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Effect of Oils with Different Fatty Acids Profile on the Physical Properties of Formulated Emulsion.

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ABSTRACT

The effects of different fatty acids composition of oils on the physical properties of formulated oil emulsions were investigated. To achieve this goal, milk fat as the choice on the basis it contains saturated fatty acids (SFAs) higher than unsaturated fatty acids (USFAs) (~ 2:1), while the choice of palm oil on the basis it contains SFAs equal to the USFAs (~ 1:1), and commercial oil consists of soybean, cottonseed and coconut oils on the basis it contains SFAs lower than USFAs (~ 1:2). Oil emulsions (20% oil w/w) were formulated with 20% melted butter oil (FBO), palm oil (FPO), oils blend (FOB), butter oil and palm oil (FBOPO) (1:1) or butter oil and oils blend (FBOOB) (1:1), and 80% reconstituted skim milk. The results revealed that surface tension, protein load and viscosity were the highest in FOB, but those were the lowest in FPO and FBOPO. Inversely, FBO and FBOPO had the best foaming capacity, while FOB had the lowest foaming capacity. The amount of freeze-thaw serum leakage resulted from frozen FOB was highest and more turbidity followed by that resulted from FBOOB. Finally, it could be concluded that as USFAs content increased, surface tension, protein load, viscosity, freeze-thaw serum leakage increased, while foaming capacity decreased.

Keywords: oils fatty acid composition, formulated oil emulsions, physical properties.

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INTRODUCTION

Foods are complex systems containing various ingredients such as proteins, fats and sugars as well as minerals and low molecular weight emulsifiers (Samant *et al*., 2007). These compounds may interact with each other in different complex ways depending on type of ingredients and their percentage, pH, heat treatment, and processing steps as well as their own properties (Sánchez *et al.*, 2005). Fat is the main ingredient in the most dairy products such as cream, butter, ice cream and butter oil. It's occurs as a free fat or as an emulsion. In both types of products the physical properties depend on the degree of crystallization, and the size and shape of crystals (Huyghebaert *et al*., 1996). The crystallization of fat in emulsion droplets can influence the overall structure and stability of the emulsion, by causing coalescence of droplets due to fat crystals that penetrate the droplets' surface and grow into adjacent droplets (Millqvist-Fureby, 2001). Also, important attributes such as smooth, creamy and rich texture; satiating effects are influenced by the droplets of fat (Rios *et al.,* 2014).

In most countries, consumption of high-fat dairy products has been on the decline in recent years due to a negative nutritional image of milk fat. The negative nutritional image of milk fat is mostly related to the current conception that the intake of milk fat is associated with an increased risk of developing coronary heart disease (CHD) or metabolic syndrome (MacRae *et al.,* 2005 and Mensink, 2006). This property is claimed to be due primarily to certain saturated medium- and long-chain fatty acids, which are considered to have atherogenic action, through increasing plasma cholesterol and low-density lipoprotein levels (Kris-Etherton & Yu, 1997). Inversely, polyunsaturated fatty acids have a hypocholesterolaemic effect in human. Deficiency of essential fatty acids such as linoleic, linolenic and arachidonic acids, growth is retarded and dermal symptoms appear (Sundram, 2003). Therefore, it is possible to reduce the saturated fatty acids (SFAs) and enhance unsaturated fatty acids (USFAs) as well as other bioactive lipids by means of animals feeding or breeding (Palmquist *et al.,* 2006; AbuGhazaleh & Holmes, 2009 and Abd El-Aziz *et al.,* 2012), milk fat fractionation (Fatouh *et al.,* 2003; Dewettinck *et al.,* 2004 and Rombaut & Dewettinck, 2006), and by lipase-catalyzed modification (Balcão & Malcata, 1998 and Kalo & Kemppinen, 2003). Another low-priced possible approach to increase the USFAs and became more suitable for manufacture of other products with desired properties, by means of direct incorporation of low-priced plant oils in high-fat dairy products.

