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Effect of Pullulan Derived from *Micrococcus luteus* on Preserving Selected Properties of Sunflower Oil

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Abstract

This study evaluates the efficacy of pullulan, a bacterial polysaccharide extracted from a local *Micrococcus luteus* isolate, in preserving the oxidative stability of unrefined sunflower oil. Using peroxide value (PV) and thiobarbituric acid value (TBA) as metrics. Five treatments were compared against a traditional antioxidant, butylated hydroxytoluene (BHT), and a control. Results demonstrated that the use of 800 ppm pullulan significantly inhibited oxidative degradation of the unrefined sunflower oil, particularly at 20°C storage. Peroxide values for 800 ppm pullulan (1.91 mEq.kg^{-1} at 20°C; 2.88 mEq.kg^{-1} at 50°C) were slightly higher than BHT (1.82 and 1.99 mEq.kg^{-1}) but markedly lower than the control (4.49 and 3.59 mEq.kg^{-1}). Thiobarbituric acid values for 800 ppm pullulan (0.38 mg MDA.kg^{-1} (malondialdehyde) at 2°C at day 40, and at 50°C at day 30, were marginally lower than BHT (0.43 and 0.47 mg MDA.kg^{-1}) and significantly reduced versus the control (0.51 and 1.42 mg MDA.kg^{-1}), respectively.

Keywords: exopolysaccharides, *Micrococcus luteus*, oil oxidation, sunflower oil

Introduction

Pullulan is a polysaccharide produced by the fungus *Aureobasidium pullulans*. This fungus is colorless, tasteless, odorless, biodegradable, and non-toxic, making it suitable for a wide range of industrial applications. Pullulan is primarily used in the food, pharmaceutical, and cosmetic industries, where it serves in the manufacture of edible films, dissolvable tablets, capsule coatings, adhesives, and biodegradable packaging. It also functions as a thickener or stabilizer in various products.

The use of natural substances with antioxidant properties, whether of plant or microbial origin, has become increasingly popular in research. Exopolysaccharides (EPS), especially those produced by bacteria, have emerged as promising alternatives to synthetic compounds due to their lower purification complexity compared to plant-derived polysaccharides, as well as their low cost, biodegradability, and non-toxicity (Chalob and Abdul-Rahman, 2018; Omar and Awda, 2024). EPSs are utilized as cleaning and emulsifying agents in various industries, including food, cosmetics, pharmaceuticals, bioremediation, petroleum, and paints, providing safe and environmentally friendly solutions (Fadhil and Mousa, 2021).

Micrococcus species, found in diverse environments including soil (Kridi and Al Zoubi, 2021), are notable for their ability to degrade xenobiotics and synthesize antioxidant and antimicrobial compounds. *Micrococcus luteus*, in particular, is highly resistant to harsh conditions (Chang et al., 2024). Microbial xopolysaccharides, whether homo- or heteropolysaccharides, are valuable for various industrial applications (Kadhum and Haydar, 2020).

Aureobasidium pullulans produces a highly water-soluble, tasteless, odorless, organic, and biodegradable polymer. Pullulan is recognized as safe, a U.S. FDA designation for substances deemed safe by experts under intended conditions of use due to its non-carcinogenic, non-toxic, non-immunogenic, and non-mutagenic properties, which have contributed to its widespread commercial acceptance. Pullulan derivatives exhibit high heat resistance and a broad range of viscosities and solubilities. Research indicates that the production of pullulan by *M. luteus* can be enhanced under optimized conditions (Chalop and Mousa, 2025). Pullulan degrades at temperatures between 250°C and 280°C, exhibits excellent film-forming ability, and possesses high mechanical strength. It can be utilized to produce thin films, nanoparticles, nanofibers, and flexible coatings. These characteristics make pullulan a crucial microbial polymer and a viable alternative to both natural and synthetic polymers (Nedunchezhiyan et al., 2022).

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Sunflower oil, the third most widely consumed edible vegetable oil globally, is a key raw material for the chemical and food industries. In Iraq, sunflower is a priority crop for meeting the nation's oil needs. However, fatty food products, especially meat products, are susceptible to degradation through lipid oxidation during processing and storage. Lipid oxidation leads to rancidity, off-flavors, discoloration, and the formation of toxic compounds such as 4-hydroxynonenal (Trabelsi et al., 2018).

