Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

The effect of adding alcoholic extract of grape seed on some indicators of oxidation and microbial contamination of cooling preserved beef

Ali J. Mohamed[1], Mustafa Adnan Idan [2,] Ali Ahmed Khalaf [3]

1- Animal Production Department Agriculture College, Misan University- Misan, Iraq.

2- Food Science Department, Agriculture College, University of Misan - Misan, Iraq.

3- Department of Agricultural Biotechnology, Kut-Technical Institute, Middle Technical University Iraq

ARTICLE INFO

Article History:

Received: 2025/09/15
Review: 2025/10/10
Accepted: 2025/10/13

Keywords:
alcohol extraction,

antioxidants,

grape seeds,

meat storage,

antimicrobials.

DOI: **10.48311/fsct.2026.84098.0**

*Corresponding Author E-Mail:

ali.jasim@uomisan.edu.iq

ABSTRACT

The study was conducted in the laboratories of the Department of Animal Production/ College of Agriculture / Misan University from 5/10/2023 to 15/11/2023 to improve the qualitative qualities of meat by reducing some standards of oxidation and microbial contamination of the cooling preserved beef. The study used 6 kg of veal, divided into four treatments, each involving 1.5 kg of meat and three repeaters at a rate of 500 grams of meat per repeater. After conducting the alcoholic extraction of grape seeds, preparing meat samples, and distributing them in plastic containers, the experimental treatments were as follows, T1: control treatment without any addition. T2, T3, T4: Treatment of immersing the meat piece with alcoholic extract concentration 0.02, 0.03, 0.04 % respectively. After all treatments were flooded for an hour, they were removed from the plastic utensils, wrapped in polyethylene bags, and cooled to 4 °C. The findings indicated that the alcoholic extract contained active ingredients such as alkaloids, phenols, flavonoids, glycosides, steroids, tannins, and saponins. The use of alcoholic extract at a concentration of 0.04% led to the extension of the shelf life of meat until the tenth day by reducing the values of peroxide, free fatty acids and thiobarbituric acid, which amounted to 4.83 (mEq/kg meat), 1.03 (%) and 1.97 (mg Malonaldehyde/Kg meat) respectively Meat's shelf life was extended until the tenth day with the use of 0.04% alcohol extract. This was achieved by lowering the levels of peroxide, free fatty acids, and thiobarbituric acid, which were 4.83 (mEq/kg meat), 1.03 (%), and 1.97 (mg Malonaldehyde /kg meat), respectively, in addition to reducing the number of total bacteria, Psychrophilic and coliform bacteria, which amounted to 84.6 x 106, 6.72 x 104 and 23.1 x 104 (CFU/g) respectively, which reflected positively on the reduction of oxidative indexes and pollution compared to treatment control.

1-Introduction

Meat is an important and essential source of animal protein of high nutritional value because of its content of essential mineral acids necessary for the growth of the human body (Stadnik,2024)

. Due to the chemical and biological nature of meat and its products, they are damaged when stored as a result of the oxidation of fat and bacterial growth, which are the main factors affecting the quality of food and its reduction in vitality, as the oxidation of fats leads to the deterioration of taste, flavor, and texture of meat and its products, which leads to a short storage life (Amaral *et al*., 2018). Despite the widespread use of many industrial preservatives in the manufacture of meat products that prevent oxidation, but to the growing health awareness among consumers of the health risks of these additives to the consumer (Luo and Fang, 2008), research has been conducted in recent years on additives of plant origin to possess both antioxidant and antimicrobial effectiveness (Falowo *et al*., 2014), which serve to sustain welding quality and prevent economic loss (Kumar *et al*., 2015).

Vitis vinifera is one of the largest fruit crops in the world and grape seeds are usually by- products of processed grape products such as grape juice and grape molasses (Chand *et al.*, 2021) contains 29% carbohydrates, 60-70% moisture, 13% fat, and 11% protein in addition to minerals and phenolic compounds (Costa *et al.,* 2015). In addition, grape seeds contain very high levels of proanthocyanidins, which constitute the superior antioxidant ability of grape seeds (Israr *et al.*, 2021; Romero *et al.*, 2022). Studies have shown that these compounds possess stronger antioxidant properties compared to those of vitamins (Bagchi *et al.*, 2014). Shi *et al.*, (2003) found that the antioxidant power of proanthocyanidins is twenty times greater than that of vitamin E and fifty times greater than that of vitamin C.

This study aimed at the possibility of exploiting grape seeds extract to the beef stored in refrigeration to prolong its storage period and preserve its qualitative properties.

