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Research Article

Role of Minor Constituents and Balanced Fatty Acids in Upgrading the Low Stability of Cooking Oils Blended with Palm Super Olein

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Abstract

Background: Vegetable oil blending is one of the most potent ways in improving and upgrading low stability cooking oils.

Objective: This study is chiefly concerned by the balance between saturated, monounsaturated and polyunsaturated fatty acids as recommended by World Health Organization (WHO) as well as improving their oxidative stability. **Methodology:** Palm super olein was blended with soybean and sunflower oils at different ratios, namely, 50:50, 55:45, 60:40, 65:35 and 75:25% w/w to identify the best cooking oil blends in terms of fatty acid balance and other specific characteristics. Bioactive minor lipid constituents of cooking oils and their blends including vitamin E, phytosterols, phytostanols, fatty acid components and oxidative stability were analyzed. **Results:** The 50:50 and 55:45% super palm olein:soybean or sunflower oil blend show the highest content of total tocopherols. While, the ratio of 65 and 75% of palm super olein blends to other oils, gave the highest amount of total tocotrienol which is the most potent antioxidants. With reference to phytosterols composition, it was found that the ratio 50:50 and 55:45% super palm olein:sunflower oil blend show highest amount of 5-, 7-stigmasterol, β -sitosterol, 7-avenasterol. While, the highest level of 5-stigmasterol and β -sitosterol was found in the blend of palm olein:soybean (50:50 and 55:45%). Most of phytosterols components exert antioxidant effects and enhance immunity in the human body. The ratio 55, 60 and 65% of palm super olein to soybean or sunflower oils show nearly ideal proportion between fatty acid groups. **Conclusion:** The addition of palm super olein to sunflower and soybean oils improves the oxidative stability of these oil blends and increases their phytonutrient contents as well as the nutritional value of oil blends via the balance between fatty acids.

Key words: Palm super olein, phytosterols, vitamin E, cooking oils, fatty acid balance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Vegetable oils are used for cooking and frying and these oils have limited technological application in their original forms because of their properties. To improving their commercial application, vegetable oils are often modified using hydrogenation, interesterification, fractionation and blending¹. Blending method is more important for vegetable oils with different properties and is consider as one of the simplest methods to create new specific characteristics. Cooking oil blending has been a common acceptable practice in many countries.

Any single cooking oil can have low physical, chemical, nutritional properties and limited oxidative stability. For example, palm olein is widely used in Malaysia as cooking oil while soybean oil in the United States is used as major cooking oil. To maintain quality of vegetable oil, mixing oil is gaining worldwide popularity due to its advantages not only can change fatty acid profile but also increase the levels of bioactive minor lipid components including powerful natural antioxidants^{2,3}. Blended oil may have been incorporated with health-improving through strengthened by minor components from the blending partner that constitute the mixture⁴. The use of blended oil is better than the single oil alone thus reducing the risks of coronary heart disease due to natural antioxidant contents and improved fatty acid composition of the new oil^{5,6}. Recently, blending of common edible oils with unconventional oils (rice bran, tomato, apricot seed, grape seed and black cumin seed oil) rich in bioactive lipid components which may leads to promote nutritional and functional characteristics to the foods as well as increase the oxidative stability⁷⁻⁹. Stability of vegetable oils is being concerned to meet consumer satisfaction¹⁰. To raise resistance of soybean and sunflower oils against oxidation, blending with specific oils having antioxidant potency is necessary¹¹.

For these reasons, palm super olein was selected to achieve this target. Palm super olein contains about 30% tocopherols and 70% tocotrienols of the vitamin E content; rendering it one of the richest specific sources of natural antioxidants. Researchers have confirmed that tocotrienols show different biological and physiological properties than tocopherols¹²⁻¹⁵.

Concerning the fatty acid balance, World Health Organization (WHO)¹⁶ recommended that the best balance as follows: 1:1:1 of saturated, monounsaturated and polyunsaturated fatty acids for generating the best LDL/HDL ratio¹⁷.

The formulation of balanced fatty acids as well as raising the antioxidation potency via phytonutrient of the oil blends have not been studied comprehensively.

The present investigation is chiefly concerned with the fatty acid balance as prerequisite, between saturated, monounsaturated and polyunsaturated (S:M:P) fatty acids (1:1:1) as recommended by WHO as far as possible. Mixing of palm super olein (rich in tocotrienols) with soybean or sunflower oils, recognized as common cooking oils in many countries especially in Egypt, was in the following calculated ratios 50:50, 55:45, 60:40, 65:35 and 75:25% w/w. The later ratios verify the balance between S:M:P fatty acids in oil blends (1:1:1).

Therefore, the main goal of this study is to prepare new oil blends formulation with palm super olein to maximize high nutritional value via fatty acid balance and oxidative potency. The palm super olein, soybean, sunflower and their blends were analyzed for its vitamin E, phytosterols, phytostanols and fatty acid profiles using HPLC and GLC analysis. In addition, the oxidative stability, expressed as induction period, was carried out by Rancimat method for all the investigated oils samples.

MATERIALS AND METHODS

Materials: Palm Super Olein (PSO) was kindly supplied from Agwaa Company Suez, Egypt. Soybean oil (SBO) and sunflower oil (SFO) were purchased from the commercial market in Poland.

All solvents of HPLC grade, 1 M methanolic KOH, sterol standards and anhydrous pyridine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of tocopherols were obtained from Calbiochem-Novobiochem (San Diego, CA, USA), FAME standards and Sylon BTZ was purchased from Supelco (Bellefonte, PA, USA).

