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Minor components and thermal stability of butter, wheat germ and corn oils in the russian market

Samh Sobhy El-Hadad[1,2,*] and Natalia Aleksandrovna Tikhomirova[1]

[1]Department of Technology and biotechnology of food products of animal origin, Moscow State University of Food Production (MGUPP), Moscow, **Russian Federation.**
[2]Dairy Science Department, National Research Centre, Dokki, Giza, **Egypt.**

***Correspondence:** samehsobhygalal@yahoo.com **Accepted:** 02 Aug.2018 **Published online:** 30 Sep2018

The content of minor components as well as fatty acid composition (saturated and unsaturated) is important indicator for judgement of nutritional value of reality fats and oils. For this purpose, the main objective of this work was to identify some of the beneficial and bioactive minor lipid components in butter oil (BO), wheat germ oil (WGO) and corn oil (CO). WGO is composed of nonpolar lipids (Minor components), glycolipids, phospholipids, tocopherols, sterols, free fatty acids, and volatile components.Phospholipids, tocopherols and sterols analysed by HPLC. Moreover, fatty acids composition by GC was identified. Oxidative stability and progression of oxidation was followed by measuring peroxide value (PV) and Rancimat test, Inverse relationships were noted between PV and OS at storage. The results were compared to three oils, namely butter, corn and wheat germ. It was found that WGO and CO contained high amounts of sterols. Total tocopherols were found in very high amounts of WGO (13.53 mg/g). On the other side, it was found that the levels of saturated fatty acids (short-chain and medium-chain fatty acids) were enriched in butter oil (BO) (5.21 and 18.15% respectively), while unsaturated fatty acids especially linoleic acid was present in higher quantity in WGO and CO 55.8 and 55.56 % respectively. Oxidative stability of oil samples was better in WGO, most likely as a consequence tocopherols level and minor lipid components (e.g., phospholipids and sterols) found higher in wheat germ oil.

Keywords: Wheat germ oil, butter oil, tocopherol, sterols, Phospholipids.

INTRODUCTION

The composition of milk fat or butterfat has been the subject of numerous studies, and some components of this fat have received specific regard. Therefore, having studied the fatty acids and unsaponifiable components of substances such as sterols and tocopherol (Patton et al., 1964). Milk fat is composed of 98% triacyglycerols (TGs) which are tri-ester of fatty acids and glycerol. More than 400 fatty acids and 200 TGs have been identified. Milk fat is characterized by short-chain (C4–C8, 8.3%), medium-chain (C10-C12, 6.6%) and long-chain (C14–C18, 81.9%) length chain fatty acids. Moreover, milk fat is a relatively high saturated fat about 65% saturated fatty acids (mainly C16:0, C18:0 and C14:0) and about 35% unsaturated fatty acids (mainly C18:1) (Grall & Hartel 1992 and Lopez et al., 2006).

Most vegetable oils and fats are generally found to contain some minor components as unsaponifiable matter. These minor components could be sterols, phospholipids, tocopherols, pigments, squalene, carotenoids and volatile components, etc. Cholesterol is present as the minor component in majority of the animal body fats as well as milk fats (Marikkar et al., 2016).

Phospholipids that represent approximately 1% of milk lipids consist of five fractions of

different structure and biological activity (Contarini and Povolo, 2013). Milk and dairy product contain: phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylinositol (PI), and phosphatidylserine (PS) (Walczak et al., 2016). Milk phospholipids play an important role in human body. Phospholipids had demonstrated a potentially favorable role in reducing the risk of cardiovascular and cancer disease (Russo et al., 2013).Tocopherols and phytosterols are important components in the oil fraction of vegetable oils and nuts. In the past few years their beneficial health effects have sparked great interest in quantifying them in different foods (Lopez-Ortiz et al., 2006). Vega et al., (2002) reported that the sterol fraction analysis showed that milk fat contains only cholesterol and margarines presented high levels of phytosterols.

The determination of sterols in all products is carried out for the following objectives: to measure the total cholesterol content to obtain nutritional information, detect the presence of vegetable fats, and measuring specific phytosterols (β-sitosterol and stigmasterol). HPLC method for the determination of cholesterol and eventually of other sterols in oils and dairy products is a fast and reliable method alternative to the GC chromatography (Contarini et al., 2002 and Borkovcová et al., 2009). Our study proposes to supply and confirmed data onto the levels of the individual minor components in the edible oils currently obtainable on the Russians market because enable an update of the Russian food composition database. In the regard, this is the first facts for minor components in the edible oils consumed in Russia. True data would demonstrate a right addition as they could be used to produce results of the health effects of minor components.

