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# The Effect of Olive Storage Treatments on Quality and Oxidative Indices of Olive Oil

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**ABSTRACT**

Storing olives in brine and vinegar to enhance flavor and extend shelf life is one of the most common methods. However, these additives can influence the quality of olive oil. In this study, the effects of salt (10% and 20%) and vinegar (3% and 5%) on the physicochemical properties of olive oil were examined over different storage periods (days 1, 30, 60, and 90). Indicators such as pH, acidity, peroxide value, anisidine value, totox index, total phenolic compounds, and antioxidant activity were measured. Results showed that pH decreased over time. The lowest pH (4.8) was observed in the treatment with 20% salt and 5% vinegar on day 90, while the highest pH (8.1) was recorded in the treatment with 10% salt and 3% vinegar on day 1. The peroxide value of the oil on day 1 in the treatment with the lowest concentrations of salt and vinegar was 2.7 meq/kg, which increased to 28.3 meq/kg by the end of the second month in the treatment with the highest concentrations. Anisidine and Totox indices also increased with higher concentrations of salt and vinegar and over time, especially during the first month. Increasing salt concentration led to a rise in phenolic compounds and antioxidant activity, whereas higher vinegar concentration and longer storage duration caused a decline in these parameters.The highest phenolic content (3480 mg/kg) was recorded in the treatment with 20% salt and 3% vinegar on day 1, while the lowest (2731 mg/kg) was observed in the treatment with 10% salt and 5% vinegar on day 90. Antioxidant activity decreased from 44% in the treatment with high salt and low vinegar on day 1 to 19% in the treatment with low salt and high vinegar on day 90. To maintain the oxidative stability of olive oil, using a high concentration of salt (20%) and a low concentration of vinegar (3%) is recommended.

## 1- Introduction

Olive (*Olea europaea*) belongs to the Oleaceae family. Iran is considered one of the oldest regions for olive cultivation in the world, with the introduction of this oilseed dating back approximately 900 years in provinces such as Gilan, Qazvin, and Zanjan. Today, in addition to these provinces, olive is also cultivated in Golestan and Fars [1]. Studies by Shavakhi et al. (2020) have shown that olives grown in the highlands of Northern provinces like Zanjan possess the highest oleic acid content and the lowest levels of saturated fatty acids [2]. In general, comparisons between domestic and foreign olives indicate that Iranian olives contain higher levels of linoleic acid, whereas foreign varieties have more oleic acid. This difference results in higher oxidative stability in foreign olives. However, due to the higher linoleic acid content in Iranian olives—which is classified as an essential fatty acid—the nutritional value of Iranian olives ranks higher. Therefore, controlling the oxidation process in Iranian olive oil requires more precise storage conditions [3].The most important phenolic compounds in olives that contribute to oxidative stability include oleuropein, phenolic alcohols and acids, flavonoids, hydroxytyrosol, and tyrosol. As the fruit ripens, the concentration of some of these compounds—especially oleuropein—decreases. In addition to ripeness, factors such as extraction method, refining processes, storage duration and conditions, shelf life, and processing techniques also influence the concentration of polyphenols [4, 5]. The contribution of fatty acid composition to oxidative stability is estimated at 24%, which is significantly lower than the 51% contribution from polyphenolic compounds. Consequently, even oils with similar fatty acid profiles may exhibit different oxidative stabilities [6].Over the past 20 years, olive oil consumption in Iran has more than tripled, currently reaching 150 grams per capita. However, this figure still falls short of the recommended intake. On the other hand, the average consumption of canned olives in Iran is 450 grams, which is significantly higher than the global average of 245 grams. These statistics suggest that Iranians prefer consuming olives over olive oil. Therefore, by understanding this preference, high-quality olive oil can be indirectly introduced into the Iranian diet through proper olive preservation methods [7]. During storage, olives undergo fermentation by their natural flora at approximately 25°C. This process occurs in brine concentrations of 10–15%, which also helps reduce bitterness. To maintain firmness and crispness, it is recommended to add 3–6% vinegar to the brine solution after processing [8]. Notably, changes in physicochemical and antioxidant properties of olives stored in vinegar and brine at 4°C are being studied and reported for the first time.

## 2- Materials and Methods

### 2.1. Selection of Raw Materials

In early October, healthy and blemish-free olives (yellow cultivar) were manually harvested from 20 trees once they turned dark green. Damaged fruits were removed, and the olives were soaked in a 2% sodium hydroxide solution for 10 hours to allow penetration into two-thirds of the fruit flesh. The olives were then washed for one to two days to completely remove residual sodium hydroxide [8]. The fruits were stored in brine tanks with two salt concentrations (10% and 20%). To enhance flavor and shelf life, vinegar was added to each brine tank at two concentrations (3% and 5%). Oil was extracted from the olives at four intervals (day 0, 30, 60, and 90) during storage at approximately 25°C. All oil-related tests were conducted at the Saman

Payesh Salamat Laboratory in Gilan Province (Viromed).

### 2.2. Preparation and Testing

Oil extraction was performed using a semi-industrial oil press. First, the olives were washed and de-leafed. They were then crushed and transferred to the malaxer (kneading unit) for 30 minutes at 25°C. The resulting paste was centrifuged (6000–8000 rpm), and the extracted oil was filtered for clarity. The olive oils were stored in dark glass bottles (three 1-liter samples) away from light for testing.