In comparison to other oils, milk fat contains short chain fatty acids and higher amount of SFAs. Palm oil is a natural product, which has numerous advantageous properties, such as its high thermal and oxidative stability and its plasticity at room temperature (Mamat *et al.,* 2005 and Rosnani *et al.,* 2007). It's incorporated into fat blends for the manufacture of a wide variety of foods products. Soybean is a vegetable oil extracted from the seeds of the soybean. It is one of the most widely consumed cooking oils. Per 100 g, soybean oil has 16 g of saturated fat, 23 g of monounsaturated fat, and 58 g of polyunsaturated fat. The major unsaturated fatty acids in soybean oil triglycerides are the polyunsaturated α-linolenic acid, 7-10%, and linoleic acid, 51%; and the monounsaturated oleic acid, 23% (Ivanov *et al.,* 2010). Cottonseed oil is cooking oil extracted from the seeds of cotton plants of various species. Its fatty acid profile generally consists of 70% USFAs, (18% MUSFAs, and 52% PUSFAs), 26% SFAs. According to the cottonseed oil industry, cottonseed oil does not need to be hydrogenated as much as other polyunsaturated oils to achieve similar results (Jones & King, 1996). Coconut oil is extensively used for food and industrial purposes. The oil is rich in medium chain fatty acids and exhibits good digestibility (Che Man & Marina, 2006).

The reducing of SFAs or increasing USFAs contents may affect not only health characteristics, but also the degree of crystallization, solid fat content, melting point, and the size and shape of crystals. The presence of solid fat, to promote fat globule rupture, and of liquid fat, to promote clumping, may be necessary for formation of stable dairy foam Pilhofer *et al.* (1994). The use of the low-melting range butter oil fraction results in emulsion formulation with a higher creaming stability and protein load than medium-melting range butter oil (Scott *et al.*, 2003 and Fatouh *et al.,* 2006). The size of fat globule was lower in Feta cheese made with low-melting range butter oil fraction than that made with medium- and high melting range butter oil fraction. herefore, the aims of this study were to investigate the effects of different fatty acids composition of oils, especially, relation between SFAs and USFAs or polyunsaturated fatty acids (PUSFAs), on the physical properties of formulated oil emulsions.

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MATERIALS AND METHODS

Materials

Fresh butter was obtained from Dairy Processing Unit, Faculty of Agriculture, Cairo University, Giza, Egypt. Butter oil (BO) was prepared from fresh butter by the method of Amer *et al*. (1985). Palm oil (PO) was obtained from the Extracted Oils and Derivatives Company (Safola Misr Company), 10th of Ramadan City, Egypt. Coconut (CNO), soybean (SBO) and cottonseed oils (CSO) as well as skim milk powder (low heat, made in the USA) were purchased from the local market at Cairo, Egypt. The SBO, CSO and CNO were mixed at rate of 2:2:1 (w/w), respectively to obtained oils blend (OB), which contains unsaturated fatty acids higher than saturated fatty acids. The fatty acids profile of BO, PO and OB are presented in Table 1.

Table 1. Fatty acids profile (g/100 g oil) of butter oil, palm oil and oils blend

Fatty acids profile	Butter oil	Palm oil	Oils blend
C_4	3.27	-	-
C_6	1.43	-	-
C_8	1.51	-	0.59
C_{10}	1.33	-	0.48
C_{12}	2.01	0.22	3.74
$C_{14:0}$	10.51	1.20	2.07
$C_{14:1}$	0.60	-	-
$C_{16:0}$	31.73	44.40	21.69
$C_{16:1}$	2.05	0.30	-
$C_{18:0}$	13.00	4.61	4.06
$C_{18:1}$	27.59	38.85	28.34
$C_{18:1\ T}$	0.87	-	-
$C_{18:2}$	2.81	9.89	33.85
$C_{18:3\ n6}$	-	0.05	1.07
$C_{18:3\ n3}$	0.53	0.22	3.74
C_{20}	0.35	0.18	0.26
$C_{20:1}$	0.37	-	-
SFAs	65.14	50.61	32.89
USFAs	34.82	49.31	67.00
- **MUSFAs**	31.48	39.15	28.34
- **PUSFAS**	3.34	10.16	38.66

SFAs, saturated fatty acids; USFAs, unsaturated fatty acids; MUSFAs, mono unsaturated fatty acids; PUSFAs, poly unsaturated fatty acids

Methods

Emulsions formulation

Table 2. Abbreviations and description of formulated oil emulsions

Abbreviations	Formulated oil emulsions
FBO	Emulsion formulated with 20% butter oil and 80% reconstituted skim milk (~ 3.2% protein).
FPO	Emulsion formulated with 20% palm oil and 80% reconstituted skim milk (~ 3.2% protein).
FOB	Emulsion formulated with 20% oil blend and 80% reconstituted skim milk (~ 3.2% protein).
FBOPO	Emulsion formulated with 10% butter oil as well as 10% palm oil and 80% reconstituted skim milk (~ 3.2% protein).
FBOOB	Emulsion formulated with 10% butter oil as well as 10% oils blend and 80% reconstituted skim milk (~ 3.2% protein).

