

ENZYMATIC DEGUMMING OF SOYBEAN, RAPESEED, AND SUNFLOWER OILS FROM UKRAINIAN CROPS USING PURIFINE ENZYMES

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<https://doi.org/10.23939/chcht19.04.761>

Abstract. This study investigates the efficiency of enzymatic degumming of crude soybean, rapeseed, and sunflower oils using Purifine® phospholipase C (PLC), phospholipase A1 (PLA₁), a lipase-modified component (LM), and the multienzyme complex Purifine® 3G (combining PLC, PLA₂ and PI-specific phospholipase C), as well as their combinations. The primary objective is to achieve low residual phosphorus content (<5 ppm), which is essential for subsequent catalytic hydrotreatment in the production of hydrotreated vegetable oil (HVO) and for improving the efficiency of transesterification in the production of fatty acid methyl and ethyl esters (FAME/FAE). The degumming performance was evaluated based on the levels of phosphorus, sulfur, free fatty acids (FFA), and the dry mass of the gum phase. The highest efficiency was demonstrated by the Purifine® 3G system and the combination of PLC + PLA₁ + LM, which provided deep purification, minimal loss of neutral oil, and stable FFA levels. Importantly, these enzymatic systems not only enabled residual phosphorus levels to be reduced below 5 ppm, but also significantly decreased sulfur content – from initial values of 60–100 ppm to final concentrations of 9–27 ppm, depending on the oil type. This sulfur reduction is particularly important for protecting hydrotreating catalysts and ensuring compliance with fuel standards such as EN 15940 and ASTM D975. The findings support the feasibility of enzymatic systems as a sustainable technology for the pretreatment of feedstocks for both second-generation (HVO) and first-generation (FAME) biofuel production, offering benefits in catalyst life, product purity, and process efficiency.

Keywords: enzymatic degumming, phospholipases, Purifine 3G, hydrotreated vegetable oil, fatty acid methyl and ethyl esters, phospholipids, vegetable oil, phosphorus, biofuel, multienzyme system.

1. Introduction

The purpose of the degumming process for vegetable oils is to remove phospholipids and other polar impurities that negatively affect the quality of the final product by reducing its stability, clarity, shelf life, and technological performance during further processing. Achieving a low phosphorus content – below 5 mg/kg – is especially important, as this is a critical threshold for the successful use of the oil in the production of hydrotreated vegetable oil (HVO).¹ Furthermore, effective degumming significantly reduces costs in subsequent refining stages, lowers emulsion formation, and minimizes oil losses, which is particularly relevant in the production of high-purity food-grade and technical oils.^{2–11}

Traditionally, degumming is carried out using physical (water) or chemical (acid or acid-enzymatic) methods.² Water degumming is effective only for hydrated phospholipids, while acid degumming requires neutralization and results in oil losses and waste formation. As a biotechnological alternative, enzymatic degumming has gained attention in recent decades, offering deeper purification with reduced losses and lower environmental impact.^{3–10}

Various enzymatic degumming processes have been investigated at laboratory and pilot scales.^{4–16} Commercially available oil-processing enzymes differ in their mechanisms of action. Phospholipase A1 (PLA₁)^{8, 11, 13} and A2 (PLA₂)^{11, 12} hydrolyse fatty acids at the sn-1 and sn-2 positions of glycerol, respectively. Phospholipase C (PLC)^{11–13} catalyses the hydrolysis of the phosphate-glycerol bond in phosphatidylcholine (PC) and phosphatidylethanolamine (PE), while lipid acyltransferase (LAT) transfers fatty acid residues. All

these enzymes contribute to reducing oil losses caused by binding to gums, thereby improving yield.^{8–16}

Enzymatic degumming relies on the action of phospholipases – enzymes that hydrolyse ester bonds within phospholipid molecules, generating lysophospholipids and free fatty acids. PLA1 and PLA2 are most frequently used, as they convert both hydrated and non-hydrated phospholipids into more polar forms that can be readily removed by centrifugation.¹¹ In some cases, PLC and Phospholipase D (PLD) are additionally applied, targeting different parts of the phospholipid molecule and enabling deeper purification – particularly for pharmaceutical or cosmetic-grade oils.¹²

Numerous studies have demonstrated the effectiveness of enzymatic degumming for a wide range of vegetable oils, including soybean, rapeseed, sunflower, rice bran, and corn oils. Each oil type has a unique phospholipid profile, necessitating an individual approach to enzyme selection and process parameters.^{13–16} For example, the combined use of PLA1 and LAT is often employed for rapeseed and rice bran oils to enhance the yield and stability.

