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MODIFICATION OF LARD'S THERMAL PROPERTIES TO IMPROVE ITS FUNCTIONALITY: POTENTIAL COCOA BUTTER SUBSTITUTE

MODIFICACIÓN DE LAS PROPIEDADES TÉRMICAS DE LA MANTECA DE CERDO PARA MEJORAR SU FUNCIONALIDAD: SUSTITUTO POTENCIAL DE MANTECA DE CACAO

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ABSTRACT

Lard is an animal fat containing specific triacylglycerols (TAGs) where the saturated fatty acids are mainly located in the sn-2 position providing it with inadequate attributes for the food industry, such as graininess. By Interesterification, a redistribution of fatty acids within the glycerol molecule takes place modifying fats and oils properties. Interesterification of lard and coconut oil (CO) blends at 70:30 and 80:20 ratios, resulted in IB_E70, IB_E80 (enzymatic procedure) and IB_C70, IB_C80 (chemical procedure). They were characterized by their acidity index (AI), iodine index (II) and thermal behavior by differential scanning calorimetry (DSC). II results showed that the highly saturated TAGs in CO affects lard only at the 70:30 ratio. DSC results made evident that the IB_E and IB_C melting profiles are not significantly different. Additionally, they showed higher crystallization and melting enthalpies compared to native lard, indicating a higher degree of intermolecular arrangement. These findings led to an application as a potential cocoa butter (CB) substitute. A mixture (CBR80) of 20% IBE70 and 80% CB, resulted in a thermal behavior that most resembled CB. Microstructure and texture showed CBR80 as a feasible CB replacer.

Keywords: Lard, Interesterification, Cocoa butter replacer

RESUMEN

La manteca de cerdo es una grasa que contiene triacilgliceroles (TAGs) específicos, en donde los ácidos grasos saturados se localizan principalmente en la posición sn-2, lo que determina atributos (textura arenosa) inadecuados para la industria alimentaria. Con la interesterificación, los ácidos grasos son redistribuídos dentro de la molécula del glicerol, modificando las propiedades de lípidos. Se interesterificaron mezclas de manteca de cerdo y aceite de coco (CO) en proporciones 70:30 y 80:20, obteniéndose IBE70, IBE80 (proceso enzimático) y IBC70, IBC80 (proceso químico). Se caracterizaron por índice de acidez, índice de yodo (II) y perfil térmico por calorimetría diferencial de barrido (DSC). Los resultados del II mostraron la influencia de los TAGs saturados del CO en las mezclas 70:30. Mediante DSC se encontró que no hay diferencia significativa entre los perfiles de fusión de IBE e IBC. Ambas presentaron entalpías de cristalización y fusión mayores que la manteca original, indicando un mayor orden de arreglo intermolecular. Estos resultados apuntaron hacia un potencial sustituto de manteca de cacao (CB). Se hicieron estudios térmicos, de microestructura y textura de una mezcla con 20% de IBE70 y 80% de CB (CBR80), como posible ingrediente para la confección de un sustituto de CB.

Palabras clave: Manteca de cerdo, Interesterificación, Sustituto de Manteca de Cacao

INTRODUCTION

Some edible fats and oils are not suitable for its application on many food products, mainly due to its difficulties for crystallization, melting temperature range and their consistency. Overall, the industry does not consider animal fats as the best ingredient for use in food formulations (Jimenez-Colmenero 2007; de Oliveira *et al*., 2015). Furthermore, some other fats with important uses in food industry, such as milk fat, cocoa butter (CB), among others, are expensive and/or its supply is uncertain (Ríos *et al*., 2014; Gregersen *et al*., 2015); therefore, there is a need to seek modification processes to make poorly functional fats applicable to a wider range of products. Modification procedures include hydrogenation, fractionation, interesterification and blends. Partial hydrogenation was for many years the traditional oil processing method, with the inconvenience of *trans* fatty acids formation and their adverse effects on human health (Ribeiro *et al*., 2009). Therefore, healthier alternatives are needed and a particular interest in the interesterification process has increased in recent years arising from the fact that the resulting modified fats do not possess undesirable byproducts (*i.e. trans* fats), and the process is relatively inexpensive (Zhang *et al*., 2015). Interesterification leads to the rearrangement of the acyl groups within or between TAGs molecules modifying the physicochemical properties (*e.g.* thermomechanical properties) of the final fat, maintaining