### 2.3. Acidity Measurement

This test was conducted according to Iranian National Standard No. 1478. Acidity was calculated based on the amount of sodium hydroxide required to neutralize the free fatty acids in the sample.

### 2.4. Peroxide Value Measurement

Performed according to Iranian National Standard No. 1479. The sample was dissolved in iso-octane and gallic acid, followed by the addition of potassium iodide. The liberated iodine, due to peroxides, was measured iodometrically using starch indicator and sodium thiosulfate solution.

### 2.5. Anisidine and Totox Value Measurement

This test followed Iranian National Standard No. 4093. Absorbance was measured at 350 nm using a Lambda spectrophotometer. The Totox index was calculated as the sum of twice the peroxide value and the anisidine value [9–11]. All results were compared against the limits set for extra virgin olive oil (Iranian National Standard No. 1446) [12].

### 2.6. Determination of Phenolic Compounds

Five grams of sample were soaked in 80 mL of 80% methanol for 48 hours. The solution was filtered and diluted to 100 mL with 80% methanol. One milliliter of the sample was transferred to a 100 mL flask, followed by the addition of 70 mL distilled water and 5 mL of 10% Folin reagent. After stirring for 8 minutes, 15 mL of 20% sodium carbonate solution was added and the volume was adjusted to 100 mL with water. The mixture was kept in the dark for 2 hours. Absorbance was read at 765 nm using a spectrophotometer, and phenol concentration was calculated using a standard curve [13].

### 2.7. Free Radical Scavenging Activity

The antioxidant capacity was measured based on the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals [14]. A 125 mM methanolic DPPH solution was prepared. Five microliters of sample were added to Falcon tubes with 5 mL distilled water and vortexed for 10 seconds. Then, 100 µL of the diluted sample was mixed with 3.9 mL DPPH solution and kept in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer. The percentage of radical scavenging was calculated using the following formula:

% Inhibition = 100 × (Absorbance of Control – Absorbance of Sample) / Absorbance of Control

### 2.8. Statistical Analysis

Analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$) were performed using SPSS software version 22. Graphs were generated using Excel 2013.

## 3-Results and Discussion

### 3.1. Acidity Index Results

The results of the study revealed that the addition of salt and vinegar, as well as the duration of storage, significantly affected the acidity level of the extracted olive oil ($P < 0.05$). Mean comparisons showed that the lowest acidity was observed in the treatment with the lowest concentrations of salt and vinegar on day one. As the concentrations of salt and vinegar increased, the acidity also rose by the end of the 90-day period, reaching 1.44% and 2.47% respectively, based on oleic acid content (Figure 1). Thus, the acidity of the oil was directly correlated with the salt and vinegar concentrations in the olive storage tanks. According to the standards, the permissible acidity for extra virgin olive oil is less than 0.8%, while for virgin and refined oils, it should not exceed 2% and 3%, respectively [12]. As shown in Figure 1, the treatment containing 20% salt and 5% vinegar reached the upper limit of acidity from day one and exceeded the acceptable range for virgin oil in subsequent days. However, the other three treatments maintained acceptable acidity levels throughout the 90-day period. Previous studies have shown that lipoxygenase enzyme activity increases during the storage period, peaking around the 15th week post-harvest. This enzyme, classified as an oxidoreductase, specifically targets three essential fatty acids: linoleic acid, linolenic acid, and arachidonic acid. Data indicate that higher salt concentrations enhance lipoxygenase activity, leading to the release of fatty acids such as linoleic acid and consequently increasing oil acidity [15]. Since the optimal pH for lipoxygenase activity is approximately 5.5, the addition of vinegar—which lowers the pH in the storage tanks—creates a favorable environment for this enzyme, thereby facilitating acidity increase [16]. Moreover, triglycerides within the olive fruit are naturally protected from atmospheric oxygen by the fruit's skin. However, if the skin is damaged during storage or if conditions allow oxygen penetration, triglyceride breakdown occurs, resulting in elevated acidity [17]. Mechanical damage to olives can also expose lipase enzymes—located in vacuoles of the pulp or pit—to the oil, triggering lipolysis reactions. Esterase enzymes also increase during the salting process, and their activity is enhanced with higher salt concentrations [18]. Both lipase and esterase contribute to oxidative reactions mediated by catalase, separating fatty acids from lipids and producing free fatty acids that serve as substrates for oxidation [19]. This process can lead to increased acidity during the storage period. Microbial activity also plays a role in acidity elevation. Although oil from freshly harvested healthy fruits contains very low free acidity, during ripening in brine, conditions become favorable for microbial growth. These microorganisms secrete significant amounts of exogenous lipase, which increases oil acidity. In damaged olives, where brine penetration is deeper, lipase activity is even more pronounced [20].To date, no studies have investigated the relationship between NaCl concentration in brine and its diffusion into olive flesh. Therefore, the exact depth of salt penetration cannot be predicted, and definitive conclusions about salt concentration effects remain uncertain. Nevertheless, higher salt concentrations increase ionic pressure, accelerating oil oxidation and raising acidity [21]. Additionally, elevated salt levels influence ion exchange between olive flesh and brine, promoting the consumption of free sugars by lactic acid bacteria, which in turn increases acid production during fermentation [22]. High salt concentrations also cause wrinkling and irritation of the olive skin tissue, activating lipoxygenase and triggering lipid hydrolysis [23]. A study by Jenisova et al. (2021) demonstrated that

exposure of olives to concentrated vinegar solutions has a similarly detrimental effect on oil oxidation as high salt concentrations, which was also evident in the present research [24].

