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Use of immobilized phospholipase A_1-catalyzed acidolysis for the production of structured phosphatidylcholine with an elevated conjugated linoleic acid content

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RESUMEN

Uso de acidólisis por fosfolipasa A_1 inmovilizada para la producción de fosfatidilcolina estructurada con elevado contenido de ácido linoleico conjugado.

Fosfatidilcolina estructurada (SPC) fue producida por acidólisis de fosfatidilcolina (PC) y ácido linoleico conjugado (CLA) usando fosfolipasa A_1 (PLA_1) inmovilizada. Los efectos de carga de enzima (2, 5, 10, 15 y 20%, respecto al peso de los sustratos), temperatura (20, 30, 40, 50 y 60 °C) y la relación molar de sustratos (1:2, 1:4, 1:6, 1:8 y 1:10, PC/CLA) fueron evaluados para alcanzar la más elevada incorporación de CLA en PC. La máxima incorporación de CLA obtenida fue de 90% a 50 °C y 200 rpm, para una relación molar 1:4 con una carga de enzima de 15% después de 24 h.

PALABRAS CLAVE: Acidólisis – Ácido linoleico conjugado – Fosfolipasa A_1 – Fosfolípidos estructurados – Fosfatidilcolina

SUMMARY

Use of immobilized phospholipase A_1-catalyzed acidolysis for the production of structured phosphatidylcholine with an elevated conjugated linoleic acid content.

Structured phosphatidylcholine (SPC) was successfully produced via immobilized phospholipase A_1 (PLA_1) – catalyzed acidolysis of phosphatidylcholine (PC) with conjugated linoleic acid (CLA). The effects of enzyme loading (2, 5, 10, 15 and 20%, with respect to the weight of substrates), temperature (20, 30, 40, 50 and 60 °C) and the molar ratio of substrates (1:2, 1:4, 1:6, 1:8 and 1:10, PC/CLA) were evaluated to maximize the incorporation of CLA into PC. The maximum incorporation of CLA achieved was ca. 90% for 24 h of reaction at 50 °C and 200 rpm, using a 1:4 substrate molar ratio and an enzyme loading of 15%.

KEY-WORDS: Acidolysis – Conjugated linoleic acid – Phosphatidylcholine – Phospholipase A_1 – Structured phospholipids

1. INTRODUCTION

Phospholipids (PL) are widely used in the food, pharmaceutical and cosmetics industries because of their ability to function as emulsifiers, stabilizers and antioxidants (Guo *et al.,* 2005). The need for developing functional and nutraceutical foods with special characteristics has led to the design of structured phospholipids (SPL). These novel PL are manufactured by incorporating into natural PL medium-chain fatty acids, of *n*-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or other fatty acids (FA) with biological functions such as conjugated linoleic acid (CLA). By replacing the existing FA asymmetrically in a naturally occurring PL molecule with desired FAs, new physical properties and special functions can also be achieved (Vikbjerg *et al.,* 2007; Hossen and Hernández, 2005; Peng *et al.,* 2002).

To meet the requirements of different industrial applications, hydrolysis, hydroxylation, acetylation and hydrogenation have been employed for chemical modification of commercial lecithin to generate lyso-PL, hydroxylated PL, hydrogenated PL and other PL (Doig *et al.,* 2003; Sinram, 1991). Enzymatic modification of PL has several advantages over chemical modification. The selectivity of enzymes makes the modification of PL simple and easy to accomplish because enzymatic reactions can often be conducted under mild conditions. This biocatalytic approach in microaqueous reaction systems greatly reduces the need for using toxic or otherwise undesirable solvents (Guo *et al.,* 2005).

One group of enzymes that can be used for the modification or synthesis of PL is phospholipases (PLA). These enzymes play crucial roles in cellular regulation, metabolism, biosynthesis and selective modification of PL (D'Arrigo and Servi, 1997). A second group of enzymes that are employed in the synthesis of PL is lipases. These enzymes are comprised of a wide variety of ubiquitous enzymes extensively employed in the processing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, paper manufactureing and the production of cosmetics. Lipases can also be used

to accelerate the degradation of fatty waste and polyurethane (Sharma *et al.,* 2001).

