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Physical and chemical properties of milkfat and phytosterol esters blends

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Abstract

Over the past few decades, consumption of butter has declined due to its high content of saturated fatty acids and cholesterol. Opposingly, phytosterol esters (PE) have been used as a food ingredient for their effectiveness in lowering plasma cholesterol levels. The objective of this work was to evaluate the effects of the addition of PE on the physical and chemical properties of milkfat (MF). The fatty acid and sterol compositions were determined by gas chromatography (GC). A constant speed texture analyser was used to evaluate consistency. Solid fat content (SFC) was determined by nuclear magnetic resonance (NMR) and crystal structure was observed with polarized light microscopy. Results showed that β-sitosterol (33.5%), campesterol (23.6%), and stigmasterol (14.9%) were the most abundant sterols in the PE blend. Blends containing MF and PE presented a softer consistency than pure MF at low temperatures. Despite the higher SFC, PE caused a decrease in the SFC of MF. The images of MF, PE, and their blends obtained by polarized light microscopy showed the influence of temperature on crystal size and number. This method revealed the particular crystalline structure of PE, formed by a solid, dense crystal network, with amorphous crystals of varied sizes, different from crystal structures generally observed in fats. The high SFC and low consistency of PE may indicate a weak crystal network, probably due to the presence of different molecular structures.
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1. Introduction

Butter manufacture dates back to some of the earliest historical records as a human food long before the Christian era. Its consumption was associated with a higher standard of living (Hui, 1996). The composition of milkfat is somewhat complex. Approximately 400 separate fatty acids have been detected in milk lipids (Hui, 1996), with wide variations in chain length (German & Dillard, 2006; Jensen, Ferris, & Lammi-Keefe, 1991; Rodrigues & Gioielli, 2003). Nutritionally, butterfat contains a high percent-

age of hypercholesterolemic fatty acids (mid-length saturated fatty acids) (Rousseau, Forestiere, Hill, & Marangoni, 1996), namely C12:0, C14:0 and C16:0, which represent 27.6 mol% of the total (Walstra, Geurts, Noomen, Jelleman, & van Boekel, 1999). During recent years, a shift in consumer preference is seen toward products containing less fat of a healthier type (Ronne, Yang, Mu, Jacobsen, & Xu, 2005).

The texture of milkfat and butter is of great importance. A major disadvantage of butter is its unspreadable consistency when cold compared to margarines which are spreadable immediately after removal from the refrigerator. Butter also possesses poor structural stability at room temperatures, and exhibits oiling off and moisture migration (Wright, Hartel, Narine, & Marangoni, 2000). Vegetable

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fats however, present better or equivalent functionality at lower prices. On the other hand, the flavor of butter cannot be achieved using vegetable products. Even the addition of flavouring to mimic the buttery flavor has been insufficient (Rousseau et al., 1996).

Phytosterol esters have recently been used as a food ingredient in modern formulations. Epidemiologic and experimental investigations suggest that dietary sterols may offer protection from the most common cancers in Western societies, such as colon, breast, and prostate cancer, but their main effect lies in reducing circulating cholesterol concentrations (López-Ortíz, Prats-Moya, & Berenguer-Navarro, 2006; Sivakumar, Bati, Perri, & Uccella, 2006). Although the hypocholesterolemic effect of phytosterols has been demonstrated for several decades, the exact molecular mechanisms underlying these processes are still poorly understood (Jolodar, Hourihane, & Moghadasian, 2005). Phytosterol compounds exhibit virtually no side effects and they have not shown any evidence of in vitro mutagenic activity or subchronic toxicity in animals (Rozner & Garti, 2006).

