Sustainable refining of vegetable oil made easy with a designer phospholipase C enzyme

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Abstract

Enzymes are extraordinary catalysts and a preferred tool for green chemistry, but they have not evolved to tolerate the harsh conditions required in large-scale chemical processes, making the replacement of inorganic catalysts extremely expensive in most cases. However, the continuous knowledge generated by protein engineers helps to realize the promise of using biocatalysts to address critical environmental and industrial challenges.

The increasing demand for food and biofuels pressures the vegetable oil industry to continuously improve its manufacturing efficiency and at the same time mitigate the environmental pollution caused by the refineries. Enzymatic degumming is a key enabling technology to obtain cleaner and more efficient methods, but advanced enzymes are needed for widespread adoption.

Here, we present an enzymatic degumming process based on chPLC, a synthetic phospholipase C with remarkable thermal stability and catalytic properties.

The process developed does not require modifications in current soybean oil factories, avoiding upfront investments, and is four times faster than the enzymatic processes available in the market. A techno-economic analysis predicts a ~ 60% cost reduction for a base-case scenario, establishing the largely expected conditions to promote the global adoption of enzymatic oil degumming by refineries of all sizes.

To the best of our knowledge, this is the first report showing the application of a synthetic enzyme designed to address an industrial need having an impact on both the global economy and the environment, in the billion-dollar range.

Introduction

Vegetable oils represent a core sector of the global economy, and their demand for food and fuel production is continuously increasing (Cerminati et al., 2017, 2018; Elena et al., 2016, 2017). Even when they are renewable, the environmental impact of deforestation to produce them and the emissions and waste generated during oil extraction and refining make the production unsustainable (Hails et al., 2020; Aguirre et. al., 2021).

South America concentrates 57 % of the world's soybean production, and the cultivated area doubled in the last two decades. The majority of this expansion occurred in the Brazilian Amazon, where the soybean area increased more than tenfold, from 0.4 to 4.6 million ha (Kuschnig et al., 2021; Song et al., 2021).

Current soybean oil refining processes are inefficient and generate around 65 kg of waste per MT of crude oil. Part of this material is sold as byproducts, but a significant fraction is often inadequately disposed-of or treated with strong acids; causing environmental pollution, particularly in countries with poor regulations and controls (Bailis et al., 2014; Selfa et al., 2015). Thus, more efficient and environmentally friendly oil refining methods can help to mitigate the environmental pollution caused by oil refining and deforestation by reducing the land needed to obtain a given amount of oil (Alexandre et al., 2016; Monoj K. Gupta, 2017).

The crude soybean oil possesses up to 3.5 % of phospholipids (PLs), equivalent to 1200 ppm of inorganic phosphorus (P), which includes phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA). The PLs are removed in the first oil refining step, known as "degumming", which causes the major losses of oil during this process. The traditional method used for this is known as "water

degumming" (Dumont & Narine, 2007). In this process, the PLs are extracted with water and retain some trapped triacylglycerol (TAGs) to form a heavy phase called "gums", which is separated by centrifugation. The resulting degummed oil contains 100-200 ppm of P, and under this specification is exported or further processed to obtain edible oil or biodiesel (Dijkstra, 2010).

In the last decade, enzymatic degumming using type C phospholipases (PLCs) was commercially unavailable. This class of enzymes increases the final yield of the refining process by hydrolyzing PLs, giving as products the corresponding polar heads and oil-miscible diacylglycerols (DAGs). At the same time, the reaction releases TAGs trapped by the PLs. The overall extra yield provided by the generated DAGs and the released TAGs is around 2% (Dijkstra, 2010; Elena et al., 2017; Sein et al., 2019). For soybean oil, microbial PLCs with dual specificity for PC and PE are the most effective enzymes, since together these PLs usually account for more than 70% of the total PLs present in soybean oil. However, the use of PLC-based technologies is far from widely adopted (Demarco et al., 2020; Sharma et al., 2019). Traditional water degumming is performed rapidly at 75-80 °C, but when enzymes are employed these harsh conditions need to be softened because commercial PLCs (coPLC) work at 55 °C and require 2 hours or more to process the substrates. Thus, the lack of robust coPLCs makes it necessary to introduce modifications in the refining plants. This capital investment comes along with an increase in the operational costs, which together are a major barrier for early adopters.

