

Evaluating the antioxidant activity of Aqueous Extract of Date pits in Improving the Chemical Stability of Freeze-Stored Beef.

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Abstract

Meat derived from older cows represents an economically important protein source; however, its reduced consumer acceptance is largely attributed to increased connective tissue cross-linking and heightened susceptibility to oxidative deterioration during storage. This study investigated the efficacy of aqueous date seed extract (from *Phoenix dactylifera* L.) as a natural antioxidant in improving the oxidative stability of aged beef stored at -18°C for 90 days. Four treatments were evaluated (0, 5, 10, and 15% extract; CRD design), and lipid and protein oxidation markers were quantified. Frozen storage significantly increased oxidative indices in all samples ($P \leq 0.05$). After 90 days, control samples exhibited the highest lipid oxidation, with FFA reaching 0.75% and TBA values 5.55 mg MDA/kg. In contrast, the 10% extract treatment reduced FFA to 0.50% ($\approx 33\%$ reduction), while the 15% treatment lowered TBA to 5.03 mg MDA/kg. Peroxide values decreased from 8.75 mEq/kg fat (control) to 7.62 mEq/kg in the 10% treatment. Protein oxidation followed a similar pattern; carbonyl content declined from 2.12 to 1.92 nmol/mg protein, whereas thiol concentration was preserved at 59.80 $\mu\text{mol/g}$ protein in the 15% treatment compared to 52.42 $\mu\text{mol/g}$ in controls. The observed protective effect is attributed to phenolic-mediated radical scavenging and potential metal-chelating activity of date seed bioactives, limiting both primary and secondary oxidation products. Collectively, supplementation with 10–15% aqueous date seed extract demonstrates significant potential as a clean-label antioxidant strategy to enhance oxidative stability and extend the storage life of frozen aged beef.

Keywords: date seed powder, free fatty acids, lipid oxidation, peroxide, protein oxidation

Introduction

Meat plays a pivotal role in the human diet due to its diverse nutritional profile particularly its high-quality protein that provide all essential amino acids required for tissue development and maintenance. Additionally, it supplies crucial micronutrients such as B complex, vitamins, iron and phosphorus contributing to overall health and metabolic function (Ursachi *et al.*, 2020). The date palm (*Phoenix dactylifera* L.) represents a fundamental agricultural crop across the Middle East, particularly due to its nutritional and economic value. One of its main by-products, date pits often overlooked and discarded has attracted growing scientific interest for its rich content of bioactive compounds such as polyphenols, dietary fiber and natural antioxidants – which offer promising applications in food preservation, cosmetic and nutraceutical (Mrabet *et al.*, 2020 : Al-Harbi *et al.*, 2021) . Powdered date seed have emerged

as a functional additive in meat preservation due to their potent antioxidant properties (Salihi and Alsaadi., 2024). Oxidative reactions are among the main factors contributing to the deterioration of meat characteristics, including flavor, color and overall quality (Liu *et al.*., 2011). The significance of date seed lies in their rich content of biological active compounds such as phenolic compounds, flavonoids and antioxidants, which have demonstrated effectiveness as both antioxidants and antimicrobial agents across various food applications (Sayas-Barberá *et al.*, 2020 : Kelany, and Yemiş., 2023). Assessing the nutritional value of meat often centers on its amino acids profile since certain essential fatty acids cannot be synthesized by the human or animal body dietary intake becomes crucial for sustaining overall health (Al-jabari and Alsaadi., 2023). Despite its richness in nutrients, meat is highly perishable due to factors like protein breakdown, lipid oxidation, enzymatic activity,

and microbial growth (Rahman *et al.*, 2023). To address these challenges, Various preservation techniques have been developed, with freezing being among the most effective (Hammad *et al.*, 2019). Freezing enable long – term storage while maintaining the freshness and integrity of the meat, often allowing preservation for six to twelve months depending on the type, industry standards recommend string meat at temperatures between -18°C and -20°C to retain its nutritional and sensory quality, a method widely used across the food sector (Soyer *et al.* 2010). Nevertheless, prolonged freezing durations may negatively impact certain meat properties, raising concerns about the long-term effectiveness of this method (Coombs *et al.*, 2017; Leygonie *et al.*, 2012).