2-Materials and Methods

Study site and sample preparation:

The study was conducted in the laboratories of the Department of Animal Production/ College of Agriculture/University of Misan, and for the period from 5/10/2023 to 15/11/2023, during which grape seeds were purchased from the local markets of Misan Governorate and cleaned and sifted from dust and washing. Drying and grinding were carried out until the process of alcohol extraction of grape seeds, and the purchase of 6 kg of veal meat, divided into four treatments , each treatment with three replicates, at a rate of 500 gm meat for each replicate.

Preparation of Alcoholic Extract for Grape Seeds:

The alcohol extraction method was adopted by Kim *et al*. (2006) In the preparation of plant extracts, weighing 100g of grape seed powder, a concentration of 98% was added to 500 ml of ethanol, mixed well, and left for 24 hours at laboratory temperature (25C). After that, the extract was filtered using filter paper (What man No.1). The filter was concentrated with a Rotary Vaccum Evaporator at a temperature of 40 C to get rid of the solvent, and the filter was left at room temperature to get rid of the solvent completely to obtain a highly viscous concentrated substance,

addition for each 2g of this substance 50 ml distilled water to prepare the concentrations used in the study.

Experiment Parameters:

After conducting the process of alcohol extraction of grape seeds preparing meat samples and distributing them in plastic containers, the experimental treatments were as follows: T1: Control treatment without any addition, T2: Treatment of immersing the meat piece with the alcohol extract by a concentration of 0.02%, T3: Treatment of immersing the meat piece with the alcohol extract at a concentration of 0.03% and T4: Treatment of immersing the meat piece with the alcohol extract at a concentration of 0.04%. All treatments were flooded for an hour after which they were taken out of plastic containers and then wrapped in polyethylene bags and stored refrigerated at a temperature of 4°C until the tests were conducted.

The traits studied:

The active compounds of the alcohol extract were detected, which included glycosides. If they followed the method described by Gizaw *et al.* (2022) in detecting them, alkaloids and phenols, the method of Harborne (1998) adopted in detecting them, tannins and soaps used the method of Shihata (1951) in detecting them, the method of Jaffer *et al*. (1983) was adopted detecting flavonoids, and steroids, the method mentioned in Al-Bid (1985) was followed to detect them.

The indicators of fat oxidation in beef samples were measured by estimating the peroxide value and free fatty acids based on the method of Dustson and Pearson (1985) and estimating the value of thiobarbituric acid (TBA) based on the Gheisari *et al.* (2010).

Microbiological analysis:

Indicators of microbial contamination in beef samples were measured by estimating the total bacterial count (TBC) using the Nutrient Agar and incubation medium at a temperature of 37c for 24 hours and then the bacterial colonies were counted according to the method of Robert and Greenwood(2008). After that, the number of psychrophilic bacteria was estimated using the nutritional medium Nutrient Agar. Then, the dishes were incubated at a temperature of 4 ± 1 C for 11 days. Then, the number of bacteria was calculated according to the method mentioned by Andrew (1992). The number of coliform bacteria was estimated using the cultivated medium Maconkey Agar. The dishes were placed in the incubator at a temperature of 30°C for 24 hours. The number of red colonies was calculated only according to the method of Elmer *et al*. (1979).

Statistical Analysis

The experiment data were analyzed statistically according to the complete random design (CRD) using the ready-made statistical program (GENSTAT, 2012), and the results were compared using the lowest significant difference (L.S.D) at a probability level ($p<0.05$).

3-Results

Active Compounds

The results of Table (1) showed that the alcohol extract of grape seeds contains most of the active compounds, which included alkaloids, phenols, flavonoids, tannins, saponins, glycosides, and steroids, as all the disclosures showed positive results when inferred through the interactions shown in the table.

Table (1): Results of chemical detection of active compounds in grape seed alcohol extract

Sequence	Active compounds	Indicating active compounds	Detection results
1	Alkaloids	The appearance of turbidity	+
		Reddish-brown precipitate	+
2	Phenols	Green color appears	+
3	Flavonoids	yellow color	+
4	Tannins	White gelatinous precipitate	+
		Blue green color	+
5	soaponins	The appearance of foam	+
6	Steroids	Dark blue color appears for a long time	+
7	Glycosides	Red precipitate	+

+: An indication of the presence of the active compound when detected.

Peroxide (P.V.)

The results of Table (2) showed the effect of using different concentrations of the alcohol extract of grape seeds on the average peroxide number (mEq /kg of meat) of the cold-preserved beef samples at a temperature of 4 C, as it was noted that there was a significant decrease ($p \leq 0.05$) in the value of the peroxide number of meat when treated with different concentrations of the alcohol extract of grape seeds compared to the control treatment during the cooling preservation period at a temperature of 4 °C, as the average peroxide number for the eighth day was 4.96, 3.82, 3.77 mEq/Kg flesh for T2, T3, and T4, respectively, compared to a control (T1) treatment with an average peroxide of 5.64 (mEq /kg meat). Hence, the control treatment exceeded the standard limits and was excluded from the experiment because of its high oxidation indicators. It was noted from the table results that the treatments to which the alcohol extract of grape seeds was added kept within the permissible limits of the peroxide value until the tenth day of the cryopreservation period.

