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Preparation of specialty fats from beef tallow and canola oil by chemical interesterification: physico-chemical properties and bread applications of the products

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Abstract Blends of beef tallow (BT)/canola oil (CO) (85:15, 65:35, w/w) were interesterified under the following conditions: 0.4% CH_3ONa, 60 °C, 30 min. Triacylglycerol (TAG) composition, polymorphic forms, crystal morphology, thermal properties, rheological properties and oxidative stability of the original and interesterified blends were evaluated. Chemical interesterification (CIE) caused a more balanced rearrangement of TAG species, reduction of trisaturated (S_3), triunsaturated (U_3) TAG content and increase in monosaturate-diunsaturated (SU_2) TAG content. X-ray diffraction (XRD) revealed that all the interesterified blends were exclusively double chain (2L) stacking β' crystal. Thermal curves confirmed slight narrow melting and crystallization temperature ranges appearing in interesterified blends, mainly due to decreased S_3 and U_3 type TAGs and increased SU_2 after CIE. Rheological analysis showed that CIE led to dropping in both hardness, storage modulus (G') and loss modulus (G'') of BT/CO blends. The oxidative stability of the interesterified fats, which was reduced compared with the substrate blends, was significantly improved using 0.02% TBHQ. A shortening and a margarine, both containing low *trans* were prepared by interesterified BT/CO 85:15, 65:35 blends, respectively, which were crystallized in a votator and tested in bread baking. The results of instrumental and sensory analyses showed similar acceptability of the two types of breads. The present study suggests that the interesterified fats fulfill the requirements of marketable bakery shortenings and margarines, respectively.

Introduction

During handling, storage and transportation, BT-based plastic fats tend to develop grainy crystal aggregates contributing to undesirable organoleptic qualities. Granular crystals also easily develop in palm oil-based [1] and lard-based blends [2]. Following IE of selected fat blends containing palm oil [3] and lard [2], these undesirable characteristics are often reduced or eliminated. Reports of such attempts have not been found regarding BT as yet.

In the studies of Foglia et al. [4] and Rodríguez et al. [5], the interesterification (IE) of BT and sunflower oil catalyzed by lipase or CH_3ONa was found to improve physical properties of BT, whereas physical blending did not significantly modify it. Ledóchowska and Wilczynska [6] have performed the IE of BT/rapeseed oil (4:6 w/w) mixture to evaluate the influence of CIE and EIE on the oxidative stability of the blend. Recently, the results of IE of BT/rapeseed oil blends performed with lipase and CH_3ONa as catalyst [7] indicated that the distribution of fatty acids was practically random after CIE, close to random when the lipase Novozym 435 was applied, and stable at *sn*-2 position regarding fatty acids composition when Lipozyme IM was used, compared with the starting blend. The above-cited reports show that softer products with lower melting points can be produced by IE of BT with a vegetable oil.

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However, the purpose of IE is to obtain not only satisfactory melting properties but also suitable crystallization behavior in order to produce higher quality plastic fats with ideal functionality.

According to Tang and Marangoni [8, 9], solid fat crystals and crystal agglomerates in plastic fats interact to form a continuous three-dimensional solid fat network, which is ultimately responsible for the macroscopic properties of the fat system, such as hardness, whippability, spreadability, graininess formation, brittleness, and aeration properties. Factors that influence the network properties of plastic fats include (i) TAG compositions; (ii) crystal habit, which includes crystal polymorphism, size, number, shape, and aggregation behavior [10, 11]. Thus, if CIE is used to improve undesirable crystallization behavior of BT, overall evaluation should be achieved in terms of TAG composition, crystal habit (polymorphism and morphology), rheological properties of the modified solid fats with reference to desired physical and chemical properties.

CO, among common dietary fats and oils, contains the lowest level of long-chain saturated fatty acids such as palmitic and stearic acids; it is also characterized by a high level of monounsaturated fatty acid, oleic acid, and is second only to olive oil in oleic acid content among the major fats and oils; furthermore, it contains relatively high level of linolenic acid content, which not only affects the oxidative stability of oil, but also complement the fatty acid composition of BT, so that the interesterified product between CO and BT is expected to have more desirable crystallization behaviors.

Within this framework, the purposes of this study were (i) to evaluate the effects of CIE on the chemical compositions, crystal habit (polymorphism and morphology), thermal properties, rheological properties and oxidative stability of BT/CO blends; and (ii) to assess the functionality of the shortening and margarine products formulated using the CIE BT/CO blends in baking applications.

Materials and methods

Materials

BT and CO were generously provided by Kerry Specialty Fats Ltd. (Shanghai, China). Both BT and CO were kept at 4 °C in the dark until used. The catalyst, CH_3ONa powder, was stored at room temperature in a desiccator to avoid decomposition. Supelco 37 Component FAME Mixture was purchased from Sigma–Aldrich China (Shanghai, China). Two commercially acceptable reference samples, i.e., a bakery shortening and a margarine (commercial shortening A and commercial margarine A) were purchased from a local store. All other reagents and solvents were of analytical or chromatographic grade to suit analytical requirements.

Chemical interesterification

BT was melted at 70 °C in an oven prior to use. The liquefied BT and CO were mixed to prepare two samples with liquefied BT accounting for 65 and 85% (w/w), respectively. Each substrate blend was subjected to CIE in a 15-kg pilot-scale batch-stirred tank reactor under the following optimum conditions: 0.4% CH_3ONa as catalyst, 60 °C, 30 min. The blends, interesterified and non-interesterified were held at 4 °C prior to analysis and application tests.

Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared according to the AOCS Official Method Ce 2-66 [12] and subsequently analyzed with a GC-14B gas chromatograph (GC) equipped with a fused-silica capillary column (CP-Sil88, 100 m × 0.25 mm × 0.2 mm) and a flame ionization detector (Shimadzu, Tokyo, Japan). The temperature of the injection port and detector was all set at 250 °C. The column was heated to 120 °C and held for 3 min, then programmed at 8 °C/min to 175 °C and held for 28 min, then the temperature was increased to 215 °C at 3 °C/min and held for 20 min. The fatty acid species were identified using the retention time of a FAME standard solution and quantified based on relative peak area.

TAGs composition

TAGs were separated by reversed-phase high-performance liquid chromatography (HPLC) using a Nova-pak RP-C18 column (150 × 4.6 mm, particle size 4 μm) (Waters, Milford, USA) with acetonitrile/dichloromethane (65:35, v/v) as the eluent at a flow rate of 1.0 mL/min and followed with an evaporative light scattering detector (ELSD). TAGs were identified by HPLC coupled to atmospheric pressure chemical ionization mass spectrometry (HPLC/APCI-MS) using same HPLC conditions as described above. A Platform ZMD 4000 (Waters, Milford, USA) mass spectrometer (MS) equipped with an APCI interface was run at an APCI source block and probe temperature of, respectively 100 and 400 °C, and an MS multiplier voltage of 700 V. The measurement range was between m/z 250 and 1,200. Quantitative determination of individual TAGs in fat blends was made using HPLC results following the procedures of Chen et al. [13].

Crystal polymorphism by XRD

The polymorphic forms of fat crystals in the blends were determined by D8 Advance X-ray diffraction (Bruker,

Karlsrube, Germany), using Cu–Ka radiation with Ni filter ($k = 1.54056$ Å, voltage 40 kV, current 40 mA, fixed 1.0-, 1.0-, and 0.1-mm divergence, anti-scatter and receiving slits, respectively). Samples were scanned from 1 to 30° (2θ scale) at a rate of 2.0°/min. The analyses were performed at ambient temperature, and duplicate analysis was carried out.

Differential scanning calorimetry (DSC)

The thermal properties of the samples were measured using a Mettler Toledo DSC 1 (Mettler Toledo, Schwerzenbach, Switzerland). Samples were hermetically sealed in an aluminum pan with an empty pan serving as a reference. The samples were heated to 70 °C and held for 5 min, and then the crystallization profiles were obtained by cooling to −20 °C at 5 °C/min. After holding for 5 min at this temperature, the melting profile was obtained by heating to 70 °C at 5 °C/min.

Rheological properties

Large deformation using a texture analyzer

A TA-XT2i texture analyzer (Stable Micro Systems Ltd., London, UK) was employed to determine hardness with a constant speed compression test. Melted samples (in 25 g portion) were each placed in a 100 mL beaker, and tempered at 5 or 24 °C for 12 h. A cone probe (P45/C) attached to a 5-kg compression load cell was penetrated twice into the sample at 0.5 mm/s to a distance of 5 mm. The maximum penetration force and the final penetration force were recorded. Hardness was reported as the maximum penetration force (g), triplicate measurements were obtained.

Small deformation using a rheometer

The rheological properties of the blends were determined using an AR1000 rheometer (TA Instruments, New Castle, DE) fixed with a water circulator at the base of a 2-cm parallel-plate geometry. Samples were melted at 70 °C in a microwave oven before being poured into moulds and stored in an incubator at 5 °C for 12 h. An oscillatory stress sweep was performed to determine the linear viscoelastic region (LVR) at a frequency of 1 Hz, followed by a frequency sweep test from 4 to 63 rad/s at constant stress (mid-point of the LVR) to evaluate the storage modulus G' and the loss modulus G''. All the tests were conducted at ambient temperature.

Oxidative stability

Oxidation induction times were measured with the Ranci-mat 743 apparatus (Metrohm AG, Herison, Switzerland) on 4-g fat sample heated to 120 °C under a purified air flow rate of 20 L/h. The volatile degradation products are trapped in distilled water, and its conductivity is followed. The induction time is defined as the time necessary to reach the inflection point of the conductivity curve.

Shortening and margarine manufacture

Shortening and margarine samples, respectively, using the interesterified blends as the base stock (hereinafter referred to as CIE-shortening and CIE-margarine, respectively) were prepared in a Gerstenberg and Agger A/S crystallizer (Copenhagen, Denmark). The unit consists of two melting tanks, one chilling system (two separate chilling units) and one pin worker. The basic recipe for shortening production includes 99.3% CIE BT/CO blend 85:15, 0.7% emulsifier (0.5% molecular distilled monoglyceride + 0.2% lecithin) and additional 0.02% TBHQ; and that for margarine production contains 82.5% CIE BT/CO blend 65:35, 15.5% water, 1.3% sodium chloride, 0.7% emulsifier (same as for shortening manufacture), and additional 0.02% TBHQ. The oil phase was prepared by melting the interesterified blend in the mixing tank at 65 °C, and then adding emulsifier premixed in melted interesterified blend in the ratio 1:4. The water phase (for margarine only) was then added slowly to the oil phase under agitation to form a good emulsion. The emulsion was held for 10 min at 65 °C under agitating prior to being pumped into the chilling unit at 45 kg/h, where it was rapidly cooled to 21 °C using scraper blade and intermediate crystallizer rotations both of 300 rpm; and then worked in the pin worker using a rotation of 200 rpm. The finished products were held for 72 h in a KBF115 climatic chamber (Binder, Tuttlingen, Germany) with the temperature maintained at 25 °C.

