Evaluation of some methods to detect milk fat adulteration by palm oil and palm kernel oil mixture

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ABSTRACT

Adulteration of milk fat with other types of fat has been an actual problem because of the economic impact of substituting highly-priced milk fat with lower-priced ones such as vegetable oils. Therefore, the aim of the present study was to examine the accuracy and sensitivity of some simple procedures to detect the milk fat adulteration with vegetable oils including palm oil (PO) and palm kernel oil (PKO) in different levels as a model of commercial adulterated milk samples. The butter oil (BO) was mixed with a mixture of vegetable oils (50% PO + 50% PKO) in order to create 4 treatments representing adulteration of butter oil in different proportions of 25, 50, 75% and 100% compared to control sample of the pure butter oil. The results showed that such adulteration didn't cause significant changes in the fat constants especially when determine the refractive index, melting point, saponification value, ester number and iodine number. Apparent Solidification Time (AST), Reichert-Meissl and Polenske number could be used to detect such milk fat adulteration with PO/PKO mixture especially in the high-level content of vegetable oil ≥ 50% of milk fat content.

Keywords: adulteration; milk fat; palm oil; butter oil.

INTRODUCTION

Natural form of milk has high nutritional value which provides various nutrients including protein, fat, vitamins, carbohydrate and minerals. Also, milk proteins provide some of amino acids needed for the proper growth of infants and adults. Buffalo and cow milk contains (fat 7.6 and 4.5%), (lactose 4.9 and 4.9%), (protein 3.8 and 3.8%), (ash 0.78 and 0.72%), (total solids 17 and 13.9%), respectively (Afzal et al., 2011).

Milk adulteration is defined as an act of intentionally degrading the quality of the foodstuff and milk-based product to increase the sale either by mixing or substitution of low-grade ingredients or by the removal of some precious substances (FDA, 1995).

Adulteration of the milk fat occurs in various ways using less expensive fat as milk fat alternative throughout homogenization of it with skim milk or by direct mixing of such alternative fat with butter oil (Destaillats et al., 2006).

Recent studies indicated that milk adulteration traders are incorporating various blends of oil or synthetic fatty acids in order to nullify the fat constants-based analyses procedure for detecting the milk fat adulteration with vegetable oil (Pathania et al., 2020).

Milk fat is a good source of fat-soluble vitamins and essential fatty acids. So, milk-based products are characterized as high nutritive value foodstuff which consumed all around the world, which providing an economic importance in the international commerce. Therefore, they are vulnerable to many types of adulterants in order to increase the profits of unscrupulous companies (Souza et al., 2011).

Control of potential adulterants is an issue mainly due to as the laboratories improve detection techniques, adulteration producers introduce novel substitutes that can't be detected by established procedures (Finete et al., 2013). Various adulteration methods reduce the nutritional value of dairy products which affects the consumer health causing nausea, diarrhea, allergies, respiratory irritation and bleeding, digestive disorders, and kidney disorders; while in severe cases may causes cancer disease and death (Handford et al., 2016).

Some milk and dairy products companies remove milk fat due to their higher price than other ingredients in order to achieve additional financial profits using vegetable oils (Azad and Ahmed, 2016). Hansen and Holroyd (2019) reported that the detection of

adulteration of milk with vegetable oils is very difficult as the fatty acid composition is very similar among milk fat and vegetable oil.

Palm oil (PO) is extracted from the middle rind of oil palm fruit and has a balance of unsaturated fatty acids (oleic acid) and saturated fatty acids (palmitic acid). Also, palm kernel oil (PKO) is extracted from the kernel of the palm fruit. PKO composition and characteristics differ from PO due to the higher content of lauric acid (Dian et al., 2017).

Therefore, the aim of the present study was to examine the accuracy and sensitivity of some simple procedures to detect the milk fat adulteration with vegetable oils including PO and PKO in different levels as a model of commercial adulterated milk samples.

MATERIALS AND METHODS

Materials:

Fresh whole buffalo's milk (7.7 % fat) supplied by faculty of Agriculture farm, Cairo University, Giza, Egypt. Palm oil and palm kernel oil which flushed with nitrogen and stored at -20 ◦C until used were obtained from the extracted oils and derivatives company (Arma Food Industries Company), 10th Ramadan City, Egypt. Other chemicals and reagents were used in analytical grade.

