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Comparison of Chemical and Enzymatic Interesterification of Fully Hydrogenated Soybean Oil and Walnut Oil to Produce a Fat Base with Adequate Nutritional and Physical Characteristics

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Summary

The optimal physical, chemical and nutritional properties of natural lipids depend on the structure and composition of triacylglycerols. However, they are not always mutually compatible. Lipid modification is a good way to give them specific functionalities, increase their oxidative stability, or improve their nutritional value. As such, chemical and enzymatic interesterification may be used to modify them and produce structured lipids. In accordance, the aim of this study is to compare chemical and enzymatic interesterification of binary blends of fully hydrogenated soybean oil and walnut oil, using sodium methoxide or Lipozyme TL IM, respectively, to produce a fat base with adequate nutritional and physical characteristics. Three different mass ratios of fully hydrogenated soybean oil and walnut oil blends (20:80, 40:60 and 60:40) were interesterified and evaluated. Total interesterification was determined by the stabilization of the solid fat content. Chemical reaction of the 20:80 blend was completed in 10 min and of the 40:60 and 60:40 blends in 15 min. Enzymatically interesterified blends were stabilized in 120 min at all of the mass ratios. Complete interesterification significantly reduced the solid fat content of the blends at any composition. Chemical and enzymatically interesterified fully hydrogenated blend of soybean and walnut oil at mass ratio of 40:60 showed the plastic curve of an all-purpose-type shortening rich in polyunsaturated fatty acids, with a high linolenic acid (C18:3n3) content and with zero *trans*-fatty acids.

Key words: chemical interesterification, enzymatic interesterification, triacylglycerol, hydrogenated soybean oil, walnut oil, solid fat content

Introduction

Lipids are the highest energy source of the three macronutrients (carbohydrates, proteins and lipids). They also add flavour, texture and satiety to foods. Slight differences in the fat produce significant changes in the food. For instance, a fat that performs well in baked products will not work well enough in ice cream, producing a pasty and waxy feeling instead of a pleasant cooling effect (*1*). On the other hand, some fats may have excellent physical properties for specific applications but are not recommended due to nutritional concerns. These lipids may not be absorbed as expected or may even have some deleterious effects on health due to the presence of certain fatty acids. Each of the three fatty acids bonded to the sn-1, sn-2 or sn-3 position of the glycerol backbone can vary in regard to chain length, number and position of double bonds, and geometrical configuration. These characteristics give lipids their physical, chemical and nutritional properties (*2*,*3*).

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The optimal physical, chemical and nutritional properties of an oil or fat are not always mutually compatible (*4*). As such, lipid modification is a good way to give them specific functionalities, increase their oxidative stability, or improve their nutritional value. The temperature at which fats crystallize and melt or the ratio and kind of solid and liquid fats that produce a specific plastic behaviour are physical properties that are specific for each application (*4*). Concerns about *trans* configuration or actions to reverse the obesity epidemic in which the aim is to reduce oil absorption stand in contrast to the need to increase the poor lipid absorption in an immature digestive system or in patients with cystic fibrosis (*5*,*6*). Our knowledge of lipid metabolism has played a part in the development of structured lipids. These lipids are obtained synthetically by changing the fatty acid composition and/or their distribution in the glycerol backbone in order to meet a specific need and improve their nutritional or functional properties (*7*). Betapol™ and Salatrim are structured lipids produced with nutritional purposes. One is a substitute for human milk fat and the other one is a reduced-calorie fat. Structured lipids may also mimic desirable physicochemical properties. Plastic fats and cocoa butter equivalents prepared from lower value fats and oils can also be synthesized (*8*,*9*).

