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Physical and textural properties of an experimental table margarine prepared from lipase-catalysed transesterified palm stearin: palm kernel olein mixture during storage

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Abstract

The storage performances of an experimental table margarine prepared from *Rhizomucor miehei*-catalysed transesterified palm stearin (PS): palm kernel olein (PKO) (40:60) mixture and a commercial margarine, stored at temperatures of 20 and 30°C were evaluated for their slip melting point (SMP), peroxide value (PV), cone penetrometry (CP), storage modulus (G') and solid fat content (SFC). All samples showed acceptable PV levels after three months of storage. Cone penetrometric, SMP and SFC results indicated that generally the experimental samples were firmer than the commercial samples. Significant correlations were observed between SFC and penetration values ($r = 0.82$; $P < 0.01$). Interestingly, the storage modulus (G') values and yield values of the samples also showed significant correlations ($r = 0.89$; $P < 0.001$). © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Margarine was originally developed in 1869 as an alternative to butter which was in short supply and expensive (Chrysam, 1996). The first margarines were made from animal fats, but today most margarines are formulated from vegetable oils. The product range now includes table margarines, bakery margarines, and specialised puff pastry margarines and has now been extended to various low calorie spreads which essentially contain much higher levels of water and lower levels of fat than those legally required in margarines.

Table margarines fall into two main types: packet margarines which are designed to be spreadable at ambient temperature, and tub margarines which are spreadable on removal from the refrigerator, at a temperature of 5–10°C. Typical solid fat content (SFC) curves for the two types show much lower SFC in the tub margarines. When packet margarines are intended for use in a tropical climate, with ambient temperatures around 30°C, higher solids contents are required (Rasid, Jaais, Yusoff & Elias, 1996).

With the availability of hydrogenated oils of various grades, the choice ingredients for blends with the desired solids content profiles is very large indeed. Individual manufacturers will usually base their choice on local availability and on price, as well as the technical consideration of a fine crystal structure to give the required spreadable character.

The rheological characteristics of finished margarine are expressed in terms such as consistency, texture, plasticity, hardness, structure and spreadability. Studies on the effect of storage conditions on the quality of retail margarine have a tendency to focus on the changes in the physical, chemical and rheological properties that occur during storage. The effect of storage on the sensory properties of the product also has great importance to the manufacturers, distributors and consumers.

In this study, margarines prepared using lipase-transesterified palm stearin: palm kernel olein (PS:PKO) feedstock (experimental margarine), and commercial margarine were stored at 20 and 30°C for 3 months and evaluated at regular intervals. One of the aims of this work was to maximise the use of palm stearin, which a cheaper fraction obtained from fractionation of palm

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oil. The minimum quantity of PS that is usually added to a standard table margarine formulation is 10% (Teah, 1982). To maximise the use of palm stearin, high levels of the fat (minimum 40%) were used in this work. The assumption is that, if a suitable table margarine formulation that is spreadable at room temperature can be obtained with a minimum of 40% PS, then we should expect to obtain softer products at lower percentages of PS in the blend. Testing techniques for evaluating the physical and textural properties of the experimental and commercial margarine included slip melting point (SMP), peroxide value (PV), penetrometry, solid fat content (SFC) and viscoelasticity.

2. Materials & methods

2.1. Materials

Refined, bleached and deodorised hard palm stearin (PS) (slip melting point, 54.5°C) was obtained from Ngo Chew Hong Oils and Fat (M) Pte. Ltd., while palm kernel olein (PKO) was obtained from Southern Edible Oil (Malaysia) Ind. Pte. Ltd. The fats were stored at 4°C. Prior to use, palm stearin was melted at 60°C in the oven. *R. miehei* lipase (Lipozyme 1M60) was obtained in the immobilized form (moisture content: 2–3%) from Novo Nordisk Ind. (Copenhagen, Denmark).

2.2. Transesterification reaction

A minimum of 50 kg of *R. miehei* lipase-catalysed transesterified blends of PS:PKO at 40:60 ratio was prepared for the fat phase of the experimental margarine. The reaction blend (100 g PS:PKO/g lipozyme) was agitated in a flask in an orbital shaker at 200 rpm at 60°C for 6 h. The same enzyme after removal from the reaction mixture, was re-used to esterify a total of five changes of fresh substrate. The transesterification reaction was according to the method described by Ghazali, Hamidah and Che Man (1995).