Baking application and sensory analysis

Breadmaking process was conducted as described by Ahmadi et al. [14] using the formulation as listed in Table 1. The bread quality attributes were evaluated after cooling for 2 h at room temperature. Bread quality analyses include weight, volume (determined by seed displacement in a loaf volume meter), and hardness (performed using a TA-XT2i texture analyzer) [15].

Sensory analysis of breads was carried out by 37 untrained consumer panelists. The selected method was a triangle test. In the triangle test, three coded samples are given to the panelist. In the questionnaire form it is stated that two of the samples are identical and the panelist is asked to identify the odd sample. The sensory evaluation test and analysis of breads were carried out 2 h after bread preparation [16].

Table 1 Formulation of the breadmaking recipe

Ingredients	Weight (g)	Source
High-gluten bread flour	500	Retail
Water	250	Retail
Salt	10	Retail
Sugar	40	Retail
Powdered milk	20	Retail
Whole egg	60	Retail
Shortening or margarine	40	Commercial or CIE BT/CO blend
Yeast	4	Retail
Baking aids[a]	0.5	Retail

[a] Enzyme alpha amylase and emulsifiers

Results and discussion

Fatty acid composition

Fatty acid compositions of the ingredient fats and their blends used in the CIE are given in Table 2. Compositions of BT and CO are in agreement with the ranges published in literature [17]. The major fatty acids in BT are palmitic acid (26.09%), stearic acid (19.50%) and oleic acid (35.03%). For CO, the predominant fatty acids are oleic acid (59.39%) and linoleic acid (21.19%). As expected, an

Table 2 Fatty acid composition (%) of the ingredient fats (BT and CO) and their blends

Fatty acid	BT	CO	BT/CO (% w/w)	
			65:35	85:15
C14:0	3.35	–	2.00	2.85
C16:0	26.09	4.82	17.72	23.03
C16:1	2.36	0.23	1.65	2.02
C17:0	1.52	–	1.00	1.26
C17:1	0.70	–	0.50	0.61
C18:0	19.50	2.03	13.49	16.84
C18:1 *trans*	4.21	–	2.80	3.45
C18:1	35.03	59.39	43.89	38.41
C18:2 *trans*	0.13	–	0.07	0.14
C18:2	1.30	21.19	8.37	4.51
C20:0	0.22	0.56	0.35	0.21
C20:1	0.17	1.06	0.40	0.56
C18:3	0.40	7.51	2.48	1.29
C22:1	–	0.58	–	–
Non-identified	5.02	2.63	5.28	4.82
Total *trans*	4.34	–	2.87	3.59
Saturated	50.68	7.41	34.56	44.19
Monounsaturated	42.47	61.26	49.24	45.05
Polyunsaturated	1.83	28.7	10.92	5.94

increased weight ratio of CO from 15 to 35% in the substrate blend resulted in a reduced content of palmitic acid, stearic acid and increased content of oleic acid in the blends. Thus, concomitantly, the saturation degree of BT was reduced from 50.68 to 44.19 and 34.56% with the addition of 15 and 35% CO, respectively. The total *trans* fatty acid level in BT/CO blend 65:35 and 85:15 was 2.87 and 3.59%, respectively, which is lower than the commercial bakery plastic fats prepared with partially hydrogenated oils. Hence, from the nutritional and functional point of view, these new substrate blends could be used for the production of low *trans* shortenings, margarine and frying fat. As CIE neither affects the degree of saturation nor causes isomerization of the fatty acid double bonds [18, 19], fatty acid compostions of the interesterified blends are not shown.

TAGs composition

The change in TAG profiles of the CIE product is obvious, correspondingly, the relative concentrations of several TAGs increased while those of others were observed to decrease (Fig. 1). The results obtained are similar to previous findings by Chen et al. [13] and Liew et al. [20]. This change indicates that CIE has occurred in all reaction products.

The molecular structures of the TAG species present in each substrate blend and its corresponding interesterified fat, were determined using reversed-phase HPLC/APCI-MS, whereby TAGs are separated on the basis of their equivalent carbon number, ECN (ECN = CN − 2 DB, where CN is the number of carbons and DB the number of double bonds). Peak identification can be achieved by combining ECN values with the information from the MS spectrum. The MS spectra of the TAGs showed sodium adducts of the molecular ions $[M + Na]^+$ and one fatty acyl moiety cleaved ions $[M-(R-COO)]^+$ ($[DG]^+$). Regarding the $[DG]^+$ ions, their intensity changes mainly in relation to the position occupied by the fatty acid that is removed. The less abundant $[DG]^+$ ion corresponds to the loss of the fatty acid from the *sn*-2 position because this is energetically less favorable than losing a fatty acid from the *sn*-1 or *sn*-3 position [21].

The HPLC/APCI-MS spectra of TAGs *sn*-OOO, *sn*-POO, *sn*-POP in CIE BT/CO 65:35 are shown in Fig. 2. The mass spectra of TAGs show relative abundance of $[DG]^+$ species in consistence with rules above, which are similar to those reported previously [21]. Table 3 reports the TAG composition of the control and interesterified product detected by HPLC-ELSD (Fig. 1) and corresponding mass identification data for the separated TAGs.

