DOI:10.29013/AJT-24-11.12-75-82

INFLUENCE OF REFINING PROCESSES ON OXIDATIVE STABILITY OF COTTONSEED OIL

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Cite: *Zufarov O., Serkayev K., Isroilova Sh., Khamidova M. (2024). Influence of Refining Processes on Oxidative Stability of Cottonseed Oil. Austrian Journal of Technical and Natural Sciences 2024, No 3 – 4.* https://doi.org/10.29013/AJT-24-11.12-75-82

Abstract

Phospholipids and metals ions such as iron (III) often produce deleterious effect when present in vegetable oils. The trace quantities of iron (III) and phospholipids reduce the oxidative stability of vegetable oils. The present study reports show the influence of refining processes on the oxidative stability of cottonseed oils. The process of water degumming effectively reduces phosphorus and iron (III) content in cottonseed oil. The oxidative stability of water degummed oil is reduced by nearly half compared to crude oil. Consequently, neutralized and bleached oils exhibit compromised oxidative stability, with an induction period of 3.18 h for extracted oil and 2.97 h for pressed oil. Tocopherol losses of approximately 30% occur during deodorization process, significantly impacting oxidative stability. Deodorized cottonseed oils demonstrate the lowest oxidative stability, with induction periods of 2.47–2.55 h.

Keywords: *cottonseed oil; refining; gossypol; oxidative stability; iron*

Introduction

Edible oils assume a central role in human dietary practices, constituting a primary reservoir of essential fatty acids and vital nutrients. Within this spectrum of oils, cottonseed oil has ascended to eminence, attributable to its versatility, nutritional significance and prospective applications in various industrial sectors.

The susceptibility of edible oils to oxidative instability presents a notable challenge, resulting in the onset of rancidity and a consequent decline in their nutritional quality. Cottonseed oil, derived from the seeds of the Gossypium plant, has garnered broad utility across culinary, food processing, and industrial domains owing to its substantial presence of saturated fatty acids and advantageous bioactive constituents (Manoj, K.·Bbaohong, Z.·Jayashree, P.·Kanika, S. Radha, C. H.·Vijay, S. Deepak, C.·Sangram, D., Abhijit, D.·Nadeem, R.·Marisennayya, S. Suman, N. Sabareeshwari, V. Pran, M. José. M.L., 2023). The oxidative stability of cottonseed oil is inherently influenced by its fatty acid composition. Cottonseed oil is characterized by the predominance of C16 and C18 fatty acids, resulting in the presence of no more than two double bonds. Approximately 50% of the fatty acid composition of cottonseed oil comprises polyunsaturated fatty acids, with linoleic acid being the primary constituent. Consequently, the crude cottonseed oil exhibits comparatively lower oxidative stability comparing with oils such as olive oil, palm oil, peanut oil. The presence of tocopherols in cottonseed oil acts as an effective inhibitor of rancidity development, thereby enhancing the stability of the resulting product and extending its shelf life. Cottonseed oil is inherently hydrogenated and possesses a favorable fatty acid composition, including significant quantities of palmitic, stearic, oleic, linoleic, and linolenic acids, rendering it suitable for cardiovascular health. Cottonseed oil has gained prominence in culinary applications due to its high smoke point, which reaches 232 °C. This characteristic sets it apart from other cooking oils, making it well-suited for the process of frying various food items. The distinctive strong yellow coloration of cottonseed oil is attributed to the presence of gossypol (Ziaa, M. A. Shahc, S. H. Shoukata, S., Hussaind, Z., Khane, S. U., Shafqatf N. 2022; O'Brien, R.D., 2002; O'Brien, R.D. et al. 2005). Oxidative stability, denoting an oil's capacity to withstand oxidation, stands as a fundamental factor influencing its storage longevity, flavor preservation, and overall quality. The oxidative stability of cottonseed oil is subject to the influence of multiple factors, encompassing its fatty acid composition, the presence of gossypol and tocopherols, the constituents acting as antioxidants, and the employed processing techniques. Gossypol, akin to numerous other aromatic phenolic compounds, exerts a robust and efficacious role as a natural antioxidant (Laughton, M. J. Halliwell, B. Evans, P. J. Hoult, J.R. 1989). As an illustration, gossypol has demonstrated its capacity to safeguard carotene from pre-existing lipid peroxides in vitro and exhibits carotene-preserving antioxidant properties in vivo. Furthermore, natural antioxidants like tocopherols and gossypol have exhibited their effectiveness in enhancing the oxidative stability of biodiesel (Hove E. L., 1944; Moser, B., 2012).

