Comparison of composition, thermal behaviour and polymorphism of pink guava *(Psidium guajava)* seed oil-palm stearin blends and lard

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Abstract

A study was carried out to compare composition, thermal behavior, and polymorphic forms of palm stearin-pink guava seed oil blends with those of lard (LD). Four blends were prepared by mixing pink guava seed oil (PGO) with and palm stearin (PS) in different ratios: PGO-1, 40:60; PGO-2, 45:55; PGO-3, 50:50; PGO-4; 55:45. The blends and lard were compared in terms of their basic physicochemical parameters, fatty acid and triacylglycerol (TAG) compositions, melting, solidification and polymorphic properties. Results showed that PGO-2 and LD were found to display similarities in terms of slip melting point value and the peak maximum of the high-melting thermal transition. In the solid fat content (SFC) profile, PGO-2 and LD were found to display the least difference. In the X-ray diffraction analysis, PGO-2 displayed both β and β' polymorphs that were similar to the polymorphic form of LD.

Introduction

Guava fruit juice as well as fresh cut fruit regularly consumed and processed widely in Malaysia. The manufacturing sector generates large amounts of seeds as waste material. According to Chek Zaini *et al.* (2009), about 100 tons of seed waste generated out of 10, 000 tons of guava fruits. Utilization of guava industry wastes can help minimize the wastes disposal problem of the industry. According to our preliminary research, guava seed can be classified as a linoleic rich oil which is good for health especially in reducing blood cholesterol. Pink guava seed oil *(Psidium guajava),* also known as PGO, still remains unexplored item in Malaysia. One way to utilize guava seed oil is to formulate halal fat as a replacement for lard, which is classified as non halal and has been used in the past for various types of products such as bread, pastries, biscuits, cakes and cookies (Marikkar and Yanty, 2014). As lard is a prohibited food item according to Islamic food laws and regulations (Che Man *et al.*, 2005), formulating an alternative is a requirement for Muslim consumers. As guava seed oil is a liquid substance at room temperature condition, it needs to be blended with a suitable solid fat derived preferably from oil palm source. Palm stearin being the solid component of palm oil that is relatively cheaper to use for fat blend formulation with unsaturated vegetable oils. Generally, palm stearin is a raw material for margarine and shortening which are used for cookies, ice cream, chocolates and cakes (Andreia *et al.*, 2009; Sahri and Idris, 2010). The production of shortening made out of palm oil has been studied extensively previously (Nor Aini, 2001). Being a less expensive product, palm stearin is very economical for shortening formulation, and it can help improve plasticity of shortenings (Nor Aini and Maimon, 1996). The use of guava seed oil-palm stearin to produce a replacement fat for lard has not been investigated previously. It is expected that blending of PGO-PS would create a good shortening with favorable nutritional characteristics due to low-level saturated and high-tri-unsaturated fatty acid content. Hence, the objective of this study is to investigate the thermophysical behavioral changes of PGO that is physically blended with PS in stimulating the properties of LD.

Materials and methods

Materials

A sample of lard was obtained from a supermarket in Malaysia. Dried seeds of pink guava were collected from Sime Darby Plantation, Sitiawan, Malaysia.

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The sample of PS was purchased from Sime Darby Plantation, Teluk Panglima Garang, Malaysia. All chemicals used in this experiment were of either analytical or HPLC grade.

Extraction of PGO and blend preparation

PGO was extracted from ground samples of dried pink guava seeds using Soxhlet extraction method with petroleum ether at 40-60°C (AOAC, 2007). The extracted oil was used to prepare blends after expelling solvent under reduced pressure using a rotary evaporator (IKA RV10 from Germany) system. A total of four blends were prepared: (PGO-1) 40:60; (PGO-2) 45:55; (PGO-3) 50:50; (PGO-4) 55:45 (w/w), and identified by the mass ratio of pink guava seed oil to palm stearin. All samples were kept in cold storage at 4°C. Prior to analyses, PGO, LD, PS and the blends were warmed at 60°C until they became completely molten.

