Nutritional enrichment of vegetable oils with long-chain n-3 fatty acids through enzymatic interesterification with a new vegetable lipase

J.S. Sousa, A.G. Torres and D.M.G. Freire

SUMMARY: The aim of the present work was to produce vegetable oils enriched with long-chain n-3 fatty acids of nutraceutical interest, through an enzyme-catalyzed interesterification with a new lipase, from physic nut (Jatropha curcas L.). The Vegetable Lipase Powder (biocatalyst) called VLP, which has never been applied in functional foods, was obtained from the physic nut seed, and efficiently hydrolyzed the 95% of waste fish oil in 24 h. Urea precipitation was used to concentrate polyunsaturated fatty acids (PUFA) and was further interesterified with oils of different sources by means of enzymatic catalysis. After the interesterification reaction, which was also catalyzed by the VLP, the PUFA content in coconut oil increased almost ten-fold from 1.8% to 17.7%. In palm oil, the PUFA content increased two-fold from 10.5% to 21.8%, while in olive oil the level of PUFA increased from 8.6% to 21.3%. The mixture of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (3.7% to 3.9%) was incorporated into the triacylglycerol fraction of each of the coconut, palm and olive oils. Through the hydroesterification (hydrolysis followed by interesterification) all the interesterified vegetable oils tested presented sufficient EPA and DHA levels to satisfy the levels recommended for intake by human adults in one tablespoon.

KEYWORDS: Interesterification; Jatropha curcas L.; Lipase; Polyunsaturated fatty acids; Structured lipids

RESUMEN: Enriquecimiento nutricional de aceites vegetales con ácidos grasos n-3 de cadena larga mediante interesterificación enzimática con una nueva lipasa vegetal. El objetivo del presente trabajo fue producir aceites vegetales enriquecidos con ácidos grasos n-3 de cadena larga de interés nutraceutico, por interesterificación catalizada mediante una nueva lipasa, una enzima de semilla de Jatropha curcas L. La lipasa vegetal en polvo (biocatalizador) llamada VLP, nunca ha sido aplicada en alimentos funcionales, se obtuvo mediante procedimientos físicos con semillas de nueces, e hidrolizó eficientemente el 95% de aceites de residuos de pescado en 24 h. La precipitación con urea se utilizó para concentrar los ácidos grasos poliinsaturados (PUFA) que fueron posteriormente interesterificados con aceites de diferentes fuentes mediante catalísis enzimática. Tras la reacción de interesterificación, que también fue catalizada por la VLP, el contenido de PUFA en el aceite de coco aumentó casi diez veces de 1.8% a 17.7%. En el aceite de palma, el contenido de PUFA aumentó dos veces desde 10.5% a 21.8%, mientras que en el aceite de oliva el nivel de PUFA incrementó de 8.6% a 21.3%. La mezcla de ácido eicosapentaenoico (EPA) y ácido docosahexaenoico (DHA) (3.7% a 3.9%) fué incorporada a la fracción de triacilgliceroles de cada uno de los aceites, coco, palma y oliva. Mediante hidroesterificación (hidrólisis seguido de interesterificación) todos los aceites vegetales interesterificados ensayados contienen en una cucharada suficientes niveles de EPA y DHA para cubrir los niveles recomendados de ingesta de humanos adultos.

PALABRAS CLAVE: Ácidos grasos poliinsaturados; Interesterificación; Jatropha curcas L.; Lipasa; Lípidos estructurados


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1. INTRODUCTION

Lipases are very versatile biocatalysts thanks to the range of different reactions they are capable of catalyzing, offering several major advantages over classic catalysts, including their specificity, regioselectivity, and enantioselectivity (Freire and Castilho, 2008). The reactions catalyzed by these biocatalysts produce fewer byproducts, less wastewater, and use milder temperature and pressure conditions, minimizing the migration of acyl groups and oxidation processes (Hernández-Martín and Otero, 2008), thereby opening up a potentially huge range of biotechnology applications (Lee et al., 2006; Wu et al., 2006; Freire and Castilho, 2008).