Optimization of the enzymatic process parameters – temperature, pH, enzyme concentration, buffer-to-substrate ratio, reaction duration, and hydration level – is essential for minimizing phosphorus content and oil loss. A typical laboratory experiment involves the treatment with a buffer solution to adjust pH, controlling reaction temperature, and adding the appropriate enzyme dosage.⁴ Different approaches to experimental design can be used. While the “one-factor-at-a-time” method is simple, it does not account for interactions between variables. In contrast, other designs of experimental approaches allow simultaneous assessment of multiple factors and their interactions.¹⁷ For example, response surface methodology has proven to be a powerful tool for degumming process optimization.^{2,13–14,18–19}

Initial enzymatic degumming studies focused primarily on rapeseed,^{4,21,22} soybean,^{2,11,21,22} and rice bran oils.^{13,20–22} Later work examined the impact of enzymatic degumming on the physicochemical properties of sunflower oil.^{15,16}

A critical success factor in enzymatic degumming is the precise optimization of operating conditions – temperature, pH, enzyme dosage, buffer / substrate ratio, reaction time, and degree of hydration.^{2,7,9,17,19}

Interest has been shown in phospholipase immobilization on solid supports (*e. g.*, chitosan, calcium alginate, polymeric gels), which enables enzyme reuse, improves thermal and pH stability, and simplifies product purification from protein residues.^{23–25} This opens new perspectives for upscaling enzymatic degumming in industrial settings that require high levels of environmental and economic sustainability.

In summary, enzymatic degumming of vegetable oils using various classes of phospholipases is a modern and promising approach for improving the quality and processability of vegetable fats. It allows tailored purification based on the type of raw material and end-product requirements, reduces processing losses, and promotes the implementation of sustainable biotechnology in the food and oil industries.

2. Experimental

2.1. Materials

Soybeans, rapeseed, and sunflowers were cultivated in the Lviv region (Ukraine) in the fields of the “Agrocenter” farming enterprise. The corresponding oils – soybean (SBO), rapeseed (RO), and sunflower (SFO) – were extracted using hexane on the production facilities of LLC “D-MIX” (Zolochiv, Lviv region, Ukraine). After extraction, the oils were stored in sealed polymer containers at 4–6 °C until enzymatic treatment.

The physicochemical properties of the raw oils were determined according to the analytical methods summarized in Table 1.

Four enzymatic preparations from the Purifine® line developed by DSM-Firmenich (DSM Food Specialties, Netherlands) were used in the study. Purifine® PLC is a preparation based on phospholipase C derived from *Pichia pastoris*, which catalyses the hydrolysis of phosphatidylcholine (PC) and phosphatidylinositol (PI), forming diacylglycerols (DAG) and phosphorus-containing residues. Optimal conditions: pH 5.5–6.0, temperature 55–60 °C, reaction time 1–2 hours, dosage 50–100 ppm. Purifine® PLA1 contains phospholipase A1 from *Aspergillus oryzae*, active toward glycerophospholipids at the sn-1 position. It produces lysophospholipids (LPL) and free fatty acids (FFA). Optimal conditions: pH 5.5–6.5, temperature 50–60 °C, reaction time 1–3 hours, dosage 50–150 ppm. Purifine® LM is a thermostable lysophospholipase that catalytically converts residual lysophospholipids into glycerophosphates. It is derived from microorganisms capable of high-temperature enzymatic hydrolysis. Optimal conditions: pH 5.5–6.0, temperature 75–85 °C, reaction time 30–60 minutes, dosage 50–100 ppm. Purifine® 3G is a complex enzymatic cocktail containing three enzymes: phospholipase C (PLC) from *Pichia pastoris*, phospholipase A2 (PLA2) from *Aspergillus niger*, and PI-specific phospholipase C from *Pseudomonas fluorescens*. The formulation targets phospholipids and enables effective formation of DAG, phosphates, FFA, and LPL. Optimal conditions: pH 6.5–7.5, temperature 50–60 °C, reaction time 1–3 hours, dosage 50–200 ppm.

Table 1. Physicochemical properties of the tested oils

Property	Unit	SFO	SBO	RO
Density ²⁷ at 15 °C	kg/m ³	918	922	910
Kinematic viscosity ²⁸ at 40 °C	mm ² /s	32.6	30.7	31.8
Flash point ²⁹	°C	320	345	315
Sulfur content ³⁰	ppm	60	90	100
Phosphorus content ³¹	ppm	7.5	9.0	5.9
Water content ³²	ppm	180	200	160
Copper strip corrosion ³³ (3 h at 50 °C)	–	Class 1	Class 1	Class 1
Acid value ³⁴	mg KOH/g	2.30	0.70	1.90
Iodine value ³⁵	g I ₂ /100 g	125	133	110

2.2. Methods

2.2.1. Enzymatic reaction

Enzymatic degumming of soybean, rapeseed, and sunflower oils was carried out using the developed protocols based on manufacturer recommendations and research data for each of the four enzymes (Purifine® PLC, PLA₁, LM, 3G).^{8,11–16,26} For combined treatment variants, technological schemes and parameters were developed within the framework of this study. All procedures were standardized for 1 kg of oil, ensuring a valid comparison of the efficiency of individual enzymes and their combinations under identical process conditions. Aqueous enzyme solutions were prepared immediately before application by dissolving the enzyme in 20–30 mL of distilled water (2–3 % of the oil mass), to which 20 mM phosphate buffer was added to stabilize pH within the target range (5.5–7.5, depending on the enzyme). Before adding the enzyme solution, the oil was preheated to the required temperature, depending on the enzyme specificity and type of raw material. The reaction mixture was stirred at 500 rpm using a laboratory overhead stirrer. The enzyme dosage in enzymatic degumming is determined by their specificity,