Determination of slip melting point (SMP) and iodine value (IV)

SMP and IV of the oil and fat samples as well as the blends were determined according to AOCS method Cc.3.2.5 and AOCS method Cd Id-92, respectively (AOCS, 1999). All analyses were performed in three replicates.

GLC analysis of fatty acid methyl esters (FAME)

Fatty acid methyl esters were prepared by dissolving 50mg of the oil in 0.8 ml of hexane and adding 0.2 mL of 1 M sodium methoxide (PORIM, 1995). GLC analysis was performed on a gas chromatograph (Agilent Technologies, Singapore) fitted with an FID detector. The polar capillary column DB-wax (with 0.25 mm internal diameter, 30 m length, and 0.25 lm film thickness; Agilent Technology, Santa Clara, CA) was used. The oven temperature was programmed as follows: initial temperature of 50°C (for 1 min), programmed to increase to 200°C at 8°C/min. Both injector and detector temperatures were maintained at 200°C throughout the analysis. The carrier gas (helium) flow rate was 1.0 mL/min and the split ratio was 58:1. Identification of the samples' peaks was done with reference to a chromatographic profile containing 37 FAME standards (Supelco, Bellefonte, PA). The percentage of fatty acid was calculated as the ratio of the partial area to the total peaks area (Yanty *et al.*, 2011).

HPLC analysis of TAG composition

The TAG composition was determined using a Waters Model 510 liquid chromatograph, equipped with a differential refractometer model 410 as a detector. The TAG separation was performed on a Merck LiChrospher RP-18 column (5 μm) (12.5 cm x 4 mm i.d.; Meck Darmstach, Germany). The mobile phase was acetone/acetonitrile (63.5:36.5, v/v), and the flow rate was 1.5 mL/min. The oven temperature was maintained at 30°C. The injector volume was 10 μL of 5 % (w/w) oil in chloroform. Each sample was chromatographed three times, and the data were recorded as area percentages (Yanty *et al.*, 2011). The identification of the peaks of the sample was done using a set of TAG standards purchased from Sigma-Aldrich (Deisenhofen, Germany) as well as the TAG profiles of LD (Yanty *et al.*, 2011), and PS (Yanty *et al.*, 2014), as reported previously.

Thermal analysis by DSC

Thermal analysis was carried out using a Mettler Toledo differential scanning calorimeter (model DSC 823), equipped with a thermal analysis data station (STARe software, version 9.09, Schwerzenbach, Switzerland). Before the analysis, calibration of the instrument was done using indium as the metallic standard, based on the onset temperature of fusion and the heat of the indium's fusion. Nitrogen (99.99 % purity) was used as the purge gas at a rate of 20 mL/min. The sample was placed in a standard DSC aluminum pan and approximately 4–8 mg of molten sample was weighed and then hermetically sealed. An empty and hermetically sealed DSC aluminum pan was used as the control. The oil and fat samples were subjected to the following temperature program: 70°C isotherm for 1 min, cooled at 5°C/min to -70°C. The samples were held at -70°C isotherm for 1 min, and heated at 5°C/min to reach 70°C (Yanty *et al.*, 2011).

Determination of SFC by NMR

SFC was measured according to AOCS method Cd 16b-93 [19] using a Bruker Minispec (model Mq 20) pulse NMR spectrometer (Karlsruhe, Germany). Before the analysis, the instrument was calibrated using 0, 31.2, and 72.1% solid SFC calibration standards. The sample in the NMR tube was melted at 70°C for 15 min, followed by chilling at 0°C for 60 min, and then held at each measuring temperature for 30 min prior to measurement. Melting, chilling, and holding of the samples were carried out in pre-equilibrated thermostated glycol-containing baths, accurate to 0.1°C. SFC measurements were taken in triplicate at 5°C intervals over a range of 5–55°C (Yanty *et al.*, 2011).