Several studies and reviews have been published concerning the modification of PL using physical, chemical and enzymatic methods (Joshi *et al.,* 2006; Guo *et al.,* 2005; Vikbjerg *et al.,* 2005; Adlercreutz *et al.,* 2002; Lilja-Hallberg and Härröd, 1995; Mutua and Akoh, 1993; Van Nieuwenhuyzen, 1981). Specific reports concerning the incorporation of n-3 FA in PL have been published by Kim *et al.* (2010), García *et al.* (2008), Kim *et al.* (2007), Vikbjerg *et al.* (2007), Peng *et al.* (2002), Haraldsson and Thorarensen (1999), Hosokawa *et al.* (1998) and Totani and Hara (1991). In addition, several authors have reported the immobilization of PLA for use in the modification of different PL via a variety of chemical reactions including hydrolysis with the phospholipase A_2 (Kim *et al.,* 2001: Madoery *et al.,* 1995), transphosphatidylation (Dittrich and Ulbrich-Hofman, 2001), phosphatidylglycerol synthesis (Wang *et al.,* 1997) with phospholipase D, (Dippe *et al.,* 2008) or acidolysis with the phospholipase A_1 (Kim *et al.,* 2010; Garcia *et al.,* 2008; Vijeeta *et al.,* 2004) or A_2 (Vikbjerg *et al.,* 2007; Yamamoto *et al.,* 2006; Lilja-Hallberg and Härröd, 1995). Hossen and Hernandez, (2005) reported the use of lipases (Lipozyme RM IM, Lipase F-AP15, Novozym 435 and Lipozyme TL IM), phospholipase (PLA_2) and the combination of both enzymes in order to increase the incorporation of FA into PL.

The aim of the present work was the production of structured phosphatidylcholine (SPC) containing CLA residues by acidolysis catalyzed by immobilized phospholipase A_1 (PLA_1). The effects of enzyme loading, temperature and molar ratio of substrates were evaluated to attain the highest incorporation of CLA into phosphatidylcholine (PC).

2. MATERIALS AND METHODS

2.1. Materials

Soybean lecithin (PC98), purchased from Shenyang Tianfeng Bioengineering Technology Co. (Shenyang, Liaoning, China) and 92% pure CLA (c-9,t-11 + t-10,c12) as a free fatty acid (a gift from Lipid Nutrition, Wormerveer, The Netherlands) were used as substrates. Lecitase® Ultra (a gift from Novozymes A/S Bagsvaerd, Denmark) was used as the biocatalyst. Duolite A568 was a gift from Rohm and Haas (Barcelona, Spain) and used as a carrier for the biocatalyst.

2.2. Methods

2.2.1. Immobilization of PLA_1

The commercially available undiluted PLA_1 solution (Lecitase® Ultra) containing ca. 1.5% protein was immobilized on a Duolite A568 according to the method reported by García *et al.* (2008), but using a different volume of enzyme (20 mL) and weight of support (4 g).

2.2.2. PLA_1 – catalyzed acidolysis reaction

For all acidolysis reaction trials, different enzyme loads (2, 5, 10, 15 and 20%, with respect to the total weight of substrates), temperatures (20, 30, 40, 50 and 60 °C) and molar ratios of substrates (1:2, 1:4, 1:6, 1:8 and 1:10, PC/CLA) were employed. 4 g of the mixture of substrates were combined with immobilized PLA_1 and allowed to react in 25 mL glass bottles. The reactors were agitated in an orbital incubator (SI 50, Stuart Scientific, UK) operating at 200 rpm for 24 h. Samples were withdrawn periodically to monitor the extent of incorporation of the CLA into PC.

2.2.3. FA analysis of SPC

Samples were withdrawn from the reaction mixture and analyzed by GC. Methyl esters of esterified fatty acids were prepared via selective derivatization of 200 μL of reaction mixture with the addition of 0.5 mL chloroform (Scharlab; Barcelona, Spain) and 0.5 mL 0.5M sodium methoxide in methanol (Sigma Chemical Company; St. Louis, MO, USA). After incubation for 5 min, 0.2 mL of water and 2 mL hexane (Scharlab) were added. After vortexing, the methyl esters extracted in the hexane layer were collected. One μL of the extract was injected into an Agilent 6890N GC fitted with a Phenomenex ZB-WAX plus column (30 m × 0.32 mm × 0.25 μm). Injector and FID temperatures were set at 250 and 300 °C, respectively. The oven temperature was held constant at 200 °C for 20 min. CLA isomers were identified by comparing their retention times with those of a known standard.

3. RESULTS AND DISCUSSION

The need for functional foods and nutraceuticals has led to the incorporation of different FA and n-3 PUFA into PL in order to develop novel compounds and to ascertain if different acyl donors influence the reactivity of these acids for the production of SPL. The replacement of acyl groups in the *sn-1* and *sn-2* positions has been carried out via hydrolysis and esterification and by direct interesterification with CLA, EPA, DPA (docosapentaenoic acid), DHA and CA (caprylic acid), (see Table 1). The reactivities of different FA are influenced by both the specificity of the lipase used as the biocatalyst and by some inhibition effects. Egger *et al.* (1997) have reported that reaction rates seem to be the same for saturated FA of 6 – 12 carbon atoms but are lower for C14 and C16 FA. The highest reaction rates were observed for C18:1 but higher degrees of unsaturation resulted in lower reaction rates.