2.3. Processing and storage conditions

The production of the experimental table margarine was carried out using Palm Oil Research Institute of Malaysia (PORIM)'s pilot plant, "Kombinator" (Schröder, Lubeck, Germany), that is basically made up of two scraped-surface heat exchanger units and a worker unit. Prepared margarine blends were stored at 49°C. From storage, the molten oil was pumped into a tank. The heat exchanger precools the molten oil to 22.3°C. In the worker unit, the temperature was maintained at 25.6°C. The oil is later chilled to −8°C and −10°C in coolers 1 and 2, respectively. The composition of the experimental table margarine was in compliance with the definition of margarine (81% transesterified fat, 16% water, 0.4% soy lecithin emulsifier, 0.003% colouring, 0.03% flavour, 2% salt and 0.02% antioxidants). Commercial margarine [Unilever (M) Holdings Pte. Ltd.] samples were purchased from a local supermarket in Kajang, Malaysia. Information on the label stated that it contained palm oil and palm kernel oil. Knowledge of the age and past history of the sample was minimal. The experimental table margarine was stored in covered plastic containers (base diameter, 9.3 cm; top diameter with cover, 11.7 cm; height, 7.5 cm) while the commercial table margarines were stored in their original metal containers (250 g/container). The samples were immediately stored at different test temperatures (20 and 30°C) for 3 months and analyzed weekly for rheological changes. The initial week of storage for both the margarines were termed as week 0 (days 0–7) and subsequent weeks as weeks 1–12 (days 8–90). The commercial margarine, stored at 20 and 30°C, was abbreviated CM20 and CM30, respectively, while the experimental margarine was designated EM20 and EM30, respectively.

2.4. Slip melting point (SMP)

The SMP test is based on the AOCS Cc 3-25 (Firestone, 1989) method. The tests were done in replicates at weeks 0, 3, 6, 9 and 12.

2.5. Peroxide value (PV)

PV was determined according to the AOCS method Cd 8-53 (AOCS, 1987). PV tests were done in replicates at weeks 0, 3, 6, 9 and 12.

2.6. Cone penetrometric (CP) measurements

The hardness of the commercial and experimental margarine was evaluated by means of a cone penetrometer at 20 and 30°C. The samples and the cone penetrometer were stored at the test temperature for 24 h prior to measurements. The CP had an angle of 40° and the weight of the cone assembly was 79.03 g. The penetrating cone was placed just above the surface of the sample before it was released. The penetration depth in 0.1 mm increments was read on the dial after 5 s of contact and four readings were taken for each sample at weekly intervals. The yield value (C value) is calculated according to the formula of Haighton (1959). To obtain good reproducibility, only penetration depths between 75 and 250 units were taken into consideration. The calculations are as shown below:

$$C = \frac{k \times W}{p^{1.6}}$$

where C = yield value (g/cm^2)
k = constant (5840 for 40° cone angle)
W = weight of cone (79.03 g)
p = penetration depth (in mm)

2.7. Solid fat content (SFC)

A Bruker Wideline NMR (Karlsruhe, Germany) using a direct measurement procedure was employed for the SFC measurements. A stainless steel plunger was used to obtain representative samples of the commercial and experimental samples stored at 20 and 30°C. The samples were then placed in tubes that had been kept at both the test temperatures. The SFC of the samples at 20 and 30°C were measured in replicates at weeks 0, 3, 6, 9 and 12.

2.8. Viscoelasticity measurements

A Haake RS100 controlled-stress and rate rheometer (Haake GmbH, Kalsruhe, Germany) was used to measure the viscosity and viscoelasticity behaviour of the experimental and commercial margarine samples. The rheometer has a stabilised low inertia air-bearing and a high resolution digital encoder. Temperature control was maintained by a Haake F3 circulator waterbath with an accuracy of ±0.02°C (Haake GmbH, Karlsruhe, Germany).

All commercial and experimental samples were carefully scooped out with a flat-based stainless steel spatula to minimise sample deformation. Samples were conditioned to the test temperatures before measurements were taken. Dynamic oscillatory measurements were performed with a 35 mm diameter with serrated parallel plate geometry (PP35) to avoid slippage of samples. Evaluation of the linear viscoelastic (LVE) range was performed at a frequency of 1 Hz. Storage modulus (G') and loss modulus (G'') parameters were obtained. Small frequency sweeps from 0.01 to 100 Hz were carried out within the LVE region to avoid the destruction of the samples. Storage modulus (G') was the parameter chosen for comparison purposes.