Table 1. Basic physicochemical characteristics and fatty acid composition (%) of Pink
guava seed oil (PGO), palm stearin (PS), PGO-PS blend and lard

Sample	SMP (°C)	Iodine Value	Fatty acid (%)										
			C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C21:0
PGO	-	111.9±0.9b	0.0±0.0f	0.07±0.0f	7.28±0.1g	0.05±0.0d	4.6±0.3e	7.2±0.8f	79.7±0.0a	0.1±0.0b	0.45±0.0a	0.11±0.0ab	0.13±0.1a
PGO -1	50.3±0.2b	42.3±0.2d	0.1±0.0c	0.8±0.0c	40.7±0.2a	0.08±0.0c	4.9±0.8bc	18.9±0.2c	33.9±0.4e	0.15±0.0a	0.4±0.5ab	0.0±0.0	0.0±0.0
PGO -2	49.7±0.3bc	42.8±0.0d	0.1±0.0d	0.7±0.0d	37.8±0.1c	0.1±0.0c	4.9±0.1cd	18.0±0.3d	37.8±0.5d	0.1±0.0b	0.4±0.0b	0.0±0.0	0.0±0.0
PGO -3	48.7±0.3c	43.2±0.80d	0.1±0.01e	0.7±0.0d	35.7±0.1d	0.1±0.0c	4.8±0.0d	17.2±0.4e	41.0±0.15 c	0.1±0.0b	0.4±0.1b	0.0±0.0	0.0±0.0
PGO -4	43.6±1.0d	50.6±4.9c	0.1±0.0f	0.6±0.0e	31.4±0.1e	0.1±0.0e	4.6±0.1e	17.0±0.1d	46.2±0.5b	0.1±0.0c	0.0±0.0d	0.0±0.0	0.0±0.0
Lard	35.8±0.7e	65.3±1.9b	0.3±0.0a	1.8±0.0a	26.1±0.0f	1.3±0.1a	16.7±0.4a	33.5±0.13 a	18.7±0.02f	0.9±0.0a	0.3±0.1c	0.2±0.1a	0.0±0.0
PS	52.7±0.2a	35.7±0.2e	0.2±0.0b	1.1±0.0b	57.5±0.2a	0.1±0.0b	5.0±0.1b	29.1±0.1b	6.3±0.1g	0.1±0.0b	0.4±0.0b	0.1±0.0bc	0.0±0.0

Each value in the table represents the mean of three replicates. Means within each row bearing different superscripts are
significantly (P, 0.05) different; C10:0, Capric acid; C12:0, Lauric acid; C14:0, Myristic acid; C16:0, Palmitic acid; C16:1,
Palmitoleic acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid, C18:3, Linolenic acid; C20:0, Arachidic acid;
C20:1, Erucic acid; C21:0, Heneicosanoic acid; C22:0, Behenic acid.
[1]Abbreviations: PGO, pink guava seed oil; PS, palm stearin; Temp, temperature

X-ray diffraction analysis

The polymorphic forms of fat crystals were determined using a wide angle X-ray diffraction (WAXD) machine (D8 Advance Bruker AXS, Karlsruhe, Germany). Before analysis, samples were held at 25°C at room temperature. The power used was 40 kV, 40 mA with the source of the beam from Cu Kαl X-ray beam (λ = 0.15406 A˚). The samples were scanned from 15°2θ to 25° 2θ, increasing with a step size of 0.025°/0.1 sec (Ribeiro *et al.*, 2009). Short spacing on the X-ray film was measured with Evaluation Diffract plus software. The short spacing of the β' form was at 4.2 and 3.8 A°, whereas those of the β form were at 4.6 A° (Dsouza *et al.*, 1991).

Statistical analysis

All results from analysis were expressed as the mean value ± standard deviation. Data were statistically analyzed by one-way analysis of variance (ANOVA) using Tukey's test of the MINITAB (version 16) statistical package at 0.05 probability level.

Results and Discussion

SMP and IV

The data presented in Table 1 compared the IV and SMP of the blends with those of LD and PS. The IV of PS was 35.71, which was higher than the findings reported by Yanty *et al.* (2014). The IV of PGO was 111.95 and was almost similar to the value reported in

a previous study (Prasad and Azeemodin, 1994). LD, on the other hand, was found to have an IV of 65.33 and SMP of 35.8°C. All blends of this study displayed an IV significantly (p<0.05) lower than those of the original PS and LD samples. According to the data presented in Table 1, the SMPs of the blends were found to range from 43.6 to 50.3°C. Among them, PGO-2 was found to display an SMP value somewhat closer to that of LD which was less than 10.0 unit difference.