The process of oil refining plays a pivotal role in the oil processing industry, with its primary objectives being the removal of impurities and the enhancement of oil stability. These refining procedures typically encompass degumming, neutralization, bleaching, and deodorization, each exerting distinct influences on the composition and stability of the oil (Gharby, S., 2022). Throughout the refining process, impurities such as phospholipids, free fatty acids, and trace metals function as prooxidants and are eliminated from the crude oil. However, it's noteworthy that natural antioxidant constituents present in crude oils, including tocopherols, squalene, and phytosterols, are also removed during the refining process (Ghazani, S. M. Marangoni, G.A. 2013; Kreps, F. Vrbiková, L. Schmidt, Š., 2014). In the refining process, compounds like gossypol and related molecules are eliminated. However, crude cottonseed oil still retains phospholipids and tocopherols. Notably, the levels of tocopherols are diminished through the high-temperature physical refining process (Gunstone, F.D., 2007). Phospholipids and trace metals are eliminated from the oil through processes involving water treatment, "Totaal Ontslijmings Process," total degumming process, acid treatment, and other related techniques. However, it's noteworthy that water-degummed oil may still retain elevated levels of iron (III). Consequently, addressing the challenge of iron removal can be addressed by developing a method that effectively breaks down these iron/phosphatide complexes, ultimately converting the resulting iron into a form that can be readily removed from the oil (Albert, J. D., Martin, V. O., 1989). The pro-oxidative properties of phospholipids stem from their anionic structural characteristics, which enable them to attract pro-oxidative metal ions. Conversely, their antioxidative effect against lipid oxidation arises from their capacity to enhance the binding affinity of antioxidants to the interfacial region, facilitate the breakdown of hydroperoxides, and form complexes with metal ions. (Chen, B. McClements, D. J. Decker, E.A., 2013). The oxidative stability of vegetable oils, as assessed by the Rancimat method, exhibited a notable decline during the neutralization stage (Zacchi, P. Eggers, R. 2008; Suliman, T.E.M.A. Jiang, J., Liu, Y.F., 2013). This phenomenon arises from the elimination of bioactive compounds, including phenolic compounds, tocopherols, and phytosterols, through their absorption by the soapstock during the neutralization stage. The most sensitive stage within the refining process is the bleaching phase. During this step, undesirable components, including pigments, trace metals, phospholipids, and specific degradation products, are eliminated. However, it's important to note that certain valuable compounds like tocopherols and sterols may also be unintentionally removed. This can lead to a substantial reduction in oxidative stability of the oil (Dubravka, K. Tomislav, D. Klara, K. Jasenka, G. Sandra, N. Marko, O. 2012). The most substantial reduction in oxidative indices was observed during the deodorization phase (Ardakani, M.S., Mohajeri, F.A., Askari, E. Javdan, G., Pourramezani, F., Fallahzadeh, H., Sadrabad, E.K., 2023). Elevated temperatures during the deodorization process are accountable for the degradation of tocopherols, which hold significance for maintaining the oxidative stability of edible oils (Jawad, I. M., Kochhar, S. P. Hudson, B.J.F. 1983). Among these variables, the employed refining techniques exert a substantial influence on the degree of oxidative stability alteration. To comprehensively assess the impact of refining approaches on oxidative stability, it is crucial to possess a profound comprehension of the underlying mechanisms involved. Cottonseed oil, characterized by its valuable nutritional characteristics and multifarious applications, necessitates careful consideration to safeguard its oxidative stability and extend its shelf life. The refining methodologies applied during processing substantially modify the oxidative stability of the oil by impacting its chemical constitution and antioxidant content. The aim of this article is the study influence of refining methods on oxidative stability of cottonseed oil.