The peroxide value is commonly used to assess oil quality and monitor oxidative degradation over time. However, it does not fully reflect the overall quality of the product. Therefore, additional tests, such as the Thiobarbituric Acid (TBA) assay, are employed for a more comprehensive evaluation of antioxidant effects (Al-Mousawi, 2021).

This study was conducted to evaluate the effect of adding pullulan to unrefined sunflower oil to improve its oxidative stability and extend its shelf life. The pullulan antioxidant properties were assessed by measuring peroxide and TBA values of oils stored at 20°C and 45°C and compared to those of oils treated with synthetic antioxidants and untreated controls. The use of bacterial pullulan in enhancing the quality of sunflower oil was determined.

Sunflower oil was chosen for its wide availability, relatively low cost, and well-characterized chemical composition, making it an ideal model substrate for evaluating biosurfactant or bio emulsifier production and activity. Its richness in triglycerides and fatty acids provides an excellent carbon source for microbial growth and the production of metabolites. Additionally, sunflower oil's hydrophobic nature makes it suitable for assessing emulsification efficiency and stability, which are critical factors in determining the effectiveness of microbial biosurfactants.

Materials and Methods

The sunflower oil used in this study was prepared through cold mechanical extraction of seeds obtained from the local market in Ghazaliya (Al-Hamza Attariya), Baghdad. Following extraction, the oil was filtered to remove any solid residues and then bottled in suitable plastic tubes. To preserve its quality during transport, the oil was kept at low temperatures and protected from light.

Pullulan, previously extracted and purified from a local isolate of the bacterium *Micrococcus luteus*, was used in this study. The purified pullulan was stored in powder form, as shown in Figure 1. Several concentrations of pullulan (100, 200, 300, 600, and 800 ppm) were prepared for treating the unrefined sunflower oil. Additionally, a treatment with the synthetic antioxidant butylated hydroxytoluene (BHT) at a concentration of 200 ppm was included as a positive control, as this is a common standard in similar studies.

The prepared oil samples were divided into two groups to study the effect of temperature on storage stability. The first group was stored at 20°C, while the second group was stored at 45°C. Both groups were monitored over 30 days. For the group stored at 20°C (±2°C), peroxide and thiobarbituric acid (TBA) tests were conducted every 10 days. For the group stored at 45°C (±5°C), these tests were performed every 7 days, following the guidelines of FAO (2019).

The peroxide value (PV) of the oil samples was determined using the American Oil Chemists Society (AOCS) method Cd 19-90 (AOCS, 2001). In this procedure, 5 grams of oil from each treatment were mixed with 30 mL of a solution containing acetic acid and chloroform (in a 3:2 ratio) in a 250 mL conical flask. After stirring, 0.5 mL of a saturated potassium iodide (KI) solution was added, and the mixture was stirred for an additional 2 minutes. The solution was then diluted with 30 mL of distilled water, and three drops of 1% starch solution were added as an indicator. The mixture was titrated with 0.01 N sodium thiosulfate until the color changed from purple or black to pale yellow. The peroxide value was calculated using the following formula:

$$Peroxide\ value(meq.kg^{-1}\ oil) = \frac{Volume\ of\ sodium\ thiosulfate\ x\ Normality\ x\ 1000}{sample\ weight\ (g)}$$

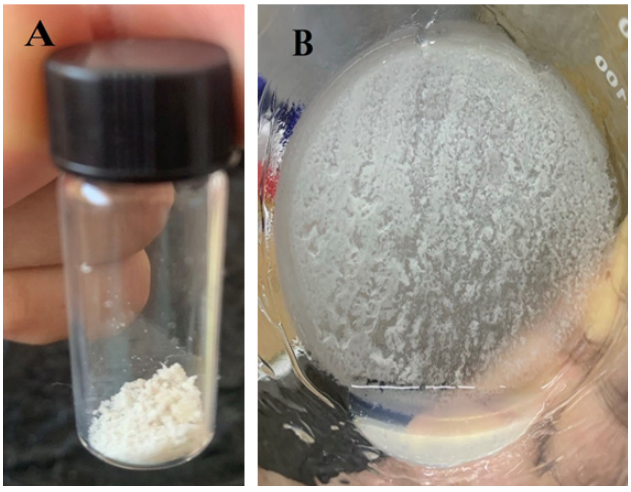

Figure 1. The purified pullulan powder (A) and unpurified wet pullulan (B).