Fatty acid composition

The fatty acid distributional patterns of the blends were compared to those of PS and LD as shown in Table 1. The PS sample was found to possess a higher proportion of palmitic acid (57.5%) but lower proportions of linoleic (6.3%) and stearic (5.01%) acids. PGO, on the other hand, was found to have a higher proportion of linoleic acid (79.69%) but lower proportions of palmitic (7.28%) and oleic (7.21%) acids. LD was found to possess higher proportions of oleic (33.46%) and palmitic (26.13%), where the proportion of oleic was the highest among other samples but the proportion of palmitic was least compare to PS and all blends. The addition of PGO (40 - 55%) into PS was found to cause a gradual increase in the proportions of linoleic acid (from 33.91 to 45.42%) and moderate reductions in the amounts of palmitic acid (from 40.72 to 26.13%). With respect to the original sample of PS, the total saturated fatty acid content increased which leads

Table 2. Triaclyglycerol (TAG) composition of Pink guava seed oil (PGO), Palm stearin (PS), PGO-PS blends and lard

	PGO-1	PGO-2	PGO-3	PGO-4	PGO	Lard	PS
LLLn	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.1±0.00ᵃ
LLL	20.1±0.1ᵉ	22.5±0.1ᵈ	24.7±0.0ᶜ	27.1±0.1ᵇ	50.7±0.3ᵃ	0.0±0.0ᶠ	1.0±0.0ᶠ
OLL	6.0±0.0ᵈ	6.7±0.0ᶜ	7.3±0.0ᵇ	8.0±0.0ᵇ	14.8±0.1ᵃ	0.0±0.0ᵉ	2.9±0.0ᵉ
OOL	0.1±0.0ᵈ	0.2±0.0ᶜ	0.2±0.0	0.2±0.0ᶜ	1.1±0.1ᵇ	0.1±0.0ᵈ	4.0±0.0ᵃ
OOO	1.6±0.0ᶜ	1.6±0.0ᶜᵈ	1.5±0.0ᶜ	1.5±0.0ᶜᵈ	0.0±0.0ᵈ	2.2±0.0ᵇ	2.8±0.0ᵃ
PLL	7.8±0.0ᵈ	8.6±0.0ᶜ	9.4±0.0ᵇ	10.3±0.0ᵇ	18.2±0.1ᵃ	0.0±0.0ᵉ	7.1±0.1ᶠ
