J Am Oil Chem Soc (2011) 88:443–446
DOI 10.1007/s11746-010-1684-9

LETTER TO THE EDITOR

Application of Enzymatic Hydrolysis on Sunflower Lecithin Using a Pancreatic PLA_2

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Received: 31 May 2010 / Revised: 20 August 2010 / Accepted: 8 September 2010 / Published online: 1 October 2010

Sir,

Lecithins are a mixture of acetone insoluble phospholipids, containing mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), minor compounds such as phosphatidic acid (PA), and other minor substances such as carbohydrates [1]. The introduction of changes in the original concentration of these phospholipids, by chemical or enzymatic modification of their structure can lead to lecithins with different physicochemical and functional properties, with respect to native lecithin. These modified compounds can be used in the development of a variety of food products, such as margarines, chocolates and bakery products [2, 3]. Enzymatic hydrolyzed lecithin may present technological and commercial advantages over native lecithins as O/W emulsifier and mold- or pan-releasing agent. Consequently, the demand for lysolecithin has been increasing in recent years [4, 5]. PLA_2 is the most employed family of enzymes for the modification of lecithins [6]. These enzymes play an important role in biochemical processes such as turnover of membrane phospholipids or the regulation of bioactive substances, e.g. lysophospholipids, fatty acids, and lipid mediators in inflammatory and digestion processes [7, 8].

In Argentina, the production of sunflower oils is of utmost importance with the consequent economic relevance [9]. Sunflower lecithin, i.e. a byproduct of the degumming process of oils [10–12], is an alternative to soybean lecithin because it is considered to be a non-GMO product, which is currently preferred by certain consumers.

The aim of the present investigation was to evaluate the influence of the main operating conditions on the application of an enzymatic hydrolysis on sunflower lecithin at laboratory scale. This study seeks to contribute to the oil industry with useful information for rescaling this modification process, in order to increase the value-added of sunflower lecithins.

The enzymatic hydrolysis process was carried out using a native sunflower lecithin provided by a local oil industry and a porcine pancreatic PLA_2 (Lecitase 10 L, 10,580 IU/mL, Novo Nordisk, Denmark). This process was carried out in a thermostated reactor at laboratory scale, using 27 g of sunflower lecithin and 18 ml of $CaCl_2$ solution (0.1 or 0.4 M). The initial pH was adjusted to 7 or 9 by adding 4 N NaOH solution. Then, the resulting mixture was set at 60 °C, and phospholipase A_2 solution was incorporated (0.4 or 2.0 ml in 100 g of lecithin). The system was kept under continuous agitation (50 rpm) for 40 or 300 min. The evolution of the hydrolysis process was followed by measuring the pH, with a pH meter for solid samples (840049 Puncture Tip, Saenz SRL). Products of enzymatic hydrolysis were subjected to a sudden decrease in temperature to stop the process of hydrolysis and then deoiled using acetone, according to AOCS Official Method Ja 4–46, procedures 1–5 [13]. After that, samples were stored at 0 °C. The hydrolysis process was carried out in duplicate.

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Phospholipid composition of samples obtained under different conditions of enzymatic hydrolysis, was determined by ^{31}P-NMR analysis using a Bruker Avance 300 MHz automatic spectrometer using triphenyl phosphate as internal standard (Spectral Service GmbH, Köln, Germany) [14]. Then 100 mg of each hydrolyzed sample was diluted in 1 ml of deuterated chloroform, 1 ml of methanol and 1 ml of 0.2 M Cs-EDTA (pH 8.0). The organic layer was separated after 15 min shaking, and analyzed by this spectroscopic technique. Phospholipid composition was expressed in terms of molar concentration.

The degree of enzymatic hydrolysis associated with each type of phospholipid was determined using a relationship between the phospholipid concentration in deoiled and hydrolyzed sunflower lecithin, as is shown in Eq. 1:

$$\%H_{PL} = \left(\frac{PL_i - PL_f}{PL_i}\right) \times 100 \quad (1)$$

where PL is PC, PI, PE or PA; PL_i is the phospholipid concentration in deoiled lecithin (PC_i 37.5%, PI_i 37.8%, PE_i 15.1%, PA_i 4.3%) and PL_f is the phospholipid concentration in the hydrolyzed lecithin.

The sunflower lecithin used as starting material presented the following composition: 43.1% phospholipids (PC 36.7%, PI 35.3%, PE 15.0%, PA 5.1%, and 7.9% others phospholipids), 33.4% oil, and 23.5% of other compounds (glycolipids, complex carbohydrates).