Methods:

Preparation of BO with mixture of PO and PKO

Buffalo's milk fat samples were extracted as described by De (2004) in order to prepare model of commercial adulterated milk samples. Then, adulterated milk fat samples with a mixture of palm oil and palm kernel oil (PO50%/PKO50%) with different levels (25%, 50%, 75% and 100%) in comparison of control sample of natural buffalo's milk fat as shown in Table 1.

Analytical methods

The physical and chemical properties were determined for adulterated BO with a mixture of PO and PKO as follow:

Physical analysis:

Refractive Index (RI) was measured according to AOAC (2016) using Zeiss Refractometer at 40 ºC. Butyro-Refractometer (BR) was determined using Abbe refractometer as described in AOAC (2016). Apparent Solidification Time (AST) which refer to the time taken by the melted fat to get apparently solidified at a given temperature. AST was determined according to the developed procedure by Kumar et al. (2009). The melting point was determined using thin wall capillary tubes (1 mm internal diameter) according to the method described in AOAC (2016).

Chemical analysis:

Acid value was determined according to IUPAC (1987). Acid value as oleic acid (%) for butter oil and palmitic acid (%) for palm oil and palm kernel oil was calculated using the following equations:

Acid value = (VxNx56.1)/sample weight (g)

Where: N = normality of KOH, V: volume (ml) of KOH.

Iodine value was determined using the Hanus method according to the method described in AOAC (2016). Blank sample was performed without the oil, and the iodine value was calculated as grams of iodine per 100 g of oil.

The saponification value was determined by the procedure of AOAC (2016), 5 g of samples were saponified using 50 ml of ethanolic KOH (5%) in a conical flask connected to an air condenser and boiled until the oil was completely saponified, cooled and then titrated with 0.5 N HCl using phenolphthalein as indicator.

The ester value was determined as described by Dileesh et al. (2013).

Reichert-Meissl value (soluble volatile fatty acids) and Polenske value (insoluble volatile fatty acids) were determined according to the method described by AOAC (2016).

Reichert-Meissl value was calculated by the following formula:

Richert-Meissl number = [(B-S) x 1.1 x 5]/W

Where: S: volume (ml) of NaOH required by sample, B: volume (ml) of NaOH required by blank, and W: weight (g) of the sample.

Polenske value was calculated by the following formula:

Polenske number = [(S-B)×5]/ W

Where: S: volume (ml) of NaOH required by sample, B: volume (ml) of NaOH required by blank, and W: weight (g) of the sample.

RESULTS AND DISCUSSION

Four samples of adulterated butter oil with a mixture of palm oil (PO) and palm kernel oil (PKO) were prepared with different

proportions of a mixture of PO and PKO, in the level of 25%, 50%, 75% and 100%, in compared of control sample of pure BO as presented in Table 1, as a model of adulterated commercial milk samples in order to examine the suitable procedure to detect such milk fat adulteration.

Refractive index

The refractive index is affected by the degree of saturation of the oil, the ratio of the Cis and Trans double bonds, as well as the degree of oxidation of the oil(Abd-ElGhany et al., 2020). Also, El-gazzar et al. (2021) mentioned that the refractive index is one of the most important physical properties used in the identification of oils and fats, as it is useful in estimating the degree of saturation. For the analysis of the refractive index of milk fat the Abbe refractometer was used which provides the true scale of refraction; or using the butyro-refractometer (BR reading) which is more convenient for interpretation (BIS, 1966).

The refractive index results of pure butter oil and adulterated butter oil with a mixture of PO and PKO at 40 °C using an Abbe refractometer are presented in Table 2. It could be noted that the refractive index of BO was 1.4524, while the refractive index of (PO+PKO)100% was 1.4532. Thus, addition of PO/PKO mixture resulted in an increase in the refractive index of BO; The increase was proportional to the level of addition PO/PKO. The refractive index of BO mixed with PO/PKO at 40 °C was in the normal range for pure BO reported by other researchers at all adulteration levels. Fatouh et al. (2005) found that the refractive index of BO was 1.4522. Also, Yahia (2018) found that the refractive index of BO was 1.4534. The results showed that there were significant ($P \leq 0.05$) difference among pure butter oil and a mixture (PO+PKO)100%. However, the refractive index could not be used as a method to detect adulteration of milk fat with a mixture of palm oil and palm kernel oil at the adulteration levels reported in Table 1, because they were all in the normal range of a refractive index of the pure butter oil mentioned by the researchers before.