For the determination of thiobarbituric acid (TBA) values, the AOCS method Cd 19-90 (AOCS, 2001)

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was also followed. A 0.2% TBA reagent solution was freshly prepared by dissolving 0.5 g of 2-thiobarbituric acid in a 250 mL volumetric flask and diluting to the mark with 1-butanol. Oil samples were dissolved in 1-butanol and adjusted to a final volume of 200 mL. From this solution, 5 mL was transferred to a boiling-resistant glass test tube with a tightly closed cap, to which 5 mL of the TBA reagent was added. The mixture was shaken using a vortex shaker. A blank control was prepared in the same manner, omitting the oil. The tubes were incubated in a water bath at 95°C for 2 hours, then cooled to room temperature. Absorbance was measured at 530 nm using a spectrophotometer, and the TBA value was calculated as milligrams of malondialdehyde per kilogram of oil.

$$Thiobarbituric\ acid\ value\ (TBA) = \frac{[50^{*}\ Absorbance\ of\ sample\ -\ Absorbance\ of\ control\ sample]}{Sample\ weight\ (mg)}$$

Results and Discussion

Peroxide Values at 20°C Storage

The peroxide test is a chemical assay used to determine the extent of lipid oxidation in oils and fats. Its primary purpose is to measure peroxides formed because of lipid oxidation, a process that leads to spoilage and deterioration of oil quality. This test is widely used in the food industry to ensure the quality of oils and fats incorporated into food products. In the oil industry, it helps assess the quality of both crude and refined oils. In scientific research, it is employed to study lipid oxidation and its effects on health and product quality. Lipid oxidation typically occurs during the processing and storage of food products, making the peroxide value (PV) a key parameter for monitoring the initiation of oxidation and expressing the presence of primary lipid oxidation products.

Table 1 in this study shows that the peroxide values of sunflower oil stored at 20°C varied depending on the concentration of pullulan added. Notably, the peroxide value decreased as more pullulan was incorporated into the oil. For example, on the seventh day, oil samples treated with 800 ppm pullulan exhibited a peroxide value of 1.1 mEq.kg^{-1}, which was lower than both the samples treated with synthetic antioxidant (BHT) and the control samples, the latter of which reached 2 mEq.kg^{-1}. This reduction is attributed to the antioxidant properties of Pullulan, a sugar polymer classified as an exopolysaccharide (EPS), which inhibits the rapid increase in peroxide values as lipid oxidation progresses (Liu et al., 2018).

Supporting evidence from other studies further underscores the effectiveness of pullulan. For instance, fish oil treatments covered with pullulan films exhibited lower peroxide values over 45 days of storage at 20°C compared to those covered with starch or lactose. pullulan forms strong, compact films with fiber structures that envelop the oil, restricting oxygen movement and reducing the frequency of oil droplet collisions (Koç et al., 2010). Additionally, polysaccharides like pullulan can chelate metal ions, thereby inhibiting lipid oxidation. For example, tragacanth gum has demonstrated radical scavenging ability due to its hydrogen-donating properties (Paraskevopoulou et al., 2007). The increased viscosity from higher pullulan concentrations further obstructs free radical propagation, preventing their reaction with unsaturated fatty acids. Sihame et al. (2024) also noted that EPSs possess antioxidant activity, functional and emulsifying properties, and strong stabilizing effects in a concentration-dependent manner.

The comparative analysis of peroxide values between control oil samples and those treated with various concentrations of pullulan reveals a consistent trend: all pullulan-treated samples exhibited lower peroxide values, even at lower concentrations, than those treated with synthetic antioxidants. This underscores pullulan's efficient inhibitory effect on oil oxidation, aligning with prior findings that EPS polysaccharides can reduce both primary and secondary oxidation in oil coatings (Liu et al., 2018). For example, polysaccharide-coated shrimp fillets maintained lower peroxide values throughout refrigerated storage compared to uncoated fillets (Balti et al., 2020). The control oil samples, in contrast, exhibited higher peroxide values and more pronounced increases in polyunsaturated fatty acid decomposition products, resulting from unmitigated oxidation.

