Round Robin Test on Free Fatty Acid (FFA) Determination of Refined Palm Oil

Ringkasan

Ringkasan cara AOCS Ca 5a — 40 boleh didapati daripada ujian round robin ini. Kesilapan yang berkemungkinan akan timbul di dalam cara ini, disebabkan oleh, berlainan darjah goncangan campuran titratan dan suhu semasa titratan. Goncangan yang terlalu kuat menyebabkan kemasukkan karbon dioksid manakala suhu yang tinggi semasa titratan menyebabkan penyabonan minyak. Kedua-dua tindakan ini menyebabkan kehilangan sementara warna indikator apabila hampir penamat titratan. Langkah-langkah bagi mengelakkan kesilapan-kesilapan tersebut ada dicadangkan.

Introduction

The determination of free fatty acids in oils and fats, is highly dependent on several factors, namely, solvent temperature, colour of the oil, acidity, time of titration as well as the amount of shaking involved in the titration.

In a round robin test on FFA determinations carried out in July 1980, certain discrepancies have been found by some collaborators. A few collaborators had difficulties with the AOCS Ca 5a-40 method recommended by PORIM, namely that at the end point the pink colour persists for only 10-15 seconds, and not as specified for 30 seconds. Further addition of sodium hydroxide to obtain the 30 seconds minimum colour persistence time would result in extremely high FFA values and errors. Indeed it was known that one laboratory had their end point colour persistence for only 5-10 seconds.

It was necessary for us to conduct another round robin test to assess the problem further as the first cross-check in 1980 was invalidated by collaborators not following the instructions given. Experiments were also conducted in the laboratory to investigate the reasons for the short end point time which other collaborators did not appear to find.

Experimental

The AOCS Ca-40 method of determination was used. A copy of the method, together with instructions and report sheets were sent to all collaborators. They were expected to carry out the analysis according to method and to report any modifications and other problems involved.

Twenty seven collaborators participated in this cross-check. Four samples of oil were sent, labelled A, B, C, and D. Sample A and D were duplicate samples of the same stock labelled differently as A and D. Duplicate analysis was carried out on each sample.

Results and Discussions

(i) The overall results are shown in *Table 1*. The maximum and minimum values obtained for each sample are indicated by double and single lines respectively. Laboratory 4 shows consistently high results in three out of four samples while laboratory 23 has consistently lower results. However they are not excluded from the analysis of variance as the results when rounded to 2 decimal places are not exactly outlying in the range of values obtained. A coefficient of variation of 3% is obtained for FFA values of about 0.09, while higher variation of 4.5% is obtained with FFA values of 0.05%.

Table 1. Overall FFA Results from 27 Laboratories

Code No.	Sample A		Sample B		Sample C		Sample D	
1	0.05	0.04	0.09	0.09	0.09	0.09	0.04	0.05
2	0.05	0.05	0.09	0.09	0.11	0.10	0.05	0.05
3	0.05	0.05	0.08	0.08	0.09	0.09	0.05	0.05
4	0.06	0.06	0.10	0.10	0.12	0.12	0.05	0.05
5	0.05	0.05	0.09	0.09	0.09	0.09	0.04	0.04
6	0.05	0.05	0.09	0.09	0.10	0.10	0.05	0.05
7	0.04	0.04	0.08	0.08	0.09	0.09	0.04	0.04
8	0.05	0.05	0.09	0.10	0.10	0.10	0.05	0.05
9	0.06	0.06	0.09	0.09	0.09	0.09	0.06	0.06
10	0.05	0.05	0.10	0.09	0.10	0.10	0.05	0.05
11	0.05	0.05	0.08	0.08	0.10	0.09	0.05	0.05
12	0.05	0.05	0.09	0.09	0.09	0.09	0.05	0.05
13	0.05	0.05	0.08	0.08	0.11	0.10	0.05	0.05
14	0.05	0.05	0.09	0.09	0.10	0.10	0.05	0.05
15	0.04	0.04	0.08	0.08	0.09	0.10	0.04	0.04
16	0.05	0.05	0.10	0.10	0.11	0.12	0.06	0.05
17	0.04	0.04	0.09	0.09	0.09	0.09	0.04	0.04
18	0.03	0.03	0.07	0.07	0.09	0.09	0.04	0.04
19	0.04	0.04	0.09	0.09	0.10	0.10	0.05	0.05
20	0.04	0.04	0.08	0.08	0.09	0.09	0.05	0.05
21	0.04	0.04	0.08	0.08	0.10	0.09	0.05	0.05
22	0.04	0.04	0.08	0.08	0.08	0.08	0.04	0.04
23	0.04	0.04	0.07	0.07	0.06	0.06	0.03	0.03
24	0.05	0.04	0.09	0.08	0.10	0.10	0.05	0.05
25	0.04	0.05	0.09	0.09	0.10	0.10	0.05	0.06
26	0.04	0.04	0.08	0.08	0.08	0.09	0.04	0.04
27	0.06	0.06	0.10	0.10	0.11	0.10	0.06	0.07
Mean	0.046		0.086		0.094		0.047	
Std. dev	0.0023		0.0026		0.0027		0.0019	
cv (%)	5.0		3.0		2.9		4.0	

