International Dairy Journal 86 (2018) 1–8

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Performance of packed bed reactor on the enzymatic interesterification of milk fat with soybean oil to yield structure lipids

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ARTICLE INFO

Article history:
Received 10 March 2018
Received in revised form
21 June 2018
Accepted 22 June 2018
Available online 30 June 2018

ABSTRACT

The interesterification reaction of milk fat with soybean oil mediated by *Rhizopus oryzae* lipase immobilised on a hybrid organic-inorganic support of polysiloxane-polyvinyl alcohol (SiO_2-PVA) was assessed in a packed bed reactor running in continuous flow (45 °C, blend of milk fat and soybean oil 65:35%, w/w). The reactor performance was quantified for different flow rates corresponding to space times (0.5 and 4 h). For each condition, the influence of the space time on the consistency, free fatty acid and solid fat content (SFC, %) was determined. Percentages of reduction on consistency (71.2%) were obtained in relation to the initial blend (1431 ± 42 gf cm^{-2}) and the SFC values ($SFC_{10\,°C}$ = 39.6%; $SFC_{20\,°C}$ = 20.5% and $SFC_{35\,°C}$ = 0.2%) were within the ranges considered ideal for spreads with satisfactory spreadability. The immobilised lipase was stable with respect to its catalytic characteristics, exhibiting a half-life of 39 days.

1. Introduction

Milk fat fractions are used in a wide range of food products, due to many favorable physical, chemical and nutritional properties (Kontkanen et al., 2011). However, sometimes it is advantageous to modify milk fat fraction composition aiming to change physical properties of the resulting fats to fit them more closely to those desired for food or other purposes (Cowan, 2018).

Many technologies are being exploited industrially for chemical modification, e.g., hydrogenation, interesterification, acidolysis and alcoholysis (Kontkanen et al., 2011). Among these, interesterification is a good tool for oil and fat modification with relation to textural properties. This reaction promotes the redistribution and interchange of fatty acids within and among the triacylglycerol molecules of the oils and fats (Kadhum & Shamma, 2017). The result is an expressive change in the physicochemical properties of the raw material employed, such as melting and crystallisation behaviour, viscosity and functionality (Lee, Tan, & Lai, 2012).

The interesterification reaction can be achieved chemically or enzymatically (catalysed by lipases; Kadhum & Shamma, 2017). The chemical approach is normally carried out in a batch operation; the enzymatic approach is normally continuous. Moreover, the enzymatic interesterification is a simpler process with fewer steps and lower operating temperatures. The temperature of operation for the enzymatic process is 70 °C, while the chemical method varies from 80 to 120 °C according to the implementation of the process (Cowan, 2018). Continuous enzymatic interesterification can be carefully controlled, allowing specific melting profiles to be accomplished. Thus, by this reaction, products with new and improved melting profiles can be made.

Previous work carried out in our lab has identified *Rhizopus oryzae* lipase as a potential lipase source to produce structured lipids from interesterification of milk fat with soybean oil (Paula, Nunes, De Castro, & Santos, 2016). Pursing our interest in developing a feasible enzymatic process, an attempt was made to perform the process in a continuous mode. For this, the packed-bed reactor (PBR) configuration was selected based on its suitability to perform typical lipase catalysed reactions (Amimi, Ilham, Ong, Mazaheri, & Chen, 2017; Choi, Kim, Kim, Lee, & Kim, 2017; Esteban et al., 2009). This configuration is a form of continuous flow reactor, in which the immobilised biocatalyst is packed in a column or as a flat bed, and the substrate and product streams are pumped in and out of the reactor at the same rate. The advantages of packed bed reactors include ease of operation, ease of scale-up, low cost and high efficiency (Zhang et al., 2016). In addition, PBRs are kinetically more favourable than continuous stirred reactors

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https://doi.org/10.1016/j.idairyj.2018.06.014
0958-6946/

(CSTRs), as the disadvantage of the high mechanical stress due to the agitation can be avoided.

The efficiency of the PBR system in this study was evaluated by the interesterification of milk fat with soybean oil, in solvent-free media, catalysed by an preparation of lipase from *R. oryzae* immobilised on a hybrid organic-inorganic support of polysiloxane-polyvinyl alcohol (SiO_2-PVA) running in continuous mode. This lipase preparation has shown consistent results for carrying out interesterification reactions of a blend of milk fat and soybean oil (65:35%, w/w) with a high half-life time when recycled in a batch reactor coupled with a basket. The continuous interesterification was carried out at lab-scale, in a packed-bed reactor running in a inert atmosphere to avoid the oxidation of the feedstock. Different flow rates were assessed to define feasible operating parameters, aiming to establish the potential for, and challenges in, scaling up the process.

2. Materials and methods

2.1. Materials

Refined, bleached, and deodorised soybean oil was bought locally. Milk fat was obtained from commercial unsalted butter, purchased in a local market, and melted to between 50 and 65 °C in a microwave oven followed by centrifugation at 1372 × *g* for 10 min to separate the aqueous phase (Paula, Nunes, De Castro, & Santos, 2015a). Commercial virgin olive oil (0.3% acidity), purchased in a local market, was used to determine the hydrolytic activity of the biocatalysts. A commercial food grade lipase from *R. oryzae* (L036P, Biocatalysts, Cardiff, England) in a powder form was used without further purification. Tetraethoxysilane (TEOS) and polyvinyl alcohol (PVA, MW 88,000) were acquired from Aldrich Chemical Co. (Milwaukee, WI, USA). A deep blue liposoluble dye (organic synthetic pigment) was obtained from Glitter Ind. Com. Imp. Exp. Ltd (Carapicuiba, São Paulo, Brazil) and used as a tracer. All solvents and reagents for analyses were analytical grade.