BR reading is one of the quality parameters covered under legal standards (Rules, 2011) for milk fat. BR for all treatments was very close to each other, as the pure butter oil sample recorded 40.0, and the results for the treatments of a mixture of BO with PO and PKO ranged between 40.3 to 41.3 (Table2). All results were in the normal range for pure butter oil reported by Anil et al. (2017) who found that BR for pure buffalo milk fat in different months throughout the year ranged between 40.40 and 41.20. Also, Rules (2011) reported that BR for pure milk fat varies from 40.0 to 43.0 for most regions. The results showed that there were no significant ($P \leq 0.05$) differences between the treatments. Although many dairy companies use BR to detect the adulteration of milk fat coming to them from suppliers, the results obtained indicated that this device is not suitable for detecting adulteration of milk with a mixture of PO and PKO.

Apparent Solidification Time (AST)

Milk fat may be liquid at temperatures above 40°C and solid at temperatures below 40°C (Fox, 2012). AST values were recorded at 18 °C for pure butter oil and mixtures of BO with PO and PKO. Figure 1 shows that AST for butter oil was 2:30 (min:sec) while the total AST values for mixtures between butter oil, palm oil, and palm kernel oil ranged from 2:55 to 12:00 (min:sec) at 18 °C. It is clear from the results that the value of AST increases with the increase in the percentage of cheating, and it is also clear that the slope of change in AST increases with the addition of PO/PKO when with the increase in the percentage of cheating especially at higher levels of addition. Kumar et al. (2009) found that the AST of buffalo milk fat at the selected temperature (18 °C) were 2:31 to 3:25 (min:sec). The results showed that there were not significant ($P \leq 0.05$) difference among pure butter oil and the treatment containing 25% vegetable oils, while there were significant differences between the control sample and the other treatments. Also, the AST results of the treatments at adulteration levels (50%, 75% and 100%) were higher than the normal range of pure butter oil, which indicates that AST could be used to detect such milk fat adulteration with PO/PKO mixture especially in the high-level content of vegetable oil ≥ 50% of milk fat content.

Melting point

The melting points of fatty acids increase with increasing chain length and decrease with increasing unsaturated fatty acid content (Sabry et al., 2020). Figure 2 shows that the melting point test results of pure butter oil and adulterated butter oil. It is clear from the results that adulterating butter oil with a mixture of PO and PKO didn't lead to high change in the melting point. In particular, the melting point of butter oil was 34.5°C and gradually increased with the level of addition

until reached 35°C in PO/PKO100% mixture. The results showed that there were no significant (P ≤ 0.05) difference among pure butter oil and other treatments. Also, the melting point results for BO adulterated samples with palm oil and palm kernel were in the normal range for the melting point of BO which other researchers reported. Celik and Bakirci (2000); EL-Hadad (2013) and Yahia (2018). This indicates that the melting point test is not valid for the adulteration detection mentioned in Table 1.

Acid value

The acid value of butter oil was 0.24, while the acidity value of PO/PKO mixtures which ranged between 0.25 and 0.28 (Table 3). The acid value of the BO was consistent with that reported by Ahmed (2021) who stated that the acid value of buffalo milk fat was 0.24 and that the acid value was not suitable for detecting adulteration of cow or buffalo milk fat with coconut oil. The results showed that there were no significant (P ≤ 0.05) difference among pure butter oil and other treatments. Acid values depend on various factors such as free fatty acids, acid phosphate, amino acids, and storage conditions. Therefore, the acidity value could not be used to detect the adulteration of butter oil with a mixture of palm oil and palm kernel oil.

Saponification number

The saponification number is one of the important chemical constants in order to confirm the fat quality. It is also considered a successful parameter for detecting the fat adulteration, especially adulteration of milk fat with vegetable oils (Zaidul et al., 2007). Table 3 shows that the saponification number of BO is 235.6 (mg KOH/g) and gradually decreased by increasing the proportion of mixture (PO+PKO) in the butter oil, where it was 227, 224.4, 222.8 and 218.8 mg KOH/g at adulteration levels of 25%, 50%, 75% and 100%, respectively. However, all samples were in the normal range (210-250 mg KOH/g) for the saponification number of pure butter oil as reported by Samet-Bali et al. (2009) and Özkanlı and Kaya (2007). El-Hadad (2013) mentioned that the saponification value of butter oil was 225 mg KOH/g. Likewise, Hamed et al. (2019) stated that the saponification value of butter oil was 226 mg KOH/g. The results showed that there were significant (P ≤ 0.05) differences between pure butter oil and adulterated treatments by 50%, 75% and 100% with a mixture of palm oil and palm kernel oil, but the results obtained confirm that the saponification value test is not effective in detecting adulteration of butter oil with a mixture of (PO+PKO) at the adulteration levels mentioned in Table 1.

