Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Food Chemistry 102 (2007) 1407–1414
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Determination of free fatty acids in palm oil samples using non-aqueous flow injection titrimetric method

Bahruddin Saad *, Cheng Woon Ling, Md Sariff Jab, Boey Peng Lim, Abdussalam Salhin Mohamad Ali, Wan Tatt Wai, Muhammad Idiris Saleh

School of Chemical Sciences, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia

Received 20 July 2005; received in revised form 5 May 2006; accepted 27 May 2006

Abstract

Flow injection (FI) non-aqueous titrimetric methods for the determination of free fatty acids (FFA) in palm oil samples are described. Single-line and two-line FI manifolds using phenolphthalein (PHP) and bromothymol blue (BTB) as indicators were developed. The method is based on the monitoring of the changes of absorbance of the indicators used from basic–acidic–basic form (pink–colourless–pink for PHP, blue–yellow–blue for BTB) as a result of the neutralization of KOH that was used as carrier stream by the injected FFA sample. FI parameters such as carrier and reagent concentration, flow-rate, length of reaction coil, size of mixing chamber and injected volume were optimized. The single-line manifold with PHT as indicator is recommended for the determination of samples with acidity degree (a.d.) higher than 0.4, but the oil samples need to be diluted with 2-propanol before their injection. For lower acidities (a.d. < 0.4), a two-line manifold with BTB as indicator is recommended. The two-line manifold allows direct injection of oil samples (no off-line dilution required). The optimized FIA method is linear over the range 0.4–10.0 a.d. (based on palmitic acid) for single-line manifold and 0.11–0.50 a.d. for the two-line manifold. Sample throughput of 35–74 and 21–46 samples h^{-1} for single-line and two-line manifolds, respectively, were achieved. Fifty different samples of palm oils were tested using the appropriate FIA manifolds, and results were compared with the standard PORIM procedure which involves manual titration. Good correlations between the two methods were obtained (r^2, at least 0.92) UV–VIS absorption spectra indicate that the absorption of these oil samples were minimum at the detection wavelengths (562 nm for PHP and 627 for BTB), indicating that the method is negligibly interfered from the background colour of the samples.

Keywords: Flow injection analysis; Spectrophotometry; Free fatty acid; Palm oil

---

1. Introduction

Palm oil is one of the 17 major oils and fats produced and traded in the world today (Jaafar & Sukaimi, 2001). Within the span of four decades, palm oil has emerged as the fastest growing oil in the world. In fact, palm oil is projected to be the world's largest oil produced, although it is currently occupying second position after soybean oil (Jaafar & Sukaimi, 2001). To meet the ever changing needs and for the industry to remain competitive, innovations in many forms are necessary.

The quality of palm oil is determined by various factors and free fatty acid (FFA) is one of the most frequently determined quality indices during production, storage, and marketing (price dictated by FFA content). Other parameters that dictate the price of a palm oil product include moisture, impurities, totox and iodine values. Currently, FFA is determined by manual titration of the sample against potassium hydroxide in hot 2-propanol solution, using phenolphthalein as indicator (PORIM Test Methods, 1995). Due to the fact that some samples contain

---

* Corresponding author. Tel.: +60 46533888x4027; fax: +60 46574854.
E-mail address: bahrud@usm.my (B. Saad).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.05.051

coloured substances (mainly carotenoids) which causes difficulty to distinguish the end-point and the labour-intensive nature of these determinations, alternative methods are clearly needed. A survey through the literature indicate that improved analytical methodology for the determination of FFA in palm oil, apart from the work on fourier transform infrared (Che Man & Moh, 1998) and near-infrared reflectance spectroscopic methods, (Che Man & Setiowaty, 1999) are scarce.

Determination of individual free fatty acids is commonly done using capillary gas chromatography, and to a lesser extent, HPLC and capillary electrophoresis (CE), where the analyte is first isolated using liquid–liquid extraction or solid-phase extraction prior to the analytical separation. Derivatization methods are necessary for these methods either to increase the volatility of analytes (the case for GC) or to improve the sensitivity as in HPLC and CE methods. CE separation using indirect UV detection has also been reported (Roldan-Assad & Gareil, 1995). Several papers have been published for the determination of FFA in biological fluids (Fuse, Kusu, & Takamura, 1997; Husek, Simek, & Tvrzicka, 2002; Miwa, 2002); in milk (Puchades, Suescun, & Maquieira, 1994; Shahin, Hamzawi, & Haggag, 1987); and in oil and fats (Canham & Pacey, 1987; Ekstrom, 1981; Ismail, Van de Voort, Emo, & Sedman, 1993; Kwon & Rhee, 1986; Puchades et al., 1994; Schooner, Simard, & Pandian, 1991; Zhi, Rios, & Valcarcel, 1996; Nouros, Georgiou, & Polissiou, 1997; Mariotti & Mascini, 2001; Shantha & Napolitano, 1992). A potentiometric method for the determination of acidity in oilseeds has also been reported (Kuselman, Tur'yan, Burenko, Goldfeld, & Anisimov, 1999). More recently, the determination of acidity in fruit juices based on sequential injection analysis using a lab-on-valve system was reported (Jakmunee, Pathimapomlert, Hardwell, & Grudpan, 2005). Biosensors based either on purified enzymes (Schoemaker, Feldbrugge, Grundig, & Spener, 1997; Schooner et al., 1991) or intact enzymes (e.g. butyrate kinase from *E. coli*) in bacteria (Schmidt, Standfuz-Gabisch, & Bilitewski, 1996) has been reported for the determination of certain FFA. These approaches, although interesting, are plagued by teething problems of short lifetime, long response times and frequently poisoned by sample components.