Materials and methods

Materials

Cottonseed oil samples were collected from local oil factory situated at the "Kattakurgan Oil Plant" (Kattakurgan, Uzbekistan). Subsequently, the collected samples were carefully transported to the laboratory, ensuring light protection and an inert nitrogen atmosphere, and were then stored at the low temperature (8 °C) until further analytical procedures were conducted.

Refining process

Apparatus

Magnetic stirrer (IKA Werk, Staufen im Breisgau, Germany), centrifuge (MPW-340, CHEMARGO, Blachownia, Poland) at 1,3 $m.s^{-2}$, spectrophotometer (UV/VIS-1601, Shimadzu, Tokyo, Japan), inductively coupled plasma optical emission spectroscopy (V Liberty 200, Victoria, Australia) and Rancimat (743, Metrohnm, Herisau, Switzerland) were used during the experiments.

Water degumming. Crude cottonseed oils were degummed by heating the oils to 70 °C, adding water (5% by volume) and stirring for 20 min by magnetic stirrer. Then, the mixture was centrifuged for 20 min.

Neutralization. Water-degummed cottonseed oils were heated to 80 °C, and a water solution including NaOH (20% weight) in a portion of 5% by volume of the oil was added and the mixture was stirred for 30 min. The mixture was kept at 80 °C up to 30 min. and transferred to a holding vessel. After settling for 60 min the mixture was centrifuged for 20 min.

Bleaching. Neutralized oils were heated to 80 °C and were initially mixed intensively with bleaching clay in a portion of 0.5% by weight of the oil. The total reaction times are only 30 min. The mixture was filtered to obtain bleached oil.

Deodorization. The deodorization process was conducted in laboratory equipment at a temperature of 230 °C and under a vacuum of 0.2 kPa for 30 min, utilizing 0.2% steam.

Determination of phosphorus, iron, gossypol, tocopherols and fatty acid composition of vegetable oils

The content of phospholipids was determined as the total phosphorus on a vegetable oil according to AOCS Official Method Ca 12–55 (AOCS. Official Method Ca 12–55. 1994). Iron was also determined following AOAC Official Method 990.08 (AOAC. Official method 990.08. 2003). The gossypol content in cottonseed oil was determined by AOCS Official Method Ca 13–56 (AOCS. Official Method Ca 13–56. 1993). The total tocopherol content in cottonseed oil was determined be AOCS Official Method Ce 8–89 1993). The fatty acid composition of cottonseed oil was determined by a capillary gas chromatography (Niklová, I., Schmidt, Š., Habalová, K., Sekretár, S., 2001; Christopherson, S. W., Glass, R., 1969).

Determination of oxidative stability

To monitor the oxidation of cottonseed oils, Rancimat (743, Metrohnm, Herisau, Switzerland) apparatus was used. Oxidation progression was tracked by measuring the increase in conductivity within a constant volume under isothermal conditions set at 110 °C. The oxidative environment was maintained with air at a flow rate of 20 $l.h^{-1}$. Each sample weighed 3 grams, and we conducted three parallel measurements for every sample.

Results and discusion

The oxidative stability of vegetable oil is significantly influenced by its fatty acid composition. The impact of the fatty acid composition of cottonseed oil, characterized by the presence of fatty acids containing no more than 2 double bonds, cottonseed oil contain 54.9% linoleic acid and 17.2% oleic acid (Tab.1), has a substantial effect on the oil's ability to resist oxidative decomposition. For cottonseed oil, characterized by a low content of 3 double bonds, specifically no more than 0.5% in its fatty acids, higher oxidative stability can be expected compared to oils containing a greater amount of polyunsaturated fatty acids, such as sunflower oil. This is because the double bonds in fatty acids are vulnerable points where oxidative reactions begin. Therefore, cottonseed oil with a low content of double bonds in its fatty acid composition has the potential to provide high oxidative stability. Crude cottonseed oil exhibits an induction period of 7.6–8.7 h, indicating a higher oxidative stability of the oil (Tab.4).