Oil blends design: To achieve the balance between saturated, monounsaturated and polyunsaturated fatty acids in oil blends, the ratios calculated as follows: 50:50, 55:45, 60:40, 60:40 and 75:25 of oil blends depending on the fatty acid composition of investigated pure oil samples. Palm super olein was added to soybean oil or sunflower oil. The mixtures of oils were placed in duplicates in 250 mL beakers for each blend and were mixed by using a mechanical stirrer at 180 rpm for 15 min. The oil blends were mentioned as follows:

| Oil blends | Ratio of PSO:SBO or SFO w/w | | | | |
|------------|-----------------------------|-------|-------|-------|-------|
| PSO:SBO | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 |
| PSO:SFO | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 |

PSO: Palm super olein, SBO: Soybean oil, SFO: Sunflower oil

Methods

Vitamin E: Vitamin E (tocopherol and tocotrienol) analysis was carried out by HPLC according to Balz *et al.*¹⁸ and

Hassanien *et al.*¹⁹, a solution of 250 mg of oil in 25 mL of n-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with aL-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm) and a D-2500 integration system. The samples in the amount of 20 mL were injected with a Merck 655-A40 auto sampler onto a Diol phase HPLC column 25 cm_4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 mL min⁻¹. The mobile phase used was n-heptane/tert-butyl methyl ether (99:1, v/v).

Phytosterols and phytostanols: Phytosterol and phytostanols content as well as composition were determined by GC following the procedure described by AOCS Official Method Ch6-91^{19,20}. Briefly, lipids (50 mg) were saponified with 1 M methanolic KOH for 18 h at RT, then water was added and the unsaponifiables extracted three times with hexane/methyl tert-butyl ether (1:1 v/v). The solvent was evaporated under a stream of nitrogen. Dry residues were dissolved in 0.2 mL pyridine and silylated with 0.8 mL of sylon BTZ. Derivatives of the sterols were separated on a HP 6890 series II Plus (Hewlett Packard, Palo Alto, USA) equipped with DB-35MS capillary column (25 m×0.20 and 0.33 mm; J and W Scientific, Folsom, CA). Sample of 1.0 mL was injected in split less mode. The column temperature was held at 100°C for 5 min and then programmed to 250°C at 25°C min⁻¹, held for 1 min, then further programmed to 290°C at 3°C min⁻¹ and held for 20 min. The detector temperature was set at 300°C. Hydrogen was used as the carrier gas at a flow rate of 1.5 mL min⁻¹. An internal standard, 5α-cholestane, was used for sterols quantification. Phytosterols and cholesterol were identified by comparison of retention data with standards and GC/MS (7890A/5975C VL MSD with Triple-Axis Detector, Agilent Technologies Inc., Santa Clara, CA, USA) using the same chromatographic conditions. Samples from autonomous series were analyzed in triplicate.

Fatty acids: Methyl esters of fatty acids (FAME) were prepared according to AOCS Official Method Ce 1 k-07^{19,21}. Diluted FAME were separated on a HP 5890 series II (Hewlett Packard, Palo Alto, USA) equipped with an innowax capillary column (30 m_0.20 mm_0.20 mm) and FID (FID). Hydrogen was used as the carrier gas at flow rate of 1.5 mL min⁻¹. The column temperature was isotherm 210°C. Detector and injector temperatures were set at 240°C. Fatty acids were identified by comparison of the retention times with authentic standards and the results were reported as weight percentages after integration and calculation using Chem. Station (Agilent technologies).

Oxidizability Cox value: The Cox value of the oils was calculated by applying the formula proposed by Fatemi and Hammond²²:

$$\text{Cox value} = \{[\text{C18:1}(\%)] + 10.3 \times [\text{C18:2}(\%)] + 21.6 \times [\text{C18:3}(\%)]\} / 100$$

Oxidative stability index: For determination of oxidative stability of oils AOCS²³ Official Method Cd 12b-92 was used. The Protective Factor (PF) of the investigated oils to indicate the susceptibility to oxidation of oils and is expressed as percentage extension of the induction period²⁴, according to the following equation:

$$\text{PF} = \frac{\text{IP sample} - \text{IP control}}{\text{IP control}} \times 100$$

Statistical analysis: The obtained data were analyzed with the statistical analysis software CoStat V6.4. Means were compared using Least Significant Difference (LSD) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Vitamin E: The palm super olein oil (PSO) can be considered as a good source of natural antioxidant (tocopherols, tocotrienols) compared to other vegetable oil. Therefore, it can be mixed with other oils to raise and improve stability.

Table 1 and 2 show the total and concentration of vitamin E in cooking oil samples and their mixtures. Total tocopherols content was the highest in SFO (768.6 ppm) followed by SBO (576.3 ppm). The PSO contained the least amount of total tocopherols (108.8 ppm) and highest amounts of tocotrienols 313.8 ppm. No tocotrienols were detected in SBO and SFO while all tocopherols and tocotrienols profiles were observed in PSO. The major vitamin E present in PSO was α-tocopherols, α, γ and δ-tocotrienols and their contribution was 25.1, 32.0, 26.5 and 11.3% respectively.

