Sustainable Chemistry

Exploitation of Soybean Oil Acid Degumming Waste: Biocatalytic Synthesis of High Value Phospholipids

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The acid degumming waste of the seeds oil refining industry is currently disposed of, but, instead, it could be exploited as an important source for the preparation of many products. In this work, the waste coming from the soybean oil refining step was first recovered and treated, allowing the isolation of a fraction enriched of phospholipids (PLs). Then, the latter was transformed, *via* an enzymatic reaction catalysed by phospholipase D (from *Streptomyces netropsis*), into more valuable products: polar head modified PLs-enriched mixtures containing phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidylhydroxybutyrate (PB). In the following we show how biocatalysis can be exploited for the production of value-added PLs, to be used as functional food and nutraceutical ingredients, from a renewable feedstock. This alternative to the industrial usual disposal procedures should impart to the whole process a greater value in terms of carbon recycling, in agreement with the paradigms of bioeconomy for a wiser reuse of renewable resources in a circular economy perspective.

Introduction

In the last years, the issues related to the environmental deterioration and the pauperization associated with the increasing world population have pushed the researchers to investigate on how to recover and to reuse the wastes available from renewable biological resources, in alternative to their direct disposal. In this mainframe, from an industrial point of view, the demand for new approaches with reduced environmental impact has become a high priority. Therefore, the possibility to transform industrial wastes, such as biomass, into high value products follows the principle guidelines of the 4R plan (Recover, Reduce, Reuse and Recycle) in a circular economy context.

The edible vegetable oils industry produces, during the different refining steps, high quantities of wastes. The global production of vegetable oils has largely increased in the last twenty years: from 92 million tons in 2000 to 191 million tons in 2017. The main products are composed of soybean, palm, canola and sunflower oils. Soybean especially constitutes the leading crop of oilseeds with a worldwide production of 54 million tons in 2017.[1]

Keeping this in mind we focused our attention on the exploitation of a carbon-rich stream coming from the edible industrial soybean oil refining in the context of a circular economy perspective. In industrial practice, crude seed oils undergo a refining cycle generally composed of six steps: degumming, neutralization, winterization, decolourization, deodorization and clarification.[2] The main goal of these processes is the removal of undesirable minor components, which could damage the quality of the final consumer product. The first refining step is constituted by two consecutive processes: water degumming and acid degumming, both have an important role in the removal of the phospholipids (PLs) from crude oils, thus improving their physical stability and facilitating further processing.[3] PLs are composed of a glycerol backbone, which is esterified with two fatty acid chains at *sn*-1 and *sn*-2 positions, and at *sn*-3 with a phosphate diester bearing the so-called polar head. The composition of acyl chains and polar heads depends on the source of PLs and is fundamental for their physical and biological properties.[4] PLs, which are the main components of natural membranes, play a pivotal role, since they are involved in many cellular processes such as differentiation, regeneration and molecule transportation

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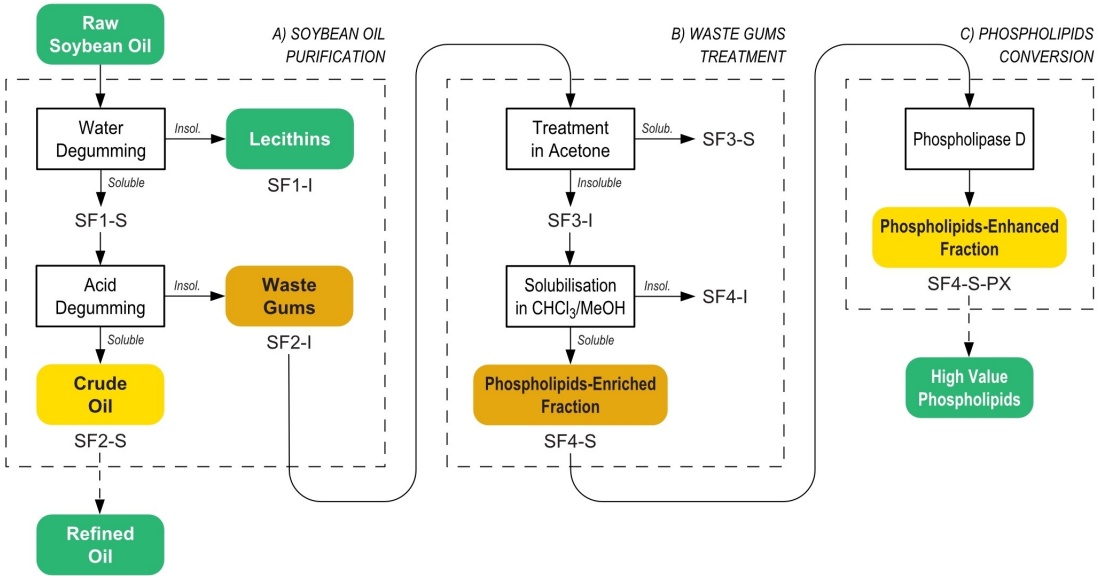


