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PHOSPHOLIPIDS IN COTTONSEED OIL AND DEGUMMING METHODS

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Abstract: *Phospholipids are categorized into two types: hydratable phospholipids and non-hydratable phospholipids. This study examines the hydratable and non-hydratable phospholipids in cottonseed oil, as well as the types of degumming and their impact on the content of phosphorus, calcium, and magnesium. During water degumming, hydratable phospholipids are removed, but non-hydratable phospholipids remain. Water-hydratable phospholipids constitute 78-83% of the total phospholipids in cottonseed oil. However, water degumming does not sufficiently reduce the cottonseed oil's phosphorus, calcium, and magnesium content. Acid degumming reduced the phosphorus content by 17.1 mg.kg^{-1}, calcium by 11.1 mg.kg^{1}, and magnesium by 14.2 mg.kg^{-1}. The total degumming process significantly reduced the levels of accompanying substances in cottonseed oil. After the total degumming process, the phosphorus content was less than 12.4 mg.kg^{-1}, calcium was 7.4 mg.kg^{-1}, and magnesium was reduced to 1.1 mg.kg^{-1}.*

Key words: *Cottonseed oil, degumming, phospholipids.*

1. Introduction
1.1. General aspects

Cottonseed oil, derived from the seeds of the cotton plant, has gained recognition as a valuable dietary fat due to its versatile nutritional profile and health benefits. Known for its mild flavor and high smoke point, it is widely used in cooking, baking, and food production. Cottonseed oil is rich in essential fatty acids, particularly linoleic acid that plays a crucial role in supporting cardiovascular health, skin health, and hormone regulation. Additionally, cottonseed oil contains antioxidants like vitamin E, which help combat oxidative stress and support the immune function. Due to its balance of unsaturated fats and beneficial compounds, cottonseed oil has become an important source of nutrition, particularly in diets seeking to replace saturated fats with healthier options [27].

Cottonseed oil is obtained using hexane and other solvents by pressing and extraction methods. Vegetable oils, depending on their nature, extraction

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method from the raw material, and storage conditions, contain not only the primary group of triacylglycerols but also structural lipids that determine the color, taste, odor, and other properties specific to the type of oil. Crude cottonseed oil contains substances such as phospholipids, free fatty acids, pigments, tocopherols, gossypol, non-saponifiable substances, oxidation products, and more. The composition and quality of oils vary depending on the method of oil production, with solvent-extracted oils containing more accompanying substances than pressed oils due to the influence of the solvent. The process of purifying oil from unwanted lipid groups and impurities is called refining, with the ultimate goal of isolating triacylglycerols from other lipid groups and impurities. Modern full refining technology involves the removal of phospholipids (degumming), free fatty acids (alkaline neutralization), coloring substances (bleaching), and substances responsible for the taste and odor of oils and fats (deodorization) [17, 27, 35].

Degumming is removing hydrophilic substances, the most important of which are phospholipids, from oil using water. During oil storage, they precipitate as readily degradable sediment, which hinders several oil processing operations. Usually, crude oil contains two different types of phospholipids: hydratable, processed by water solutions during refining, and non-hydratable, processed during acid refining. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol are the main types of phospholipids in vegetable oils. In general, vegetable oils contain two types of phospholipids: hydratable and non-hydratable [8, 31, 32].

These compounds can trap metal ions (copper + iron) and prevent their catalytic activity associated with the formation of free radicals in crude oil [6]. Today, there are several types of degumming processes: water degumming, acid degumming, Total degumming process degumming, Soft degumming, enzymatic degumming, ultrafiltration degumming, and degumming with ethanolamine [5, 34, 39].

1.2. Water Degumming

This degumming involves mixing heated oil with a measured amount of water. During this process, phospholipids form hydrophilic particles with water, lose their ability to dissolve in fat, and precipitate as a hydration fuse [12, 18, 23, 24, 36]. Water-degummed oil contains 50-200 $mg.kg^{-1}$ of phosphorus, depending on the content and type of NHPL in the crude oil [25]. Calcium and magnesium contents also decrease through water degumming [11].