The hydrolyzed lecithins present a high concentration of major lysophospholipids, with respect to native (1.1%) and deoiled sunflower lecithin (2.4%). In this way, Fig. 1 shows the ^{31}P-NMR spectra of the deoiled sunflower lecithin and two hydrolyzed sunflower lecithin obtained under different processing conditions.

The effect of phospholipase A_2 on each phospholipid (PC, PE, PI and PA) can be analyzed following the evolution of the degree of hydrolysis. In Table 1, it is possible to observe that PC presented a degree of hydrolysis higher than 91.4% for all the conditions assayed. When 2.0% of PLA_2 was used, the residual PC concentration in the hydrolyzed lecithin was lower than the detection limit of the ^{31}P NMR equipment (<0.1%) (see Fig. 1). Values of $\%H_{PE}$ were also high (>80.9%), although slightly lower than $\%H_{PC}$, in all experiments. These results can be correlated with those described by Penci [15] using a very low amount of substrate (1 mg).

Values obtained for the different degrees of hydrolysis ($\%H_{PL}$) and total lysophospholipid content (LPL) were analyzed using ANOVA, and the influence of each variable was associated with the percentage of the explained variance (Table 2).

Phospholipase A_2 content and reaction time were significant for all the analyzed parameters. A rise of these variables showed a highly significant increase ($p < 0.01$)

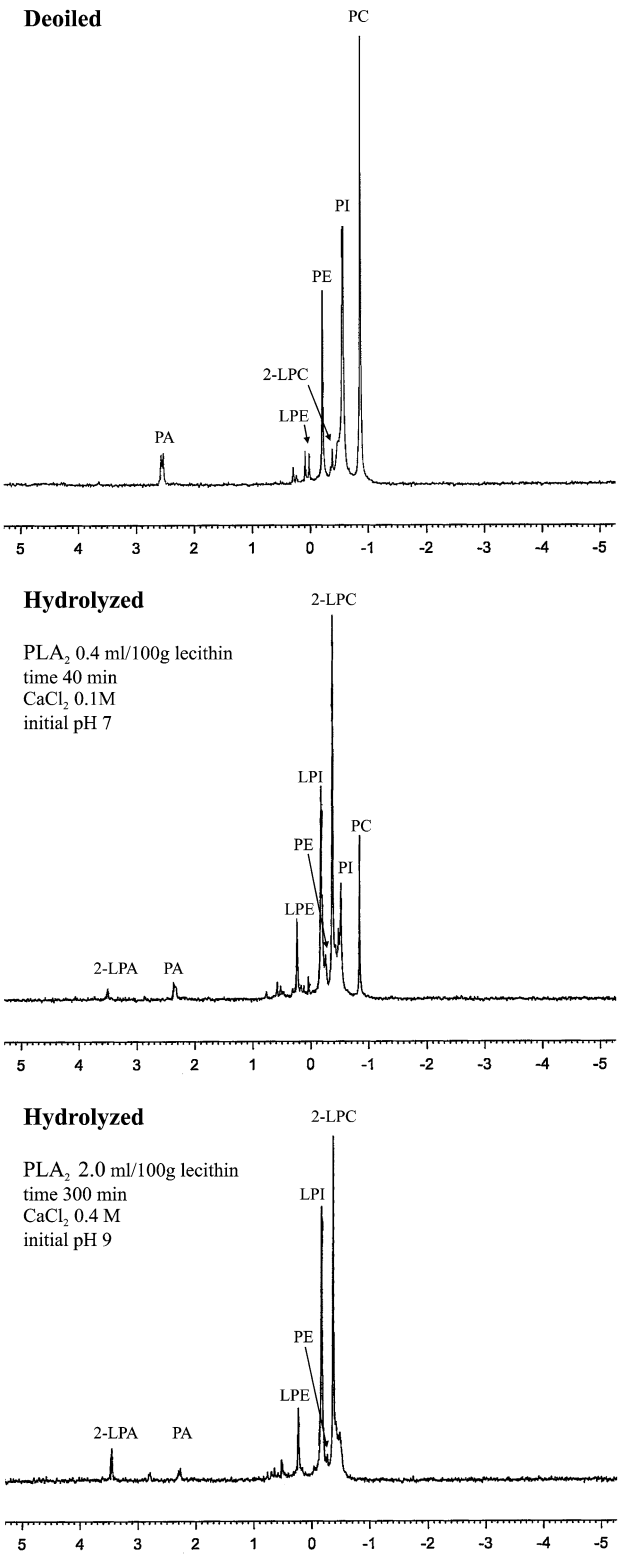

Fig. 1 ^{31}P-NMR spectra at 300 MHz of different modified sunflower lecithins

of the different degrees of hydrolysis obtained for the main phospholipids (see Table 1). Phospholipase content was the variable that presented the highest effect on the explained