Alternative analytical methods for the determination of palm oil quality parameters have recently been initiated by our research group. In our approach, low cost instrumentation that are within the budget even for small palm oil operators, simple and one that can potentially be adopted for process control are given special consideration. The FTIR method reported by Ismail et al. (1993) to determine the FFA in olive oil, fats and oils by measuring the C=O band at 1711 cm^{-1}, although does not require solvents and short analysis time (~2 min) was not considered due to the higher cost in the instrumentation involved. FIA methods, in tandem with suitable flow through detectors seem attractive as they can be easily automated and are able to meet the objectives outlined above. A few reports on the FIA determination of FFA in other food samples were reported (Canham & Pacey, 1987; Ekstrom, 1981; Zhi et al., 1996). These earlier FIA methods contain undesirable features such as involving on-line extractions (Ekstrom, 1981; Kwon & Rhee, 1986; Zhi et al., 1996) uses toxic organic solvents, complex flow lines (2–8 channels), and involving phase separators (Canham & Pacey, 1987; Puchades et al., 1994).

An automated non-aqueous titrimetric method for the determination of FFA in olive oil (Nouros et al., 1997) and extra virgin olive oil (Mariotti & Mascini, 2001) have been developed. The method is simple, involving only a one-line manifold. Thus, the objective of this study is to develop FIA methods for the determination of the FFA in palm oil and its fractions, and palm kernel oil samples, by adopting the pioneering work of Nouros et al. (1997). Apart from that, a two-line manifold using the reagents phenolphthalein (PHP) and bromothymol blue (BTB) was also investigated. As will be shown later, these methods offer interesting alternatives for the determination of various types of palm oil samples.

2. Terminology

Low acidity oil – refer to commercial palm olein that had been established using standard titrimetry to contain low FFA (<0.06 a.d.).

Diluted oil – prepared by weighing 100 g low acidity oil and diluting to the 500 mL mark with 2-propanol.

Neutralised oil – prepared according to the method of Ismail et al. (1993). The oil was extracted four times with 0.01 M KOH solution. The aqueous layer was removed and the oil fraction was centrifuged (3600 rpm) for 60 min.

Acid degree (a.d.) – mass of palmitic acid in a given mass of oil, expressed as %.

3. Materials and methods

3.1. Reagents and solutions

Palmitic acid (99–101%) was purchased from BDH, potassium hydroxide and 2-propanol from R&M Chemicals; phenolphthalein (PHP) and bromothymol blue (BTB) were from Hopkins & Williams. Stock solutions of 0.10 M KOH, 1.0×10^{-3} M PHP and BTB were prepared by weighing appropriate amounts and dissolving in 2-propanol. Carrier solution containing KOH and indicator was prepared daily by dilution. KOH of approximately 0.1 M was standardized with potassium hydrogen phthalate before used.

Palmitic acid standards were prepared in diluted palm oil so that the matrix is similar to the actual samples. All standards were prepared in diluted oil. Stock palmitic acid (90.0 a.d.) was prepared by weighing the required amount of palmitic acid and dissolving in diluted oil solution. Standards 1.0–4.0 a.d. and 0.1–0.8 a.d. were prepared by appropriate dilution of the 5.0 and 1.0 a.d. palmitic acid solutions, respectively.

3.2. Sample

Palm oil, palm kernel oil, and palm kernel oil fractions were obtained from Intertek Testing Services and Keck Seng Ltd., Johor. A few cooking oil samples were bought from local market outlets.

3.2.1. Preparation of sample

Samples with liquid fraction only (refined bleached deodourised (RBD) olein, crude palm kernel oil, palm fatty acid distilled (PFAD), crude palm olein) were prepared by dissolving the sample (5.0 g) in 2-propanol in 25 mL volumetric flasks.

Samples with both liquid and solid fraction at room temperature were weighed (5.0–10.0 g) in 100 mL conical flasks. 2-propanol (25 mL) were added to the sample. The mixtures were heated to 60–70 °C and thoroughly homogenized by shaking. The mixtures were cooled and filtered. The residues were washed twice with 10 mL of 2-propanol. The filtrates were then diluted with diluted oil in 50 mL volumetric flasks. PFAD samples with high acidity (>75 a.d.) were further diluted with diluted oil solution so that its acidity lies in the working analytical range.

3.3. PORIM method

0.5–2.0 g of samples (based on the expected acidity) were weighed into Erlenmeyer flasks. Neutralized 2-propanol (50 mL) was added. The flask was placed on a hot plate and the temperature was regulated to about 40 °C. The sample was shaken gently while being titrated against standard KOH (0.1 M) to the first permanent pink colour using phenolphthalein as indicator. The colour must persist for 30 s. The standard alkali used was 0.1 M for crude oils and 0.02 M for refined oils.