Table (2): Effect of alcohol extract of grape seeds on the value of peroxide (mEq / kg meat) for cold-preserved beef at a temperature of 4°C

Treatments	Refrigerated storage periods/ day							
	1	3	5	7	8	9	10	11
T1	0.35	1.42	3.70	4.85	5.64	-	-	-
T2	0.36	1.11	2.62	3.87	4.96	4.73	5.20	-
T3	0.33	0.92	1.51	2.76	3.82	4.66	5.13	-
T4	0.37	0.85	1.43	2.63	3.77	3.95	4.83	5.06
L.S.D.	0.015	0.250	0.092	0.054	0.061	0.088	0.074	-

T1: Treatment of control without any addition. T2: Treating the meat piece with alcohol extract at a concentration of 0.02%. T3: Treating the meat piece with alcohol extract at a concentration of 0.03%. T4: Treating the meat piece with alcohol extract at a concentration of 0.04%. (L.S.D.): The lowest significant difference at a probability level (p≤0.05).

Free fatty acids (FFA):

The results of Table (3) show the effect of using different concentrations of the alcoholic extract of grape seeds on the percentage of free fatty acids (%) of cold-preserved beef at a temperature of 4 °C. There is a significant decrease ($p \leq 0.05$) in the percentage of free fatty acids in beef processed at different concentrations of the alcoholic extract of grape seeds compared to the control sample, as the average percentage of free fatty acids for the eighth day was 0.9 7, 0.92, 0. 84% when adding 0.02, 0.03 and0.04%, respectively, compared with the control treatment in which the percentage of free fatty acids reached 1.27%, and thus exceeded the acceptable limits and was excluded from the experiment. It was noted that the coefficients to which the extract was added at different concentrations were kept within the permissible limits for free fatty acids until the tenth day of cryopreservation.

Table (3): Effect of the use of different concentrations of alcohol extract of grape seeds in the percentage of free fatty acids (%) of cold-preserved beef at a temperature of 4 C

Treatments	Refrigerated storage periods/ day							
	1	3	5	7	8	9	10	11
T1	0.12	0.35	0.67	1.19	1.27	-	-	-
T2	0.14	0.30	0.54	0.75	0.97	1.16	1.28	-
T3	0.16	0.22	0.50	0.70	0.92	1.05	1.21	-
T4	0.15	0.20	0.39	0.51	0.84	0.91	1.03	1.25
L.S.D.	0.001	0.021	0.057	0.082	0.078	0.126	0.081	-

T1: Treatment of control without any addition. T2: Treating the meat piece with alcohol extract at a concentration of 0.02%. T3: Treating the meat piece with alcohol extract at a concentration of 0.03%. T4: Treating the meat piece with alcohol extract at a concentration of 0.04%. (L.S.D.): The lowest significant difference at a probability level (p≤0.05).

Thiobarbituric Acid (TBA)

The results of Table (4) showed: The effect of using different concentrations of grape seed alcohol extract on thiobarbituric acid value (mg malonaldehyde/kg meat) for cold-preserved beef at a temperature of 4°C. There was a significant decrease ($p \leq 0.05$) in the TBA value of beef treated with different concentrations of grape seed alcohol extract compared to the control treatment, as the average TBA value in the control sample was 0.17 (mg malonaldehyde/kg meat) on the first day and rose with the continuation of the period of cryopreservation to reach an average of 2.33 (mg malonaldehyde/kg meat) on the eighth day of preservation and thus exceeded the limits of the standard specifications that confirm the maximum value of TBA in preserved meat as 2 (mg malonaldehyde/kg meat) (Central Organization for Standardization, Control and Quality), which is considered unfit for consumption if it exceeds these limits and thus excluded control samples from the experiment. As for the samples of processed meat in concentrations of 0.02, 0.03 and0.04%, they were kept within the permissible limits until the eighth day of preservation, as the averages reached 1.51, 1.42, and 1.19 (mg malonaldehyde /kg meat), respectively, with the continued moral superiority of the fourth treatment until the tenth day of the preservation period, as they were excluded on the eleventh day due to the high value of thiobarbituric acid from the standard.

Table (4): Effect of the use of different concentrations of the alcohol extract of grape seeds on the value of thiobarbituric acid (mg malonaldehyde /kg meat) for cold-preserved beef at a temperature of 4 C.