Phytosterols are commonly found in foods such as wheat germ, soybeans and corn oil, representing important components in the oil fraction of vegetable oils and nuts. They are constituents of the unsaponifiable lipid fraction of oils, along with other compounds present in relatively important amounts, such as tocopherols, squalene, and other hydrocarbons. Phytosterols cannot be synthesized in the human body (López-Ortíz et al., 2006; Rozner & Garti, 2006). The average daily intake of plant sterols in adults ranges between 150 and 400 mg/day (3–6 mg/kg body weight) and mainly consists of β-sitosterol, campesterol and stigmasterol (Ntanios, 2001; Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000). The addition of phytosterols to the diet in the range of 1.2–2.0 g/day is known to lower total serum and LDL cholesterol by up to 20% (Soupas, Huikko, Lampi, & Piironen, 2005).

Phytosterols are plant based compounds with chemical structures similar to that of cholesterol, however they contain an additional hydrophobic carbon chain. As a result, they are more hydrophobic than cholesterol and are poorly soluble both in water and oil phases (Rozner et al. 2006). Plant sterols are generally delivered in a fat medium as this increases their solubility and improves absorption. Most studies have used margarines and mayonnaise as sources of plant sterols; however other functional fat sources should be considered (Rudkowska, Roynette, Dilip, & Jones, 2006). A low-fat yoghurt-based drink moderately enriched with plant sterols was developed by Volpe et al. (2001) and was shown to lower total and LDL cholesterol levels in patients with primary moderate hypercholesterolemia.

For the production of phytosterol esters, sterol molecules are esterified to fatty acids molecules. PE are classified as lipids, however they present crystallization characteristics very different from general edible fats and oils. These are composed mainly by TAGs and minor components (Gunstone & Padley, 1997), while PE correspond to a complex mixture of many different types of sterols and fatty acids, most of them esterified to each other (Leeson & Floter, 2002). This complex structure turns crystallization into a difficult phenomenon to happen, as asymmetric molecules present more difficulty to pack in a crystalline way (Narine & Marangoni, 1999).

Current literature provides a great amount of information on the effects of phytosterol esters in cholesterol lowering, but research on the influence of their physical and chemical properties and their technological application in food products are limited. The objective of this work is to characterize a commercial blend of phytosterol esters and observe the effects of its addition on the physical and chemical properties of milkfat.

2. Materials and methods

2.1. Material

MF was obtained from butter, purchased from a local store (São Paulo, Brazil), totally melted at 60–70 °C in a microwave oven for 30 min and filtered through a 3 μm filter in an oven at approximately 50 °C. Samples of a commercial blend of PE (L&P Food Ingredient Co. Ltd., Guangdong Food Industry Institute) were donated by Tovani Benzaquen Co. Ltd.

2.2. Blend preparation

Two binary mixtures of MF and PE were prepared. MF and PE were blended in 0.875:0.125 and 0.750:0.250 weight proportions. The blends were prepared to match the phytosterol ester content of commercial margarines enriched with this functional ingredient. The samples were prepared after complete melting of the fats at 70–80 °C for 30 min under stirring and the mixtures were stored under refrigeration at 5 °C.

2.3. Sterol composition

The sterol composition of PE was determined using gas chromatography as described by Naeemi, Ahmad, AlSharrah, and Behbahani (1995). The instrument used was a Shimadzu/Class GC 10 (Shimadzu do Brazil) equipped with a 30 m fused silica capillary column with internal diameter of 0.25 mm (J&W DB-5). Helium carrier gas flow rate was 1.5 ml/min at a split ratio of 1:50. Injector temperature was 290 °C and detector temperature was 300 °C. The oven temperature was initially set at 180 °C, then programmed to heat at 20 °C/min to 280 °C, and held at 280 °C for 10 min. Qualitative sterol composition was determined by comparing the retention times of the peaks with the respective standards of sterols. Quantitative composition was accomplished by area normalization and expressed as mass percentage. Samples were run in duplicate and the reported values are the average of the two runs.