Materials and Methods

Preparation of Date Seed Powder:

The Zahdi variety was chosen for the aqueous extract due to its availability and low cost. 13 kg of Zahdi dates were used. The date seeds were removed from the fruit, then soaked in water for 24 hours. The outer shell was then removed. The peeled date seeds were then dried at room temperature for 48 hours. The dried date seeds were then ground using an electric grinder (CLA TRONIC), made in China (Model No. CT-1288) (**Figure 1**).



Figure 1. Sun drying of date pit

Preparing the aqueous extract

Date seed powder was soaked in distilled water at a ratio of 7 distilled water to 1 powder(W/V). The soaking period was 24 hours. The infusion was then filtered using a fine sieve. The powder infusion was prepared and placed in a centrifuge to form a concentrated extract. The experimental parameters were then prepared based on the concentrations required for the experiment. 5%, 10%, and 15% ml of the extract were added to 100g of meat, respectively.

Meat Samples

Meat from an older cow was purchased from local markets in Samarra. Samples were prepared and cut into three groups, which were then divided into experimental groups. The meat was refrigerated for 24 hours. The experimental groups were then prepared and kept at the specified concentrations for another 24 hours in the refrigerator. Finally, they were dried with gauze and frozen at -18°C for three months.

Experimental Treatments

The experiment was divided into 4 experimental treatments with 3 replicates, as shown in the **table 1**.

Table 1. shows the experimental treatments used in the study.

Treatment	Addition rate
1	0% aqueous extract of date seed powder.
2	5% aqueous extract of date seed powder.
3	10% aqueous extract of date seed powder.
4	15% aqueous extract of date seed powder.

Estimating the Peroxide Value (P.V)

This estimate was based on (Egan ., 1981). 2 grams of extracted fat were weighed using a saxolite apparatus, and 30 ml of a mixture containing (3 parts glacial acetic acid + 2 parts chloroform) was added, along with 0.5 ml of saturated potassium iodide, 30 ml of distilled water, and 1 ml of starch indicator (1%). The mixture was then sieved with a 0.01% sodium thiosulfate solution until the blue color disappeared. It was estimated based on the following equation:

Peroxide value (mEq) = number of milliliters of sodium thiosulfate x 0.01 x 1000 / sample weight.

Estimation of Thiobarbituric Acid (TBA):

We found out how much fat was being burned in the sample by following the steps in (Witte *et al.* ,1970) to figure out the thiobarbituric acid level. In short, this is what we found: For two minutes, a homogenizer was used to mix one gram of the sample with 25 ml of a cold solution that contained 20% trichloroacetic acid (TCA) dissolved in 2 M phosphoric acid. The mix was put into a 50-ml volumetric flask, and until the mark was hit, pure water was added. It was mixed, and then 25 ml was taken out and spun for 30 minutes at 30,000 speeds. Then, filter paper No. 1 was used to separate the two parts. Five milliliters of the filtrate were put into a test tube, and then five milliliters of a 0.005 M TBA solution were added. Dissolving the TBA in purified water made the blank. The sample to be measured was left out of the tube and everything else was mixed together. The stuff was put into test tubes,

mixed, and then the tubes were tightly closed. It took 15 to 16 hours in a dark room at room temperature or 30 minutes in a water bath to heat them up (Tarladgis *et al.*, 1964). At a wavelength of 530 nm, a spectrophotometer was used to find out how much color A was absorbed. 5.2 times the absorption value was used to find the TBA number. There was mcg of malondialdehyde (MDA) in every kg of seeds, as shown by the following equation: TBA value (mg/MDA kg = $A_{530} \times 5.2$)

Find out how many free fatty acids (FFA) are there.