Table 1
Enzyme-catalyzed interesterification reactions with different fatty acids for the production of structured phosphatidylcholine

Reference	Extent of incorporation	Molar ratio of substrates	Enzyme load (w/w)	Temperature
Kim *et al.* (2010)	43% n-3 PUFA into PC	1:8 (PC/n-3 PUFA)	15% immobilized PLA_1	50 °C
Chojnacka *et al.* (2009)	28% ALA into the *sn*-1 position of egg-yolk PC	1:5.5 (PC/ALA)	20% Novozyme 435	52 – 55 °C
	25% ALA into position *sn*-2	1:13 (PC/ALA)	60% excess PLA_2	25 °C for 48 h and 40 °C for next 48 h
Garcia *et al.* (2008)	35% n-3 PUFA into PC	1:8 (PC/n-3 PUFA)	10% immobilized PLA_1	50 °C
Kim *et al.* (2007)	28% n-3 PUFA into PC	1:10 (PC/n-3 PUFA)	10% PLA_1	55 °C
Vikbjerg *et al.* (2007)	25% CA into PC	1:9 (PC/CA)	30% immobilized PLA_2	45 °C
	30 and 20% CLA and DHA into PC, respectively	1:3 (PL/FA)		
Hossen and Hernandez (2005)	16% CLA into soy PC	1:5 (PL/CLA)	20% Lipozyme TL IM	57 °C
Vikbjerg *et al.* (2005)	49% CA into PC	1:6 (PC/CA)	40% Lipozyme RM IM	55 °C
Peng *et al.* (2002)	35% CA into PC ca. 30% CLA into PC 18.9% EPA and DHA into PC	1:5.5 (PL/FA)	20% Lipozyme TL IM	60 °C

PUFA: polyunsaturated fatty acid**, PC:** phosphatidylcholine, **PLA_1:** phospholipase A_1, **ALA:** α-linolenic acid, **PLA_2:** phospholipase A_2, **CA:** caprylic acid, **CLA:** conjugated linoleic acid, **DHA:** docosahexaenoic acid, **FA:** fatty acid, **PL:** phospholipid, **EPA:** eicosapentaenoic acid.

Analysis of the entries in Table 1 indicates that, Kim *et al.* (2007, 2010) and Garcia *et al.* (2008), have successfully incorporated n-3 PUFA (defined as the sum of EPA + DPA + DHA) into PC. These authors noted that EPA was the most reactive FA followed by DPA and DHA. In a separate study Vikbjerg *et al.* (2007), found CLA to be the most reactive FA when it was compared with caprylic acid and DHA. These authors achieved the incorporation of 30, 25.3 and 20.2% of these FA respectively. On the other hand Peng *et al.* (2002), found caprylic acid as the most reactive FA when it was evaluated with CLA and the sum of EPA + DHA. The Incorporation of these FA reached 35, 30 and 18.9%, respectively.

In addition to being aware of the reactivities of the different acyl donors, one must also note that the purity of the substrates can affect the efficacy of the reaction and the resulting improved acyl exchanges. In this work, 98% pure PC and 92% pure CLA (Clarinol A-95) as free FA were used as substrates. We were able to achieve 90% incorporation of CLA into PC. This result is the highest extent reported in the open literature for the incorporation of any FA into PL.

As for the entries in Table 1, we believe that the rather low extents of incorporation produced were most likely influenced by a) the purity of the substrates employed and b) the reactivity of the FA employed. Kim *et al.* (2007, 2010), described the use of granulated PC (purity >95%) and a preparation of free FA from a fish oil concentrate by saponification as their substrates. Chojnacka *et al.* (2009), referred to a 70% content of ALA in the free FA used as their substrate. Garcia *et al.* (2008) used lecithin (95% soy PC) and concentrated fish oil (ca. 70% n-3) as substrates. Vikbjerg *et al.* (2007), reported Epikuron 200 (PC, 93%), CLA (purity 80%) and DHA (> 99%) as their substrates. Hossen and Hernandez (2005), employed Lipoid S100 (PC ≈ 94%) and safflower oil for the synthesis of CLA as their substrates. Vikbjerg *et al.* (2005), reported Soybean PC (Epikuron 200, purity 93%) and caprylic acid (purity 97%) as their substrates. Peng *et al.* (2002), worked with a PL profile (area %) of PC (54.8), phosphatidylethanolamine (15.6), phosphatidylinositol (15.3), phosphatidic acid (4.3), lysoPC (0.4) and other phospholipids (9.6) together with a mixture of EPA (35%) and DHA (25%); CLA (purity 80%) and CA (purity 97%) as their substrates.