2.4. Fatty acid composition

Fatty acids were converted into fatty acid methyl esters (FAMES), as done by Hartman and Lago (1973). Analyses of FAMES were performed on a Varian GC, model 3400CX gas chromatograph (Varian Ind. Com Ltda. – Brazil), equipped with split-injection port, flame-ionization detector, star chromatography workstation version 5.5 and a 30 m fused silica capillary column (ID = 0.25 mm) coated with 0.25 μm of CP-Wax 52CB (Chrompack). Helium carrier gas flow rate was 1.5 ml/min at a split ratio of 1:50. Injector temperature was 250 °C and detector temperature was 280 °C. The oven temperature was initially set at 75 °C for 3 min, then programmed to increase at 37.5 °C/min to 150 °C followed at 3 °C/min to 215 °C. After drawing up air into the filled syringe (sample volume 1 μl) and inserting the needle into the heated injector, samples were injected manually after a dwell-time of 2 s. Qualitative fatty acid composition was determined by comparing the retention times of the peaks with the respective standards of fatty acids. Quantitative composition was accomplished by area normalization and expressed as mass percentage, according to AOCS Official Method Ce 1-62 (AOCS, 1997a). Samples were run in duplicate and the reported values are the average of the two runs.

2.5. Softening point

Softening point was determined using the open tube melting point method, according to the AOCS official method Cc 3-25 (AOCS, 1997b). This analysis was performed in triplicate.

2.6. Consistency

Consistency was determined by penetration tests using a 45° acrylic cone fitted to constant speed texture analyser TA-XT2 (Stable Micro Systems). Consistency was calculated as "yield value" (kgf/cm^2), according to the equation proposed by Haighton (1959):

$$C = K \frac{W}{p^{1.6}} \tag{1}$$

where

C yield value (kgf/cm^2),
K a constant depending on the cone angle (4700 – undimensional),
W compression force (kgf), and
p penetration depth (cm).

Test parameters were as follows: penetration depth: 1.0 cm; speed: 0.2 cm/s; time: 5 s.

The samples were heated between 70 °C and 80 °C in a microwave oven for complete melting of the crystals, and conditioned in 50 ml glass beakers (Pyrex, USA). Tempering occurred for 24 h in a common refrigerator (5–8 °C) and then for 24 h in an oven with controlled temperature (5, 10, 15, 20, 25, and 30 °C ± 0.5 °C). Measurements were performed in triplicate.

2.7. Solid fat content (SFC)

SFC was determined by nuclear magnetic resonance, using a 20 MHz Maran Ultra Bench Top NMR (Oxford Instruments, England), according to AOCS method Cd 16b-93 (AOCS, 1999). Two replicates were performed, and the reported value is the average of the two.

2.8. Polarized light microscopy

The samples were heated at 80 °C in an oven and kept at this temperature for 30 min to destroy crystal memory. A drop of the molten fat was placed on a pre-heated microscope slide, and covered with a pre-heated coverslip. The slides were prepared in an oven at 80 °C and stored at 10 °C and 25 °C for 5 h. Crystal morphology was studied under isothermal conditions with a polarized light microscope (Olympus System Microscope, model BX 50-Olympus America Inc.) fitted with a digital camera (Media Cybernetics). Visual magnifications were 40 and 200×. Temperature control was maintained by a thermal microscope stage model TS-4 (Physitemp Instruments Inc.). The images show a typical field for each sample and they were analyzed by using the Image Pro-Plus Version 4.5.1 for Windows (Media Cybernetics) software.

3. Results and discussion

3.1. Sterol and fatty acid compositions of the samples

The sterol composition of PE is presented in Table 1. The predominant sterols found in this commercial blend were β-sitosterol, campesterol, and stigmasterol, which together represent 72% of total sterols.

