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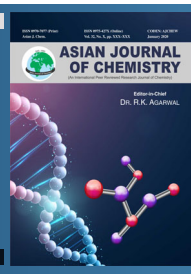


# Determination of Vitamin A and Vitamin E Contents in Fortified Cooking Oil using Visible Spectrophotometry

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For measuring vitamins A and E in fortified palm oil, trichloroacetic acid (TCA) through visible spectrophotometry and total antioxidant capacity (TAC) through phenanthroline method were developed. Using TCA determination method, vitamin A was directly measured at λ = 620 nm, with 92.84 % ± 0.74 accuracy, 0.79 % RSD, 2.15 IU/g limit of detection (LOD) and 7.17 IU/g limit of quantitation (LOQ). Using the mole difference calculation method of $Fe^{2+}$ after $Fe^{3+}$ reduction by total antioxidants and vitamin A, vitamin E content was indirectly measured. Total antioxidant capacity required for reducing $Fe^{3+}$ was determined using phenanthroline method at λ = 510 nm. The temperature and reaction time for the saponification process were also evaluated. Optimum saponification was obtained at 70 ºC in 20 min with a recovery percentage of 93.81 %. Determined Vitamin E had 93.94 % ± 0.63 accuracy, 0.68 % RSD, 0.11 IU/g LOD and 0.76 IU/g LOQ.

**Keywords: Palm oil, Vitamin A, Vitamin E, Trichloroacetic acid, Phenanthroline.**

## INTRODUCTION

Vitamin A fortification of food was attempted to overcome various diseases caused by vitamin A deficiency. The World Health Organization launched this programme globally in 1999 and was published by the International Agency for the Prevention of Blindness in Vision 2020: Right to Sight. Some countries produce and use palm oil as a medium vitamin A fortification [1]. Palm oil is a suitable medium for vitamin A fortification because its natural vitamin E content can inhibit vitamin A degradation during the processes of distribution, storage and manufacture. In addition to fulfil the vitamin needs, vitamin A and E contents are crucial as an antioxidant to inhibit oxidative damage caused by unsaturated fatty acids [2]. Furthermore, consuming vitamins A and E in excess leads to health issues such as osteoporosis, liver damage, prostate cancer, cancer risk and birth defect risk [3]. Therefore, efforts are needed to control vitamin A and E content in fortified palm oil.

Currently, chromatography-based methods are used to measure vitamin A and E content of various food and biological materials [2,4,5]. These methods are accurate and precise, but they require expensive equipments and reliable personnel. Therefore, spectrophotometry is a promising new method for measuring the vitamin A and E content.

Determination of vitamin A content using visible spectrophotometry is based on blue colour formation by carbonium ions originating from anhydro-vitamin A protonation by using trichloroacetic acid (TCA) solution with dichloromethane (DCM). This method involves Carr-Price method development, where the blue colour fades quickly and is influenced by carotene. Using the TCA method, vitamin A content can be measured directly by adding TCA solution to DCM and dissolving it in oil. The absorbance of blue colour obtained is measured at λ = 620 nm [6-9].

Total vitamin A and E in fortified cooking oil was determined through visible spectrophotometry based on the total antioxidant capacity (TAC) in reducing metal ions through the electron transfer mechanism [10]. As an antioxidant, vitamins A and E donate two electrons to reduce metal ions [11,12]. The decolourization is associated with the total antioxidant concentration of the sample [10]. The phenanthroline method is one of the approaches for TAC measurement that uses $Fe^{3+}$ as an oxidant probe. Using this method, an antioxidant capacity of oil and fat-soluble compounds can be measured because it

works for fat solvents such as methanol, ethanol and acetone. This method has another advantage that red-orange colour of Fe(II)-phenanthroline complex {$[Fe(C_{12}H_8N_2)_3]^{2+}$} is stable at pH 2-9, with $\lambda_{max}$ 512 nm in water solvents [13] and $\lambda_{max}$ 510 nm in ethanol solvents [14]. However, a saponification process to separate vitamins A and E from triglycerides is required for this measurement because metal ions can oxidize unsaturated fatty acids that are part of triglycerides, thus disrupting the measurement process [15]. The optimum conditions (temperature and time) required for saponification vary for each oil type. The factors influencing the saponification process are alkali concentration, stirring, excess alkali [16,17], and the fatty acid type that compose triglycerides [18].