Tab1. *Fatty acid composition (% of area) of cottonseed oil*

Fatty acids		%
Myristonic acid	(14:0)	0. 8
Palmitic acid	(16:0)	23.1
Stearic acid	(18:0)	2.3
Oleic acid	(18:1)	17.2
Linoleic acid	(18:2)	54.9
a-linolenic acid	(18:3–9,12,15)	0.3
y-linolenic acid	(18:3–6,9,12)	0.2
Arachic acid	(20:0)	0.1
Behenic acid	(22:0)	–
Other fatty acids		1.1

Water degumming reduces the phosphorus content to 15.5–22.9% in cottonseed oil (Tab. 2 and Tab. 3), mainly removing easily hydratable phospholipids during water degumming. Phospholipids can be a cause of oxidative processes in the oil, leading to a loss of its stability. Additionally, water degumming also has a positive effect on reducing the iron content in the oil. The water degumming process promotes the formation of complexes between iron and phospholipids, increasing the efficiency of their removal from the oil during refining. As a result, the iron content is reduced to 42.1% in extracted and 30.1% in pressed oil (Tab. 2 and Tab. 3), which contributes to improving the quality of the oil and its long-term stability. During water degumming, some tocopherols can transfer to the aqueous phase and be removed along with phospholipids and other impurities. A reduction in tocopherol content by 3–4% can weaken the oil's antioxidant capacity and make it more vulnerable to oxidative processes. Also, during water degumming, the content of gossypol decreases by 15–18%, which has a positive effect on its quality but also reduces its oxidative stability. The oxidative stability of water degummed oil decreased by almost 2 times comparing with crude oil, with induction periods of 4.06 h for extracted oil and 4.24 h for pressed oil (Tab. 4).

Table 2. *Influence of water degumming, neutralization, bleaching and deodorization processes on phosphorus, iron, tocopherols and gossypol removal from cottonseed extracted oil*

Vegetable oils	Cottonseed extracted oil							
	Phosphorus		Iron		Tocopherols		Gossypol	
	[mg.kg⁻¹]	[%]	[mg.kg⁻¹]	[%]	[mg.kg⁻¹]	[%]	[mg.kg⁻¹]	[%]
Crude oil	713.4	100	7.6	100	776.7	100	2364.1	100
Water degummed	110.4	15.5	3.2	42.1	752.1	96.8	1938.5	82.1
Neutralized	19.5	2.7	0.9	11.8	691.3	89.0	312.1	13.2
Bleached	12.1	1.7	0.4	5.3	663.2	85.3	120.6	5.1
Deodorazed	11.1	1.5	0.3	3.9	432.1	55.6	119.1	5.0

Table 3. *Influence of water degumming, neutralization, bleaching and deodorization processes on phosphorus, iron, tocopherols and gossypol removal from cottonseed pressed oil*

Vegetable oils	Cottonseed pressed oil							
	Phosphorus		Iron		Tocopherols		Gossypol	
	[mg.kg⁻¹]	[%]	[mg.kg⁻¹]	[%]	[mg.kg⁻¹]	[%]	[mg.kg⁻¹]	[%]
Crude oil	312.4	100	5.3	100	530.7	100	1891.2	100
Water degummed	71.5	22.9	1.6	30.1	514.3	96.9	1607.5	85.1
Neutralized	14.1	4.5	0.6	11.3	477.1	89.9	270.4	14.3
Bleached	9.1	2.9	0.3	5.6	453.4	85.4	115.4	6.1
Deodorized	8.6	2.7	0.2	3.7	294.7	55.5	114.1	6.0