MATERIALS AND METHODS

Wheat germ oil, corn oil and fresh butter were purchased from a local market in Moscow, Russia. Fresh butter was obtained, melted at 60°C and centrifuged at 2000 g for 5 min to separate the protein and other materials. The top butter oil (BO) layer was decanted and stored at -20°C until use.

Chemicals

Standards used for vitamin E (ά-, β-, γ- and δ-tocopherol), sterols and phospholipids were purchased from Merck (Darmstadt, Germany). All solvents and reagents from various suppliers were of the highest purity needed for each application and used without further purification.

Preparation of fatty acid methyl esters (FAMEs)

Preparation of FAMEs was carried out according to the method recommended by AOAC (2005) was used for preparation of fatty acid methyl esters. About 0.2 g of the oil was mixed with 30 ml sulphuric acid/methanol mixture (1:24 v/v) in a 250 ml round bottom flask. The content of the flask were then heated under reflux for about three hrs. The methyl esters were twice extracted with petroleum ether (40-60°C) then it was washed several times with distilled water till the washings were neutral to phenolphthalein. The combined fatty acids methyl esters layers were dried over anhydrous sodium sulphate and filtered. The petroleum ether was then removed using a rotary evaporator and aliquots of the fatty acid methyl esters were analysed by gas chromatography.

Chromatographic conditions:

The methyl ester of the fatty acids standard compounds were analysed with a Perkin Elmer Auto System XL (GC) gas chromatography Equipped with flame ionization detector (FID), Fused silica capillary column ZB-Wax (60 m x 0.32 mm id). the oven temperature was programmed in two stages as follows: First the column temperature was increased at 40°C , held at 40°C for 5 min and then from 40 to 220°C at rate 3°C /min. Detector and injector temperature generally 250, 230°C; respectively. The carrier gas (helium) flow rate was 1 ml/min.

Unsaponifiable matter (Tocopherols and sterols analysis)

Sample preparation

Tocopherols (ά, β, γ and δ form) and sterols in oils were prepared according to the method of Lopez-Ortiz., et al., (2006). Approximately 0.2–0.3 g of the oil sample was weighed into a dark bottle. Ascorbic acid solution 0.1M (5 mL) and potassium hydroxide solution 2M (20mL) were added. The samples were saponified shaking the mixture at 125 rpm in an incubation system at 60◦C for 45 min. After cooling at room temperature the resulting mixture was then filtered and treated with 10 mL of saturated sodium chloride solution, 10 mL of a solution of BHT in *n-hexane* (5 mg/L) and 100 mL of hexane solution of tocopherols (100 ppm), added as internal standard. Samples were

then stirred for 1 min in the Vortex mixer. The hexane phase containing tocopherol and sterols was collected and dried by passing through anhydrous sodium sulfate. The aqueous layer was then re-extracted with a new aliquot of 5mL *n-hexane*. This new fraction was dried and then combined with the first hexane extract. The entire hexane solution was evaporated to dryness in a Rota vapor at 50° C. After wards a stream of nitrogen was passed over the residue until it was totally dry in order to avoid tocopherol oxidation. The resulting residue was dissolved in 1mL of methanol and filtered through a 0.45 mm nylon syringe membrane from Phenomenex (Torrance, CA, USA). Finally, the resultant solution was kept in a dark vial and stored at -20° C until analysis. Aliquots of this solution were injected into the HPLC system.

Chromatographic conditions:

Analysis was carried out at room temperature on the liquid chromatograph HPLC (Knauer, Germany) equipped with UV detector at 250 nm. Gemini-Nx 5u, C18, 250×4.6 mm column was used. Mobile phase was a mixture of hexane and isopropanol (99:1 v/v) at flow rate 1.5 ml/min. The concentrations of tocopherol and sterol fractions in the samples were obtained by comparing their peak areas with the peak area of standards in relation to concentration.