Reconstituted skim milk, ~ 3.2% protein, was prepared by reconstituted the required amount of skim milk powder in distilled water under continuous stirring at room temperature. Oil emulsions (20% oil w/w) were formulated with 20% melted butter oil (BO), palm oil (PO), oils blend (OB), BO and PO (1:1) or BO and OB (1:1), and 80% reconstituted skim milk as described in Table 2. All formulated oil emulsions were preheated to

60ºC, homogenized at 13.6/3.5 MPa (stage 1/stage 2) using laboratory double stage homogenizer (Rannie, Copenhagen, Denmark), then heated to 78ºC and cooled to 38ºC. Sodium azide was added at the rate of 0.02% as preservative and all formulated emulsions were aged overnight at 5.0±2°C before analysis. All reformulated oil emulsions were prepared in triplicates.

Methods of analysis

Fatty acids profiles: The method recommended by AOAC (2007) was used for preparation of fatty acid methyl esters. The methyl ester of the fatty acids compounds were analyzed 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 i.d). The oven temperature was programmed in two stages as follows: first held at 40°C for 5 min and then from 40 to 220°C at rate 3°C/min. Detector and injector temperature generally were 250, 230°C; respectively. The carrier gas (helium) flow rate was 1 ml/min.

Surface tension: The surface tension of the formulated oil emulsions was measured as described by Arbuckle (1986). A tube of uniform bore is used and the number of drops of the sample falling per time is compared with that of water. The surface tension of water is 72-73 dynes.

Protein load: The adsorbed protein on the surface of fat globules was determined by measuring the protein content (AOAC, 2007**)** of the aqueous phase of formulated oil emulsions before and after centrifugation at 10,350 xg (Sigma Laborzentri Fugen, 2 K15, Germany) for 30 min at 20ºC (Cano-Ruiz & Richter, 1997) and freezing at -30°C for 45 min. The adsorbed protein was calculated from the difference between the initial and final proteins of the aqueous phase and expressed as protein load**.**

Creaming rate*:* Creaming rate of the formulated oil emulsions was measured as the rate of creaming over 15 days of storage as described by Scott *et al.* (2003). Fresh formulated emulsions were placed in 100 ml graduated cylinders up to mark, capped, and stored at 5±2°C. Initial fat content was determined and fat content of the lower phase of each emulsion was analyzed in duplicate after 1, 7 and 15 days of storage using the Gerber method (AOAC, 2007).

Apparent viscosity: Apparent viscosity of the formulated oil emulsions was determined using a Brookfield Synchro-Lectric viscometer (Model LVT; Brookfield Engineering Inc. Stoughton, MA). Apparent viscosity (mPas) were measurements at shear rate of 5 to 60 s^{-1} using spindle - 00 at 6°C for upward curve.

Foaming capacity and instability: Foaming capacity of the formulated oil emulsions measurement was made at 5 min intervals for a total of 20 min. A 200 ml formulated emulsion was whipped in a mixer (Heidolph No. 50 111, Type RZRI, Germany) at speed setting 10 under freezing conditions according to procedure described by Smith *et al.* (2000). Foaming capacity was expressed as foam expansion immediately after whipping (ml/100 ml emulsion). Foaming instability of the whipped emulsion was measured as described by Mangino *et al.* (1987). A 100 ml whipped emulsion (after 20 min) was left in graduated cylinders at 25±2ºC and the decrease in the volume was measured at 30 min intervals for a total of 90 min. the percentage of foaming instability was calculated using the following equation:

$$\text{Foaming instability (\%)} = [(V-V_0)/V]\ 100$$

Where; V, the volume of foam immediately after whipping and V_0, the volume of foam after the time