We carried out acidolysis reactions under a wide variety of conditions, varying temperatures, enzyme loading and molar ratio of substrates while employing high purity substrates and very reactive acyl donors in order to produce SPC with an elevated CLA content. Our first task was the selective derivatization of the substrates employed. We confirmed that they were 92 and 98% pure, respectively. A GC analysis of the derivatized

soybean lecithin indicated that the c-9,t-11 + t-10,c12 CLA isomers were not present at detectable levels; then, we monitored the extent of the incorporation of the CLA into PC for all of the acidolysis reaction trials.

3.1. Effect of the molar ratio of substrates

Adlercreutz *et al.* (2002) have reported that increased yields for both esterification and transesterification reactions were obtained by increasing the free FA concentration; changes in polarity or viscosity in the reaction medium while increasing free FA concentration (Egger *et al.,* 1997) have been documented. We were able to obtain an increase in the incorporation of CLA into PC when the molar ratio of substrates was highest for enzyme loadings of 2, 5 and 10% at all temperatures employed (Figure 1). The highest incorporation of CLA into PC achieved was 90.1% in a trial at a molar ratio of 1:4 (PC/CLA), with immobilized PLA_1 as biocatalyst (Figure 1B). This extent of incorporation is the maximum of FA into PL reported to date in the literature and contrasts strongly with the levels of the studies listed in Table 1.

In our experimental studies, the smallest extent of incorporation were those for the trials at