Table 2 shows the fatty acid composition of MF, PE and their blends. Butyric (C4:0) and caproic (C6:0) acids were present in relatively smaller amounts compared to literature. Butyric acid content generally ranges from 3% to

Table 1
Sterol composition of the phytosterol esters

Sterol	Average ± SD (%)
Cholesterol	0.3 ± 0.2
Brassicasterol	8.9 ± 2
Campesterol	23.6 ± 3
Campestanol	1.3 ± 0.6
Stigmasterol	14.9 ± 3
Clerosterol	3.6 ± 2
β-Sitosterol	33.5 ± 5
β-Sitostanol	3.2 ± 2
Δ^5-Avenasterol	0.1 ± 0.1
$\Delta^{5,24}$-Stigmastadienol	5.0 ± 2
Δ^7-Stigmastenol	2.0 ± 1
Δ^7-Avenasterol	3.6 ± 2

Table 2
Fatty acid composition of the samples as wt %

Fatty acid	Samples (MF:PE)			
	1	2	3	4
	(1:0)	(0.875:0.125)	(0.750:0.250)	(0:1)
4:0	<0.1	<0.1	0.1 ± 0.0	0.0 ± 0.0
6:0	0.3 ± 0.0	0.9 ± 0.0	1.3 ± 0.3	0.0 ± 0.0
8:0	0.8 ± 0.0	0.9 ± 0.0	1.1 ± 0.3	0.0 ± 0.0
10:0	2.4 ± 0.0	2.3 ± 0.0	2.3 ± 0.4	0.0 ± 0.0
12:0	3.2 ± 0.0	3.0 ± 0.0	3.0 ± 0.3	0.0 ± 0.0
14:0	12.2 ± 0.1	11.7 ± 0.0	11.5 ± 0.4	0.9 ± 0.1
14:1	1.3 ± 0.0	1.3 ± 0.0	1.2 ± 0.1	0.0 ± 0.0
15:0	1.4 ± 0.0	1.4 ± 0.0	1.3 ± 0.0	0.0 ± 0.0
16:0	33.6 ± 0.1	32.4 ± 0.0	32.0 ± 0.3	6.2 ± 0.1
16:1	1.9 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	0.0 ± 0.0
17:0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.0 ± 0.0
18:0	12.1 ± 0.0	11.8 ± 0.0	11.6 ± 0.4	2.3 ± 0.0
18:1	23.7 ± 0.1	24.3 ± 0.1	24.3 ± 0.8	32.5 ± 0.9
18:1 *Trans*	3.2 ± 0.0	3.2 ± 0.0	3.1 ± 0.1	1.6 ± 0.1
18:2 ($n-6$)	1.6 ± 0.0	2.1 ± 0.0	2.4 ± 0.0	17.1 ± 1
18:3	0.3 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	3.4 ± 0.1
18:2 (CLA)	1.2 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	0.0 ± 0.0
20:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	4.7 ± 0.5
22:1	0.0 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	31.3 ± 1
Saturated	66.8 ± 0.0	65.2 ± 0.1	65.0 ± 0.0	14.1 ± 0.5
Monounsaturated	30.1 ± 0.1	31.0 ± 0.1	31.0 ± 0.0	65.4 ± 0.4
Polyunsaturated	3.1 ± 0.0	3.8 ± 0.0	4.0 ± 0.0	20.5 ± 1

Mean value of two replicates ± standard error of the mean.

4% of milkfat's total fatty acid content (Gunstone, Harwood, & Padley, 1986; Hui, 1996; Molkentin & Precht, 2000; O'Brien, 1998; Wright et al., 2000). Butyric acid exclusively occurs in milkfat and its quantification can be used to estimate the milkfat content in food products (Molkentin & Precht, 2000). However, a maximum of 0.1% was found in MF and their blends analyzed in this work. It is probable that this fatty acid has been lost during MF extraction from butter, performed at high temperatures, and during the preparation of fatty acid methyl esters for chromatography, considering that butyric acid is very volatile and water soluble. Caproic acid is also volatile and a portion present in raw milkfat was probably lost due to the same reason.

The results also show that saturated fatty acids represent $66.8 \pm 0.0\%$ of MF total fatty acids. The predominant fatty acids found were palmitic (C16:0), oleic (C18:1), myristic (C14:0), and stearic (C18:0) acids. The other fatty acids were present in amounts from $0.3 \pm 0.0\%$ to $3.2 \pm 0.0\%$ each.