From the results recorded in Table 1 and 2, it was noticed that mixed oil samples contains higher level of total tocopherols and total tocotrienol. Concerning tocotrienols profiles in mixtures, it was found that when add 50, 55, 60, 65 and 75% of PSO to 50, 45, 40, 35 and 25 of SBO α-tocotrienol appears at a level of 23.2, 26.8, 28.6, 28.8 and 28.5% respectively. In addition, γ-tocotrienols found at concentration of 9.9, 10.6, 13.4, 15.3 and 18.3% respectively. However, δ-tocotrienol appears in mixture samples at concentration of 4.3, 4.8, 5.8, 5.8 and 6.0% respectively. Also, β-tocotrienol present in reasonable content in mixture PSO:SBO. The same observations occur in case of increasing addition of PSO to SFO, it was noticed gradual increase in the levels of α, γ and δ-tocotrienols which are not found in single SFO. It can be

Table 1: Vitamin E profiles for palm super olein, soybean oil and their blends

| Vitamin E | Ratio of palm super olein:soybean oil (w/w%) | | | | | | |
|--------------------------|--|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | 100% SBO | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 | 100% PSO |
| Tocopherol (ppm) | | | | | | | |
| α-tocopherol | 69.5±0.16 ^{g*} | 77.0±0.26 ^f | 79.4±0.17 ^e | 83.6±0.18 ^d | 86.8±0.22 ^c | 91.2±0.22 ^b | 106.1±0.12 ^a |
| β-tocopherol | 5.9±0.21 ^a | 2.6±0.08 ^b | 2.6±0.07 ^b | 2.0±0.10 ^c | 1.4±0.06 ^d | 1.2±0.05 ^d | 0.0 ^e |
| γ-tocopherol | 314.5±0.14 ^a | 128.0±0.31 ^b | 103.7±0.36 ^c | 84.8±0.24 ^d | 80.1±0.20 ^e | 72.6±0.15 ^f | 2.7±0.03 ^g |
| δ-tocopherol | 186.4±0.24 ^a | 44.2±0.32 ^b | 43.2±0.31 ^c | 23.5±0.09 ^d | 19.1±0.07 ^e | 16.4±0.06 ^f | 0.0 ^g |
| Total tocopherol | 576.3 ^a | 251.8 ^b | 228.9 ^c | 193.9 ^d | 187.4 ^e | 181.4 ^f | 108.8 ^g |
| Tocotrienol (ppm) | | | | | | | |
| α-tocotrienol | 0.0 ^g | 97.1±0.24 ^f | 110.9±0.32 ^e | 112.4±0.12 ^d | 114.8±0.33 ^c | 117.9±0.25 ^b | 135.6±0.14 ^a |
| β-tocotrienol | 0.0 ^g | 9.2±0.08 ^f | 9.5±0.25 ^e | 10.7±0.14 ^d | 11.4±0.11 ^c | 12.9±0.10 ^b | 18.2±0.24 ^a |
| γ-tocotrienol | 0.0 ^g | 41.4±0.14 ^f | 43.9±0.31 ^e | 52.4±0.25 ^d | 60.6±0.21 ^c | 75.8±0.15 ^b | 112.3±0.31 ^a |
| δ-tocotrienol | 0.0 ^f | 18.2±0.14 ^e | 19.8±0.25 ^d | 22.9±0.25 ^c | 23.1±0.32 ^c | 25.1±0.14 ^b | 47.7±0.11 ^a |
| Total tocotrienol | 0.0 ^g | 165.9 ^f | 184.1 ^e | 198.4 ^d | 209.9 ^c | 232.5 ^b | 313.8 ^a |
| Total vitamin E (ppm) | 576.3 ^a | 417.5 ^c | 413.0 ^d | 392.3 ^f | 397.3 ^e | 413.9 ^d | 422.6 ^b |

PSO: Palm super olein, SBO: Soybean oil, *Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test

Table 2: Vitamin E profiles for palm super olein, sunflower oil and their blends

| Vitamin E | Ratio of palm super olein:sunflower oil (w/w%) | | | | | | |
|--------------------------|--|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | 100% SFO | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 | 100% PSO |
| Tocopherol (ppm) | | | | | | | |
| α-tocopherol | 744.5±0.13 ^{g*} | 336.0±0.39 ^b | 307.3±0.25 ^c | 289.8±0.24 ^d | 257.1±0.22 ^e | 241.4±0.14 ^f | 106.1±0.12 ^g |
| β-tocopherol | 13.0±0.15 ^a | 6.6±0.08 ^b | 5.7±0.02 ^c | 4.9±0.31 ^d | 4.5±0.11 ^e | 3.6±0.14 ^f | 0.0 ^g |
| γ-tocopherol | 11.2±0.08 ^a | 6.3±0.36 ^b | 5.6±0.25 ^c | 4.7±0.16 ^d | 4.4±0.16 ^e | 4.1±0.15 ^f | 2.7±0.03 ^g |
| δ-tocopherol | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Total tocopherol | 768.6 ^a | 348.9 ^b | 318.6 ^c | 299.4 ^d | 266.0 ^e | 249.1 ^f | 108.8 ^g |
| Tocotrienol (ppm) | | | | | | | |
| α-tocotrienol | 0.0 ^g | 70.9±0.19 ^f | 76.2±0.29 ^e | 79.3±0.31 ^d | 84.4±0.12 ^c | 99.3±0.14 ^b | 135.6±0.14 ^a |
| β-tocotrienol | 0.0 ^f | 9.1±0.16 ^e | 10.3±0.11 ^d | 10.8±0.41 ^c | 10.5±0.14 ^{cd} | 12.6±0.21 ^b | 18.2±0.24 ^a |
| γ-tocotrienol | 0.0 ^g | 43.2±0.25 ^f | 49.5±0.25 ^e | 52.5±0.15 ^d | 60.5±0.21 ^c | 65.1±0.34 ^b | 112.3±0.31 ^a |
| δ-tocotrienol | 0.0 ^g | 15.6±0.21 ^e | 14.9±0.24 ^f | 16.3±0.11 ^d | 19.2±0.11 ^c | 20.7±0.22 ^b | 47.7±0.11 ^a |
| Total tocotrienol | 0.0 ^g | 138.8 ^f | 150.9 ^e | 158.9 ^d | 174.6 ^c | 197.7 ^b | 313.8 ^a |
| Total vitamin E (ppm) | 768.6 ^a | 487.7 ^b | 469.5 ^c | 458.3 ^d | 440.6 ^f | 446.8 ^e | 422.6 ^g |