Key:

Results have been rounded off to 2 decimal places.

Samples A and D are of the same palm oil stock

maximum values obtained

minimum values obtained

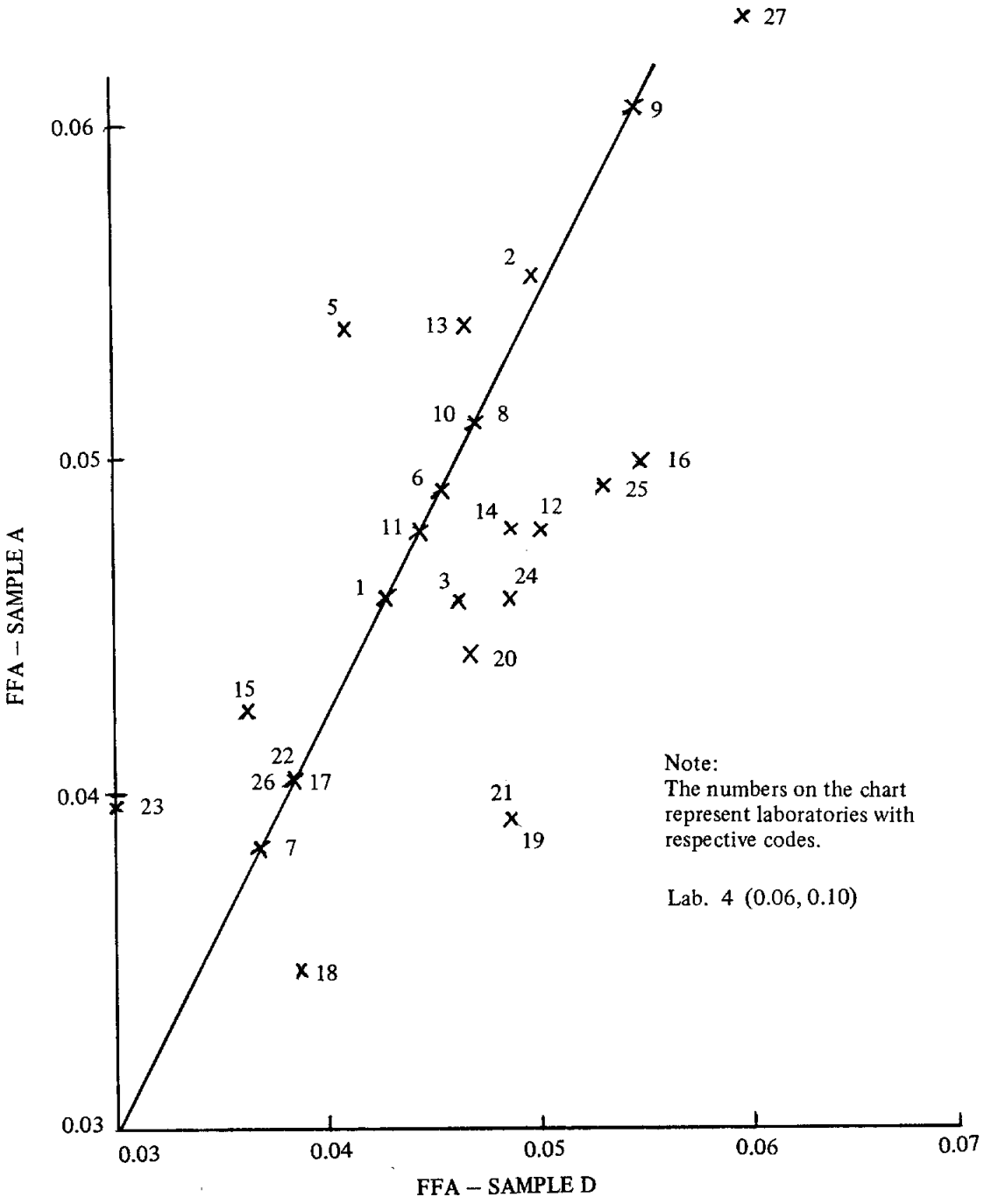

Fig. 1. FFA Sample Chart for Samples A and D.