Vegetable lipases are widely available in nature and are more readily accepted by the food and pharmaceutical industries than microbial lipases. They normally show particular selectivity, often for a substrate, and can be used profitably for the specific enrichment or isolation of a particular type or class of lipid (Yankah and Akoh, 2000).

Vegetable lipases are a less studied alternative to the use of microbial lipases by the food industry. In this field, the synthesis of structured lipids (SL), which are for example triacylglycerols (TAGs) that have been restructured or modified to alter their fatty acid composition and/or their distribution in the glycerol molecule (Lee and Akoh, 1998; Sreenivasan, 1978) are very attractive products in the market. These modifications can alter their bioactivity and modify or improve their physical and/or chemical characteristics, thus offering potential health benefits (Akoh, 1995), by changing the nutritional properties of food (Yankah and Akoh, 2000), for example, to reduce the caloric value of lipids (Gunstone, 1998; Auerbach et al., 2001).

With new findings concerning the positive effects that PUFA have on humans, there is increasing interest in different oils and fats for treating and preventing several diseases and improving health (Molendi-Coste; Legry; Leclercq, 2011; Connor, 2000; Stillwell, 2006). Structured lipids might be effective means of providing specific PUFA for nutritional and therapeutic purposes, like the treatment of specific diseases or abnormal metabolic conditions. They are also regarded as functional foods, which are foods or ingredients that have a positive impact on health above and beyond the basic nutrients they contain (Yankah and Akoh, 2000).

The global market offers a broad range of dietary supplements of PUFA (Mu et al., 1998), diet or low-calorie products (Salatrim and Olestra) (Nagao et al., 2000; Kasai et al., 2003), drugs for hyperlipidemia (MaxEPA®; Sevenseas Healthcare Ltd., UK) and cholesterol reduction (Omacer®; Pronova Bioacre, Norway), among others. These products are not used just to supplement the daily intake of specific PUFA, but also for clinical purposes (Mu et al., 1998).

Structured lipids may be synthesized by a transesterification reaction, either chemically or enzymatically. Chemical interesterification is promoted by heating mixtures of oils and free fatty acids (FFA) in the presence of a chemical catalyst, such as alkali metals or metal alkylates (Osborn and Akoh, 2002). During this process, all the fatty acids present in the mixture of lipids change position randomly in the TAG molecules (Akoh, 1995), making it difficult to control the nutritional or functional properties of the oils and fats obtained (Macrae and Hammond, 1985). However, when enzymes are used as catalysts for the interesterification, these problems might be overcome due to the regiospecificity of some lipases.

Coconut oil (Cocos nucifera L.) is a natural source of medium chain fatty acids (MCFA). 90% of its composition is saturated fatty acids which makes it resistant to oxidative rancidity. The MCFA have numerous functional applications because they can stimulate the immune system, are used in weight control and as antimicrobial agents, such as dietary in cases of chronic degenerative disease, cardiovascular disease, liver disease and cancer (Gopala et al., 2010). Associated with this, the incorporation of PUFAs such as EPA and DHA proposed in this paper generates a structured lipid with a single MCFA and PUFA composition, which provides benefits to the sum of such effects in the immune system by increasing the intake of EPA and DHA in addition to the nutritional advantages of MCFA.

Palm oil (Elaeis guineensis) and its derivatives are commonly recognized in the food industry as having high oxidative stability due to its composition of fatty acids, carotenoids and tocotrienols; thus, it can be used at high temperatures and supports a long life of products (Foster et al., 2009; Embrandiri et al., 2013). It has low levels of polysaturated, particularly omega-3 fatty acids and its enrichment with these properties improves its nutritional fatty acids.

Olive oil (Olea europaea L.) has a privileged position among the edible oils because of its high consumption by the inhabitants of the Mediterranean basin (Oh et al., 2009). Over the past decades, nutritional and epidemiological studies have provided evidence that the consumption of virgin olive oil contributes to human health, due to the presence of antioxidant compounds and their derivatives (Frankel, 2011). However, in many cultures olive oil is more commonly used as a condiment (seasoning) to salads. The prerogative in this research would be to make it even more attractive from the point of view of nutrition by inserting PUFAs and consequently increasing the beneficial effects for the immune system by increasing the intake of EPA and DHA.