Lipid auto-oxidation is initiated by the direct reaction of molecular oxygen with unsaturated fatty acids, resulting in the formation of free radicals and hydroperoxides. The presence of EPS polysaccharides, such as pullulan, reduces the formation of oxidation products by limiting gas transport and providing antioxidant protection (Balti et al., 2020). Pure pullulan and agar films have demonstrated antioxidant activity due to bioactive functional groups within their biopolymer chains (Roy and Rhim, 2023). Pullulan has also been shown to stabilize emulsions and enhance the stability of cooked beef sausages during storage (Trabelsi et al., 2018). Generally, lower storage temperatures and shorter storage times help preserve the fat fraction, while high-quality unsaturated fatty acids remain susceptible to oxidative rancidity, leading to the formation of primary and secondary oxidation products that negatively affect quality (Suárez-Medina et al., 2024).

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Further research supports the role of pullulan as an oxygen barrier. For instance, gelatin and pullulan films applied to fish liver oil samples resulted in lower peroxide values compared to uncoated samples, as the membrane restricted oxygen access (Li et al., 2022). Since hydrogen peroxides are relatively unstable and decompose into secondary oxidation products, the presence of pullulan helps maintain lower peroxide values. The results of this study confirm that increasing pullulan content in oil treatments leads to a smaller rise in peroxide values during storage. Additionally, sunflower oil's natural antioxidants, such as beta-sitosterol and α-tocopherol, contribute to its oxidative stability during storage.

It is also worth noting that the addition of honey to cream and meat has been reported to decrease peroxide values, highlighting honey's role in preventing the formation of oxidative compounds (Mousa et al., 2024). Enzymatic degradation of triglycerides and phospholipids by lipases and phospholipases increases the percentage of free fatty acids, which are more prone to oxidation. The oxidation of free fatty acids leads to the formation of hydroperoxides and peroxides, which are primary products of lipid oxidation and precursors to secondary products such as aldehydes, ketones, and alcohols—many of which are responsible for off-flavors (Suárez-Medina et al., 2024).

Throughout storage, all oil treatments exhibited a gradual increase in peroxide values, which is typical for sunflower oil due to its high content of unsaturated fatty acids, such as oleic and linoleic acids. Oxidation in high-linoleic acid sunflower oil occurs primarily at the linoleic position, while high-oleic acid sunflower oil sees a similar depletion of oleic acid. The breakdown of these unsaturated fatty acids leads to increased formation of hydrogen peroxides and hydroperoxides, resulting in higher peroxide values (Marmesat et al., 2009). Nevertheless, in this study, the peroxide values of all oil samples remained within acceptable limits throughout the storage period. According to the Iraqi standard specification for vegetable oils, the permissible limit is 10 mEq.kg^{-1} oil (Al-Moussawi, 2021), while the Codex standard for virgin oils is 15 mEq.kg^{-1} (Moigradean et al., 2012).

Peroxide Values at 45°C Storage

Values in Table 2 reflect a direct variation of the percent composition of Pullulan added to sunflower oil. Stored at 45°C, the peroxide value of the sunflower oil decreased. The first few days saw the peroxide value rise to 1.60 (mEq.kg^{-1} oil) for the samples of oil to which 800 ppm of pullulan had been added. This was then recorded at a level below that, 1.30 and 2.46 mEq.kg^{-1} oil, for the unrefined sunflower oil and the control synthetic antioxidant, respectively. The polysaccharide pullulan had lowered the oxidation processes in the oil, and this fact is further supported by the increase in peroxide values, which are much

Table 1. Peroxide values for oil stored at 20°C

Storage period (in days)	Peroxide values (mEq.kg^{-1} oil)						
	Control samples oil without additives	Synthetic antioxidant sample (200 ppm)	Pullulan-added samples (ppm)				
			800	600	300	200	100
0			0.8				
7	2.00	1.19	1.10	1.07	1.10	1.187	1.18
15	2.80	1.39	1.35	1.60	1.80	1.220	1.98
21	3.59	1.66	1.60	1.79	2.19	1.600	2.78