To find out how many free fatty acids there were, the method in (Egan., 1981) was used. The cold method is used to get the fat out of this recipe. Ten grams of fat are mixed with ether, ethanol, and phenonaphthalene solution, each of which is 25 ml. It was then given 0.1 N sodium hydroxide and left alone until it turned pink. The amount of free fatty acids was found using oleic acid as a guide.

Thiol content

Ellman's reagent or 5,5-Dithiobis (z-nitro benzoic acid) (DTNB) was used to measure thiol groups according to the steps outlined in (Soyer *et al.*, 2010). Mix 50 ml of buffer Tris 0.10 mol pH 8 with 5% SDS to make 2 grams of meat, then add 100 μ l of BHT at a concentration of 0.01%. The mix was heated to 80 °C in a water bath for 30 minutes and then spun at 5,000 rpm for 20 minutes. Using the Biuret Kit, the solution was filtered and its protein level was found using the biuret method. As a first step, mix 0.5 mL of the filtrate with 2 mL of the buffer solution that was used for grinding. Then, add 0.5 mL of the 10 mM reagent (DTNB) and mix again. A frequency of 412 nm was used to test the absorption after the samples were left to sit in the dark for 30 minutes. To make a blank sample, water was used instead of the sample. The total Thiol Group was shown using the thiol content from the equation below (Salman *et al.*, 2018).

Concentration of SH (μ mol/g protein) = 109

Because:

AS stands for the sample's absorbance.

The Planck absorption is ab.

e is the molar factor. 13600 moles per centimeter

Number of times diluted (D): 2 to 9 Finding out how much carbonyl is present. We took one gram of meat and mixed it with 10 ml of Tris-HCL buffer solution 50 nmol, which had a pH of 7.4 and contained 1 mM of EDTA and 0.1 g/L butylated hydroxytoluene (BHT). We then used a homogenizer to separate the suspensions and found the carbonyl content (Nakamura and Goto ., 1996) by adding 20% TCA to 1 ml of the protein suspension

and making a blank sample. After putting the tubes on ice for 5 minutes, they were centrifuged at 10,000 rpm for another 5 minutes. To separate the solutions, 1 ml of 20% TCA was used to wash the precipitated protein, and the mixture was then centrifuged at 10,000 rpm for 5 minutes. The suspension was split up, and the sediment was mixed with 2 ml of 10 mM DNPH made in 2N of HCL. A blank sample, made up of 2 ml of 2N of HCL with no DNPH added, was then added. The samples were mixed with a vortex and kept at room temperature for an hour in the dark. A 1:1 mix of ethanol and ethyl acetate was used to wash the protein that had crystallized. To get rid of the (DNPH) dye, the process was done three times. After centrifugation, the protein that had made clumps was mixed with 8 M Urea. The absorbance was recorded at 370 nm, and the carbonyl content (nmol / mg protein) was given by the following equation (Salman *et al.*, 2018):

109 nmol/mg of protein for carbonyl

In which case:

AS stands for the sample's absorbance.

Ab = Planck's molar factor for absorbance
22,000 moles per centimeter = ϵ

Statistical Analysis

The Statistical Analysis System (SAS) (2012) tool was used to look at the data and see how the different methods affected the traits that were being looked at using a completely randomized design (CRD). Duncan's (1955) multinomial test was used to find significant changes between means.

Model in mathematics:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} is the value of the jth data that is linked to treatment i. μ is the trait's general mean.

It stands for the effect of treatment i. e_{ij} is the chance error, which has a mean of 0 and a range of $\pm 2\sigma$.

Results and Discussion

Figuring out the amount of free fatty acids (FFA)