Table 1
Slip melting point (SMP) of experimental and commercial table margarines during storage

	Slip melting point (°C)			
	Storage temperatures (°C)			
	20°C		30°C	
Storage weeks	Experimental margarine (EM20)	Commercial margarine (CM20)	Experimental margarine (EM30)	Commercial margarine (CM30)
0	41.0	39.5	40.5	38.5
3	41.0	40.0	41.0	39.0
6	43.0	40.0	42.0	39.5
9	44.0	40.0	43.0	39.5
12	47.0	40.5	46.5	39.5

2.9. Statistical analyses

The statistical results presented were obtained using the general linear model (GLM) procedure available in the Statistical Analyses System (SAS) software package and separations of the means were carried out using the New Multiple Range Duncan test at a confidence level of 95 and 99%.

3. Results and discussion

3.1. Changes in slip melting point (SMP)

During SMP measurements, the sample temperature is raised and the solid fat melts. The progressive reduction of crystalline matter means that, at a certain temperature, the fat crystal network lacks sufficient cohesion to hold onto its matrix and becomes sufficiently soft until it suddenly rises (Rousseau, Hill & Marangoni, 1996). The temperature of a SMP usually corresponds to a stage of melting in which there is about 4% residual solid fat (Pocklington & Hautfenns, 1986).

Table 1 shows the SMP of the experimental and commercial samples at storage weeks of 0–12. Experimental samples stored at both 20°C (EM20) and 30°C (EM30) showed the greatest increase [6°C (14.6%) over week 0 (control) samples] in SMP after 3 months of storage. The rate of increase was greatest during the last 3 weeks of storage (weeks 9 to 12). In the first 9 weeks of storage, experimental margarine stored at 20 and 30°C showed an increase of SMP of about 3°C (7.3%) while, in the last 3 weeks of storage, the SMP increased by another 3°C (7.3%), indicating that the experimental samples were getting firmer, probably due to the formation of β crystals which would make the end-product undesirable. As for samples CM20 and CM30, an increase of only 1°C (2.6%) was observed after almost 13 weeks of storage, indicating that the SMP of the commercial samples were constant and stable.

Separation of means using the New Multiple Range Duncan test showed that the CM20 and CM30 samples were not significantly different from each other; neither were CM20 and EM30 ($P > 0.001$). Significant differences in SMP with storage were observed at the 95% confidence interval. All the samples at storage weeks 0, 3, 6 and 9 were not significantly different from one another; neither were the samples at week 6, 9 and 12.

3.2. Changes in peroxide value (PV)

The most common cause of oil deterioration is rancidity, and the most common cause of rancidity is oxidation. It is generally accepted that the first products formed by oxidation of an oil are hydroperoxides. The usual method of assessment is by the determination of the peroxide value (PV), which is reported in units of milli-equivalents of oxygen per kg of fat (Rossell, 1986).

Oxidative stability is important for obvious reasons. It is undesirable for products to develop rancidity during storage before use and it is important to avoid rancidity in the finished products for as long as possible. Freshly deodorised oil should have a PV of less than 1 unit (Rossell, 1986).

Table 2 shows the PV of the commercial and experimental margarines at different storage times. After three months of storage, all the samples (commercial and experimental) showed acceptable levels of PV (<2 meq O_2/kg) with both the experimental and commercial samples placed at 20°C exhibiting lower PV than their counterparts at 30°C. The slight increase in PV at higher temperatures (30°C) could be due to the higher tendency of a "softer" fat to oxidise as compared to "harder" fats (Lusas & Riaz, 1996). The PV for EM20 and CM20 samples were similar. However, for the samples kept at 30°C, the experimental sample exhibited slightly higher PV than the commercial samples. It has been shown that peroxides develop very slowly at 21.1°C but increase markedly at 37.8°C, probably because oxygen diffuses more rapidly into the reaction interface at higher temperatures (Mounts & List, 1996). The oxidation rate doubles for every 11.1°C increase in temperature, the practical significance of this is obvious. To minimise peroxide formation and the flavour problems that accompany it, products should be stored at as low a temperature as practical (Mounts & List). The generally higher PV values obtained in the experimental samples compared to the commercial samples could be due to the higher iron content in palm stearin as compared to palm oil in the commercial samples.

Statistical ANOVA analysis indicates significant differences between the commercial and experimental samples ($P<0.001$). As expected, the New Multiple Range Duncan test shows that the means of the CM20 and EM20 samples were not significantly different from each other. Samples CM30 and EM30, however, were each significantly different from the others. This is probably due to the fat being kept at higher temperatures.