POL	8.0±0.0ᶠ	8.3±0.1ᵉ	8.7±0.0ᵈ	9.3±0.0ᶜ	12.7±0.1ᵇ	5.7±0.0ᵍ	18.0±0.0ᵃ
POO	8.2±0.1ᶜ	7.6±0.0ᵈ	7.0±0.1ᵉ	6.9±0.0ᵉ	0.0±0.0ᶠ	13.6±0.0ᵇ	18.4±0.1ᵃ
OOS	0.6±0.0ᵇ	0.5±0.0ᵇᶜ	0.4±0.1ᶜ	0.4±0.0ᶜ	0.0±0.0ᵈ	1.9±0.1ᵇ	2.8±0.0ᵃ
PPO	19.0±0.1ᵇ	17.3±0.1ᶜ	16.0±0.03ᵈ	16.0±0.04ᵈ	0.0±0.0ᶠ	33.7±0.0ᵃ	14.3±0.1ᵉ
MPL	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.2±0.01ᵃ	0.8±0.2ᵇ
POS	2.5±0.00ᵈ	2.2±0.0ᵉ	2.0±0.0ᵉ	2.3±0.0ᵈ	0.0±0.0ᶠ	5.6±0.1ᵇ	16.6±0.1ᵃ
PPL	4.6±0.0ᵇ	4.2±0.0ᶜ	4.0±0.01ᶜ	4.0±0.0ᶜ	0.0±0.0ᵉ	8.0±0.04ᵃ	2.4±0.1ᵈ
SOS	0.2±0.0ᵇ	0.2±0.0ᵇ	0.2±0.0ᵇᵈ	0.2±0.02ᵇᵈ	0.0±0.0ᵉ	0.5±0.0ᵇ	1.1±0.0ᵃ
MPP	1.2±0.02ᵃ	1.1±0.02ᵃ	1.0±0.01ᵇ	0.7±0.0ᶜ	0.0±0.0ᵈ	1.6±0.0ᵃ	0.0±0.0ᵈ
MMP	1.0±0.0ᵇ	1.0±0.0ᵇ	1.0±0.0ᵇ	1.1±0.0ᵇ	0.0±0.0ᶜ	0.9±0.0ᵇ	0.0±0.0ᶜ
PPP	15.9±0.1ᵇ	14.7±0.0ᶜ	13.5±0.0ᵈ	9.6±0.0ᵉ	0.0±0.0ᶠ	20.9±0.0ᵃ	0.5±0.0ᶠ
MMM	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.0ᵃ	0.0±0.0
PPS	3.0±0.0ᵇ	2.8±0.0ᶜ	2.5±0.1ᵈ	1.8±0.0ᵉ	0.0±0.0ᶠ	4.1±0.1ᵃ	1.9±0.0ᵉ
PSS	0.4±0.0ᵇ	0.3±0.0ᵇᵈ	0.2±0.0ᵈ	0.2±0.1ᵈ	0.0±0.0ᵉ	0.4±0.1ᵇ	2.7±0.0ᵃ
UK1	0.4±0.0ᵇ	0.4±0.0ᵇᵃ	0.4±0.0ᵇ	0.4±0.0ᵇ	0.9±0.0ᵃ	0.0±0.0ᶜ	0.0±0.0ᶜ
UK2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.5±0.6ᵃ	0.0±0.0	0.0±0.0
Others	0.4±0.00ᵇ	0.4±0.00ᵇ	0.4±0.0ᵇ	0.4±0.0ᵇ	2.5±0.6ᵃ	0.0±0.0ᶜ	0.0±0.0ᶜ
UUU	27.8±0.1ᵉ	30.9±0.0ᵈ	33.7±0.0ᶜ	36.8±0.1ᵇ	66.6±0.5ᵃ	2.3±0.0ᶠ	11.7±0.0ᶠ
UUS	24.5±0.04ᶠ	25.0±0.04ᵉ	25.5±0.1ᵈ	26.9±0.0ᶜ	30.9±0.2ᵇ	20.8±0.1ᵃ	46.2±0.2ᵃ
USS	26.3±0.2ᵇ	23.9±0.1ᵈ	22.1±0.0ᵉ	22.5±0.0ᵉ	0.0±0.0ᶠ	48.9±0.1ᵃ	37.0±0.2ᵇ
SSS	21.4±0.2ᵇ	19.8±0.0ᶜ	18.3±0.0ᵈ	13.4±0.0ᵉ	0.0±0.0ᶠ	28.2±0.1ᵃ	5.0±0.0ᶠ