Treatments	Refrigerated storage periods/ day							
	1	3	5	7	8	9	10	11
T1	0.17	0.42	0.80	1.89	2.33	-	-	-
T2	0.18	0.37	0.63	1.17	1.51	1.84	2.18	-
T3	0.19	0.26	0.51	1.00	1.42	1.78	2.15	-
T4	0.17	0.23	0.48	0.76	1.19	1.63	1.97	2.12
L.S.D.	0.001	0.035	0.041	0.221	0.098	0.071	0.050	-

T1: Treatment of control without any addition. T2: Treating the meat piece with alcohol extract at a concentration of 0.02%. T3: Treating the meat piece with alcohol extract at a concentration of 0.03%. T4: Treating the meat piece with alcohol extract at a concentration of 0.04% (L.S.D.): The minimum moral difference at a probability level ($p \leq 0.05$).

Total Bacteria Count (TBC):

The results of Table (5) indicate that the total number of bacteria in the control treatment has increased significantly at the level of ($p \leq 0.05$) during the period of cryopreservation compared to the treatments to which the alcohol extract of grape seeds was added, as the total number of bacteria in the control sample reached 76.1× CFU/g 10^6 for the eighth day of preservation, which was excluded due to the high oxidation indicators in it. As for the treatments to which the alcohol extract of grape seeds was added in different concentrations, the average number of total bacteria on the eighth day of cryopreservation reached 60.5, 57.3, 50.8 × 10^6 (CFU/g) for the second, third, and fourth treatment, respectively. From the table, we note that the total number of bacteria in the control sample exceeded the limits of the standard specifications and became unfit for consumption, and it was noted that the treatments to which the alcohol extract of grape seeds with different grapes kept within the permissible limits of the total bacterial numbers for the tenth day of cryopreservation.

Table (5): Effect of the use of different concentrations of alcohol extract of grape seeds in the preparation of total bacteria (CFU/g) for cold-preserved beef at a temperature of 4 C

Treatments	Refrigerated storage periods/ day							
	1	3	5	7	8	9	10	11
	10^4				10^6			
T1	21.3	38.5	36.5	52.4	76.1	-	-	-
T2	22.5	28.7	32.4	47.1	60.5	83.6	93.4	-
T3	21.9	25.9	28.5	41.3	57.3	72.9	90.5	-
T4	20.4	23.6	25.9	36.5	50.8	67.5	85.6	91.8
L.S.D.	0.810	2.001	2.666	4.801	3.210	5.412	2.992	-

T1: Treatment of control without any addition. T2: Treating the meat piece with alcohol extract at a concentration of 0.02%. T3: Treating the meat piece with alcohol extract at a concentration of 0.03%. T4: Treating the meat piece with alcohol extract at a concentration of 0.04%. (L.S.D.): The lowest significant difference at a probability level ($p \leq 0.05$).

Psychrophilic Bacteria:

The results of Table (6): The effect of using different concentrations of the alcohol extract of grape seeds in the preparation of Psychrophilic Bacteria (CFU/g) for cold-preserved beef at a temperature of 4 °C showed a significant decrease ($p \leq 0.05$) in the numbers of Psychrophilic Bacteria in the samples of the meat baker that were treated with different concentrations of the alcohol extract of grape seeds compared to the control sample during the cooling preservation period at a temperature of 4 °C, the average numbers of Psychrophilic Bacteria for the eighth day were 7.76, 6.54, 5.42 x 10^4 CFU/g for experimental treatments , respectively, compared to the control treatment, which amounted to 8.23 ×10^4 CFU/g. Thus, the control treatment exceeded the standard limits and was excluded from the experiment due to the high pollution indicators in it. It was noted that the treatments to which the alcohol extract of grape seeds was added in different concentrations kept within the permissible limits of the numbers of Psychrophilic Bacteria for the tenth day of cryopreservation. The third and fourth treatments were morally superior to the rest of the treatments due to the decrease in the number of Psychrophilic Bacteria until the end of the cryopreservation experiment, where the lowest treatments were.

Table (6): Effect of the use of different concentrations of alcohol extract of grape seeds in the preparation of Psychrophilic Bacteria (cfu/g) for cold-preserved beef at a temperature of 4 C

Treatments	Refrigerated storage periods/ day							
	1	3	5	7	8	9	10	11
					10^4			
T1	4.39	5.83	6.44	7.87	8.23	-	-	-
T2	4.31	4.77	5.22	6.49	7.76	8.45	9.21	-
T3	4.27	4.71	5.17	5.32	6.54	7.81	8.95	-
T4	4.24	4.33	4.65	4.96	5.42	5.67	6.72	8.45
L.S.D.	0.030	0.076	0.081	0.370	1.200	0.601	0.251	-

T1: Treatment of control without any addition. T2: Treating the meat piece with alcohol extract at a concentration of 0.02%. T3: Treating the meat piece with alcohol extract at a concentration of 0.03%. T4: Treating the meat piece with alcohol extract at a concentration of 0.04%. (L.S.D.): The lowest significant difference at a probability level ($p \leq 0.05$).