Developing thermostable and more efficient PLCs, represents therefore the major upgrade needed to facilitate the widespread adoption of enzymatic oil degumming. However, PLCs with improved catalytic efficiency and capable of tolerating temperatures above 75 °C have not been reported so far.

Natural enzymes have not evolved to acquire the properties often required by industrial processes, and therefore searching for natural sequences may not provide the solution. The current knowledge is not sufficient to rationally design unnatural proteins that fit into the industrial constraints (Louis & Abriata, 2021). On the other hand, advanced protein engineering technologies provide libraries with countless variants, but a method for the selection of a few mutants with the expected properties hidden in billions of useless sequences is not always available (Arnold & Moore, 1997).

Consensus-guided mutagenesis (CGM) is a semi-rational approach, where amino acid sequences are inferred from the alignment of a group of homologous enzymes. Often, the recreated sequence shows increased thermal stability (Spence et al., 2021; Thornton, 2004), a property required by the ideal catalysts to be used in oil degumming. However obtaining thermostable enzymes that retain at the same time the catalytic efficiency of their mesophilic counterparts represents an additional challenge (Furukawa et al., 2020).

In this work, we propose a new oil degumming process using a synthetic PLC designed for high thermostability. Mimicking at lab scale the harsh conditions of industrial plants, low doses of chPLC are shown to rapidly complete the degumming reaction at 80 °C. The obtained results indicate that a chPLC-based process is suitable for its easy implementation in water degumming plants, avoiding the need for capital investments while reducing operational costs. We provide an assessment of the expected impact on the oil industry and the environment based on a brief techno-economic analysis.

Materials And Methods

Cloning

The chPLC ORF was codon-optimized and synthesized as described previously (Menzella, 2011). Then, the ORF was digested with *NdeI-EcoRI* and inserted into identical sites of the pTGR100 (Ravasi et al., 2012). *E. coli* XL1Blue was used to propagate plasmids.

Production of chPLC in *C. glutamicum* ATCC13869 strain

Seed cultures of *C. glutamicum* ATCC13869 harboring pTGR100-chPLC were prepared in 1-L Erlenmeyer flasks with 0.1 L of semi-defined medium and grown at 30 °C in a shaking incubator. The medium contains 10 g of glucose, 9 g of K₂HPO₄, 4 g of KH₂PO₄, 10 g of (NH₄)₂SO₄, 2 g of yeast extract, 2 g of MgSO₄, 200 µg of biotin, 5 mg of thiamine, 10 mg of FeSO₄, 1 mg of MnSO₄, 1 mg of ZnSO₄, 200 µg of CuSO₄, and 10 mg of CaCl₂ per liter, supplemented with 25 mg/L of kanamycin.

Fed-batch fermentations were carried out in a 2-L bioreactor (New Brunswick BioFlo 115, USA) containing 1 L of the same medium. Temperature and agitation were maintained at 30 °C and 1200 rpm, respectively. The pH was maintained at 7.0 by adding 25% NH₄OH, and the dissolved oxygen level was controlled at 30 % of air saturation by adding pure oxygen when necessary. The feeding process was initiated when the glucose initially present in the medium was exhausted with a solution containing 600 g/L glucose, 20 g/L MgSO₄·7H₂O at the rate (mL/h) was determined by a balance mass equation (Menzella et al., 2003, 2005) to maintain the specific growth rate at 0.15 h⁻¹.