mechanism of action, and the phospholipid content of the feedstock. Phospholipase C (Purifine® PLC)^{12, 13} hydrolyzes phosphatidylcholine (PC) and phosphatidylethanolamine (PE) into diacylglycerols and phosphates, ensuring consistently high efficiency for any type of oil; therefore, it is used at a dosage of 100 ppm. Phospholipase A₁ (Purifine® PLA₁)^{8,11,13} cleaves the bond at the sn 1 position of glycerophospholipids to produce lysophospholipids and free fatty acids; its universal activity allows the application of 125 ppm for all oil types. The lipase-modified enzyme (Purifine® LM) acts as a lysophospholipase, removing residual lysophospholipids, and is applied at 75 ppm regardless of the feedstock. In the multi-enzyme complex Purifine® 3G (PLC, PLA₁, LM),^{11,26} dosages are adjusted according to the type of oil: 100 ppm for soybean oil with a high phospholipid content, 75 ppm for rapeseed oil with a lower phospholipid content and better hydration, and 60 ppm for sunflower oil with minimal phospholipid load. In combined schemes (PLC with LM, PLC with PLA₁ and LM), the dosage of each enzyme is selected based on its function: PLC performs primary phospholipid hydrolysis, PLA₁ cleaves at the sn 1 position, and LM ensures final removal of lysophospholipids, achieving maximum degumming in a single process cycle.

Table 2. Enzymatic process conditions for each enzyme preparation and oil type

Enzyme type	pH	T, °C	Duration, h	Enzyme dosage, ppm
Purifine® PLC	5.5–6.5	60	2.0	100 (all oil types)
Purifine® PLA ₁	5.5–6.5	60	2.0–2.5	125 (all oil types)
Purifine® LM	5.5–6.0	80	2.0	75 (all oil types)
Purifine® 3G	5.5–6.5	60	2.0	100 (soybean), 75 (rapeseed), 60 (sunflower)
Purifine® PLC + LM	5.5–6.5	60/80	3.0	PLC – 100, LM – 75
Purifine® PLC + PLA ₁ + LM	5.5–6.5	60/80	1.0	PLC – 100, PLA ₁ – 125, LM – 75

The treatment duration ranged from 1 to 3 hours, depending on the type of enzyme and the characteristics of the feedstock. Upon completion of the reaction, all samples were subjected to thermal enzyme inactivation by heating to 80 °C and maintaining this temperature for 30 minutes. Afterward, the mixture was transferred to a separatory funnel for phase separation of the gum layer (hydrophilic hydrolysis products) from the purified oil. The upper oil layer was collected for further analytical evaluation.

In the combined enzymatic treatment variants (PLC + LM, PLC + PLA1 + LM), phospholipases (PLC, PLA1) were first added at a temperature of 60 °C, and the reaction was maintained for the appropriate duration (1–2 hours), after which a lysophospholipase treatment stage was conducted at 80 °C for 30–45 minutes. Stirring was continuous throughout the entire process.

Control samples of all three oil types were subjected only to standard thermal treatment (60 °C, 1 hour) without enzyme addition – simulating conventional water degumming.

2.2.2. Physicochemical properties of raw materials and products

The physicochemical properties of the raw materials and processed oils were determined according to the methods described in references 27–35.

3. Results and Discussion

To evaluate the efficiency of enzymatic degumming of vegetable oils for their preparation for the production of hydrotreated vegetable oil (HVO), crude soybean, rapeseed, and sunflower oils were treated using

the enzymatic preparations Purifine® PLC, PLA1, LM, and the multi-enzyme complex Purifine® 3G, as well as their combinations. The efficiency of purification was assessed by determining the residual phosphorus (P) and sulfur (S) contents, which are key criteria for suitability for catalytic hydrotreatment (since phospholipids cause deactivation of hydrogenation catalysts—particularly those based on Ni, Mo, Co, Pt, Pd—and contribute to deposit formation), as well as free fatty acids (FFA) and the mass fraction of gum phase (on a dry basis).

3.1. Features of the enzymatic regime and degumming results for sunflower oil

The initial phosphorus content in crude sunflower oil was 7.5 ppm, and the FFA content was 1.15 %. Water degumming proved ineffective, reducing phosphorus only to 7.0 ppm. The use of Purifine® PLC, PLA1, and LM individually provided only partial degradation of phospholipids (final P: 4.7–5.2 ppm). The most effective purification was achieved with Purifine® 3G (2.6 ppm)—a ready-to-use multi-enzyme cocktail containing phospholipase C, PI-specific PLC, and PLA2—as well as with the combination of PLC + PLA1 + LM (2.5 ppm) (see Table 3).

A reduction in gum mass from 4.7 % to 3.5–4.1 % is the result of a decrease in oil content within the gum phase: the enzymes break down the phospholipid structure, allowing the previously bound oil (which would be lost during water degumming) to return to the neutral fraction as DAG and FFA. This mechanism has been confirmed by DSM laboratory studies, where the oil content in the gum phase was reduced by almost half following the action of Purifine® 3G (from ~45 % to ~20 %). The FFA levels remained in the range of 1.17–1.35 %, which is acceptable for biofuel applications.