Table 2. Peroxide values for oil stored at 45°C

Storage period (in days)	Peroxide values (mEq.kg^{-1} oil)						
	Control samples 0il without additives	Synthetic antioxidant sample (200 ppm)	Pullulan-added samples (ppm)				
			800	600	300	200	100
0			0.6				
7	1.54	1.03	0.80	0.80	0.90	1.13	1.35
15	2.46	1.44	0.81	1.17	0.96	1.65	1.53
21	2.38	1.29	1.50	1.89	1.50	1.50	1.98
30	2.50	1.58	1.51	1.50	1.58	1.84	2.56

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slower with higher pullulan addition up to 800 ppm. The chemical composition, storage temperatures, oxygen, and even packaging materials, in some cases, can initiate rancid oxidative changes in oils, as well as light. Indeed, rancidity in oils originates from free radical reactions; these reactions can also be triggered by light, utilizing an extremely active oxygen atom. It has been proven that, for at least 90 days at room temperature, fats will decompose to form their oxidation products, thereby increasing the peroxide value. Oils decompose to release ketone acids at a temperature above 30°C; autoxidation and the decomposition of free radical products.

Temperature, light, and oxygen-dependent; in other words, these factors accelerate the process of lipid oil microencapsulation by stimulating the formation of free radicals, thereby promoting the decomposition and aggregation of hydrogen peroxide, a strong prooxidant. In this study, these factors had a short induction period before the oxidation process sharply attacked the oil microcapsules. On the storage aspect, however, EPSs, the expanded polysaccharides, contributed a good protective effect to the coating system, as evidenced by a lower peroxide value (PV) (Liu et al., 2018). For 12 days of storage at 40°C, the walnuts coated with polysaccharide films gave lower values in terms of peroxides, thiobarbituric acid reactive substances, and free fatty acids than those coated with polyethylene films, which may further support the ability of polysaccharide coatings in inhibiting lipid oxidation in walnuts (Wang et al., 2023). In addition, Kodali and Sen (2008) found that bacterial exopolysaccharides from probiotics possess antioxidant activities that prevent damage caused by free radicals. This activity was revealed to be comparable to that of vitamin E. The results of our work agree with those of Al-Moussawi (2021). The latter author found that levan polysaccharides retarded the increase in peroxide values for the oil on storage at 65°. Levan is a type of fructan polysaccharide of fiber found in plants and microorganisms. It is an antioxidant that can reduce the peroxide formed during storage, as well as lose the free radicals that might be formed in the oil by complexing with metal ions. Moreover, the increase in peroxide values was retarded in all by further concentrations of levan in the oil. The relationship between temperature and the kinetic energy and viscosity of the liquid is assumed to be indirect for kinetic energy and direct for viscosity. The viscosities of vegetable oil, surface tension, and dynamic viscosity decrease with an increase in temperature (Gandova et al., 2024). In many such cases, polysaccharides protect lipids from peroxides, most likely by enabling these compounds to be adsorbed on the surface of the oil droplet, thus forming a high-viscosity and surface shear-strength layer. Lipe peroxides are surfactants that are easily activated due to catalyzed oxidation reactions (Paraskevopoulou et al., 2007).

Since fats and oils are less soluble in oxygen and water, less oxygen will be consumed. Therefore, room temperature, time, and light should be considered in storing oil. There should also be a minimum of outside air contact. Temperature, pressure, humidity, concentration, and flow rate all affect viscosity, and consequently, the quality of sunflower oil. In their study, Choe and Min (2009) noted that the rapid increase to its maximum level in their samples stored at elevated temperatures may be attributed to the influence of heat, as thermal oxidation occurs. Very high temperatures result in many chemical reactions inside the oils, including oxidation. Considering the total oil parameters provided, it can be concluded that the use of a high temperature of 45°C in the storage of unrefined sunflower oil is not suitable since a gradual increment in peroxide value characterized it as the level of oxidation, viscosity, and kinetic energy of molecules including fat-dissolving enzymes oxidation products, and free radicals, increased. However, the addition of pullulan to the oil retards this increase in peroxide values, making it a significant factor in hindering oxidation. Therefore, the peroxide values of the oil parameters remained within acceptable limits until the last day.