Conjugated linoleic acid (CLA) is a group of fatty acids found predominantly in the milk and meat of ruminant animals, such as cows, goats, and sheep (Chin, Liu, Storkson, Ha, & Pariza, 1992). The level of CLA in milkfat commonly falls between 0.3% and 1.0%, depending on seasonal fluctuations, feeding practices, and processing steps (O'Shea, Lawless, Stanton, & Devery, 1998). It is considered to be important in terms of anticarcinogenic activity, besides influencing in decreases in body fat concurrent with

increases in lean body mass. Other nutraceutical benefits of CLA include protection against atherosclerosis, cachexia, and treatment of non-insulin-dependent diabetes (Campbell, Drake, & Larick, 2003). The samples analyzed here presented $1.1 \pm 0.0\%$ to $1.2 \pm 0.0\%$ of CLA.

The predominant fatty acids found in PE were oleic, linoleic and erucic acids. The other fatty acids are present in amounts from $0.9 \pm 0.1\%$ to $6.2 \pm 0.1\%$. Due to the presence of more than 30% of erucic acid (C22:1), it is probable that the sterols have been obtained in the free form and then esterified to free fatty acids from rapeseed oil. Rapeseed oil contains approximately 40% of erucic acid and it is produced in large amounts in China, country of origin of PE, besides India, Europe and Canada (USDA, 2006). As the solubility of phytosterols in edible oil is very low (Vu, Shin, Lim, & Lee, 2004), the esterification of these compounds may improve efficacy because it allows greater solubilisation within the margarine and optimal dispersion within fat containing micelles during the intestinal metabolism of lipids (Nestel, Cehun, Pomeroy, Abbey, & Weldon, 2001).

3.2. Softening point

Table 3 shows the softening point results for all samples. Pure MF presented a softening point of 33.1 ± 0.2 °C, which was in agreement with the results found by Rousseau and Marangoni (1999). These authors found a value of 33.4 °C for the softening point of milkfat. No values of

Table 3
Softening points of the samples

Sample	Softening point (°C)
MF	33.1 ± 0.2^a
$(0.875:0.125)^A$	31.5 ± 0.5^b
$(0.750:0.250)^A$	31.1 ± 0.1^b
PE	24.3 ± 0.0^c

[A] (MF:PE). Mean value of three replicates $\pm$ standard error of the mean. Different superscript letters (a–c) indicate significant differences ($p < 0.05$), according to the Tukey test.

softening or melting points of phytosterol esters have been found in literature to date. Experimentally, the commercial blend of PE studied here presented a softening point of 24.3 ± 0.0 °C.

The addition of PE to MF caused a decrease in the softening point, although the amount of PE added did not result in statistically different softening points ($p < 0.05$).

3.2.1. Consistency

Table 4 presents the consistency of the samples at temperatures ranging from 5 °C to 30 °C. Among all samples, pure MF presented the highest values in all temperatures, ranging from 12.7 ± 0.3 kgf/cm^2 at 5 °C to 0.2 ± 0.01 kgf/cm^2 at 25 °C. The consistency of the samples decreased as a function of temperature, all values being statistically different from each other ($p < 0.05$) from 5 °C to 20 °C, as observed by different letters in each column. This decrease can be due to the gradual melting of the crystals, leading to a structurally weaker network (Rousseau, Hill, & Marangoni, 1996) which is, in turn, responsible for the plasticity of fats. Between 5 °C and 10 °C, only a small decrease in consistency could be observed, indicating that the samples could accommodate a larger proportion of oil without losing structural integrity (Deman, 1983). On the other hand, from 10 °C to 25 °C, a sharper decline in consistency could be noticed in 5 °C intervals. This has been previously interpreted as a structurally weaker network (Rousseau et al., 1996). None of the samples exhibited measurable consistency at 30 °C. The zero value for consistency physically represents a product with very low consistency, in a way that the equipment is not capable

to detect it. These products generally showed themselves as a high viscosity fluid.