Table 3. Enzymatic degumming conditions and results for sunflower oil

Sunflower oil with 7.5 ppm P, 60 ppm S, 1.15 % FFA	pH	Reaction / hydration temperature, °C	Reaction / hydration time, h	Final P degummed oil, ppm	Final S degummed oil, ppm	Final FFA degummed oil, %	Gums, dry basis, %
Water degumming, no enzymes used	N	60	1.0	7.0	50	1.22	4.7
Purifine® LM enzyme degumming	N	80	2.0	5.0	12	1.31	5.5
Purifine® PLC enzyme degumming	5.5–6.5	60	2.0	4.7	11	1.19	4.3
Purifine® PLA1 enzyme degumming	5.5–6.5	60	2.0	5.2	14	1.52	4.9
Purifine® PLC, Purifine® LM enzyme degumming	5.5–6.5	60/80	3.0	3.5	10	1.43	5.3
Combination of Purifine® LM, Purifine® PLC and Purifine® PLA1 enzyme degumming	5.5–6.5	60	1.0	2.5	9	1.35	4.1
Purifine® 3G enzyme degumming	5.5–6.5	60	2.0	2.6	10	1.17	3.5

3.2. Features of the enzymatic regime and degumming results for rapeseed oil

The initial phosphorus content was 5.9 ppm, and FFA was 0.70 %. The best results were again obtained using Purifine® 3G and the PLC + PLA1 + LM combination, both achieving a residual phosphorus level of 2.8 ppm. Other enzymes demonstrated only partial

effectiveness: PLC – 4.9 ppm, LM – 5.0 ppm, while water degumming reduced phosphorus to 5.1 ppm (see Table 4).

A reduction in the gum dry matter content from 3.7 % to 2.5–3.1 % is associated with a decrease in phospholipid content and the corresponding amount of bound oil in the gum phase. The FFA levels remained in the range of 0.77–0.85 %, making such treatment suitable for physical refining or further hydrotreating without additional neutralization.

Table 4. Enzymatic degumming conditions and results for rapeseed oil

Rapeseed oil with 5.9 ppm P, 100 ppm S, 0.70 % FFA	pH	Reaction / hydration temperature, °C	Reaction / hydration time, h	Final P degummed oil, ppm	Final S degummed oil, ppm	Final FFA degummed oil, %	Gums, dry basis, %
Water degumming, no enzymes used	N	60	1.0	5.1	70	0.92	3.7
Purifine® LM enzyme degumming	N	80	2.0	5.0	54	1.11	3.5
Purifine® PLC enzyme degumming	5.5–6.5	60	2.0	4.9	47	0.89	3.3
Purifine® PLA1 enzyme degumming	5.5–6.5	60	2.0	5.1	51	1.12	2.9
Purifine® PLC, Purifine® LM enzyme degumming	5.5–6.5	60/80	3.0	3.7	45	1.13	3.3
Combination of Purifine® LM, Purifine® PLC and Purifine® PLA1 enzyme degumming	5.5–6.5	60	1.0	2.8	27	0.85	3.1
Purifine® 3G enzyme degumming	5.5–6.5	60	2.0	2.8	25	0.77	2.5

3.3. Features of the enzymatic regime and degumming results for soybean oil

Soybean oil was characterized by the highest initial phosphorus content (9.0 ppm) and FFA (1.90 %). Water degumming only reduced phosphorus to 7.1 ppm. The most effective options again were Purifine® 3G (3.4 ppm) and the combination of PLC + PLA1 + LM (3.5 ppm) (see Table 5).

The gum content decreased from 5.7 % to 3.5–4.1 %. As in the previous cases, this reduction was due not only to the removal of polar compounds but primarily to the decrease in neutral oil content in the gum phase, which typically remains lost during conventional water

degumming. After enzymatic treatment, FFA levels slightly increased (1.97–2.05 %), which is acceptable for biofuel production.

The results of the enzymatic degumming of crude soybean, rapeseed, and sunflower oils confirmed the high efficiency of applying multi-enzyme systems, particularly the Purifine® PLC, PLA1, LM enzymes, and the complex formulation Purifine® 3G. The reduction of residual phosphorus levels below 5 ppm in the degummed samples allows these technologies to be considered suitable for the pre-treatment of feedstocks for catalytic hydrotreatment in the production of Hydrotreated Vegetable Oil (HVO) and for improving the quality of feedstock used in the production of Fatty Acid Methyl Esters (FAME) biofuel.¹