There are technological and biological methods of modifying oils and fats that may expand their uses. The biological methods include genetic engineering and crop or animal control (*4*). The technological methods include blending and fractionation, which are physical processes that produce value-added fats and oils (*1*). However, it is not always easy to predict what will happen to minor components that could affect the oxidative stability of products (*4*). Hydrogenation solved this problem, allowing for the production of fats with creaming properties, frying stability, sharp melting properties, or other functional characteristics for specific applications while enhancing oxidative stability (*1*). However, hydrogenated fats contain the two least desirable fatty acids: saturated and *trans* (*10*,*11*). Full hydrogenation is an alternative that produces hard fats, which may be used to prepare low to zero-*trans* commercial fats through interesterification (*12*, *13*). An interesting raw material that can be used in this process is fully hydrogenated soybean oil. This is a relatively low-cost product with a high C18:0 content (around 85 %). It is not atherogenic and has none of the adverse effects on cardiovascular diseases reported for shorter fatty acids (C12:0, C14:0 and C16:0) (*13*).

Interesterification is a reaction through which it is possible to rearrange the fatty acids in the triacylglycerol molecule so that its composition changes but the fatty acid profile is preserved (*14*–*16*). There are two ways to carry out interesterification: chemical and enzymatic. Chemical interesterification requires an alkaline catalyst. It is relatively inexpensive, readily available, and easy to use and scale-up. However, it lacks specificity, offering little or no control over the position in which fatty acids are distributed in the final product (*7*,*17*). Enzymes can be used to increase the amount of control that one can have over the nature of the product as a consequence of the specificity shown by many lipases. As such, enzymatically interesterified lipids have a more defined structure (*11*,*18*). The enzymatic process offers milder reaction conditions and thus lowers degradation of long-chain polyunsaturated fatty acids. Moreover, it also produces fewer by-products than the chemical process (*19*–*21*). In this respect, raw materials such as walnut oil are of great interest. Walnuts are unique within the nut family due to their high polyunsaturated fatty acid content, specifically C18:3n3, and a ratio of C18:2n6/C18:3n3 of 4:1, which has shown to decrease the risk of heart disease (*22*).

In accordance, the aim of this study is to compare chemical and enzymatic interesterification of binary blends of fully hydrogenated soybean oil and walnut oil and evaluate them in the synthesis of a zero *trans*-fat and high C18:3n3 fat bases.

Materials and Methods

Materials

Raw materials for chemical and enzymatic interesterification were fully hydrogenated soybean oil supplied by Watt's S.A. (Santiago, Chile) and walnuts donated by Valbifrut S.A. (Santiago, Chile). Walnut oil was obtained by cold pressing. The product was also neutralized and bleached (*23*). Fully hydrogenated soybean oil and walnut oil were stored at 4 °C in a nitrogen atmosphere until the experiments were conducted. Raw materials were chosen because of the saturated fatty acid content of fully hydrogenated soybean oil and the associated high melting point (68–75 °C), and the high C18:3n3 content of walnut oil (14 %), as shown in Table 1. For chemical interesterification, analytical grade sodium methoxide (95 %, Sigal Ltda., Santiago, Chile) and citric acid monohydrate

Table 1. Fatty acid composition of raw materials: fully hydrogenated soybean oil (FHSBO), walnut oil and FHSBO/walnut oil mixes

Fatty acid	*w*/%				
	FHSBO	Walnut oil	FHSBO/walnut oil		
			20:80	40:60	60:40
not determined	0.38	0.59	0.51	0.46	0.42
C12:0	0.98	–	0.39	0.59	0.78
C14:0	0.44	0.03	0.19	0.28	0.36
C15:0	0.05	–	0.02	0.03	0.04
C16:0	10.06	7.65	8.61	9.10	9.58
C16:1/17:0	0.20	0.16	0.18	0.18	0.19
C18:0	87.18	2.4	36.31	53.27	70.22
C18:1 isomer	–	0.31	0.19	0.12	0.06
C18:1n9-*trans*	0.03	–	0.01	0.02	0.02
C18:1n9-*cis*	0.17	16.36	9.88	6.65	3.41
C18:2 isomer	0.05	1.86	1.14	0.77	0.41
C18:2n6	0.06	56.52	33.94	22.64	11.35
C18:3 isomer	–	0.32	0.19	0.13	0.06
C18:3n3	–	13.78	8.27	5.51	2.76
C22:0	0.37	0.02	0.16	0.23	0.30
C24:0	0.04	–	0.02	0.02	0.03

(Merck, Santiago, Chile) were used. For enzymatic interesterification, Granotec Chile S.A. (Santiago, Chile) donated Lipozyme® TL IM, an immobilized and sn-1 and sn-3 stereospecific enzyme obtained from *Thermomyces lanuginosus*.