Figure 1. Schematic representation of raw soybean oil purification (Panel A), treatment of the waste coming from the acid degumming step (Panel B), and phospholipids conversion (Panel C).

through the membranes and are promoters of the biological activities of various membrane-linked proteins and receptors.[5] PLs are also studied as diagnostic markers for several diseases and as components of many nutraceutical preparations for neurological and cholesterol-linked diseases.[6] Dietary PLs have been demonstrated to be essential in the prevention of a large number of human diseases such as cancer, coronary heart issues, cholesterol metabolism and inflammations.[7] In addition, due to their peculiar structure, PLs are amphiphilic molecules leading to their spontaneous aggregation in aqueous environments, allowing the formation of micelles, bilayers and liposomes. Such supramolecular assemblies are very appealing for the cosmetic sector and for drug delivery applications.[8] The investigation of new methods of preparation of a large variety of PLs with different chemical structures and composition is mandatory for all studies on their biological activity.[9] Natural PLs could be obtained from animal sources such as egg yolk and milk, but vegetable sources are largely preferred because they avoid the potential risks of transmissible diseases.[10]

In this mainframe, natural PLs, coming from agricultural wastes, could be a source of phosphatidylcholine (PC), which can be further transformed into more valuable natural products such as phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) or even in non-natural PLs for new biomedical applications.[11] For this purpose, the authors have recently exploited an analogue residue coming from corn oil processing.[12] However, in this work, the attention is focused on the recovery and the enhancement of the water degumming waste coming from soybean oil refining plants for high value PLs production. The final aim is to establish a simple procedure as a valid alternative to the currently employed expensive and wasteful disposals.

Results and Discussion

The first step of the refining cycle of crude edible seed oils and, especially that of soybean oil, is constituted by water degumming (Figure 1, Panel A), which allows the obtainment of an insoluble fraction SF1-I. The latter is an abundant mixture of PLs rich in phosphatidylcholine (PC) and phosphatidylinositol (PI), usually directly commercialized as "lecithins" without further treatments (see Figure 2, Panel A, for physical appearance). This procedure implies the addition of water (1–5 %) to the oil at 60–75 °C, leading to the gentle hydration of some PLs components forming an emulsion, which is then separated by centrifugation affording a viscous liquid with a colour ranging from yellow to brown. The recovered solid product SF1-I (usually reported as "gums") is then dried to obtain lecithins for food and industrial purposes.[14] However, the liquid oily phase SF1-S still contains a small fraction of some PLs, and therefore it has to be submitted to further purification process. The second step is the acid degumming procedure: addition of phosphoric or citric acid to the crude oil (60–90 °C). This step

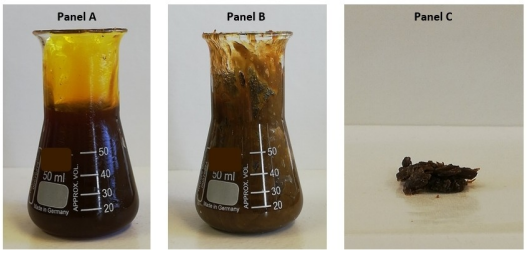


Figure 2. Picture of the solid fractions recovered from water degumming and acid degumming in soybean refining plant (SF1-I (Panel A), SF2-I (Panel B), and SF4-S (Panel C)).