Freeze-thaw stability: The whipped formulated oil emulsions (after 20 min) were filled into plastic cups, covered and hardened in a deep freezer at –20°C for at least 24 hr. The cup was placing onto the wire mesh over a glass funnel fitted on conical flask, and weighing the amount of serum drained into the conical flask at 25±2°C every 10 min intervals for a total of 40 min**.** The percentage of freeze-thaw serum leakage of frozen whipped emulsion was calculated using the following equation:

$$\text{Freeze-thaw serum leakage (\%)} = (W_S/W_{FWE})\ 100$$

Where: W_S, the weight of serum leakage and W_{FWE}, the weight of frozen whipped emulsion.

Serum turbidity: The melted serum resultant from frozen whipped formulated oil emulsions was diluted by distilled water (1:9). The serum turbidity was measured at 540 nm by a UV visible spectrophotometer (Schimadzu spectrophotometer 1201, Japan) according to the method of Goff & Jordan (1989). The higher optical density means the higher turbidity.

Statistical analysis: Analysis of variance (ANOVA) and Duncan's test were conducted using a Statistical Analyses System (SAS, 2004). A probability to $P \leq 0.05$ was used to establish the statistical significance.

RESULTS AND DISCUSSION

Surface tension and protein load

The surface tension and protein load of oil emulsions formulated with butter oil (FBO), palm oil (FPO) or oils blend (FOB) and their blends (FBOPO or FBOOB) are presented in Table 3. The results showed that the FOB had the highest ($P < 0.05$) protein load (55.09%), while the FBO and FBOPO had the lowest ($P < 0.05$) protein load (36.77 or 36.70%, respectively). Protein load of FPO (43.90%) was comparable to the protein load of FBOOB (43.71%). The higher protein load in FOB could be attributed to the lower melting point and the higher USFAs content (0.2%) in OB compared with the other oils. The affinity of hydrophobic protein segments to lipids in the liquid state is much stronger than crystallized lipids (Korg, 1991). These results are agreement with that found by Abd El-Aziz (2008) in emulsion formulated with low-melting butter oil fraction. Fatouh *et al.* (2006) found that protein load increased as fat globule size decreased. The fat globule size decrease as the slip melting point decreased and USFAs increased in BO fractions.

A similar, the surface tension of FOB or FBOOB was significantly higher ($P < 0.05$) than that of FBO, FPO or FBOPO. Also, the surface tension of FOB was higher ($P < 0.05$) than that of FBOOB. There was no significant difference in the surface tension of FBO, FPO or FBOPO ($P > 0.05$). The higher surface tension in FOB and FBOOB may correlated with the higher protein load and/or higher viscosity (Fig 1). Arbuckle (1986) booked that the grater the attraction between the molecules of the liquid, the higher the surface tension value.

Table 3. Surface tension and protein load of oil emulsions formulated with butter oil, palm oil, oils blend, and their blends

Reformulated oil emulsions	Surface tension (dyne)	Protein load (%)
FBO	52.73 ^{C}	36.77 ^{C}
FPO	52.66 ^{C}	43.90 ^{B}
FOB	58.35 ^{A}	55.09 ^{A}
FBOPO	53.46 ^{C}	36.70 ^{C}
FBOOB	55.46 ^{B}	43.71 ^{B}

Means with different superscripts letters are significant difference ($P \leq 0.05$); FBO, emulsion formulated with 20% butter oil; FPO, emulsion formulated with 20% palm oil; FOB, emulsion formulated with 20% oils blend; FBOPO, emulsion formulated with 10% butter oil and 10% palm oil; FBOOB, emulsion formulated with 10% butter oil and 10% oils blend.