1.3. Acid Degumming

For acid-degumming technology, water-degummed or crude oils can be used. In this process, strong acids break down NHPL structures. Citric and phosphoric acid can be used for oil degumming [15, 19]. Typically, citric acid is added to crude or water-degummed oil and vigorously mixed. Then, the necessary amount of water is added, and the mixture is cooled, promoting the transition of phosphatides to a semicrystalline phase. This phase contains acid, added water, wax, calcium, and magnesium, all bound to NHPL [29].

1.4. The Total Degumming Process

In the total degumming process, crude or water-degummed oil is heated and vigorously mixed with phosphoric acid. After a short interval (2-3 min.), an aqueous alkaline solution is added to the mixture and stirred for 6 min. Phosphatides and other impurities are removed during the first separation with minimal oil loss. Following the first separation, hot water is added to achieve complete hydration of phospholipids, and impurities are separated in a second separator. With this technology, the phosphorus content in oils decreases by less than 10 $mg.kg^{-1}$, and there is also a possible reduction in the oil's acid value [20, 28].

1.5. Soft Degumming

The use of ethylenediaminetetraacetic acid in the degumming process of vegetable oils was described in 1948 and named Soft degumming. This method involves the removal of a maximum amount of phosphatides and metals using ethylenediaminetetraacetic acid in the presence of emulsification additives. During Soft degumming, water-degummed oil or crude oil is mixed with an aqueous solution of ethylenediaminetetraacetic acid or its salts [7, 29, 37]. Non-hydratable phospholipids (NHPL) are salts of phosphatidic acid (PA/M^{+2}) and phosphatidylethanolamine (PE/M^{+2}). Ethylenediaminetetraacetic acid, during contact with NHPL, forms complexes with metals (PA/M^{+2} and PE/M^{+2}) [10, 13].

1.6. Enzymatic Degumming

In this process, enzymes convert NHPL into HPL forms [28, 30]. The process can be divided into 4 consecutive steps: creating optimal conditions for enzymatic reactions (optimizing pH and temperature); adding enzymes to the aqueous phase (citric acid solution is used as a buffer to bind calcium for subsequent enzyme deactivation); enzymatic reaction; separation of lysolecithins from the oil. Currently, two types of enzymes are used for oil degumming: pancreatic phospholipase A2 - Lecitase 10L and phospholipase A1 - microbial lipase [21, 26, 33].

1.7. Ultrafiltration Degumming

Membrane technologies can be used to remove phosphatides from crude oils [16]. Despite the small difference in molecular mass between TAGs and phospholipids, they can be separated by membrane filtration. The difference in mass between TAGs, FFAs, and phospholipids allows for the use of ultra-filtration membranes in the degumming process [1, 9]. Some membranes are considered unfavorable because they are unstable in solvents such as hexane [22].

1.8. Degumming with ethanolamine

Kaimal et al. [14] employed ethanolamines for the refining of rice hull oil. Zufarov et al. [38] was also employed for the degumming of sunflower and rapeseed oils, demonstrating the effectiveness of ethanolamines in reducing the phosphorus, calcium, and magnesium content in oils.

The study of phospholipids in cottonseed oil and the effectiveness of various degumming methods – water, acid, and total (TOP) degumming – represents a novel approach that addresses a significant gap in current research. While considerable work has been done on the role of phospholipids in other oils, such as soybean and sunflower, limited research exists on cottonseed oil specifically. This study uniquely focuses on how different phospholipids, like phosphatidylcholine and others, react to the degumming method, which influences both the quality and the nutritional profile of the oil.

The novelty of this research is further underscored by its comparative analysis of calcium and magnesium removal rates among degumming processes, something scarcely explored in recent studies. With cottonseed oil becoming increasingly popular due to its nutritional profile, understanding the impact of advanced degumming processes on oil purity and nutrient retention is essential. This research not only fills a critical gap in the literature but also introduces practical insights that could optimize cottonseed oil refining for enhanced nutritional quality, setting a foundation for future studies to build upon.