The results in Table (2) show that the percentage of free fatty acids is significantly lower ($P \leq 0.05$) For experimental treatments T2, T3, and T4, which were treated with an aqueous extract of date kernel powder, compared to the control treatment T1, this ratio increased significantly with increasing storage time of the meat pieces after treatment with the aqueous extract. This is likely attributed to the presence of important chemicals in the extract, such as flavonoids and phenolic compounds, which inhibit bacterial growth by limiting lipolysis through enhanced stability of fat membranes and reduced

susceptibility to oxidative damage (Kilani and Yemesh, 2023). During the first 30 days of storage, the free fatty acid content increased in treatments T1 (0.47), T2 (0.39), and T4 (0.36). A continued increase in the free fatty acid content (0.54) was observed in the control treatment T1 during the second storage period (60 days). When compared to the baseline treatment, the amount of free fatty acids decreased in experimental treatments T2, T3, and T4 (0.42, 0.32, and 0.43, respectively). During the 90-day storage period, the free fatty acid level in the control group (T1) continued to rise (0.75). In contrast, the free fatty acid values in groups T2, T3, and T4 were lower (0.56, 0.50, and 0.57, respectively) than the control group. This indicates a lack of oxidative protection, highlighting the importance of using natural antioxidants. The free fatty acid content decreased significantly in group T3 throughout the storage period. This demonstrates that the aqueous extract of the antioxidant powder effectively acts as a natural antioxidant. This finding aligns with the results of a study by Juda et al. (2024), which showed that aqueous extracts do not contain any active chemicals such as anthanins, titinins, and saponins, which are commonly found in cholesterol-lowering diets. It also supports the findings of a study by Shabeeb & Makki (2021), which demonstrated a decrease in free fatty acid levels in beef samples treated with frozen flaxseed gel and stored at -18°C for four months. Consistent with the study by Shia (2019), the aqueous extract of date kernel powder exhibits a high capacity for removing DPPH free radicals and slowing the aging process of beef patties stored at low temperatures. The reduction in free fatty acid content may be due to the presence of chemicals in the plant extracts, particularly carotenoids and flavonoids, which inhibit the growth of lipase-producing bacteria. This enzyme accelerates fat rancidity, leading

to increased free fatty acid production. This aligns with the findings of Alwani (2017), which demonstrated that storage duration affects the percentage of free fatty acids. Table 2 shows that the effect was significant ($P \leq 0.05$), with the percentage of free fatty acids being low during the initial storage period (30 days), then gradually increasing over time, reaching its peak during the third storage period (90 days). According to Al-Dalali, Li and Xu (2021) Suh (2021), the percentage of free fatty acids increases with increasing storage duration. This is because lipolytic enzymes, such as lipase and phospholipase, release free fatty acids, which produce an unpleasant odor and lose their flavor over time (Table 2)

Measuring the ratio of thiopartic acid TBA

The results in Table 3 show that the percentage of thiopartic acid TBA is significantly lower ($P \leq 0.05$) in the experimental treatments that were treated with the aqueous extract of date kernels powder (T2, T3, and T4) compared to the control treatment (T1). This percentage has also gone up significantly as the storage time has increased. It takes 30 days for the value of TBA in the control deal T1 2.11 (malone dihydes/ kg meat) to reach a high level. When the trial transactions T2, T3, and T4 were treated with the water-based extract of date kernel powder, the levels of TBA 2.05, 1.94, and 1.83 (malone dihydes/kg meat) went down compared to the control treatment. The value of TBA continued to rise in the control treatment T1 over the next two storage periods (60 and 90 days), giving 4.86 and 5.55 (malone dehyde/ kg meat), respectively. On the other hand, treatment T4 had the lowest values of TBA over the two storage periods (90 and 60 days), giving 3.23 and 5.03 (malone dehyde/ kg meat), respectively. Here we can see that the water-based extract of date kernel powder works well as an

Table 2. The effect of the use of aqueous extract of date kernel powder on the free fatty acids of aged beef stored in freezing

Treatment	Period			Mean
	30	60	90	
T1	0.472 ^{ab} ±0.0083	0.543 ^{ab} ±0.0063	0.752 ^a ±0.0048	0.598 ^a ±0.0026
T2	0.394 ^{bc} ±0.0081	0.424 ^{bc} ±0.0074	0.574 ^{ab} ±0.0052	0.464 ^b ±0.0057
T3	0.284 ^d ±0.0062	0.317 ^{cd} ±0.0070	0.499 ^{bc} ±0.0044	0.365 ^c ±0.0057
T4	0.365 ^{bc} ±0.0066	0.435 ^{bc} ±0.0073	0.565 ^{ab} ±0.0055	0.455 ^b ±0.0042

The means of each group followed by a different letter indicate significant differences between them at the probability level ($P \leq 0.05$) according to Duncan's multiple range test.