Apparent viscosity

As shown in Fig 1, all oil formulated oil emulsions exhibit non-Newtonian and shear thinning behaviors; decreasing viscosity as shear rate increases. Similar observation were found by Abd El-Aziz *et al.* (2013) in milk fat emulations formulated with different protein types, Scott *et al.* (2003) in cream formulated with fractionated milk fat and milk-derived components and by Wan Rosnani *et al*. (2007) in ice cream mix prepared from palm oil or/and milk fat blends. The decreasing in the viscosity can be attributed to the breakdown of colloidal aggregate particles as increasing shear is applied during the first stage of viscosity measurement (Fox & McSweeney, 1998). In general, oil composition significantly ($P < 0.05$) influenced viscosity of formulated oil emulsions. The apparent viscosity of the FOB was the highest compared with other oil formulated emulsions. Such results are agreement with that found by Abd El-Aziz (2008), but differ from that found by Scott *et al.* (2003) in emulsion formulated with low-melting butter oil fraction. A similar, but less marked, the apparent viscosity of the FPO and FBOOB was higher than that of FBO and FBOPO at the same shear rate. Bear *et al.* (1995) reported that an increase in the level of USFAs in the fat used for manufacturing a

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low-fat cheese resulted in smaller fat droplet size, which enhanced the rheological properties of the product. Apparent viscosity for FBO was comparable to that of FBOPO at different shear rates (P > 0.05).

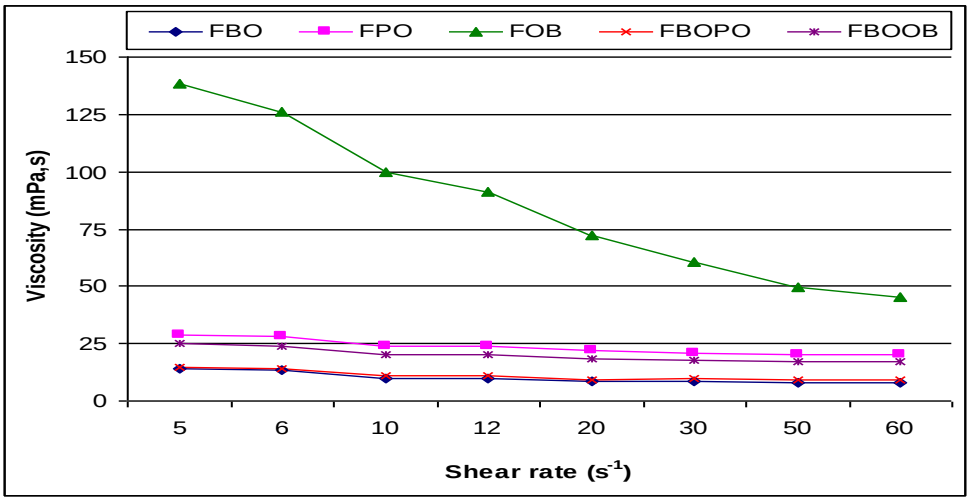

Fig 1. Apparent viscosity of oil emulsions formulated with butter oil, palm oil, oils blend, and their blends at different shear rate (5 to 60 s^{-1}).

Creaming rate

Fig 2 exhibited the effect of oil composition on the creaming rate of formulated oil emulsions during storage at 5±2°C for 15 days. At day 1, there was no significant difference in creaming rate in different formulated oil emulsions (P > 0.05). During storage period for 15 days, all formulated oil emulsions showed continued increase in the creaming rate, however, the increasing was not significant only in FPO (P > 0.05). This means that, the FPO was more stable compared with other formulated oil emulsions. The creaming rate was the highest in FBO at day 7 (P < 0.05), while at day 15, the creaming rate was the highest in FOB followed by in FBO. The higher creaming rate in FOB, which had higher protein load (free-protein content), is agreement with Lent *et al.* (2008), they found that low free-protein content can cause formation of homogenization clusters; it will lead to both higher viscosities and apparent particle sizes, the latter leading to higher creaming rates. On the contrary, Wiking (2005) mentioned that with increasing emulsion viscosity and decreasing the droplet size, the rate of droplet move upward decreased. Both FBOPO and FBOOB had medium creaming rate and no significant difference between each other (P < 0.05) during storage period.