Table 1 Degree of hydrolysis of main phospholipids (%H_{PL}) and total lysophospholipid content (LPL) of hydrolyzed sunflower lecithins under different operating conditions

PLA_2[a]	Time (min)	$CaCl_2$ (M)	Initial pH	$\%H_{PC}$[b]	$\%H_{PI}$[b]	$\%H_{PE}$[b]	$\%H_{PA}$[b]	LPL[b]
0.4	40	0.1	7	91.7	44.7	80.9	69.8	63.2
0.4	40	0.1	9	91.4	44.8	81.6	44.8	61.9
0.4	40	0.4	7	93.3	45.1	82.0	58.8	65.2
0.4	40	0.4	9	95.5	46.4	84.5	39.9	66.5
0.4	300	0.1	7	94.9	62.0	85.4	91.9	74.9
0.4	300	0.1	9	94.4	61.6	86.1	60.2	69.4
0.4	300	0.4	7	97.9	63.6	85.7	80.3	74.3
0.4	300	0.4	9	97.6	65.0	86.5	53.8	72.6
2.0	40	0.1	7	100.0	92.4	88.8	100.0	87.8
2.0	40	0.1	9	100.0	93.4	85.8	83.0	88.1
2.0	40	0.4	7	100.0	100.0	88.1	90.8	91.4
2.0	40	0.4	9	100.0	97.2	90.1	60.5	88.7
2.0	300	0.1	7	100.0	100.0	96.7	100.0	93.8
2.0	300	0.1	9	100.0	96.0	97.7	100.0	92.0
2.0	300	0.4	7	100.0	100.0	97.1	100.0	93.9
2.0	300	0.4	9	100.0	100.0	96.2	88.4	92.5

[a] ml PLA_2 solution in 100 g of lecithin

[b] Average values ($n = 2$). The coefficient of variation was lower than 6%

Table 2 Percentage of explained variance according to ANOVA for the degree of hydrolysis of main phospholipids (%H_{PL}) and total lysophospholipid content (LPL)

Variables[a]	Explained variance (%)				
	$\%H_{PC}$	$\%H_{PI}$	$\%H_{PE}$	$\%H_{PA}$	LPL
PLA_2	74.0	91.3	59.2	47.3	90.1
Time	6.6	5.4	31.7	15.3	7.1
$CaCl_2$	*nsv*	*nsv*	*nsv*	5.7	*nsv*
pH	*nsv*	*nsv*	*nsv*	24.6	0.5
PLA_2 × time	6.6	2.6	5.3	*nsv*	0.9
PLA_2 × $CaCl_2$	5.6	*nsv*	*nsv*	*nsv*	*nsv*
PLA_2 × pH	*nsv*	*nsv*	*nsv*	1.8	*nsv*

nsv not significantly variable ($p < 0.05$)

[a] time × $CaCl_2$, time × pH, and $CaCl_2$ × pH interactions were *nsv* for all parameters analyzed

variance of %H_{PL} values (>47.3%), especially for $\%H_{PC}$ and $\%H_{PI}$.

Calcium chloride concentration and pH were not significant variables on the hydrolysis of PC, PI and PE ($p > 0.05$). However, a significant increase of $\%H_{PA}$ values when decrease the levels of both variables was recorded.

On the other hand, a highly significant effect of the PLA_2 × time interaction on the hydrolysis of PC, PE and PI was observed. At high concentrations of PLA_2, the reaction time did not present an important effect on the parameters $\%H_{PC}$ and $\%H_{PI}$, due to the high degree of hydrolysis exhibited by both phospholipids (>96.0%). In addition, a wide range of $\%H_{PE}$ values was determined for a major level of PLA_2 in the period studied.

The hydrolyzed sunflower lecithins presented a LPL content ranging from 61.9–93.8% (Table 1). The influence of the different variables on the total lysophospholipid concentration of the hydrolyzed sunflower lecithin was according to the degree of hydrolysis discussed above. A highly significant increase ($p < 0.01$) of LPL content was recorded as a function of the rise of the variables PLA_2 content and reaction time which represent 90.1 and 7.1% of the explained variance for this parameter, respectively.

Acknowledgments This work was supported by grants from CONICET (PIP 1735), ANPCyT (PICT 1085), and UNLP (11/X502). Sunflower lecithin was provided by Vicentin SAIC; Thorsten Buchen and Sandro Goñi are acknowledged for their technical assistance.

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