Each value in the table represents the mean of three replicates. Means within each row bearing different superscripts are significantly (P< 0.05) different; O: oleic; P: palmitic; L: linoleic; Ln: linolenic; M: myristic; U: unsaturated; S: saturated; UK, unknown.
[1]Abbreviations: PGO, pink guava seed oil; PS, palm stearin; Temp, temperature.

to a decline in the IVs of blends as seen in Table 1. Among the blends, PGO-2 showed the closer value to lard which gave less than 15.0 points differences in palmitic acid.

TAG composition

TAG distributional patterns of the blends were compared with those of PS and LD as shown in Table 2. The major TAG molecular species of PS were POO, POL, POS and PPO, comprising 67.33% of the total. The proportion of the monosaturated (PLL, POL, POO and OOS) and disaturated (PPO, MPL, POS, PPL and SOS) TAG contents of PS were 46.22% and 37.00%, respectively. PGO, on the other hand, was found to possess LLL (50.72%) as the most dominant TAG, followed by PLL (18.21%), OLL (14.75%) and POL (12.72%). For LD, the content of tri-unsaturated TAG was 2.34%, whereas the contents of monosaturated, disaturated and trisaturated were 20.58, 47.88 and 28.22%, respectively. With the increasing concentration of PGO in the blend series, the proportions of LLL, OLL, PLL and POL increased. The increases in the proportions of tri-unsaturated TAG molecular species were remarkable as the proportion of PGO in PS was tended to increase from 27.79 to 36.79% (Table 2). For instance, the proportion of tri-unsaturated TAG molecules of PGO-4 was 36.79%, which was higher than PGO-1 (27.79%). With the increase of PGO, a significant increase in the monosaturated TAG molecular species could also be noticed from 24.49 to 26.93% in the blend (Table 2). Further, the proportion of monosaturated TAG molecular species of PGO-4 was 26.93% which were nearly 2.5% higher than that of PGO-1. The increasing proportions of disaturated as well as trisaturated TAGs in the fat blends compare to the sample of PGO could be remarkable as the increasing of PS in the blend as noticed in Table 2. All values of blends were not close to that of LD, but with the incorporation of PGO and PS, it produced the values disaturated as well as trisaturated which led to the occurrence of palmitic as can be observed in the overall fatty acid distribution.

Thermal Characteristics

Melting curves

DSC thermal profiles of the blends are compared with those of PS, PGO, and LD as shown in Figure 1. The melting curve of PS was characterized by the appearance of three endotherms; two smaller lower melting transition at f1 (8.21°C) and f2 (41.60°C) and a large higher melting transition at f3 (53.59°C). The high melting transition was due to the high proportion of saturated TAG in PS as noticed in previous Table 2. On the other hand, the melting curve of PGO was characterized by a major transition at -17.51°C with a shoulder peak at -7.18°C, and minor transition at -50.60°C. As the completion of the endothermic thermal transition is marked at -4.51°C, no further thermal transition was observed beyond that point. For the sake of comparison, there is hardly any report that deals with the DSC thermal characteristics of PGO. Once PS has been blended with PGO, the amount of disaturated (37.00%), as well as trisaturated (5.06%) TAG molecular species, was found to change significantly in the blends with the concurrent reductions in the proportions of disaturated TAG molecular species (Table 2). Meanwhile, blending PGO with PS steadily changed the unsaturated to saturated ratio. These changes in fatty acid and TAG compositions would affect the thermal profile of blends so that all blends displayed high melting thermal transition in the temperature region above 10°C. In a previous study by Norizzah *et al*. (2004), mixing palm olein with PS gave rise to the appearance of a broad peak in the high-melting region of the thermal curve of PS. This difference could be due to fatty acid and TAG compositional differences between palm olein and PGO. The enthalpy and peak temperature of the high-melting peak of the thermal curve in the present study were found to decrease gradually with increasing proportion of PGO in blends. According to Figure 1, the peak maxima of the high-melting thermal transition for PGO-1, PGO-2, PGO-3 and PGO-4 were b3 (50.32°C), c3 (49.82°C), d3 (49.41°C) and e_3 (46.26°C), respectively. It was clear that the peak maxima of PGO-4 showed the closest value to that LD which had peak at 44.20°C (g4). When compared to the original PGO sample, the end of the melting curves' temperatures in the blends were also found to increase significantly with the increasing saturated contents in the blends. The polymorphic behavior of blends could also be predicted from DSC thermograms (Zeiton *et al*., 1993). According to Figure 1, the profile patterns of PGO-1, PGO-2 and PGO-3 were similar within ranges of -36.39 to 0.16°C and 39.07 to 52.32°C.
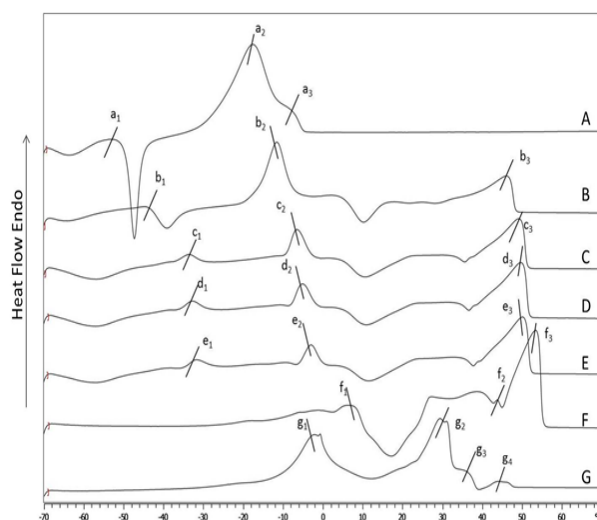