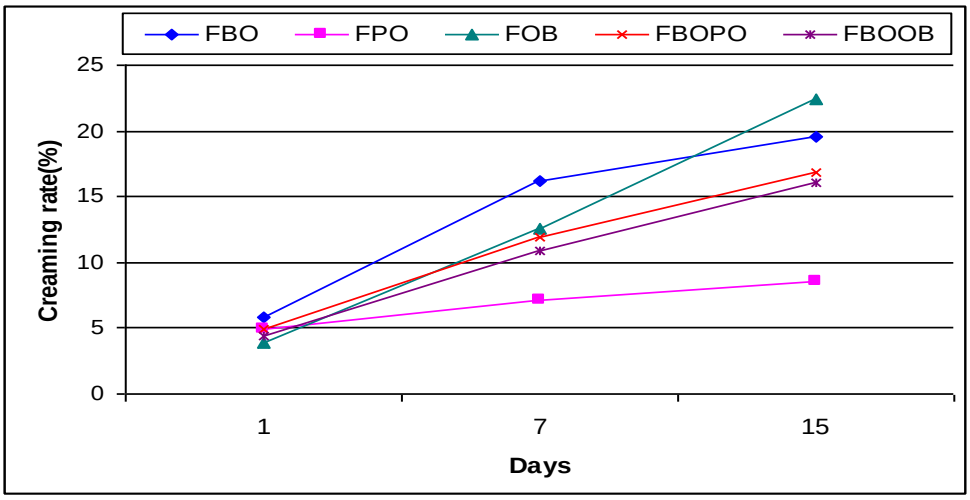

Fig 2. Creaming rate of oil emulsions formulated with butter oil, palm oil, oils blend, and their blends during storage at 5±2°C for 15 days.

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Foaming capacity and stability

Foaming capacity determines the ease with which air can be incorporated into the emulsion (Berger *et al* 1972). Foaming capacity and stability of formulated oil emulsions as affected by types of oils under freezing condition for 5, 10, 15 and 20 min are represented in Fig 3 and 4, respectively. In general, foaming capacity and stability of formulated oil emulsions were influenced by fatty acids composition of oil. In particular, the greater value of foaming capacity was obtained in the FBO, while the lower foaming capacity ($P < 0.05$) was obtained in the FOB throughout the foaming times. The higher air phase in formulated oil emulsion may be correlated with the higher free protein content (lower protein load). Zhang & Goff (2004) reported that the serum proteins may stabilize more air serum interface, promoting aeration and foam stability. Also, an inverse relationship between the foaming capacity and the apparent viscosity was observed in the all formulated oil emulsions. Smith *et al.* (2000) reported that an increased viscosity of the serum phase is known to reduce the ability to incorporate air during whipping. Abd El-Aziz (2008) mentioned that the high content of USFAs affect the fat destabilization (fat coalescence), which acts as a foam depressant. Similar observations were found by Stanley *et al.* (1996) in ice cream mixes containing carbohydrate-based fat replacers, and Abd El-Aziz *et al.* (2015) in ice cream containing cress seed and flaxseed mucilage's. The increase in the emulsion viscosity could have been the primary reasons for decrease the whipping abilities (Schmidt and Smith, 1992). However, there was no significant difference in the foaming capacity obtained in both FBO and FPO or in both FPO and FBOOB ($P > 0.05$). During foaming time, the higher foaming rate was observed in all formulated oil emulsions whipped during the first 5 min. Thereafter, formulated oil emulsions showed continued increase in the foaming capacity until 15 min for FBO, FPO FOB and FBOPO and until 20 min for FBOOB.

As shown in Fig 4, FPO was higher in the foam instability percentage than other formulated emulsion after 30 min; however, there was no significant difference in foam instability induced in both FPO and FBO. At 60 min, there was no statistical difference among the FBO, FPO, FBOPO and FBOOB in the foam instability ($P > 0.05$). The FBO, FPO, FBOPO and FBOOB had foam instability percentage of 77, 77, 99 and 90%, respectively. Inversely, the whipped FOB was more stable compared with other formulated oil emulsions. Negative correlation was found between emulsion viscosity and foam instability**.** Adapa *et al.* (2000) reported that high viscous systems do not favor foaming capacity but do favor foam stability. In addition, the results show that, the lower foam instability might be negatively correlated with the higher foaming capacity.