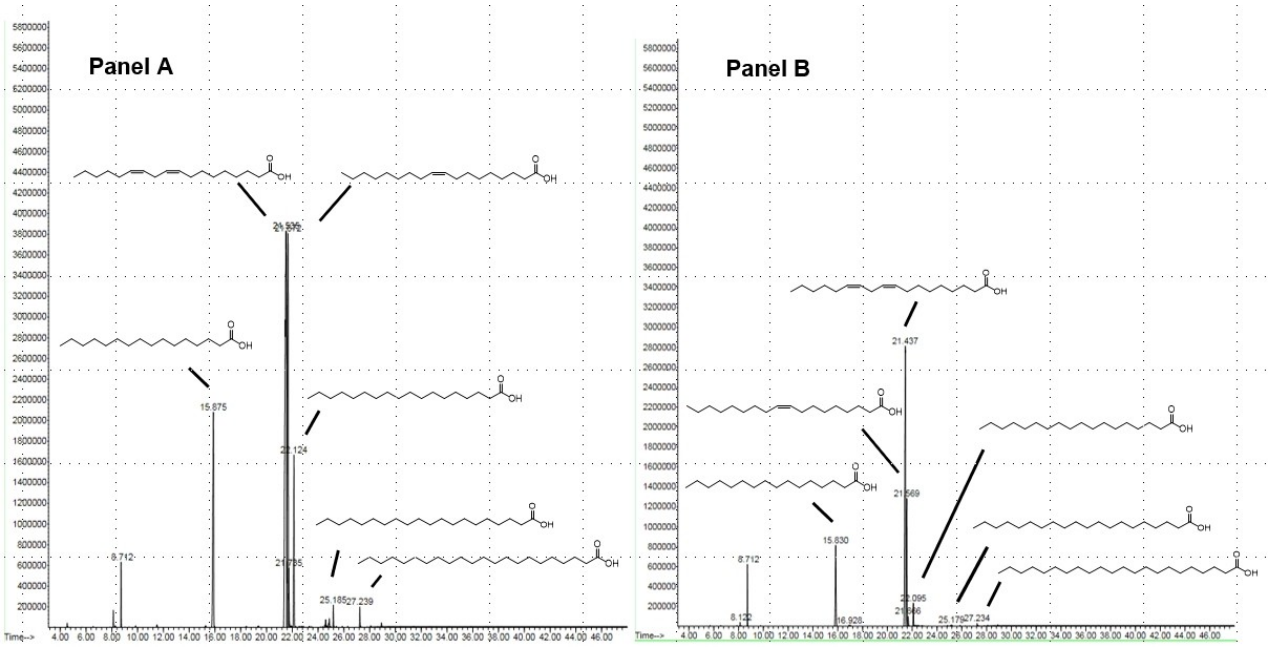


Figure 3. GC/MS analysis chromatogram of hydrolysed crude soybean oil SF2-S (Panel A) and SF4-S (Panel B).

yields the SF2-S fraction (crude oil), which is usually further refined for the food market, and a solid fraction (SF2-I) constituting the so-called "waste gums". The latter is a semi-solid residue that requires expensive disposal (see Figure 2 Panel B for the appearance of SF2-I). Indeed, the solid residue SF2-I can no longer be used directly as lecithin for food applications, because this material is usually considered too difficult to be treated for further exploitations. Its high-water content leads to an easy degradability that makes it unsuitable for long-term storage with the necessity, from an industrial point of view, to be disposed of as special waste. These gums, coming from the degumming steps, are mainly composed of PC, PI and PE, combined with various amounts of other substances, such as triglycerides, fatty acids, and carbohydrates. The PLs composition and fatty acid profiles are highly dependent on the raw material sources from which they are obtained and will be here analysed.

Biomass Treatment and Analysis

The set-up of the processing of the waste gums SF2-I reported in Panel B of Figure 1 has been performed. The extraction of PLs from the acid degumming was obtained by performing precipitation followed by a solubilisation process using a mixture of solvents. In detail, SF2-I was precipitated in cold acetone as SF3-I, exploiting the fact that PLs possess a very low solubility in this solvent, whereas the other components of the waste (SF3-S) are very soluble therein (see Experimental Section in Supplementary Information for details).