Thiobarbituric Acid Assay for Oil Stored at 20°C

Thiobarbituric acid (TBA) values are widely regarded as a reliable indicator for monitoring the extent of lipid peroxidation in oils and oil-rich foods. This test specifically measures the concentration of malondialdehyde (MDA), a secondary product formed from the breakdown of primary lipid hydroperoxides. As MDA is a final product of primary hydroperoxides, its quantification offers insight into the degree of oxidative deterioration occurring within the oil.

The results presented in Table 3 demonstrate that TBA values in sunflower oil stored at 20°C varied according to the amount of pullulan added. Notably, TBA values were inversely proportional to pullulan concentration. For instance, on the tenth day, oil samples with 800 ppm of pullulan exhibited a TBA value of 0.81 mg malondialdehyde per kg oil, which was the same as on day one and also matched the value for samples with 600 ppm pullulan. This suggests that these concentrations of pullulan were optimal in minimizing lipid peroxidation. In contrast, oil samples treated with a synthetic antioxidant reached a level of 1.44 mg malondialdehyde per kg oil, while the control samples peaked at 2.46 mg malondialdehyde.kg^{-1} oil.

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These findings are consistent with previous studies. For example, shrimp treated with polysaccharide (EPS) coatings exhibited significantly lower TBA values compared to uncoated shrimp. The EPS coatings act as barriers, slowing the diffusion of oxygen, water, and other volatile compounds, thereby retarding lipid peroxidation and enhancing both oxidative stability and shelf life. Similarly, in cooked beef sausages, the addition of EPSs slowed the increase in TBA values during refrigerated storage, often outperforming vitamin C as an antioxidant.

In the present study, the control sunflower oil samples exhibited a rapid increase in TBA values, rising from 0.37 on the first day to 0.41 mg malondialdehyde.kg^{-1} oil by the tenth day, and continued to increase more quickly than samples supplemented with pullulan or synthetic antioxidants. This can be attributed to the unrefined nature of the sunflower oil, which contains high levels of unsaturated fatty acids that are particularly susceptible to oxidation (Gandova et al., 2024). Factors such as the presence of unsaturated fatty acids, vitamin E, and catalytic metals like iron can further accelerate lipid oxidation, resulting in the formation of hydroperoxides, aldehydes, and ketones, which degrade the color, flavor, and nutritional value of the oil (Awad, 2019).

Pullulan proved effective in retarding the rise in TBA values, even at low concentrations, compared to the control samples. As an extracellular polysaccharide, pullulan may act as a potent reductant, supplying electrons needed to neutralize oxygen radicals such as hydrogen peroxide or superoxide, thus inhibiting lipid peroxidation during storage (Trabelsi et al., 2018). Other studies have shown that Chito oligosaccharide polymers with alpha-tocopherol can suppress the degradation of polyunsaturated fatty acids, resulting in high oxidative stability and low TBA values over extended storage periods

The mechanism by which polysaccharides inhibit lipid oxidation involves their emulsifying properties, reduction of surface tension, and ability to chelate metal ions—particularly Fe^{2+}, which catalyzes lipid peroxidation. For example, xanthan gum has been shown to inhibit Fe^{2+}-dependent lipid oxidation by chelating iron at its negatively charged sites. Thus, the addition of polysaccharides, such as Pullulan, to oils can lower both peroxide and TBA values.

Regardless of the proportion of Pullulan added, the rise in TBA values during cold storage indicated that some lipid oxidation still occurred, albeit at a slower rate. The extent of oxidation depends on various factors, including the type of oil, storage conditions, and duration. Oils rich in polyunsaturated fatty acids are especially prone to oxidation and the formation of spoilage products. However, the addition of polysaccharides effectively delayed these changes, primarily by forming a protective polymer layer that limits oxygen diffusion (Suárez-Medina et al., 2024).