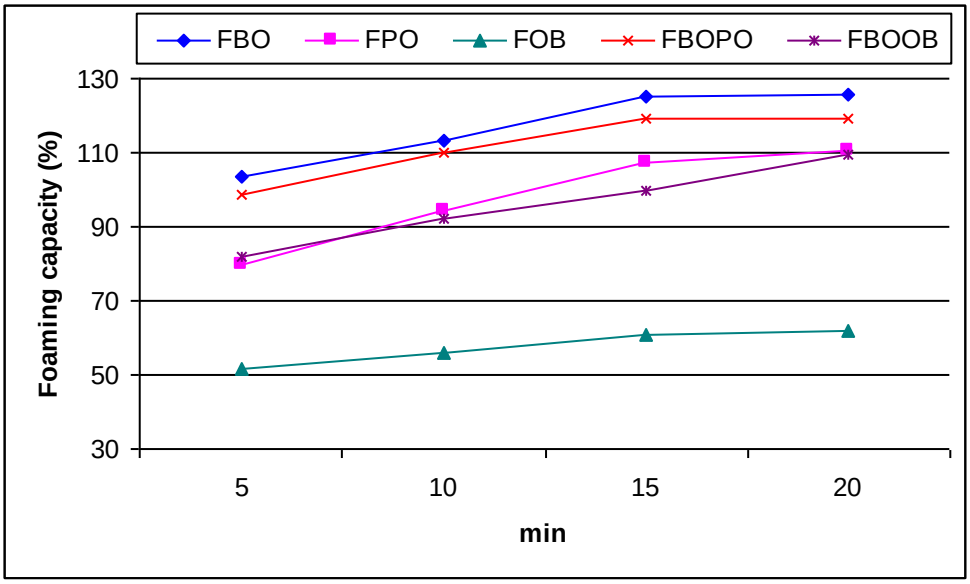

Fig 3. Foaming capacity of oil emulsions formulated with butter oil, palm oil, oils blend, and their blends at 5 min intervals for 20 min.

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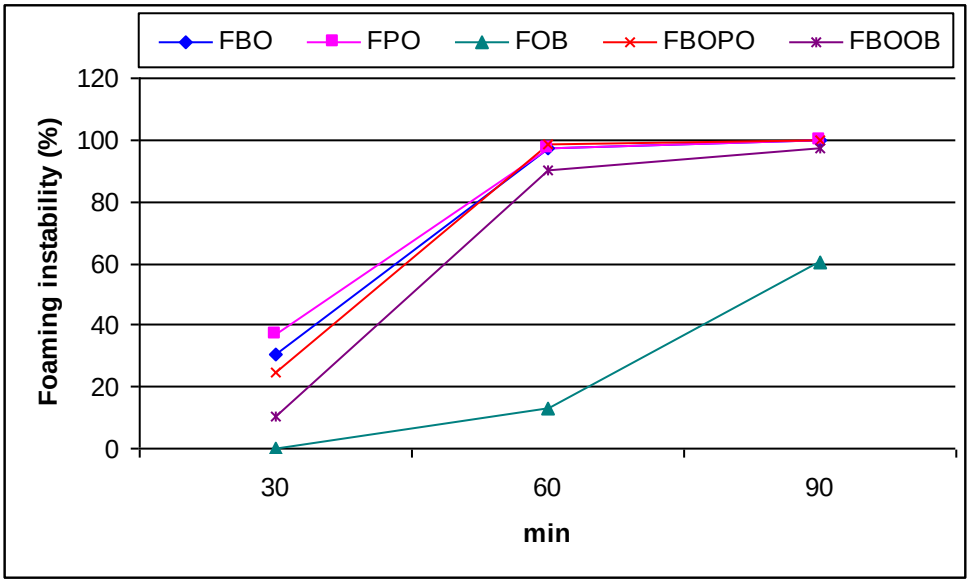

Fig 4. Foaming instability of oil emulsions formulated with butter oil, palm oil, oils blend, and their blends at 30 min intervals for 90 min.

Freeze-thaw stability

The percentage of freeze-thaw serum leakage of frozen whipped formulated oil emulsions is presented in Fig 5. After 10 min, no serum leakage was observed in all frozen whipped formulated oil emulsions, except FOB. After 20 min onward, the percentage of the serum leakage obtained from frozen FOB was the highest ($P < 0.05$) followed by frozen FBOOB, but that obtained from frozen FPO was the lowest compared with other frozen whipped formulated oil emulsions. The serum leakage of frozen FBO was comparable to that of the frozen FBOPO. The percentage of freeze-thaw serum leakage might be negatively correlated with slip melting point of oil or/and USFAs content, especially PUSFAs content (Table 1). A similar, frozen whipped FOB had the higher serum turbidity (higher optical density), but whipped FPO had the lowest serum turbidity ($P < 0.05$) compared with other frozen formulated oil emulsions. However, there was no significant difference in serum turbidity resulted from frozen FBOPO and FBOOB ($P > 0.05$). The higher serum turbidity could be attributed to the higher protein and oil contents in the serum leakage.