The aim of the present work was to produce vegetable oils enriched with long-chain n-3 fatty acids of

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nutraceutical interest, through an enzyme-catalyzed interesterification with a new lipase, from the physic nut (Jatropha curcas L.).

2. MATERIALS AND METHODS

2.1. Source of lipase and oils

The Vegetable Lipase Powder (biocatalyst) called VLP, used for the hydrolysis and interesterification reactions, was obtained from physic nut seeds (Jatropha curcas L.) as described by Cavalcanti et al. (2007) and characterized by Sousa et al. (2010).

This VLP showed activity (96±4 U·g⁻²) using olive oil as substrate. One unit of lipase activity (U) was defined as the amount of enzyme necessary to produce 1 μmol of FFA per minute, under assay conditions (Freire et al., 1997). This assay was done using 0.05 M Tris-HCl buffer, pH 8.0, at 40 °C, as described by Sousa et al. (2010).

The refined coconut and palm oils had acidity of 0.4±0.1% and 6.2±0.2%, respectively; the virgin olive oil had acidity of 1.2±0.2%; and the fish oil (sardine waste) of 3.9±0.1%. The vegetable oils were purchased from a local supermarket, and the fish oil was kindly supplied by the Guabi Nutrição Animal co. (Campinas-SP, Brazil). The solvents were of analytical or chromatographic grade, as appropriate.

In the present study we used a low cost substrate, which consisted of fish oil residue, and also a low-cost waste raw material and a crude biological catalyst (VLP), rendering the proposed method a greater applicability in industrial processes.

2.2. Fatty acid composition of the raw materials and products

The different lipids in the raw materials and products were separated by TLC and the bands related to the TAG were identified by comparing the relative elution of commercial standards. The samples were applied in a silica gel plate (10 μL·cm⁻¹) and lipid classes were separated with hexane:diethyl ether:acetic acid (70:30:1, v:v:v) as the mobile phase. The spots were revealed non-destructively with a 0.2% solution of 2,7-dichlorofluorescein in methanol, and then sprayed to make the spots visible under UV light.

The TAG spots corresponding to structured lipids were identified and scraped off the TLC onto a TLC sheet. The different lipids in the raw materials and products were separated by TLC and the bands related to the TAG were identified by comparing the relative elution of commercial standards. The samples were applied in a silica gel plate (10 μL·cm⁻¹) and lipid classes were separated with hexane:diethyl ether:acetic acid (70:30:1, v:v:v) as the mobile phase. The spots were revealed non-destructively with a 0.2% solution of 2,7-dichlorofluorescein in methanol, and then sprayed to make the spots visible under UV light.

The TAG spots corresponding to structured lipids were identified and scraped off the TLC onto a derivatization tube. The oils and FFA were methylated according to the method described by Lepage and Roy (1986). The analysis of fatty acid methyl esters was performed on a gas chromatograph (CG2010; Shimadzu, Japão) equipped with a capillary column Omegawax 320 (30 m×0.32 mm×0.25μm; Sigma, São Paulo, Brazil). All tests were performed under the following conditions: injector temperature: 260 °C; carrier gas: helium at 4.0 mL·min⁻¹; the sample injection volume was 1 μL with split ratio: 1:20; column temperature gradient: 150 °C for 5 min, increased at 2 °C·min⁻¹ to 210 °C, and remained constant for 30 min; flame ionization detector temperature: 280 °C.

2.3. Production and concentration of PUFA

The reaction of hydrolysis of fish oil 50% (v/v) in 0.1 M Tris-HCl buffer pH 8.0, without the addition of emulsifiers, was carried out with the VLP (10% w/v; 100 U/g of substrate) at 40 °C for 48 h.