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their fatty acid composition (De Clercq *et al*., 2011). Interesterification can be performed chemically or enzymatically. The chemical reaction involves sodium alkylates as main catalysts, which are easy to handle and remove, and can initiate the reaction in low concentrations, causing a random fatty acid redistribution. However, the application of chemical catalysis can cause the formation of soap and methyl esters. On the other hand, the enzymatic interesterification takes place under relatively mild conditions, reducing the oil/fat loss with fewer unit operations. This technique has the great advantage of allowing a specific redistribution of fatty acids, for design products (Zhang *et al*., 2015; Paula *et al*., 2014). Consequently interesterification is a promising tool that can be used to modify poorly functional fats, that when combined with vegetable oils, could enhance some of their thermomechanical properties (*e.g.* melting point, hardness) (Wang *et al*., 2016).

Lard is an animal fat obtained from the adipose tissue of pig by a rendering process. Lard is distinctive from other fats because the saturated fatty acids are mainly located in the *sn*-2 position and the unsaturated fatty acids in the external positions. It is also known that lard crystallizes generally in the most stable polymorph of fats (*i.e.* β). Unfortunately, this provides it with inadequate functional characteristics such as graininess and poor creaming, limiting its use in the food industry (Steen *et al*., 2015; Seriburi and Akoh, 1998; Campos *et al.,* 2002). On the other hand, coconut oil (CO) has gained importance in food and cosmetic industries over recent years. The unique fatty acid composition of this oil, containing more than 65% medium-chain fatty acids (fatty acids of 6 to 12 carbon atoms), provides higher water solubility and better digestibility than fats with TAGs containing long-chain fatty acids (Cassiday, 2016; Nugrahini *et al*., 2015). CO crystallizes mostly in the desired polymorphic β′ form (Chaleepa *et al*., 2010) which delivers a smooth texture (Campos *et al*., 2002). Hence, for the purpose of this investigation, we chose CO as blending oil for the interesterification process to improve the physicochemical and functional properties of lard. In previous studies, blends of lard and vegetable oil (*i.e.* soybean oil) have been modified through enzymatic interesterification in order to obtain structured lipids to be applied as plastic fats and human milk fat substitutes, with a successful outcome (Silva *et al*., 2012; da Silva *et al*., 2013). Moreover, there are studies focused on alternatives for fats used as important ingredients in food products, such as CB, due to its high price and increasing demand. The basis of these alternatives are the modification of commercial vegetable oils and some animal fats through enzyme-catalyzed interesterification to obtain CB substitutes and/or equivalents (Kadivar *et al*., 2013).

In this study, two blends of lard and CO (80:20 and 70:30) were enzymatically and chemically interesterified resulting in an interesterified blend. Their physicochemical properties and possible application as a CB substitute were evaluated.

MATERIALS AND METHODS

Materials

The interesterified blends (IB) chemically (IB_C) and enzymatically (IB_E) were produced from blends of lard and CO in a 70:30 (IB_C70, IB_E70) and 80:20 ratio (IB_C80, IB_E80). Lard was obtained from a local butchery in Aguascalientes, Ags., Mexico and CO from Hain Pure Foods (The Hain Celestial Group, USA); and CB from Pealpan. San Luis Potosi, Mexico.

Interesterification

Chemical interesterification was achieved with sodium methoxide as a catalyst (Sigma-Aldrich) following Roy and Bhattacharyya (1993) method, with modifications. Samples of 100 g with 0.4 g of catalyst were held at 90 °C for 40 min with constant stirring (300 rpm) with a magnetic stirrer and under reduced pressure conditions. Then, the catalyst was neutralized with citric acid and the reaction mixture was washed with boiling water. Afterwards, the product was separated using a sedimentation funnel. Finally, product´s moisture was removed with reduced pressure. IB_C samples were bottled and kept under refrigeration at 0 °C. For the enzymatic interesterification, the methodology of DeClerq *et al.* (2011) was applied, *sn*-1,3 specific lipase from *Candida antarctica B* (Novozyme) was used. The reaction mixture consisting of 100 g sample with 1% of enzyme was held at 70 °C for 30 h with constant stirring (200 rpm) under vacuum conditions. At the end of the reaction time, the mixture was filtered to recover the enzyme. Finally, the IB_E samples were bottled and kept under refrigeration at 0 °C.

Chemical characterization

The acidity and iodine index were determined through the official Mexican Normative procedures: NMX-F-101-1987 and NMX-F-152-SCFI-2011 respectively. Each determination was carried out with the corresponding replicate.