Coliform Bacteria

The results of Table (7) indicate that the number of coliform bacteria in the control treatment has increased significantly at the level of ($p \leq 0.05$) during the period of cryopreservation compared to the treatments to which the alcohol extract of grape seeds was added, as the numbers of coliform bacteria in the control sample reached 34.8 × 10^4 CFU/g for the eighth day of preservation, which was excluded due to the high oxidation indicators in it. As for the treatments to which the alcohol extract of grape seeds was added in different concentrations, the average number of total bacteria on the eighth day of cryopreservation reached 25.7, 21.9, 19.7 × 10^4 (CFU/g) for the second, third, and fourth treatment, respectively. From the table, we note that the number of coliform bacteria in the control sample exceeded the limits of the standard specifications and became unsuitable for cryopreservation. it was noted that the samples to which the alcohol extract of grape seeds was added in different treatments remained within permissible limits during the cryopreservation.

Table (7): Effect of the use of different concentrations of alcohol extract of grape seeds in the preparation of coliform bacteria (CFU/g) for cold-preserved beef at a temperature of 4 C

Treatments	Refrigerated storage periods/ day							
	1	3	5	7	8	9	10	11
	10^4							
T1	14.5	17.9	13.7	28.5	34.8	-	-	-
T2	12.7	16.3	21.4	22.4	25.7	28.9	30.6	-
T3	14.2	16.1	19.5	20.6	21.9	23.8	26.2	-
T4	13.4	16.0	17.9	18.3	19.7	21.5	13.7	25.6
L.S.D.	0.022	0.211	1.550	1.912	3.006	2.307	3.100	-

T1: Treatment of control without any addition. T2: Treating the meat piece with alcohol extract at a concentration of 0.02%. T3: Treating the meat piece with alcohol extract at a concentration of 0.03%. T4: Treating the meat piece with alcohol extract at a concentration of 0.04%. (L.S.D.): The lowest significant difference at a probability level ($p \leq 0.05$).

4-Discussion

Biological activity of the active compounds in grape seed extract:

Active compounds are defined as compounds with low molecular weights containing an aromatic ring carrying one or more hydroxyl (OH) aggregates, produced in the plant by secondary metabolism when the plant is exposed to a viral or bacterial infection (Nyambe-Silavwe *et al.*, 2015). Effective compounds have an important role in plant health functions. They are necessary to regulate growth and participate in reproduction in plants by attracting insects that pollinate the plant. In addition, they protect plants from UV rays and protect the plant from harmful insect infections (Nyambe-Silavwe *et al.*, 2015). Phytochemical active compounds are chemically heterogeneous and include 10,000 individual compounds, some of which are soluble in water and others dissolved only in organic solvents. Another group is large insoluble polymers (Edreva *et al.*, 2008). In addition, water-soluble compounds tend to be coupled with sugar as in glycosides and are often found in a cell gap, as the presence of phenolic compounds in plants in a conjugate form increases their solubility and makes them suitable for storage in plant gaps (Oudah *et al.*, 2014; Tlili *et al.*, 2017). The mechanism of action of active compounds is due to their reduction property, which makes them able to give a hydrogen atom to the free radicals resulting from the oxidation of the compounds and inhibiting their action (Nakatani, 2000). As between Mohsin *et al.* (2019) The active compounds have antioxidant effectiveness through their oxidative and reductive properties, which play an important role in neutralizing free radicals and capturing oxygen or breaking down hydrogen peroxide into water, as well as their effect as antioxidants. The active compounds have a wide variety of other biological effects that

are beneficial to cells and the organism.