The acidity, which indicates the percentage of FFA (w/w) in the oil, was titrated with a 0.04 N NaOH solution until pH 11.0 using an automatic titrator. The samples (0.1 g) were solubilized in 40 mL of a solution composed of acetone and ethyl alcohol (1:1). The blanks were made by titrating the solution with no sample. The acidity was established according to equation (01):

\[ Acidity (\%) = \frac{N \cdot (V_b - V_s) \cdot MM}{10 \cdot m} \]  

Where:
N=NaOH normality; Vs=volume of base used in sample titration (mL); Vb=volume of base used in blank titration (mL); MM=molecular mass of the predominant fatty acid (g); m=sample mass (g).

After the hydrolysis reaction, the FFA were extracted with hexane and PUFA were concentrated with urea precipitation, as described by Gámez-Meza et al. (2003) (Wanasundara and Shahidi, 1999).

2.4. Enzymatic interesterification of vegetable oils with FFA enriched in n-3 PUFA

The interesterification reaction was catalyzed by VLP with the following substrates: oils of coconut, palm and olive, and FFA enriched with n-3 PUFA obtained from the urea-complexation of hydrolyzed fish oil (item 2.3). This reaction was catalyzed by VLP 10% (w/v) in a 3:1 (mol:mol) ratio of FFA: oil, at 40 °C for 24 h in a thermostatted batch stirred reactor (BSR) under magnetic stirring. At the end of the reaction, the samples were diluted with 2.0 mL of n-hexane for extraction in a conical glass tube with PTFE-lined screw caps, and analyzed by thin layer chromatography (TLC). After the interesterification of the vegetable oils with FFA enriched with n-3 PUFA, lipid classes (TAG, DAG, MAG and FFA) the oils were separated by TLC (data not shown), but were not quantified. The TAG bands were scraped off and methylated for analysis according to the method described by Lepage and Roy (1986).
3. RESULTS AND DISCUSSION

3.1. Hydrolysis of fish oil and urea concentration of n-3 PUFA by complexing with urea

The VLP was not purified once the initial idea was to prove that this vegetable lipase (not studied yet) would be capable of carrying out the interesterification reaction, which was the aim of the present work. In this case, it can be seen as a positive factor, once the biocatyst performance can be improved through purification (higher lipase activity) and immobilization technics (allowing lipase reuse by a larger number of cycles) like those already applied in commercial enzymes.

After 24 h of hydrolysis reaction the VLP had converted 95% of the fish oil into FFA, reaching 99% in 48 h. This conversion efficiency was higher than that obtained by Hee-Guk et al. (2007), who studied the hydrolysis of sardine oil by six lipases, from porcine pancreas, Candida rugosa, Candida cylindracea, Rhizopus niveus, Mucor meihei and Pseudomonas sp. In this study, the reactions were conducted for 24 and 48 h in an ideal emulsion system, the highest yields of hydrolysis were obtained with the lipases from Pseudomonas sp. and porcine pancreas, with maximum yields of 80% FFA after 48 h, which were lower than what we found for the VLP from the physic nut.

The fatty acid composition (Table 1) of fish oil was consistent with the date published data for fish oil fatty acids (Haraldsson and Hjaltason, 2001; Jennings and Akoh, 2001), with relatively high contents in the n-3 PUFA of nutritional interest, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). After hydrolysis for 24 h, followed by urea complexation, the contents of EPA and DHA increased, respectively, approximately by 40% and 50% compared to the original fish oil.

Klinkerson et al. (2004) observed enrichments of approximately 80% and 200% in EPA and DHA contents, respectively, through the urea complexation of hydrolyzed refined tuna oil. Gámez-Meza et al. (2003) obtained 220% enrichment with EPA and DHA in sardine oil hydrolysates with an immobilized lipase from Pseudomonas cepacia and 130% and 210% with EPA and DHA, respectively, when the hydrolysis was conducted with a chemical catalyst. Possibly, these results of higher enrichment with n-3 PUFA, compared with the present work, can be explained by their use of refined, bleached and deodorized (RBD) fish oils.