Ester number

The ester number is an indicator of saponifiable fatty acids excluding the free fatty acids (Aremu et al., 2015). As shown in Table 3, the ester number of BO is 235.14 (mg KOH/g), while the ester number in the BO and PO/PKO mixtures were gradually decreased with the increase of PO/PKO ratio, which ranged from 218.4 to 226.7 (mg KOH/g). The results indicated that there were significant (P ≤ 0.05) differences between the pure butter oil and the treatments to which the mixture of vegetable oils was added, especially when the levels of addition were increased by ≥ 50% of milk fat content.

Iodine value

The values of iodine (I2/100g) for BO and its mixtures with (PO+PKO) are presented in Figure 3. The iodine value of pure butter oil was higher than the iodine value of a mixture of palm oil and palm kernel oil. In particular, the iodine value obtained for pure butter oil is consistent with that reported by the researchers El-Hadad (2013) who reported the iodine value of butter oil was 34.3 (I2/100g); Yahia (2018) found the iodine value of butter oil was 34.68 (I2/100g); Hamed et al. (2019) said the iodine value of butter oil was 34.2 (I2/100g). The results indicated that the proportion of unsaturated fatty acids in butter oil, a mixture of palm oil, and palm kernel oil are close, as well as the degree of hardness. This agrees with what was mentioned by Zaidul et al. (2007) they found that the iodine value is the percentage of iodine to which lipids can bind and it is taken up by the binary bonds of unsaturated fatty acids. The iodine value is useful for measuring the degree of unsaturation which high iodine value expresses that the fat or oil contains high level of unsaturated fatty acids that contribute to the softness of butter fat. There were not significant (P ≤ 0.05) differences between the results obtained for adulterated butter and pure butter oil samples. Also, the results were within the normal range for the iodine value of pure butter oil (27-35 I2/100g) reported by the researchers Fatouh et al. (2005); Kumar et al. (2009); El-Aziz et al. (2012) and Yr et al. (2012).

Reichert-Meissl and Polenske values

Sandulachi et al. (2014) reported that the Reichert Meissl and Polenske values are an

indicator of short-chain fatty acids (SCFAs) that may be present in some oils such as milk fat, coconut oil, and palm oil. Hence, Reichert-Meissl value expresses the water-soluble volatile fatty acids, but Polenske value expresses water-insoluble volatile fatty acids. The value allows one to distinguish between butter and butter substitutes made from vegetable oils. The hydrolysis of the butter fat gives C4:0, C6:0 and C8:0 acids which are volatile in steam, while no acid below C10:0 is obtained from vegetable oils.

Reichert-Meissl which defined as the volume (mL) of 0.1N NaOH required to neutralize steam volatile and water-soluble fatty acid distilled from 5 g of fat under specified conditions (BIS, 1966). This constant is a measure of butyric acid and caproic acid in milk fat. The value of Reichert-Meissl for BO is 28.1 while it was decreased with the level of vegetable oils (PO+PKO) were increased (Table 4). Although there were differences in the results between the treatments, the Reichert-Meissl value of the cheating percentage was 25% in the normal range (17-34) which mentioned by El-Aziz et al. (2012); Samet-Bali et al. (2009); Yr et al. (2012); El-Hadad (2013), Yahia (2018) and Hamed et al. (2019). Also, there were no significant ($P \leq 0.05$) difference among this treatment and pure butter oil. It is clear from the results that the test was not useful in detecting adulteration of butter oil at a rate of 25% of (PO+PKO) mixture, however, the test can be used to detect adulteration of milk fat with this mixture at high level content of vegetable oil ≥ 50% of milk fat content.

