturated triglycerides. Randomization is therefore not an obvious process for producing low cloud point oil.

Introducing additional oleic acid into a palm olein, which already contains more oleic acid than palm oil, lowers the saturated fatty acid content, reduces the content of high melting triglycerides on randomization but it does not avoid their formation. Fractionation still remains necessary and this considerably reduces the yield of the low cloud point oil. Cost calculations may have indicated that this oil would still be cheaper than high oleic acid sunflower seed oil but the authors do not mention to what extent the prices used in these calculations depend on local circumstances. They realize that the short path distillation is the most expensive step in the process but do not mention that the process requires two distillation steps as shown in Fig. 1.

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