Table 1. Fatty acid composition (g·100 g⁻¹ of total fatty acids) of oils: fish (FO), coconut (CO), palm (PO), olive (OO) and interesterified vegetable oils with the hydrolyzed and concentrated fish oil. The interesterification reaction was catalyzed by VLP 10% (w/v) in a 3:1 (mol:mol) ratio of FFA:oil, at 40 °C for 24 h

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Fish Oil (FO)</th>
<th>FOHC</th>
<th>Coconut Oil (CO)</th>
<th>Interesterified CO+FOHC</th>
<th>Palm Oil (PO)</th>
<th>Interesterified PO+FOHC</th>
<th>Olive Oil (OO)</th>
<th>Interesterified OO+FOHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capric acid    - C10:0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>5.14</td>
<td>3.56</td>
<td>0.01</td>
<td>0.12</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Lauric acid    - C12:0</td>
<td>0.14</td>
<td>n.d.</td>
<td>46.85</td>
<td>31.94</td>
<td>0.16</td>
<td>0.15</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Miristice acid - C14:0</td>
<td>4.24</td>
<td>2.12</td>
<td>19.40</td>
<td>14.85</td>
<td>0.65</td>
<td>1.42</td>
<td>n.d.</td>
<td>1.03</td>
</tr>
<tr>
<td>Palmitic acid  - C16:0</td>
<td>15.64</td>
<td>3.96</td>
<td>9.86</td>
<td>7.49</td>
<td>36.04</td>
<td>27.89</td>
<td>11.11</td>
<td>9.58</td>
</tr>
<tr>
<td>Palmitoleic acid - C16:1</td>
<td>5.93</td>
<td>6.05</td>
<td>n.d.</td>
<td>3.45</td>
<td>n.d.</td>
<td>2.73</td>
<td>0.85</td>
<td>3.53</td>
</tr>
<tr>
<td>Stearic acid   - C18:0</td>
<td>4.33</td>
<td>0.81</td>
<td>3.59</td>
<td>2.00</td>
<td>5.22</td>
<td>3.47</td>
<td>3.20</td>
<td>2.14</td>
</tr>
<tr>
<td>Oleic acid     - C18:1n-9</td>
<td>25.38</td>
<td>26.48</td>
<td>7.19</td>
<td>14.58</td>
<td>46.97</td>
<td>41.37</td>
<td>75.74</td>
<td>61.50</td>
</tr>
<tr>
<td>Linoleic acid  - C18:2n-6</td>
<td>15.72</td>
<td>20.02</td>
<td>1.77</td>
<td>11.26</td>
<td>10.55</td>
<td>15.51</td>
<td>8.04</td>
<td>14.53</td>
</tr>
<tr>
<td>Linolenic acid - C18:3n-3</td>
<td>2.21</td>
<td>2.77</td>
<td>n.d.</td>
<td>1.50</td>
<td>n.d.</td>
<td>1.24</td>
<td>0.63</td>
<td>1.58</td>
</tr>
<tr>
<td>Eicosenoic acid - C20:1n-9</td>
<td>2.33</td>
<td>2.50</td>
<td>n.d.</td>
<td>0.86</td>
<td>0.41</td>
<td>1.02</td>
<td>0.44</td>
<td>0.97</td>
</tr>
<tr>
<td>Eicosatetraenoic acid - C20:4n-3</td>
<td>1.07</td>
<td>1.61</td>
<td>n.d.</td>
<td>0.19</td>
<td>n.d.</td>
<td>0.18</td>
<td>n.d.</td>
<td>0.18</td>
</tr>
<tr>
<td>Araquidonic acid - C20:4n-6</td>
<td>0.69</td>
<td>1.01</td>
<td>n.d.</td>
<td>0.17</td>
<td>n.d.</td>
<td>0.20</td>
<td>n.d.</td>
<td>0.21</td>
</tr>
<tr>
<td>Eicosapentaenoic acid - C20:5n-3</td>
<td>8.99</td>
<td>12.89</td>
<td>n.d.</td>
<td>2.28</td>
<td>4.51</td>
<td>2.27</td>
<td>n.d.</td>
<td>2.45</td>
</tr>
<tr>
<td>Docosapentaenoic acid - C22:5n-3</td>
<td>3.97</td>
<td>5.64</td>
<td>n.d.</td>
<td>0.82</td>
<td>0.92</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.91</td>
</tr>
<tr>
<td>ΣSFA</td>
<td>24.2</td>
<td>6.9</td>
<td>91.0</td>
<td>63.4</td>
<td>42.0</td>
<td>33.1</td>
<td>14.3</td>
<td>12.7</td>
</tr>
<tr>
<td>ΣMUSFA</td>
<td>33.6</td>
<td>35.1</td>
<td>7.2</td>
<td>19.0</td>
<td>47.4</td>
<td>45.1</td>
<td>76.9</td>
<td>66.0</td>
</tr>
<tr>
<td>ΣPUSFA</td>
<td>42.0</td>
<td>58.0</td>
<td>1.8</td>
<td>17.7</td>
<td>10.5</td>
<td>21.8</td>
<td>8.6</td>
<td>21.3</td>
</tr>
<tr>
<td>Ratio n-6:n-3</td>
<td>0.67</td>
<td>0.59</td>
<td>–</td>
<td>1.85</td>
<td>–</td>
<td>2.57</td>
<td>13.33</td>
<td>2.23</td>
</tr>
</tbody>
</table>