Likewise, the addition of PE to MF caused a statistically significant decrease in consistency from 5 °C to 15 °C, as can be observed by different letters in each line. The addition of 12.5% and 25.0% of PE to MF caused a decrease in consistency at all temperatures. The decrease in consistency is always higher than the percentage addition of PE at all temperatures, revealing the formation of a eutectic system. The consistency of the PE could only be measured at 5 °C. From 10 °C on, the product visually seemed to present a high degree of crystallization, due to the high opacity; however, the crystals did not seem to aggregate in such a way to form a crystal network similar to the ones generally found in plastic fats (Rodrigues, Mancini-Filho, Torres, & Gioielli, 2004). This crystal structure may be weak due to the structural differences between sterols and fatty acids molecules that will not pack well. Thus, the product did not present enough structure to make possible its determination.

According to Haighton (1959), a fat can be considered spreadable in a range of consistency from 0.1 to 1.5 kgf/cm^2. Therefore, MF presented a very hard consistency from 5 °C to 15 °C, and showed spreadability from 20 °C to 25 °C. With the addition of PE to MF, samples became more spreadable at lower temperatures. However, there are other factors influencing the texture of a spread, like crystallization procedure including cooling rate, degree of supercooling, mechanical working, and tempering, the solid fat content of the fat used, and the presence of non-fat materials (Marangoni & Rousseau, 1998).

3.3. Solid fat content (SFC)

The SFC of the samples as a function of temperature is shown in Table 5. At 10 °C, PE had $65.8 \pm 0.6\%$ SFC, which decreased as a function of temperature, presenting a sharp drop from $56.0 \pm 0.6\%$ to $14.4 \pm 0.9\%$ between 20 °C and 25 °C range of temperature, where their softening point is located (24.3 ± 0.0 °C). PE presented no SFC at 40 °C. Rousseau et al. (1996) determined the SFC of milkfat from 5 °C to 40 °C and in their work, milkfat

Table 4
Consistency of the samples

Temperature (°C)	Consistency (kgf/cm^2)			
	MF	$(0.875:0.125)^A$	$(0.750:0.250)^A$	PE
5	12.7 ± 0.3^a	7.6 ± 0.3^c	4.6 ± 0.1^e	$0.4 \pm 0.1^{i,j}$
10	11.7 ± 0.8^b	6.4 ± 0.4^d	3.7 ± 0.05^f	0.0 ± 0.0^j
15	4.7 ± 0.06^e	2.5 ± 0.06^g	1.2 ± 0.05^h	–
20	$0.9 \pm 0.01^{h,i}$	$0.4 \pm 0.02^{i,j}$	0.0 ± 0.0^j	–
25	$0.2 \pm 0.01^{i,j}$	0.0 ± 0.0^j	–	–
30	0.0 ± 0.0^j	–	–	–

[A] (MF:PE). Mean value of three replicates $\pm$ standard error of the mean. Different superscript letters (a–j) indicate significant differences ($p < 0.05$), according to the Tukey test.

Table 5
Solid fat content of the samples

Temperature (°C)	SFC (%)			
	MF	$(0.875:0.125)^A$	$(0.750:0.250)^A$	PE
10	47.8 ± 0.4^d	37.3 ± 0.2^e	28.9 ± 0.3^g	65.8 ± 0.6^a
15	34.7 ± 0.6^f	26.6 ± 0.3^h	20.5 ± 0.1^i	63.4 ± 0.5^b
20	19.0 ± 0.0^i	14.4 ± 0.1^j	10.7 ± 0.1^k	56.0 ± 0.6^c
25	10.9 ± 0.8^k	8.5 ± 0.6^l	$6.6 \pm 0.6^{l,m}$	14.4 ± 0.9^j
30	5.9 ± 0.4^m	3.4 ± 0.1^n	2.9 ± 0.6^n	6.4 ± 0.5^m
35	0.3 ± 0.4^o	0.0 ± 0.0^o	0.0 ± 0.0^o	$5.1 \pm 1^{m,n}$
40	0.0 ± 0.0^o	–	–	0.0 ± 0.0^o