The Polenske number is a measure of the water-insoluble volatile fatty acids and is useful to detect oils with intermediate chain fatty acids (Sheppard et al., 1985). Polenske number results are presented in Table 4. The Polenske number for BO was less than the adulterated treatments and gradually increased with the increase of the adulteration mixture (PO+PKO) and the increase was proportional to the increase in the adulteration rates until it reached 4.0 for the adulteration mixture (PO+PKO). In particular, the adulteration rate was 25% within the normal range (1.30-2.90) for pure butter oil reported by other researchers (Celik and Bakirci, 2000; Park *et al.* 2007). It is evident from the results that the test can be used to detect adulteration with PO/PKO mixture, especially in the high-level content of vegetable oil ≥ 50% of milk fat content.

CONCLUSION

It could be concluded that the tested simple procedures including Apparent Solidification Time (AST), Reichert-Meissl, and Polenske number could be used to detect such milk fat adulteration with palm oil and palm kernel oil mixture especially in the high level content of vegetable oil ≥ 50% of milk fat content.

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Table 1: Formulations of butter oil with PO and PKO mixture.

Treatments	Butter oil (%)	PO_{50}/PKO_{50} (%)
$BO_{100\%}$	100	0
$BO/(PO+PKO)_{25\%}$	75	25
$BO/(PO+PKO)_{50\%}$	50	50
$BO/(PO+PKO)_{75\%}$	25	75
$(PO+PKO)_{100\%}$	0	100

BO: Butter oil; (PO+PKO): a mixture of palm oil and palm kernel oil in a ratio of 50:50

Table 2: Refractive index and Butyro-Refractometer reading of BO and mixture of PO/PKO.

Treatments	Refractive index at 40 °C	Butyro-Refractometer reading at 40 °C
$BO_{100\%}$	1.4524 ± 0.003^{a}	40.0 ± 0.7^{a}
$BO/(PO+PKO)_{25\%}$	1.4526 ± 0.002^{ab}	40.3 ± 1.2^{a}
$BO/(PO+PKO)_{50\%}$	1.4528 ± 0.003^{ab}	40.7 ± 1.2^{a}
$BO/(PO+PKO)_{75\%}$	1.4531 ± 0.002^{ab}	41.0 ± 0.5^{a}
$(PO+PKO)_{100\%}$	1.4532 ± 0.006^{b}	41.3 ± 0.8^{a}

BO: Butter oil; (PO+PKO): a mixture of palm oil and palm kernel oil in a ratio of 50:50

a, b: Means with the same letter among treatments are not significantly different at $P \leq 0.05$.

Table 3: Acid value, saponification number and ester number of BO and mixture of PO/PKO.

Treatments	Acid value	Saponification number (mg KOH/g)	Ester number (mg KOH/g)
$BO_{100\%}$	0.24 ± 0.04^{a}	235.6 ± 5.1^{a}	235.4 ± 5.1^{a}
$BO/(PO+PKO)_{25\%}$	0.25 ± 0.04^{a}	227.0 ± 5.2^{ab}	226.7 ± 5.2^{ab}
$BO/(PO+PKO)_{50\%}$	0.26 ± 0.02^{a}	224.4 ± 5.8^{b}	224.1 ± 5.8^{b}
$BO/(PO+PKO)_{75\%}$	0.27 ± 0.02^{a}	222.8 ± 2.6^{b}	222.6 ± 2.5^{b}
$(PO+PKO)_{100\%}$	0.28 ± 0.02^{a}	218.8 ± 5.4^{b}	218.4 ± 5.5^{b}

BO: Butter oil; (PO+PKO): a mixture of palm oil and palm kernel oil in a ratio of 50:50

a. b: Means with the same letter among treatments are not significantly different at $P \leq 0.05$.

Table 4: Reichert-Meissl number and Polenske number of BO, and mixture of PO/PKO.

Treatments	Reichert-Meissl number	Polenske number
$BO_{100\%}$	28.1 ± 1.8^{a}	1.5 ± 0.4^{a}
$BO/(PO+PKO)_{25\%}$	23.1 ± 2.9^{ab}	2.0 ± 0.5^{a}
$BO/(PO+PKO)_{50\%}$	16.1 ± 1.6^{c}	3.0 ± 0.4^{b}
$BO/(PO+PKO)_{75\%}$	9.8 ± 1.6^{d}	3.5 ± 0.5^{bc}
$(PO+PKO)_{100\%}$	2.2 ± 0.8^{e}	4.0 ± 0.3^{c}

BO: Butter oil; (PO+PKO): a mixture of palm oil and palm kernel oil in a ratio of 50:50

a. b, c, d, e: Means with the same letter among treatments are not significantly different at $P \leq 0.05$.