Values are means of duplicate analyses (deviation lower than 5% for all samples); n.d.: not detected, ΣSFA: sum of saturated fatty acids, ΣMUSFA: sum of monounsaturated fatty acids; ΣPUSFA: sum of polyunsaturated fatty acids.
3.2. Fatty acid composition and nutritional properties of the interesterified oils

The fatty acid profiles of the vegetable oils, the waste fish oil and the interesterified oils after 24 h of reaction are presented in Table 1. Coconut oil has been incriminated as being atherogenic because of its lipid profile, which contains 90% saturated fatty acids, mostly composed of lauric acid. Coconut oil is rich in medium-chain fatty acids, but low in PUFA. However, after interesterification with the fish oil hydrolysate, the PUFA percentage increased from undetectable levels to final contents of 3.7% EPA+DHA. However, this nutritional property of the interesterified oil is yet to be tested. Palm oil is the most important edible oil in the world’s food market and one of the world’s top commodities (Gunstone et al., 2007). As a consequence of its high saturated fatty acid and solid fat contents, palm oil is normally mixed with other liquid oils to improve its plasticity and melting properties, as it normally occurs in a semi-solid form (Fajardo et al., 2003). Palm oil is rich in natural antioxidants, especially tocotrienols, tocopherols and carotenoids (Goh et al., 1985). The initial fatty acid composition of palm oil consisted basically of palmitic and oleic acids, which accounted for approximately 80% of the total fatty acids (Table 1). After interesterification, the total PUFA contents increased two-fold, and the final contents of EPA+DHA were 3.8%.

Olive oil is widely consumed in the Mediterranean basin and its regular consumption may bring health benefits, such as the prevention of cardiovascular diseases, and the modulation of immune and inflammatory states. It has been suggested that these positive effects of olive oil might be promoted by its antioxidant components, and high levels of oleic acid (Owen et al., 2000; Stark and Madar, 2002), which account for up to 75% of the total fatty acids in the oil. In the case of the interesterified olive-fish oil, a 2.5-fold increase in PUFA was observed, with a final content of EPA+DHA of 3.9%.

The interesterified oils produced contain a more balanced mixture of all fatty acid classes: medium-chain, long-chain, saturated and polyunsaturated. This combination is capable of assuring both the provision of essential fatty acids and aiding in the absorption and digestion of medium-chain fatty acids, as described by Osborn and Akoh, (2002).

Klinkerson et al. (2004) studied the interesterification of RBD tuna oil and n-3 FAME, with the chemical catalyst sodium methoxide, at 80 °C with an oil:n-3 FAME ratio of 1:4, and 5 h of reaction. The EPA content increased from 41 to 60.4 mg·g⁻¹ oil and the DHA content increased from 266 to 388 mg·g⁻¹ oil.