(ii) Figure 1: Two Samples Chart

The data for this chart is obtained by sending two similar samples designated as A and D to each collaborator. The two results then provide a pair of co-ordinates that determine a point for each laboratory. The pattern made by these points conclusively demonstrate the major role played by the differing systematic errors. If random errors are the cause of the scatter then the two determinations may err equally in either being both low, both high, A low and D high or D low and A high and points would be scattered all over the average value. Here the points generally lie on or form an elliptical pattern with the major axis of the ellipse running diagonally at an angle of 45° to the D axis. Points 4, 5, and 23, being far out from the general pattern would indicate larger random errors for these laboratories. Indeed, the lengths of perpendicular drawn from the points to the 45 degree line are directly related to the random errors.

(iii) Analysis of Variance

An analysis of variance was conducted on the results obtained *(Table 2)*. Tests of significance and the component variance was estimated *(Table 3)*. This analysis shows that variances for samples, laboratories and laboratory — sample interaction are very significant.

The repeatability standard deviation is estimated as $\sqrt{\sigma^2} = \pm 0.0024$ while reproducibility standard deviation is $\sqrt{\sigma^2 + \sigma^2_{LS} + \sigma^2_{L}} = \pm 0.0090$. Hence at 95% confidence level, the agreement of two determinations performed in one laboratory shall not differ by more than 0.01 (Least significant difference within laboratory) And agreement of single determinations in two different laboratories shall not differ by more than 0.02. These results indicate that the method as recommended give acceptable variations between laboratories.

Table 2. Analysis of Variance

Source	df.	s.s.	m.s.	
Samples (S)	3	0.104038	0.034679	
A vs D (S_1)	1	0.000015	0.000015	NS
diff samples (S_2)	2	0.104023	0.052012	***
Labs (L)	26	0.011750	0.000452	***
L x S	78	0.003875	0.000050	
L x S_1	26	0.000847	0.000033	***
L x S_2	52	0.003028	0.000058	***
Error	108	0.000640	0.0000059	

Note:

*** significant at 0.1% level

Table 3. Table of Variance, Agreement Within and Between Laboratories

$\sigma^2 = 0.0000059$

$\sigma^2_{LS} = 0.000026$

$\sigma^2_{L} = 0.000049$

Repeatability std. deviation = ± 0.0024

Reproducibility std. deviation = ± 0.0090

Least significant difference (LSD) within lab. = 0.01

LSD between lab. = 0.02

The agreement obtained in the present cross-check are comparable if not better than those obtained in the July 1980 FFA cross-check, (LSD within laboratory being 0.02 and LSD between laboratories 0.03).

(iv) Summary of Operational Conditions and Observations Noted by Collaborators

Of the 27 laboratories taking part in the cross-check, 19 use IPA as solvent, while the rest use ethanol. While 2 ml. of phenolphthalein has been recommended in the AOCS method, it has been observed that many collaborators use a smaller amount (about 0.5 ml.) in their routine analysis. The amount of indicator used does not appear to affect the titration or the end point persistence as noted by the pink colour change to colourless after several seconds. Two collaborators reported a colour change in less than 30 seconds.

(v) Factors Affecting The Pink Colour Persistence of The End Point

The factors affecting the colour persistence were investigated with the following experiments as shown in *Tables 4 and 5*. A titration of pure IPA (without oil sample) with one drop 0.1N sodium hydroxide using 1 drop of phenolphthalein as indicator was conducted under different conditions and with different degrees of shaking.

Table 4. Factors Affecting End Point Colour Persistence (b) of Isopropanol (IPA) Titrated with Sodium Hydroxide (c)

	Amount of Heat	Gentle Shaking	Vigorous Shaking
1)	No heat	1 minute	15 seconds
2)	Heat (40°C)	3 minutes	1 minute
3)	Heat (80°C)	> 3 minutes	2 minutes

Note (b) time for which pink colour obtained reverts back to colourless.

(c) 50 ml. of IPA and one drop of indicator phenolphthalein titrated with one drop of 0.1N sodium hydroxide.