It is essential to note that the oil samples in this study did not contain antimicrobial agents, which allowed for a gradual yet steady increase in TBA values. Although low-temperature storage is not conducive to rapid microbial growth, it does not completely halt the activity of spoilage bacteria, such as Pseudomonas, which secrete lipolytic enzymes like lipases and

Table 3. Thiobarbituric acid values for oil stored at 20°C

Storage period (in days)	Peroxide values (mEq.kg^{-1} oil)						
	Con. samples	BHT sample	Pullulan-added samples (ppm)				
			800	600	300	200	100
0	0.37	0.37	0.37	0.37	0.37	10	0.37
10	0.41	0.40	0.37	0.37	0.38	20	0.40
20	0.44	0.41	0.37	0.39	0.40	30	0.42
30	0.47	0.41	0.38	0.40	0.40	40	0.44

Table 4. Thiobarbituric acid values for oil stored at 45°C

Storage period (in days)	Peroxide values (mEq.kg^{-1} oil)						
	Con. samples	BHT sample	Pullulan-added samples (ppm)				
			800	600	300	200	100
0	0.37	0.37	0.37	0.37	0.37	10	0.37
10	0.42	0.37	0.37	0.38	0.38	0.39	0.40
20	0.48	0.38	0.37	0.40	0.34	0.40	0.41
30	0.50	0.42	0.37	0.40	0.42	0.44	0.46

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phospholipases. These enzymes hydrolyze esters, increasing the proportion of free fatty acids and thus the oil's susceptibility to oxidation (Golvardzadeh and Yasini, 2016).

Other research supports these findings. For example, burger samples treated with modified cellulose exhibited a decreasing trend in TBA values, whereas control samples showed significant increases over time. This trend is attributed to the breakdown of peroxides and the subsequent formation of aldehydes and ketones, which reduce peroxide numbers but raise TBA values as secondary oxidation products accumulate.

In summary, the addition of pullulan to sunflower oil significantly retarded the increase in TBA values during storage, confirming its effectiveness as a natural antioxidant. This protective effect is due to Pullulan's ability to limit oxygen diffusion, chelate pro-oxidant metals, and inhibit the propagation of lipid peroxidation, thereby preserving the quality and shelf life of oil-rich foods.

Thiobarbituric Acid Assay for Oil Stored at 45°C

Table 4 presents the variation in thiobarbituric acid (TBA) values observed in unrefined sunflower oil stored at 45°C, depending on the percentage of pullulan added. The results clearly demonstrate that the addition of pullulan inhibited the increase in TBA values, indicating a reduction in lipid peroxidation. This effect was evident from the earliest days of storage: on the seventh day, oil samples with 800 ppm Pullulan exhibited a TBA value of 0.37 mg malondialdehyde per kg oil, which was similar to the value on day one and nearly identical to the 600 ppm pullulan samples (0.38 mg malondialdehyde.kg^{-1} oil). These findings suggest that higher concentrations of pullulan are particularly effective at suppressing oxidative degradation at elevated temperatures. Notably, these TBA values were also comparable to those observed in samples treated with synthetic antioxidants (0.37 mg malondialdehyde.kg^{-1} oil), while the control samples without any antioxidant reached 0.42 mg malondialdehyde.kg^{-1} oil. This highlights pullulan's potential as a natural alternative to synthetic antioxidants, offering protection without compromising the sensory or qualitative properties of the oil.

Over the course of storage at 45°C, the inhibitory effect of Pullulan became even more pronounced. By the end of the storage period, oil samples with 800 ppm pullulan had a TBA value of just 0.38 mg malondialdehyde.kg^{-1} oil, compared to 0.47 mg for the synthetic antioxidant-treated samples and 1.42 mg for the control. This trend is consistent with findings by Le Priol et al. (2022), who reported that the inclusion of polysaccharides in oil formulations increased viscosity, improved emulsion droplet size, and reduced oxygen diffusion, thereby enhancing oxidative stability during long-term storage. The formation of a glassy polysaccharide layer acts as a barrier, slowing down oxygen penetration and protecting the oil from rapid oxidation.

Recent studies have further demonstrated that combining polysaccharides with tocopherols can inhibit lipid oxidation, even under accelerated high-temperature conditions, thereby improving the oxidative stability of crude oils (Ma et al., 2021). Tocopherols, which are naturally present in unrefined sunflower oil, are recognized as effective antioxidants (Baqir et al., 2024). The relatively low TBA values observed in pullulan-treated samples, especially at 800 ppm, suggest a synergistic antioxidant effect between pullulan and the natural tocopherols in the oil, often outperforming synthetic antioxidants.