The insoluble fraction SF3-I was then dissolved in a mixture of $CHCl_3/CH_3OH$ (3/1) affording the fraction SF4-S, which is rich of PLs. This fraction, as well as the crude soybean oil SF2-S, have been analyzed in terms of acyl chains after basic hydrolysis (see Experimental section for details). The results of the determination of acyl chains (reported in Figure 3 and Table 1) indicate that palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) are the predominant fatty acids in SF4-S with the percentages of ~15%, 18% and 57% respectively. Similar percentages are present in the crude oil with a slight difference for the linoleic and the oleic acids contents. These data are in accordance with those reported in the literature.[15]

PLs composition of SF4-S has been determined by ESI/MS analysis. This analysis has been performed also on SF1-I in order to compare the composition of the crude lecithins with that of waste gums. The ESI/MS spectra are shown in Figure 4, and the results are reported in Table 2. These data indicated that the main PLs present in the two samples were PC, PI, phosphatidylethanolamine (PE) and phosphatidic acid (PA). In SF4-S, the presence of phosphatidylglycerol (PG) has been also detected. For the same polar head, ESI/MS analysis showed the presence of the following major acyl chains combinations (reported in Figure 5): palmitoyl/linoleoyl (C16:0/C18:2), mole-

Table 1. Fatty acid composition of SF2-S (crude soybean oil) and SF4-S fractions (results obtained by GC/MS analysis).

Fatty acid	Symbol	% in SF2-S	% in SF4-S
Palmitic	C16:0	14.5	14.9
Stearic acid	C18:0	7.1	2.9
Oleic	C18:1	27.8	17.9
Linoleic	C18:2	46.1	56.8
Arachidic	C20:0	0.6	0.2
Behenic	C22:0	0.5	0.2

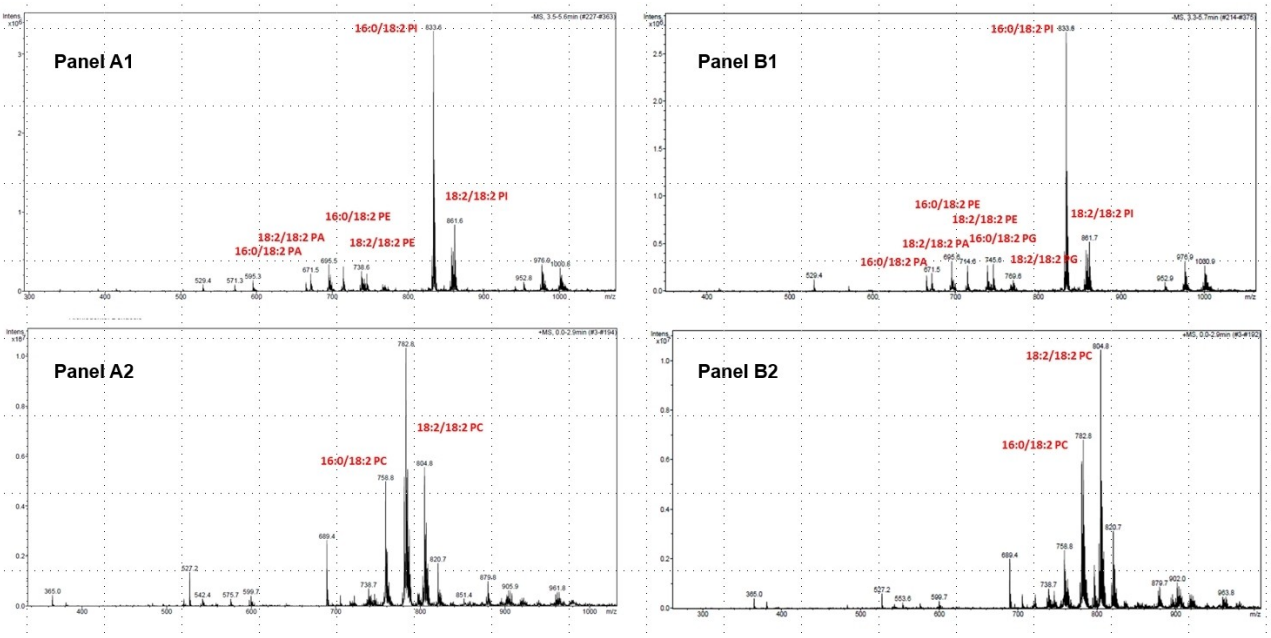


Figure 4. ESI/MS spectra of crude soybean oil SF2-S (Panel A1 and A2) and SF4-S (panel B1 and B2).