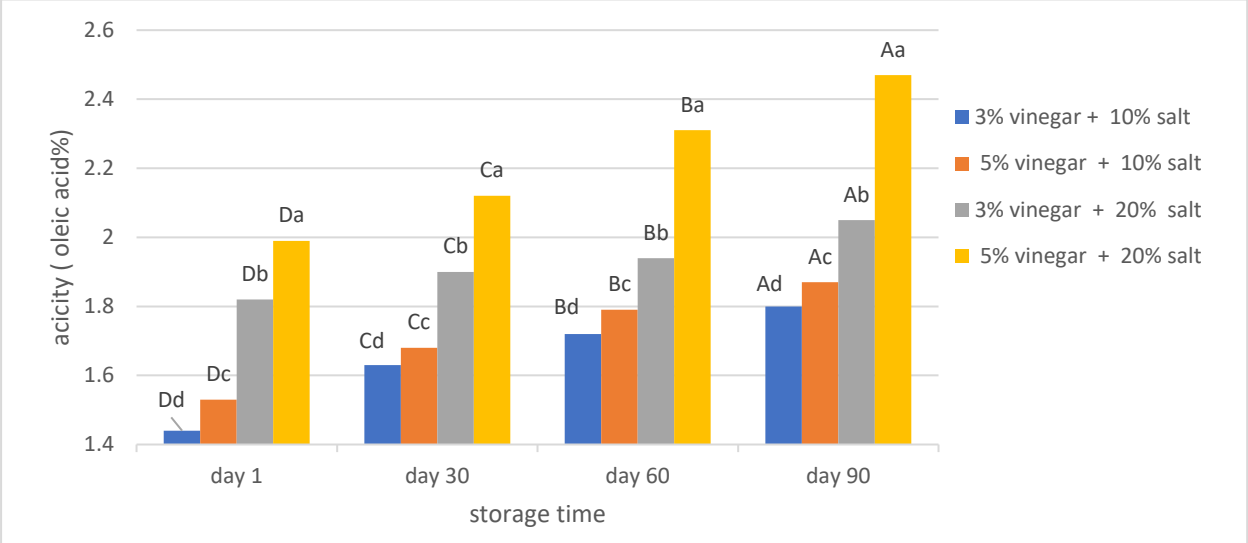


Figure 1: The Effect of Adding Different Concentrations of Vinegar and Salt during Storage on the Acidity of Olives ($p<0.05$)

The difference in uppercase letters represents in each column a significant difference in each storage period, while the difference in lowercase letters indicates a significant difference at each additive salt and vinegar treatment

### 3.2. pH Measurement Results

The results of the study indicated that the addition of salt and vinegar, as well as the duration of storage, significantly affected the pH level of the oil ($P < 0.05$). Specifically, increasing salt concentration and adding vinegar led to a decrease in pH (Figure 2). The lowest pH was observed in the treatment with the highest concentrations of salt and vinegar at the end of the 90-day period (4.8), while the highest pH was recorded in the treatment with the lowest concentrations on day one (8.1).The salt concentration in the brine is one of the most critical factors influencing olive preservation. During the processing and ripening stages, changes in the pH of the brine solution can affect microbial growth and fermentation dynamics. At lower salt concentrations, while *Lactobacillus* species responsible for olive ripening can grow, other undesirable microorganisms may also find favorable conditions for proliferation. In contrast, higher salt concentrations promote the growth of lactic acid bacteria by inhibiting thermophilic bacterial groups through the production of antimicrobial compounds such as diacetyl, hydrogen peroxide, and bacteriocin proteins during lactic fermentation. This creates optimal conditions for the growth of beneficial bacteria like *Lactobacillus plantarum* and *Lactobacillus brevis*, whose metabolic activity leads to the production of organic acids and results in the lowest pH values in these treatments [25, 26]. Research by Rezaei Khalaj et al. (2020) demonstrated that to prevent the growth of harmful bacteria, the salt concentration during fermentation should be maintained at a minimum of 4%. Additionally, the presence of vinegar significantly reduces pH levels [27]. Acidic conditions in the fermentation environment accelerate the dominance of lactic acid bacteria, thereby contributing to a more rapid pH decline. During the initial days of fermentation, the relatively high pH

allows for the growth of various microbial groups, including *Enterobacteriaceae*, yeasts, and lactic acid bacteria. However, as the pH gradually drops below 8, conditions become more favorable for lactic acid bacteria to thrive and produce acids, leading to a sharp decline in pH by approximately day 90. Furthermore, since sugars serve as the primary carbon source for fermentative microorganisms, the breakdown of organic compounds during fermentation increases sugar availability, which in turn enhances microbial activity and organic acid production. As a result, pH decreases progressively throughout the storage period [28].