The main TAGs of BT are POO, POP, POS, accounting for 60.89% of total TAG composition. BT also contains
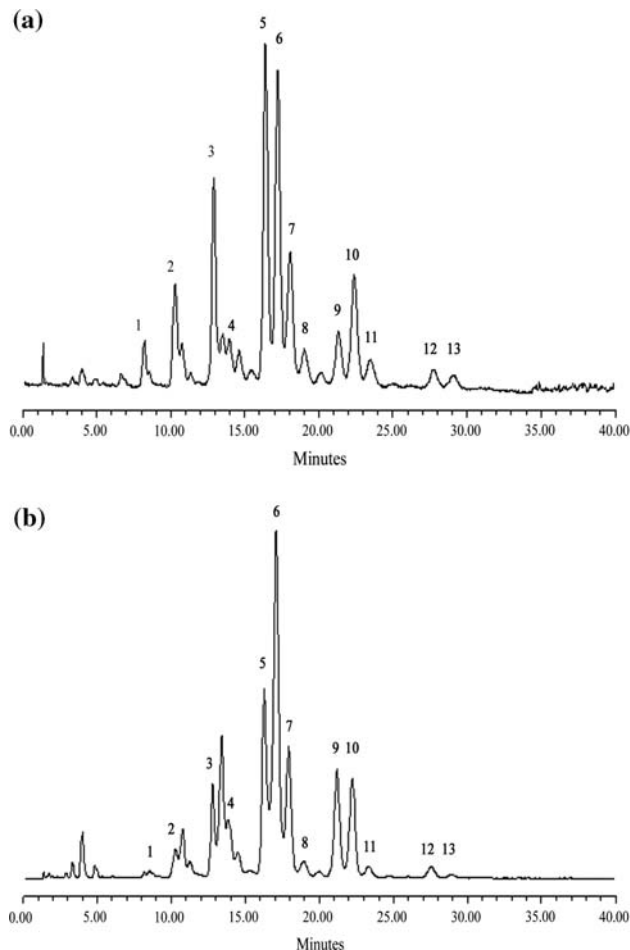
Fig. 1 HPLC profiles of BT/CO blend (65:35 w/w) before (**a**) and after (**b**) CIE (peak number identity to be given in Table 3)

Fig. 2 HPLC/APCI-MS mass spectra of *sn*-OOO (**a**), *sn*-POO (**b**), *sn*-POP (**c**)

appreciable amounts of SOO, PPP, PLO, PPS, SOS, OOO, and PSS. For CO, the major TAGs are triunsaturated species, with OOO predominating (38.39%), followed by LOO (26.32%) and LnOO (15.84%) (Table 3). These results were in accordance with those presented by Scrimgeour and Harwood [22] for the TAG composition of CO. The TAG composition of the binary fat blends represents a linear combination of the ingredient fat in the blends (Table 3). For example, as the proportion of BT was increased from 65 to 85% in the blend, the proportion of POP, POO, POS, PPP, and other TAG present in BT was increased. CIE induced large changes in the TAG composition of BT/CO blends, as shown in Table 3. In CIE BT/CO blend 65:35, the proportion of the TAG, i.e., triunsaturated (U_3) LnOO, LOO, and OOO, was reduced from 6.14, 10.67, and 20.60% in BT/CO blend 65:35 to 1.74, 5.25, and 13.03%; trisaturated (S_3) PPP, PPS, was also decreased from 2.79, 1.75% in BT/CO blend 65:35 to 1.22, 0.78%, respectively. Concomitantly, the proportion of other TAGs, such as monosaturate–diunsaturated (SU_2) PLO, POO and SOO, was increased from 2.82, 20.94, and
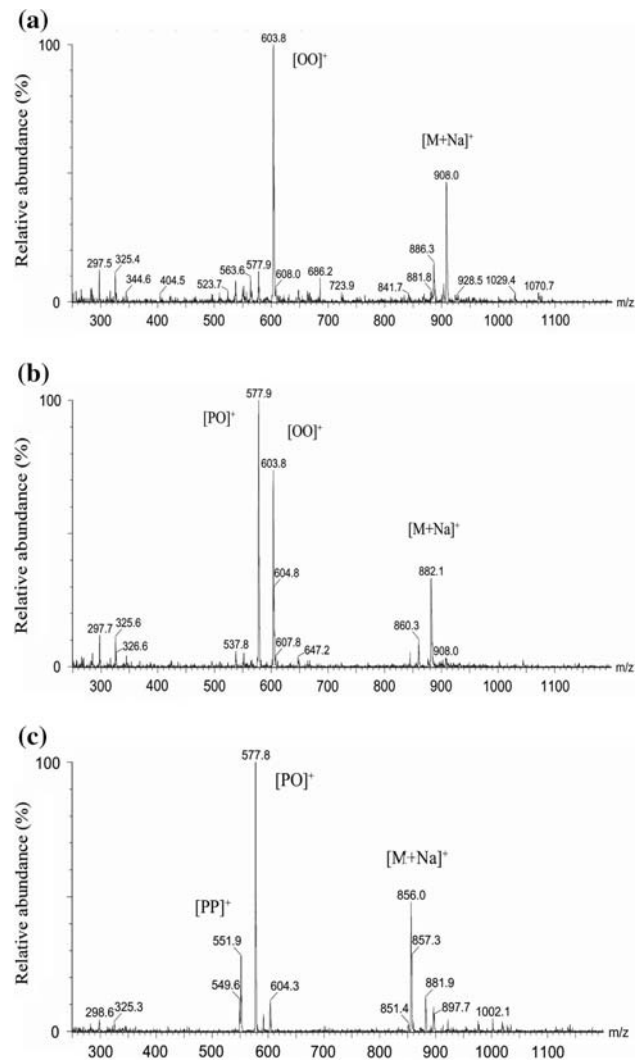
3.74% to 4.42, 29.69, and 8.66%, respectively. The TAG composition of CIE BT/CO blend 85:15 shows the similar changes as compared to BT/CO blend 65:35. These variations indicate that the TAG composition of the interesterified blends had a more balanced or even peak distibution than the substrate blends, as the relative concentration of several TAG (U_3, S_3) decreased, while that of others (SU_2) increased, and several new TAGs might also have been synthesized. This result is consistent with findings reported in the literature [23, 24].