Chemical interesterification

Blends of fully hydrogenated soybean oil and walnut oil made at three mass ratios (20:80, 40:60 and 60:40) were chemically interesterified following the procedure described by Rodríguez *et al*. (*24*). Briefly, each blend was dried under vacuum conditions (100 mm Hg) and heated with constant stirring (at 150 rpm) in a thermoregulated bath until it reached (90±2) °C. Next, 0.5 % (by mass) sodium methoxide was added and the reaction was carried out for 10, 15 or 60 min. Citric acid monohydrate was added to stop the reaction (1.78 g per g of sodium methoxide) and kept for 5 min. The interesterified blend was then washed three times with distilled water to remove the produced soap and any residue of sodium methoxide or citric acid monohydrate.

Enzymatic interesterification

Blends of fully hydrogeneated soybean oil and walnut oil made at three mass ratios (20:80, 40:60 and 60:40) were enzymatically interesterified following the method reported by Abigor *et al*. (*25*). Each blend was dried under vacuum conditions (100 mmHg) and heated with constant stirring (at 150 rpm) in a thermoregulated bath until it reached (70±2) °C, a lower temperature than the one required for chemical interesterification. Next, 5 % (by mass) Lipozyme TL IM was added. The reaction was carried out for 30, 120 or 240 min. In order to stop the reaction, the enzyme was removed by filtration. All of the chemically and enzymatically interesterified blends were stored at 4 °C in a nitrogen atmosphere.

Solid fat content

Because interesterification modifies the melting profile of lipids, which becomes constant when equilibrium is reached (*10*), the solid fat content was measured and used as an indicator of this. The solid fat content of interesterified blends was measured using pulsed nuclear magnetic resonance (p-NMR) according to the AOCS Official Method Cd 16–81 (*26*). Briefly, dry and filtered samples were placed in glass tubes and completely melted (at 60 °C and 10 min) and then solidified (at 0 °C and 30 min). Samples were then allowed to dissolve in water at 10.0, 21.1, 26.7, 33.3 and 40.0 °C for 15 min. Finally, the solid fat content was measured at each temperature in a Bruker Minispec PC120s p-NMR analyzer (Bruker Analytische Mestechnik, Rheinstetten, Germany).

Fatty acid profile

Methylated fatty acids of the triacylglycerols were analyzed in an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA). A fused silica capillary column BPX70 (0.25 μm film thickness, 50 m length, 0.33 mm i.d.; SGE Analytical Science, Austin, TX, USA) was used. Samples were run with hydrogen as the carrier gas between 160 and 230 °C at a rate of 2 °C/min. Standard fatty acid methyl esters from Merck (Darmstadt, Germany) were used for identification purposes.

Statistical analysis

All of the analyses were carried out in triplicate and the results are expressed as mean values±standard error of the mean (SEM). Statistical differences between the times and mass ratios were determined using one-way analysis of variance and Fisher's test. Differences were considered significant at $p<0.05$. Statistical analysis was performed using Statgraphics v. 4.0 (StatPoint, Inc., Warrenton, VA, USA).

Results and Discussion

Chemical and enzymatic interesterification were carried out to obtain a fat base with a high content of C18:3n3 and zero *trans*-fats, as shown in Table 1.