2. Materials and Methods

2.1. Materials

Cottonseed oil samples were collected from the local oil factory Kattakurgan Oil Plant (Kattakurgan, Uzbekistan).

Magnetic stirrer (IKA Werk, Staufenim Breisgau, Germany), centrifuge (MPW-340, CHEMARGO, Blachownia, Poland) at 1.5m.s^{-2}, spectrophotometer (UV/VIS-1601, Shimadzu, Tokyo, Japan), inductively coupled plasma (ICP) optical emission spectroscopy (V Liberty 200, Victoria, Australia) were used during the experiments.

2.2. Methods

2.2.1. Water Degumming

The crude cottonseed oils, obtained through extraction and pressing, underwent a degumming process. This involved heating the oils to 70°C, adding a 6% volume of water, and stirring the mixture using a magnetic stirrer for 25 min. Subsequently, the blend was centrifuged for 20 min.

2.2.2. Acid Degumming

The water solution containing 30% citric acid was introduced into the heated (80°C) cottonseed oil, constituting 2% of the oil's volume for the mixer. The resulting blend was stirred for 20 min. Maintaining the oil-acid mixture at 80°C for 30 more min., it was then gradually cooled down to 22°C. To this mixture, water (1%) was added before transferring it to a holding vessel. Following a settling period of 60 min, the mixture underwent centrifugation for 20 min.

2.2.3. Total Degumming Process

Water-degummed oil was vigorously mixed with (15 wt-%) phosphoric acid in a portion of 0.1%, based on the weight of the oil under 80 °C. After a 5-min. reaction, the acid was partially neutralized using a 23% sodium hydroxide (NaOH) solution, amounting to 0.3% by weight of the oil. The entire reaction process lasted for 10 min. Subsequently, the hydrated phosphatides were separated by centrifugation, which took 20 min.

2.2.4. Determination of Phosphorus, Calcium, and Magnesium in Vegetable Oils

The amount of phospholipids was determined as the total phosphorus in a vegetable oil according to the AOCS Official Method Ca 12-55 [2].The phospholipid content was determined under Thin-Layer Chromatography according to the AOCS Ja-86 [4]. The amount of calcium and magnesium was determined by inductively coupled plasma (ICP) optical emission spectroscopy (V Liberty 200, Victoria, Australia) by following the AOCS official method Ca 17-01 [3].

2.3. Statistical Analysis

All measurements were triplicate. The statistical analysis was carried out with the program Statgraphics Plus, Version 1.4 for Windows (Manugistic, Rockville, USA). The significance of differences between mean values was determined at the p = 0.05 (5%) level, using a one way analysis of the variance and the t-test.

3. Results and Discussion

3.1. Water Degumming

In crude cottonseed oils, there is a high phosphorus content. Specifically, in solvent-extracted cottonseed oil, the phosphorus content reaches 654.2 $mg.kg^{-1}$ in pressed oil, this value is significantly lower at 341.3 $mg.kg^{-1}$. This difference can be explained by the fact that a significant portion of phospholipids and other accompanying substances transfer to the solvent-extracted oil during the extraction process using a solvent (Tab.1). Among the phospholipids in cottonseed oil, a significant proportion consists of water-hydratable phospholipids, such as phosphatidylcholine and phosphatidylinositol.