Differential Scanning Calorimetry (DSC)

Hermetically sealed samples in aluminum pans were analyzed with a TA Instruments (New Castle, DE, USA) Model Q1000 equipment to determine their crystallization and melting thermograms. Samples were heated at 80 °C for 15 min, cooled to -40 °C at a rate of 10 °C/min. After 2 min at -40 °C, samples were heated to 80 °C, at a rate of 5 °C/min. Thermal parameters were obtained through a TA Instruments Universal Analysis 2000 (v. 4.5A) software. All determinations were carried out with a replicate.

IB blends with CB

Samples of IB_E70 were blended with CB at 20% (CBR20), 30% (CBR30), 50% (CBR50) and 80% (CBR80).

Solid Fat Content (SFC)

SFC was determined by AOCS official method (Cd 16b-93 1997); using a low resolution nuclear magnetic resonance equipment (the minispec mq20 BRUKER NMR Analyzer, Brucker Analytik; Rheinstetten, Germany). As CB is a stabilizing fat,

it must be tempered before measuring SFC profile. Test tubes were filled to a height of 4 cm and melted at 100 ºC for 15 min, followed by the tempering at 60 ºC for 5 min, 0 ºC for 90 min, 26 ºC for 40 h, 0 ºC for 90 min. Finally, kept for 60 min at the measuring temperatures: 0, 5, 10, 20, 30, 40 and 60 ºC. Each determination was carried out with a replicate.

Polarized light Microscopy

The microstructure was analyzed using an Olympus BX51 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) equipped with a color video camera (KP-D50; Hitachi Digital, Tokyo, Japan) and a Linkam TP94 heating/cooling stage (Linkam Scientific Instruments, Ltd., Surrey, England) connected to an LTS 350 temperature control station (Linkam Scientific Instruments, Ltd.) and a liquid nitrogen tank. A droplet of melted fat was placed between a glass slide and a glass slip. The sample was first melted at 60 ºC, followed by the same thermic program used for SFC determination. After the 40 h incubation period at 26 ºC, samples were placed on the heating/cooling stage and kept at 20 or 30 ºC for 60 min. After the 60 min incubation time, polarized light microphotographs (PLM) were obtained at 20x.

Texture analysis

The texture was analyzed using a TA-XTPlus texture analyzer equipment (Stable Microsystems, Surrey, UK) with an aluminum 20 mm cylinder probe (P/20), using 5 Kg load cell. Samples were penetrated to a depth of 12 mm with a constant speed of 1.0 mm/s. Blends were analyzed at 0, 10, 20 and 30 ºC respectively; and firmness (Kg) was recorded using the equipment software (Texture Exponent 32; Stable Microsystems). Five independent determinations for each sample were carried out.

Statistical analysis

The data was analyzed with the Statistica software (V 12.0 StatSoft Inc., Tulsa, OK, USA). A one-way ANOVA followed by a Tukey's significance test for comparison was applied to determine significant differences.

RESULTS AND DISCUSSION

Chemical characterization

Acidity index (AI) serves as an indicator of fats and oils quality since it represents the free fatty acids proportion in a system and it can be used as an estimation of fat's stability. According to official Mexican normative (NMX-F-110-1999), regarding lard's specifications, AI should not be higher than 1% (expressed as oleic acid). On another note, the international normative institution- Codex Alimentarius, establishes that lard can present AI values up to 1.25% (CODEX STAN 211-1999, 2015). Within this context for the IB_E80 and IB_E70, the AI resulted higher than the acceptable normative values, NMX-F-101-1987; NMX-F-110-1999 (4.51%±0.07 and 4.22%±0.1 respectively) indicating a considerable amount of free fatty acids in the studied samples. On the contrary, for the IB_C80 and IB_C70 the AI was 0.82%±0.07 and 1.09%±0.06 respectively, which is within the acceptable parameters. In interesterification studies (performed enzymatically and/or chemically), it has been noticed that higher rates of diacilglycerides and free fatty acids are produced by the enzymatic process in comparison with the chemical interesterification, and that lipases are able to hydrolyze the TAGs to free fatty acids, mono and diacylglycerols. This can be associated with the presence of water in the sample, even with the enzyme preconditioning and water/moisture removal before the initiation of the interesterification process (Lopes *et al*., 2016; Wirkowska-Wojdyla *et al*., 2016). Therefore, this indicates that the interesterification of fatty acids in the chemical procedure is more effective than in the enzymatic procedure, under this study conditions. In spite of these differences, none of the IB samples presented any visible evidence of sensorial defects in terms of odor, color and consistency (results not shown).