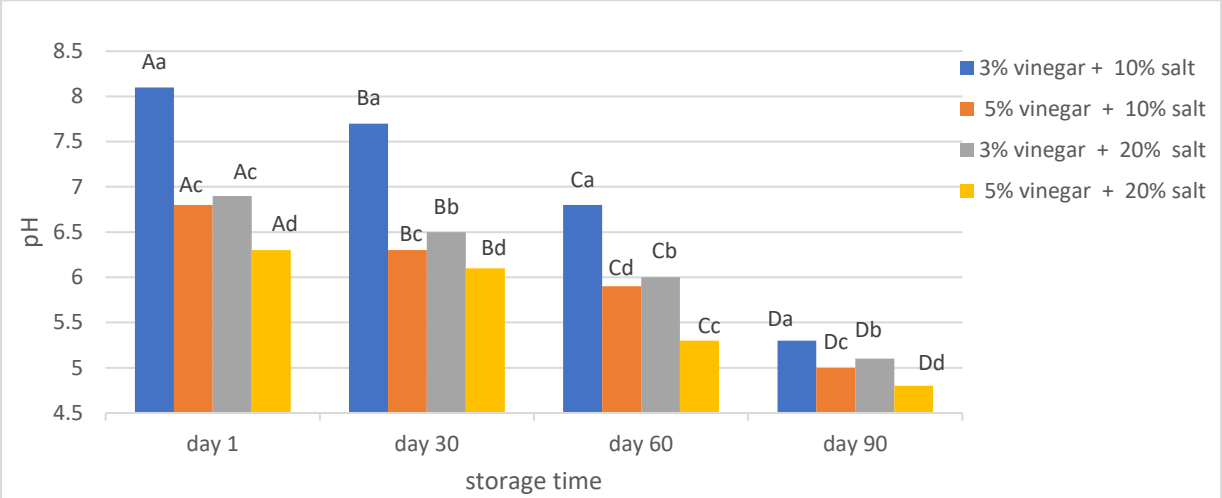


Figure 2: The effect of adding different amounts of vinegar and salt during storage on the pH of olives ($p<0.05$)

The difference in uppercase letters in each column represents a significant difference in each storage period, while the difference in lowercase letters indicates a significant difference at each additive salt and vinegar treatment

A major portion of the pH reduction during the storage period was attributed to the activity of lactic acid bacteria, particularly during the initial stages of fermentation. The pH dropped sharply within the first three months of storage, followed by a more gradual decline [29]. During fermentation, the conversion of simple sugars in olives into organic acids leads to a significant decrease in pH. This decline occurs more rapidly in the early months and then stabilizes between 4.0 and 4.5 until the end of the one-year period, which aligns with the findings of the present study [27].

### 3.3. Peroxide Value Results

The results showed that salt and vinegar addition, storage duration, and their interaction had a significant effect on the peroxide index ($P < 0.05$). Lipid oxidation leads to the formation of hydroperoxides, and a peroxide value of 5–10 meq/kg indicates the onset of lipid oxidation [30]. According to national and international olive oil standards, the maximum allowable peroxide value is 20 meq/kg [31]. Mean comparisons revealed that peroxide levels increased with storage time, although this trend was not consistent throughout the entire storage period. This may be due to the transformation of primary oxidation products into secondary derivatives. Momenian et al. [6] reported a slight decrease in oleic acid and a minimal increase in linoleic acid during storage. Consequently, the ratio of monounsaturated to polyunsaturated fatty acids slightly declined, making the oil more susceptible to oxidation. The increase in linoleic acid

content may be attributed to the activity of oleate desaturase, which converts oleic acid into linoleic acid [32]. This study found that treatments containing 10% salt remained within the acceptable peroxide limit (20 meq $O_2$/kg oil) after three months of storage [12]. The lowest and highest peroxide values were recorded on day one in the tank with the lowest salt and vinegar concentrations (2.7 meq/kg), and at the end of the second month in the tank with the highest concentrations (28.31 meq/kg), respectively. Moreover, tanks with high vinegar concentration (5%) exceeded the standard peroxide limit earlier (from day 30), while tanks with salt showed a slower increase (Figure 3). A comparison of peroxide and acidity indices suggests that low acidity alongside elevated peroxide levels may indicate the progression of oxidation and the conversion of free radicals into secondary oxidation products, signaling oxidative spoilage. The decrease in peroxide value during the third month may be due to the transformation of hydroperoxides into secondary oxidation products such as aldehydes, ketones, and conjugated dienes, which are responsible for the pungent aroma and flavor of oxidized oil [19].

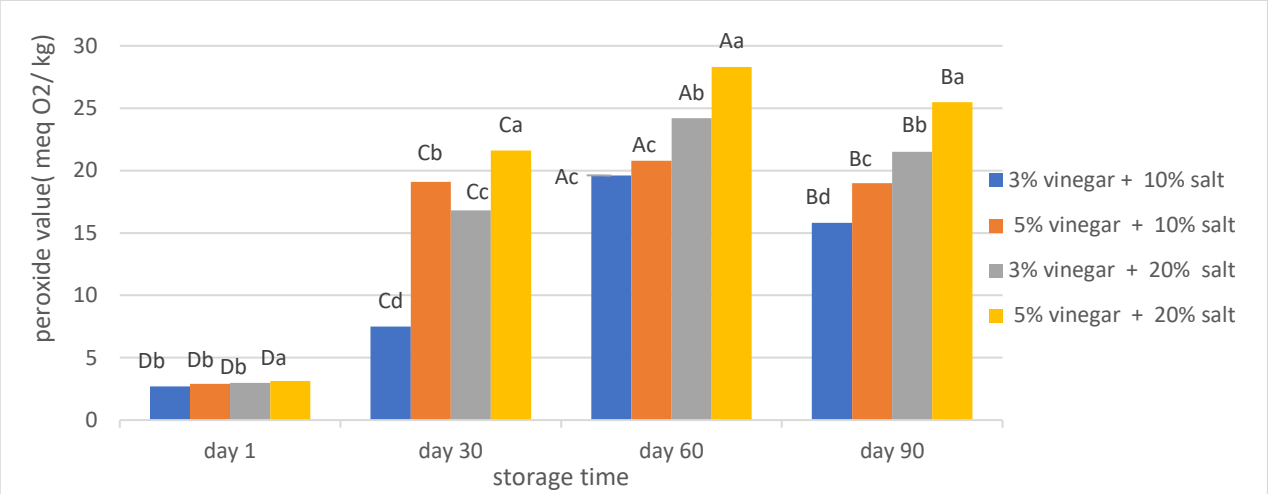


Figure 3: The effect of adding different amounts of vinegar and salt during storage on peroxide value of olives ($p<0.05$)