The functional properties of shortenings and margarines can be related to the TAG composition of their fat phase [25]. TAGs S_3, with melting points between 54 and 65 °C, are mainly responsible for the structure of products, whereas TAGs SU_2 are important with regard to their sensorial and functionality properties at room temperature. The TAGs U_3, with melting points from −14 to 1 °C, are

Table 3 Main TAG species present in BT/CO blends before and after CIE as separated by reversed-phase HPLC and characterized by APCI-MS

Peak[a]	TAG[b]	BT	CO	BT/CO (% w/w)				[M + Na][+d]	[DG][+d]	[DG][+d]	[DG][+d]
				65:35	65:35-I[c]	85:15	85:15-I[c]				
1	LLL	–	4.87	1.56	–	0.42	0.40	902	[LL]+ 600	–	–
2	LnOO	–	15.84	6.14	1.74	1.44	1.18	904	[LnO]+ 600	[OO]+ 604	–
3	LOO	–	26.32	10.67	5.25	3.31	2.32	906	[LO]+ 602	[OO]+ 604	–
4	PLO	4.64	3.50	2.82	4.42	3.75	5.84	880	[PL]+ 576	[LO]+ 602	[PO]+ 578
5	OOO	3.12	38.39	20.60	13.03	9.02	8.37	908	[OO]+ 604	–	–
6	POO	27.02	3.31	20.94	29.69	27.56	28.09	882	[PO]+ 578	[OO]+ 604	–
7	POP	17.47	–	9.96	10.07	14.66	15.27	856	[PO]+ 578	[PP]+ 552	–
8	PPP	4.99	–	2.79	1.22	3.93	2.79	830	[PP]+ 552	–	–
9	SOO	5.71	–	3.74	8.66	5.18	6.17	910	[SO]+ 606	[OO]+ 604	–
	SSL							910	[SS]+ 608	[SL]+ 604	–
10	POS	16.40	–	8.39	8.22	14.22	13.66	884	[PO]+ 578	[SO]+ 606	[PS]+ 580
11	PPS	4.33	–	1.75	0.78	3.42	2.98	858	[PP]+ 552	[PS]+ 580	–
12	SOS	3.25	–	0.83	0.88	1.95	1.94	912	[SO]+ 606	[SS]+ 608	–
13	PSS	2.80	–	1.06	0.27	1.47	1.27	886	[PS]+ 580	[SS]+ 608	–
14	SSS	0.47	–	–	–	0.15	–	914	[SS]+ 608	–	–

[a] Peak numbers correspond to those shown in the HPLC chromatogram shown in Fig. 1a, b

[b] Proposed TAG structure: *Ln* linolenyl, *O* oleoyl, *L* linoleoyl, *P* palmitoyl, *S* stearoyl

[c] Interesterified blend

[d] Sodium adduct molecular ion and fragment ions as detected by APCI-MS

important for the softness of the product [26, 27]. Hence, the increase of the SU_2 contents of BT/CO blends, promoted by CIE, is associated to the increase of technological functionality, the betterment of sensorial characteristics, and, therefore, to a greater potential of these interesterified bases for food application [28, 29].

Polymorphism

XRD, used to identify crystal polymorphs, is based on the determination of the long and short spacings of crystals. The short spacing of the α form appears near 4.15 Å, of the β' form at 4.2 and 3.8 Å and of the β form at 4.6 Å (single strong spacing). Levels of β' and β crystals in mixtures were estimated by the relative intensity of the short spacings at 3.8, 4.2, and 4.6 Å [30]. The long spacings of the fat samples indicated the lamellar packing structure of the fats [31].

XRD patterns (Fig. 3) were recorded on samples cooled 24 h in a refrigerator at 5 °C. BT contained mixtures of both α and β' polymorphic forms with the β' form dominating due to the presence of fatty acids of various chain lengths. Incorporation of CO into BT increased the diversity of the fatty acids and TAGs in the blends and thus retained the β' form of BT. The α forms were not found in BT/CO blend 65:35 or 85:15 due to the dilution effect (Fig. 3a), because the amount of α polymorphic forms is

too low in concentration in BT to exert any influence. After CIE, only the β' crystals were observed to be present in all the blends. This is consistent with deMan's observation that a diversification of fatty acid chain lengths in fat samples tends to promote β' stability [21]. There was a long spacing observed in both BT/CO blend 65:35 and 85:15 at 41.7 and 40.1 Å, respectively. The long spacing near 41.1 Å is most likely due to double (2L) stacking of the triglycerides and most likely from the orthorhombic perpendicular (O⊥) subcell of the β' polymorph [31]. There was the same double packing for the CIE BT/CO blend 65:35 and 85:15. Little effects of CIE was found on the TAG packing. This is in agreement with the report of Zhang et al. [32]. To summarize, the interesterified fat displayed 2L stacking of the β' polymorph. This form of crystal is important in bakery fats such as shortenings and margarines because β' crystals give a good aeration to bakery dough and smooth texture to the bakery products. Therefore, the produced fats in the present study can be applicable as alternatives for bakery fats.