Fat stability can also be evaluated by II (iodine index), which allows us to measure the degree of unsaturation among the fatty acid chains present in oils and fats (Adewale *et al*., 2014). Lipids with a higher II have a tendency to oxidation due to the higher degree of double bounds leading to less stability. For the same percentage of CO in the IBs results, there are no significant differences between the samples II (61.03 ±0.6 for IB_E80; 60.91±0.5 for IB_C80- and 34.45±0.6 for IB_E70 and 34.26±0.5 for IB_C70 ($p>0.01$). This, since during an interesterification process the fatty acid composition remains the same, including the degree of unsaturation, regardless of the process type *(i.e.* chemical or enzymatic). According to the official normative (NMX-F-110-1999), the acceptable II for lard and byproducts is 45-70, and for this particular lard used in the blends, the II was 62.63 ± 1.6, therefore for blends IB_E80 and IB_C80, the amount of CO used was not sufficient to significantly change their II ($p>0.01$). On another note, values of II for blends IB_E70 and IB_C70 were lower than the II normative values of lard but higher than the CO (6.3-10.6), and so the higher proportion of CO in IBs, largely affects lard, due to the highly saturated TAGs in CO (NMX-F-110-1999; CODEX STAN 211-1999., 2015) making it more stable in terms of oxidation.

Thermal properties

For discussion purposes of this study, as shown in Table 1, regarding the crystallization profile, the only crystallization temperature analyzed is the start of crystallization (*i.e.* beginning of the first exotherm) denominated T_{cr} for all samples. For the melting profile, all melting temperatures were analyzed at the maximum of the endotherms (T_{M1}, T_{M2}, T_{M3}). As for the crystallization enthalpy (ΔH_{cr}) and melting enthalpy (ΔH_M) only one value was determined as the sum of all transitions in the crystallization and melting profile correspondently. Moreover, uninteresterified blends (UIBs) of lard and CO were prepared in the same proportions of the IBs for comparison purposes (UIB70, UIB80).

First, it is evident that the general melting profile of all IBs as seen in Figure 1 is very similar. For the same proportion but different interesterification method the main melting parameters *(i.e.* T_{M1}, T_{M2}, T_{M3} *and* ΔH_M) are not significantly

Table 1. Crystallization (T_{cr}) and melting (T_{M1} T_{M2} T_{M3}) temperatures for raw materials (CO and Lard); Interesterified blends (IB_E70 and IB_E80) and Uninteresterified Blends (UIB70 and UIB80).
Tabla 1. Temperaturas de cristalización (T_{cr}) y fusión (T_{M1} T_{M2} T_{M3}) de materias primas (CO y manteca de cerdo); Mezclas interesterificadas (IB_E70 and IB_E80) y Mezclas no interesterificadas (UIB70 and UIB80).

SAMPLES	T_{cr1} (°C)	ΔH_{cr}(J/g)	T_{M1} (°C)	T_{M2} (°C)	T_{M3}(°C)	ΔH_M(J/g)
CO	[a] 5.4 ±0.3	[a] 106.7 ±0.07	-	[a] 22.5 ±1.4	-	[a] 130.5 ±5
Lard	[b] 13.4 ±3.8	[b] 71.7 ±0.9	[a] -5.0 ±1.2	-	[a] 28.5 ±0.5	[b] 71.6 ±1.1
IB_E 70	[c] 11.8 ±0.0	[c] 64.9 ±0.8	[b] -2.3 ±0.3	[b] 13.7 ±1.4	[a] 28.1 ±2.1	[c] 78.3 ±0.9
IB_E 80	[b]14.2 ±4.7	[b] 72.5 ±0.6	[a] -5.2 ±0.7	[b] 12.3 ±3.0	[a] 30.6 ±2.9	[d] 87.9 ±2.2
UIB 70	[a] 5.1 ± 0.2	[d] 41.3 ±0.1	[b] -2.6 ± 0.1	[b] 11.4 ±0.3	[b] 26.7 ±0.3	[e] 44.5 ±1.1
UIB 80	[d] 7.2 ±0.2	[e] 32.5 ±0.8	[c] -3.6 ±0.06	[b] 12.9 ±0.07	[a] 27.9 ±0.01	[e] 39.3 ±0.3

Means ± SD n=2. Different letters mean significant differences ($p<0.05$)