2.2. Support synthesis and lipase immobilisation

The support was prepared by sol–gel technique and the resulting SiO_2–PVA particles were neutralised and used to immobilise the *R. oryzae* lipase according previous procedure (Paula et al., 2015b). To perform this work, ten batches of immobilised derivatives were prepared, and the average measured hydrolytic activity was 4150 Ug^{-1} biocatalyst. Moisture content was lower than 10% to reduce by-product formation.

2.3. Continuous runs

Interesterification of milk fat with soybean oil was conducted in a jacketed glass column (internal diameter, 15 mm; height, 200 mm; total volume, 32 cm^3) connected to a circulating water bath to maintain the temperature at 45 °C. The continuous run was started by loading the reactor with the biocatalyst, and the substrate was continuously pumped (peristaltic Perista Pump SJ-1211; Atto Bioscience & Biotechnology, Tokyo, Japan) from a reservoir through marprene tubing (913.AJ05.016; Watson Marlow, Modugno, Italy) to the bottom end of the bioreactor at the required flow rate. For each run, 23.4 g (d = 2.5168 ± 0.0521 g cm^{-3}) of biocatalyst was used. The substrate was prepared by mixing 65% milk fat with 35% soybean oil and flow rates from 4.8 to 48 mL h^{-1} corresponding to space-times from 4 h to 0.5 h were used to determine the reactor limit performance. Fig. 1 shows the experimental setup for the reaction system.

During the continuous runs, output stream samples were collected for analysis of the relevant variables, such as free fatty acids, consistency and solid fat contents. For each tested space-time, the parameters were determined when the concentrations within the reactor remained relatively constant over a period corresponding for at least three space times. The space-time was calculated according to Levenspiel (1999) as described in Equation (1):

$$\tau = \frac{V}{v_0} \tag{1}$$

where τ is the space time (h), V is the working volume of the reactor (mL) and v_0 is the flow rate (mL h^{-1}).

2.4. Operational stability of the immobilised derivative

The biocatalyst stability was assessed by measuring the hydrolytic activity of the immobilised derivatives at the end of each continuous run, taking the original activity as 100%. The recovered immobilised lipase was then washed with *tert*-butanol to remove any substrate or product retained in the matrix. Hydrolytic activity was determined by the olive oil emulsion method according to the methodology described by Paula, Nunes, Santos, and De Castro (2011). The inactivation constant (kd) and half-life ($t_{1/2}$) for the immobilised lipase were calculated as described by Costa-Silva, Teixeira, Carvalho, Mendes, and De Castro (2014).

2.5. Hydrodynamic characterisation of the PBR system

Tracer response analysis was used to characterise and model of the flow through the reactor. The PBR was filled with 23.5 g of previously denatured immobilised *R. oryzae*. Then, the system was operated under a continuous flow rate of substrate (11.4 mL h^{-1}), which corresponded to 2.1 h of space time. A deep blue liposoluble dye (8.5%, w/w) was solubilised in substrate and used as a tracer. A pulse input experiment was performed, and the residence time distribution (RTD) was experimentally determined by injecting the tracer into the reactor at time zero and then by spectrophotometrically measuring the tracer concentration, C, at 650 nm (Varian Cary 50 UV/Vis Spectrophotometer; Varian Australia Pty Ltd, Mulgrave, Australia) in the output stream as a function of time. The experiments were performed in duplicate. The residence time distribution function, $E(t)$, was calculated by Equation (2) (Fogler, 2009):

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \tag{2}$$

where $C(t)$ is the tracer concentration at time t.

The mean residence time, t_m, for a constant volumetric flow, was calculated using Equation (3) (Fogler, 2009):

$$t_m = \int_0^\infty t.E(t)dt \tag{3}$$

The integration in equation (3) was calculated using the ORIGIN 8.0 software (OriginLab Corporation, Washington, United States).

2.6. Analytical methods

2.6.1. Free fatty acid content

The AOCS method Ca 5a-40 (AOAC, 2004) was used to determine total free fatty acids (FFAs), which was expressed in terms of the percentage of free oleic acid.

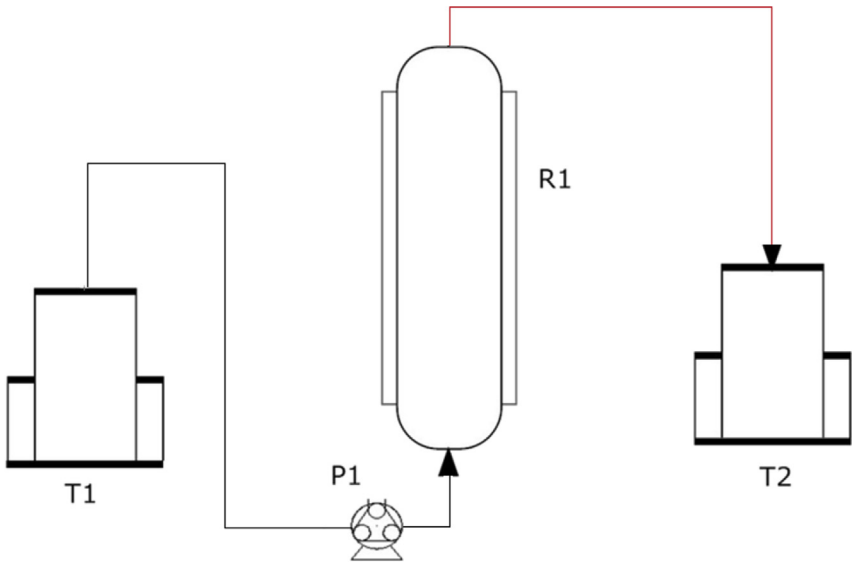

Fig. 1. Simplified flowchart of the packed bed reactor used in the interesterification reactions of milk fat with soybean oil: T1, substrate reservoir; T2, product reservoir; R1, PBR column; P1, peristaltic pump; black line, feed; red line, products. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

2.6.2. Consistency

Consistency of the raw materials and interesterified products were determined using a texture analyser (QTS-25 Brookfield, Middleboro, United States).Samples were heated in a microwave oven (55–60 °C) to completely melt crystals and conditioned in a glass beaker (100 mL) for 48 h at 10 °C. The TA15 probe was used, which is an acrylic cone with an angle of 45°. Tests were performed under the conditions described by Paula et al. (2016) and the measurements were used to calculate the "yield value", according to Haighton (1959).