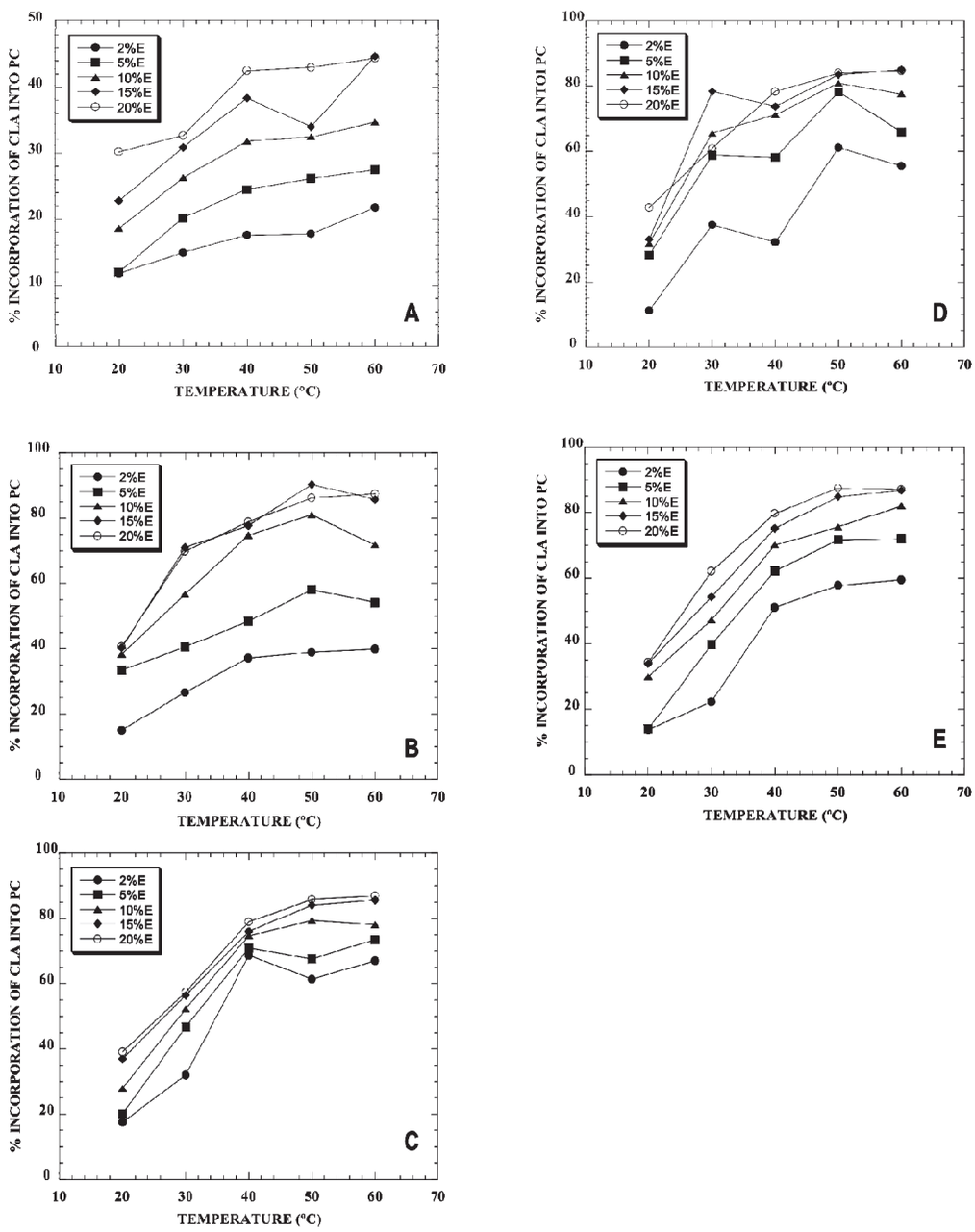

Figure 1
Effect of the molar ratio of substrates PC/CLA on the production of SPC. A. ratio=1:2, B. ratio=1:4, C. ratio=1:6, D. ratio=1:8, E. ratio=1:10. Conditions: reaction for 24 h at 20, 30, 40, 50 and 60 °C; orbital shaker at 200 rpm; with 2, 5, 10, 15 and 20% enzyme loadings with respect to the weight of substrates.

a ratio of substrates equal to 1:2 PC/CLA (Figure 1A). The influence of higher enzyme loadings and temperatures is reflected as increases in the extent of incorporation of CLA. However, the degree of incorporation never exceeded 50%. When a 1:4 molar ratio of substrates was employed (Figure 1B), maximum incorporation of CLA into PC was achieved. The effect of higher ratios for the entire enzyme loads was also noted in all the experiments where incorporations were almost 2-fold compared to those obtained in the previous ratio of substrates. For a 1:6 substrates molar ratio (Figure 1C), we observed a very interesting behavior and promising area for further optimization because the reactions showed faster rates and very similar incorporations were obtained at 40 °C. Beyond this point the increments reached somewhat different values but still no significant differences could be observed when 15 and 20% enzyme loads were used or 5 and 10% enzyme loads at the end of the trials. Using the same ratio of substrates, Vikbjerg *et al.* (2005) achieved 49% incorporation of caprylic acid (CA) into PC, while in our trials we achieved a much higher percentage, even at an enzyme loading of 2%. When testing a 1:8 substrates molar ratio (Figure 1D), we noted a rapid increase in the kinetics for reactions at 20 and 30 °C over the entire enzyme loadings. However, smaller extents of incorporation were obtained at 60 °C than at 50 °C. All the yields for this ratio were higher than those reported by Kim *et al.* (2010), who attained 43% and Garcia e*t al.* (2008), who reached 35% incorporation of n-3 PUFA into PC. It is important to note that for a 1:10 molar ratio of substrates (Figures 1E), the highest temperatures (40, 50 and 60 °C) and the highest enzyme loadings (10, 15 and 20%) favored the incorporation of CLA into PC even when an increment was always observed throughout the reaction time. Using a similar substrates ratio Kim *et al.* (2007) reported 28% incorporation of n-3 PUFA into PC with a loading of 10% PLA_1. In this work and under the same conditions we attained 80% incorporation.

In general, better acyl exchanges were obtained by increasing the molar ratio of substrates even for the lowest enzyme loading. Vikbjerg *et al.* (2005), reported lower incorporation of CA into PC and higher incorporation of CA into lysoPC as the molar ratio of substrates increased. On the other hand, Peng *et al.* (2002) reported that a molar ratio of substrates equal to 5.5 was best for attaining the highest incorporation of CA into PC. Higher ratios reduced the incorporation of caprylic acid.

3.2. Effect of temperature

Given the nature of enzyme-catalyzed reactions, temperature is a relevant variable to be considered for acidolysis. Several reports in the literature indicated an optimum temperature for the production of SL. In the present work, we carried out the reaction at five different temperatures (20, 30, 40, 50, 60 °C). As the temperature was increased, larger extents of incorporation were obtained. This effect was favored by high enzyme loadings.