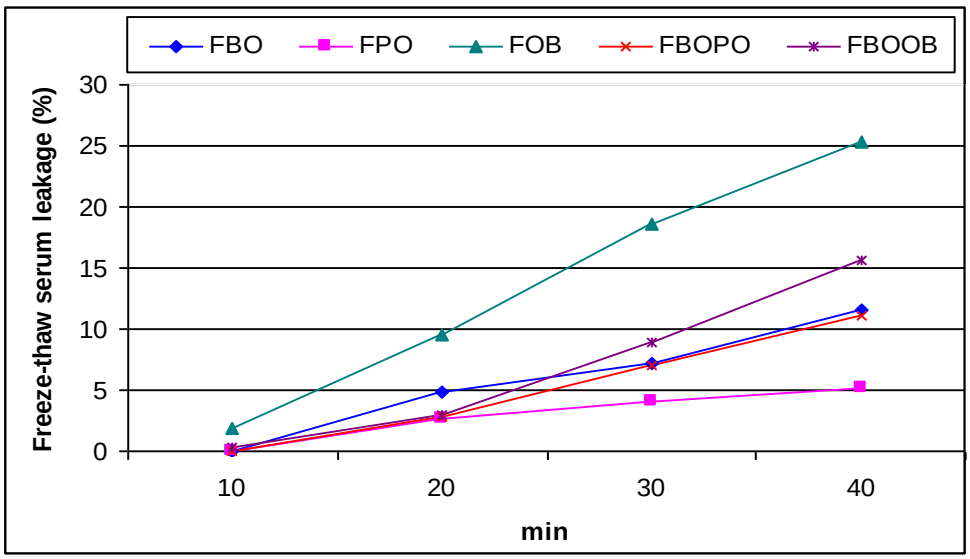

Fig 5. Freeze-thaw serum leakage of frozen whipped emulsions formulated with butter oil, palm oil, oils blend and their blends at 10 min intervals for 40 min.

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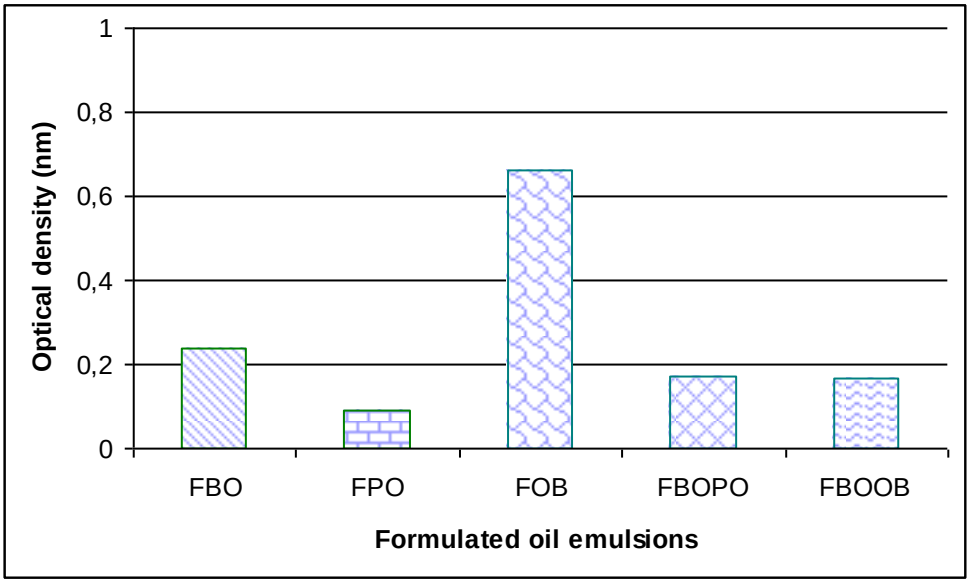

Fig 6. Optical density of serum leakage of frozen whipped emulsions formulated with butter oil, palm oil, oils blend and their blends after 40 min

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