3.4. Flow injection analysis set-up

Two types of FI units depicted in Fig. 1 were used. Palmitic acid standards for the single-line and two-line FIA manifolds were prepared in diluted oil and neutralized oil, respectively. The mixing chamber was prepared using polyethylene disposable syringe (5 mL) where one end was cut off (Fig. 2). An outlet was drilled on one side to be connected to the line to the detector. Its volume can be adjusted by moving the plunger. A Hitachi U-1000 spectrophotometer equipped with a Uvonic ultramicroflow cell (20 µL and 1.0 mm pathlength), a Rheodyne type 500 rotary injection valve, an *x*–*y* recorder (Kipps & Zonen) was used to record the FIA output. Solutions were propelled by a multi-channel peristaltic pump (Gilson Minipuls 3). PTFE tubing (0.8 mm i.d.) was used throughout, except for the peristaltic pump tubing where Viton was used. The reaction coil used in the two-channel manifold was coiled (0.8 cm i.d. and 50 cm long). Indicator solutions and representative palm oil samples were scanned from

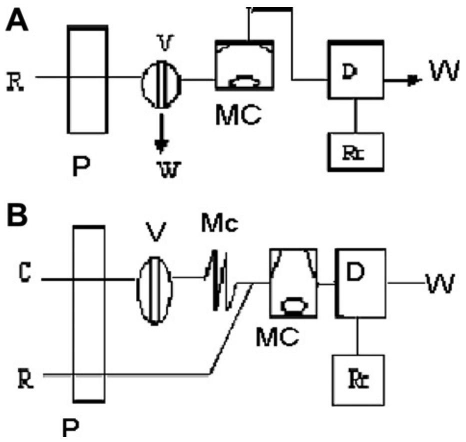

Fig. 1. Single-line (A) and two-line (B) FIA manifolds used for the determination of FFA. Pump; R – Reagent stream; C – carrier stream; V – Injection valve; Mc – Mixing coil; MC – Mixing chamber; D – Detector; Rc – Recorder; and W – Waste.

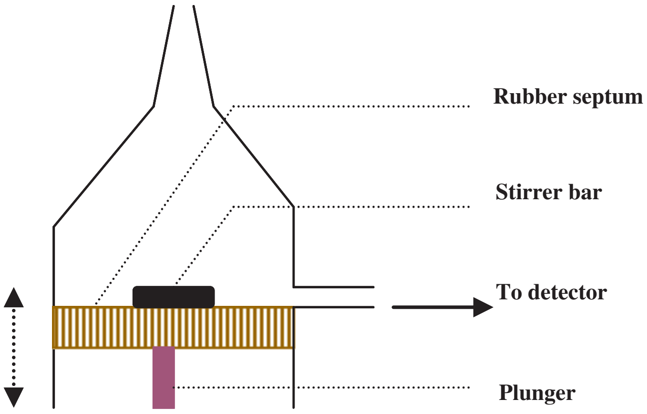

Fig. 2. Details of the mixing chamber.

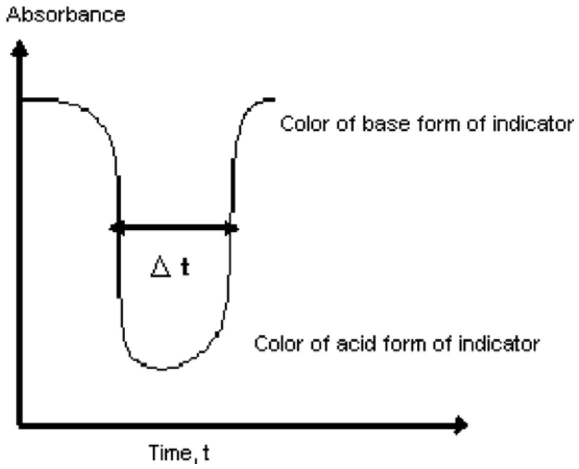

Fig. 3. FIA titration peak.

400–800 nm using a Hitachi U-2000 double-beam spectrophotometer with a 1.0 cm corex glass cuvette.

4. Results and discussion

4.1. *Basis of analytical determination*

The underlying principles in the FIA determination is based on the monitoring of the baseline absorbance of the indicator in the presence of KOH (pink for PHP, λ_{max}, 562 nm; blue for BTB, λ_{max}, 627 nm) when reacted with FFA which changes to colourless and yellow of the acidic form of the PHP and BTB indicators, respectively (Fig. 3). Peak widths of the negative peaks, measured at fixed height, is proportional to the logarithm of the concentration of acid (Ruzicka & Hansen, 1988). Throughout this studies, peak widths at half the peak height were used.

4.2. *Choice of tubing materials and organic solvents as FI carrier stream*

Since the method is based on non-aqueous titrimetry, the choice of tubing material and its durability when continuously exposed for long periods of time as pumped with the organic solvent was studied. An ideal solvent should be able to dissolve the palm oil sample, indicator and KOH, is cheap, of low volatility and environmentally friendly. Ethyl acetate, cyclohexane, acetone, carbon tetrachloride, *n*-hexane and benzene were able to dissolve the palm oil sample but did not dissolve KOH, while ethanol, methanol and petroleum spirit were able to dissolve KOH but did not solubilise the palm oil very well. Diethyl ether was able to solubilise both the sample and KOH but was not considered due to its volatility. 2-propanol was found to be the best solvent and thus used for the entire FIA work.