When acidolysis reactions took place at 20 °C (Figure 2A), for 1:2 and 1:4 substrate molar ratios, the incorporation produced was less than 40%. Conversely, for greater ratios the incorporation was higher. A similar trend was observed when 30 °C (Figure 2B) was tested and we noted similar incorporations when 1:6 and 1:10 ratios were used. When 40 °C was evaluated (Figure 2C), 30% incorporation was reached for a 1:2 substrate molar ratio and we observed no significant differences when 10, 15 and 20% enzyme loads were used. The other ratios produced greater incorporations, with the highest at a 1:10 ratio. Very similar extents of incorporation were obtained for 1:4, 1:6 and 1:8 ratios for 15% enzyme load. In a previous study Vikbjerg *et al.* (2007), evaluated 45 °C to carry out their experiments and they reported a 36% incorporation of CA into PC and 30 and 20% of CLA and DHA into PC respectively. At 50 °C (Figure 2D), again the smallest incorporations were attained for a 1:2 ratio. When 1:6 and 1:10 ratios were tested, they had a similar trend, but surprisingly the same percentage of incorporation was reached for 1:4, 1:6, 1:8 and 1:10 ratios when a 20% enzyme load was used. Previous reports have mentioned this reaction temperature. Kim *et al.* (2010), obtained 43% incorporation of n-3 PUFA into PC while in their conditions we produced 84% incorporation. Garcia e*t al.* (2008), cited 35% incorporation of n-3 PUFA into PC while in their conditions we produced 73.83% incorporation. When 60 °C was evaluated (Figure 2E), 1:4, 1:6, 1:8 and 1:10 substrates molar ratios had the same incorporation rates, reaching the same values for a 10% enzyme load and very close incorporation for 15 and 20% enzyme loads. Again, the 1:2 ratio produced the lowest incorporation and 40% incorporation was the highest for 10, 15 and 20% enzyme loads. Using the same temperature, Peng *et al.* (2002), reported 35% esterification of CA into PC, ca. 30% of CLA into PC and 18.9% of EPA and DHA into PC. Employing a close temperature (57 °C), Hossen and Hernandez (2005) obtained 16% incorporation of CLA into soy PC.

Other works evaluating the effect of temperature have been reported by Kim *et al.* (2007), who obtained an increment of n-3 PUFA into PC and lysoPC incorporation when the temperature was increased from 25 to 65 °C. Vikbjerg *et al.* (2007), suggested 45 °C as the temperature for reaching the maximum incorporation of CA into PC; when evaluating higher temperatures the acyl exchange was smaller. In a similar work Vikbjerg *et al.* (2005), also reported a decrease in the incorporation of

CA into PC when the temperature changed from 40 to 60 °C but for lysoPC the behavior was the opposite. In the experiments carried out by Peng *et al.* (2002), 55 °C appeared to be the optimal temperature to attain the maximum incorporation of CA into PL; when evaluating higher temperatures the incorporation was smaller.

In general, in all our data we observed a positive effect of temperature on the incorporation of CLA into PC and it was clearly favored by the enzyme load. At the same time, a higher substrate molar ratio also increased the extent of incorporation.

3.3. Effect of enzyme loading

This is the main variable in the enzyme-catalyzed incorporation of FA into PL. When we evaluated the different enzyme loadings, higher incorporations were attained as the enzyme load increased, and this effect was favored by temperature. It has been reported that high enzyme loads are needed for an effective incorporation of novel FA into PL by acidolysis in a solvent-free system (Haraldsson *et al.,* 1999; Aura *et al.,* 1995). However, the use of high enzyme loadings produced problems with agitation and decreased mass transfer.