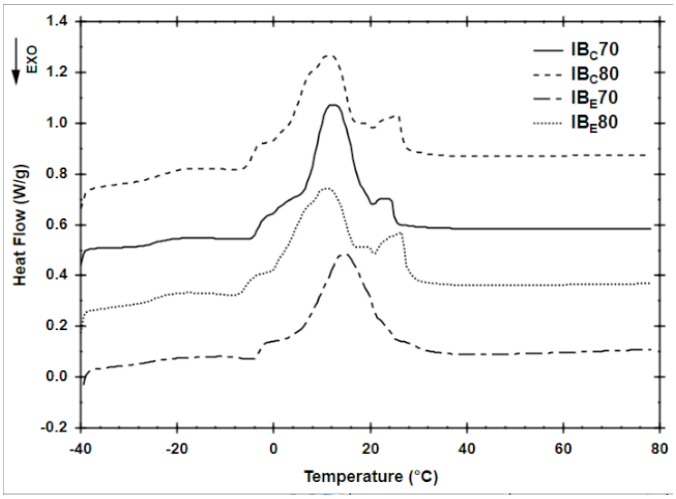

Figure 1. Melting thermograms of IB_E70, IB_C70, IB_E80, IB_C80 (see supporting information for crystallization thermograms).
Figura 1. Termogramas de fusión de IB_E70, IB_C70, IB_E80, IB_C80 (ver en información complementaria los termogramas de cristalización).

different ($p>0.05$) (see supporting information). This could be attributable to the small proportion of used enzyme (*i.e.* 1%), perhaps the use of a greater amount of enzyme could result in a different TAGs profile sufficient to differentiate the thermal behavior of the IB_E products from the IB_C products (De Clercq *et al*., 2011). Additional investigation is necessary to establish the relationship between enzyme concentration used in the interesterification process and the resultant TAGs profile. Since the interesterification method had no meaningful effect on the thermal behavior of the IBs only the IB_E70 and IB_E80 were chosen for further discussion.

The crystallization and melting profile are similar to those previously reported for a variety of native lard and CO (Fig. 2). The thermal behavior of these raw materials is of multiple transitions due to their complex TAGs profile and their polymorphic nature (Shen *et al*., 2001; Steen *et al*., 2015). In general, interesterification procedures led to modifications in the thermal properties (*i.e.* T_{cr}, T_{M1-3}, ΔH_{cr}, ΔH_M) of the original raw materials (*i.e.* Lard and CO) and the UIBs (Table. 1, Fig. 2). UIBs thermal profiles are the result only of the mixed TAGs of

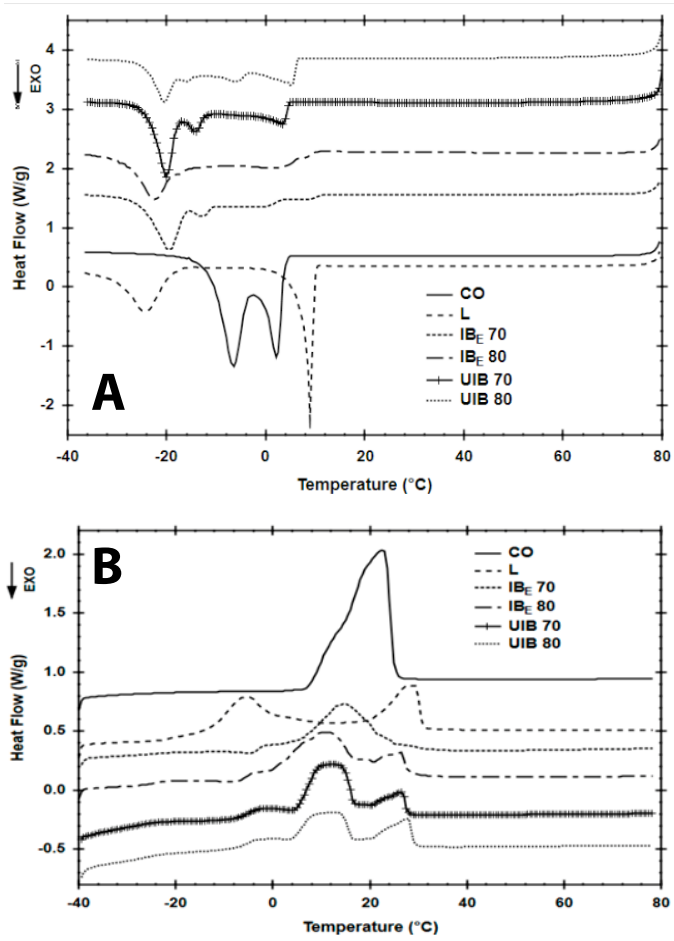

Figure 2. Thermal behavior of CO, Lard (L), IB_E 70, IB_C 70, IB_E80, IB_C80, UIB70 and UIB80*)* A) Crystallization, B) Melting.
Figura 2. Comportamiento térmico de CO, manteca de cerdo (L), IB_E 70, IB_C 70, IB_E80, IB_C80, UIB70 y UIB80*)* A) Cristalización, B) Fusión.