At the same time, crystal morphology analysis revealed that both blending of CO and CIE substantially altered the crystal network of BT. Crystal network structure in terms of crystal network density, crystal size, and crystal morphology of the interesterified blends shown smaller and more loosely packed spherulites (photographs not shown for sparing page space).
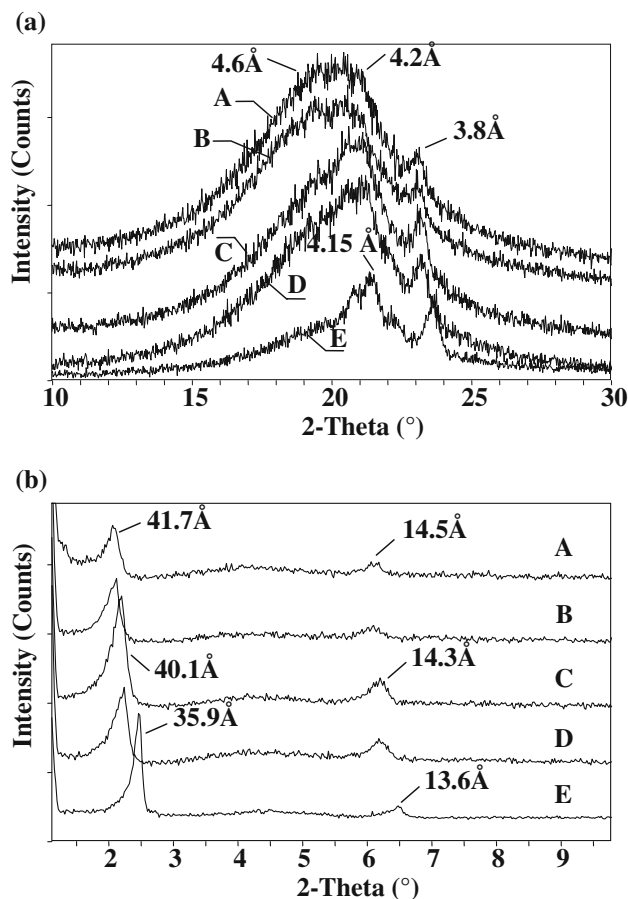
(a)

(b)

Fig. 3 Diffractograms for short spacings (**a**), long spacings (**b**) of BT, BT/CO blends before and after CIE determined by XRD. *A* BT/CO blend 65:35, *B* CIE BT/CO blend 65:35, *C* BT/CO blend 85:15, *D* CIE BT/CO blend 85:15, *E* BT alone

Melting and crystallization behaviors

The thermal behavior of edible fats is normally characterized by two physical events, namely melting and crystallization [33]. The melting and cooling curves of the BT, BT/CO blend 65:35, and BT/CO blend 85:15 before and after CIE obtained by DSC analyses are presented in Fig. 4.

The melting thermogram of BT shows three endotherms (3.95, 9.70, 42.60 °C) and two exotherms (7.3, 22.65 °C). The broadening effect between the three peaks was due to the broad TAG distribution in BT (Fig. 4a). After addition of 15 or 35% CO, the overall thermogram shifts slightly to the left. The maximum peak temperature shifts from 42.60 to 41.55 and 39.65 °C, respectively, and the minimum peak temperature also decrease, which indicated the dilution effect of the CO. CIE of BT/CO blend 85:15 and 65:35 narrows temperature range (difference between minimum and maximum peak temperature) from 1.55–41.55 °C to 2.05–41.55 °C, −1.30 to 39.65 °C to −1.05 to 38.95 °C,
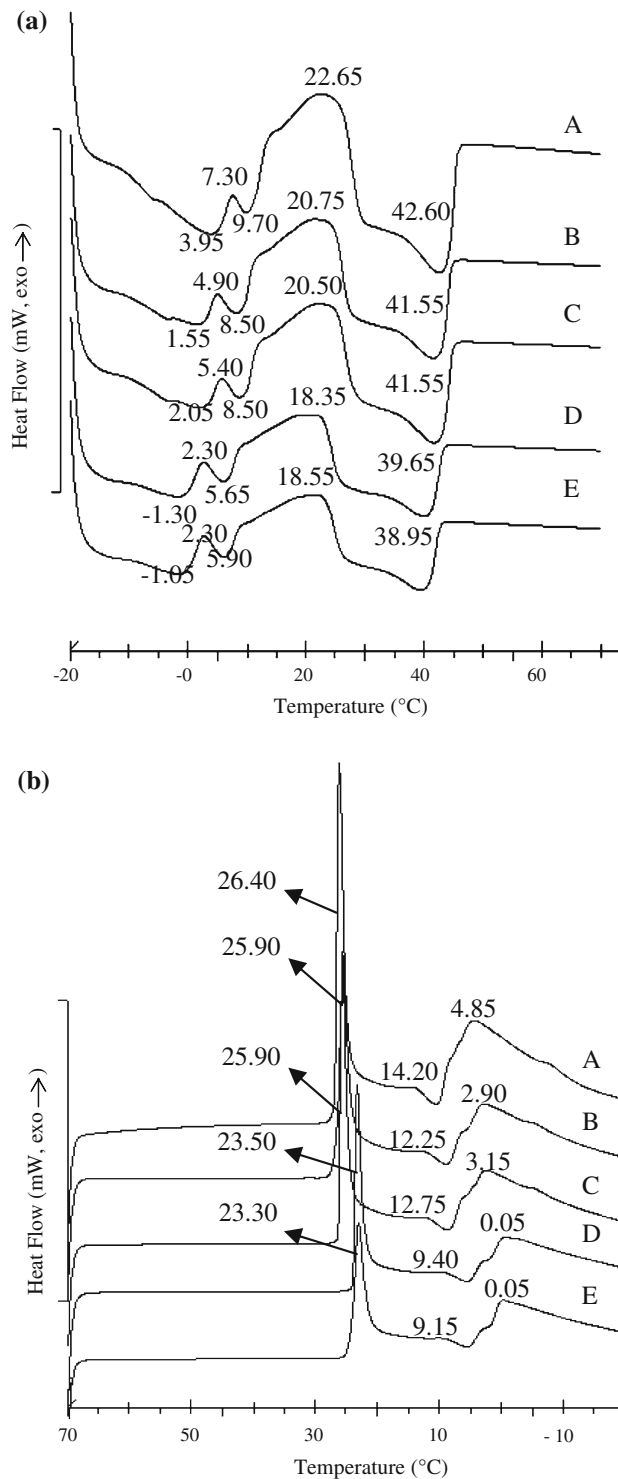
(a)