The control samples, which lacked added antioxidants, exhibited the highest TBA value (1.42 mg malondialdehyde.kg^{-1} oil) after 10 days of storage at 45°C. This is expected, as unrefined sunflower oil is rich in unsaturated fatty acids and, when stored at high temperatures, is highly susceptible to oxidation (Koç et al., 2010). Despite the protective effects of pullulan and synthetic antioxidants, all oil samples stored at 45°C showed increased TBA values by the end of the storage period, underscoring the challenges of maintaining oil stability under such harsh conditions. The high content of polyunsaturated fatty acids in sunflower oil, combined with exposure to heat, oxygen, and light, accelerates oxidative degradation. The structure of these fatty acids, particularly the presence of methylene bridges between double bonds, makes them especially vulnerable to free radical attack and subsequent lipid peroxidation.

These results align with those of Al-Moussawi (2021), who found that the polysaccharide levan could inhibit the rise in TBA values in sunflower oil at even higher temperatures (65°C), performing comparably to synthetic antioxidants. However, the effect of polysaccharides is concentration-dependent, with higher concentrations providing greater inhibition of TBA value increases. As storage progresses, hydroperoxides decompose into smaller molecules, and their reactions with acids or bases yield various organic compounds, which may explain the observed trend of low peroxide values but high TBA values at the end of storage.

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Temperature is a critical factor influencing oil quality, with even slight increases leading to significant changes in physical and chemical properties. In this study, the highest TBA values were observed in control samples, while samples treated with synthetic antioxidants or higher concentrations of pullulan consistently showed lower TBA values. For samples treated with only 100 ppm pullulan, the TBA values increased more rapidly than those with higher pullulan concentrations. Still, they remained lower than the control throughout the storage period, reaching 0.48 mg malondialdehyde.kg^{-1} oil by the end. This is slightly higher than the synthetic antioxidant-treated samples (0.47 mg), reflecting the limited protective effect at low pullulan concentrations.

The ability of polysaccharides to inhibit lipid oxidation is linked to their capacity to increase viscosity, which slows the movement of reactants and retards the oxidation process (Paraskevopoulou et al., 2007). Under conditions of increased viscosity, the stability of lipids against oxidation is enhanced. However, high temperatures also accelerate the decomposition of peroxides, leading to increased TBA values at the end of storage (Farhoosh and Moosavi, 2009).

In summary, the addition of pullulan, particularly at higher concentrations, significantly enhanced the oxidative stability of unrefined sunflower oil when stored at elevated temperatures. These findings support the use of pullulan as a natural antioxidant in food oils, contributing to improved product quality, enhanced food safety, and reduced health risks associated with lipid oxidation.

Conclusions

This study has demonstrated that the addition of pullulan polysaccharide to sunflower oil can significantly retard undesirable changes due to its potent antioxidant activity, with benefits observed across microbiological, chemical, and physical parameters. The enhancement of oxidative stability was directly proportional to the concentration of pullulan added, with 800 ppm proving the most effective and 100 ppm proving the least effective. This approach offers a safe and effective method for preserving the quality of oils and the foods to which they are added. Sunflower oil fortified with exopolysaccharide pullulan consistently exhibited lower peroxide and thiobarbituric acid (TBA) values throughout storage, indicating improved resistance to oxidative degradation. The use of pullulan thus represents a promising strategy for inhibiting the thermal oxidation of sunflower oil, particularly under elevated storage temperatures such as 45°C. This work highlights the potential for replacing synthetic antioxidants with a natural, biodegradable biopolymer. Pullulan not only preserves oil quality but also aligns with the growing demand for health-conscious, environmentally friendly food additives. The presence of vitamins, minerals, and functional groups within the pullulan structure further broadens its application prospects. In summary, the findings support the feasibility of using pullulan-based edible coatings in food packaging and preservation. Pullulan's eco-friendly nature and effectiveness in maintaining oil stability make it a valuable addition to the food industry, paving the way for safer and more sustainable food preservation solutions.

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