Table 1

Influence of water degumming on the phospholipids content in cottonseed oil

Processing stages	Phospholipids			
	Solvent-extracted cottonseed oil		Pressed cottonseed oil	
	[%]	[$mg.kg^{-1}$]	[%]	[$mg.kg^{-1}$]
Crude oil	100.0	654.2	100.0	341.3
Phosphatidylethanolamine	35.9	234.8	37.1	126.6
Phosphatidylcholine	50.7	331,7	42.9	146.4
Phosphatidylinositol	5.3	34.7	9.2	31.4
Phosphatidic acid	3.4	22.2	1.1	3.8
others	4.7	30.8	9.7	33.1
Water degummed oil	100.0	111.1	100.0	72.3
Phosphatidylethanolamine	64.1	71.2	76.3	55.2
Phosphatidylinositol	12.3	13.6	16.3	11.8
Phosphatidic acid	19.0	21.1	4.9	3.6
Others	4.6	5.2	2.5	1.7

As described by Sharma et al. [28], the relative rate of hydration for phosphatidylcholine is 100%, while phosphatidyl acid is 8.5%, as shown by the analyses of water degummed of cottonseed oil (Table 1).

In solvent-extracted oil, phosphatidylcholine makes up for about 50.7% of the total phospholipid content, while in pressed oil, its share is 42.9%. Phosphatidylethanolamine is also present in significant amounts, with a phosphorus content of 35.9% in solvent-extracted oil and 37.1% in pressed oil. Other types of phospholipids, such as phosphatidylinositol and phosphatidic acid, are also present in the oils, but their content is around 10% (Figures 1 and 2). The high content of water-hydratable phospholipids in cottonseed oil makes it suitable for further processing through a water degumming method. Non-hydratable phospholipids, such as salts of phosphatidic acid, phosphatidylethanolamine, and others, are also present in crude oils.

After water degumming, the majority of phospholipids are removed. In solvent-extracted oil, the removal of phospholipids accounts for 82.1%, while in pressed oil, it's 78.2%. Phosphatidylcholine is fully hydratable (100%), whereas the hydratability of phosphatidylethanolamine is 69.7% in solvent-extracted oil and 56.4% in pressed oil.

The hydratability of phosphatidylinositol is also high, at around 60-62%. Phosphatidic acid exhibits the lowest hydratability, at approximately 4-5%. The non-hydratable portion of phospholipids accounts for 16.9% in solvent-extracted oil and 21.8% in pressed oil (Table 2).

After water degumming, the calcium and magnesium content in solvent-extracted oil decreased (Tables 4 and 5). Calcium decreased to 60.1%, amounting to 45.7 mg.kg^{1}, while magnesium decreased to 33.8%, equaling 62.2 mg.kg^{-1} in the oil. In pressed oil, following water hydration, the calcium content decreased to 27.1%, totaling 14.6 mg.kg^{1}, and the magnesium content decreased to 11.9%, amounting to 13.1 mg.kg^{-1}.

The residual calcium content in solvent-extracted cottonseed oil after water degumming is 60.1% (Table 4), which is slightly higher than in water-degummed solvent-extracted sunflower oil at 49.6%, based on the content in crude oils [18]. The residual magnesium content in solvent-extracted cottonseed and sunflower oils after water degumming in the same manner, it is approximately 33-35% (Table 5), based on the magnesium content in crude oils [28].

The residual amounts of calcium and magnesium in water-degummed oils indicate the presence of non-water-hydratable phospholipids. Reducing the residual phosphorus content in water-degummed oils is necessary as it can have a negative impact on subsequent refining processes.