Enzymatic oil degumming

Oil degumming experiments were performed as previously described (Elena et al., 2017) using 3 mg/Kg of crude soybean oil with 1200 ppm of P. Briefly, crude oil was preincubated at the given temperature in a VP710 stirrer (V&P Scientific Inc.) and the enzyme added in 3 % of water and the mixture incubated with continuous stirring.

NMR analysis of crude and PC-PLC-treated oil

NMR analysis was performed essentially as described by Ravasi et al (Ravasi et al., 2015). Briefly, 300 mg of control or enzyme-treated oil samples were extracted with 900 µL of NMR solution (100 mM Tris-HCl pH 10.5, 50 mM EDTA, 2.5 % sodium deoxycholate), incubated for 1 h at 37 °C, and the heavy phase was extracted with 600 µL hexane. Finally, 50 µL of D₂O were added, and the ³¹P NMR spectrum was acquired using Bruker 300 Ultrashield equipment.

Inorganic phosphate release from PLC-treated oil

For phosphate determination, 100 mg of the homogenized oil was mixed with 0.5 mL of 100 mM Tris-HCl pH 8.0, incubated for 1 h at 37 °C, and centrifuged for 5 min at 14000 *g*. 50 µL of the aqueous phase were recovered and treated with 0.3 U of calf intestinal phosphatase (Promega, WI, USA) for 1 h at 37 °C. 50 µL of the obtained mixture were mixed with 450 µL of 5 % TCA and with 500 µL of color reagent (4 % FeSO₄, 1 % (NH₄)₆MoO₂₄·H₂O, 3.2 % H₂SO₄), and absorbance recorded at 700 nm in a Novaspect III (Amersham Bioscience). The concentration of inorganic phosphate in the samples was calculated using a standard curve containing 2.5 to 25 mM of inorganic phosphate.

Techno-economic analysis

Direct fixed capital cost considers: (i) equipment purchasing cost, (ii) other direct costs including piping, installation, insulation, and electrical facility, and (iii) indirect costs: design and construction, and was calculated using a Lang factor of 3.0, the standard value for refineries techno-economic analysis multiplied by the equipment purchasing cost (Kuschnig et al., 2021). The total investment was calculated by adding the startup cost and the working capital to the direct fixed capital cost. The equipment purchasing cost considered is \$ 380,000 for the agitated tank and \$ 60,000 for two heat exchangers, cost of labor \$ 30/h for a total of 2800 h-year, steam (\$ 23/ton), electricity (\$ 0.17/Kwatt), and process cooling water (0.04/m³) were obtained from default values of SuperPro Designer and formation provided by oil producers. The amortization period considered is 10 years, and the annual interest rate considered is 6 %.

The same procedure was used to calculate the total investment for other size refining plants. For these cases, the equipment purchasing cost was estimated using the costs of the equipment for the 100 thousand MT /year plant as an input for the formula $\text{Cost1/Cost2} = (\text{Price 1/Price 2})^{0.6}$.

NPV and IRR were calculated by the SuperPro Designer software (Canizales et al., 2020).

Results And Discussion

1- Cost analysis of enzymatic oil degumming

Figure 1A shows a layout of the operations used today to remove the PLs in most of the soybean oil refining plants. In the refining line, the oil from flaked soybeans is extracted with hexane, which is then distilled to give the crude oil with up to 1200 ppm of P. Next, the oil stream at 80 °C is mixed with 3 % of water in a stirred tank with a residence time of 30 minutes, sufficient to form the gums. The light phase composed of degummed oil, with less than 200 ppm of P, is separated by centrifugation at temperatures above 72 °C, required to avoid the formation of water/oil emulsion.

The coPLCs in the market work at 55 °C and require 2 h to digest the target PLs, making necessary the insertion of a first heat exchanger reduce the temperature from 80 to 55 °C, a high shear mixer, a stirred tank at least four times larger to provide the residence time to complete the hydrolysis, and a second heat exchanger to raise the temperature back to above 72 °C (Figure 1 B).