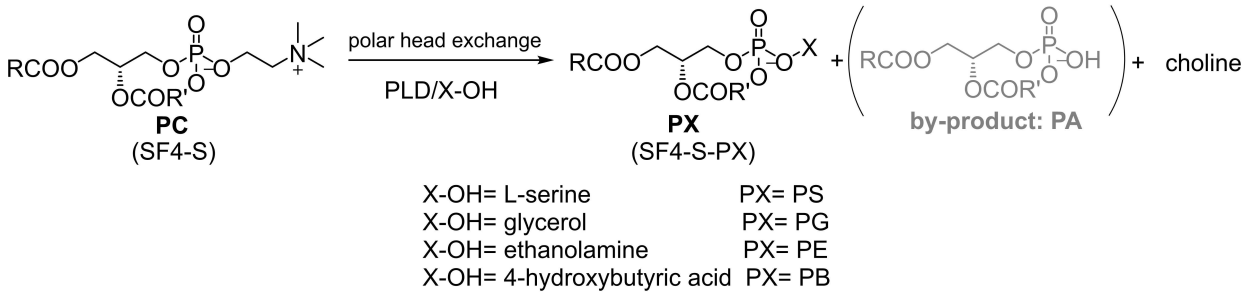
Table 2. Identification of the main PLs in SF4-S by ESI/MS analysis.

Identified phospholipids	Chains *sn*-1/*sn*2	Positive m/z	Negative m/z
PC-A	16:0/18:2	$[757.8+23]^+$	–
PC-B	18:2/18:2	$[781.8+23]^+$	–
PG-A	16:0/18:2	–	$[745.6]^-$
PG-B	18:2/18:2	–	$[769.6]^-$
PA-A	16:0/18:2	–	$[671.5]^-$
PA-B	18:2/18:2	–	$[695.6]^-$
PI-A	16:0/18:2	–	$[833.6]^-$
PI-B	18:2/18:2	–	$[857.6]^-$
PE-A	16:0/18:2	–	$[714.6]^-$
PE-B	18:2/18:2	–	$[738.6]^-$

cules referred to as group A and linoleoyl/linoleoyl (C18:2/C18:2), molecules referred to as group B. This feature can be used as a defined fingerprint of this seed source. HPLC analysis confirmed the identification of the PLs detected by ESI/MS and provided a rough evaluation of the mixture composition: PA 57%, PG 14%, PI 5%, PE 9%, PC 15%.

Conversion of SF4-S in Selected PLs

After recovery and analysis of the fraction SF4-S, a sample was submitted to the enzymatic transphosphatidylation in such a way to transform all the PC present in the mixture, into a final product highly enriched of PL with defined polar head. In fact, the preparation of polar head modified PLs is at the basis of the exploitation and investigation of their several interesting properties. In general, their preparation is highly challenging, because such products are usually obtained by chemical synthesis from appropriate glycerol-based precursors with very complex sequences.[16] For this reason, a selective biocatalytic approach would be highly recommended. The enzymatic transformations were carried out using the phospholipase D (PLD, EC 3.1.4.4) from *Streptomyces netropsis* which is an enzyme able to catalyse, in a biphasic solvent system, the exchange of the polar head of PLs in the presence of an appropriate nucleophile X-OH (see Scheme 1).[12,17] It should be pointed out that this microorganism produces, as reported also



Scheme 1. Phospholipase D-catalyzed transformations of PC to PX.