2.6.3. Solid fat content assay

The solid fat content (SFC) of the reaction medium and interesterified products at 10, 20 and 35 °C were assayed in a in differential scanning calorimeter (DSC; Model 6220 from SII Nanotechnology-Seiko, Northridge, USA). DSC analysis was performed based on the official method Cj 1-94 of the American Oil Chemist's Society (AOCS, 2004). The solid fat content was calculated for each temperature considering DCS melting curves by partial integration (Kaisersberger, 1989; Nassu & Gonçalves, 1995), according to Equation (4):

$$SFC(\%) = \frac{\int_{T_0}^{T} H(T)dT}{\int_{T_0}^{T_f} H(T)dT} \quad (4)$$

where: T is temperature; T_0 is the onset temperature of melting; T_f is the temperature at which the sample is completely melted; Cp is the specific heat.

3. Results and discussion

3.1. Hydrodynamic characterisation of the PBR system

The RTD is an essential tool to assess fluid velocity patterns, which can be used to describe the flow regime in the reactor. Since flow regime affects the reactor performance, its description may enable better process control (Fogler, 2009; Levenspiel, 1999). In this work, for the experimental determination of mean residence time of the reactants in the reactor, as well as the characterisation of the reaction mixture in the bed, a pulse input experiment was performed.

For this, a dark blue dye substance (liposoluble dye CI 61554) was used as a tracer and a calibration curve (absorbance = 1.0098*C, $R^2 = 0.999$) was built, in which C is the concentration of the tracer in reaction medium (blend of milk fat and soybean oil, 65:35%, w/w).

In ideal reactors, without preferred path or dead zones, the average residence time, calculated according to Equation (3) (Fogler, 2009) based on distribution function of time of residence, should match the space-time (time required to process a volume of reactor, calculated with Equation (1) for closed systems (Fogler, 2009). The greater the number of preferential paths and dead zones in the reactors the greater the difference between the mean residence time values (tm) and the space-time (τ).

Illustration of the experiment over time after the tracer was injected at the base of the reactor can be observed in Supplementary material Fig. S1. The results allow the residence-time distribution curve E (t) to be plotted as a function of time, according to Equation (2) (Supplementary material Fig. S2).

Using Equation (3), the mean residence time was calculated as 135 min. This value was approximately 8.74% higher than the calculated value (taking into account the experimental conditions and Equation (1) and can be considered acceptable in this type of test, indicating no preferential paths or back mixing in the bed, demonstrating good quality of packing. The data obtained were used to calculate the variance (Fogler, 2009), or square of the standard deviation, that indicates the spread of distribution; the greater that value, the greater the distribution spread (Supplementary material Table S1). Asymmetry coefficient was also determined (Fogler, 2009) and it measures the extent that a distribution is skewed in one direction or another in reference to the mean (Supplementary material Table S1).

3.2. Influence of the space-time on the interesterification enzymatic reactions of milk fat and soybean oil in continuous flow

The reactor was filled with immobilised lipase forming a well packed column and substrate (65:35%, milk fat/soybean oil) was

feed at increasing flow rates from 0.08 to 0.80 mL min^{-1}, corresponding to 4 to 0.5 h space time, respectively, for a total period of 16 days.

Fig. 2 shows the concentration of free fatty acids for different space times. In the early stage the free acid content reached values as high as 25% and then decreased sharply attained a virtually stable value (1.12 ± 0.31%) at 72 h, until the end of the run. Such behaviour may be related to the amount of water present in the immobilised derivative (10.3%), which favoured the hydrolysis at the beginning of the continuous run.

The consistency of the interesterified products was assessed (Fig. 3). The change of space time along the continuous run had a strong influence on the consistency of the interesterified product, resulting in a variation between the 412 to 1182 gf cm^{-2} corresponding to consistency reduction from 71.2 to 17.4% in relation to the original consistency of the blend (1431 ± 42 gf cm^{-2}).

Average values for consistency and reduction achieved for each space-time are displayed in Table 1. Over the range of the flow rate studied, the best reactor performance was found to be at space-time of 4 h (flow rate = 0.08 mL min^{-1}). Such conditions

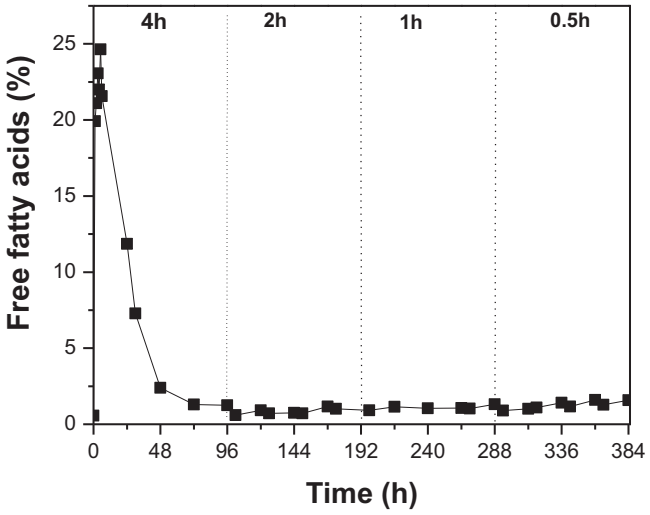

Fig. 2. Free fatty acid content (%) obtained in the interesterification reaction of milk fat with soybean oil in packed bed reactor using different space-times.