Phospholipids

Phospholipids fraction (Phosphatidylethanolamine, Phosphatidylinositol, Phosphatidylserine and Phosphatidylcholine) were carried out according to the analytical method described by Eisenmenger et al., (2008). The oil samples were dissolved in chloroform (15 mg oil/mL) and filtered through 0.2-µm Iso Disc filters (Supelco, Bellefonte, PA, USA) for further analysis. A normal phase silica column, µ Porasil 10 µm (3.9 mm i.d 9 300 mm) from Waters (Milford, MA, USA) was used for the analytical separation of the compounds. The mobile phase consisted of two mixtures: A: hexane: water: isopropyl alcohol (40:58:2) and B: hexane: water: isopropyl alcohol (40:50:10). The solvent gradient system was as follows: 100% A to 100% B in 7 min, then held for 6 min followed by returning to 100% A in 1 min and held for 11 min. Total run time was 25 min. The detector system consisted of a photodiode array detector (PDA) (Model 2996, Waters Milford, MA, USA) in series with an evaporative light scattering detector (ELSD) (Model 2000, All tech associates Inc., Deerfield, IL, USA). The ELSD set points were as follows: nitrogen flow rate 3.5 mL/min, impactor ON and drift tube temperature of 80° C. Identification and quantification of chromatographic peaks were based on external standard curves prepared for individual standards. Phospholipid standards were purchased from Sigma Inc. (St. Louis, MO, USA). Standards were dissolved in chloroform. All solvents utilized for HPLC mobile phase were HPLC grade and filtered using a GH Polypro (47 mm, 0.45 lm) hydrophilic polypropylene membrane filter (Pall Life Sciences, Ann Arbor, MI, USA) before use.

Oxidative Stability

Peroxide value:

WGO, BO and CO (100 g) was estimated by stored in 200 ml conical flask in oven at 60℃. PV was determined at regular intervals for 15 days according to Egan et al., (1981).

Induction Period:

The oxidative stability of the samples was estimated as the induction period (h) according to the method described by Tsaknis et al., (1999) using a Rancimat Metrohm instrument (Ud.CH-9100 Herisau, Switzerland, Model 679) at 110°C with air flow at 20 L/h^{-1}.

RESULTS AND DISCUSSION

Fatty Acid Compositions

Fatty acid composition of BO, CO and WGO is presented in Table 1.From the results, it could be noticed that, BO was distinctive by short chain fatty acids (SCFAs) medium chain fatty acids (MCFAs) and long chain fatty acids (LCFAs). In CO and WGO SCFAs and MCFAs were not found, while LCFAs were the most abundant especially, oleic and linoleic acids. Moreover, CO and WGO contained a higher portion of USFAs (78.1 and 88.38 % respectively) and lower portion of SFAs (11.57 and 22.21 %) than BO (30.76 and 68.75%, respectively).Similar observations were found by Calvo et al., (2007) in Egyptian processed cheese containing palm oil and by Abd El-Aziz et al., (2013) in blend of milk fat with PO. Butyric and myristic acids were the most abundant SCFAs and MCFAs in BO, respectively. Palmitic acid as a SFAs and oleic acid as a USFAs were the most abundant all three oils, But BO contain a higher palmitic acid percentage (31.11%) compared with CO and WGO (8.63 and 19.2 % respectively).

Table 1.Fatty acid compositions of fats and oils consumed in Russia.

Fatty acids	Samples of oil		
	BO	WGO	CO
	(%)		
SCFAs			
C4:0 (Butyric acid)	1.93	-	-
C6:0 (Caproic acid)	1.83	-	-
C8:0 (Caprlyic acid)	1.45	-	-
Total	5.21		
MCFAs			
C10:0 (Capric acid)	2.61	-	-
C12:0 (Capric acid)	3.63	-	-
C13:0 (Tridecylic acid)	0.1	-	-
C14:0 (Myristic acid)	10.96	-	-
C14:1 (Myristoleic acid)	0.85	-	-
Total	18.15		
LCFAs			
C15:0 (Pentadecylic acid)	1.13	-	-
C15:1 (Pentadecenoic acid)	0.24	-	-
C16:0 (Palmitic acid)	31.11	19.2	8.63
C16:1 (Palmitioleic acid)	1.51	1.08	0.1
C17:0 (Palmitioleic acid)	0.67	0.44	0.06
C17:1 (Heptadecenoic acid)	0.31	0.29	0.04
C18:0 (Stearic acid)	10.42	1.20	2.74
C18:1 (Oleic acid)	26.64	14.0	31.36
C18:2 (Linoleic acid)	2.35	55.8	55.56
C18:3 (Linolenic acid)	0.86	8.3	0.5
C20:0 (Arachidic acid)	0.5	-	0.35
C20:1 (Arachidic acid)	0.27	-	0.22
C22:0 (Behenic acid)	0.14	-	0.39
Total	76.15 100	100	100
SFAs	68.75	22.21	11.57
USFAs	30.76	78.1	88.38

BO: Butter oil, CO: Corn oil: WGO: wheat germ oil

SCFAs: Short chain fatty acids, MCFAs: Medium chain fatty acids, LCFAs: Long chain fatty acids, SFAs: Saturated fatty acids, USFAs: Unsaturated fatty acid.