Table 2
Peroxide value (PV) of experimental and commercial table margarines during storage

	Peroxide value (meq O_2/kg)			
	Storage temperature (°C)			
	20°C		30°C	
Storage weeks	Experimental margarine (EM20)	Commercial margarine (CM20)	Experimental margarine (EM30)	Commercial margarine (CM30)
0	1.38	1.28	1.70	1.50
3	1.38	1.34	1.70	1.50
6	1.40	1.34	1.72	1.52
9	1.40	1.40	1.72	1.52
12	1.40	1.40	1.74	1.54

3.3. Changes in consistency

The cone penetrometer (CP) is a rapid, yet empirical method used in the evaluation of texture (de Man, 1983). It measures the depth of penetration of a cone assembly of specific dimensions and weight into a given sample after 5 s. During penetration of the cone assembly, the cone sinks into the fat sample until the stress exerted by the increasing contact surface of the cone is balanced by the hardness of the fat (de Man, 1983). In so doing, the crystal aggregate network is partially and irreversibly destroyed (Le Fèbvre, 1983).

Table 3 displays the yield value (g/cm^2) of samples analysed from week 0 to 12. The experimental samples placed at 20°C showed an increase in yield value (hardness) in the first 5 weeks of storage. Thereafter, the yield values remained rather constant. Statistical ANOVA analyses show that there was no significant difference in the hardness of all the samples during storage, including the slight increase in the yield value of the EM20 samples. All the other samples (EM30, CM20 and CM30) had rather constant yield values throughout the 3 months of storage (Table 3). Among all the samples, EM20 generally had the highest yield value, indicating a firmer product, followed by EM30, CM20 and CM30

Table 3
Yield value (g/cm^2) of experimental and commercial table margarines during storage

	Yield value (g cm^2)			
	Storage temperatures (°C)			
	20°C		30°C	
Storage weeks	Experimental margarine (EM20)	Commercial margarine (CM20)	Experimental margarine (EM30)	Commercial margarine (CM30)
0	985.8.	272.3	656.5	76.9
1	985.5	288.9	624.4	71.8
2	981.7	270.2	612.0	80.2
3	985.8	241.9	610.1	70.3
4	991.8	195.5	601.0	70.7
5	991.8	265.3	589.4	66.6
6	996.0	256.3	616.7	71.3
7	996.0	260.2	624.4	66.4
8	996.0	270.2	616.7	68.1
9	991.8	242.9	636.1	66.7
10	996.0	248.9	612.0	67.1
11	996.0	243.6	621.5	71.5
12	996.0	246.4	621.5	69.4

samples. Haighton (1959) studied the relationship between margarines and shortenings and he test-ranked them subjectively from very soft to very hard. Based on the subjective test (thumb test) of Haighton, sample EM20 is considered hard but still satisfactorily spreadable. Samples EM30 and CM20 were plastic and spreadable while sample CM30 was very soft.

Statistically, the mean yield values of all the samples were significantly different from one another ($P < 0.001$). The higher yield value for the EM20 samples suggested a more structured, denser crystal network than the others while placing the experimental samples at 30°C (EM30) gave a substantially softer product. This was interpreted as a structurally weaker network, given the lower SFC value obtained as well (Table 4). Significant correlation was also observed between SFC and penetration values ($r = 0.82$; $P < 0.01$). Most of the work done on the influence of the SFC concerns the relationship between penetration hardness and SFC (de Man & Beers, 1987). Haighton (1976) found the yield value (as determined by penetrometry) to be inversely proportional to the second power of the SFC, which would thus be directly related to the spreadability of margarines. Interestingly, the storage modulus (G') values (Table 5) obtained from our viscoelastic studies and yield values of the experimental and commercial margarine samples showed significant correlation ($r = 0.89$; $P < 0.001$). While this may be true only for this system, it nonetheless constitutes an interesting result. According to van der Tempel (1961), two types of bonds are present in fat systems, namely primary bonds and secondary bonds. Primary bonds result from crystals growing together and are irreversible because they do not reform after rupture. Secondary bonds, on the other hand, are due to the reversible London–van der Waals forces and can reform after being ruptured (Chawla, de Man & Smith, 1990). The former are responsible for hardness in fats, while the latter represent the forces at work during viscoelastic measurements. Since the rheological examination was carried out in the non-destructive region, the effects of the primary and secondary bonds may be additive.