lard and CO with three very distinctive endotherms (Fig. 2B). The slight differences in values in T_{M1} and T_{M3} from lard and T_{M2} from CO, are due to dilution since they are in a mixture (da Silva *et al*., 2013; Miklos *et al*., 2013). The lower results in UIBs ΔH_{cr} and ΔH_M values than those for lard and CO, $p<0.05$ (Table 1); are related to the intermolecular arrangement (*i.e.* chain-

chain interactions) of TAGs species from the different crystalline forms (Silva *et al*., 2012). The incompatibility among the different TAGs (e.g. due to a steric effect) (Kiyotaka, 2001) of lard and CO could be causing the energetic reduction. Thus, the differences of thermal parameters between the UIBs and the IB_Es indicate changes in the TAGs composition achieved by interesterification (Silva *et al*., 2012), this because the modification in the TAGs profile leads to changes in crystallization and polymorphic form of fats. Both IB_Es resulted in a higher T_{cr} (Table 1, Fig. 2A) than the correspondent UIBs, this could indicate that interesterification led to a higher percentage of trisaturated TAGs (da Silva *et al*., 2013), which need less degree of supercooling to nucleate, nevertheless this should be confirmed by a qualitative chromatographic analysis. In the same line ΔH_{cr} and ΔH_M values for the IB_Es (Table 1) also showed higher values than their corresponding UIBs ($p<0.05$). Thus the resultant TAGs composition in the IB_Es could be leading to crystals with higher degree of intermolecular arrangement (Shen *et al*., 2001; Silva *et al*., 2012; da Silva *et al*., 2013) (*i.e.* releasing/absorbing more energy).

On the other hand, when comparing IB_Es thermal parameters directly with the original lard (Table 1, Fig. 2), the most noticeable difference is the appearance of T_{M2}. Since raw lard crystallizes in a β form (Campos *et al*., 2002), T_{M3} should correspond to the melting point of that crystal lattice. The tendency of the IBs to have only one major exotherm at lower temperature (*i.e.* T_{M2}) could be due to the formation of a less stable polymorph, most likely β' since the melting temperature is much closer to T_{M2} for the CO (Chaleepa *et al*., 2010). Considering that the crystallization of β form results in a brittle and grainy texture and β' in a smooth texture (Campos *et al*.,2002), the formation of the new type of aggregation is most likely towards the disappearance of crystals responsible of the graininess flaw of lard (Miklos *et* al., 2013; Meng *et* al., 2010). In other studies, vegetable oils low in saturated long-chain fatty acids, have been used in blends to reduce graininess of animal fats. Granular crystals are easily developed in lard and in other fats such as palm oil products, since long-chain fatty acids such as stearic and palmitic, have been linked with graininess formation; the addition of CO rich in medium-chain fatty acids, by interesterification, could reduce granular crystallization (Meng *et al*., 2010). Even though the IB_Es presented three melting temperatures (*i.e.*T_{M1-3}), it is evident the tendency to have one major endotherm decreasing the preponderance of very distinctive multiple endotherms observed in lard and even in the UIBs (Fig. 2B). This indicates modifications in crystal aggregation to a more homogenous type of crystals. The latter is concomitant with the higher ΔH_M values observed in IB_E samples ($p<0.05$), indicative of not only the incorporation of the saturated fatty acids of CO, but also a more orderly crystal aggregation (da Silva *et* al*.,* 2013*;* Meng *et al*., 2010). Finally the only differences on thermal parameters between IB_E70 and IB_E80 samples rely mostly on the higher ΔH_{cr} and ΔH_M values for IB_E80 ($p<0.05$), indicating that the lower amount of fatty acids of CO, incorporated during interesterification, resulted in TAGs with higher degree of molecular interactions (Shen *et* al*., 2001)*.

The analysis and observation of this data, led us to find an application for the IBs, since their behavior resembled the one of CB. Therefore, some blends in different proportions of IB_E70 and CB were chosen; IB_E was chosen over the IBc due to the milder conditions of its modification process, assuming that the integrity of this blend components was better and IB_E70 was chosen over the IB_E80 due to the higher oxidation stability shown by the II analysis.