The durability of a few commercial tubing types when contacted with 2-propanol was investigated by soaking overnight in 2-propanol and its mass changes noted. Viton, by virtue of its availability in our lab was chosen even though other materials such as Tygon silicon, silicon peroxide, Tygon lab were also found to be suitable. The use of Pharmed and C-Flex tubings, however, should be avoided due to the extensive swelling of these materials when contacted with 2-propanol.

Table 1
Adopted FIA operating parameters

Parameter	Single-line FIA		Two-line FIA	
	PHP	BTB	PHP	BTB
KOH concentration, 10^{-4} M	5.0	2.5	5.0	5.0
Indicator concentration, 10^{-5} M	2.0	2.0	2.0	2.0
Mixing chamber, mL	1.0	1.0	1.0	1.0
Injection volume, μL	25	25	50	50
Flow rate, mL min^{-1}	4.2	4.2	R: 2.6 C: 1.4	R: 2.6 C: 1.4
Detection wavelength, nm	562	627	562	627

Key: PHP, phenolphthalein.
BTB, bromothymol blue.
R – Reagent (KOH with indicator in 2- propanol).
C – Carrier (2-propanol).

4.3. *Single-line manifold*

A single-line manifold was first used for the studies (Fig. 1). The effect of FIA parameters such as indicator concentration (1.0, 1.5, 2.0, 2.5 × 10^{-6} M), volume of mixing chamber (1.0, 1.5, 2.0 mL), injection volume (25, 50, 100, 250 mL) and flow rate (1–5.0 mL min^{-1}) were investigated by injecting 1.0 a.d. palmitic acid standard. The use of another indicator, BTB, apart from the PHP indicator investigated earlier (Mariotti & Mascini, 2001; Nouros et al., 1997) was also studied. In dilute solutions,the pH transition of PHP (pH transition range 6.2–7.6) falls outside the steep portion of the titration curve, and an indicator such as BTB (pH transition range 8.3–10.0) must be used (Christian, 2004). Thus, it is reasoned that the use of BTB might lead to better sensitivity as compared to the use of PHP.

The FIA operating conditions chosen were a compromise between peak sensitivity (peak widths) and sample throughput, and are summarized in Table 1. Apart from

Table 2
Analytical characteristics of FIA methods[a]

Variable		Single-line FIA		Two-line FIA	
		PHP	BTB	PHP	BTB
Regression equation[b]		$Y = 38.7$ logC + 23.2	$Y = 36.3$ logC + 23.2	$Y = 122.2$ logC + 135.0	$Y = 133.3$ logC + 133.7
Correlation coefficient, r^2		0.999	0.997	0.997	0.997
Detection limit, a.d.		0.06	0.06	0.10	0.10
Linearity range, a.d.		0.4 – 10.0	0.4 –10.0	0.20 – 6.0	0.20 – 6.0
Sample throughput, samples h^{-1}		35 – 74	55–66	15 – 40	15 – 21
Reagent consumption, mL		3.4 – 7.2	3.8–4.6	6 – 16	11 – 16
Recovery, %[c]	1.0 a.d. spiked	100.3 ± 0.9	90.0 ± 1.7	96.1 ± 1.1	103.9 ± 0.9
	4.0 a.d. spiked	102.0 ± 1.0	103.0 ± 1.8	100.1 ± 3.6	100.8 ± 0.9

[a] Operated under conditions stated in Table 1.
[b] *Y* and C refer to time (Δt, s) and concentration (a.d.), respectively.
[c] Express as mean ± standard deviation ($n = 3$).

the concentration of KOH used, the FIA conditions were all similar for both reagents. The analytical characteristics of the FIA method when operated under the optimized condition is shown in Table 2. A linear range of 0.4–10 a.d. FFA with good linearity (regression coefficient ~0.99) was found. The detection limit was established by injecting low levels of standard palmitic acid and gradually increasing its concentration. The detection limit was obtained when the signal:noise was 3. The FIA method is comparable to the FTIR method with respect to sensitivity and linear range (0.4–6 a.d.) (Che Man & Setiowaty, 1999), but clearly superior over the reference PORIM method especially in terms of reduction in consumption of reagent and speed of analysis. The sample throughput is comparable to the report of Nouros et al. (1997) for olive oils. The method was further evaluated for its feasibility to determine FFA in oil that had been spiked with palmitic acid. Good recoveries (90.0–104.0%), especially when PHP was used, were found. The method is also characterized by having good precision as reflected in the low relative standard deviation (0.88–3.63%, $n = 24$).

The single line FIA manifold using PHP as indicator was used for the analysis of FFA in several types of palm oil samples. The results are shown in Table 3 and are compared to the reference titrimetric method of PORIM. As anticipated, better reproducibility of the FIA method (RSD 2.2%) was found over the reference method (RSD, 4.1%). The correlation of results between the FIA and the reference methods, especially at lower acidities was good (Fig. 4). The FIA method is clearly superior when used to determine highly coloured samples (e.g., samples # 3 and 30) which are not possible to determine their end-points using the reference titrimetric method. A paired student-t test was performed on the data to compare the means obtained from the two methods. The calculated t value at the 95% confidence level is 1.872 while the tabulated value is 1.960 (Christian, 2004), suggesting that there is no significant difference of the mean between the two methods. The FFA values of these samples are somewhat higher due to their prolonged storage in the lab before the analysis. It is known that differences in the matrix and viscosity of the solution injected into FI system can give rise to gross errors (Nouros et al., 1997). Thus, the use of 2-propanol as solvent which is completely miscible with the palm oil samples had managed to overcome this problem.