PSO: Palm super olein, SFO: Sunflower oil, *Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test

concluded that the ratio of mixture 75 PSO:25 SBO or SFO is preferable than that the other mixtures since gave higher content total tocopherols and tocotrienols. It is noteworthy that tocotrienols are strong antioxidant and health benefits²⁵. Now, tocotrienols have received more attention than tocopherols, because they show a different biological activities²⁶. They also demonstrate great potential as an anti-osteoporotic agent²⁷. The PSO is especially rich in tocotrienols, what could be a purpose to enrich of cooking oils (SBO and SFO). To improve the vitamin E of cooking oils (SBO and SFO), it can be mixed with PSO as a source of α, γ and δ-tocotrienol beside α-tocopherols. Cooking vegetable oils which contained γ and δ-tocotrienol beside α, γ and δ-tocopherols improve bioavailability of these oils. The two isomers (γ and δ-tocotrienol) were reported to exert the most

potent anti-cancer effects in several studies^{28,29}. Blending of PSO with SBO or SFO positively influenced on the vitamin E content especially tocotrienols and can be recommended as a safe and economic method of increasing the concentration of these compounds in cooking oils. Increased the level of tocotrienols in blended oils could be beneficial since tocotrienols have been shown to inhibit cholesterol synthesis, reduce plasma cholesterol levels as well as influencing other risk factors for cardiovascular disease and suppress tumor-cell proliferation^{28,29}.

Phytosterols and phytostanols: The contents of desmethylsterols, 4-monomethylsterols and 4, 4-dimethylsterols as well as phytostanols were identified in cooking oils samples (Table 3, 4). The total phytosterols

Table 3: Phytosterols and phytostanols compositions for palm super olein, soybean oil and their blends

| Phytosterols | Ratio of palm super olein:soybean oil (w/w%) | | | | | | |
|--|--|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|
| | 100% SBO | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 | 100% PSO |
| Desmethylsterols ($\mu\text{g g}^{-1}$) | | | | | | | |
| Campesterol | 181.1 \pm 11.8 ^{a*} | 130.9 \pm 5.1 ^b | 127.0 \pm 4.9 ^c | 122.1 \pm 3.8 ^d | 115.9 \pm 4.6 ^e | 103.3 \pm 1.4 ^f | 80.9 \pm 1.3 ^g |
| Campestanol | 11.9 \pm 4.2 ^a | 5.4 \pm 0.2 ^c | 5.1 \pm 0.2 ^d | 4.4 \pm 0.1 ^e | 9.0 \pm 0.3 ^b | 1.6 \pm 0.1 ^f | 0.0 ^g |
| Δ 5-stigmasterol | 146.7 \pm 8.5 ^a | 101.0 \pm 1.9 ^b | 94.3 \pm 1.2 ^c | 91.1 \pm 0.9 ^d | 84.0 \pm 1.0 ^e | 77.0 \pm 0.8 ^f | 50.6 \pm 0.4 ^g |
| β -sitosterol | 557.9 \pm 32.1 ^a | 384.5 \pm 23.0 ^b | 369.2 \pm 14.0 ^c | 346.9 \pm 12.0 ^d | 332.0 \pm 15.1 ^e | 296.7 \pm 11.7 ^f | 203.0 \pm 9.7 ^g |
| Sitostanol | 46.6 \pm 1.7 ^a | 22.0 \pm 0.7 ^b | 20.0 \pm 0.7 ^c | 19.2 \pm 0.8 ^d | 15.8 \pm 0.5 ^e | 11.9 \pm 0.4 ^f | 0.0 ^g |
| Δ 5-avenasterol | 0.0 ^g | 8.0 \pm 0.2 ^f | 8.5 \pm 0.2 ^e | 9.6 \pm 0.1 ^d | 10.0 \pm 0.2 ^c | 12.0 \pm 0.3 ^b | 15.7 \pm 0.5 ^a |
| 4, 4-dimethylsterols ($\mu\text{g g}^{-1}$) | | | | | | | |
| Cycloartenol | 82.8 \pm 7.3 ^a | 40.5 \pm 1.6 ^b | 35.4 \pm 1.3 ^c | 34.0 \pm 1.5 ^d | 30.5 \pm 1.1 ^e | 22.4 \pm 0.8 ^f | 0.0 ^g |
| 2, 4 methylene-cycloartenol | 12.8 \pm 0.5 ^a | 6.0 \pm 0.3 ^b | 6.0 \pm 0.3 ^b | 5.0 \pm 0.2 ^c | 3.9 \pm 0.2 ^d | 3.5 \pm 0.1 ^e | 0.0 ^g |
| Total phytosterols and phytostanols ($\mu\text{g g}^{-1}$) | | | | | | | |
| Total | 1039.8 ^a | 697.8 ^b | 665.3 ^c | 632.3 ^d | 601.1 ^e | 528.4 ^f | 350.2 ^g |