The role of plant extracts in reducing oxidation indicators

Oxidation is one of the main causes of meat damage, which occurs during the transformation of muscles into meat or in the stage of meat processing or the stages of storage (Kumar *et al.*, 2015). This phenomenon leads to undesirable changes such as changing color and flavor and decreasing nutritional quality, thus reducing the shelf life of the product and forming secondary compounds that are harmful to human health (Falowo *et al.*, 2014). The oxidation of fats is one of the natural reactions that occur in foods, causing a reduction in the quality of food. These reactions begin shortly after the slaughter of the animal, and they are one of the most important problems facing food products with high-fat content during preservation, such as meat and its products (Van Hecke *et al*. 2017), oxidative rancidity, which occurs as a result of several factors, the most important of which is the level of unsaturated fatty acids (PUFA) Unsaturated Fatty Acid Poly in meat, which is an essential substance for the oxidation process, the industrial antioxidants (BHT) Butuloted Hydroxy Toluene and BHA Butuloted Hydroxy Anisol can inhibit the primary and secondary oxidation of meat at a good speed, but the risks lie in the possibility that they are carcinogens that disrupt the effectiveness of body cells and the structure of the DNA molecule and cell membrane. The most important free radicals are oxygen derivatives such as peroxides (O_2), hydrogen peroxide (H_2O_2), hydroxyl root (OH), and single or incomplete oxygen (O_2) (Mitsumoto *et al*., 2005). Oxidation is the transfer of electrons from a substance to the oxidizing agent, as the oxidizing agent is the substance capable of receiving electrons, oxidation is one of the basic and important reactions in the human body, and the results of this oxidation are the release of free radicals (Free radicals), which in turn destroy and destroy cell molecules through a series of reactions and also destroy fatty acids in the cell, which makes the human body vulnerable to many infections (Dawidowicz *et al.*,2006). Accordingly, technologies such as packaging and natural antioxidant workers have been developed with an understanding of the fat oxidation mechanisms responsible for reducing the sensory and nutritional quality of meat and its products. Fat oxidation can be controlled by different strategies such as additives and the use of special packages to prevent negative effects on the sensory properties of meat (Amaral *et al.*, 2018). Several studies have shown the possibility of replacing industrial antioxidants with natural antioxidants (Franco *et al.*, 2018), as the most important factors that led to the increase in the use of natural substances are the low cost and compatibility with the

diet (Anbudhasan *et al.*, 2014) and their great impact on inhibiting the oxidation of fats and their safety in influencing the consumer (Foroudi *et al.*, 2014).

The role of plant extracts in reducing microbial pollution indicators:

Meat and its products are exposed to damage and microbial corruption because they contain all the nutrients necessary for the growth of microorganisms, the most important of which are Salmonella, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and others that begin to spread from the stage of the live animal and during the stages of slaughter that pose a threat to consumer health. The possibility of controlling the reduction of the level of microorganisms in meat products when processed in certain ways, such as the use of refrigeration that extends the shelf life of meat, reduces microbial contamination in carcasses and reduces the growth and reproduction of microorganisms when processing meat or using natural inhibitors, which have a significant role in preventing the deterioration, safety and quality of food products (Pal *et al.*, 2018). Salmonella bacteria are one of the most important pathogens that may be transmitted to food and meat (Bennett *et al*., 2015), and Jackson et al. (2013) pointed out that the main cause of poisoning and deaths resulting from food contamination is due to salmonella bacteria, as 1.2 million infections occur annually in the United States of America. Blount (2015) confirmed that most of the infections are due to the consumption of meat products contaminated with E. coli bacteria, in addition to the fact that these bacteria are naturally present in the intestines of animals and humans and are harmless, but there are pathogenic strains such as *E. coli* H157 that cause spasm and bloody diarrhea and may even reach death in cases.

The mechanism of action of active compounds is still not fully understood, but it can cause damage to the walls of bacterial cells and thus affect the formation of biofilms. Polyphenols also affect the biosynthesis of protein within the bacterial cell, alter metabolic processes, and prevent the synthesis of ATP and DNA. Therefore, medicinal plants are an alternative to chemical preservatives used in the meat industry, especially nitrates that can inhibit the growth of pathogenic microbes (Efenberger-Szmechtyk *et al.*, 2020).

Conclusion:

Based on the results obtained from this study, we can conclude that the alcohol extract of grape seeds contains active compounds with a biological effect. Adding grape seed extract to cold- preserved beef extended the shelf life of meat compared to the control treatment through its action as a natural antioxidant, which led to a decrease in oxidation indicators (thiobarbituric acid, peroxide value, and free fatty acids). The addition of alcohol extract for grape seeds also contributed to reducing the high total number of bacteria, Psychrophilic Bacteria, and Coliform Bacteria that cause meat corruption and damage.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were

conducted in accordance with institutional and laboratory biosafety guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This research received no external funding.

Data Availability

All data supporting the findings of this study are available within the manuscript.

5-References

[1] Al-Abid, M. R. (1985). Zurrzusamme mse turungder Abschla B membrane in Phoenix dactylifera. Wurzburg University. Wuzzburg, FR of Germany. 153-140.

[2] Amaral, A. B., Silva, M. V. da and Lannes, S. C. da S. (2018) 'Lipid oxidation in meat: mechanisms and protective factors – a review', Food Science and Technology, 38(1), 1–15.