Table 4
% Solid fat content (% SFC) of experimental and commercial table margarines during storage

	% Solid fat content (% SFC)			
	Storage temperatures (°C)			
	20°C		30°C	
Storage weeks	Experimental margarine (EM20)	Commercial margarine (CM20)	Experimental margarine (EM30)	Commercial margarine (CM30)
0	25.3	10.4	14.8	5.3
3	30.8	12.7	14.6	9.9
6	31.3	10.0	13.3	7.2
9	30.8	9.3	12.6	7.9
12	37.8	22.1	22.9	14.9

3.4. Changes in solid fat content (SFC)

The solid fat profile gives an indication of the melting properties of a fat. SFC is one of the determining factors in the texture of plastic fats. In general, the SFC of the fat phase is responsible for many of the magarines' characteristics including general appearance, ease of packing, organoleptic properties (flavour release, coolness and thickness), ease of spreadability and oil exudation. For example, the SFC at 20°C determines the tendency towards oil exudation and a value of not less than 10% is essential to prevent oiling off.

Table 4 shows the SFC at 20 and 30°C for the commercial and experimental samples measured at weeks 0, 3, 6, 9 and 12. Based on the results, it is noted that among all the samples, the EM20 samples generally had the highest initial SFC and consequently the highest SFC at all the storage weeks studied, followed by EM30, CM20 and CM30 samples. For the EM20 samples, SFC from week 0 to 12 increased by 12.5% while samples CM20, CM30 and EM30 increased by 11.7, 9.6 and 8.1%, respectively (Table 4). Similar to the SMP results (Table 1), the largest increase in % SFC occurred during the last weeks of storage (weeks 9 to 12). The experimental margarine placed at 20°C, also showed the highest SMP at week 12 (Table 1) and was the firmest as measured by the CP method. CM30, having the lowest % solids, showed the lowest SMP (Table 1) and

Table 5
Storage Modulus (G') at 1 Hz of experimental and commercial table margarines during storage

	Storage modulus (G') (Pa)			
	Storage temperatures (°C)			
	20°C		30°C	
Storage weeks	Experimental margarine (EM20) ($\times 10^5$)	Commercial margarine (CM20) ($\times 10^4$)	Experimental margarine (EM30) ($\times 10^5$)	Commercial margarine (CM30) ($\times 10^4$)
0	6.27	9.23	3.33	4.60
1	5.75	10.50	2.75	4.58
2	5.28	8.62	2.27	–
3	6.39	6.47	2.26	4.21
4	7.32	6.14	2.22	4.24
5	8.50	8.00	2.21	2.90
6	9.57	7.51	2.32	4.42
7	10.70	7.70	2.75	2.76
8	9.47	8.08	2.40	3.15
9	9.43	6.63	2.90	2.94
10	11.60	7.25	2.28	2.94
11	11.70	6.75	2.90	4.57
12	12.00	6.92	2.81	3.94

firmness, indicating that this product is the softest. The SFC and SMP of the experimental margarine can be still further reduced as a rather high amount (minimum 40%) of palm stearin was used in the blends. Therefore, by lowering the amount of palm stearin in the blends, more liquid oil can be incorporated into the blends and the nutritional and physical quality of the experimental margarine can be further improved. However, samples CM20 and EM30, although having rather similar SFC, exhibited different hardness values. This is because the strength of crystal network depends not only on the amount of solids present (% SFC) but also by the polymorphic behaviour and the crystal size formed (de Man, de Man & Blackman, 1989).

Correlation of SFC value with CP data appear to indicate that a small difference in SFC can lead to a large variation in rheological behaviour ($r = 0.82$; $P < 0.01$); the role of solid fat appeared secondary. The small changes in SFC were not solely responsible for the substantial differences in CP value. Other mechanisms such as the polymorphic behaviour and the size of the crystals, could also be involved. As stated by de Man and Beers (1987), fats with identical SFC can have very different rheological properties.