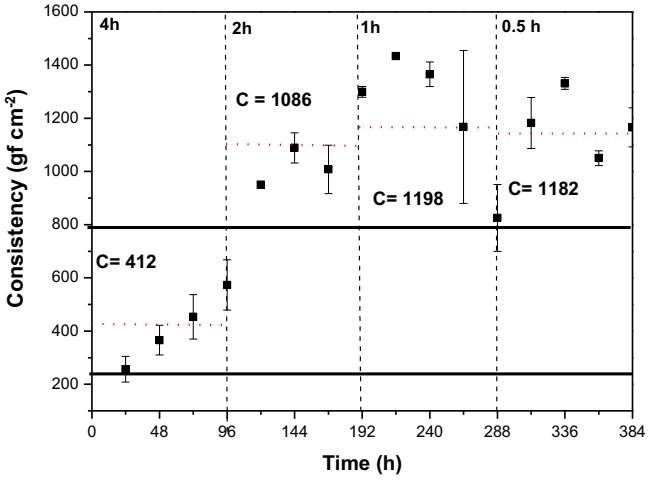

Fig. 3. Consistency of the products obtained in the interesterification reaction of milk fat with soybean oil in packed bed reactor, using different space-times: C, average value of consistency in each studied space-time.

Table 1
Consistency values and reduction percentage of the interesterified products in relation to the substrate (reaction blend) during continuous run in packed bed reactor using different space times.[a]

Space time (h)	Consistency (gf cm^{-2})	Consistency reduction (%)
4	412 ± 134	71.2
2	1087 ± 153	24.1
1	1198 ± 273	16.3
0.5	1182 ± 115	17.4
0	1431 ± 42	–

[a] Space time 0 = milk fat:soybean oil blend; milk fat = 6074 ± 407 gf cm^{-2}.

produced products having consistency within the range of 200–800 gf cm^{-2}, considered, according to the classification proposed by Haighton (1959), as ideal for a satisfactory product properties and plasticity spreadability. The other evaluated space times resulted in products having consistency values higher than upper limit (800 gf cm^{-2}).

The highest percentage reduction occurred using the space-time of 4 h (71.2%). Decreasing the space-time to 2 h resulted in 24% reduction in the consistency; while values attained using lower space times (1 h and 0.5 h) provide similar reduction of around 16%. Such similarity in the consistency values verified at space-time lower than 4 h suggested that only slight modifications of the blend occurred under these conditions and as expected, no difference in the consistency values were recorded.

DSC technique was used in our work to determine the SFC and thermal profile of the different evaluated fats. Although NMR techniques have been more commum used for SFC determination and the values are different when compared with those obtained by DSC, this latter technique can be a flexible way for tempering the fat prior determination of thermal properties. In addition, thermograms show a thermal fingerprint of the fat, allowing for distinguishing among fats with identical SFC values (Aguedo et al., 2009; Nassu & Gonçalves, 1995; Ribeiro, Basso, Grimaldi, Gioielli, & Gonçalves, 2009a; Ribeiro et al., 2009c). Through the DSC technique, the thermal phenomena observed for these materials can be verified by monitoring the enthalpy and the phase transition of various triglycerides in the mixture (Ribeiro et al., 2009a), which is considered to be an advantage of this technique over other calorimetry techniques (Tan & Che Man, 2000).

Fig. 4a shows the thermograms for both raw materials (milk fat and soybean oil) in comparison with the thermogram obtained for the blend (65% milk fat and 35% soy oil) before interesterification. Fig. 4 b shows the effect of the enzyme modification on the thermal profile of the interestified products collected at the output of the PBR at different space-times after attaining the steady states (72 h at $\tau = 4$ h; 168 h at $\tau = 2$ h; 264 h at $\tau = 1$ h; 360 h at $\tau = 0.5$ h).

It is possible the different thermal behaviours observed for these samples are due to their different compositions in triglycerides. Temperature fusion of fat contributes to the expansion of sample volume, characterised by the presence of endothermic peaks. In general, oils and fats may be showed extremely complex thermal behaviour, which will depend on chemical composition and procedures of the DSC analysis (Ribeiro et al., 2009a).

For milk fat (Fig. 4a) three endothermic regions can be observed, the first from −40 to 7 °C, the second from 7 to 20 °C, in which peak "E" is present at 15 °C, and the third from 20 to 40 °C. Such results are consistent with those reported by Hunt and Buckin (2000); interpretation of the measurements for milk fat by DSC can be due to the existence of three predominant species, low fusion (around 8 °C), medium fusion (between 8 and 20 °C) and high fusion (above 20 °C). For the soybean oil is possible to notice the presence of a main endothermic event, the peak "B" at −27 °C. Milk fat showed a wide range of fusion from −35 °C to 36 °C, while soybean oil has a