Table 5. Enzymatic degumming conditions and results for soybean oil

Soybean oil with 9.0 ppm P, 90 ppm S, 1.90 % FFA	pH	Reaction / hydration temperature, °C	Reaction / hydration time, h	Final P degummed oil, ppm	Final S degummed oil, ppm	Final FFA degummed oil, %	Gums, dry basis, %
Water degumming, no enzymes used	N	60	1.0	7.1	40	1.92	5.7
Purifine® LM enzyme degumming	N	80	2.0	6.0	27	2.11	5.5
Purifine® PLC enzyme degumming	5.5–6.5	60	2.0	5.7	25	2.09	4.3
Purifine® PLA1 enzyme degumming	5.5–6.5	60	2.0	7.2	28	2.12	4.9
Purifine® PLC, Purifine® LM enzyme degumming	5.5–6.5	60/80	3.0	4.7	19	2.13	4.3
Combination of Purifine® LM, Purifine® PLC and Purifine® PLA1 enzyme degumming	5.5–6.5	60	1.0	3.5	12	2.05	4.1
Purifine® 3G enzyme degumming	5.5–6.5	60	2.0	3.4	11	1.97	3.5

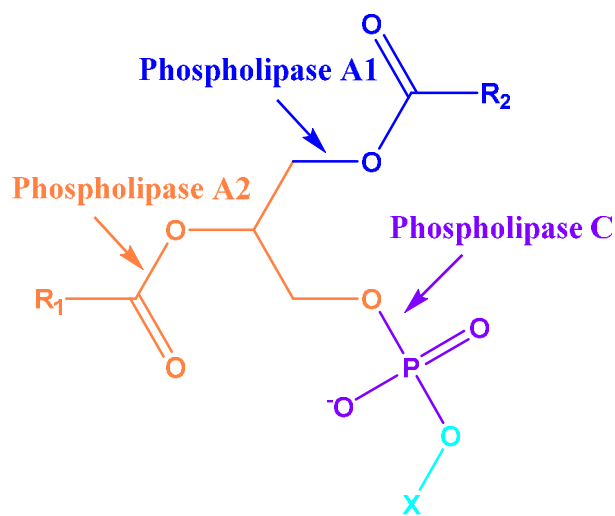


Fig. 1. Phospholipases mode of action. A generalized depiction of a phospholipid, where X = H, choline, ethanolamine, inositol, etc. The various sites of attack for hydrolytic cleavage of ester bonds of the phospholipase types A1, A2, and C are shown with arrows^{3,8,10,26}

Phospholipids in vegetable oils can exist in hydrated or non-hydrated forms. While hydrated phospholipids can

be removed by water washing and centrifugation, non-hydrated ones require a more specific approach – either chemical (*e. g.*, citric acid or EDTA) or enzymatic. The obtained results confirm that enzymatic degumming, based on the action of phospholipases such as PLA1, PLA2, and PLC, is an effective alternative to traditional methods.^{3,10} It not only significantly reduces the phospholipid content but also increases the yield of neutral oil by converting phospholipids into diacylglycerols (DAG) and free fatty acids (FFA) (see Fig. 1).

Purifine® 3G proved to be particularly effective, as it contains phospholipase C, PI-specific PLC, and PLA2, enabling the simultaneous breakdown of major phospholipids – PC, PE, and PI. Thanks to this multi-enzyme composition, the lowest residual phosphorus levels (2.6–3.4 ppm) were achieved across all types of oils. Moreover, the use of Purifine® 3G or its functional analogues led to a significant reduction in the mass fraction of the gum phase – not due to mechanical removal, but because of decreased neutral oil content in the emulsified gums. This enzymatic action facilitates the return of a substantial portion of the oil into the main fraction in the form of DAG and FFA, thereby directly increasing the total yield of purified product.

Table 6. Summary of enzymatic treatment results for vegetable oils

Type of oil	Initial phosphorus, ppm	Residual phosphorus, ppm	Most effective systems	Gums, dry basis, %	FFA, %
Sunflower oil	7.5	2.5–2.6	<i>Purifine</i> ® 3G, PLC + PLA1 + LM	3.5–4.1	1.17–1.35
Rapeseed oil	5.9	2.8	<i>Purifine</i> ® 3G, PLC + PLA1 + LM	2.5–3.1	0.77–0.85
Soybean oil	9.0	3.4–3.5	<i>Purifine</i> ® 3G, PLC + PLA1 + LM	3.5–4.1	1.97–2.05

Experimental data clearly demonstrate that multi-enzyme systems allow achieving residual phosphorus levels below 5 ppm, which meets the technical requirements for feedstock intended for catalytic hydrotreatment in HVO production¹ and in the production of Fatty Acid Methyl Esters (FAME)¹ biofuel. At the same time, the FFA levels remained within acceptable limits, indicating a controlled hydrolysis process and the absence of excessive degradation of the triglyceride fraction (see Table 6).

Thus, enzymatic degumming using multi-enzyme systems, such as *Purifine*® 3G or experimental phospholipase cocktails, ensures not only the achievement of target phosphorus levels but also a significant reduction in neutral oil losses through the gum phase. These advantages make such approaches economically viable and environmentally safe for integration into the production chain of next-generation biofuels.