(b)

Fig. 4 Differential scanning calorimetry (DSC) melting (**a**) and cooling (**b**) curves of BT, BT/CO blends before and after CIE. *A* BT alone, *B* BT/CO blend 85:15, *C* CIE BT/CO blend 85:15, *D* BT/CO blend 65:35, *E* CIE BT/CO blend 65:35

respectively. These observations are supported by the changes of TAG composition after CIE as discussed in section "TAGs composition"—the high-melting TAG (S_3)

and low-melting TAG (U_3) decreased, middle-melting TAG (SU_2) increased.

The crystallization curves of BT show three major exotherms: a sharp high-temperature peak (26.40 °C), a middle-temperature peak (14.20 °C), and a broad low-temperature peak (4.85 °C). Thermograms indicated a slight left shift on the overall peaks and a decrease in peak size accompanied with the addition of CO and formation a new shoulder peak around 7 and 4 °C in both BT/CO blend 85:15 and 65:35 (data not shown in curves), respectively. The corresponding temperature ranges became narrower in interesterified blends than their original blends which is also an indication of TAG composition alteration after CIE. These results are in agreement with those obtained by Aguedo et al. [34].

The CIE of BT/CO blends had only slight impacts on the DSC profiles—no peaks disappearance and no new peaks formation. The consequences on DSC profiles were dependent on TAG composition, the relative concentration of U_3, S_3 TAG decreased, SU_2 increased, and little new TAG have been synthesized.

Rheological properties

Hardness is a critical factor in determining the functionality and consumer acceptance of semi-solid fat system, associated with TAG composition, solid fat content (SFC), crystal patterns (both polymorphism and morphology). Modification of TAG, SFC and crystalline network by IE affects the sensorial properties of fats, thus directing their application in food products [35]. The hardness of BT, substrate and interesterified blends stored at 5 °C (refrigeration temperature) and 24 °C (room temperature) is presented in Fig. 5. The hardness was negatively correlated
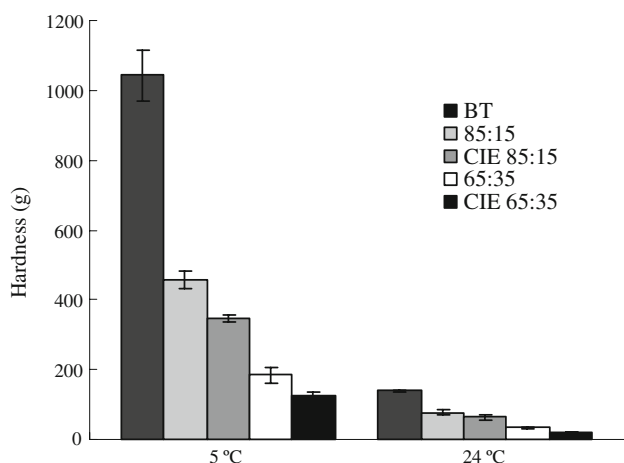
by the storage temperature since higher hardness values appear at 5 °C as compared to that at 24 °C. At the same temperature of storage, the hardness of BT, BT/CO blend 85:15 and 65:35 gradually decreased as the proportion of CO in the mixture increased. This was interpreted as a structurally weaker network, producing a decrease in SFC [36, 37].

Differences in crystal pattern and aggregation behavior due to CIE could lead to an alteration in the structure of the fat crystal network in BT, resulting in altered rheological properties such as the hardness [38]. The hardness of interesterified BT/CO blend 85:15 and 65:35 was lower than that of the original BT/CO blends at 5 and 24 °C, respectively. This can probably be due to the higher amounts of SU_2 TAGs and lower amounts S_3 TAGs produced after the CIE reactions.