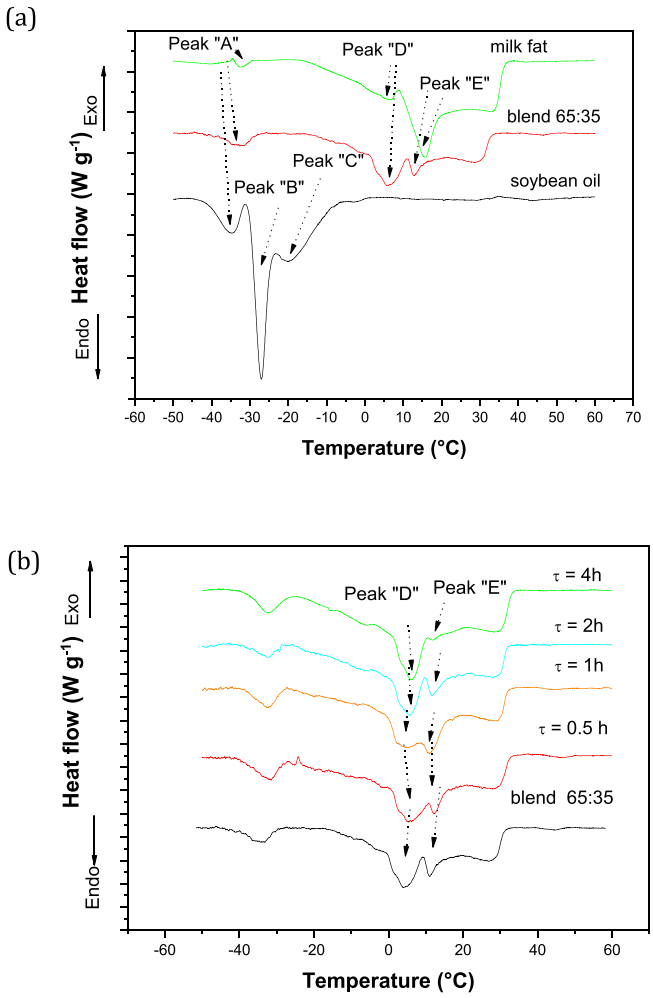

Fig. 4. Heat flow curves as a function of temperature for the (a) milk fat, soybean oil and blend of milk fat and soybean oil (65:35%, w/w) and (b) products obtained from the interesterification reaction of the blend in packed bed reactor at different space-times (72 h at $\tau = 4$ h; 168 h at $\tau = 2$ h; 264 h at $\tau = 1$ h; 360 h at $\tau = 0.5$ h).

more restricted fusion range from −42 °C to 0 °C, which is directly related to more complex composition in TAGs of milk fat compared with soybean oil.

For the blend (65:35%) the endothermic event in the region "A" was intensified, in comparison with that observed on the thermogram for milk fat. In addition, the peaks at regions of "B" and "C" could not be observed in the thermogram of the blend. The width of peak "D" was intensified, suffering a slight offset at lower temperatures, compared with that noted on the thermogram for milk fat. This behaviour of reduction on the temperature was also observed for the endothermic event in the region of the peak "E". However, in this case, it was observed that the width of this peak was decreased due to the addition of oil to the milk fat. The melting curves of the mixtures differ, depending on the amount of fat (milk fat in this case) and oil (soybean oil) in the blends. The more complex DSC melting patterns with the higher numbers of peaks and shoulders can be attributed to complicated interactions among the mixture constituents, including different mixed crystal formation (Bell, Gordon, Jirasubkunakorn, & Smith, 2007; Fauzi, Rashid, & Omar, 2013).

The analysis of the thermograms exhibited in Fig. 4b revealed that, after the 72 h ($\tau = 4$ h) reaction, there was a decrease in the endothermic peak "E" (characteristic of milk fat), promoting the formation of a slightly more extended peak. For the other studied space-times only small variations were observed in comparison with the thermogram obtained for the original blend (65:35%, w/w).

In addition to the thermal profile, another important parameter in the characterisation of oils and fats is the solid fat content (SFC), which indicates the percentage of fat that is in the solid form at a given temperature (Gunstone, Harwood, & Dijkstra, 2007). This property is responsible for many important attributes of milk fats, for example, physical appearance, sensory characteristics, spreadability, melting characteristics, and plasticity or consistency of the food product. The variation of solid fat content and the melting temperatures, along with other factors, such as crystalline morphology, determines the temperature range in which a fat can be considered as plastic (Otero, Lópes-Hernandez, Garacía, Hernández-Martín, & Hill, 2006).

SFC values calculated using thermograms of the interesterified products are displayed in Fig. 5 as a function of temperature in comparison with the values obtained for the milk fat and blends before and after the interesterification. Special features of the fats are obtained at different temperature ranges (Grimaldi, Gonçalves, & Esteves, 2000).

The solid fat content between 4 and 10 °C determines the dispersion of the product at cooling temperature. SFC not more than 32% at a temperature of 10 °C is recommended to ensure good spreadability at this temperature. The SFC of the product between 20 and 22 °C determines the stability and resistance to oil exudation. In this case, the ideal content shall not be less than 10% (O'Brien, 2004; Ribeiro, Basso, Grimaldi, Gioieli, & Gonçalves, 2009b). The SFC between 33 and 38 °C influences the impressions and sensations of sandiness of a fat in the mouth (O'Brien, 2004; Otero, Lópes-Hernandez, Garacía, Hernández-Martín, & Hill, 2006). In foods such as margarine, it is desirable to have high solid fat content to provide suitable crystalline structure at room temperature, and low solid fat content at high temperatures,

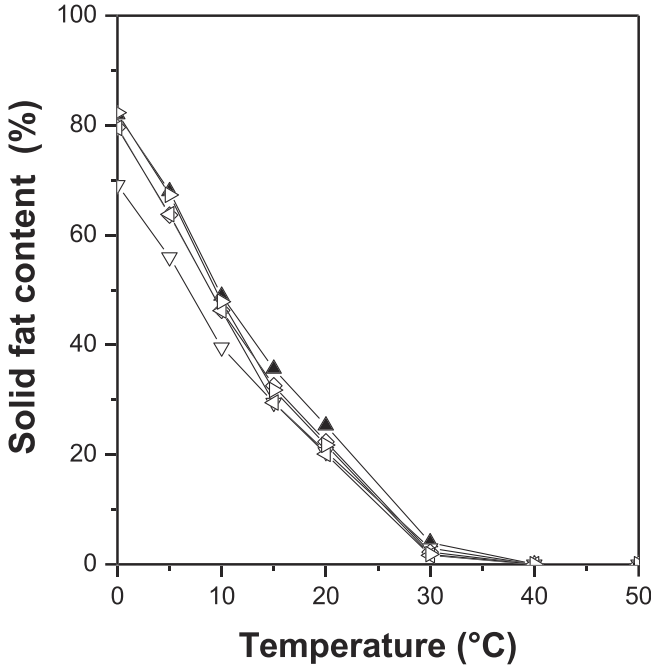

Fig. 5. Solid fat content as a function of temperature for milk fat, blend of milk fat and soybean oil (▲: 65/35% w/w) and products obtained in the interesterification reaction in packed bed reactor using different space-times (▽, 72 h at $\tau = 4$ h; ◇, 168 h at $\tau = 2$ h; ◁, 264 h at $\tau = 1$ h; ▷, 360 h at $\tau = 0.5$ h).

so that the fusion easily occurs in the mouth (Grimaldi, Gonçalves, Gioieli, & Simões, 2001).