PSO: Palm super olein, SBO: Soybean oil, *Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test

Table 4: Phytosterols and phytostanols compositions of palm super olein, sunflower oil and their blends

| Phytosterols | Ratio of palm super olein:sunflower oil (w/w%) | | | | | | |
|--|--|-------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|
| | 100% SFO | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 | 100% PSO |
| Desmethylsterols ($\mu\text{g g}^{-1}$) | | | | | | | |
| Campesterol | 160.7 \pm 4.8 ^{a*} | 119.9 \pm 3.7 ^b | 117.5 \pm 4.1 ^c | 112.3 \pm 4.0 ^d | 110.3 \pm 3.3 ^e | 98.8 \pm 2.9 ^f | 80.9 \pm 1.8 ^g |
| Δ 5-stigmasterol | 119.1 \pm 3.8 ^a | 88.2 \pm 3.1 ^b | 83.4 \pm 2.9 ^c | 79.7 \pm 3.0 ^d | 74.8 \pm 2.8 ^e | 70.1 \pm 2.8 ^f | 50.6 \pm 1.4 ^g |
| β -sitosterol | 778.4 \pm 11.2 ^a | 493.7 \pm 19.6 ^b | 467.1 \pm 18.5 ^c | 433.7 \pm 15.1 ^d | 398.3 \pm 9.9 ^e | 347.8 \pm 10.4 ^f | 203.0 \pm 6.9 ^g |
| Sitostanol | 141.8 \pm 1.3 ^a | 67.2 \pm 2.0 ^b | 61.3 \pm 2.1 ^c | 56.4 \pm 1.8 ^d | 46.6 \pm 1.0 ^e | 32.8 \pm 0.4 ^f | 0.0 ^g |
| Δ 5-avenasterol | 5.8 \pm 0.6 ^g | 9.9 \pm 0.2 ^f | 11.0 \pm 0.2 ^e | 11.8 \pm 0.2 ^d | 12.1 \pm 0.3 ^c | 13.6 \pm 0.2 ^b | 15.7 \pm 0.4 ^a |
| Δ 7-stigmasterol | 137.8 \pm 3.6 ^a | 66.8 \pm 1.6 ^b | 59.4 \pm 1.4 ^c | 52.8 \pm 1.3 ^d | 45.9 \pm 1.1 ^e | 31.2 \pm 1.3 ^f | 0.0 ^g |
| Δ 7-avenasterol | 36.1 \pm 1.8 ^a | 16.7 \pm 0.4 ^b | 15.2 \pm 0.3 ^c | 13.4 \pm 0.3 ^d | 11.6 \pm 0.2 ^e | 8.0 \pm 0.2 ^f | 0.0 ^g |
| 4, 4-dimethylsterols ($\mu\text{g g}^{-1}$) | | | | | | | |
| Cycloartenol | 93.3 \pm 5.3 ^a | 45.3 \pm 1.1 ^b | 43.1 \pm 0.9 ^c | 36.0 \pm 0.6 ^d | 32.2 \pm 0.7 ^e | 21.8 \pm 0.3 ^f | 0.0 ^g |
| 2, 4 methylene-cycloartenol | 26.9 \pm 2.1 ^a | 12.5 \pm 0.3 ^b | 11.2 \pm 0.3 ^c | 9.6 \pm 0.2 ^d | 9.1 \pm 0.1 ^e | 6.5 \pm 0.1 ^f | 0.0 ^g |
| 4-monomethylsterols ($\mu\text{g g}^{-1}$) | | | | | | | |
| Citrostadienol | 65.2 \pm 2.8 ^a | 31.3 \pm 1.1 ^b | 27.2 \pm 1.1 ^c | 25.8 \pm 1.0 ^d | 21.4 \pm 0.7 ^e | 15.3 \pm 0.5 ^f | 0.0 ^g |
| Total phytosterols and phytostanols ($\mu\text{g g}^{-1}$) | | | | | | | |
| Total | 1565.1 ^a | 951.5 ^b | 896.4 ^c | 831.5 ^d | 762.3 ^e | 645.9 ^f | 350.2 ^g |

PSO: Palm super olein, SFO: Sunflower oil, *Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test

and phytostanols content in SFO were at the highest level (1565.1 $\mu\text{g g}^{-1}$). The SBO was the second-richest source of total phytosterols and phytostanols content (1039.8 $\mu\text{g g}^{-1}$), followed by PSO (350.2 $\mu\text{g g}^{-1}$). Incorporating of 50% PSO with 50% of each SBO or SFO gave surpasses total phytosterols and phytostanols than the other cooking oil blends. The total phytosterols and phytostanols in all oil blends were higher than that of individual PSO and less than single SBO and SFO. From the results recorded in Table 3 and 4, it was noticed that β -sitosterol, campesterol and Δ 5-stigmasterol were the major phytosterols in all investigated oil samples. When PSO was incorporated with each SBO or SFO in different ratios, it was noticed that there is an increase in Δ 5-stigmasterol and campesterol than that in single PSO. Concerning β -sitosterol, it was found that the addition of 50% from PSO to SBO or SFO (50%), causes an increase in the level of this sterol than that the other cooking oil blends. Moreover, the levels of β -sitosterol in all cooking oil blends are higher than that in individual PSO.