[3] Anbudhasan, P.; Surendraraj, A.; Karkuzhali, S. and Sathishkumaran, P. (2014). Natural antioxidants and its benefits. International Journal of Food and Nutritional Sciences, 3(6): 225-232.

[4] Andrew, W. (1992). Manual of quality control 4. Rev. 1. Microbiological analysis.

[5] FAO. Food and Nutrition Paper]. Washington, USA: FAO.

[6] Bagchi, D., Swaroop, A., Preuss, H. G., & Bagchi, M. (2014). Free radical scavenging, antioxidant and cancer chemoprevention by grape seed proanthocyanidin: an overview. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 768, 69-73.

[7] Bennett, S. D.; Littrell, K. W.; Hill, T. A.; Mahovic, M. and Behravesh, C. B. (2015). Multistate foodborne disease outbreaks associated with raw tomatoes, United States, 1990–2010: a recurring public health problem. Epidemiology & Infection, 143(7), 1352-1359.

[8] Blount, Z. D. (2015). The natural history of model organisms: The unexhausted potential of E. coli. Elife, 4, e05826.

[9] Chand, N., Ali, P., Alhidary, I. A., Abdelrahman, M. A., Albadani, H., Khan, M. A., & Khan, R. U. (2021). Protective effect of grape (Vitis vinifera) seed powder and zinc-glycine complex on growth traits and gut health of broilers following Eimeria tenella challenge. Antibiotics, 10(2), 186.

[10] Chedea, V. S., Braicu, C., Chirilă, F., Ober, C., & Socaciu, C. (2011). Antibacterial action of an aqueous grape seed polyphenolic extract. African Journal of Biotechnology, 10(33), 6276-6280.

[11] Costa, A., Pegas Pereira, E. S., Assumpção, E. C., Calixto dos Santos, F. B., Ota, F. S., de Oliveira Pereira, M., & Abildgaard, E. N. (2015). Assessment of clinical effects and safety of an oral supplement based on marine protein, vitamin C, grape seed extract, zinc, and tomato extract in the improvement of visible signs of skin aging in men. Clinical, cosmetic and investigational dermatology, 319-328.

[12] Dawidowicz, A. L.; Wianowska, D. and Baraniak B. (2006). The Antioxidant properties of alcoholic extracts from sambucus nigra l. lebensmittel - wissenschaft und –technology. 39: 308–315.

[13] Edreva, A.; Velikova, V.; Tsonev, T.; Dagnon, S.; Gürel, A.; Aktaş, L. and Gesheva, E. (2008). Stress-protective role of secondary metabolites: diversity of functions and mechanisms. Gen Appl Plant Physiol., 34(1-2): 67-78.

[14] Efenberger-Szmechtyk, M.; Nowak, A. and Czyzowska, A. (2020). Plant extracts rich in polyphenols: antibacterial agents and natural preservatives for meat and meat products. Critical Reviews in Food Science and Nutrition, 1-30.

[15] Elmer, W. K., Stephen D. A., Dowell, V. R., Herbert, Jr. & Sommers, M. (1979). Color Atlas and text book of Diagnetic Microbiology. Priinted in the united starter of America.

[16] Falowo, A. B., Fayemi, P. O. and Muchenje, V. (2014). 'Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review', Food Research International. Elsevier, 64, pp. 171–181.

[17] Foroudi, S.; Potter, A. S.; Stamatikos, A.; Patil, B. S. and Deyhim, F. (2014). Drinking orange juice increases total antioxidant status and decreases lipid peroxidation in adults. Journal of Medicinal Food, 17(5): 612-617.

[18] Franco, D.; Rodriguez-Amado, I.; Agregán, R.; Munekata, P. E.; Vázquez, J. A.; Barba, F. J. and Lorenzo, J. M. (2018). Optimization of antioxidants extraction from peanut skin to prevent oxidative processes during soybean oil storage. LWT, 88: 1-8.

[19] GENSTAT (2012). GenStat Statistical Software, version 15.01. GenStat CO, UK.

[20] Gheisari, H. R., Møller, J. K., Adamsen, C. E., & Skibsted, L. H. (2010). Sodium chloride or heme protein induced lipid oxidation in raw, minced chicken meat and beef. Czech Journal of Food Sciences, 28(5), 364-375.

[21] Gizaw, A., Marami, L. M., Teshome, I., Sarba, E. J., Admasu, P., Babele, D. A. Abdisa, K. (2022). Phytochemical screening and in vitro antifungal activity of selected medicinal plants against candida albicans and aspergillus niger in west shewa zone, Ethiopia. Advances in Pharmacological and Pharmaceutical Sciences, 2022(1), 3299146.