The results attained for solid fat content indicated that the blend and interesterified products showed lower values as the temperature increases. The blend before interesterification showed high SFC at 10 °C (49%), which explains the high value of consistency (1431 ± 42 gf cm^{-2}; Table 1) at this temperature.

It was observed that interesterified products showed lower SFCs compared with the blend at all studied temperatures. This reduction was observed for the product obtained at 72 h ($\tau = 4$ h), while the remaineing products resulted in slight or practically no decrease in the SFC in relation to the blend. According to the criteria established by O'Brien (2004), the product that showed better plasticity was obtained at the 72 h reaction (space time = 4 h) due to the value of SFC considered to be ideal for a "spread" at 10 °C (closer to 30% in comparison with the others), and it also had good resistance to the migration of oil at 20 °C (solids > 10%) and level of solid fat near zero at 30 °C (3%), indicating suitability for applications at this temperature, since in this case no sandiness in the mouth is present. Again, the results of SFC showed that space times from 2 to 0.5 h were not efficient, resulting in a product without satisfactory dispersion properties.

Actually, small changes in the solid fat content usually results in low variation in texture properties, as was the case of the products obtained for the space-time of 0.5 h, 1 h and 2 h. However, for the product obtained for the space-time of 4 h, the variation of the solid fat content in relation to the non-interesterified blend was around 10%, which is considerable. This explains why the texture variation for this product was higher in relation to the other products. Furthermore, texture properties of fats are dependent not only on the SFC of the product, or in the other words, the amount of crystals; the texture of a fat is also influenced by the crystal size, by the strength and the morphology of the fat crystal network, and the different forms it might adopt at a given temperature (Arana, 2012; Marangoni & Narine, 2002). Interesterification promotes significant alterations in the microstructure of fats, since it modifies the morphology and density of the crystalline network, modifying the properties of texture and functionality of the interesterified fats (Fauzi et al., 2013).

3.3. Reactor operational stability

After establishing the suitable conditions for carrying out the interesterification of milk fat with soybean oil (run 1), further investigation was made to determine the reactor stability performance. For this, a space-time of 4 h was chosen, keeping fixed the other conditions for 15 days. To monitor the process the following parameters were quantified: levels of free fatty acids (%), consistency of the products and the SFC by DSC. In addition, the biocatalyst half-life time was determined and results are summarised in Table 2.

With respect to the fatty acid content, similar behaviour was observed as previously verified. At the early stages the activation of the enzyme produced high amounts of free acids (about 12%), from 48 h on free fatty acid content was virtually stable (1.2 ± 0.2%) until the end of the reaction. As previously discussed, the interesterification of oils and fats involves, at a molecular level, sequential reactions of hydrolysis and esterification (re-synthesis) of triglycerides (Marangoni, 2002; Willis & Marangoni, 2008). As the water acts as a substrate in hydrolysis reactions, this reaction is favored with the increased amount of water provided by the biocatalyst (Aires-Barros & Range, 2003).

Consistency of interesterified products was evaluated in relation to the consistency of the original blend. In the first 24 h the consistency value reached values close to the lower limit (200 gf cm^{-2}) according to the classification proposed by Haighton (1959). This is explained by considering the formation of free fatty acids at high levels (12%), indicating that hydrolysis of triacylglycerols occurred along with formation of monoacylglycerols and diacylglycerols, compounds that are responsible for lowering the consistency of interesterified products. Actually, the presence of small amounts of products from partial hydrolysis, mono- and diacylglycerols, can change the texture of the material, since these substances act as emulsifiers (Kaewthong, Sirisansaneeyakul, Prasertsan, & H-Kittikun, 2005).

After 48 h, the consistency values of the interesterified products were similar to each other and close to the upper limit in the order of 800 ± 63 gf cm^{-2} corresponding a decrease 39 ± 5% in relation to the initial blend consistency (1431 ± 42 gf cm^{-2}).

Reduction in consistency through this run (run 2) was lower than previous one (run 1), but this result can also be attributed to moisture of biocatalyst which play a role in the formation of free fatty acids and small amounts of emulsifiers.

Analysis of the thermograms (not shown) revealed that during the interesterification reaction, there was a shift of endothermic peaks "A" and "B" to slightly higher temperatures. Furthermore, almost no variation was observed when comparing thermograms of interesterified products at different times; this was as expected, since the continuous run is operated under steady state conditions.

The SFC results indicated that both blend and products showed lower values with increasing temperature of analysis. For all the assessed temperatures, the products had lower SFC compared with the blend. In addition, the results obtained at different reaction time (72 and 242 h) were quite similar, corroborating that the reaction was run under stable conditions. It appears that according to the criteria established by O'Brien (2004), the interesterified products showed good resistance to migration of oil at 20 °C (solid > 10%) and SFC near zero at 30 °C (solid < 4%), indicating suitability for applications at this temperature, since in this case present no sandiness in the mouth. With respect to the plasticity, the products displayed CGS around 48%, lower than the value obtained for the blend (54%), but higher than the ideal (around 30%) for a "spread" at 10 °C.

Finally, the operational stability of the biocatalyst was also assessed. At the end of continuous run 2 the biocatalyst was recovered and washed with *tert*-butanol and residual hydrolytic activity was determined according to the methodology described in section 3.4.6. A loss of 28% was found in relation to the initial activity for the reactor running on milk fat and soybean oil blend. The deactivation coefficient (*kd*) and half-life ($t_{½}$) were evaluated, giving values of 0.0175 day^{-1} and 39 days, respectively.