On the other hand, Linolenic acid was a significant higher in WGO (8.3 %) as compared BO and CO (0.86 and 0.5 % respectively). Similar observations were found by Eisenmenger and Dunford (2008), Ramadan and Wahdan (2012) and Callaghan et al., (2016). In addition, the lower limit of linoleic acid in BO compared with WGO was useful in detecting WGO in BO. Lopez et al. (2006) and Heussen et al., (2007) reported that butter fat did not contain more than 2.0% linoleic acid.

Tocopherol fractions

The tocopherols are one of the main groups of the portion of the minor compounds in vegetable oils. Oils originated from the same species can have variable content and composition of these minor compounds, due to climatic and agronomic conditions; oil extraction system and refining procedure (Cert et al., 2000). Tocopherols content of butter oil, corn oil and wheat germ oil which determined by HPLC as another component of the unsaponifiable fraction are presented in Table (2). It is evident from the results that, BO had the lowest tocopherols fractions as compared to CO and WGO. In particular, α-tocopherols, *β*-tocopherol, γ-tocopherol and δ-tocopherol of BO were 0.031, 0.0, 0.004 and 0.001 mg/g while were 0.411, 0.019, 0,445 and 0.029 mg/g fat for CO and 12.4, 0.61, 0.08 and 0.27 mg/g fat for WGO respectively. These results are accordance with Alonso et al., (1997), Minar and Abedel-Razek (2009) and Ghafoor et al., (2017).Also, it could be seen that the total tocopherol (α-tocopherols, *β*-tocopherol, γ-tocopherol and δ-tocopherol) in BO was accounted 0.036 mg/g fat, while the total tocopherol in CO and WGO were 0.904 and13.26 mg/g oil respectively. Chas et al., *(*1994) reported that γ-tocopherol was the major tocopherol detected in the vegetable oils and ranged from 24.1-93.3 mg/100 g which was markedly high. In addition, WGO and CO had a large amount of α-tocopherol andγ-tocopherol which was traces in BO (0.031 and 0.004 mg/g respectively). These results are agreement with the data obtained by Lopez -Ortı´z et al., (2006) and Minar & Abedel-Razek (2009).

Sterol fractions

Sterols are nutritionally important lipids that need to be routinely determined in foods. Cholesterol, sitosterol and stigmasterol are polycyclic steroid compounds with similar chemical structure (Borkovcová et al., 2009). Table (3) shows the sterol fractions (cholesterol as the major sterols in milk fat and β-sitosterol as the major sterols in most vegetable oils) of BO, CO and WGO. The total content of cholesterol and all sterols in BO, CO and WGO were 2.125, 4.56 and 21.7 mg/g of total sterols. These results are agreement with other researchers as well Alonso et al., (1997); Contarini et al., (2002), Sambanthamurthi et al*.*, (2003) and Minar & Abedel-Razek (2009). However, β-sitosterol as the major phytosterols in CO and WGO Sambanthamurthi et al., 2003 and Ghafoor et al., (2017), was 20.98 and 3.38 (m/g) for WGO and CO respectively, while was 0.015 mg/g for BO. These results are means that, BO had highest cholesterol content and lowest β-sitosterol content, while WGO and CO had highest β-sitosterol content and lowest cholesterol content. These mean that, the addition of vegetable oils (wheat germ and corn oils) caused gradual decrease in cholesterol percentage of BO.

Phospholipids

Phospholipids fraction of BO, CO and WGO which determined by HPLC are presented in Table (4). It is obvious that, WGO had higher phospholipids fraction as compared to CO and BO. Specially, Phosphatidylethanolamine, Phosphatidylinositol, Phosphatidylserine and Phosphatidylcholine of WGO were3.01, 2.96, 0.88 and 9.5; while in CO were 0.2, 1.3, 0.0 and 0.0 mg/g fat respectively. On the other side, in BO were traces. The obtained herein results are generally in harmony with those reported by (Avalli and Contarini 2005; Walczak et al., 2016; Ferreiro et al., 2017). As well as, it could be seen that the total phospholipids: Phosphatidylethanolamine, Phosphatidylinositol, Phosphatidylserine and Phosphatidylcholine in BO was accounted 0.1 mg/g fat, while the total phospholipids in WGO and CO were 16.35 and 1.5 mg/g oil respectively. In comparison BO contained Phosphatidylethanolamine as abundant components (30 %) while it exhibited lower amount of Phosphatidylinositol (8.2 mg/g). On the contrary, WGO contained high amount of Phosphatidylcholine (9.5mg/g), also which was formed high amount in BO (23.4 %) and low amount of Phosphatidylserine (0.88 %). On the other side, CO contained low amount of Phosphatidylethanolamine (0.2 mg/g) and Phosphatidylinositol (1.3 mg/g). These results are in agreement with Eisenmenger and Dunford (2008).