The engineering work to modify the refining line and install the equipment required for enzymatic degumming is expensive and challenging since room for a larger tank is not always available. A total investment of 1.32 million dollars was estimated, as described in the Methods section, to adapt a plant with a production capacity of 100 thousand MT of soybean oil per year for enzymatic degumming. Working with crude oil at 55 °C has also an impact on the operational costs, which include energy, and maintenance of the additional equipment. Another costly problem stems from the delays provoked by the frequent fouling of the first heat exchanger, caused by the

PLs and sediments are present in the crude oil, which is labor-intensive and eventually impacts plant productivity.

To get insights into the obstacles that discourage the adoption of the technology, the costs associated to implement and maintain a PLC-based enzymatic oil degumming process for a plant that processes 100 thousand MT of crude oil per year were calculated as described in the Methods section (Table 1) and cross-checked with the information provided by an oil manufacturer that recently implemented the enzymatic degumming technology and others using the traditional method.

To be used in plants with standard water degumming installations the enzyme should hydrolyze the target PLs present in soybean oil for 30 minutes at 80 °C. An enzyme fulfilling these constraints would avoid capital investments and reduce the operational cost of enzymatic degumming, making therefore a significant contribution to achieving widespread implementation of this green technology.

2- Design and testing of a synthetic PLC for oil degumming experiments

Searching for solutions to foster the adoption of enzymatic oil degumming, a synthetic PLC enzyme named chPLC, was custom-designed by a consensus-guided protein engineering method. The library of sequences used to create the synthetic enzyme with higher thermal stability was narrowed by applying specific constraints; to preserve near-native catalytic properties. The designed catalyst differs in 16 % of its amino acid residues as compared to the closest natural enzyme.

As described in an accompanying paper, this enzyme shows a much higher melting temperature and superior catalytic properties than all the natural and engineered PLCs described so far.

A synthetic codon-optimized DNA sequence encoding for chPLC was expressed in fed-batch cultures of the *C. glutamicum* 13869 strain, and gram quantities of the enzyme were purified by ultra/diafiltration and used for oil degumming experiments.

To test the performance of chPLC, we used a previously validated method (Cerminati et al., 2017; Elena et al., 2017), where industrial reaction conditions are reproduced at lab scale, and an assay to measure the inorganic P released that highly correlates with the extra yield of oil obtained.

First, 3 % of a 0.1 g/L solution of chPLC in water was added to pre-heated crude soybean oil with 1200 ppm of P (3 mg/Kg final) and incubated with stirring at temperatures ranging from 50-90 °C. In parallel, BcPLC (5 mg/Kg), the best-characterized enzyme of this class, and coPLC, at the dose recommended by the manufacturer (200 ppm of formulation, equivalent to 5 mg/Kg), were tested under identical conditions.

Figure 2A shows that after 2 h chPLC completely hydrolyze the PC and PE present in the oil at temperatures between 55 and 80 °C, as measured by the amount of P released. As expected, the BcPLC and the coPLCs capacity to remove P decayed at incubation temperatures above 60 °C. Remarkably, at 80 °C chPLC removes 100 % of PC and PE with a dose 40 % lower than that used for coPLC (3 vs 5 mg/kg), as indicated by the inorganic phosphate contained in the excised PLs polar heads.

³¹P NMR quantitative analysis shows that at 80 °C the coPLC preparation could process only one-third of the amount of PLs hydrolyzed by chPLC. The decline of the signals corresponding to PC and PE, with the concomitant appearance of peaks corresponding to phosphocholine and phosphoethanolamine, can be observed in the spectra shown in Figure 2B.

To determine the minimum amount of enzyme required to degum crude oil in 30 minutes, which corresponds to the residence time of crude oil in water-degumming plants, a time-course analysis of PLs hydrolysis at 80 °C treated with variable quantities of chPLC was carried out. Figures 3 A and B show that 3 mg/(kg crude oil) of

chPLC completely hydrolyzed the PC and ~ 93% of the PE present in the oil in 30 minutes, as determined by ^{31}P NMR analysis.