The difference in uppercase letters in each column represents a significant difference in each storage period, while the difference in lowercase letters indicates a significant difference at each additive salt and vinegar treatment

Zolfaghari et al. (2020) reported that immersion in salt concentrations above 10% leads to higher peroxide formation compared to low-salt treatments. They attributed this to the pro-oxidant effects of certain phenolic compounds and the promotion of oxidation under high-salt conditions [34]. However, adding salt at very low levels (0.5–2.5%) can reduce the rate of lipid oxidation [33]. Storage duration plays a more influential role in peroxide value than the type of oil itself, due to the spontaneous progression of oxidation during extended storage [35]. The interaction effects of salt and vinegar over time also revealed that treatments with the highest concentrations of both led to increased peroxide levels until the end of the second month. However, due to the transformation of peroxide compounds into other derivatives during the third month, a reduction in peroxide concentration was observed.

### 3.4. Anisidine Value Results

The anisidine value is an indicator of unsaturated aldehydes (especially 2-alkenals), which are typically formed during the progression of secondary lipid oxidation. The results showed that salt and vinegar addition, storage duration, and their interaction significantly affected the anisidine value of olive oil ($P < 0.05$). Mean comparisons revealed that anisidine values increased with storage time. The lowest and highest values were recorded on day one in the tank with the lowest salt and vinegar concentrations (0.4), and at the end of the third month in the tank with the highest concentrations (4.93), respectively (Figure 4). Although no specific limit for anisidine value in virgin olive oil is defined in Iranian or international standards, the value of 4.93 after three months suggests that secondary oxidation and its byproducts progressed slowly. High salt concentrations, especially in humid environments, accelerate oxidation. The presence of metal ions in salt (particularly copper and iron) can act as catalysts, promoting aldehyde formation and indirectly influencing anisidine values. Salt may also destabilize hydroperoxides (primary oxidation products), converting them into secondary compounds such as aldehydes and ketones, thereby increasing the anisidine value [33]. Momenian et al. (2021) demonstrated that salt, through its osmotic effect, enhances water absorption, volume, and porosity of olives, increasing oil-water contact and consequently raising anisidine levels. High vinegar concentrations also showed a direct correlation with increased anisidine values, with the highest values observed in treatments containing the highest salt and vinegar levels [6]. The low pH of vinegar (approximately 2–3) during prolonged contact with olive oil may degrade natural antioxidants such as polyphenols and vitamin E, accelerating secondary oxidation. Additionally, the water content in vinegar enhances oxygen penetration, promoting ester bond cleavage in acidic conditions and further increasing anisidine values [26]. De Leonardis et al. (2022), in their study on vinegar-formulated olive oil-based sauces, observed enhanced oxidation, resulting in a reduction of polyunsaturated fatty acids (up to 21%) and an increase in the ratio of monounsaturated to polyunsaturated fatty acids (by 25%) [36].