It showed that WGO is a rich source of minor components such as α-tocopherol which is a high

bioactive minor component. Oils with high tocopherol content can be used in applications where high level of antioxidant protection is needed. On the other hand among the tocopherols present in foods alpha-tocopherol shows the highest V.E activity, therefore making it the most important tocopherol for human health and biological activity (Abidi., 2003).

Oil stability index

Unsaturated fatty acids are susceptible to oxidation and this leads not only to loss of bioactivity but also the production of undesirable off-flavors and off-odors. The direct incorporation of oils rich in USFAs often results in unacceptable ingredients. Therefore, it is necessary to addition of antioxidants to protect the unsaturated fatty acids (Jacobsen, 2008). Table 5 represents oxidative stability, as measured by the time before a dramatic increase in rate of lipid oxidation (induction period), of CO, BO and WGO which were 5.33, 7.47 and 12.25 (h) respectively. The oxidative stability of BO, which has low content in USFAs, was higher than CO, but lower than from WGO. The CO contains high amount of USFAs; therefore, it exhibits low resistance to oxidative reactions. Scott et al., (2003) mentioned that fat with high degree of USFAs, it has a greater potential form oxidation. But WGO is stable due to high amount of tocopherols (Table 2). WGO is rich in tocopherols particularly vitamin E. Butter oil is in general in poorer relation to the phytochemicals composition when compared to WGO. Yoon et al., (1987) reported that phospholipids acted as antioxidants only in the presence of Fe^{2+} by chelating iron.

Storage oxidative stability results

The control of oil oxidation in food is important to prevent foods from deterioration and protect human health, Therefore, determination of the peroxide value is one of the most important quality control measurements for food systems specially edible oils, because it is an indicator of the primary oxidation state of the oils. This indicator measures the concentration of hydroperoxides which are unstable and can easily break down to form low molecular weight oxygenated constituents such as alcohols, aldehydes, free fatty acids, and ketones, ultimately leading to rancidity (Akinoso et al, 2010; Pizarro et al., 2013).Peroxide value (PV) of BO, CO and WGO as other indicators of oxidation rate during oxidation process at 60 °C is presented in Fig. 1. At day 1, PV of BO was lower than these of CO and WGO. . During oxidation process, the oxidation rate of BO (PV) was lower than those of CO and WGO until day 13, while that of BO was higher than those of CO and WGO from day 16th onwards. The higher oxidation rate of BO than CO and WGO may be able to be attributed to the high content of free fatty acids for BO than CO and WGO. Free fatty acids act as prooxidants in edible oil (Mistry and Min 1987). Yoon and Min (1987) reported that phospholipids (Table 4) acted as antioxidants only in the presence of Fe2+ by chelating iron. In purified soybean oil, which did not contain any metals, phospholipids worked as prooxidants. Ramadan and Wahdan (2012) found that oxidative stability of oil blends (*coriander or black cumin* with corn oil) were better than corn oil, most likely as a consequence of changes in fatty acids and tocopherols' profile, and minor bioactive lipids (e.g., sterols and phenolics). Yoon et al., (1987) reported that phospholipids acted as antioxidants only in the presence of Fe^{2+} by chelating iron. From the results could be concluded that BO was stable in oxidation (Low induction period), while WGO was more stable in oxidation than BO. In general, our results revealed that an increase in the concentration of minor components of wheat germ oil and corn oil, significantly extend induction period in WGO and CO than BO. This was contrary to the results of Rancimat and PV, possibly due to different mechanisms of oxidation reactions at the temperatures of 110° C and 60° C. These results were in agreement with Taghvaei et al., (2014).

Table 2. Levels of tocopherol (mg/g) in fats and oils consumed in Russia.