Results in *Table 4* indicate that when heat was applied, the colour at the end point persisted for a longer time than when no heat was applied. This occurs with both gentle and vigorous shaking. However the colour disappears faster with vigorous shaking. Such results are indicative of the solubility of carbon dioxide gas in isopropanol. Vigorous shaking causes a greater amount of carbon dioxide to be incorporated into the solution, hence the increased acidity results in the pink colour reverting back to colourless after a few seconds. When heated, the solubility of the gas in the solvent decreases, and a longer colour persistence time is observed.

Table 5. Factors Affecting End Point Colour Persistence (a) of FFA Analysis of RBD Palm Oil

	Amount of Heat	Gentle Shaking		Vigorous Shaking	
		(Seconds)	(FFA)	(Seconds)	(FFA)
1)	No heating	55	0.05	15	0.04
2)	Heat (40 — 50°C)	170	0.04	25	0.04
3)	Heat (70 — 80°C)	20	0.04	7	0.04
4)	No heat (with nitrogen blanket)	> 7 minutes	0.04	—	—

Note (a) time for which the pink colour obtained at end point reverts back to colourless.

A similar experiment was conducted with a palm oil sample in isopropanol, as in a normal FFA titration. *(Table 5).* Results showed that the temperature and the degree of shaking play an important role in the colour persistence. Under cold conditions, the pink colour reverts back to colourless after 55 seconds with gentle shaking and 15 seconds with vigorous shaking. The time increases to 170 seconds and 25 seconds respectively when the mixture was heated to 40-50°C, showing a similar trend as in the former experiment. This trend breaks down when the mixture was heated to 70-80°C. In this case, the colour only last for 20 seconds with slow shaking and 7 seconds with vigorous shaking, indicating that saponification occurs rapidly under these conditions. The saponification occurs at a rate sufficient to produce an error especially if the 30 seconds condition as specified in the method is followed strictly. At ambient temperature and with vigorous shaking the inclusion of carbon dioxide from the air occurs at a rate sufficient to cause the colour change in 15 seconds. This theory is further strengthened by using a nitrogen blanket during titration, which results in the pink colour remaining permanently.

Hence it is necessary to carry out the titration under suitable temperature preferably at 40°C with moderate shaking.

(vi) Effects of Different Solvent Systems

Two major solvent systems will be mentioned here, in view of its popularity. Most standard methods specify the use of ethyl alcohol; however there are some disadvantages in its use. These are:-

- The free fatty acids are soluble, but

the triglycerides are not soluble in ethyl alcohol; therefore the mixture must be well shaken to ensure complete solubility of the free fatty acids and uniform distribution of the indicator.

- The two phase system makes it inconvenient to observe the end point.
- While vigorous shaking is essential, this can be overdone, resulting in an excessive introduction of carbon dioxide from the atmosphere, and hence effecting a premature discolouration of the phenolphthalein indicator.
- For more accurate results, particularly if the acidity is low (below 0.1%), the change in colour should be observed in the upper alcohol layer, after the sample has been allowed to stand long enough for separation to take place.

Higher alcohols such as isopropanol (IPA) have been used as an alternative solvent and have been in favour in view of their lower prices compared with ethanol. However their disadvantage lies in the complete solubility of the oil. The improved solubility of the fat leads to a greater ease of saponification. This danger is especially greater under higher temperatures and vigorous shaking as observed in the forementioned experiments. Hence whichever solvent is used, ethanol or IPA, the proper conditions of the titration are important. These are:-

- A temperature of about 40-50°C is sufficient.
- A gentle shaking is preferred.
- The use of a proper sized flask for the contents so as to minimise introduction of carbon dioxide from the atmosphere.

Summary and Conclusions

(i) That 2 single determinations performed in one laboratory shall not differ by more than 0.01%.

(ii) That agreement of single determinations performed in two laboratories shall not differ by more than 0.02%.

(iii) That the premature colour disappearance at the end point is due to either titration of the cold mixture with vigorous shaking resulting in excessive incorporation of carbon dioxide, or titration at high temperatures resulting in saponification of the glycerides.

Recommendations

The following conditions should be followed for better accuracy in the FFA determination

(i) Moderate heating of the sample — solvent mixture during titration. A temperature of about 40°C is considered sufficient.

(ii) Gentle shaking or swirling is needed to prevent carbon dioxide being incorporated into the mixture.

(iii) Fast titration is necessary to avoid prolonged heating resulting in saponification.

(iv) A proper size flask be used to avoid incorporation of excessive amount of carbon dioxide.

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