[22] Harborne, A. J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. Springer science and business media.
[23] Israr, M., Chand, N., Khan, R. U., Alhidary, I. A., Abdelrahman, M. M., Al- Baddani, H. H., & Tufarelli, V. (2021). Dietary Grape (Vitis vinifera) Seed Powder and Zn–Gly Chelate Complex for Mitigating Heat Stress in Broiler Chickens: Growth Parameters, Malondialdehyde, Paraoxonase-1, and Antibody Titer. Agriculture, 11(11), 1087.
[24] Jackson, B. R.; Griffin, P. M.; Cole, D.; Walsh, K. A. and Chai, S. J. (2013). Out break-associated Salmonella enterica serotypes and food commodities, United States, 1998–2008. Emerging infectious diseases, 19(8): 1239.
[25] Jaffer, H. J.; Mohamed, M. J.; Jawad, A. M.; Naj, A. and Al-Naib, A. (1983). Phytochemical and biological screening of some Iraqi plant. Fitoterapialix (in Arabic), 59: 229-238.
[26] Kim, S. Y., Jeong, S. M., Park, W. P., Nam, K. C., Ahn, D. U., & Lee, S. C.
[27] (2006). Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. Food chemistry, 97(3), 472-479.
[28] Kumar, Y., Yadav, D. N., Ahmad, T., & Narsaiah, K. (2015). Recent trends in the use of natural antioxidants for meat and meat products. Comprehensive Reviews in Food Science and Food Safety, 14(6), 796-812.
[29] Luo, D., & Fang, B. (2008). Structural identification of ginseng polysaccharides and testing of their antioxidant activities. Carbohydrate Polymers, 72(3), 376-381.
[30] Mitsumoto, M.; O'Grady, M. N.; Kerry, J. P. and Buckley, D. J. (2005). Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties. Meat Science, 69(4): 773-779.
[31] Mohsin, R. H.; Nasser, A. K. and Al-Abadi, J. K. (2019). Effect of Quinoa seeds on enzymatic and non-enzymatic antioxidants in serum of stressful local rabbits by hydrogen peroxide. Jornal of Al-Muthanna for Agricultural Sciences, 7(3): 244-252.
[32] Nakatani, N. (2000). Phenolic antioxidants from herbs and spices. Biofactors, 13(1- 4): 141-146.
[33] Nyambe-Silavwe, H.; Villa-Rodriguez, J. A.; Ifie, I., Holmes, M.; Aydin, E.; Jensen, J. M. and Williamson, G. (2015). Inhibition of human α-amylase by dietary polyphenols. Journal of Functional Foods, 19: 723-732.
[34] Oudah, S. K.; Al-Salih, R. M. and Gusar, S. H. (2014). Study The Role of Polyphenolic Extract of Capparis Spinosa L. Leaves As a Hypoglycemic agent. International Journal of Scientific & Engineering Research, 5(5): 1561-1575.
[35] Pal, M.; Ayele, Y.; Patel, A. S. and Dulo, F. (2018). Microbiological and hygienic quality of meat and meat products. Beverage and Food World, 45(22): 7-21.
[36] Roberts, D., & Greenwood, M. (2008). Practical food microbiology. John Wiley & Sons.
[37] Romero, C., Arija, I., Viveros, A., & Chamorro, S. (2022). Productive performance, egg quality and yolk lipid oxidation in laying hens fed diets including grape pomace or grape extract. Animals, 12(9), 1076.
[38] Shi, J., Yu, J., Pohorly, J. E., & Kakuda, Y. (2003). Polyphenolics in grape seeds biochemistry and functionality. Journal of medicinal food, 6(4), 291-299.
[39] Shihata, I. M. (1951). A pharmacological Study of Anagallis Arvensis. MD Thesis, Cairo University, Eygept.
[40] SPSS: Statistical Package for the Social Sciences (2018). Quantitative data analysis with IBM SPSS, version 24: A guide for social scientists. New York, Routledge. ISBN, 978-0-415-57918-6.
[41] Stadnik, J. (2024). Nutritional Value of Meat and Meat Products and Their Role in Human Health. Nutrients, 16(10), 1446.
[42] Tlili, N.; Feriani, A.; Saadoui, E.; Nasri, N. and Khaldi, A. (2017). Capparis spinosa leaves extract: Source of bioantioxidants with nephroprotective and hepatoprotective effects. Biomedicine & Pharmacotherapy, 87: 171-179.
[43] Van Hecke, T.; Van Camp, J. and De Smet, S. (2017). Oxidation during digestion of meat: interactions with the diet and helicobacter pylori gastritis, and implications on human health. Comprehensive Reviews in Food Science and Food Safety, 16(2): 214-233.