In some formulations, coPLCs are offered in a cocktail with a second PLC specific for PI, which represents 13-17 % of the PLs found in soybean oil. Intriguingly, we found that even when the hydrolysis of PI can be verified by NMR, the contribution to the extra yield (~0.15%) is below the predictions, based on the fraction of PI found in the crude oil total PLs. A likely explanation is that hydrolysis of PC and PE by the first PLC is sufficient to release the TAGs trapped in the gums, reducing in this way the contribution of the PI-PLC to the overall extra yield. This means that if the cost of the PI-specific additional PLC is similar to that of the coPLC, the former enzyme would be paid back by the value of such a small increment in the extra yield of oil that generates, only at oil prices above ~\$3300/MT.

A second unexpected finding is that, at the optimal temperature for each enzyme, the P released by chPLC was consistently higher than that obtained with coPLC (Figure 2A). ^{31}P NMR spectra reveal that the latter enzyme does not completely hydrolyze PE, as can be observed by comparing the peaks of PE and phosphoethanolamine generated by each enzyme (Supplementary Figure 1). This higher efficiency to hydrolyze PE is also evidenced by the amount of P released (Figure 2A), which corresponds to an amount of extra oil of near 10% of that provided by coPLC. Since this amount is similar to or higher than the increment provided by the addition of a PI-specific enzyme, a 2% extra yield was assumed for all the enzymes in the calculations presented in the next section.

In summary, our experiments indicate that adding chPLC to a concentration of 0.1 g/L to the water used for degumming in traditional oil refining plants, where 3 % of water is added to degum the oil, should provide at least the same extra yield given by the current coPLC enzymes, avoiding the capital expenditures and the associated extra operational costs.

3- Techno-economic analysis of chPLC-based industrial oil degumming

With the data obtained in the above-described experiments, the impact of the chPLC-based degumming process in different scenarios was determined based on a techno-economic analysis. In the base scenario, the international five-year average oil price of \$ 822/MT is considered (January 2017-2022, The World Bank). We assume that the chPLC is offered in the market with an identical formulation and at the same selling price as coPLCs. The dose of coPLC enzyme per MT is 5 g and its cost is \$ 5. We assume the same cost (1\$/g) for chPLC, and since 3 g is sufficient to eliminate the PLs in 30 minutes, we assign a cost of \$3 to the dose needed to treat one MT of crude oil.

Aside from the cost of the enzyme, the capital amortization and extra operational costs shown in Table 1 indicate that for a 100 thousand MT per year plant, the total cost associated with the additional installation needed to treat the oil with coPLC for 2 h at 55 °C is \$ 2.95 per MT of crude oil. Since the additional installation is not required for the chPLC-based process at 80 °C, this cost is not considered in the corresponding analysis.

For this case scenario, the data obtained in this work indicate that chPLC based processes will reduce the cost of enzymatic degumming by 62 %, from \$ 7.95 to \$ 3 per MT, increasing in this way the expected net benefit by 58 % (\$ 8.49 to \$ 13.44) (Table 2).

Moreover, these estimates do not consider critical factors that could multiply the incremental benefit of the chPLC process in many cases. For example, Argentina is the major soybean oil exporter in the world, but less than 10 % of the oil is enzymatically degummed. The actual price paid to the oil producers, the FAS price, is 32 % lower due to internal taxes applied to the gross value. In this scenario, the net benefit of using chPLC is expected to be 153 % higher (Table 2).

In the base scenario, and assuming a 20 % discount rate, the total investment required to use coPLC will be recovered after 3 years, the NPV of the project is 1.4 million dollars and the IRR is nearly 40 %. However, at oil prices below \$ 671, the NPV of the project is negative, indicating that the investment is not recommended. On the other hand, oil prices above \$ 150 justify the use of chPLC, and if it falls below that value, the enzymatic treatment can be eventually suspended until oil prices return to above that break-even value (Supplementary Table 3).