When the liquid fraction (olein) samples were directly injected (no dilution) into the FIA manifold, noisy erratic peaks of poor reproducibility were obtained. This was probably due to the rather viscous samples that were not adequately mixed with the carrier solution under the FIA conditions. Furthermore, it was found that the samples adhered to the walls of the flow-through cell, requiring prolong washing with KOH and surfactant. Thus, it become mandatory for the sample to be diluted off-line before injecting into the single line FIA unit. Another strategy to overcome this problem is to use a two-line FIA manifold where the injected sample is mixed with the reagent stream on-line before reaching the detector.

4.4. Two-line FIA manifold

The two-line FIA method (Fig. 1) was optimized by investigating the effects of injection volume, carrier flow rate, reagent concentration and KOH concentration, similar to those for the single-line FIA manifold. The conditions shown in Table 1 was chosen as compromise between sensitivity and sample throughput. The method was found to be linear over 0.2–6.0 a.d. ($r^2 = 0.997$) (Table 2). FFA concentrations >6.0 a.d. cannot be prepared due

Table 3
Determination of FFA in palm oil samples using single-line manifold with PHP

Sample no.	Sample type	FFA content, a.d. (mean ± SD, $n = 3$)	
		FIA method	PORIM method
1	Crude palm oil	6.66 ± 0.04	6.68 ± 0.24
2	Crude palm oil	6.13 ± 0.05	6.44 ± 0.26
3	Crude palm oil	5.05 ± 0.02	-[a]
4	Crude palm oil	6.30 ± 0.16	6.20 ± 0.08
5	Crude palm oil	5.53 ± 0.02	5.65 ± 0.28
6	Crude palm oil	2.35 ± 0.02	2.34 ± 0.11
7	Crude palm kernel oil	5.101 ± 0.05	5.10 ± 0.22
8	Crude palm kernel oil	7.11± 0.42	6.93 ± 0.35
9	Crude palm kernel oil	8.55 ± 0.42	8.84 ± 0.47
10	Crude palm kernel oil	10.68 ± 0.10	10.6 ± 0.45
11	Crude palm kernel oil	0.78 ± 0.03	0.78 ± 0.05
12	RBD palm stearin	1.83 ± 0.09	1.91± 0.03
13	RBD palm stearin	1.05 ± 0.01	1.09 ± 0.04
14	RBD palm stearin	1.61 ± 0.02	1.63 ± 0.08
15	RBD palm stearin	1.89 ± 0.05	1.95 ± 0.06
16	RBD palm stearin	0.65 ± 0.03	0.64 ± 0.02
17	RBD palm oil	0.47 ± 0.01	0.49 ± 0.02
18	RBD palm oil	0.81 ± 0.04	0.83 ± 0.05
19	RBD palm oil	0.65 ± 0.01	0.64 ± 0.04
20	RBD palm oil	0.43 ± 0.03	0.44 ± 0.02
21	RBD palm oil	0.65 ± 0.01	0.66 ± 0.03
22	PFAD	69.94 ± 1.29	68.56 ± 2.10
23	PFAD	73.09 ± 1.28	77.27 ± 0.87
24	PFAD	74.18 ± 1.31	81.44 ± 3.74
25	PFAD	74.18 ± 0.73	85.66 ± 3.25
26	PFAD	76.97 ± 0.68	76.79 ± 2.46
27	PFAD	74.18 ± 0.72	83.73 ± 3.94
28	RBD palm kernel oil	2.46 ± 0.03	2.55 ± 0.11
29	RBD palm kernel oil	1.35 ± 0.02	1.32 ± 0.06
30	Crude palm olein	4.78 ± 0.04	–[a]
31	Crude palm olein	5.66 ± 0.15	5.73 ± 0.16
32	Crude palm olein	1.79 ± 0.02	1.82 ± 0.08
33	Crude palm olein	4.46 ± 0.04	4.34 ± 0.15
34	Crude palm olein	2.60 ± 0.01	2.68 ± 0.15
35	Crude palm olein	1.60 ± 0.02	1.69 ± 0.08
36	Crude palm olein	1.66 ± 0.02	1.66 ± 0.08
37	Crude palm stearin	11.38 ± 0.19	10.91 ± 0.47
38	Crude palm stearin	1.10 ± 0.07	1.15 ± 0.04
39	Soft stearin	0.90 ± 0.05	0.91 ± 0.01
	Mean RSD	2.17	4.06

Key: RBD – refined bleached deodorized; PFAD – palm fatty acid distilled.

[a] End-point cannot be determined; FIA conditions as stated in Table 1.