Figure 1. Melting curve profile of PGO (A), PGO-4 (B), PGO-3 (C), PGO-2 (D), PGO-1 (E), PS (F) and Lard (G). PGO, Pink guava seed oil; PS, palm stearin

All three blends displayed two peaks in the range of -36.39 to 0.16°C and a single peak in the range of 39.07 to 52.32°C.

SFC Profiling

Solid fat content is an important test that could help to determine the application of fat blends. The SFC profiles of the fat blends were compared to those of LD, PGO and PS as shown in Figure 2. The SFC of PGO at 5°C was found to be 0%, while those of LD and PS at the same temperature were 43.26 and 84.82%, respectively. The SFC of LD was found to drop to 23.01 at 25°C and tended to decrease until becoming zero at 45°C. According to our literature search, there was hardly any previous study to show the values SFC of PGO. Owing to the high content of unsaturated fatty acid in PGO (Table 1), it would not solidify even at 4°C. The SFC of PS, on the other hand, was found to be 84.83% at 5°C and became to almost zero at 55°C. The SFC values of the blends were found to decrease significantly with the increasing proportion of PGO. At 5°C, the SFC values of PGO-1, PGO-2, PGO-3 and PGO-4 were 47.19, 47.19, 38.16 and 34.38%, respectively. The decreasing SFC values were found to correlate well with the increasing proportion of unsaturated TAG molecules of the fat blends (Table 2). Of these, blend PGO-2 was found to display closer to the SFC values of LD at several temperatures, which include 15, 20, and 25°C. At 30°C, SFC value of LD (12.434%) was found to be in between PGO-3 (13.70) and PGO-4 (11.81%). Among the four blends, PGO-1 and PGO-2 showed better compatibility to LD at most temperatures. This was further confirmed by the calculations done with respect to least difference to LD where PGO-1 and PGO-2 were found to have
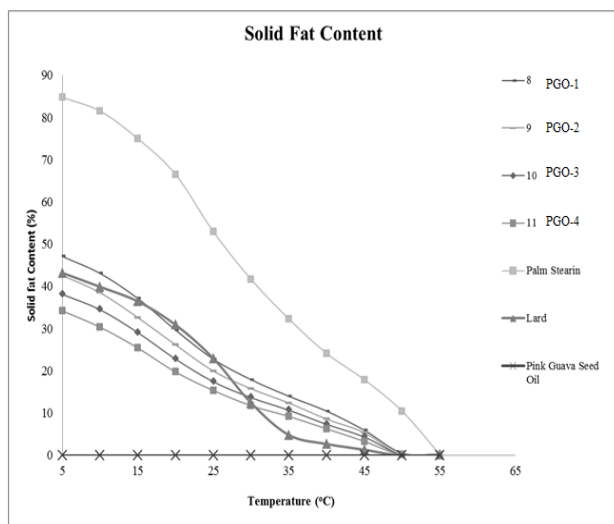
Figure 2. Solid fat content (SFC) profiles of lard, Pink guava seed oil (PGO), Palm stearin and blends of PGO-PS.

the least difference to lard in terms of SFC values throughout the temperature range. This was due to the fact that the SFC values of PGO-1 and PGO-2 were found to become closely similar to that of LD at 10, 15, 20, 25 and 30°C.