Table 3. The effect of the use of aqueous extract of date kernels powder on thiopartic acid (TBA) for frozen aged beef

Treatment	Period			Mean
	30	60	90	
T1	2.11 ⁱ ±0.0088	4.86 ^e ±0.0177	5.55 ^a ±0.0115	4.17 ^a ±1.023
T2	2.05 ⁱ ±0.0177	4.71 ^f ±0.0174	5.43 ^b ±0.0177	4.06 ^b ±0.997
T3	1.94 ^k ±0.0088	3.84 ^g ±0.0146	5.34 ^c ±0.0120	3.71 ^c ±1.001
T4	1.83 ⁱ ±0.0088	3.23 ^h ±0.0146	5.03 ^d ±0.0200	3.63 ^d ±0.933

The means of each group followed by a different letter indicate significant differences between them at the probability level ($P \leq 0.05$) according to Duncan's multiple range test.

antioxidant, as demonstrated by (Gouda *et al.*, 2024) . He said that the water-based extract of date kernels has active compounds like anthocyanin, lutein, terpenoids, and saponin that stop free radicals and slow down fat oxidation. As time goes on, the TBA values go up, which is a clear sign that more fat is being burned and more toxic chemicals are being made. This fits with (Al-Dalali, Li and Xu (2021) Suh.,2021), which showed that storing something cold causes oxidation markers, especially TBA, to slowly rise. When meat goes bad in terms of taste and color because of rotten chemicals building up, the length of time it is stored is very important. These signs can be successfully lowered by using natural antioxidants, which is in line with the results we found. Treatment with T3 has worked well, but not as well as treatment with T4. This suggests that the quantity of the extract or the way it was made may have a big effect on how well it works. As the holding time for all activities gets longer, TBA's value also goes up. This could be because aldehydes kept being made while the product was being stored (Chauhan *et al.*, 2018). This study's results were also the same as those of the (Al-Athari., 2017) study, which looked at how a group of plant products affected the TBA ratio in frozen beef pies. The study found that the TBA ratio was lower in processed beef pies with different amounts of plant extracts than in control treatment pies. These values also went up as the pies were frozen for longer periods of time, based on the concentration used.

Peroxide Valuation

Through the results obtained through **Table 4**, it is noted that in the first storage period (30 days), the treatment T2 showed a value of less than peroxide 5.32 (mEq/kg fat) compared to the control treatment T1 6.36 (mEq /kg fat). As for the treatment T3, T4, it is noted that there are no significant differences between them, as they were 5.56,5.62 (mEq/kg fat) respectively. This reflects the efficiency of the extract in inhibiting the onset of the fat oxidation chain. In the second storage period (60 days), the protective effect continued in the treatment T2 6.05 (mEq/kg fat) while the value of peroxide increased in the control treatment T1 8.11 (mEq/kg fat). In the third

storage period (90 days), the value of peroxide was higher for all experimental transactions, but the treatments T2, T3, T4 in which the aqueous extract of date nuclei was used were better compared to the control treatment T1, as the storage of meat by freezing for long periods affects internal enzymes and heme iron, which increases the chances of oxidation, but the use of plant extracts such as date nuclei improves the activity of antioxidant enzymes and reduces lipolysis This is what(Evuen *et al.*, 2025) found(Alia and Ward .,2021) also pointed out that thermal extracts from date nuclei retain high antioxidant activity, but this activity may be affected by the passage of time or the degree of freezing, which leads to a decrease in potency in the late stages. In addition, during the storage of meat, fat oxidation occurs and therefore peroxides are formed, but the use of plant-based extracts will reduce the formation of peroxides because they contain compounds with inhibitory activity against the formation of peroxides. This is consistent with (Alia and Warda, 2021), which indicated that the use of sumac fruit extract as a natural antioxidant compound helps to prolong the life of meat products by reducing the value of peroxide for the concentration used during the 90-day storage period.