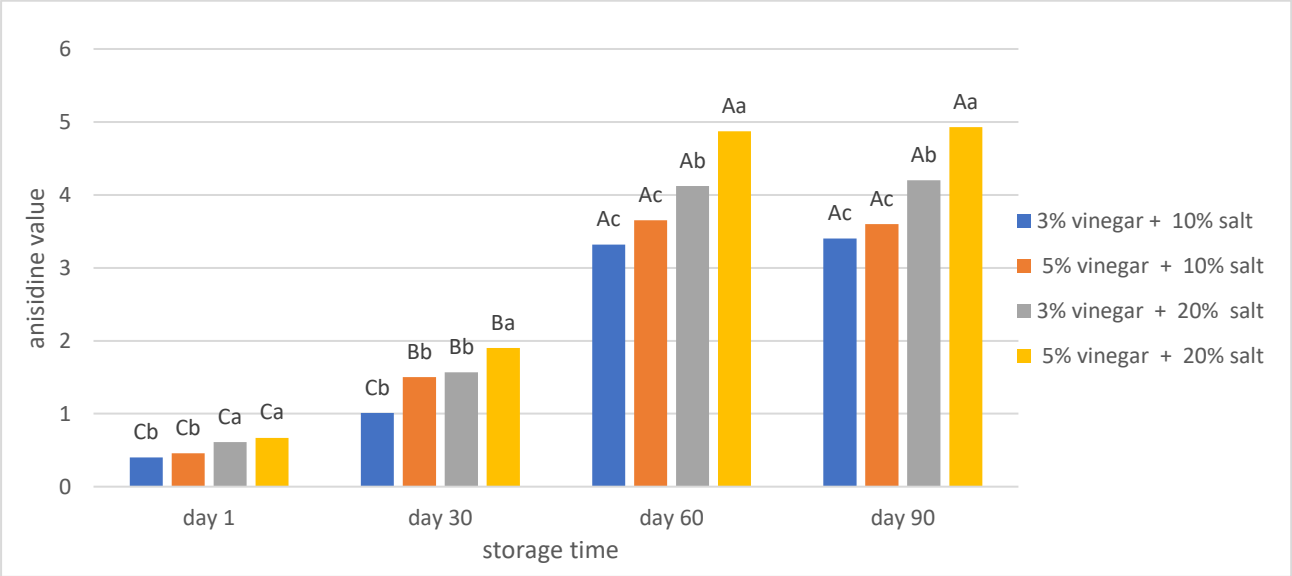


Figure 4: The effect of adding different amounts of vinegar and salt during storage on anisidine value ($p<0.05$)

The difference in uppercase letters in each column represents a significant difference in each storage period, while the difference in lowercase letters indicates a significant difference at each additive salt and vinegar treatment

Wang et al. (2017), in their study on the impact of salt concentration on oil oxidation in potato chips, reported that higher salt levels significantly increased the anisidine value. Specifically, when potato slices were immersed in salt solutions as a pre-treatment, greater amounts of glyceride esters and 3-monochloropropane-1,2-diol (3-MCPD) were formed in the oil, contributing to elevated anisidine values [37].Similarly, Fahim Danesh et al. (2018) found that during the frying process of eggplant slices, the highest levels of conjugated and carbonyl compounds were observed in oils from samples pre-treated with brine, while the lowest levels were found in samples without salt pre-treatment. These conjugated and carbonyl compounds are considered products of both primary and secondary oxidation, which can lead to increased anisidine values [38].

### 3.5. Totox Value Results

The Totox value is a comprehensive indicator of total oil oxidation, calculated as the sum of twice the peroxide value plus the anisidine value. Therefore, any factor that increases either the peroxide or anisidine index will contribute to a higher Totox value. The results showed that salt and vinegar addition, storage duration, and their interaction significantly affected the Totox value ($P < 0.05$). Mean comparisons revealed that Totox values increased with longer storage periods and higher concentrations of salt and vinegar (Figure 5). The lowest and highest Totox values were recorded on day one in the tank with the lowest salt and vinegar concentrations (5.8), and at the end of the third month in the tank with the highest concentrations (61.49), respectively. Hojjati (2019), in a study on oil extraction from oilseeds in retail settings, demonstrated that storage duration is one of the most critical factors in oxidation development. Despite the use of cold-pressing methods without thermal processing, increases in acidity, peroxide, anisidine, and Totox values were observed during storage. For example, the Totox values of sesame, canola, and sunflower oils on day one were 4.08, 2.7, and 5.11, respectively, which rose to 29.51, 27.12,

and 38.64 by the end of the second month. All samples became unsuitable for consumption by the final day of storage [39].

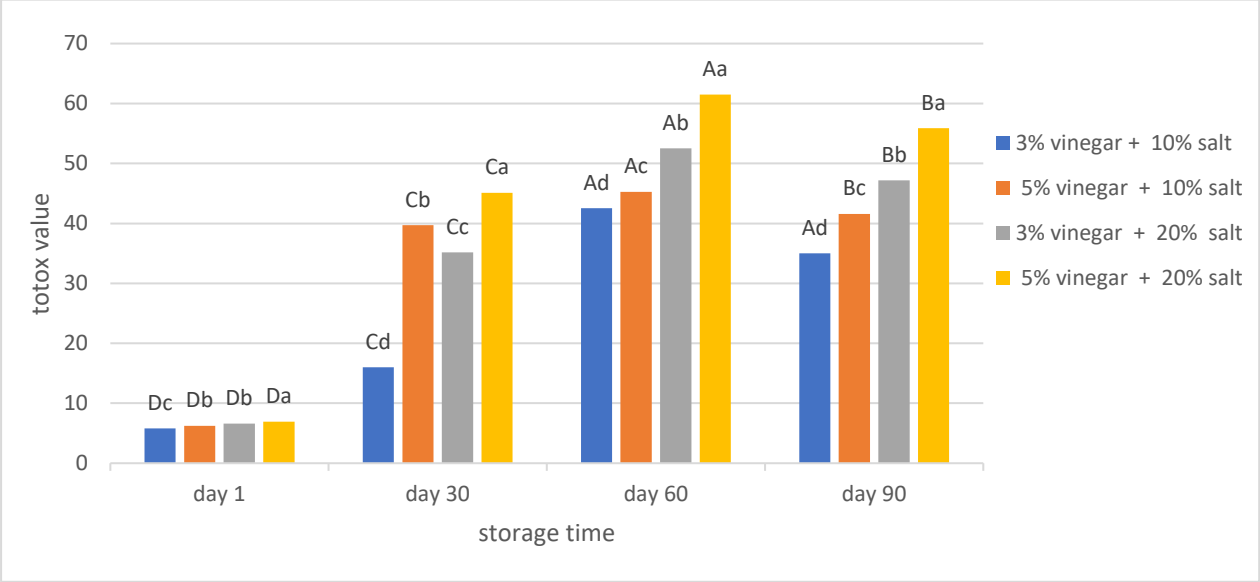


Figure 5: The effect of adding different amounts of vinegar and salt during storage on totox value($p<0.05$)