In an industrial sector reluctant to the adoption of new technologies, the impact of an upfront investment for the insertion of the additional installations and services required to implement an unknown technology is accompanied by other risks, including oil price volatility and changes in enzyme prices. For example, there are only two PLC suppliers in the world. Hence, after making the capital investment there is a risk to become captive clients with limited room to negotiate the enzyme contracts, or (especially for small producers) that the enzyme product could be eventually discontinued by the manufacturers. On the other hand, signing long-term contracts may expose the producers to scenarios where the volatility of the oil prices brings the benefit near or below the operational costs.

To determine the weight of these two factors, simple sensitivity models of the exposure of the net benefit of enzymatic oil degumming to oil prices and enzyme costs were prepared. For oil, the range of FOB prices recorded in the period 2017-2022 was considered. Figure 4 A and Supplementary Table 1, show that the incremental benefit of chPLC over the current coPLC span from 22 % to 240 %, at the highest and lowest oil prices recorded respectively.

For enzyme costs, in the range from +/- 60 % of the current selling price, the incremental benefit of the technology proposed here spans from 33 to 112 %. (Figure 4 B and Supplementary Table 2).

The sensitivity analysis results show that, for unexpected variations in oil prices and enzyme costs, the incremental benefit of the chPLC over the coPLC enzymatic degumming process offered today will grow under conditions unfavorable for oil producers. Thus, the adoption of chPLC is expected to shield the oil producers from the two main risks described above, contributing in this way to favor the adoption of enzymatic degumming.

As expected in any scale economy, the incremental benefit offered by chPLC over coPLC inversely correlates with the size of the plants and is, therefore, higher for small producers. This is crucial in developing countries where small producers are less controlled by environment protection agencies.

Offering increments in the business margin at no upfront expenses to small and medium-size plants is undoubtedly the preferred avenue to expedite the adoption of environmentally friendly technologies and one of the drivers for undertaking the work presented here. Giving oil producers the possibility to explore and validate the new technology, and the freedom to suspend its use in low-profit scenarios, or to devote the gums to other uses that could be circumstantially more convenient for the business, like producing PL-based lecithin, is another key advantage of the chPLC method.

The forecast for soybean oil production in 2025 is 65 million MT. Thus, even when accounting for soybean oil only, replacing the current water degumming methods with PLC-based degumming with an average extra yield of 2 %, would provide 1.3 million MT of extra oil per year, with a value well above one billion dollars. Alternatively, the 3.3 million ha of land required to produce such an amount of oil might be saved from deforestation or used to produce enough food for up to 28 million people (Cassidy et al., 2013; Castanheira & Freire, 2013; Flachsbarth et al., 2015; Rulli et al., 2013).

Life Cycle Assessment (LCA) shows that enzymatic degumming of one MT of soybean oil reduces the emission of CO₂ by 45 kg compared to chemical refining (Jegannathan & Nielsen, 2013). The social cost of CO₂ emission was estimated in the range of \$40- \$417 per MT of CO₂ (Diaz & Moore, 2017; Ricke et al., 2018). Under these estimates, enzymatic degumming would reduce the social impact of emissions by up to one extra billion dollars per year.

The vegetable oil industry is a major player in the world economy because of its impact on food and fuel prices. To the best of our knowledge, this is the first report showing the direct application of a synthetic enzyme specifically designed to address an industrial need with an impact on both, the global economy and the environment, in the billion-dollar range.

Conclusion

Replacing inorganic catalysts with enzymes is one of the major tools available to establish green industrial processes. However, it is not always possible to find natural enzymes with the catalytic efficiency needed to achieve a cost-effective process and at the same time capable of resisting extreme conditions. The new oil degumming method shown here proves that the synthetic chPLC enzyme largely outperforms the coPLC enzyme currently used for oil degumming at 55 °C with only 60 % of the dose used in the current industrial process. This experimental assessment establishes the basis for promoting to industrial-scale a novel enzymatic degumming process, that we anticipate will provide an environmentally friendly alternative and a valuable economic contribution to manufacturers of edible oil and sustainable fuels.