Samples of oil	Tocopherols composition (mg/g)				
	***α*-Tocopherol**	***β*-Tocopherol**	***γ*-Tocopherol**	***δ*-Tocopherol**	**Total Tocopherol**
BO	0.031	-	0.004	0.0012	0.036
WGO	12.4	0.61	0.08	0.27	13.26
CO	0.411	0.019	0.445	0.029	0.904

BO: Butter oil, CO: Corn oil: WGO: wheat germ oil

Table 3.Sterol compositions (mg/g) of fats and oils consumed in Russia.

Samples of oil	Sterols composition (mg/g)				
	Cholesterol	β-sitosterol	Stigmasterol	Campsterol	Total Sterols
BO	2.11	0.015	-	-	2.125
WGO	-	20.98	0.57	0.15	21.7
CO	-	3.38	0.40	0.78	4.56

BO: Butter oil, CO: Corn oil: WGO: wheat germ oil

Table 4. Phospholipids Fraction of fats and oils consumed in Russia.

Phospholipids fractions	Samples of oil		
	BO (%)	WGO (mg/g)	CO (mg/g)
Phosphatidylethanolamine	30.0	3.01	0.2
Phosphatidylinositol	12.9	2.96	1.3
Phosphatidylserine	16.3	0.88	-
Phosphatidylcholine	23.4	9.5	-
Total Phospholipids (mg/g)	0.1	16.35	1.5

BO: Butter oil, CO: Corn oil: WGO: wheat germ oil

Table 5.Induction period of BO, CO and WGO by Rancimat method at 110°C.

Samples of oil	Induction period (h)
BO	7.47
CO	5.33
WGO	12.25

BO: Butter oil, CO: Corn oil: WGO: wheat germ oil

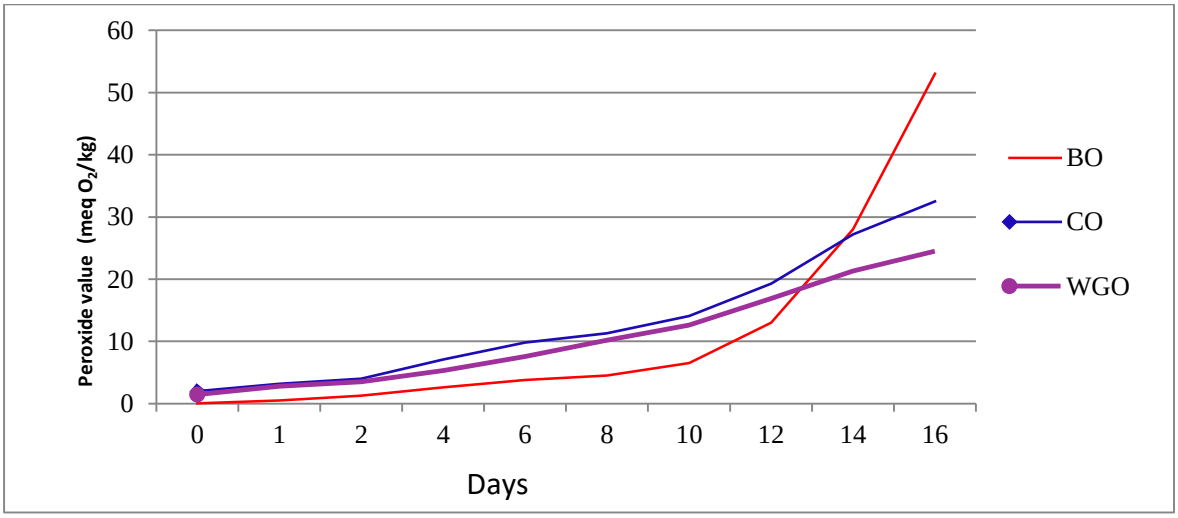

Figure 1: Peroxide value of BO: Butter oil, CO: Corn oil: WGO: wheat germ oil during incubation at 60°C/16 days.

CONCLUSION

Despite to contains unsaturated fatty acids; WGO is stable due to high amount of minor components. WGO is rich in tocopherols particularly vitamin E. WGO is being used in medicine, cosmetic, agricultural, and food industry. More studies are required for producing better quality WGO such as application of more innovative and optimized techniques for increasing its utilization after exploring more health benefits. It is concluded that the WGO and CO has the highest concentrations of α-tocopherol, where the vegetable oils with higher concentrations of γ-tocopherol were the WGO and CO. Moreover, the BO was not considered as a food source of vitamin E and phospholipids, while the WGO had been classified as a food source and was considered as an excellent source of minor components (e.g., phospholipids and sterols).

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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