Table 2
Free fatty acids, consistency and biocatalyst half-life time in continuous run in packed bed reactor using a fixed space-time of 4 h.[a]

Time (h)	Free fatty acids (%)	Consistency (gf cm^{-2})	Biocatalyst activity (units g^{-1} dry weight)
0	0.5	1431 ± 42	3034
96	1.5	785 ± 7	–
336	0.9	804 ± 18	2379

[a] Time 0 = milk fat:soybean oil blend; $K_d = 0.0175$ day^{-1}; half-life time ($t_{1/2}$) = 39 days.

These results were satisfactory and showed good operational stability of the biocatalyst. Such high operational stability can be assigned to the support used (SiO_2-PVA), a hybrid composite that combines the chemical and physical properties of the guest with the excellent optic, thermal, and chemical stability of the host silicon oxide matrix. This simple but effective procedure gave an immobilised lipase preparation with a greatly improved activity and stability as previously demonstrated in reactions carried out in both batch and continuous runs (Paula et al., 2015a,b; Simões et al., 2015; Souza, Mendes, & Castro, 2016). In addition, results compare favourable with those reported in the literature. Osorio, Gusmão, Fonseca, and Ferreira-Dias (2005), for example, assessed the use of Novozym 435® (a commercial preparation of immobilised lipase) on the interesterification reaction of palm stearin with soybean oil running on a fluidised bed reactor and a half-life time of 17 days was attained.

4. Conclusions

Among all possible applications for immobilised lipases, their use on an industrial scale is the most important. Use of these biocatalysts in laboratory scale processes has been carried out in different reactor configurations, but the continuous process is more advantageous compared with the batch. A continuous process normally employs a packed bed reactor, which was the configuration chosen to be studied here. Reduction in consistency was obtained in relation to the initial blend and the SFC values ($SFC_{10\ ^\circ C} = 39.6$; $SFC_{20\ ^\circ C} = 20.5$ and $SFC_{35\ ^\circ C} = 0.2\%$) were within the ranges considered ideal for spreads with satisfactory spreadability when a value of 4 h of spatial time was used. The immobilised lipase was stable with respect to its morphological and catalytic characteristics, exhibiting a half-life time of 39 days. The results obtained were satisfactory and showed the potential for this biocatalyst for milk fat enzymatic interesterification.

Acknowledgements

The authors acknowledge financial assistance from Fundação de Amparo à Pesquisa do Estado de São Paulo FAPESP (Process 2017/11482-7) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Process 446371/2014-9).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.idairyj.2018.06.014.

References

Aguedo, M., Giet, J., Hanon, E., Lognay, G., Wathelet, B., Destain, J., et al. (2009). Calorimetric study of milk fat/rapeseed oil blends and their interesterification products. *European Journal of Lipid Science and Technology, 111*, 376–385.

Aires-Barros, M. R., & Fernandes, P. (2003). Imobilização dos biocatalisadores. In J. M. S. Cabral, M. R. Aires-Barros, & M. Gama (Eds.), *Engenharia enzimática* (pp. 121–140). Lisboa, Portugal: Lidel.

Amini, Z., Ilham, Z., Ong, H. C., Mazaheri, H., & Chen, W. H. (2017). State of the art and prospective of lipase-catalyzed transesterification reaction for biodiesel production. *Energy Conversion and Management, 141*, 339–353.

AOCS. (2004). *Official methods and recommended practices of the American oil Chemists' Society* (5th ed.). Urbana, IL, USA: AOCS Press.

Arana, I. (2012). *Physical properties of foods: Novel measurement techniques and applications*. CRC Press.

Bell, A., Gordon, M. H., Jirasubkunakorn, W., & Smith, K. W. (2007). Effects of composition on fat rheology and crystallization. *Food Chemistry, 101*, 799–805.

Choi, N., Kim, H., Kim, B. H., Lee, J., & Kim, I. H. (2017). Production of phytosteryl ester from echium oil in a recirculating packed bed reactor using an immobilized lipase. *Journal of Oleo Science, 66*, 1329–1335.

Costa-Silva, W., Teixeira, L. F., Carvalho, A. K. F., Mendes, A. A., & De Castro, H. F. (2014). Influence of feedstock source on the biocatalyst stability and reactor performance in continuous biodiesel production. *Journal of Industrial and Engineering Chemistry, 20*, 881–886.

Cowan, W. D. (2018). *Enzymatic interesterification*. Urbana, IL, USA: AOCS Lipid Library.

Esteban, L., Muñío, M. M., Robles, A., Hitta, E., Jiménez, M. J., González, P. A., et al. (2009). Synthesis of 2-monoacylglycerols (2-MAG) by enzymatic alcoholysis of fish oils using different reactor types. *Biochemical Engineering Journal, 44*, 271–279.

Fauzi, S. H. M., Rashid, N. A., & Omar, Z. (2013). Effects of chemical interesterification on the physicochemical, microstructural and thermal properties of palm stearin, palm kernel oil and soybean oil blends. *Food Chemistry, 137*, 8–17.

Fogler, H. S. (2009). *Elementos de engenharia das reações químicas* (4th ed.). Rio de Janeiro, Brazil: LTC.

Grimaldi, R., Gonçalves, L. A. G., & Esteves, W. (2000). Características de gorduras comerciais Brasileiras. *Brazilian Journal of Food Technology, 3*, 159–164.

Grimaldi, R., Gonçalves, L. A. G., Gioielli, L. A., & Simões, I. S. (2001). Interactions in interesterified palm and palm kernel oils mixtures. I-Solid fat content and consistency. *Grasas y Aceites, 52*, 349–354.

Gunstone, F. D., Harwood, J. L., & Dijkstra, A. J. (2007). *The lipid handbook* (3rd ed.). Boca Raton, FL, USA: CRC Press.

Haighton, A. J. (1959). The measurement of the hardness of margarine and fats with cone penetrometers. *Journal of the American Oil Chemists' Society, 36*, 345–348.

Hunt, N., & Buckin, V. (2000). Temperature dependence and the effects of heat treatment on the rheological properties of various butters. *Trends in Colloid and Interface Science, 14*, 320–324.