The difference in uppercase letters in each column represents a significant difference in each storage period, while the difference in lowercase letters indicates a significant difference at each additive salt and vinegar treatment

### 3.6. Total Phenolic Compounds Measurement Results

The addition of salt, vinegar, and the duration of storage significantly affected the phenolic content of olive oil ($P < 0.05$). Specifically, increasing salt concentration led to higher levels of phenolic compounds in the oil, while higher vinegar concentrations and longer storage periods resulted in a reduction of these compounds. The highest phenolic concentration was observed in the treatment with the highest salt and lowest vinegar levels on day one (3480 mg/kg), whereas the lowest concentration was recorded in the treatment with the highest vinegar and lowest salt levels at the end of the third month (2731 mg/kg) (Table 1). During storage, Ghanbari et al. (2018) identified a significant decline in biophenolic compounds, particularly secoiridoid derivatives such as oleuropein, ferulic acid, and caffeic acid. This reduction was attributed to the active participation of these compounds in oxidative processes. In the same study, luteolin was reported as the most abundant biophenolic compound in virgin olive oil, which decreased by 10% during storage. Other biophenolic compounds also showed notable reductions over time [40]. Romeo (2012) found that increasing vinegar concentration at the end of the fermentation period led to a decrease in polyphenol content in olive fruits. This reduction may be linked to enhanced activity of lactic acid bacteria in more acidic environments [28]. Additionally, in samples with higher vinegar concentrations,

the decline in phenolic compounds may be due to increased solubility and migration of phenols from the olive tissue into the vinegar solution during storage [41].

Table 1: The effect of adding different amounts of vinegar and salt during storage on Total phenolic compound and Radical Scavenging Percentage ($p<0.05$)

| sample | Day 1 | | Day 30 | | Day 60 | | Day 90 | |
|---|---|---|---|---|---|---|---|---|
| | Radical Scavenging Percentage | Phenolic compounds (mg/kg) | Radical Scavenging Percentage | Phenolic compounds (mg/kg) | Radical Scavenging Percentage | Phenolic compounds (mg/kg) | Radical Scavenging Percentage | Phenolic compounds (mg/kg) |
| vinegar 3% + salt 10% | $44^{Aa}$ | $3322^{Ab}$ | $38^{Bb}$ | $3140^{Bb}$ | $33^{Cc}$ | $3009^{Cb}$ | $29^{Db}$ | $2827^{Db}$ |
| vinegar 5 %+ salt 10% | $39.9^{Aa}$ | $3092^{Ad}$ | $26^{Bc}$ | $3001^{Bd}$ | $22^{Cd}$ | $2902^{Cc}$ | $19^{Dd}$ | $2731^{Dd}$ |
| vinegar3%+salt 20% | $44.5^{Ba}$ | $3480^{Aa}$ | $48^{Aa}$ | $3212^{Ba}$ | $42^{BCa}$ | $3120^{Ca}$ | $34^{Ca}$ | $2913^{Da}$ |
| vinegar5 % + salt 20% | $44^{Aa}$ | $3170^{Ac}$ | $39^{Bb}$ | $3067^{Bc}$ | $37^{Bb}$ | $2981^{Cc}$ | $21^{Cc}$ | $2790^{Dc}$ |

The difference in uppercase letters in each column represents a significant difference in each storage period, while the difference in lowercase letters indicates a significant difference at each additive salt and vinegar treatment

Studies have shown that prolonged storage and fermentation lead to a significant reduction in phenolic compounds in olive fruits. This decline is primarily attributed to increased microbial activity in the environment, which causes hydrolysis of polyphenols, especially oleuropein [42]. Results from Mosallaei and Mazaheri Asadi (2021) also indicated that total phenolic content in olives decreased with longer storage duration and higher salt and vinegar concentrations [26]. Ghadiri et al. (2011) identified prolonged soaking and the high solubility of phenolic compounds as the main reasons for their loss over time. They noted that the migration of phenolic compounds from olive tissue into vinegar solution is directly related to vinegar concentration. The formation of phenolic oligomers is another factor contributing to the reduction in measurable phenolic content at high vinegar concentrations, as these insoluble compounds remain trapped within plant tissues and cannot be quantified using conventional methods [43]. Additionally, under acidic conditions, enzymatic oxidation of phenolic compounds occurs at a slower rate, while hydrolysis of polymeric phenolics and the release of membrane-bound compounds are accelerated, leading to a decrease in phenolic concentration. The reduction of phenolics in vinegar-containing solutions may also be due to the loss of cell wall integrity in acidic environments, which enhances solubility and diffusion of these compounds from plant tissues into the surrounding medium. Jenisova et al. (2021) compared the effects of salt and vinegar solutions on tocopherols and found that the greatest reduction—particularly in α-tocopherol—occurred in olives stored in vinegar [24]. Ghadiri et al. (2011) also reported that higher salt concentrations help preserve phenolic compounds. However, the efficiency of different salts in retaining phenolics varies depending on their ability to interfere with ionic bonds between these compounds and fruit tissue components. Divalent salts such as calcium chloride or magnesium chloride were found to be less effective than sodium chloride in preserving phenolics and preventing their leaching from olives [43].