IBs blend with CB as potential substitute for CB

For the DSC results regarding CB, the melting profile resulted in one major exotherm with a characteristic melting temperature (T_M) of 21.0 °C ± 0.2 similar to what has been reported in other studies (Fig. 3) (Kadivar *et* al., 2016). For the IB_Es mixtures with CB (CBR20, CBR50, CBR80), results showed that the amount of CB in the mixture is proportional to their T_M (14.23 ±0.5, 17.01 ±0.4, 18.01 ±0.2 °C respectively). This corresponds to the contribution of the higher melting TAGs (*i.e. saturated-oleoyl-saturated)* contained in CB (Kadivar *et* al., 2013). Consequently the CBR80, containing 80% of CB and 20% of IB_E, resulted on the thermal behavior that most resembles CB among the CBR mixtures (Fig. 3).

In addition to DSC, SFC profile is used to describe the melting behavior of fats, which influences highly in their

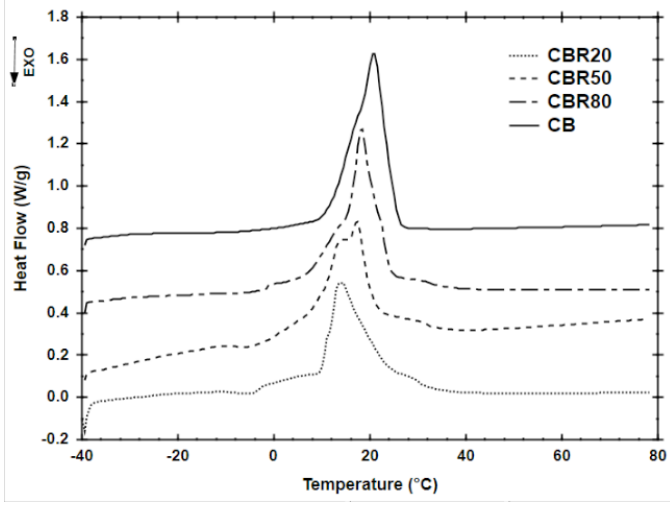

Figure 3. Melting thermograms of CBR20, CBR50, CBR80 and CB (see supporting information for crystallization thermograms).
Figure 3. Termogramas de fusión de CBR20, CBR50, CBR80 y CB (ver en información complementaria los termogramas de cristalización).

functionality. Depending on their SFC profile, a fat's suitability for a specific application can be established (Kadivar *et* al., 2016). After recording the SFC of the CBR mixtures at different temperatures, an iso-solid diagram was built (Fig. 4). These iso-solid curves can provide information about the state and compatibility of a mixture at a given temperature (Kadivar *et* al., 2016; Wang *et al*., 2011). According to SFC results, the blend that most resembled CB behavior was once again the CBR80, at temperatures below 10 °C, showing a SFC above 70% (Fig. 4). This indicates that CBR80 could be a promising

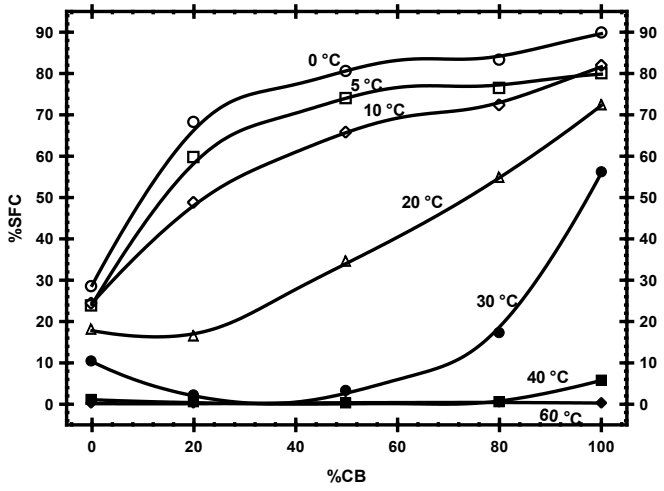

Figure 4. Iso-solid curves of CBR mixtures (see supporting information for %SFC values).
Figura 4. Curvas de iso-sólidos de mezclas de CBR (ver información complementaria para los valores de %SFC).

replacer ingredient at these temperatures for products that require low temperature processes and storage. Additionally it is important to mention that CBR80 at 20°C has the same amount of SFC as CB at 30°C (55.3% ± 1.6, p>0.2) making it suitable for wider applications.