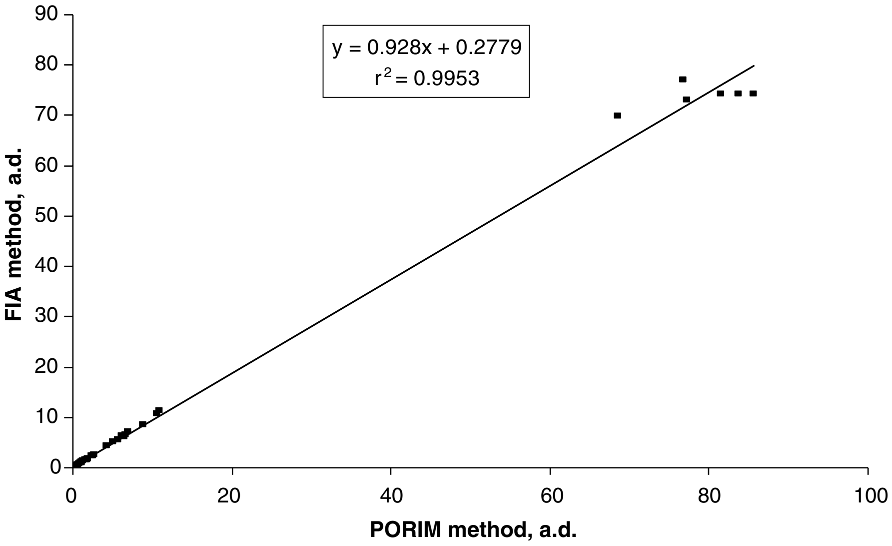

Fig. 4. Correlation of single line FIA method with standard PORIM method.

Table 4
Adopted FIA parameters for lower concentration of FFA using BTB

Parameter	Single-line FIA	Two-line FIA
KOH concentration, 10^{-4} M	1.5	2.0
Injection volume, μL	50	25
Flow rate, mL $\min^{-1}$	4.2	R: 2.4 C: 1.6

Indicator concentration and mixing chamber for all were 1.5×10^{-5} M and 1.0 mL, respectively.
R- Reagent (propanoic KOH with indicator).
C- Carrier (2-propanol).

to its limited solubility in palm oil samples. Compared to the single-line FIA manifold, the two-line method is inferior in terms of shorter linear range, lower sample throughput and uses more reagent (Table 2). The method, nevertheless, offers good recoveries (96.1–103.9%) when used to analyse spiked oil samples.

4.5. Determination of low FFA content in palm oil samples

Since the single-line and two-line FIA methods offer only moderate sensitivity (lower linear range of 0.2 a.d.), FIA methods that can detect lower concentrations of FFA such as in fresh refined oil (0.05–0.2 a.d.) was attempted. The strategy was to use lower concentration of KOH in anticipation of its ability to neutralize lower concentration of FFA. Using BTB as reagent, the single line and double line FIA conditions outlined in Table 4 was adopted. These conditions are similar to the earlier ones except that lower concentrations of KOH and indicator were used.

Analytical characteristics of the FIA methods using the adopted conditions are summarized in Table 5. Although the two methods exhibit similar detection limits, the two-line FIA is preferred due to its better sensitivity as reflected in its higher calibration slope. Reasonable recoveries for the spiked oil samples were also found (Table 5). Since the method involved direct injection of sample, olein samples are readily applicable to the system. Stearin (solid fraction) samples, however, cannot be analysed directly as stearin does not dissolve readily in 2-propanol under room conditions.

The two-line manifold using BTB as indicator when operated under the modified conditions (Table 4) was applied to the determination of eleven palm oil samples

Table 5
Analytical characteristics of FIA for lower concentrations of FFA using BTB

Parameter		Single-line FIA	Two-line FIA
Regression equation		$Y = 77.22 \log C + 98.4$	$Y = 447.2 \log C + 497.0$
Correlation coefficient, r^2		0.997	0.998
Detection limit, a.d.		0.06	0.06
Linearity range, a.d.		0.12 – 2.0	0.11 – 0.50
Sample throughput, samples h^{-1}		35 – 100	21 – 46
Reagent consumption, mL		2.5 – 14.0	5.2 – 11.4
Recovery, %[a]	0.02 a.d. spiked	107.1 ± 3.4	96.7 ± 1.5
	0.06 a.d. spiked	92.6 ± 2.6	104.0 ± 1.1

[a] Express as means ± standard deviation ($n = 3$).

Table 6
Determination of FFA in palm oil samples of low acidity using two-line manifold with BTB as indicator

Sample no.	Sample type	FFA content, a.d.	
		FIA	PORIM
1	Palm olein	0.16 ± 0.01	[a]
2	Palm olein	0.31 ± 0.01	0.31 ± 0.02
3	Palm olein	0.19 ± 0.01	0.19 ± 0.01
4	Palm olein	0.23 ± 0.01	0.24 ± 0.01
5	Palm olein	0.16 ± 0.01	0.18 ± 0.01
6	Palm olein	0.12 ± 0.01	0.14 ± 0.01
7	Palm olein	0.31 ± 0.01	[a]
8	Palm olein	0.13 ± 0.01	0.15 ± 0.01
9	RBD palm kernel oil	0.11 ± 0.01	0.10 ± 0.01
10	RBD palm kernel oil	0.18 ± 0.01	0.17 ± 0.01
11	RBD palm kernel oil	0.13 ± 0.01	0.11 ± 0.01
	Mean RSD	3.46	5.74

Express as means ± standard deviation ($n = 3$), FIA conditions shown in Table 4.