Chemically interesterified fat

Fig. 1 shows the solid fat content of blends of fully hydrogenated soybean oil and walnut oil with mass ratios of 20:80, 40:60 and 60:40 interesterified for 0 (non-interesterified), 10, 15 and 60 min. As we expected, the blends with higher fully hydrogenated soybean oil content had the highest solid fat content. Fully hydrogenated soybean oil is composed exclusively of saturated fatty acids and may contain traces of unsaturated fatty acids (Table 1). As the content of walnut oil in the blend increased, the solid fat content decreased due to the high content of unsaturated fatty acids. The chemical interesterification had the same effect on all blends: the reaction reduced the solid fat content at all temperatures. Our results echo those reported in other studies (*15*,*24*,*27*), of reduced melting point after interesterification; however, the contrary effect – an increase of the solid fat content produced by interesterification – may also happen (*14*,*28*). The chosen conditions, temperature and catalyst concentration, allowed thermodynamic equilibrium to be reached after a few minutes. This effect was reflected in the stabilization of melting temperature. No significant differences in solid fat content in the 20:80 blend were found with subsequent measurements after 10 min and in blends 40:60 and 60:40 after 15 min. Furthermore, the beginning of the reaction was retarded with increasing content of walnut oil. While the 20:80 blend had interesterified in 10 min, the melting point of the 40:60 blend after 10 min of interesterification was found at significantly lower temperatures than at the beginning of the reaction, although it was still not stabilized. Interestingly, this particular blend (Fig. 1b) shows evident variability after 10 min (high standard error of the mean), probably denoting a transition state, which reflects how unstable the solid fat content of a blend may be during the interesterification. It can also be noticed that after 10 min of interesterification, the melting point of the 60:40 blend did not change significantly from that observed in the non-interesterified blend. These results suggest that the beginning of the reaction is delayed with an increase in the content of fully hydrogenated soybean oil. Comparing our results to those reported, the reaction time may not be relevant because it might vary widely (5

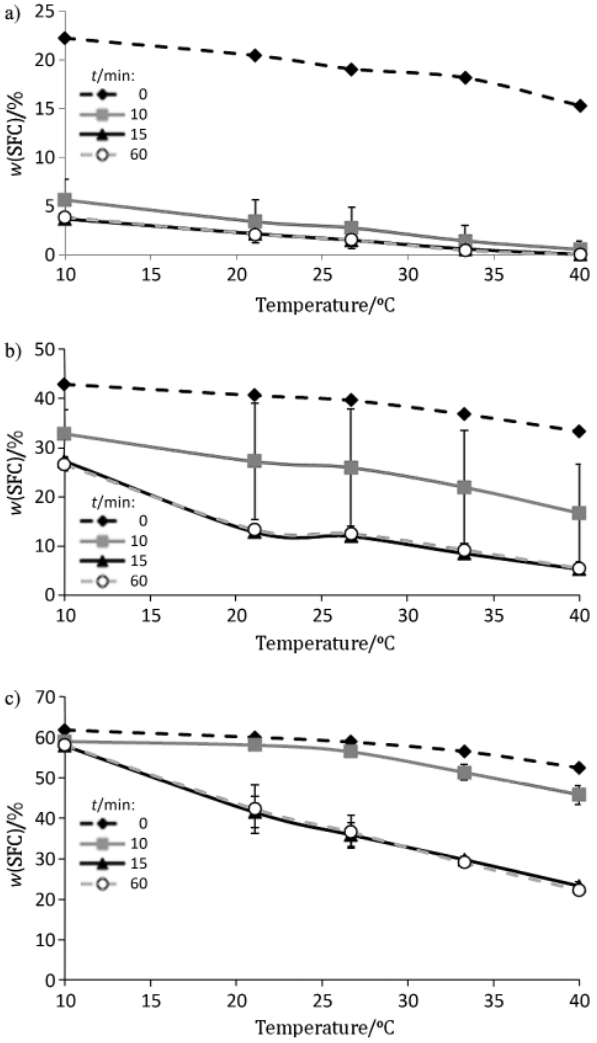

Fig. 1. Solid fat content (SFC) of non-interesterified blends (0 min) and chemically interesterified blends of fully hydrogenated soybean oil and walnut oil at mass ratios of: a) 20:80, b) 40:60 and c) 60:40, measured for 10, 15 and 60 min at different temperatures