[A] (MF:PE). Mean value of two replicates $\pm$ standard error of the mean. Different superscript letters (a–o) indicate significant differences ($p < 0.05$), according to the Tukey test.

presented lower SFC at low temperatures (40% at 10 °C and 32% at 15 °C), than this study. This may due to stage of lactation, feed, grain intake, amount and composition of dietary fat and seasonal effects (Jensen, 2002).

All samples were affected by the increase of temperature and the increasing proportion of PE in the blends, similarly to what happened with consistency, i.e., their SFC decreased with increasing temperature and higher proportions of PE. For example, the SFC of the 0.875:0.125 MF:PE blend decreased from $37.3 \pm 0.2\%$ to $0.0 \pm 0.0\%$ when temperature increased from 10 °C to 35 °C, respectively. Likewise, pure MF presented SFC of $47.8 \pm 0.4\%$ at 10 °C, which decreased to $28.9 \pm 0.3\%$ when it was blended with 25.0% of PE. The interesting fact is that PE presented higher SFC than MF at all temperatures. This

phenomenon reveals that an antagonistic interaction occurred between MF and PE, as SFC of the blends presented lower values than expected.

When different fats and oils composed mainly of triacylglycerols are blended, it is usually possible to establish a correlation between consistency and SFC, as the former is generally a function of the latter. However, at temperatures close to their melting points, fats do not present consistency because the low SFC is not enough to form the crystals. Pure MF, for example, did not present consistency at 30 °C, despite containing $5.9 \pm 0.4\%$ of SFC. At 10 °C, milkfat presented $47.8 \pm 0.4\%$ of solid fat and consistency of 11.7 ± 0.8 kgf/cm^2, representing a very solid fat, with low plasticity. At the same temperature, PE presented a SFC of $65.8 \pm 0.6\%$ and no measurable consistency. Con-
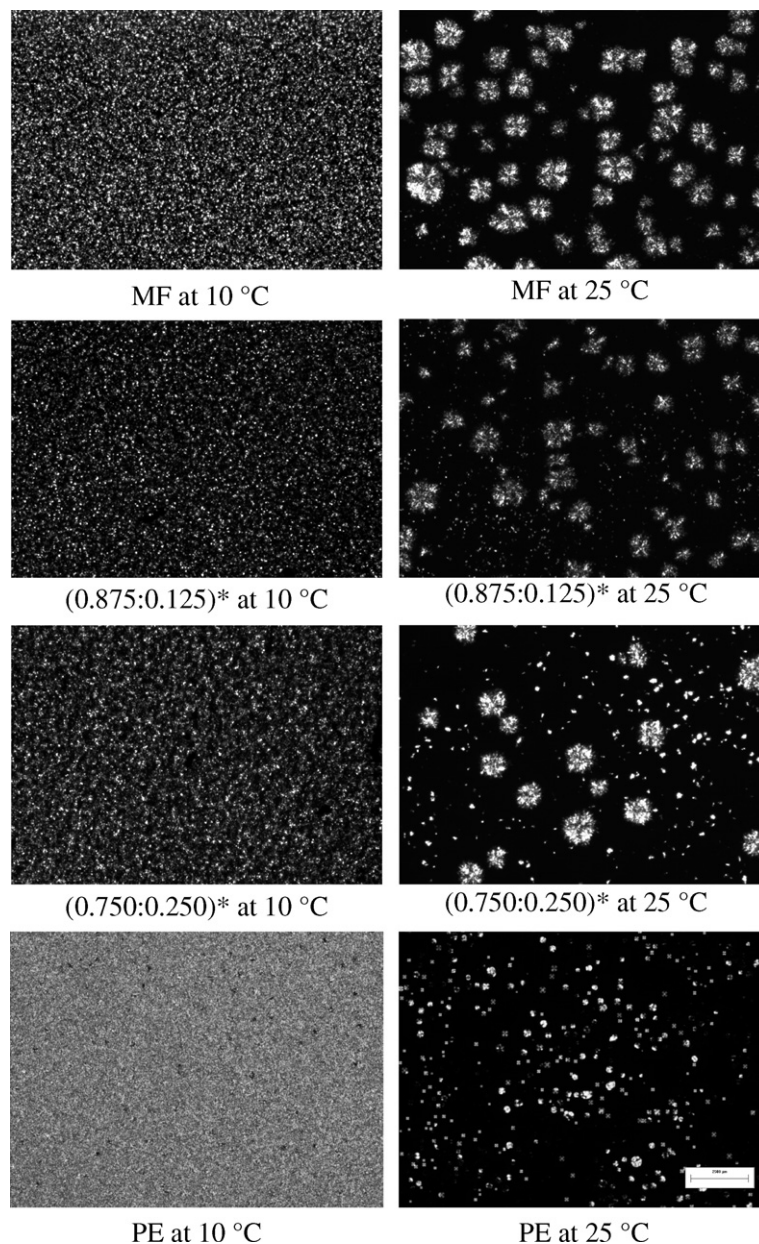