This is in line with(Bouhlali *et al.*, 2020) on the ability of phenolic extracts in date cores to inhibit the formation of hydroperoxides. In general, the oxidation of fat increases in the value of frozen beef peroxide throughout the storage periods. It is noted that the second transaction gave the lowest values during the storage periods compared to the rest of the transactions in general. The transactions that were treated with the aqueous extract of date kernels powder were better than the control treatment because of its high ability to prevent the formation of free radicals and thus it works to reduce the oxidation process(Zhang *et al.*, 2020). As for the lack of superiority of the treatment T4, although it contains the highest concentration of the extract, this is explained by (Maqsood and Benjakul.,2014) who explained that the high concentration of phenolic compounds may lead to an adverse effect (Pro- oxidant effect), especially in the presence of heme iron and proteins, which explains the lack of improvement in performance in T4.

Table 4. The impact of the use of aqueous extract of date kernel powder on the value of peroxide for frozen aged beef

Treatment	Period			Mean
	30	60	90	
T1	6.36 ^{cd} ±0.046	8.11 ^b ±0.023	8.75 ^a ±0.006	7.74 ^a ±0.685
T2	5.32 ^e ±0.012	6.05 ^d ±0.017	7.72 ^a ±0.017	6.36 ^b ±0.980
T3	5.56 ^c ±0.017	6.95 ^b ±0.017	7.62 ^a ±0.017	6.71 ^b ±0.470
T4	5.62 ^c ±0.006	7.09 ^b ±0.035	8.71 ^a ±0.035	7.14 ^a ±0.455

The means of each group followed by a different letter indicate significant differences between them at the probability level ($P \leq 0.05$) according to Duncan's multiple range test.

Carbonyl Measurement

The production of the carbonyl group is a well-known process that explains chemical changes in amino acids, and is commonly used as an indicator of the oxidation of the protein (Zou *et al.*, 2018). From the results obtained from **Table 5**, it is noticed that there is a significant decrease ($P \leq 0.05$) in carbonyl for the experimental treatments treated with the aqueous extract of date kernel powder T2, T3, T4 compared to the control treatment T1, whose value increased significantly by increasing the storage period compared to the meat pieces treated with the aqueous extract of date kernel powder, reflecting the protein oxidation gradually. The concentration of carbonyl in the treatment T1 at the end of the storage period (90 days) was 2.12(nmol/ mg protein) compared to the treatment T4, whose carbonyl value was 1.92(nmol/ mg protein). This indicates the effect of the aqueous extract of date kernel powder in reducing protein damage resulting from oxidation. The carbonyl content is closely related to the oxidation of sensitive amino acid residues such as (proline, arginine, and threonine), which causes structural changes to proteins and a decrease in the nutritional and functional quality of meat, as indicated by (Lund *et al.*, 2007). These results are also in line with what (Basuny *et al.*, 2011), pointed out, who proved that the extract of date kernels can reduce the oxidation of protein and fat in typical meat systems, which supports the possibility of using it as a natural ingredient in the preservation of meat during freezing storage. The use of plant extracts also limits the rise in the value of carbonyl as indicated by (Horbańczuk *et al.*, 2021). The results of this study are also consistent with (Alia and Warda, 2021), who indicated that the use of the aqueous extract of sumac fruits in different concentrations in beef that was stored in the freezer for 90 days to show

the extent to which this extract inhibited the oxidation of fat and protein led to a significant inhibition of carbonyl content, peroxide value and thiol content. The study also indicated the possibility of using the aqueous extract of sumac fruits as a natural antioxidant compound that helps prolong the life of meat products.