The macrostructure of fats is manifested by its plasticity, which means that it retains its shape under slight pressure but will yield under increased pressure during rolling and spreading. The microstructure of plastic fats consists of a three-dimensional network consisting of solid fat crystals which are held together by primary (non-reversible) and secondary (reversible) bonds. The strength of this crystal network depends, not only on the level of fat crystals or in other words the % SFC, but also by the polymorphic behaviour and size of the fat crystals. Two products having the same level of solid fat may show different strengths of crystal network. For instance, fat crystals in the β form can align into large needle-like agglomerates which result in a strong network and brittle product, especially if the solid level is high, while if those crystals were in the β' form, the crystal network would be weaker resulting in a smooth pliable product (de Man et al., 1989).

de Man (1963) has also shown that a plastic fat is usually workable at room temperature (25°C) when the SFC lies between 20 and 38%. In the experimental margarines at week 12, the SFC at 30°C was 22.9% and at 20°C was 37.8%. Thus, at room temperature, the margarine can be considered a plastic fat. The relationship between SFC and yield value, as determined in the equation below, is graphically displayed in Fig. 1. The linear correlation coefficient was 0.82 ($P < 0.01$):

$$\text{Yield value} = 30.309\ (\text{SFC}) - 55.043$$

From this equation, for a calculated yield value of zero, an SFC value of 1.82% can be derived. In other words, a minimum of 1.82% of solids was required in the margarines to form a texture or a crystal structure.

SFC values showed an increasing trend on storage, particularly during the last weeks of storage (weeks 9 to 12), indicating a higher degree of firmness of the samples probably due to the rearrangement of the crystals into a three-dimensional scaffolding network on storage. In fresh samples (week 0), fat probably exists as loose crystal chains which slowly form ramifications due to weak van der Waals attraction forces on setting. This crystal network is later reinforced by strong primary bonds, slowly formed between the larger crystals resulting in harder samples (Haighton, 1965). Statistically, the SFC values during storage weeks 0 to 9 were not found to be significantly different with the exception of the SFC value at week 12, which was found to be significantly different from the others ($P < 0.001$).

SFC values were also found to have a significant correlation with the G' value (Table 5) obtained from the viscoelastic studies ($r = 0.89$; $P < 0.001$) and SMP ($r = 0.79$; $P < 0.01$). In plastic products like margarine and butter, rheological properties are determined mainly by the degree of crystallinity of the fat phase they contain (Rohm & Weidinger, 1993). The fat phases in both margarines are mixtures of various triglycerides with varying melting points. At a specific temperature, part of it exists in the crystalline state and imparts solid-like elastic characteristics with a certain % SFC to the product (Shukla & Rizvi, 1995). At a higher temperature, melting of crystalline fat takes place resulting in a more liquid-like behaviour of samples, as was observed, and a lower SFC value. Differences in rheological behaviour

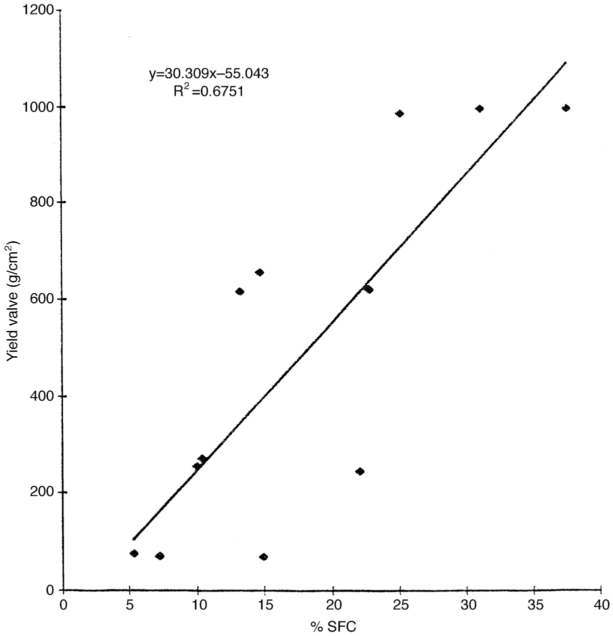

Fig. 1. Regression correlation of yield value (g/cm^2) and % solid fat content (% SFC) of table margarine during storage.

of the experimental and commercial margarines could be attributed to their distinct chemical composition. The margarine samples containing 40% palm stearin are rich in high melting triglycerides compared to the commercial margarine and thus had greater amounts of crystalline fat and higher % SFC (Shukla, Bhaskar, Rizvi & Mulvaney, 1994) which resulted in its more solid-like behaviour.

Generally, the CP, SMP and SFC results indicated that the experimental margarine was firmer than the commercial margarine and may find application as an industrial shortening or pastry margarine which contains higher solids than the standard table margarine. For standard table margarine formulations, fat blends containing lower amounts of palm stearin, possibly in the range of 20–30% may be more suitable.

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