In further physicochemical characterization, the texture of a fat is determined by their SFC and by its microstructure (Gregersen *et al*., 2015). Generally firmness in fats depends on intra-particle (crystal-crystal) and inter-particle (cluster-cluster) junctions of crystal clusters where their relative strength can be evaluated based on the density of packed crystals as they appear in microscopic images (Gregersen *et al*., 2015). Within this context and since so far, results indicated that the CBR80 mixture most resembles CB, PLMs were taken of samples with the same %SFC (CBR80 at 20°C and CB at 30 °C) (Fig. 5). Since both samples had the same amount of solids, differences in texture should be solely due to their microstructure. PLM of CBR80 (Fig. 5A) shows a higher crystal density and a more orderly packed crystal network with larger crystal clusters than the looser network with lower degree of order developed by CB (Fig. 5B). The greater degree of order of the crystal network developed by CBR80 resulted from higher intra-particle junctions (*i.e.* bigger clusters) and higher inter-particle junctions (*i.e.* higher density) than CB (Fig. 5B). This explains the results from the texture analysis where CBR80 resulted in superior firmness than CB (27.3 ±2.7, 24.8 ±1.3 kg respectively). Therefore, this shows that CBR80 is capable of developing more firmness with less solids than CB, which can be useful in the tailoring of a CB substitute.

CONCLUSIONS

Results of the present study showed that interesterification (*i.e.* chemical or enzymatic) of lard in blends with CO successfully modified their physicochemical properties, indicating the development of a more functional fat. First, special attention was directed to their thermal profile, where it was clear that the resulting IBs where independent of the interesterification method used (*i.e.* chemical or enzymatic). IB_E thermal properties indicated that interesterification could be leading to a higher proportion of more saturated TAGs forming crystals with higher degree of intermolecular arrangement. Additionally, IB_E presented a more homogeneous crystallization with a lower major melting temperature (*i.e.* T_{M2}) probably by the formation of a less stable polymorph β' causing the disappearance of lard's granular crystals. With this in mind, in future work, quantitative chromatographic analysis is necessary to elucidate the specific modified TAGs profile. In addition, a detailed texture analysis along with X-ray determination is required to confirm the disappearance of the graininess flaw.

Finally, although no precise CB substitute was found in this study, one mixture of IB_E and CB (*i.e.* CBR80) was proved useful in the tailoring of a CB substitute by the similarities in the thermal parameters but with some differences in their texture.

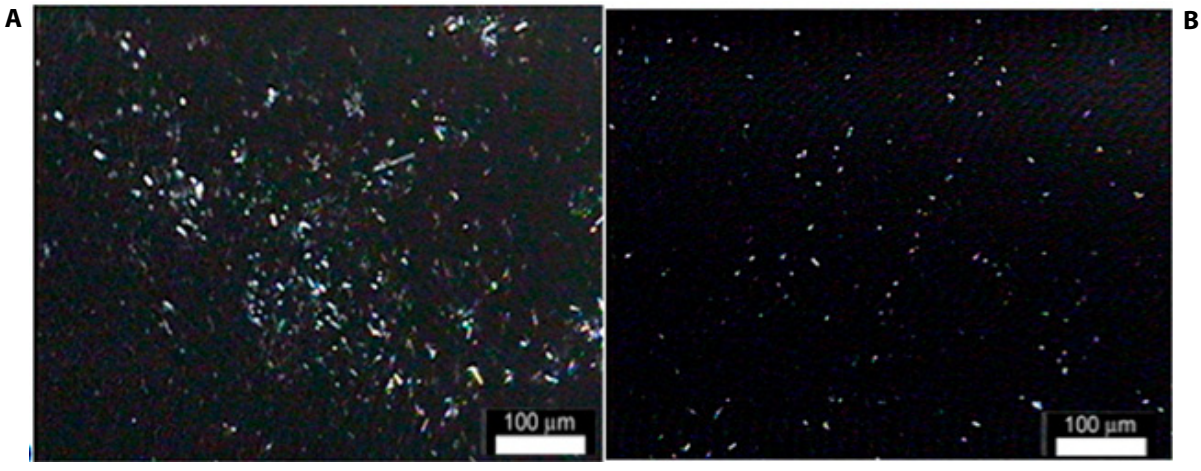

Figure 5. Microstructure of CBR80 (A) and CB (B).
Figura 5. Microestructura de CBR80 (A) y CB (B).

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All the authors declare not to have any conflict of interests.

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