Polymorphism

According to the diffractogram presented in Figure 3, PS was found to display crystals in β form where the XRD pattern displayed a strong peak at around 4.60Å. The three major polymorphic forms of fats are α, β' and β (de Man, 1992). The α form is the least stable, β the most stable and conversion of one form to another is only possible in the direction of more stable forms. The chain packing of the α form to another is only possible in the direction of more stable forms. The chain packing of the α form is hexagonal, the β' form orthorhombic and the β form triclinic (de Man, 1992). According to de Man (1992), the short spacing of the major polymorphs are; α with a single spacing at 4.15Å, β' with two strong spacings at 3.80 and 4.20Å or three spacings at 4.27, 3.97 and 3.71Å, and β with a very strong spacing at 4.60Å. The sharp peak of PS as shown in Figure 3 was similar to those reported previously by Campbell *et al.* (2002). This could be due to the TAG molecular species of PS which are dominated by POO, POL and POS molecular species (Table 2). As shown in Figure 3, LD displayed both β' and β form polymorphs, of which both were found to be dominant. This could be due to the content of disaturated TAG molecular species such as PPO, POS and PPL. As shown in Table 2, this TAG molecular group becomes an important component of LD, contributing up to 33% of the total. According to Timms (1984), POS (5.56%) could
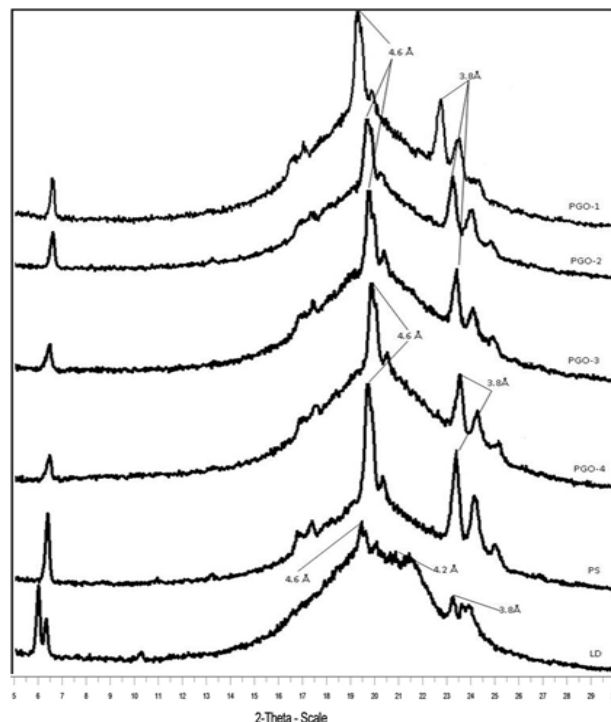

Figure 3. WAXD pattern of lard (LD), palm stearin (PS) and blends of pink guava seed oil and palm stearin (PGO-1, PGO-2, PGO-3 and PGO-4) at 25°C.

significantly contribute to the β' crystal polymorph's development in LD. Crystallization depends on the chemical composition of fats; thus LD crystallized in either β' form in rapid crystallization or both β' and β forms in slow crystallization (Campos *et al.*, 2002). As LD was exposed to a change in temperature from 70 to 4°C, crystallization has been very effective. With the decrease in crystallization temperature, the rate of nucleation becomes rapid, leading to the formation of a large number of nuclei, which resulted in smaller crystals. The tendency for this smaller crystal formation was observed as β' form polymorph in LD.

The short spacing reflections of fat blends (PGO-1, PGO-2, PGO-3 and PGO-4) were detected at 4.6Å as well as at 3.8Å which represent both β' and β form (D'Souza *et al.,* 1991; Ribeiro *et al.,* 2009). The presence of both β and β' indicates that the blend of PGO-PS has created a tendency to display both forms, which is the same polymorphic form as LD owing to the increasing proportion of the disaturated TAG molecular group. Raising the disaturated TAG molecular group in the blend will cause the rate of nuclei formation to become more effective and enhance the small crystal formations at crystallization treatment of 70 to 4°C. The ease with which this transformation will take place is related to the composition of the fat in terms of component fatty acids as well as in the structure of the triglycerides

(deMan, 1992).

Conclusions

This study showed the possibility of utilizing pink guava seed oil (PGO) to produce a fat blend with PS in order to imitate some physical properties of LD. The results of SMP and SFC, as well as fatty acid composition of PGO-2 (PGO:PS; 45:55), would produce a fat blend that has closer to LD. This result was supported by X-ray diffractograms which showed both β and β' that indicates as LD polymorphic form. In order to verify these findings, further studies should be done to evaluate the compatibility of blends as food ingredients for cookies, pie crust and cakes.

Acknowledgements

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