[a] End-point cannot be determined.

that were known to have low acidities. A comparison of this FIA and standard PORIM method is summarized in Table 6. The ability of the FIA method to determine FFA in coloured samples (sample 7) is readily seen from Table 6. A reasonably good correlation ($r^2 = 0.92$) between the FIA and PORIM method was found (Fig. 5). Application of the paired t-test indicate that there is no significant difference between the two methods at the 95% confidence level (calculated $t = 0.983$, tabulated $t = 2.228$) (Christian, 2004).

4.6. VIS spectrum

The absorbance of the red and yellow-coloured representative palm oil samples were scanned from 400 to 800 nm. It was found that all these coloured samples absorb minimally at the detection wavelengths (562 nm for PHP and 627 nm for BTB (Fig. 6), suggesting that the background colour of these samples were not sources of spectral interference.

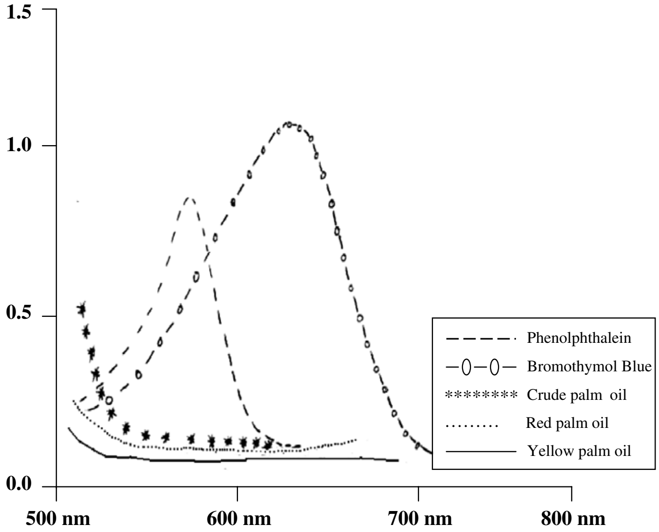

Fig. 6. Visible spectra of indicators and palm oil samples. Palm oil samples (7.13 a.d.) were prepared in 2-propanol. PHP and BTB (2.0×10^{-5} M) were mixed with KOH (5.0×10^{-4} M) in 2-propanol.

5. Conclusions

A rapid, precise and accurate FIA method for the determination of FFA in palm oil samples using single-line and two-line FIA manifolds employing PHP and BTB as indicators was developed. The single-line manifold requires off-line pre-dilution of samples and is more suitable for higher concentrations of FFA. The two-line manifold, although allowing direct injection of oil samples, resulted in lower sample throughput, and consumes larger amounts

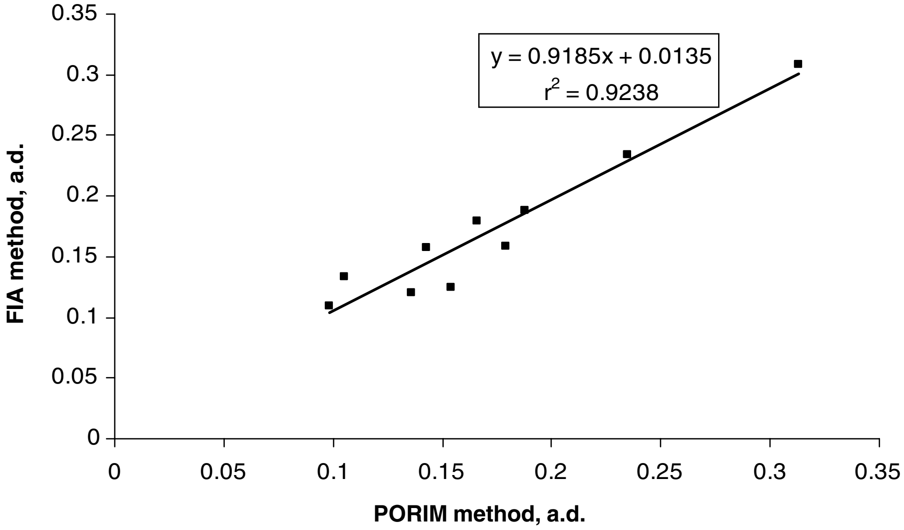

Fig. 5. Correlation between two-line FIA with standard PORIM method for low acidity palm oil.

of reagent. In summary, the single-line FIA manifold utilizing PHP as indicator and two-line manifold using BTB as indicator were recommended for the determination of sample with FFA >0.4 a.d. and <0.4 a.d., respectively. The two-line FIA method can be further adapted to allow lower acidity samples (such as RBD palm kernel and palm olein) to be determined. The superiority of the FIA methods in determining FFA in certain highly coloured samples, not possible in manual titrimetric method, is evident.

The sensitivity of the FIA method is comparable to the FTIR methods (Che Man & Moh, 1998; Che Man & Setiowaty, 1999), but the proposed FIA is cheaper and with minor modifications can be adapted for the on-line monitoring of FFA in process lines. The negligible absorption of the background colour of the sample at the detection wavelengths and the minimization of viscosity variations and matrix effects by the use of 2-propanol have also contributed to the success of the FIA determination. Further experiments with the FIA–FFA determinations as well as the automation of other palm oil parameters are being actively pursued in our laboratories.