The storage modulus (G') is a measure of the energy stored and recovered per cycle of sinusoidal deformation and is indicative of the solidlike or elastic nature of a substance, while the loss modulus (G'') is a measure of the energy lost per cycle of sinusoidal deformation and is indicative of the liquidlike or viscous nature of a substance [37]. Figure 6 depicts the change of G', G'' as a function of frequency for BT, BT/CO blends before and after CIE. For all substrates examined, G' was substantially higher than G''. This indicates that the blends were more elastic than viscous in character, and highly structured. The G' of BT/CO blend 85:15 and 65:35 was consistently higher than that of their CIE plastic fats, indicating that CIE led to a loss of elasticity, which appears to be correlated with hardness [37]. G'' was also lower for the CIE blends than for their original blends. Examination of the evolution of G'' in Fig. 6 revealed that G'' diminished more than G' for both substrate blends and CIE blends as a function of BT (w/w). According to the study of Kokini et al. [30], G'
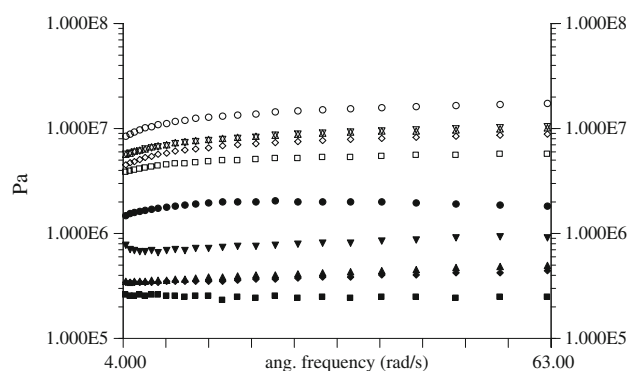

Fig. 5 Hardness of BT, BT/CO blends before and after CIE stored at 5 and 24 °C

Fig. 6 Storage (G', empty) and loss moduli (G'', solid) as a function of frequency for BT, BT/CO blends before and after CIE. BT—G' (*open circle*), G'' (*closed circle*); BT/CO blend 85:15—G' (*open inverted triangle*), G'' (*closed inverted triangle*); CIE BT/CO blend BT/CO blend 85:15—G' (*open triangle*), G'' (*closed triangle*); BT/CO blend 65:35—G' (*open diamond*), G'' (*closed diamond*); CIE BT/CO blend 65:35—G' (*open square*), G'' (*closed square*)

Table 4 Oxidation induction times of BT, BT/CO blends before and after CIE

Blend	Oxidation induction time (h)
BT	2.31 ± 0.01
BT/CO 65:35	9.84 ± 0.02
BT/CO 85:15	8.15 ± 0.01
CIE 65:35	7.98 ± 0.02
CIE 85:15	7.67 ± 0.03
CIE 65:35 + 0.02% TBHQ	13.98 ± 0.13
CIE 85:15 + 0.02% TBHQ	13.14 ± 0.18

Table 5 Effect of fat type on the specific volume and hardness of breads

	CIE-shortening	Commercial shortening	CIE-margarine	Commercial margarine
Specific volume (cm^3/g)	5.2 ± 0.2	5.0 ± 0.1	4.1 ± 0.1	4.6 ± 0.2
Hardness	578 ± 15.1	637 ± 18.5	542 ± 14.0	547 ± 15.4

dominates over G'' because the network bonding forces prevent translational movement. In a plastic fat, the intertwined crystal aggregates perform a similar function, leading to increased G' over G'' values. The shapes of the curves were quite similar for both substrate blends and CIE samples, indicating that all samples exhibited similar viscoelastic behavior, but on a different scale. All these effects were non-solid fat content-related, since solid fat content did not change substantially upon CIE.

Oxidative stability

Oxidation of unsaturated fatty acid residues results in rancidity, off-flavors, and musty odors [33, 34]. In many cases, steps must be taken to prevent the oxidation of oils and fats. The induction time is used as a measure of the oxidative stability of the semi-solid fat products. Table 4 shows the values of oxidation induction times of BT, BT/CO blends before and after CIE. The oxidation induction time of BT is 2.31 h. CO contains approximately 700 ppm tocopherol, so its application in formulation could improve oxidative stability. While BT contains higher amounts of saturated fatty acid than CO (50.68% for BT, 7.41% for CO), tocopherol could be more relevant than the nature of fatty acid composition to oxidative stability. So when 15 and 35% CO is added to BT, the induction time is increased to 8.15, and 9.84 h, respectively. In contrast, the induction time for CIE BT/CO blend 65:35 and 85:15 is lowered to 7.98 and 7.67 h, respectively, which may be incompatible with the marketable product. The CIE process reduced the oxidizability of initial blends. Results obtained in the present study were in accordance with findings of other researchers [6, 39], who attributed their results to a decrease in the levels of antioxidants (such as tocopherols) and alteration of fatty acid position in TAGs acompanying the CIE process [33, 40, 41]. Addition of 0.02% TBHQ to CIE BT/CO blend 65:35 and 85:15 increased the oxidation induction time of interesterified fats to 13.98 and 13.14 h,

respectively, which can meet oxidative stability requirements for products.

Instrumental and sensory analysis

The specific volume and hardness of breads made using the different fats are shown in Table 5. Compared with breads made with commercial shortening A and commercial margarine A, respectively, that with CIE-shortening and CIE-margarine had almost the same specific volume, but somewhat lower hardness.

Triangle test results showed that 26 of the 37 tasters could neither distinguish between breads made using the CIE-shortening and commercial shortening nor that using CIE-margarine and commercial margarine. Therefore, although the breads show differences in hardness to some extent, they have rather close sensory attributes which is a decisive criterion for consumers' acceptability.

Conclusion

CIE of the BT/CO blend causes a more balanced rearrangement of TAG species, and the resultant interesterified fat is exclusively double chain (2L) stacking β' crystal, has narrower melting and crystallization temperature ranges mainly due to decreased S_3 and U_3 type TAGs and increased SU_2 after CIE, and lower hardness, storage modulus (G') and loss modulus (G''); however, it shows reduced oxidative stability. By appropriate formulation in addition to using 0.02% TBHQ, the interesterified fat well suits bread baking applications as high stability low *trans* shortening and margarine, and is also expected to find more applications, e.g., in various types of bakery foods.

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