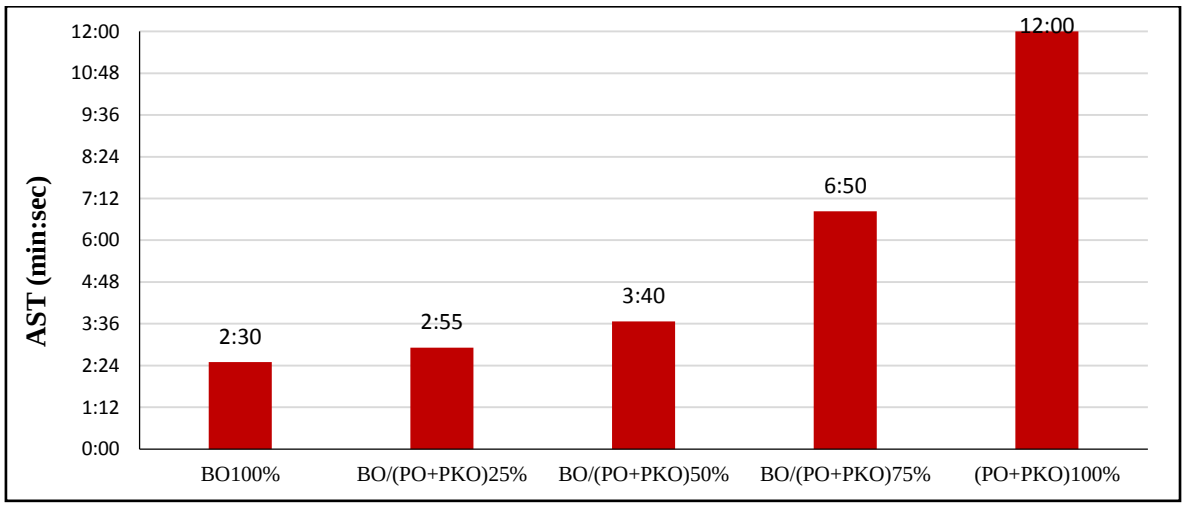

Figure1: Apparent Solidification Time (AST) of BO and mixture of PO/PKO.
BO: Butter oil; (PO+PKO): a mixture of palm oil and palm kernel oil in a ratio of 50:50

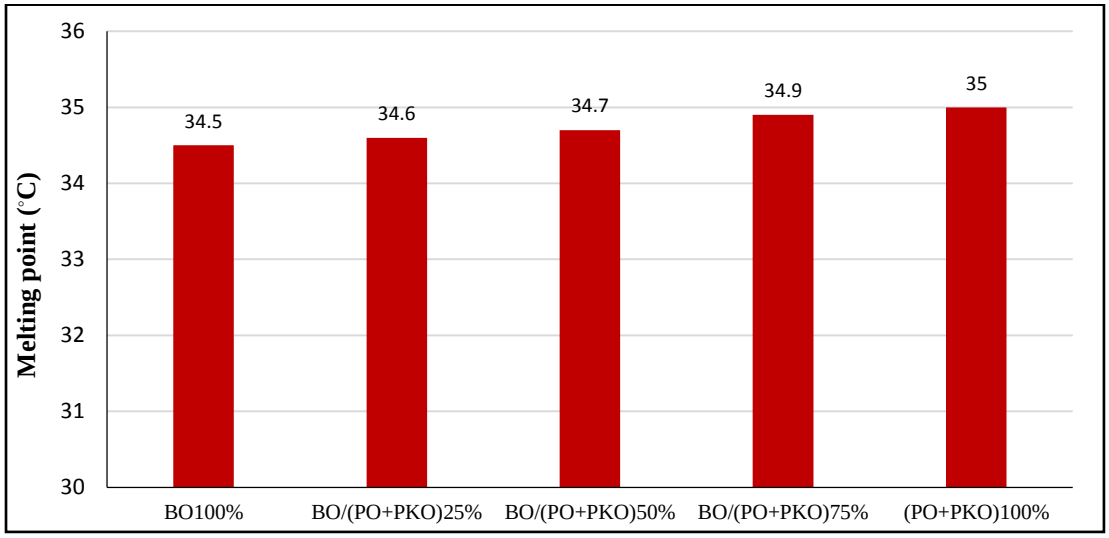

Figure 2: Melting point of butter oil and mixture of PO/PKO.
BO: Butter oil; (PO+PKO): a mixture of palm oil and palm kernel oil in a ratio of 50:50