4. Conclusions

Enzymatic degumming using *Purifine*® preparations ensures the effective removal of both hydrated and non-hydrated phospholipids, allowing residual phosphorus levels below 5 ppm – a critical threshold for subsequent catalytic hydrotreatment (HVO) or efficient alkaline transesterification (FAME). The highest efficiency was demonstrated by the multi-enzyme preparation *Purifine*® 3G, which simultaneously acts on PC, PE, and PI, converting phospholipids into DAG, FFA, LPL, and phosphates, thereby increasing the yield of neutral oil and reducing losses through the gum phase. The PLC + PLA1 + LM enzyme combination showed comparable performance to *Purifine*® 3G and is well-suited for adaptation to various feedstocks and process lines in both the food and energy sectors. The FFA levels after the treatment remained within acceptable limits for further use in biofuel technologies, and the degumming degree met the requirements for feedstock used in both HVO and FAME/FAE production. In addition to phosphorus reduction, the enzymatic systems also contributed to a substantial decrease in sulfur content – from initial concentrations of 60–100 ppm to final values ranging between 9 and 27 ppm, depending on the oil type. This sulfur reduction is essential for prolonging catalyst

life during hydrotreatment and for ensuring compliance with ultra-low sulfur fuel standards.

These findings confirm that enzymatic degumming is a versatile, environmentally friendly, resource-efficient, and technologically effective approach to preparing vegetable oils for conversion into first- and second-generation biofuels.

Funding

This research was funded by the Ministry of Education and Science of Ukraine: Development of biodiesel production technology by cavitation method with preliminary fermentation of raw materials (DB / BIODISEL).

Acknowledgements

The authors are sincerely grateful to the DeepTech Prototyping Laboratory (Lviv Polytechnic National University, Ukraine) for the opportunity to use modern laboratory equipment, which ensured the quality of the experimental part of the research within the scope of the scientific project. Special thanks are also expressed to the Center for the collective use of scientific equipment “Laboratory of advanced technologies for the creation and physico-chemical analysis of new substances and functional materials” (Lviv Polytechnic National University, Ukraine) for support in conducting analytical research, which made it possible to obtain reliable and representative results.

Abbreviations

HVO Hydrotreated Vegetable Oil – second-generation biofuel obtained via catalytic hydrotreatment
 FAME Fatty Acid Methyl Ester – first-generation biodiesel produced by transesterification
 FFA Free Fatty Acids – byproducts of lipid hydrolysis, affect oil quality and stability
 DAG Diacylglycerol – a neutral lipid formed by phospholipid hydrolysis, retained in the oil phase
 LPL Lysophospholipids – partially hydrolysed phospholipids, more polar and water-soluble

PC Phosphatidylcholine – a major phospholipid, often targeted during enzymatic degumming

PE Phosphatidylethanolamine – an amino phospholipid presents in crude vegetable oils

PI Phosphatidylinositol – an acidic phospholipid, difficult to remove without specific enzymes

PLA1 Phospholipase A1 – hydrolyses the ester bond at the sn-1 position of glycerophospholipids

PLA2 Phospholipase A2 – hydrolyses the ester bond at the sn-2 position of glycerophospholipids

PLB Phospholipase B – has both PLA1 and PLA2 activity; completely hydrolyses phospholipids

PLC Phospholipase C – cleaves the phosphodiester bond, forming diacylglycerols and phosphate esters

PLD Phospholipase D – cleaves the terminal group, forming phosphatidic acid and alcohol

PL Phospholipids – a group of amphiphilic lipids present in crude oils and gums

LAT Lipid Acyltransferase – transfers fatty acid chains between lipid molecules, aids in oil recovery