Acknowledgement

Financial support from the Universiti Sains Malaysia through a Short Term Research Grant is gratefully acknowledged.

References

Canham, J. S., & Pacey, G. E. (1987). Automated free fatty acid determination using flow injection analysis solvent extraction. *Journal of American Oil Chemists Society, 64*, 1004–1007.

Che Man, Y. B., & Moh, M. H. (1998). Determination of free fatty acids in palm oil by near-infrared reflectance spectroscopy. *Journal of American Oil Chemists Society, 75*, 557–564.

Che Man, Y. B., & Setiowaty, G. (1999). Application of Fourier transform infrared spectroscopy to determine free fatty acid contents in palm olein. *Food Chemistry, 66*, 109–114.

Christian, G. D. (2004). *Anal. Chem.* (sixth ed.). Singapore: Wiley.

Ekstrom, L. G. (1981). Automatized method for determination of free fatty acid. *Journal of American Oil Chemists Society, 58*, 935–938.

Fuse, T., Kusu, F., & Takamura, K. (1997). Determination of higher fatty acids in oils by high-performance liquid chromatography with electrochemical detection. *Journal of Chromatography A, 764*, 177–182.

Husek, P., Simek, P., & Tvrzicka, E. (2002). Simple and rapid procedure for the determination of individual free fatty acids in serum. *Analytica Chimica Acta, 465*, 433–439.

Ismail, A. A., Van de Voort, F. R., Emo, G., & Sedman, J. (1993). Rapid quantitative determination of free fatty acid in fats and oils by Fourier Transform Infrared Spectrocopy. *Journal of American Oil Chemists Society, 70*, 335–341.

Jaafar, M., & Sukaimi (2001). The future of palm oil in the new milenium in Malaysia. *Borotrop Bulletin, 16*, 10–13.

Jakmunee, J., Pathimapomlert, L., Hardwell, S. K., & Grudpan, K. (2005). Novel approach for non-segmented flow micro-titration with sequential injection using a lab-on-valve system: a model study for the assay of acidity in fruit juices. *Analyst, 130*, 299–303.

Kuselman, I., Tur'yan, Y. I., Burenko, T., Goldfeld, I., & Anisimov, B. (1999). pH-metric determination of acid values in oilseeds without titration. *Talanta, 49*, 629–637.

Kwon, D. Y., & Rhee, J. S. (1986). A simple and rapid colorimetric method for determination of free fatty acid for lipase assay. *Journal of American Oil Chemists Society, 63*, 89–92.

Mariotti, E., & Mascini, M. (2001). Determination of extra virgin olive oil acidity by FIA-titration. *Food Chemistry, 73*, 235–238.

Miwa, H. (2002). High-performance liquid chromatographic determination of free fatty acids and esterified fatty acids in biological materials as their 2-nitrophenylhydrazides. *Analytica Chimica Acta, 465*, 237–255.

Nouros, P. G., Georgiou, C. A., & Polissiou, M. G. (1997). Automated flow injection spectrophotometric non-aqueous titrimetric determination of the free fatty acid content of olive oil. *Analytica Chimica Acta, 351*, 291–297.

PORIM Test Methods (1995). Kuala Lumpur: Palm Oil Research Institute of Malaysia (PORIM).

Puchades, P. R., Suescun, A., & Maquieira, A. (1994). Determination of free fatty acids in foods by flow injection. *Journal of Science and Food Agriculture, 66*, 473–478.

Roldan-Assad, R., & Gareil, P. (1995). Capillary zone electrophoretic determination of C2 – C18 linear saturated free fatty acids with indirect absorbance detection. *Journal of Chromatography A, 708*, 339–350.

Ruzicka, J., & Hansen, E. H. (1988). *Flow Injection Analysis* (second ed.). New York: Wiley.

Schmidt, A., Standfuz-Gabisch, C., & Bilitewski, U. (1996). Microbial biosensor for free fatty acids using an oxygen electrode based on thick film technology. *Biosensors and Bioelectronics, 11*, 1139–1145.

Schoemaker, M., Feldbrugge, R., Grundig, B., & Spener, F. (1997). The lipoxygenase sensor, a new approach in essential fatty acid determination in foods. *Biosensors and Bioelectronics, 12*, 1089–1099.

Schooner, F., Simard, R. E., & Pandian, S. (1991). Colorimetric assay for free fatty acids in butter using flow-injection and immobilized enzymes. *Journal of Food Science, 56*, 1229–1232.

Shahin, Y., Hamzawi, L. F., & Haggag, H. F. (1987). Fatty acid composition of fat globule membrane neutral lipids from Egyptian buffalo, goat and cow's milk. *Food Chemistry, 24*, 11–19.

Shantha, N. C., & Napolitano, G. E. (1992). Gas chromatography of fatty acids. *Journal of Chromatography, 624*, 37–51.

Zhi, Z. L., Rios, A., & Valcarcel, M. (1996). An automated flow-reversal injection/liquid-liquid extraction approach to the direct determination of total free fatty acid in olive oils. *Analytica Chimica Acta, 318*, 187–194.