The complex Fe(II)-phenanthroline measured using the TAC method is associated with vitamin A and E concentrations in the sample. Meanwhile, using TCA method, number of $Fe^{2+}$ moles produced by vitamin A from the reduction reaction of $Fe^{3+}$ is calculated, which is further used to determine vitamin A content. Thus, difference in the number of $Fe^{2+}$ moles determined through TAC ($Fe^{2+}_{TAC}$) measurement and that of $Fe^{2+}$ moles determined based on vitamin A content calculated ($Fe^{2+}_{VA}$) is equal to $Fe^{2+}$ produced through the reduction reaction of $Fe^{3+}$ by vitamin E ($Fe^{2+}_{VE}$).

Herein, two methods were developed for measuring vitamin A and E contents in fortified cooking oil, first determination of trichloroacetic acid (TCA) through visible spectrophotometry and second, saponification of fortified palm oil for measuring TAC using phenanthroline method. The results indicated an accuracy of more than 93 % can be obtained with the spectrometry method and hence can be adopted.

## EXPERIMENTAL

Reference oils $VA_{16}VE_{1,2}$; $VA_{20}VE_{1,5}$ and $VA_{24}VE_{1.8}$ of 100 g each were prepared through addition of non-antioxidant oil (placebo) with vitamin A palmitate (10,000 IU/0.15 g) and vitamin E tocopherol (100 IU/0.4 g) for 0.024, 0.48, 0.03, 0.6, 0.036 and 0.72 g. The solution was stirred at 200 rpm for 15 min. Trichloroacetic acid (TCA), dichloromethane (DCM), potassium hydroxide, ethanol absolute, diethyl ether, iron(III) chloride hexahydrate, iron(II) sulphate heptahydrate and 1,10-phenanthroline were of analytical grade and procured from Merck.

**Vitamin A content measurement using TCA method:** A trichloroacetic acid solution was prepared by dissolving TCA (25 g) in 50 mL of DCM. A standard solution of 2-20 IU/mL of 10 mL vitamin A was prepared by pipetting 0.2, 0.4, 0.6, 0.8, 1, 1.5, and 2 mL of 100 IU/mL vitamin A solution in DCM and then adding upto 10 mL of DCM. A sample solution of vitamin A 10 mL sample solution was prepared by dissolving 2 g of oil sample in DCM. Vitamin A content was measured through absorbance measurement by pipetting 1 mL of the standard solution or sample into a cuvette, adding 1 mL of blank solution and TCA reagent each, stirring it for 3 s, leaving it for 5 s, and then reading its absorbance at $\lambda$ = 620 nm [6-8].

**Measurement of TAC using phenanthroline method:** A standard solution of 10 mL $Fe^{2+}$ with a 0.01-0.08 µmol/mL concentration was obtained by pipetting 0.1 mM $FeSO_4$ solution into ethanol for 1, 3, 4, 5, 6 and 8 mL and then adding 1 mL of 0.5% 1,10-phenanthroline in 95 % ethanol upto 10 mL. Saponification was optimized by adding 0.1 g $VA_{20}VE_{1,5}$ reference oil, 0.6 mL of 60 % KOH, 1.5 mL ethanol and 0.05 g ascorbic acid in a test tube. The tube was then closed tightly after passing nitrogen gas into it. It was then placed in a water bath heated at 50, 60 and 70 ºC for 20, 30 and 40 min. During saponification process, contact with light was avoided. Further, test-tube was cooled and 2 mL of 1% NaCl and diethyl ether each were added. Then, the mixture was stirred to extract vitamin A and E. The obtained diethyl ether layer was separated and stored in a different test tube. The extraction was repeated twice with 1 mL diethyl ether in the same test tube. Then, extracted solution was dried [19], dissolved with 5 mL ethanol and transferred in a 10 mL flask. Further, 1 mL 0.2 % $FeCl_3$, 1 mL 0.5% 1,10-phenanthroline and absolute ethanol were added until the limit mark was reached. The solution was then briefly stirred, allowed to rest based on the optimum reaction time and transferred into a cuvette. The absorbance was read at $\lambda$ = 510 nm. The same treatments were performed to measure the TAC of $VA_{16}VE_{1,2}$, $VA_{24}VE_{1,8}$ and cooking oil samples with optimum saponification conditions.