When PSO incorporated with SFO at different ratios, it was noticed that the appearance of Δ 7-stigma and Δ 7-avenasterol in all SFO:PSO mixtures (which are not found in single PSO). Concerning Δ 5-avenasterol, increase the addition of PSO to SFO cause a gradual increases of this sterol. As a result of mixing between cooking oils, 4, 4-dimethylsterols (cycloartenol and 24 methylene-cycloartenol) and 4-monomethylsterols (citrostadienol) started to appear at reasonable amounts which are not detected in individual PSO. It is known that β -sitosterol control the levels, reduce the activity of cancer cell, promote prostate gland health and enhances immunity in the human body³⁰. Moreover, β -sitosterol, campesterol and stigmasterol have antioxidant effects. The Δ 5-avenasterol has an essential anti-polymerization effect, which could conserve oils from oxidation during cooking process³¹. Recently, increased interest in consumption of phytosterols is mainly responsible for many health benefits products³². Enrichment of human food in natural phytosterols is convenient for the decreasing

Table 5: Fatty acid profiles for palm super olein, soybean oil and their blends

| Fatty acids | Ratio of palm super olein:soybean oil (w/w%) | | | | | | |
|--|--|------------------------|------------------------|------------------------|-----------------------|-----------------------|------------------------|
| | 100% SBO | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 | 100% PSO |
| Saturated Fatty Acid (SFA) | | | | | | | |
| C12:0 | 0.1±0.0 ^{d*} | 0.2±0.03 ^c | 0.2±0.01 ^c | 0.2±0.00 ^c | 0.3±0.11 ^b | 0.3±0.02 ^b | 0.4±0.02 ^a |
| C14:0 | 0.1±0.0 ^f | 0.4±0.01 ^e | 0.4±0.01 ^e | 0.5±0.02 ^d | 0.8±0.02 ^c | 0.9±0.03 ^b | 1.0±0.03 ^a |
| C16:0 | 10.2±0.31 ^g | 22.9±1.03 ^f | 24.5±1.1 ^e | 25.0±1.0 ^d | 26.6±1.0 ^c | 29.2±1.2 ^b | 34.9±1.3 ^a |
| C18:0 | 3.4±0.14 ^a | 3.1±0.13 ^b | 3.1±0.1 ^b | 2.8±0.12 ^e | 2.8±0.11 ^e | 3.0±0.13 ^c | 2.9±0.12 ^d |
| ΣSFA | 13.8 ^g | 26.6 ^f | 28.2 ^e | 28.5 ^d | 30.5 ^c | 33.4 ^b | 39.2 ^a |
| Monounsaturated fatty acid (MUFA) | | | | | | | |
| C16:1 | 0.4±0.01 ^a | 0.2±0.0 ^b | 0.0 ^c | 0.0 ^c | 0.0 ^c | 0.0 ^c | 0.0 ^c |
| C18:1 | 24.2±1.0 ^g | 35.0±1.35 ^f | 36.3±1.2 ^e | 37.5±1.3 ^d | 38.8±1.1 ^c | 40.5±1.3 ^b | 46.1±1.5 ^a |
| ΣMUFA | 24.6 ^g | 35.2 ^f | 36.3 ^e | 37.5 ^d | 38.8 ^c | 40.5 ^b | 46.1 ^a |
| Polyunsaturated fatty acid (PUFA) | | | | | | | |
| C18:2 | 56.4±2.15 ^a | 35.8±1.26 ^b | 33.3±1.33 ^c | 31.8±1.54 ^d | 29.0±1.2 ^e | 24.8±1.3 ^f | 14.6±0.66 ^g |
| C18:3 | 5.2±0.13 ^a | 2.4±0.1 ^b | 2.2±0.09 ^c | 2.2±0.08 ^c | 1.7±0.08 ^d | 1.3±0.06 ^e | 0.1±0.0 ^b |
| ΣPUFA | 61.6 ^a | 38.2 ^b | 35.5 ^c | 34.0 ^d | 30.7 ^e | 26.1 ^f | 14.7 ^g |
| Relations | | | | | | | |
| PUFA/SFA | 4.5 ^a | 1.4 ^b | 1.3 ^c | 1.2 ^d | 1.0 ^e | 0.8 ^f | 0.7 ^g |
| PUFA/MUFA | 2.5 ^a | 1.0 ^b | 0.97 ^c | 0.9 ^d | 0.79 ^e | 0.64 ^f | 0.31 ^g |
| Cox value | 7.2 ^a | 4.6 ^b | 4.3 ^c | 4.1 ^d | 3.7 ^e | 3.2 ^f | 2.0 ^g |
| Total vitamin E/PUFA | 9.3 ^f | 10.92 ^e | 11.63 ^d | 11.5 ^d | 12.9 ^c | 15.85 ^b | 28.74 ^a |

PSO: Palm super olein, SBO: Soybean oil, *Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test