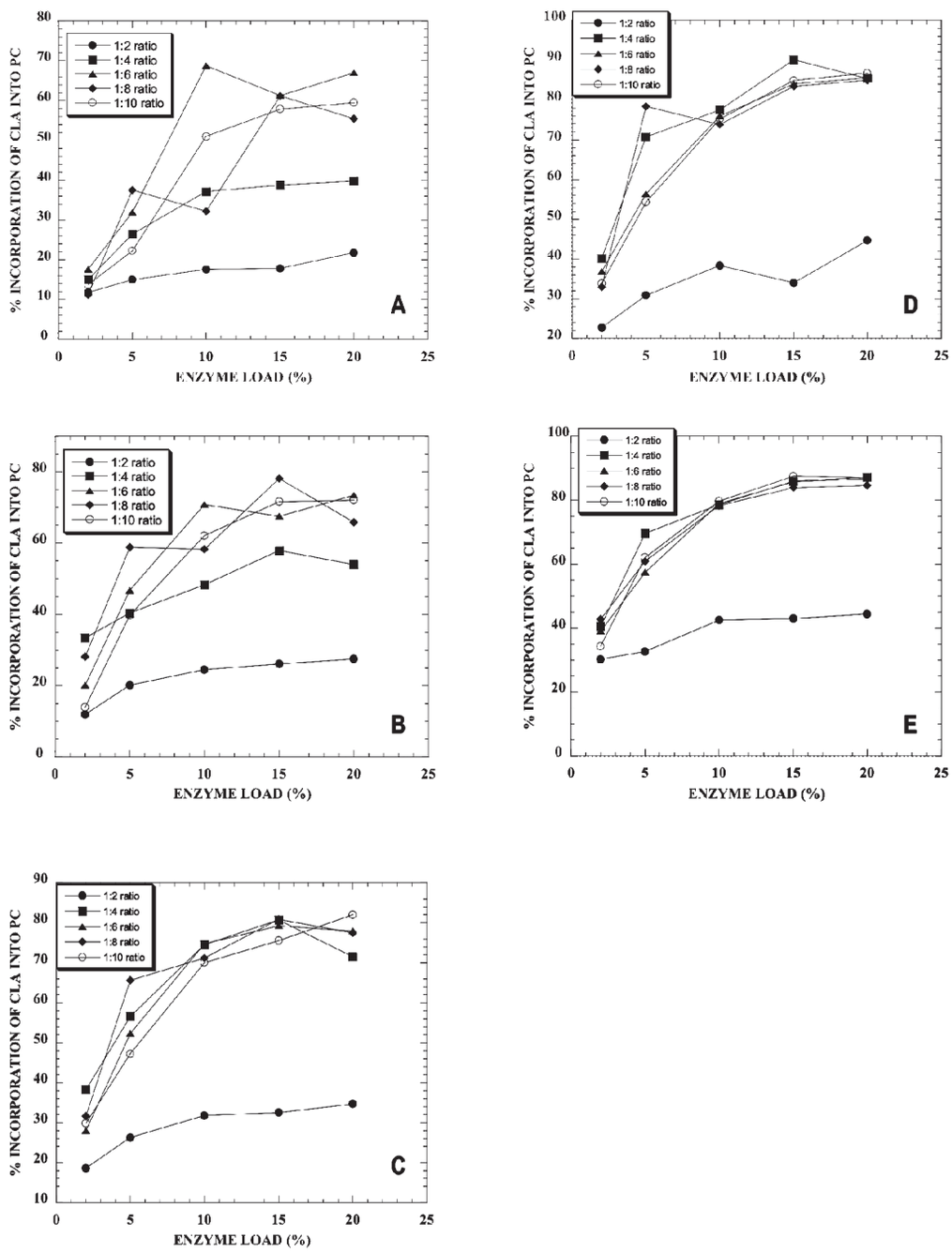

Figure 2
Effect of temperature on the production of SPC. A. 20 °C, B. 30 °C, C. 40 °C, D. 50 °C, E. 60 °C. Conditions: reaction for 24 h at 20, 30, 40, 50 and 60 °C; orbital shaker at 200 rpm; with 2, 5, 10, 15 and 20% enzyme loadings with respect to the weight of substrates.

When 2% enzyme loading was used (Figure 3A), 40% incorporation of CLA into PC was measured for a 1:8 substrate molar ratio at 60 °C. In fact, this relatively high temperature favored the incorporation for the entire substrate molar ratio at this load. At 20 °C the lowest incorporations were produced and very important increments were observed when the substrate ratio changed to 1:4 for all temperatures. With 5% enzyme loading (Figure 3B) we noted that the highest substrate molar ratio (1:10) yielded the smallest incorporations. At this loading, the trials carried out at 20 and 30 °C produced an increase in yield when the substrate molar ratio increased. Better acyl exchanges were observed for 10% enzyme loading (Figure 3C). With this loading, the reactions carried out at 40, 50 and 60 °C for all the substrate molar ratios had the highest incorporations, being the best at 60 °C. Using this same enzyme load, Garcia e*t al.* (2008), reported 35% incorporation of n-3 PUFA into PC for a 1:8 substrate molar ratio and by employing their conditions we were able to attain 73.83% incorporation of CLA into PC. When 15% enzyme loading was tested, we attained the highest incorporation of CLA into PC for a 1:4