## RESULTS AND DISCUSSION

**Measurement of vitamin A content using TCA method:** The linear relationship between the concentration and absorbance of the standard vitamin A solution is indicated using the regression equation *i.e.* y = 0.0625x + 0.0081, with r = 0.999. Thus, an increase in the intensity of blue colour with anhydro-vitamin A protonation corresponds with increase in vitamin A concentration in the standard solution according to the Lambert-Beer law as conducted by Bayfield [6], Bayfield and Cole [7]. The percentage measurement and vitamin A content recovery from reference oils $VA_{16}VE_{1,2}$, $VA_{20}VE_{1,5}$ and $VA_{24}VE_{1.8}$ are presented in Table-1, with the average recovery percentage of 92.84 % ± 0.74. This result corresponds with the standards of acceptable recovery percentage, *i.e.* 80-110 % [20]. Thus, a blue colour obtained through the reaction between vitamin A and TCA was not significantly affected by vitamin E and other antioxidants [6].

TABLE-1
AVERAGE RECOVERY PERCENTAGE OF VITAMIN A

| Sample | Initial concentration | | Measured concentration | | Recovery (%) |
|---|---|---|---|---|---|
| | IU/g | µg/g | IU/g | µg/g | |
| $VA_{16}VE_{1,2}$ | 16 | 8.8 | 14.75 | 8.11 | 92.18 |
| $VA_{20}VE_{1,5}$ | 20 | 11 | 18.62 | 10.24 | 93.10 |
| $VA_{24}VE_{1.8}$ | 24 | 13.2 | 22.38 | 12.31 | 93.23 |

While manufacturing reference oil, stirring process may decrease the vitamin A content by 6.58-7.28 %. The contact of vitamin A with light and oxygen in the air increases during stirring, which causes vitamin A degradation [3]. Moreover, a contact between oxygen and oil increases, which accelerates the formation of fat peroxyl radicals, whereas an antioxidant, vitamin A donates hydrogen atoms to bind with fatty peroxyl radicals [21]. The precision, which is denoted as RSD (n = 3), shows a good value (RSD ≤ 2 %) for each reference oil *i.e.*

$VA_{16}VE_{1,2}$ = 0.98 %, $VA_{20}VE_{1,5}$ = 0.55 % and $VA_{24}VE_{1,8}$ = 0.32 %. Total RSD average (n = 9) of vitamin A is 0.79 %. The limit of detection (LOD) and limit of quantitation (LOQ) of this method were 2.15 and 7.17 IU/g, respectively.

**Measurement of TAC using phenanthroline method:** Using a standard solution with a concentration of 0.03 mol/mL that has been rested for 15 min, obtained $\lambda_{max}$ is 510 nm, which is similar to that obtained by Oktavia *et al.* [14] using ethanol solvents. Using a vitamin A and E mixture in ethanol requires an optimum reaction time of 85 min. An increased intensity of red-orange colour obtained owing to the complex Fe(II)-phenanthroline in the standard solution shows a good linear relation at 0.1-0.8 µmol/mL concentration with the r value of 0.999 and regression equation, *i.e.* y = 11.954x + 0.0179.