Measuring Free Thiol Content

Through the results obtained from **Table 6**, it is clear that the T1 treatment recorded the lowest average concentration of thiol with a clear decrease in the value of thiol throughout the storage period. This indicates the absence of oxidative protection, which led to the gradual oxidation of proteins. These results are in line with the findings of (Xia *et al.*, 2021), which showed that the decrease of thiol is related to the formation of disulfurous bonds (-S-S-) as a result of the interaction of free radicals with the (SH-) group and the loss of vital activity of proteins and thus the loss of protein function. With regard to the treatment T2, it showed a slight improvement compared to T1 with a less severe decrease in the value of thiol. This may be due to the fact that it contains a low concentration of date nuclei. This is in line with what (Basuny *et al.*, 2011) found, which proved that the aqueous extract of date nuclei reduces the formation of hydroperoxides and slows down the oxidation of proteins in meat. As for the treatment T3, it is noted that it maintained a high concentration in the two periods (30 and 60 days) with a slight decrease in the last period (90 days). This indicates the high effectiveness granted by the aqueous extract in resisting oxidation or perhaps because of its higher concentration of phenolic compounds. According to (Grassi *et al.*, 2023), beef has a higher thiol than poultry meat and ground meat. This content can be enhanced by adding natural antioxidants such as herbal extracts or

Table 5. The effect of the aqueous extract of date kernels powder on the carbonyl content in frozen aged beef

Treatment	Period			Mean
	30	60	90	
T1	1.54 ^d ±0.015	1.80 ^e ±0.017	2.12 ^a ±0.011	1.82 ^a ±0.166
T2	1.45 ^j ±0.013	1.71 ^f ±0.012	2.02 ^b ±0.018	1.72 ^b ±0.168
T3	1.39 ^k ±0.008	1.65 ^g ±0.014	1.97 ^c ±0.012	1.67 ^c ±1.167
T4	1.34 ^l ±0.001	1.60 ^h ±0.015	1.92 ^d ±0.014	1.62 ^d ±0.167

The means of each group followed by a different letter indicate significant differences between them at the probability level ($P \leq 0.05$) according to Duncan's multiple range test.

Table 6. The effect of the aqueous extract of date kernels powder on the content of thiols in the meat of aged cows stored in freezing

Treatment	Period			Mean
	30	60	90	
T1	58.18 ^d ±0.015	55.44 ^f ±0.017	52.42 ^e ±0.011	55.35 ^d ±1.668
T2	59.36 ^c ±0.013	56.79 ^e ±0.012	55.77 ^f ±0.018	57.31 ^c ±1.097
T3	61.46 ^b ±0.008	61.23 ^b ±0.014	57.68 ^d ±0.012	60.12 ^b ±1.219
T4	63.39 ^a ±0.001	61.45 ^b ±0.015	59.8 ^c ±0.014	61.55 ^a ±1.037

The means of each group followed by a different letter indicate significant differences between them at the probability level ($P \leq 0.05$) according to Duncan's multiple range test.

dates. As for the treatment T4, it is noted that it is the most effective treatment, as it is noted that it gave the highest concentration of thiol in all periods, which indicates the occurrence of excellent oxidative stability. This may be due to the fact that it contains the highest concentration of the aqueous extract of date nuclei rich in phenols and flavonoids. This is in line with what (Horbańczuk *et al.*, 2021) found, which confirmed that natural antioxidants such as date nuclei contribute to improving the quality of meat and reducing protein oxidation and fat. This is the same thing that [39] found, which indicated that plant extracts, especially the aqueous extract of date nuclei, contain phenolic compounds that are effective in reducing protein oxidation and fat during storage. It also proved that the extract reduces the damage of BSA protein by free radicals, which reflects its effectiveness in maintaining the safety of proteins during storage.

Conclusions

The results of this study showed the effectiveness of the aqueous extract of date pits in preserving the chemical and functional properties of aged beef stored in the freezer. The experimental treatments treated with the aqueous extract recorded a clear decrease in the oxidation indicators of fat, protein, free fatty acids, peroxide value, and TBA, which reflects better functional stability of the meat during the freezing storage period compared to the control treatment. Therefore, we conclude that the aqueous extract of date pits can be relied upon as a natural and effective antioxidant in meat preservation systems, which contributes to extending the storage life of frozen meat products while maintaining their nutritional and sensory quality.

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