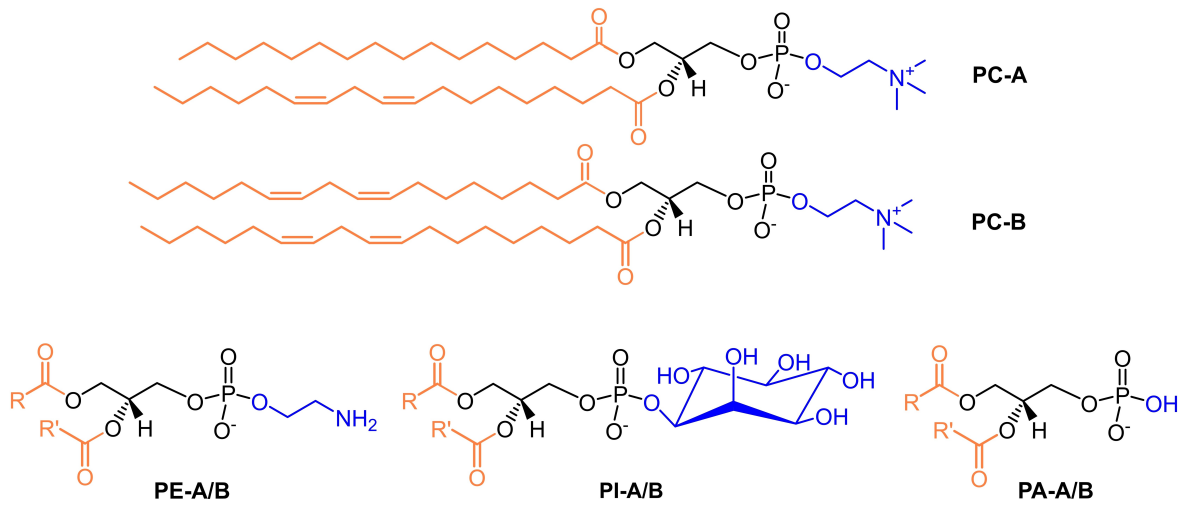


Figure 5. Structures of identified PLs in SF1-I and SF4-S obtained by ESI/MS analysis (molecules labelled A possess C16:0/18:2 chains; molecules labelled B possess C18:2/18:2 chains).

for different members of the *Streptomyces* family, a protein displaying a quite unique property among hydrolytic PLDs:[18] the capacity to selectively catalyse the transesterification PC even in the presence of water, leading to a new PL, indicated as PX in Scheme 1.[17a,19] Indeed, the presence of water should lead to the formation of the undesired phosphatidic acid (PA).

For that purpose, SF4-S was dissolved in toluene and treated with a buffered aqueous solution containing PLD, $CaCl_2$ 0.1 M, and the desired nucleophile X-OH, as reported in Scheme 1. Four reaction protocols were set up in order to obtain 4 different PLs: PS, PG and PE, which represent three of the most important natural PLs with different biological functions, and the non-natural PB, which has been recently prepared by Li *et al.* for applications in the field of anaesthetics and sedatives.[20] The different reactions allowed to obtain four enriched PLs mixtures, namely SF4-S-PS, SF4-S-PG, SF4-S-PE and SF4-S-PB containing PS, PG, PE and PB respectively. These four products were analyzed by ^{31}P NMR, ESI/MS and HPLC (see Figures 6 and 7 and Figures S1 to S4 in the Supporting Information). In all cases, quite all the PC contained in SF4-S was efficiently transformed into the desired PX leading to a final PX-enriched natural product (see Figure 6). These completely biobased final products could be exploited as starting materials for food additives or nutraceuticals preparations.

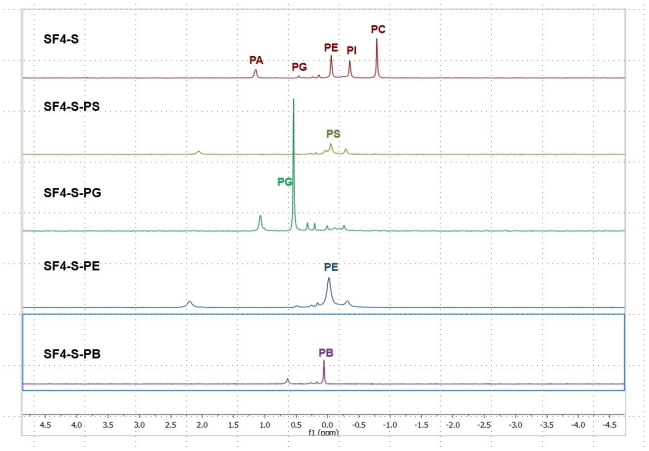


Figure 6. ^{31}P NMR of PLs-enriched mixtures starting from SF4-S.