Based on the reaction mechanism [11,12]

$$AOxH + 2Fe^{3+} \longrightarrow AOx^{\bullet} + 2Fe^{2+} + 2H^{+} \quad (1)$$

where AOxH: antioxidant, the concentration of $Fe^{2+}$ from the measurement of total antioxidants ($Fe^{2+}_{TAC}$) is the sum of $Fe^{2+}$ from the reduction of $Fe^{3+}$ by vitamin A ($Fe^{2+}_{VA}$) and vitamin E ($Fe^{2+}_{VE}$).

$$Fe^{2+}_{TAC} = 2Fe^{2+}_{VE} + 2Fe^{2+}_{VA} \quad (2)$$

For determining the optimum conditions for saponification, initial mole of $Fe^{2+}$ was theoretically determined through the amount of vitamins A and E added to the reference oil $VA_{20}VE_{1,5}$ (4.68 mol/g). The results of the optimum saponification condition is given in Fig. 1.

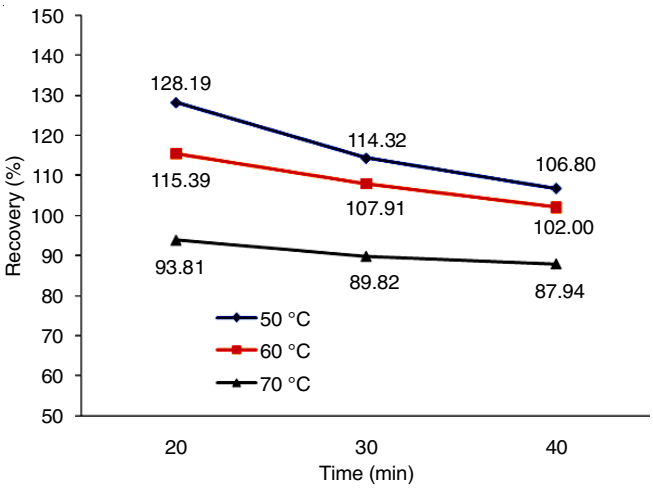


Fig. 1. Recovery percentage of saponification optimization

The conditions required for optimum saponification with a recovery percentage of 93.81 % are 70 ºC and 20 min. Thus, triglycerides are saponified completely, and the decrease in or damage of the unsaponifiable fraction is minimal. Similarly, Kaparthi and Chari [22] mentioned that almost all oil types are soluble in ethanol with 1:1 ratio at 70 ºC. Moreover, Mukarromah *et al.* [23] stated that oils which perfectly dissolves at high temperatures appear to accelerate the reaction because molecules stretch and the charges in oil move freely at high temperatures, increasing the kinetic energy of the particles and effective collisions with KOH.

**Calculation of vitamin E content:** Trichloroacetic acid (TCA) measurement of the reference oil and the calculation of vitamin E content using eqn. 2 provide vitamin E content recovery percentages of $VA_{16}VE_{1,2}$, $VA_{20}VE_{1,5}$ and $VA_{24}VE_{1.8}$ shown in Table-2. The average total recovery is 93.94 % ± 0.64, which is less than the standard recovery percentage of 95-105 % [20].

TABLE-2
AVERAGE OF RECOVERY PERCENTAGE OF VITAMIN E

| Sample | Initial concentration | | Measured concentration | | Recovery (%) |
|---|---|---|---|---|---|
| | IU/g | µg/g | IU/g | µg/g | |
| $VA_{16}VE_{1,2}$ | 1.2 | 800 | 1.12 | 747.23 | 93.40 |
| $VA_{20}VE_{1,5}$ | 1.5 | 1000 | 1.41 | 938.21 | 93.82 |
| $VA_{24}VE_{1,8}$ | 1.8 | 1200 | 1.70 | 1135.16 | 94.59 |

During the saponification process, vitamin E content of the reference oil may decrease from 5.40 to 6.59 %. Unsuitable time and temperature degrade vitamin E, which may occur owing to long interactions with alkali and heat. The right temperature for the saponification process to separate unsaponifiable fractions is 70 ºC as oil is completely soluble in ethanol at this temperature [22], but a further assessment is needed to note the actual time required for the saponification process. Moreover, stirring during the reference oil manufacturing process can decrease vitamin E content, since it increases the contact between oxygen and oil, accelerating the formation of fat peroxyl radicals, whereas an antioxidant, vitamin E donates hydrogen atoms that bind with fatty peroxyl radicals [21].