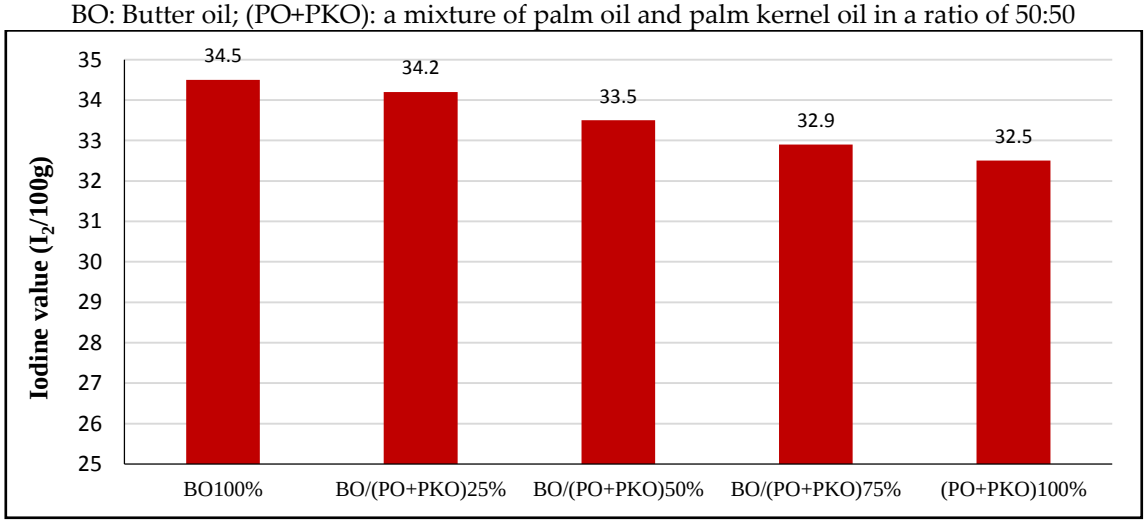

Figure 3: Iodine value of butter oil and mixture of PO/PKO.
BO: Butter oil; (PO+PKO): a mixture of palm oil and palm kernel oil in a ratio of 50:50

تقييم بعض طرق الكشف عن غش دهن اللبن بزيت النخيل وزيت نواة النخيل

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الملخص العربي

لقد كان غش دهن الحليب بأنواع أخرى من الدهون مشكلة حقيقية بسبب الأثر الإقتصادي لإستبدال دهون الحليب عالية الثمن بأخرى منخفضة الثمن مثل الزيوت النباتية. لذلك كان الهدف من هذه الدراسة هو فحص دقة وحساسية بعض الإجراءات البسيطة لكشف غش دهن الحليب بالزيوت النباتية بما في ذلك زيت النخيل وزيت نواة النخيل بمستويات مختلفة كنموذج تجاري لعينات الحليب المغشوشه. تم خلط دهن اللبن مع خليط من الزيوت النباتية (50% زيت النخيل + 50% زيت نواة النخيل) من أجل تحضير 4 معاملات تمثل غش دهن اللبن بنسب مختلفة 25% ، 50% ، 75% ، 100٪ مقارنة بعينة من دهن اللبن الطبيعي. أظهرت النتائج أن هذا الغش لم يسبب تغيرات معنوية في ثوابت الدهون خاصة عند تحديد معامل الإنكسار ودرجة الإنصهار وقيمة التصبن ورقم الإستر والرقم اليودي. يمكن استخدام إختبار وقت التصلب الظاهر وإختبار ريخرت ميسل ورقم بولنسكي للكشف عن غش دهن الحليب بخليط زيت النخيل وزيت نواة النخيل ، خاصة في المحتوى عالي المستوى من الزيت النباتي ≤ 50٪ من محتوى دهون الحليب.

الكلمات الإسترشادية: الغش, دهن الحليب, زيت النخيل, السمن.