Conclusion

In this work, the valorisation of a carbon-rich, phospholipids-containing waste coming from the acid degumming step of soybean oil during the industrial refining process has been addressed. In particular, the acid degumming waste was at first enriched in PLs by a double treatment with solvents, and then the resulting fraction was elaborated through a PLD-catalysed biocatalytic conversion aiming to transform the most abundant PL (PC) in high value natural PLs such as PS, PE and PG or in a non-natural compound such as PB. The described multi-step sequence allows the conversion of a hardly disposable industrial waste in high value raw PLs products, with very interesting potential applications in the field of functional foods and dietary supplements for nutraceutical applications. This study, in conclusion, paves the way for the creation of new alternative fates for acid degumming wastes of edible oil refining and it is of great value in terms of carbon recycling, accordingly to the paradigms of bio-economy for the wise use and re-use of renewable biological resources.

Supporting Information Summary

The experimental section, containing the procedures, the analytical details and the characterization of the synthesised products is available in the Supporting Information of this article.

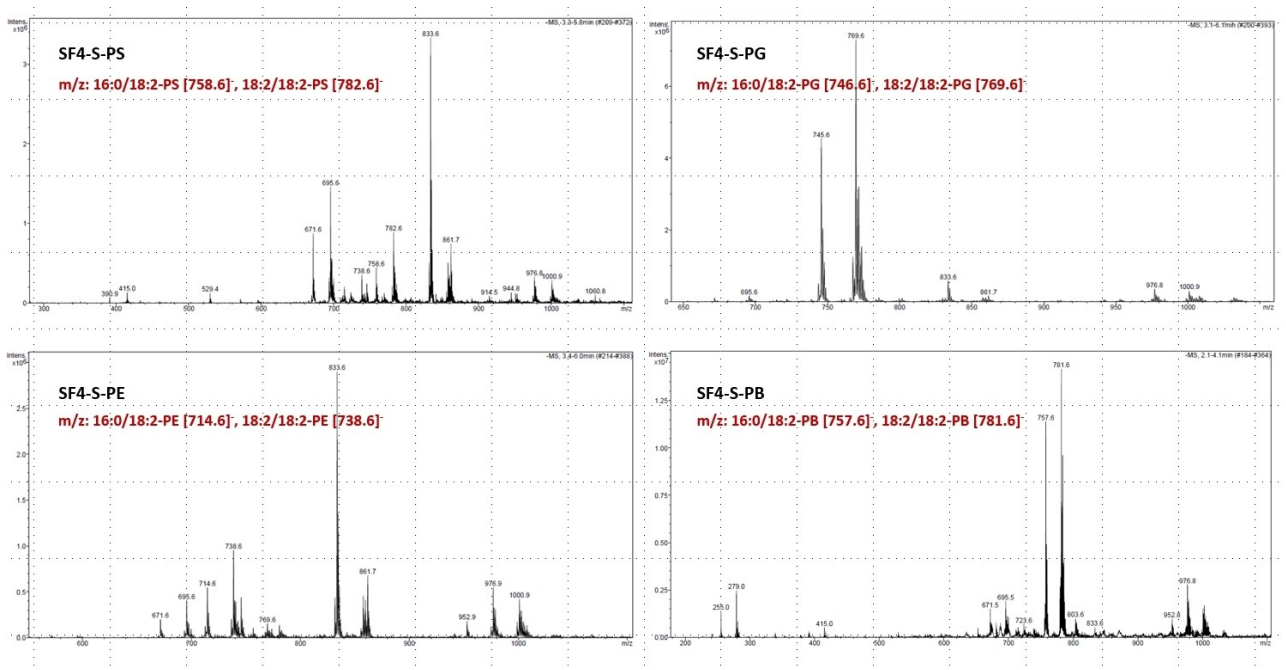


Figure 7. ESI/MS spectra of PLs-enriched mixtures from SF4-S.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: biomass valorization · biocatalysis · phospholipids · recycling · phospholipase D · circular economy

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