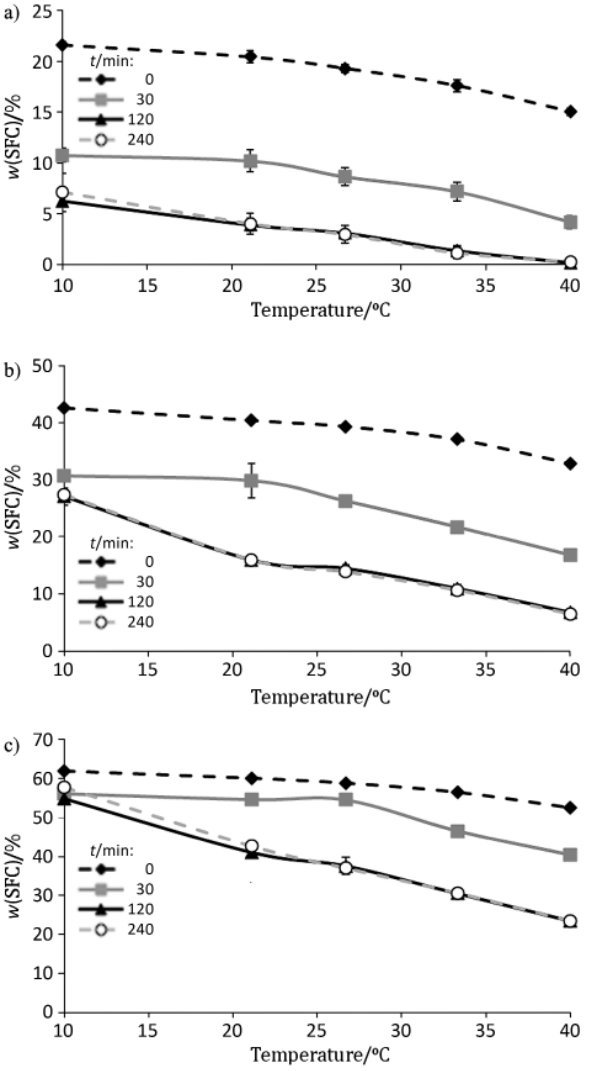

Fig. 2. Solid fat content (SFC) of non-interesterified blends (0 min) and enzymatically interesterified blends of fully hydrogenated soybean oil and walnut oil at mass ratios of: a) 20:80, b) 40:60 and c) 60:40, measured for 30, 120 and 240 min at different temperatures

min to 6 h, or even longer) depending on the conditions such as the catalyst concentration, temperature and solubility of catalysts in the reactants (*15*). In general, the reaction is accelerated with the increase in catalyst concentration and temperature, but the effect of solubility of the catalyst depends on the raw materials, mass ratios and the catalyst itself.

Enzymatically interesterified fat

Fig. 2 shows the melting profiles of fully hydrogenated soybean oil and walnut oil blends at mass ratios of 20:80, 40:60 and 60:40 interesterified for 0 (non-interesterified), 30, 120 and 240 min. As we observed with chemical interesterification, blends with higher content of fully hydrogenated soybean oil present higher melting temperatures, which are reduced with the addition of walnut oil. Also, the solid fat content of all blends decreases as the reaction progresses. Díaz Gamboa and Gioelli (*29*) synthesized functional triacylglycerols using chemical and enzymatic interesterification. They reported that the addition of a solid fat to liquid oil increases the solid fat content of the blend, which decreases after interesterification, as we found in our experiments. They also reported solid fat content at lower temperatures after interesterification of pure palm kernel fat. The thermodynamic equilibrium of all the fully hydrogenated soybean oil and walnut oil blends was achieved at 120 min when the solid fat content was stabilized. This coincides with the results of the study by Undurraga *et al.* (*8*), who reported total interesterification after 80–120 min under similar conditions (65 °C, Lipozyme TL IM). However, as in chemical interesterification, the temperature and the catalyst concentration affected the rate of the enzymatic reaction. Interesterification speeds up when temperature increases until it reaches a maximum level. At that point, it starts to slow down as the temperature increases due to the loss of enzyme activity (*30*). The catalyst concentration generally has the same effect. As the enzyme load increases, the reaction rate is accelerated, but above a certain amount, there is no effect (*31*).