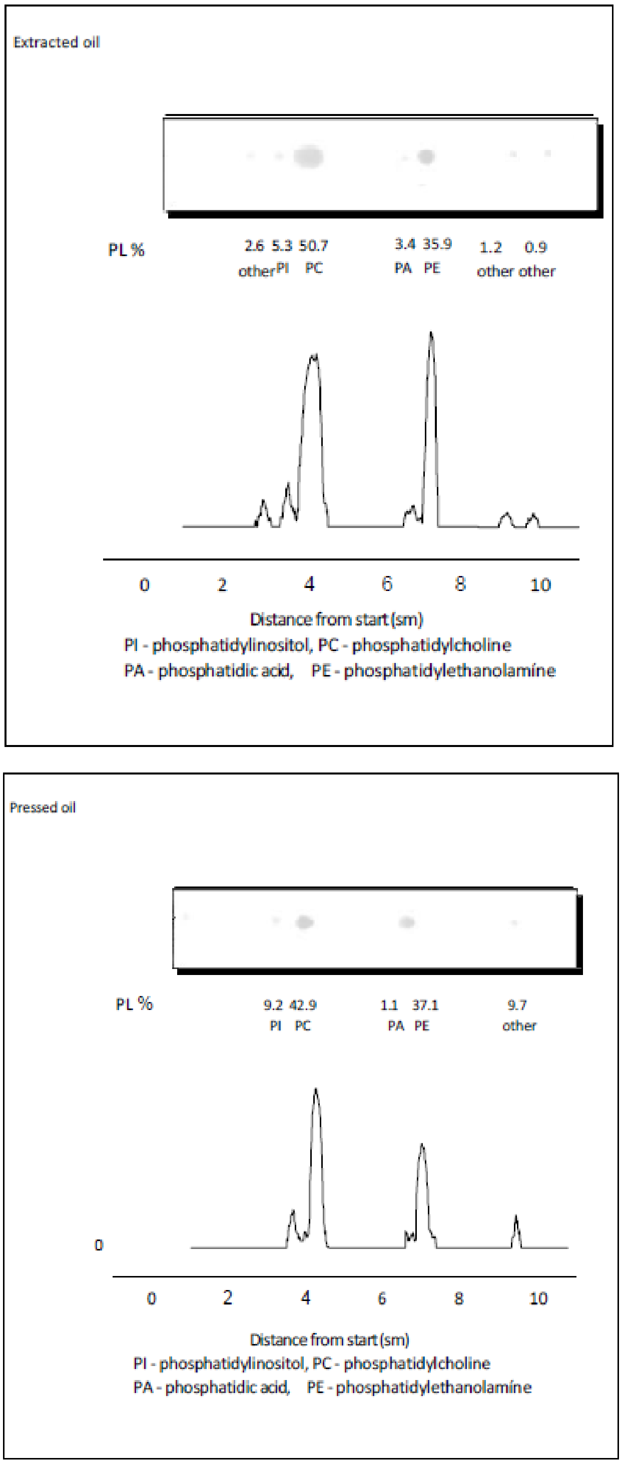

Fig. 1. *Phospholipids content in crude solvent-extracted and pressed cottonseed oils*