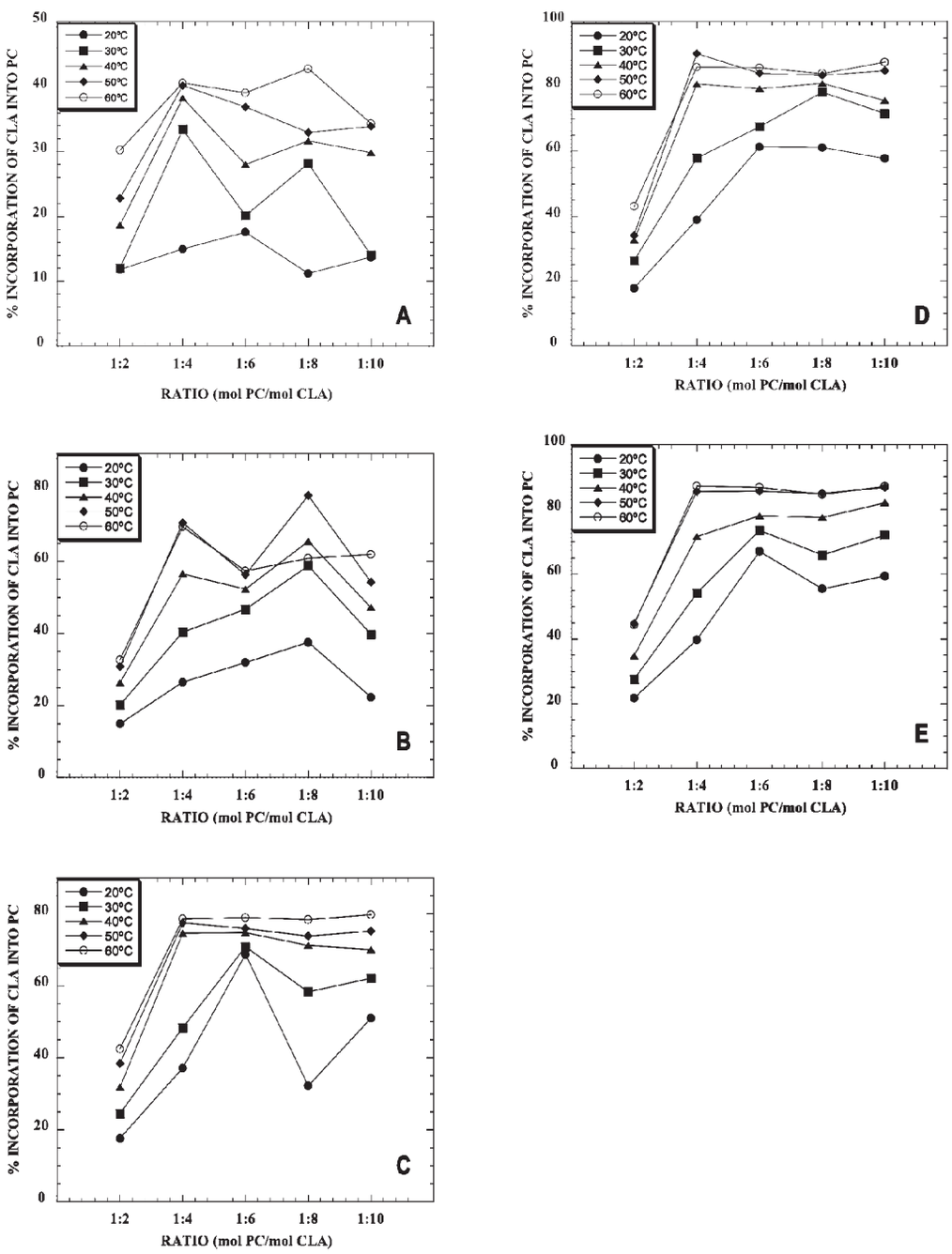

Figure 3
Effect of enzyme loading (w/w) on the production of SPC. A. 2%, B. 5%, C. 10%, D. 15%, E. 20%. Conditions: reaction for 24 h at 20, 30, 40, 50 and 60 °C; orbital shaker at 200 rpm; with 2, 5, 10, 15 and 20% enzyme loadings with respect to the weight of substrates.

substrate molar ratio (Figure 3D). With this loading, for 40, 50 and 60 °C, there was a significant increment when the substrate molar ratio changed to 1:4 but for higher ratios the incorporation was almost constant. For the trials at 30 °C the increment was observed up to 1:8 substrate molar ratio and at 20 °C only up to 1:6 substrate molar ratio. By employing the same enzyme loading, Kim *et al.* (2010) obtained 43% esterification of n-3 PUFA into PC for a 1:8 substrate molar ratio and with their conditions we were able to attain 83.45% incorporation of CLA into PC. Evaluating 20% enzyme loading (Figure 3E) we noted an increment in the percentages of incorporation from 1:2 to 1:6 substrate molar ratios for the experiments carried out at 20, 30 and 40 °C. The same kinetic trend was observed at 50 and 60 °C, producing almost the same incorporation for 1:4, 1:6, 1:8 and 1:10 substrate molar ratios. For this particular treatment, the 1:6 substrate molar ratio appears to be best ratio to reach the highest incorporation of CLA. In the literature Chojnacka *et al.* (2009), reported 28% incorporation of α-linolenic acid (ALA) into the *sn*-1 position of egg-yolk PC using a 20% enzyme load. Hossen and Hernandez (2005), obtained 16% incorporation of CLA into soy PC using a 5:1 substrate molar ratio and 20% enzyme loading. Peng *et al.* (2002), reported 35% incorporation of CA into PC, ca. 30% of CLA into PC and 18.9% of EPA and DHA into PC employing a 5.5 substrate molar ratio and 20% enzyme loading.

4. CONCLUSIONS

Several reports have been published on the modification of natural PL for novel applications. The effects of temperature, substrate molar ratio, enzyme loading and reaction time have been widely reported for that purpose. We were able to yield 90.1% incorporation of CLA into PC at 50 °C and 200 rpm, for a 1:4 substrate molar ratio with 15% immobilized PLA_1 enzyme loading after 24 h. This is highest incorporation of FA into PL ever obtained and reported in the literature. The production of SPC with an elevated content of CLA was clearly favored by the high purity of the substrates employed, which affected the equilibrium of the reaction.

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