Fig. 1. Digital images of the fat crystal networks of the samples (MF:PE). The white bar represents 250 μm. *(MF:PE).

sequently, it was not possible to set a correlation between these two properties. This difference in the behaviors of MF and PE can be due to the chemical composition of PE, which is different from most edible oils and fats, composed mainly of triacylglycerols. PE are composed of fatty acid molecules esterified to sterol molecules, presenting a particular chemical and structural composition that, in the presence of the triacylglycerols from MF, complicates the formation of a crystalline structure, decreasing the SFC of the blends (Applewhite, 1985; Shahidi, 2005).

3.4. Crystal structure

Fig. 1 presents digitalized images of samples thermostatically crystallized at 10 °C and 25 °C. At 10 °C, the addition of PE did not cause morphological differences in the crystalline structure of MF. Pure MF and its blends with PE presented a similar granular crystal structure, composed by crystals in great amounts and small size (Campos, Narine, & Marangoni, 2002). Because the nucleation rate increases at a roughly exponential rate with increasing supersaturation caused by cooling, whereas the growth rate is only proportional to supersaturation, the number of crystals increase and the crystal size falls when crystallization occurs at a lower temperature. Rapid cooling to a low temperature followed by intense stirring leads to the microscopically small crystals found in margarine, in the range of 0.1 to 20 μm (Hamilton, 1995).

As observed in the images, PE formed a solid, dense crystal network, with amorphous structure, different from crystals generally observed in fats.

The addition of PE did not seem to drastically modify the structure of MF crystals, though a smaller number of them and small crystals found in PE seemed to be present. The viscosity increases when temperature falls (Hamilton, 1995), which explains the larger crystals found in samples crystallized at 25, when comparing to samples crystallized at 10 °C.

4. Conclusions

This study has shown that the addition of PE caused a decrease in the degree of saturation of MF, improving its nutritional properties, especially regarding blood cholesterol lowering, also due to the presence of sterols like β-sitosterol, campesterol, and stigmasterol. Also, blends containing MF and PE presented a softer consistency than pure MF at low temperatures, which could result in an emulsion softer than the original butter. A correlation between SFC and consistency could not be established because PE presented high SFC and low consistency at all temperatures. Despite the higher SFC, PE caused a decrease in the SFC of MF. The interactions between the MF triacylglycerols and the PE were antagonistic and their blends presented lower softening points, consistency and SFC due to the formation of a eutectic system. The images of MF, PE, and their blends obtained by polarized light

microscopy showed the influence of temperature on crystal size and number. This method revealed the particular crystalline structure of PE, formed by a solid, dense crystal network, with amorphous crystals of varied sizes, different from crystal structures generally observed in fats.

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