Declarations

Author contributions

DV, FM, and LD conducted degumming experiments, LP calculated industrial costs, and AA and SP designed methods for gene expression in *C. glutamicum*, RMR, LAA, and MEC designed the synthetic enzyme. HGM elaborated the techno-economic analysis and wrote the manuscript with contributions from all authors. All authors participated in the scientific discussions and approved the final manuscript.

Conflict of interest

LAA, MEC, SP, and HGM are co-inventors of a patent that includes the chPLC enzyme sequence.

Ethical statement

This article does not contain any studies on humans or animals.

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Tables

Enzymatic degumming costs per MT	coPLC 55C
Investment amortization	\$ 1,68
Maintenance (2%)	\$ 0,03
Energy	\$ 0,35
Labor	\$ 0,86
other operational costs	\$ 0,03
Total installation dependent cost	\$ 2,95
Enzymes	\$ 5,00
Total enzymatic degumming cost per MT	\$ 7,95

Table 1. The current cost of soybean oil enzymatic degumming for a plant with a capacity of 100 thousand MT per year.

	coPLC 55C 1 million MT	coPLC 55C 0.1 million MT	chPLC 80C
Total installation dependent cost	\$ 1,51	\$ 2,95	Saved
Enzymes	\$ 5,00	\$ 5,00	\$ 3,00
Total enzymatic degumming cost per MT	\$ 6,51	\$ 7,95	\$ 3,00
Oil price	\$ 822	\$ 822	\$ 822
Extra yield	2%	2%	2%
Gross benefit per MT	\$ 16,44	\$ 16,44	\$ 16,44
Net benefit per MT	\$ 9,93	\$ 8,49	\$ 13,44
FAS oil price	\$ 559	\$ 559	\$ 559
Gross benefit per MT	\$ 11,18	\$ 11,18	\$ 11,18
Net benefit per MT	\$ 4,67	\$ 3,23	\$ 8,18

Table 2. Costs and benefits of enzymatic degumming with coPLC and chPLC. Values are calculated for plants with capacities of 1 and 0.1 million MT per year at the oil five-year average price.

Figures

Figure 1. Scheme of degumming units of soybean oil production plants. A- Water degumming in with a tank with 30 minutes' residence time. **B-** The by-pass inserted for enzymatic degumming, containing heat exchangers and a 4X larger tank to treat oil for 2 h.

Figure 1

See figure for legend.

A

B

Figure 2. Soybean oil degumming with PLC enzymes. A- mMoles of P release per kg of crude oil with 1200 ppm treated with 3 mg/kg of BcPLC, blue bars; 5 mg/Kg of coPLC; orange bars and 3 mg/Kg of chPLC, purple bars at the indicated temperatures for 2 h. Mean values for three experiments are shown, in all the cases SD was below 10%. B- ^{31}P NMR analysis of the phospholipids and polar heads present in oils treated at 80 C.

Figure 2

See figure for legend.

A

B

Figure 3. Time course of soybean oil degumming at 80 C. A- P release from crude oil (mMoles per kg of oil) containing 1200 ppm of P treated with 1.5, 3 and 4.5 mg/Kg of chPLC. Mean values for three experiments are shown, error bars correspond to the SD 10%. B- ^{31}P NMR analysis of the phospholipids and polar heads present in the treated oils.

Figure 3

See figure for legend.

A

B

Figure 4. Incremental benefit of the 80 C oil degumming process with chPLC compared to coPLC at 55 C. A- Sensitivity to oil price. Blue line, FOB price, orange line, FAS price. B- Sensitivity to PLC enzyme cost.

Figure 4

See figure for legend.

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