Kadhum, A. A., & Shamma, M. N. (2017). Edible lipids modification processes: A review. *Critical Reviews in Food Science and Nutrition, 57*, 48–58.

Kaewthong, W., Sirisansaneeyakul, S., Prasertsan, P., & H-Kittikun, A. (2005). Continuous production of monoacylglycerols by glycerolysis of palm olein with immobilized lipase. *Process Biochemistry, 40*, 1525–1530.

Kaisersberger, E. (1989). DSC investigations of the thermal characterization of edible fats and oils. *Thermochimica Acta, 151*, 83–90.

Kontkanen, H., Rokka, S., Kemppinen, A., Miettinen, H., Hellström, J., Kruus, K., et al. (2011). Enzymatic and physical modifi cation of milk fat: A review. *International Dairy Journal, 21*, 3–13.

Lee, Y. Y., Tang, T. K., & Lai, O. M. (2012). Health benefits, enzyme production and application of medium-chain triglycerides and long (MLCT) in food industry: A review. *Journal of Food Science, 77*, 137–144.

Levenspiel, O. (1999). *Chemical reaction engineering* (3rd ed.). Hoboken, NJ, USA: Wiley.

Marangoni, A. J. (2002). Lipases: Structure, function, and properties. In T. M. Kuo, & H. W. Gardner (Eds.), *Lipid biotechnology*. New York, NY, USA: Marcel Dekker.

Marangoni, A. G., & Narine, S. S. (2002). *Physical properties of lipids*. Boca Raton, FL, USA: CRC Press.

Nassu, R. T., & Gonçalves, L. A. G. (1995). Solid fat content determination: Comparison between pNMR and DSC techniques. *Grasas y Aceites, 46*, 337–343.

Osório, N. M., Gusmão, J. H., Fonseca, M. M., & Ferreira-Dias, S. (2005). Lipase-catalysed interesterification of palm stearin with soybean oil in a continuous fluidised-bed reactor. *European Journal of Lipid Science and Technology, 107*, 455–463.

Otero, C., López-Hernandez, A., García, H. S., Hernández-Martín, E., & Hill, C. G., Jr. (2006). Continuous enzymatic transesterification of sesame oil and a fully hydrogenated fat: Effects of reaction conditions on product characteristics. *Biotechnology and Bioengineering, 94*, 877–887.

O'Brien, R. D. (2004). *Fats and oils: Formulating and processing for applications* (2nd ed.). New York, NY, USA: CRC Press LLC.

Paula, A. V., Nunes, G. F. M., De Castro, H. F., & Santos, J. C. (2015a). Synthesis of structured lipids by enzymatic interesterification of milk fat and soybean oil in a basket-type stirred tank reactor. *Industrial & Engineering Chemistry Research, 54*, 1731–1737.

Paula, A. V., Nunes, G. F. M., De Castro, H. F., & Santos, J. C. (2016). Assessing the reaction conditions to mediate the milkfat-soybean oil enzymatic interesterification. *Brazilian Journal of Food Technology, 19*, 11615.

Paula, A. V., Nunes, G. F. M., Osório, N. M., Santos, J. C., De Castro, H. F., & Ferreira-Dias, S. (2015b). Continuous enzymatic interesterification of milk fat with soybean oil produces a highly spreadable product rich in polyunsaturated fatty acids. *European Journal of Lipid Science and Technology, 117*, 608–619.

Paula, A. V., Nunes, G. F. M., Santos, J. C., & De Castro, H. F. (2011). Interesterification of milkfat with soybean oil catalysed by *Rhizopus oryzae* lipase immobilised on SiO_2-PVA on packed bed reactor. *International Journal of Food Science and Technology, 46*, 2124–2130.

Ribeiro, A. P. B., Basso, R. C., Grimaldi, R., Gioielli, L. A., & Gonçalves, L. A. G. (2009a). Instrumental methods for the evaluation of interesterified fats. *Food Analytical Methods, 2*, 282–302.

Ribeiro, A. P. B., Basso, R. C., Grimaldi, R., Gioielli, L. A., Santos, A. O., Cardoso, L. P., et al. (2009c). Influence of chemical interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of canola oil and fully hydrogenated cottonseed oil blends. *Food Research International, 42*, 1153–1162.

Ribeiro, A. P. B., Grimaldi, R., Gioielli, L. A., Santos, A. O., Cardoso, L. P., & Gonçalves, L. A. G. (2009b). Thermal behavior, microstructure, polymorphism, and crystallization properties of zero trans fats from soybean oil and fully hydrogenated soybean oil. *Food Biophysics, 4*, 106–118.

Simões, A. L., Ramos, L., Freitas, L., Santos, J. C., Zanin, G. M., & De Castro, H. F. (2015). Performance of an enzymatic packed bed reactor running on babassu oil to

yield fatty ethyl esters (FAEE) in a solvent-free system. *Biofuel Research Journal, 6*, 242–247.

Souza, L. T. A., Mendes, A. A., & Castro, H. F. (2016). Selection of lipases for the synthesis of biodiesel from *Jatropha* oil and the potential of microwave irradiation to enhance the reaction rate. *BioMed Research International, 2016*, 1404567.

Tan, C. P., & Che Man, Y. B. (2000). Differential scanning calorimetric analysis of edible oils: Comparison of thermal properties and chemical composition. *Journal of the American Oil Chemists' Society, 77*, 143–156.

Willis, W. M., & Marangoni, A. G. (2008). Enzymatic interesterification. In C. C. Akoh, & D. B. Min (Eds.), *Food lipids: Chemistry, nutrition, and biotechnology* (pp. 807–840). Boca Raton, FL, USA: CRC Press.

Zhang, Y., Xi, X., Duan, M., Han, Y., Liu, J., Luo, M., et al. (2016). Green deep eutectic solvent assisted enzymatic preparation of biodiesel from yellow horn seed oil with microwave irradiation. *Journal of Molecular Catalysis B Enzymatic, 123*, 35–40.