Neutralization. The process of neutralization in cottonseed oil plays a pivotal role in reducing the content of various components, such as phospholipids, iron, tocopherols, and gossypol. Typically carried out using NaOH, the neutralization process aids in the removal of phospholipids from the oil. Caustic soda breaks down phospholipids into insoluble precipitates that can be easily separated from the oil. During the neutralization of extracted cottonseed oil, the phosphorus content decreases to 19.5 mg.kg^{-1}, while in pressed oil, it reduces to 14.1 mg.kg^{-1}. Additionally, the neutralization process contributes to the removal of iron. Caustic soda used in neutralization can form insoluble complexes with iron, making it more suitable for removal from the oil. The iron content in extracted oil decreases to 0.9 mg.kg^{-1}, and in pressed oil, it drops to 0.6 mg.kg^{-1}. However, the neutralization process also reduces the content of tocopherols by 10–11% compared to crude oil since they are removed along with other impurities such as phospholipids.

During neutralization process of cottonseed oils, gossypol is removed by 86–87% (Tab. 2 and Tab. 3). The reduction in tocopherol content, along with the presence of gossypol and high temperatures during oil neutralization, decreases the induction period of extracted oil to 3.61 h and pressed oil to 3.37 h (Tab. 4).

Table 4. *Influence of refining processes on oxidative stability of cottonseed oils*

Cottonseed oils	Oxidative stability of cottonseed oils	
	Extracted oil IP (h)	Pressed oil IP (h)
Crude oil	8.27	7.67
Water degummed	4.24	4.06
Neutralized	3.61	3.37
Bleached	3.18	2.97
Deodorized	2.55	2.47

Bleaching. Bleaching of cottonseed oil involves the use of adsorbents, such as bleaching clay. These materials are capable of adsorbing and removing phospholipids from the oil. After the bleaching process, the phosphorus content in the oils decreased by less than 12.1 $mg.kg^{-1}$. The bleaching process using adsorbents also contributes to the removal of iron and reduces the iron content to less than 0.4 $mg.kg^{-1}$. However, the bleaching process reduces the content of tocopherols in the oil by 4–5%. This is because bleaching involves the extraction of oil using adsorbents, and during this extraction, some bioactive components, including tocopherols, may be removed. The bleaching process partially removes gossypol from the oil, which can affect its color and oxidative stability. Bleached oils contain no more than 5–6% gossypol based on crude oil (Tab. 2 and Tab. 3). The removal of gossypol and tocopherols during the bleaching process reduces the oxidative stability of cottonseed oil. The induction period of extracted cottonseed oil was 3.18 h, while that of pressed oil was 2.97 h (Tab. 4).

Deodorization. The deodorization process typically results in the slight removal of iron from the oil, around 0.1 $mg.kg^{-1}$, which contributes to improved oxidative stability. However, during the deodorization process, which involves heating the oil at high temperatures and steam treatment, tocopherols can undergo decomposition. High temperatures and steam exposure can lead to the thermal degradation of tocopherols, resulting in their loss in the oil. Tocopherol losses are significant, accounting for approximately 30% of the total tocopherol content in crude oil. The substantial loss of tocopherols leads to a reduction in the oxidative stability of the oils. Deodorized cottonseed oils have the lowest oxidative stability, with an induction period of 2.47–2.55 h (Tab. 4).

Conclusion

The goal of this paper is analyse the effects of refining processes on the cottonseed oil oxidative stability with the Rancimat device. At each stage of refining process, undesirable components such as iron, phospholipids and gossypol are removed, but natural antioxidants like tocopherol are also eliminated. According to the results, we have found that crude cottonseed oil exhibits three times greater oxidative stability compared to deodorized cottonseed oils. The degumming process reduces the oxidative stability of oils by nearly half. Neutralization and subsequent oil bleaching have a minor impact on the oxidative stability of oils. During the deodorization process, the most significant loss occurs in tocopherols, resulting in the lowest oxidative stability of the oils. The oxidative stability of cottonseed oil is profoundly affected by its fatty acid composition and various refining stages. Understanding these factors is crucial for optimizing the quality and shelf life of cottonseed oil-based products.

Acknowledgement

This project was supported by Tashkent Institute of Chemical Technology and the Uzbek Academy of Sciences (Grant No. 0412/23).

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submitted 30.10.2024;
accepted for publication 14.11.2024;
published 30.01.2025;
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