Precision, denoted as RSD (n = 3), shows a good value (RSD ≤ 2 %) for all reference oils: $VA_{16}VE_{1,2}$ = 0.51 %, $VA_{20}VE_{1,5}$ = 0.15 % and $VA_{24}VE_{1,8}$ = 0.57 %. The average of RSD total (n = 9) of vitamin E measurement is 0.68 %. For this method, the LOD and LOQ are 0.114 and 0.759 IU/g, respectively.

**Mathematical approach on the measurement of vitamin A and E contents in fortified palm oil containing synthetic antioxidants (BHA and BHT):** Oluwalana *et al.* [24] showed that vitamin E content of fortified palm oil decreases when stored in polyethylene terephthalate bottles at 27 ºC. During the storage period, vitamin E content decreases at the rate of 35.015 % per month but its peroxide value increases. During the storage period, peroxide value increases by 15.71, 11.49 and 12.58 % without and with addition of BHT (100 ppm) and BHA (200 ppm), respectively (Fig. 2). Comparing the data of previous studies [24,25], vitamin E content of palm cooking oil decreases by 25.61 and 28.04 % with the addition of BHT and BHA, respectively.

Marteau *et al.* [26] showed that synergies occur between vitamin E and synthetic antioxidants, where synthetic antioxidants are the regenerator agents for radical vitamin E. This regeneration process occurs because tocopherol is more reactive than BHT to fat radicals, as the dissociation energy of O-H bonds in tocopherol (BDE = 69.1 kcal $mol^{-1}$) is lower than that in BHT (BDE = 72.4 kcal $mol^{-1}$) and the steric resistance of hydrogen atom in the *t*-butyl group is bound to benzene ring of BHT. The same applies to BHA (BDE = 72.3 kcal $mol^{-1}$) and TBHQ (BDE = 74.3 kcal $mol^{-1}$) [27]. Thus, a difference between the decrease in vitamin E percentage without adding synthetic antioxidants and the decrease in vitamin E percentage with the addition of synthetic antioxidants is the vitamin E

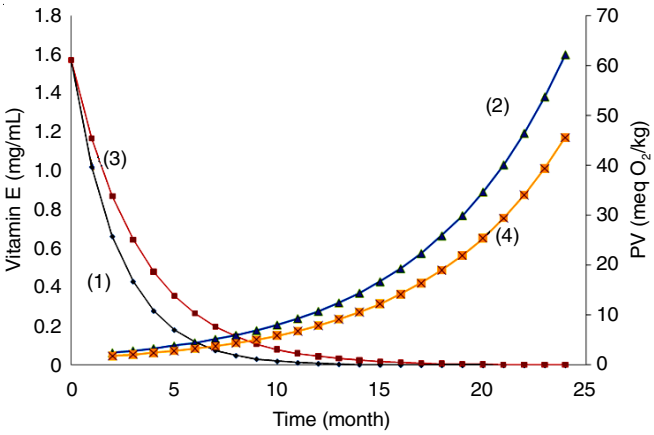


Fig. 2. Vitamin E decrease and PV increase in cooking oil during the storage period (in month). (1) decrease of vitamin E, (2) increase of PV without synthetic antioxidants, (3) decrease of vitamin E and (4) increase of PV with the addition of 100 ppm of BHT

percentage regenerated by BHT or BHA during the storage period. Thus, vitamin E percentage regenerated is the percentage of BHT or BHA decayed during the storage period. A decrease in the percentages of BHT and BHA during 1-month storage period are 9.41 % and 6.98 %, respectively. Therefore, vitamin E content of oils containing synthetic antioxidants can be calculated using eqn. 3:

$$Fe^{2+}_{TAC} = 2Fe^{2+}_{VE} + 2Fe^{2+}_{VA} + 2Fe^{2+}_{AOx} \quad (3)$$

where $Fe^{2+}_{AOx}$ is the $Fe^{2+}$ concentration obtained from the reaction between $Fe^{3+}$ and synthetic antioxidants, and theoretically $Fe^{2+}_{AO}$ can be calculated using the following decay eqn. 4:

$$Fe^{2+}_{AOx} = AOx_n = AOx_0 (1 - b)^n \quad (4)$$

where $Fe^{2+}_{AOx} = AOx_n$ = concentration of BHT ($M_r$ = 220.36 g/mol) or BHA ($M_r$ = 180.25 g/mol) after n periods; $AOx_0$ = initial concentration of synthetic antioxidants; n = storage period (difference between production month and acquisition month); and b = decay or decline rate (%) (BHT = 9.41 % and BHA = 6.98 %).

Several measurements on vitamin A and vitamin E content in fortified palm cooking oil samples with and without synthetic antioxidant addition are shown in Table-3.

TABLE-3
CALCULATION OF VITAMIN A AND VITAMIN E CONTENT OF OIL SAMPLES

| Palm oil samples | Vitamin A (IU/g) | Vitamin E (IU/g) |
|---|---|---|
| A[1] | 16.77 | 1.13 |
| B[1] | 14.43 | 0.92 |
| C[1,2] | 5.65 | 0.52 |
| D[1,2] | – | 0.11 |

All samples were obtained from local markets (1) with addition of BHT (2)

## Conclusion

Vitamin A content was measured using trichloroacetic acid (TCA) method with dichloromethane solvent at λ = 620 nm. It showed 92.84 % ± 0.74 accuracy and 0.79 % precision (RSD), fulfilling the validation test requirements with 2.15 IU/g LOD and 7.17 IU/g LOQ. A good linearity (r = 0.999) was observed using the TAC measurement at λ = 510 nm. When vitamins A and E were mixed in ethanol with $Fe^{3+}$ solution, an optimum time required for the reaction is 85 min. The optimum conditions required for the saponification process with $VA_{20}VE_{1,5}$ is 70 ºC for 20 min with the recovery percentage of total moles for vitamin A and vitamin E in $VA_{20}VE_{1,5}$ being 93.81%. Determining vitamin E content through by-difference method between TAC moles and vitamin A moles has 93.94 % ± 0.64 accuracy, 0.68 % precision (RSD), 0.114 IU/g LOD and 0.76 IU/g LOQ.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