### 3.7. Free Radical Scavenging Activity Results

Salt and vinegar addition, storage duration, and their interaction significantly affected the free radical scavenging activity of olive oil ($P < 0.05$). Mean comparisons showed that higher salt concentrations enhanced antioxidant capacity, while increased vinegar concentrations reduced it. The lowest scavenging activity was observed in the treatment with the lowest salt and highest vinegar concentration at the end of the third month (19%), and the highest was recorded in the treatment with the highest salt and lowest vinegar concentration on day one (44%). Oleuropein, the most potent antioxidant compound in olive oil, increases up to 14% of fruit weight during early growth stages on the tree. However, as the fruit darkens and anthocyanin levels rise, oleuropein content declines, especially during post-harvest ripening. Thus, the reduction in antioxidant capacity during storage can be attributed to the decreasing concentration of oleuropein. Salimi et al. (2017) demonstrated that salt stress significantly increases phenolic secondary metabolites in plants, which are highly effective in scavenging free radicals. Therefore, a direct relationship exists between salt-induced stress and antioxidant activity [44]. Phenolic compounds with higher molecular weights are more effective in neutralizing free radicals due to their greater reducing potential and ability to donate protons. Their efficacy depends on the number of aromatic rings and the nature of hydroxyl substituents. Jenisova et al. (2021) also showed that vinegar storage, compared to salt solutions, led to greater losses in tocopherols and chlorophyll. The acetic acid ($H^+$) in vinegar replaces magnesium ions in chlorophyll with hydrogen ions, causing significant hydrolysis. Since chlorophyll has antioxidant properties, this explains the reduced antioxidant activity in vinegar environments [24]. There is also a direct relationship between chlorophyll and carotenoid concentrations and the protective effect of α-tocopherol. Although tocopherols have stronger antioxidant activity than chlorophyll and carotenoids, degradation of these pigments in acidic vinegar conditions significantly reduces the antioxidant function of α-tocopherol. Abedifar et al. (2016) investigated the effect of salt concentration on the viability of probiotic *Lactobacillus* bacteria and the quality of fermented olives. Their results showed that higher salt concentrations acted as a "hurdle" for microbial survival and had a positive impact on the antioxidant properties of olive oil [45].

## 4. General Conclusion

Olive oil, as a native oil source in Iran, holds a special nutritional status due to its high concentration of unsaturated fatty acids and diverse phenolic compounds. Given that statistics show Iranians prefer consuming olives over olive oil, proper preservation methods can indirectly introduce the nutritional benefits of olive oil into the national diet. Using salt and vinegar solutions to enhance flavor and microbial resistance is a common practice in olive preservation. The findings of this study suggest that to control oxidative indices and maintain phenolic compounds during storage, it is recommended to use 20% salt and vinegar concentrations below 5%. This approach not only reduces

oxidative spoilage of olive oil but also helps preserve its phenolic content and antioxidant properties more effectively.

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# تأثیر تیمارهای نگهداری زیتون بر شاخص‌های کیفی و اکسیداتیو روغن زیتون

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**چکیده**

نگهداری زیتون در آب نمک و سرکه با هدف بهبود طعم و افزایش ماندگاری یکی از مرسوم‌ترین روش‌ها می‌باشد، اما این مواد افزودنی می‌توانند بر کیفیت روغن زیتون تأثیرگذار باشند. در این پژوهش، تأثیر نمک (۱۰٪ و ۲۰٪) و سرکه (۳٪ و ۵٪) بر ویژگی‌های فیزیکوشیمیایی روغن زیتون در دوره‌های نگهداری (روزهای ۱، ۳۰، ۶۰ و ۹۰) بررسی شد. شاخص‌هایی مانند pH، اسیدیته، پراکسید، آنیزیدین، توتوکس، ترکیبات فنلی کل و فعالیت آنتی‌اکسیدانی اندازه‌گیری گردید. نتایج نشان داد که pH در طول زمان کاهش یافت؛ کمترین مقدار pH (۴/۸) در تیمار با ۲۰٪ نمک و ۵٪ سرکه در روز ۹۰ و بیشترین مقدار (۸/۱) در تیمار با ۱۰٪ نمک و ۳٪ سرکه در روز نخست مشاهده شد. مقدار پراکسید روغن در روز اول در تیمار با کمترین غلظت نمک و سرکه برابر با ۲/۷ meq/kg بود و در پایان ماه دوم در تیمار با بالاترین غلظت‌ها به ۲۸/۳ meq/kg رسید. شاخص‌های آنیزیدین و توتوکس نیز با افزایش غلظت نمک و سرکه و گذر زمان به ویژه در ماه اول افزایش یافتند. افزایش غلظت نمک موجب افزایش ترکیبات فنلی و فعالیت آنتی‌اکسیدانی شد، در حالی‌که افزایش سرکه و مدت نگهداری باعث کاهش آن‌ها گردید. بیشترین ترکیبات فنلی (۳۴۸۰ mg/kg ) در تیمار با ۲۰٪ نمک و ۳٪ سرکه در روز نخست و کمترین مقدار (۲۷۳۱ mg/kg) در تیمار با ۱۰٪ نمک و ۵٪ سرکه در روز ۹۰ ثبت شد. فعالیت آنتی‌اکسیدانی نیز از ۴۴٪ در تیمار با نمک بالا و سرکه پایین در روز اول به ۱۹٪ در تیمار با نمک پایین و سرکه بالا در روز ۹۰ کاهش یافت. بنابراین به‌طورکلی برای حفظ پایداری اکسیداتیو روغن زیتون، استفاده از نمک با غلظت بالا (۲۰٪) و سرکه با غلظت پایین (۳٪) توصیه می‌شود.