Table 6: Fatty acid profiles for palm super olein, sunflower oil and their blends

| Fatty acids | Ratio of palm super olein:sunflower oil (w/w%) | | | | | | |
|--|--|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | 100% SFO | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 | 100% PSO |
| Saturated Fatty Acid (SFA) | | | | | | | |
| C12:0 | 0.2±0.01 ^{c*} | 0.2±0.03 ^c | 0.2±0.01 ^c | 0.2±0.00 ^c | 0.2±0.11 ^c | 0.3±0.02 ^b | 0.4±0.02 ^a |
| C14:0 | 0.2±0.01 ^e | 0.6±0.01 ^d | 0.6±0.01 ^d | 0.7±0.01 ^c | 0.7±0.05 ^c | 0.8±0.02 ^b | 1.0±0.00 ^a |
| C16:0 | 6.7±0.30 ^g | 21.4±0.10 ^f | 22.9±0.41 ^e | 24.6±0.65 ^d | 25.3±0.84 ^c | 28.8±1.01 ^b | 34.9±0.21 ^a |
| C18:0 | 2.8±0.07 ^b | 2.7±0.08 ^c | 2.4±0.16 ^f | 2.5±0.16 ^e | 2.6±0.02 ^d | 2.6±0.10 ^d | 2.9±0.02 ^a |
| ΣSFA | 9.9 ^g | 24.9 ^f | 26.1 ^e | 28.0 ^d | 29.3 ^c | 32.5 ^b | 39.2 ^a |
| Monounsaturated fatty acid (MUFA) | | | | | | | |
| C18:1 | 31.3±1.01 ^g | 38.2±1.05 ^f | 39.5±1.84 ^e | 40.4±1.98 ^d | 40.8±1.63 ^c | 43.0±1.68 ^b | 46.1±3.08 ^a |
| ΣMUFA | 31.3 ^g | 38.2 ^f | 39.5 ^e | 40.4 ^d | 40.8 ^c | 43.0 ^b | 46.1 ^a |
| Polyunsaturated fatty acid (PUFA) | | | | | | | |
| C18:2 | 58.6±2.61 ^a | 36.7±1.45 ^b | 34.3±1.12 ^c | 31.5±1.33 ^d | 29.8±1.28 ^e | 24.4±1.10 ^f | 14.6±0.14 ^g |
| C18:3 | 0.2±0.01 ^a | 0.2±0.01 ^a | 0.1±0.01 ^b | 0.1±0.01 ^b | 0.1±0.01 ^b | 0.1±0.0 ^b | 0.1±0.00 ^b |
| ΣPUFA | 58.8 ^a | 36.9 ^b | 34.4 ^c | 31.6 ^d | 29.9 ^e | 24.5 ^f | 14.7 ^g |
| Relations | | | | | | | |
| PUFA/SFA | 5.9 ^a | 1.5 ^b | 1.3 ^c | 1.1 ^d | 1.0 ^d | 0.7 ^e | 0.3 ^f |
| PUFA/MUFA | 1.87 ^a | 0.96 ^b | 0.87 ^c | 0.78 ^d | 0.63 ^e | 0.56 ^f | 0.31 ^g |
| Cox value | 6.4 ^a | 4.2 ^b | 3.9 ^c | 3.7 ^d | 3.5 ^e | 3.0 ^f | 2.0 ^g |
| Total vitamin E/PUFA | 13.12 ^f | 13.2 ^f | 13.64 ^e | 14.5 ^d | 14.7 ^c | 18.2 ^b | 28.74 ^a |

PSO: Palm super olein, SFO: Sunflower oil, *Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test

of plasma cholesterol level and coronary mortality³³. Blending between cooking oils positively influenced on the phytosterols content and can be recommended as safe and cheap way of these compounds in other edible fats and oils.

Fatty acid: The main fatty acids in the PSO were oleic and palmitic acids, 46.1 and 34.9% respectively. The SBO and SFO were characterized by a high percentage of linoleic acid

56.4 and 58.6% followed by oleic acid, 24.2 and 31.3% respectively. However, linolenic acid in only SBO amounted to 5.2%^{34,35} (Table 5, 6).

The addition of 50, 55, 60, 65 and 75% of PSO to SBO or SFO causes a gradual increase in the amount of C16:0 and C18:1. At the same time, a decrease in the content of C18:2. In addition, C18:3 appeared in reasonable amount when adding PSO to SBO. In the cooking oil samples investigation,

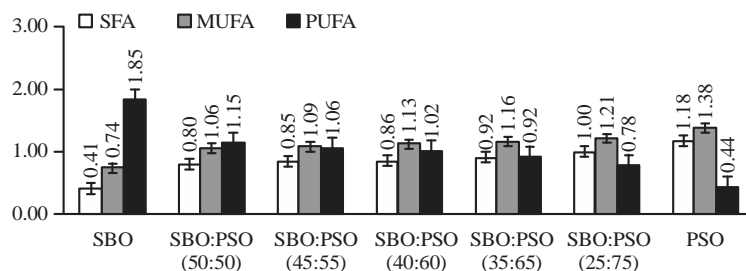


Fig. 1: Saturated fatty acid (SFA):monounsaturated fatty acid (MUFA):polyunsaturated fatty acid (PUFA) of PSO, SBO and their blends

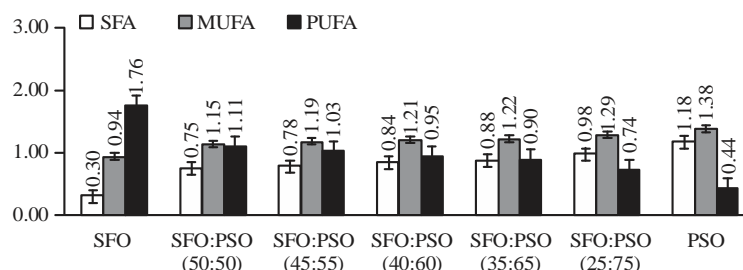


Fig. 2: Saturated fatty acid (SFA):monounsaturated fatty acid (MUFA):polyunsaturated fatty acid (PUFA) of PSO, SFO and their blends