SFO Sunflower oil

RO Rapeseed oil

SBO Soybean oil

References

- [1] Mussa, N.-S.; Toshtay, K.; Capron, M. Catalytic Applications in the Production of Hydrotreated Vegetable Oil (HVO) as a Renewable Fuel: A Review. *Catalysts*. **2024**, *14*, 452. <https://doi.org/10.3390/catal14070452>
- [2] Khamies, M.; Hagar, M.; Kassem, T. S.; Moustafa, A. H. E. Case Study of Chemical and Enzymatic Degumming Processes in Soybean Oil Production at an Industrial Plant. *Sci. Rep.* **2024**, *14*, 4064. <https://doi.org/10.1038/s41598-024-53865-9>
- [3] Cerminati, S.; Paoletti, L.; Aguirre, A.; Peirú, S.; Menzella, H. G.; Castelli, M. E. Industrial Uses of Phospholipases: Current State and Future Applications. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 2571–2582. <https://doi.org/10.1007/s00253-019-09658-6>
- [4] Yang, B.; Wang, Y. H.; Yang, J. G. Optimization of Enzymatic Degumming Process for Rapeseed Oil. *J. Am. Oil Chem. Soc.* **2006**, *83*, 653–658. <https://doi.org/10.1007/s11746-006-1253-4>
- [5] Aloulou, A.; Rahier, R.; Arhab, Y.; Noiriél, A.; Abousalham, A. Phospholipases: An Overview. In *Lipases and Phospholipases. Methods in Molecular Biology*, vol. 1835; Sandoval, G., Ed.; Humana Press: New York, NY, 2018; pp. 69–105. https://doi.org/10.1007/978-1-4939-8672-9_3
- [6] Dijkstra, A. J. Enzymatic Degumming. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 1178–1189. <https://doi.org/10.1002/ejlt.201000320>
- [7] Al Sharqi, S.; Dunford, N. T.; Goad, C. Enzymatic Wheat Germ Oil Degumming. *Trans. ASABE*. **2015**, *58*, 1867–1872. <https://doi.org/10.13031/trans.58.11032>
- [8] Jiang, X.; Chang, M.; Jin, Q.; Wang, X. Application of Phospholipase A1 and Phospholipase C in the Degumming Process of Different Kinds of Crude Oil. *Process Biochem.* **2015**, *50*, 432–437. <https://doi.org/10.1016/j.procbio.2014.12.011>
- [9] Luo, S.; Wang, W.; Zhang, H.; Liu, C.; Wang, N.; Wang, L.; Yu, D. A New Strategy for Magnetic Immobilized Phospholipase A1 and its Application in Soybean Oil Degumming: Multipoint Covalent Binding *LWT*. **2023**, *186*, 115181. <https://doi.org/10.1016/j.lwt.2023.115181>
- [10] Schaloske, R. H.; Dennis, E. A. The Phospholipase A2 Superfamily and its Group Numbering System. *Biochim Biophys Acta*. **2006**, *1761*, 1246–1259. <https://doi.org/10.1016/j.bbali.2006.07.011>
- [11] dos Passos, R. M.; da Silva, R. M.; de Almeida Pontes, P. V.; Morgano, M. A.; Meirelles, A. J.; Stevens, C. V.; Sampaio, K. A. Phospholipase Cocktail: A New Degumming Technique for Crude Soybean Oil. *LWT*. **2022**, *159*, 113197. <https://doi.org/10.1016/j.lwt.2022.113197>
- [12] Mansfeld, J. Plant Phospholipases A2: Perspectives on Biotechnological Applications. *Biotechnol Lett.* **2009**, *31*, 1373–1380. <http://dx.doi.org/10.1007/s10529-009-0034-1>
- [13] Manjula, S.; Jose, A.; Divakar, S.; Subramanian, R. Degumming Rice Bran Oil Using Phospholipase-A1. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 658–664. <https://doi.org/10.1002/ejlt.201000376>
- [14] Jahani, M.; Alizadeh, M.; Pirozifard, M.; Qudsevali, A. Optimization of Enzymatic Degumming Process for Rice Bran Oil Using Response Surface Methodology. *LWT – Food Sci. Technol.* **2008**, *41*, 1892–1898. <http://dx.doi.org/10.1016/j.lwt.2007.12.007>
- [15] Lamas, D. L.; Crapiste, G. H.; Constenla, D. T. Changes in Quality and Composition of Sunflower Oil During Enzymatic Degumming Process. *LWT – Food Sci. Technol.* **2014**, *58*, 71–76. <https://doi.org/10.1016/j.lwt.2014.02.024>
- [16] Lamas, D. L.; Constenla, D. T.; Raab, D. Effect of Degumming Process on Physicochemical Properties of Sunflower Oil. *Biocatal. Agric. Biotechnol.* **2016**, *6*, 138–143. <https://doi.org/10.1016/j.bcab.2016.03.007>
- [17] Nikolaeva, T.; Rietkerk, T.; Sein, A.; Dalgliesh, R.; Bouwman, W. G.; Velichko, E.; van Duynhoven, J. Impact of Water Degumming and Enzymatic Degumming on Gum Mesostructure Formation in Crude Soybean Oil. *Food Chem.* **2020**, *311*, 126017. <https://doi.org/10.1016/j.foodchem.2019.126017>
- [18] Marrakchi, F.; Kriaa, K.; Hadrich, B.; Kechaou, N. Experimental Investigation of Processing Parameters and Effects of Degumming, Neutralization and Bleaching on Lampante Virgin Olive Oil's Quality. *Food Bioprod. Process.* **2015**, *94*, 124–135. <https://doi.org/10.1016/j.fbp.2015.02.002>
- [19] Yang, B.; Zhou, R.; Yang, J. G. Insight into the Enzymatic Degumming Process of Soybean Oil. *J. Am. Oil Chem. Soc.* **2008**, *85*, 421–425. <https://doi.org/10.1007/s11746-008-1225-y>
- [20] Roy, S. K.; Rao, B. V. S. K.; Prasad, R. B. N. Enzymatic Degumming of Rice Bran Oil. *J. Am. Oil Chem. Soc.* **2002**, *79*, 845–846. <http://dx.doi.org/10.1007/s11746-002-0568-5>
- [21] Clausen, K. Enzymatic Oil-Degumming by a Novel Microbial Phospholipase. *Eur. J. Lipid Sci. Technol.* **2001**, *103*, 333–340. [https://doi.org/10.1002/1438-9312\(200106\)103:6<333::AID-EJLT333>3.0.CO;2-F](https://doi.org/10.1002/1438-9312(200106)103:6<333::AID-EJLT333>3.0.CO;2-F)
- [22] Sampaio, K. A.; Zyaykina, N.; Wozniak, B.; Tsukamoto, J.; Greyt, W. D.; Stevens, C. V. Enzymatic Degumming:

- Degumming Efficiency Versus Yield Increase. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 81–86. <https://doi.org/10.1002/ejlt.201400218>
- [23] de Sousa, R. R. D.; dos Santos, M. M.; Medeiros, M. W.; Manoel, E. A.; Berenguer-Murcia, A.; Freire, D. M. G.; Ferreira-Leitão, V. S. Immobilized Lipases in the Synthesis of Short-Chain Esters: An Overview of Constraints and Perspectives. *Catalysts*. **2025**, *15*, 375. <https://doi.org/10.3390/catal15040375>
- [24] Bolivar, J. M.; Woodley, J. M.; Fernandez-Lafuente, R. Is Enzyme Immobilization a Mature Discipline? Some Critical Considerations to Capitalize on the Benefits of Immobilization. *Chem. Soc. Rev.* **2022**, *51*, 6251–6290. <https://doi.org/10.1039/d2cs00083k>
- [25] Gupta, M. N.; Uversky, V. N. Enzymology: early insights. In *Structure and Intrinsic Disorder in Enzymology*; Academic Press, 2023; pp. 1–29. <https://doi.org/10.1016/B978-0-323-99533-7.00013-3>
- [26] Polovkovych, S.; Karkhut, A.; Gunka, V.; Blikharskyu, Y.; Nebesnyi, R.; Khomyak, S.; Selejdak, J.; Blikharskyu, Z. Enzymatic Degumming of Soybean Oil for Raw Material Preparation in BioFuel Production. *Appl. Sci.* **2025**, *15*, 8371. <https://doi.org/10.3390/app15158371>
- [27] DSTU EN ISO 3675:2012. Crude petroleum and liquid petroleum products. Laboratory determination of density. Hydrometer method.
- [28] DSTU EN ISO 3104:2022. Petroleum products. Transparent and opaque liquids. Determination of kinematic viscosity and calculation of dynamic viscosity.
- [29] DSTU ISO 2719:2006. Determination of flash point. Pensky. Martens closed cup method.
- [30] DSTU ISO 20846:2009. Petroleum products. Determination of sulfur content of automotive fuels. Ultraviolet fluorescence method.
- [31] DSTU 7082:2009. Vegetable oils. Methods for determination of mass concentration phosphorated content.
- [32] DSTU EN ISO 8534:2019. Animal and vegetable fats and oils. Determination of water content. Karl Fischer method (pyridine free).
- [33] DSTU EN ISO 2160:2012. Petroleum products. Corrosiveness to copper. Copper strip test.
- [34] DSTU EN 14104:2009. Fat and oil derivatives. Fatty acid methyl ester (FAME). Determination of acid value.

- [35] DSTU EN ISO 3961:2019. Animal and vegetable fats and oils. Determination of iodine value.

Received: June 30, 2025 / Revised: August 13, 2025 / Accepted: September 01, 2025

ФЕРМЕНТАТИВНЕ ДЕГУМУВАННЯ СОСВОЇ, РІПАКОВОЇ ТА СОНЯШНИКОВОЇ ОЛІЙ З УКРАЇНСЬКИХ КУЛЬТУР ІЗ ВИКОРИСТАННЯМ ФЕРМЕНТІВ PURIFINE

Анотація. У дослідженні проаналізовано ефективність ферментативної дегумації сирової соєвої, ріпакової та соняшникової олій з використанням фосфоліпази С (PLC), фосфоліпази А₁ (PLA₁), модифікованого ліпазного компонента (LM) препарату Purifine®, а також мультиферментного комплексу Purifine® 3G, який поєднує активності PLC, PLA₂ і LM, та їхніх комбінацій. Основна мета — досягнення низького залишкового вмісту фосфору (<5 ррт), що критично важливо для подальшої каталізаторної гідрообробки у виробництві гідроочищеної рослинної олії (HVO) та для підвищення ефективності реакції переестерифікації у виробництві метилових і дегумації етилових естерів жирних кислот (FAME/FAE). Оцінювання ефективності ґрунтувалось на показниках вмісту фосфору, сірки, вільних жирних кислот (FFA) та сухої маси фосфоліпідного осаду. Найефективнішими виявились ферментна система Purifine® 3G і комбінація PLC + PLA₁ + LM, які забезпечили глибоке очищення, мінімальні втрати нейтральної олії та стабільний рівень FFA. Важливо, що ці ферментні системи не лише дали змогу знизити вміст фосфору до рівня <5 ррт, а й істотно змінили вміст сірки — із початкових значень 60–100 ррт до кінцевих концентрацій 9–27 ррт залежно від типу олії. Таке зменшення вмісту сірки особливо важливе для захисту каталізаторів гідрообробки та дотримання вимог до палива згідно зі стандартами EN 15940 та ASTM D975. Результати підтверджують доцільність використання ферментативних систем як сталого технології попередньої підготовки сировини як для біопалива другого покоління (HVO), так і для першого покоління (FAME), забезпечуючи підвищення строку служби каталізаторів, чистоти продукту й ефективності процесу.

Ключові слова: ферментативна дегумація, фосфоліпази, Purifine® 3G, гідроочищена рослинна олія, метилові та етилові естери жирних кислот, фосфоліпіди, рослинна олія, фосфор, біопаливо, мультиферментна система.