Fajardo et al. (2003) studied the interesterification of n-3 PUFA with RBD palm oil. The reaction was catalyzed by immobilized lipases from Rhizomucor miehei and Alcaligenes sp. After 24 h incubation in hexane, the average incorporation of EPA and DHA in the oil was 21% and 15%, respectively. The authors observed that there was a 29% and 12% reduction in the palmitic and oleic acid content, respectively, and that more EPA and DHA was incorporated when EPAX (fish oil concentrate high in n-3 PUFA) was used in the form of an ethyl ester than when it was used in the form of a free fatty acid in the presence of a lipase from Rhizomucor miehei. In this case, the nutritional enrichment was lower than that obtained in the present work, despite the use of a raw material of superior quality and immobilized lipases. Rao et al. (2002) studied the incorporation of EPA and DHA from cod liver oil and linoleic acid (18:2n-6) from safflower oil in coconut oil, catalyzed by an immobilized lipase from Rhizomucor miehei. The contents of EPA and DHA increased from 0 to 14% (10% EPA and 4% DHA), with a 1:4 molar ratio for the coconut oil:n-3 PUFA, at 54 °C for 34h. Likewise, the maximum incorporation of linoleic acid was obtained at a 1:3 molar ratio of coconut oil:FFA at 39 °C for 48 h, obtaining 45.5% of linoleic acid.

In this case there was a greater incorporation of n-3 PUFA in coconut oil, however, it must be considered that an immobilized lipase, a higher molar ratio of PUFA coconut oil:n-3, higher temperature and longer reaction time (48 h) were used.

3.3. interesterified oils as nutritional sources of long-chain n-3 PUFA

The dietary reference intake of long-chain n-3 PUFA is based on the current intake by a healthy population, and consists of 135–270 mg a day of EPA+DHA (Institute of Medicine, 2005).

The intake of reduced levels of long-chain n-3 fatty acids has negative consequences on human health, and might occur relatively frequently in situations of irregular fish consumption. The hydroesterification method developed in the present study produced interesterified oils that would provide the adequate levels of intake with a daily intake of one tablespoon equivalent to 8.0 g (Figure 1) that would
contain up to approximately 300 mg of EPA+DHA, depending on the oil in question. When passed in appropriate tests for safety as food, these nutritionally enriched interesterified oils could help increase long-chain n-3 fatty acid consumption, or they could be used as a dietary supplement for specific clinical situations.

Diets that follow the pattern of eating fast food are rich in the omega-6 family and poor in omega-3 fatty acids, resulting in a ratio of ω-6/ω-3 of around 9:1. This ratio can be increased to 17:1, when taking into account the western diet in general (Simopoulos, 2001). With the interesterification reaction it was possible to obtain a ratio n-6/n-3 ranging from 1.85 to 2.57 (Table 1) in all products. In olive oil this ratio could be reduced from 13 to 2. Restoring the n-6/n-3 ratio to a better balance will help reduce inflammatory reactions and decrease the risk of chronic disease (Brigelius-Flohé and Traber, 1999). Improving the n-6/n-3 ratio can be achieved by decreasing the intake of omega-6 fats, increasing the intake of omega-3 fats, or both.

This result is significant in view of the recommendations of the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO), which recommends an n-6/n-3 ratio of 5:1 to 10:1.

4. CONCLUSIONS

A novel vegetable lipase found in germinated physic nut seeds (Jatropha curcas L.) proved to be efficient in waste fish oil hydrolysis, adding value to this waste by obtaining n-3 polysaturated fatty acids (mainly EPA and DHA) and enriching edible vegetable oils with these acids.

The developed interesterification process enabled the production of three nutritionally valued oils rich in EPA and DHA and of high commercial value from waste fish oil and vegetable oils using lipase from Jatropha curcas L. seed (VLP) as a biocatalyst which was never before applied in nutritional foods.

The produced oils can be used to enrich foods with PUFA, especially from the n-3 PUFA family. Our results open up promising new horizons for the use of the biocatalyst presented in the production of both free fatty acids and structured lipids of nutritional interest, since the produced lipids contain a mixture of different fatty acid classes, as a result of the versatility of this biocatalyst, which might be of interest for potential use in varied food products.

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