PSO contained the highest amount of Saturated Fatty Acid (SFA) as compared to other two single oils. For PSO, SFA was 39.2%, monounsaturated fatty acid (MUFA) 46.1% and polyunsaturated fatty acid (PUFA) 14.7%, these results were agreed with Azrina *et al.*³⁶. By virtue of the values of PUFA/SFA, PUFA/MUSFA, Cox value and total vitamin E/PUFA³⁷, the stability of blends can be determined clearly from these values which confirm best results of keeping quality of the blends. From the results recorded in Table 5 and 6, it was found that, when adding higher amount of PSO to SBO or SFO, the oil blends have low Cox value, PUFA/SFA and PUFA/MUFA. The ratio of PUFA/SFA and Cox value are commonly proved as a measure of oxidative stability^{22,38}. Karupaiah and Sundram³⁹ found that decrease in PUFA/SFA ratios in human food were associated with increase post-prandial level of HDL-C in plasma. It is also noticed that the proportion of PUFA/MUFA ratios were decreased by increase the addition of PSO to other oils and hence, increased the oxidative stability of the cooking oil blends. The SFA:MUFA:PUFA in individual cooking oils (PSO, SBO and SFO) do not reach to ideal ratio according to WHO¹⁶. The WHO¹⁶ recognized the best ratio at approximately 1:1:1 for SFA:MUFA:PUFA, the importance of this balance for generating the best LDL/HDL ratio¹⁷. This balance is critical at any level of fat intake, therefore the WHO¹⁶ recommends slightly less SFA and PUFA than MUFA in the balance. The

addition of PSO for SBO or SFO especially 55, 60 and 65% nearly exhibit balance between SFA:MUFA:PUFA, this leads to improvement the nutritional benefits. Moreover, the addition of PSO (75%) to other cooking oils causes the slightly decrease the PUFA, which leads to improvement the oxidative stability (Fig. 1, 2). The oil blends namely, 55, 60, 65 and 75% of PSO:SBO or SFO are more effective not only on nutrition benefits but also on their oxidative stability.

Oxidative stability: The susceptibility of the studied cooking oils and their blends to oxidation was measured by the Rancimat test, the inflection point in the oxidation curve is defined as IP³⁸. The length of the IP is considered a relative measure of the stability of oils. The results of oxidative stability (IP) of cooking oils and their mixture were showed in Fig. 3. It was found that PSO had the most stable against oxidation and highest IP (24.1 h). The results also showed that the IP of the mixtures gradual increased as addition of PSO ratio increased to each SBO or SFO as shown in the Fig. 3. The results of oxidative stability revealed an overall increase in IP of the blended oils than that of individual SBO and SFO. Generally, blending PSO with either SBO and/or SFO at any ratio gave a markedly higher IP than the individual oils. The Protective Factor (PF) expresses the susceptibility to oxidation of oils as percentage extension of the IP. The highest PF% was found in

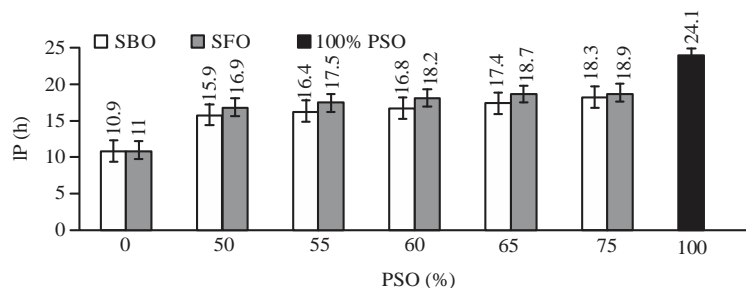


Fig. 3: Oxidative stability of individual cooking oils and their blends

Table 7: Protective factor of cooking oil blends

| Oil blends | Ratio of PSO:SBO or SFO w/w | | | | |
|-------------------------------|-----------------------------|-------------------|-------------------|-------------------|-------------------|
| | 50:50 | 55:45 | 60:40 | 65:35 | 75:25 |
| Protective Factor (PF) | | | | | |
| PSO:SBO | 45.9 ^{a*} | 50.5 ^d | 54.1 ^c | 59.6 ^b | 67.9 ^a |
| PSO:SFO | 53.6 ^e | 59.0 ^d | 65.5 ^c | 70.0 ^b | 71.8 ^a |

*Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test

case of adding 65 and 75% of PSO to other two oils (Table 7). If the value of PF is greater than one, raise the stabilizing effect of the sample. The greater value of PF lead to the higher in the antioxidant effectiveness of the added oil⁴⁰.

CONCLUSION

It was concluded that, the addition of PSO to SFO or SBO maximizing the oxidative stability of blends as well as the expected nutritional value. In addition, some cooking oil blends verified the balance between SFA:MUFA:PUFA as recommended by WHO. Moreover, the best combination of cooking oil blends was 55, 60, 65 and 75% PSO to other oils. The results of oxidative stability revealed an overall marked increase in IP of the blended oils than that of individual oils. In particular, the ratio of 65 and 75% of PSO blended with other two oils, gave the highest amount of total tocotrienol which is perceived as the most potent antioxidants. Concerning the phytosterols and phytostanols, 50:50% and 55:45% PSO with SFO oil blends show highest amount of 5-stigmasterol, β -sitosterol, 7-stigmasterol, 7-avenasterol. While, the highest level of 7-stigmasterol and β -sitosterol was found in the blends of PSO to SBO (50:50 and 55:45%). β -sitosterol, campesterol and stigmasterol exert antioxidant effects and enhance immunity in the human body. This will open new application domains particularly in the oil blending processes.

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