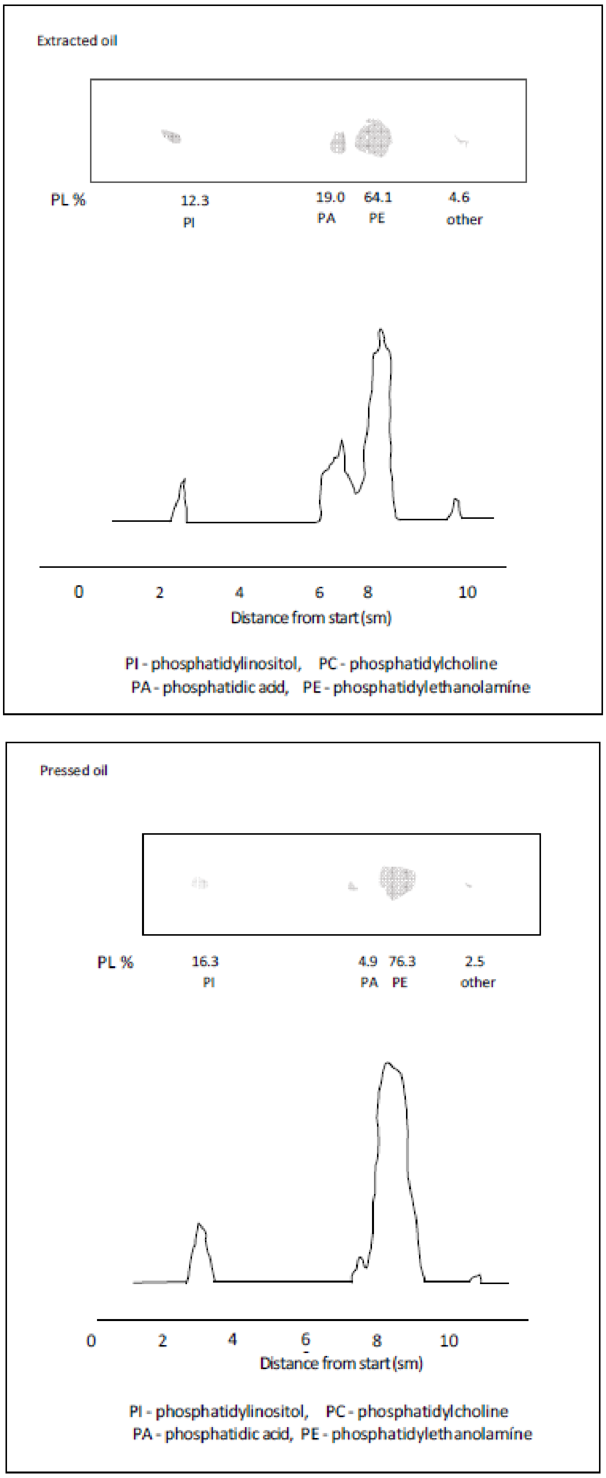

Fig. 2. *Phospholipids content in water degummed solvent-extracted and pressed cottonseed oils*

Table 2

Relative rate of hydration of phospholipids in cottonseed oil

Phospholipids	Phospholipids	
	Solvent-extracted cottonseed oil [%]	Pressed cottonseed oil [%]
Total phospholipids	83.1	78.2
Phosphatidylethanolamine	69.7	56.4
Phosphatidylcholine	100.0	100.0
Phosphatidylinositol	60.8	62.4
Phosphatidic acid	4.9	5.3
others	83.1	94.8

3.2. Acid Degumming

In this process, the structure of NHPL is broken down using citric acid. Undissociated phospholipids, calcium ions, magnesium ions, and other metals are products of this reaction. A citric acid aqueous solution is used to facilitate the separation of impurities from the oil. During the separation, most divalent impurities are removed, but undissociated ions remain in the oil. After the removal of divalent ions, the oil is washed, and the pH is increased, resulting in the formation of HPL, which is then removed. The phosphorus content after acid degumming decreased to 2.6% and amounted to 17.1 $mg.kg^{-1}$ in solvent-extracted cottonseed oil. In pressed oil, the phosphorus content decreased to 12.2 $mg.kg^{-1}$ (Table 3). Acid degumming also reduced the levels of calcium and magnesium in cottonseed oil. The calcium content in the oils amounted to less than 11.2 $mg.kg^{-1}$, while magnesium was less than 14.2 $mg.kg^{-1}$. (Tables 4 and 5) Also, the residual calcium levels after acid degumming in both solvent-extracted cottonseed and sunflower oils show approximately the same results, around 15% [28].

Table 3

Influence of degumming methods on the phosphorus content in cottonseed oil

Processing stages	Phosphorus			
	Solvent-extracted cottonseed oil		Pressed cottonseed oil	
	[$mg.kg^{-1}$]	[%]	[$mg.kg^{-1}$]	[%]
Crude oil	654.2 ± 1.3	100.0	341.3 ± 2.1	100.0
Water degummed	111.1 ± 1.4	16.9	72.3 ± 1.3	21.2
Acid degummed	17.1 ± 1.2	2.6	12.2 ± 0.3	3.5
Total degumming process	12.2 ± 1.9	1.8	5.6 ± 0.8	1.5

Table 4

Influence of degumming methods on the calcium content in cottonseed oil

Processing stages	Calcium			
	Solvent-extracted cottonseed oil		Pressed cottonseed oil	
	[mg.kg^{-1}]	[%]	[mg.kg^{-1}]	[%]
Crude oil	76.0 ± 2.3	100.0	53.7 ± 2.6	100.0
Water degummed	45.7 ± 0.7	60.1	14.6 ± 0.4	27.1
Acid degummed	11.1 ± 1.2	14.6	5.1 ± 0.2	9.5
Total degumming process	7.4 ± 0.4	9.7	2.4 ± 0.2	4.5