## REFERENCES

1. A. Laillou, D. Panagides, G.S. Garrett and R. Moench-Pfanner, *Food Nutr. Bull.*, **34(Suppl. 2)**, S72 (2013); https://doi.org/10.1177/15648265130342S109
2. L. Garai, *J. Food Technol. Res.*, **3**, 99 (2016); https://doi.org/10.18488/journal.58/2016.3.2/58.2.99.104
3. G.F. Combs Jr. and J.P. McClung, The Vitamins Fundamental Aspects in Nutrition and Health, Elsevier Inc., edn 5 (2017).
4. J. Jakobsen, A. Melse-Boonstra and M. Rychlik, *Curr. Dev. Nutr.*, **3**, nzz086 (2019); https://doi.org/10.1093/cdn/nzz086
5. M. Katsa, C. Proestos and E. Komaitis, *Curr. Res. Nutr. Food Sci.*, **4**, 92 (2016); https://doi.org/10.12944/CRNFSJ.4.Special-Issue-October.12
6. R.F. Bayfield, *Anal. Biochem.*, **39**, 282 (1971); https://doi.org/10.1016/0003-2697(71)90416-7
7. R.F. Bayfield and E.R. Cole, Colorimetric estimation of vitamin A with TCA, In: Methods in Enzymology, Academic Press, Inc., vol. 67 (1980).
8. Y. Zhang, W. Zhou, J. Yan, M. Liu, Y. Zhou, X. Shen, Y.-L. Ma, X.-S. Feng, J. Yang and G.-H. Li, *Molecules*, **23**, 1484 (2018); https://doi.org/10.3390/molecules23061484
9. P. Makhumula, M. Guamuch and O. Dary, Manual for Internal Monitoring of Oil Fortified with Vitamin A, East, Central and Southern African Health Community (ECSA-HC) (2007).
10. R. Apak, S. Gorinstein, V. Böhm, K.M. Schaich, M. Özyürek and K. Güçlü, *Pure Appl. Chem.*, **85**, 957 (2013); https://doi.org/10.1351/PAC-REP-12-07-15
11. J.A. Lucy and F.U. Lichti, *Biochem. J.*, **112**, 231 (1969); https://doi.org/10.1042/bj1120231
12. I. Matijevic, S. Bischof, A. Sutlovic and T. Pusic, Determination of α-Tocopherol in Cosmetotextiles-UV/Vis Spectrophotometric Method; 8th Central European Conference on Fiber-Grade Polymers, Chemical Fibers and Special Textiles (2015).
13. N.A. Agustina, Undergraduate Thesis, Pengaruh Ion $As^{3+}$ pada Analisa Besi(III) dengan Ligan 1,10- Fenantrolin dan Tiosianat menggunakan Metode Spektrofotometri UV-Vis, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia (2017) (In Indonesian).
14. B. Oktavia, L.W. Lim and T. Takeuchi, *Anal. Sci. Int. J. Japan Soc. Anal. Chem.*, **24**, 1487 (2008). https://doi.org/10.2116/analsci.24.1487
15. M. Ahmed, J. Pickova, T. Ahmad, M. Liaquat, A. Farid and M. Jahangir, *Sarhad J. Agric.*, **32**, 230 (2016); https://doi.org/10.17582/journal.sja/2016.32.3.230.238
16. R.D. O'Brien; Fats and Oils Formulating and Processing for Applications, CRC Press, edn 3 (2009).
17. R.F.K. Almilly, *IASJ J. Eng.*, **20**, 144 (2014).
18. C.A.S. da Silva, G. Sanaiotti, M. Lanza, L.A. Follegatti-Romero, A.J.A. Meirelles and E.A.C. Batista, *J. Chem. Eng. Data*, **55**, 440 (2010); https://doi.org/10.1021/je900409p
19. R. Eitenmiller, L. Ye and W.O. Landen Jr., Vitamin Analysis for The Health and Food Sciences, edn 2, CRC Press (2008).
20. Riyanto, Ph.D. Thesis, Validasi & Verifikasi Metode Uji: Sesuai dengan ISO/IEC 17025 Laboratorium Pengujian dan Kalibrasi, Yogyakarta: Deepublish, June (2014) (In Indonesian).

21. D.Q. Dao, T.C. Ngo, N.M. Thong and P.C. Nam, *J. Phys. Chem. B*, **121**, 9348 (2017); https://doi.org/10.1021/acs.jpcb.7b07065
22. R. Kaparthi and K.S. Chari, *J. Am. Oil Chem. Soc*., **36**, 77 (1959); https://doi.org/10.1007/BF02540248
23. L. Mukarromah, B.E. Cahyono and M. Misto, *Berkala Sainstek*, **6**, 41 (2018); https://doi.org/10.19184/bst.v6i1.7770
24. I.B. Oluwalana, M.O. Oluwamukomi, B.O. Toriola and O.R. Karim, *Adv. Res.*, **4**, 191 (2015); https://doi.org/10.9734/AIR/2015/12092
25. B. Pimpa, D. Kanjanasopa and S. Boonlam, *Kasetsart J. (Nat. Sci.)*, **43**, 370 (2009).
26. C. Marteau, D. Favier, V. Nardello-Rataj and J.M. Aubry, *Food Chem.*, **160**, 190 (2014); https://doi.org/10.1016/j.foodchem.2014.03.070
27. R. Guitard, V. Nardello-Rataj and J.M. Aubry, *Int. J. Mol. Sci.*, **17**, 1220 (2016); https://doi.org/10.3390/ijms17081220