Interesterified blends as a fat base for margarine

In order to obtain an interesterified fat similar to a fat base for margarine, the melting profiles of a commercial fat base and interesterified blends were compared. Fig. 3 shows the melting profiles of the interesterified mixtures and the commercial base. Although differences between the curves of the commercial base and the studied mixtures are observed, the 40:60 blend shows physical behaviour similar to that of the commercial base. Chemically and enzymatically interesterified blends exhibited a spreadability as good as a commercial fat with 27 % of solid fat at 10 °C; a solid fat content not greater than 32 % is essential for good spreadability at refrigeration temperature. Interesterified blends also showed good stability and resistance to oil exudation at room temperature with a solid fat content over 10 % at 21.1 °C (13 and 16 % in chemically and enzymatically interesterified blends, respectively). The poorest behaviour of interesterified blends was at body temperature. Under these conditions, the contents of 9 and 11 % of solids at 33 °C for chemically and enzymatically obtained products produced a waxier sensation in the mouth. Ideally, the solid fat content should be less than 3.5 % at 33.3 °C (*32–34*). However, the solid fat content of both interesterified blends had plasticity curves that fall within the range of all-purpose-type shortening fats. According to List *et al*. (*35*), the solid fat content required at 10, 21.1, 26.6, 33.3 and 40 °C is 18–23, 14–19, 13–14, 12–13 and 7–11 %, respectively. Overall, the interesterified blends achieved these requirements. However, at 10 °C, the solid fat content was slightly higher than that of shortening fats.

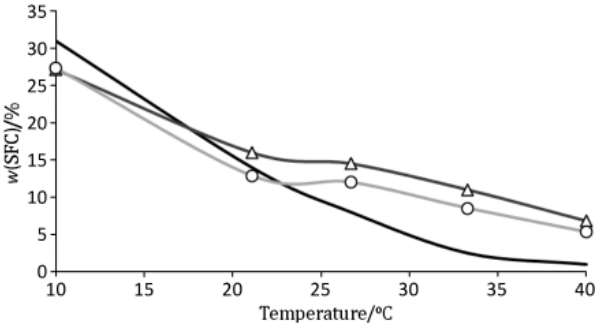

Fig. 3. Solid fat content (SFC) of chemically (–○–) and enzymatically interesterified (–△–) blends of fully hydrogenated soybean oil and walnut oil at mass ratio of 40:60, and of a commercial fat base (—), measured at different temperatures

There are no physical differences between the chemically and enzymatically interesterified blends. Other differences between chemically and enzymatically interesterified fats may be produced by the process itself. The main advantage of the enzymatic process over the chemical one is that the latter produces complete randomization of fatty acids, while enzymatic interesterification may be either substrate specific, differentiated by chain length, or stereospecific. The stereospecificity of commercial enzymes allows them to produce structured lipids with mainly nutritional advantages. Essential fatty acids are usually bonded at the sn-2 position of triacylglycerol; if these oils are enzymatically interesterified, the essential fatty acids remain at the sn-2 position, where they are more easily absorbed as sn-2 monoacylglycerol than free fatty acids (*36*). This improved absorption characteristic is not maximized in chemical interesterification. Enzymatic interesterification is mainly used when a very specific structure is required, as in Betapol®, the natural standard for mimicking human milk.

Conclusions

This study evaluated the suitability of chemical and enzymatic interesterification to produce a fat base rich in polyunsaturated fatty acids. Specifically, fully hydrogenated soybean oil and walnut oil were shown to be adequate raw materials to produce a fat base with good physical and nutritional characteristics, with a high concentration of linolenic acid (C18:3n3), and with zero *trans*-fat. In addition, fully hydrogenated soybean oil may have additional advantages compared to other solid fats, due to the high concentration of palmitic acid (C18:0), which does not have as many detrimental effects as shorter saturated fatty acids. Overall, both chemically and enzymatically interesterified blends of fully hydrogenated soybean oil and walnut oil at 40:60 mass ratio resulted in the plasticity of the shortening fat and no significant differences were found between both technologies. Additional studies related to the fatty acid bioavailability of the different mixes could be done in the future to determine if there could be any drawbacks in terms of fatty acid availability when comparing a blend with its interesterified mix. This analysis could also be reinforced through the study of the molecular distribution of the different fatty acids along the glycerol backbone after interesterification.

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