3.3. Total Degumming Process

In the Total degumming process, phosphoric acid is used. Phosphoric acid is generally considered stronger than citric acid due to its lower pKa values. After adding phosphoric acid to the mixture, an aqueous solution of alkali is added, which ultimately destroys and removes residual salts of phospholipids. All these factors contribute to the conversion of the remaining NHPL into HPL form.

Table 5

Influence degumming methods on the magnesium content in cottonseed oil

Processing stages	Magnesium			
	Solvent-extracted cottonseed oil		Pressed cottonseed oil	
	[mg.kg^{-1}]	[%]	[mg.kg^{-1}]	[%]
Crude oil	183.7 ± 3.7	100.0	109.5 ± 4.2	100.0
Water degummed	62.2 ± 1.2	33.8	13.1 ± 0.3	11.9
Acid degummed	14.2 ± 1.3	7.7	6.1 ± 0.2	5.5
Total degumming process	1.1 ± 0.2	0.5	0.6 ± 0.1	0.6

After the Total degumming process, the phosphorus content in solvent-extracted oil decreased to 1.8%, while in pressed oil, it reduced to 1.5% (Table 3). The calcium content decreased by less than 9.7% in the oils. It should be noted that the magnesium content in the oils after the Total degumming process is present in minimal quantities, i.e. less than 1.1 mg.kg^{-1} (Tables 4 and 5) [18].

The degumming process of cottonseed oil-encompassing water, acid, and total degumming (TOP) methods – has significant implications for human nutrition due to its impact on the oil's purity and nutritional quality. Water degumming effectively removes a large proportion of phospholipids, including phosphatidylcholine and phosphatidylinositol, which, if retained, could influence the oil's flavor stability and shelf life. Acid and TOP degumming further enhance the oil's nutritional value by reducing residual calcium and magnesium levels, which are essential for producing a high-quality, refined oil, suitable for consumption.

These findings underscore the importance of choosing effective degumming methods to produce a nutritionally valuable cottonseed oil. By lowering impurities that could affect both the safety and digestibility of the oil, acid and TOP degumming processes ensure a higher level of refinement. This level of refinement is crucial for making cottonseed oil a healthier choice in cooking and food preparation, ultimately enhancing its role in a balanced, health-supportive diet.

4. Conclusion

The goal of this paper is to analyze the effect of water, acid, and TOP degumming processes on cottonseed oil, with particular focus on how different phospholipid types influence water degumming and their implications for human nutrition. Understanding the hydration behavior of phospholipids is crucial because these compounds can impact the stability, clarity, and nutritional value of the oil used in cooking and food preparation. The study revealed that phosphatidylcholine is fully hydratable (100%), making it the easiest phospholipid to remove in water degumming processes, which is beneficial for producing a cleaner, more stable oil product. In contrast, phosphatidylethanolamine showed varying degrees of relative rate of hydration of phospholipid species, at 69.7% in solvent-extracted oil and 56.4% in pressed oil. Phosphatidylinositol demonstrated a hydration rate between 60-62%, while phosphatidic acid exhibited the lowest hydration rate. These findings are significant because they highlight the potential of water degumming in removing specific phospholipids, which in turn improves the oil's quality and nutritional profile by reducing impurities. Moreover, the acid and TOP degumming processes showed even greater efficacy in removing phosphorus, calcium, and magnesium impurities, which are essential for refining cottonseed oil to meet food-grade standards. Lowering these impurity levels not only enhances the oil's nutritional value but also extends its shelf life, making it a more reliable and healthier choice for consumers. By better understanding these refining processes, this research supports the development of improved methods for producing high-quality cottonseed oil, thereby contributing to healthier dietary options for consumers.

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