# Study of the effect of methanolic and nano extract of pumpkin peels on the physicochemical properties of ground beef during cold storage

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#### **ABSTRACT**

This study demonstrated the effect of methanolic and nano-extracts of pumpkin peels on beef stored refrigerated for 12 days. A concentration of 200 mg/ml was adopted for the methanolic extract of pumpkin peels and a concentration of 32 mg/ml for the nano-extracts Add the extracts separately as follows: Treatment A0 (control treatment), Treatment A1 (3% methanolic pumpkin peel extract), Treatment A2 (6% methanolic pumpkin peel extract), Treatment A3 (0.3% nano extract), and Treatment A4 (0.6% nano extract). The results showed that the nano extract was excelled on the methanolic extract of pumpkin peels. The peroxide value at the beginning of storage was 3.25, 3.20, 3.18, 3.20, and 3.20 mmEq/kg fat, respectively, and the final values reached 8.20, 7.00, 6.12, 5.88, and 5.12 mmEq/kg fat. The values of thiobarbituric acid at the beginning of storage were 0.032, 0.032, 0.033, 0.033 mg malondehyde/kg, respectively, and the final values were 0.079, 0.068, 0.060, 0.052, 0.049 mg malondehyde/kg, respectively. As for the free fatty acids, they were at the beginning of storage, it was 0.36, 0.35, 0.36, 0.35, and 0.36 mmEq/kg fat, respectively. The final values were 0.85, 0.69, 0.60, 0.53, and 0.50 mmEq/kg fat, respectively. As for the pH at the beginning of storage, it was 5.89, 6.33, 6.58, and 6.00, 6.09, respectively, and the final values were 4.84, 5.74, 5.80, 5.33, and 5.48, respectively. As for the total nitrogen values at the beginning of storage, they were 4.22, 4.21, 4.22, 4.21, 4.21 mg/100 g, and the final values were 10.89, 8.62, 8.02, 7.11. 6.55 mg/100 gm, respectively. The results confirmed that the nano-extract of pumpkin peels can be very effective as a natural antioxidant, as it preserved the quality of Ground beef by reducing fat oxidation when stored in cold storage for 12 days, where the values of peroxide, thiobarbituric acids, and acids decreased. Free fat, pH, and total volatile nitrogen in meat samples treated with 0.3 and 0.6% concentrations of nano-extract of pumpkin peels.

key words: Antioxidant, beef, peroxide value, nano

#### INTRODUCTION

Food is the first human need, and modern technologies are used to develop it and ensure its safety. Among these modern and advanced technologies that have become widespread are nano foods, as nano technology has been used to preserve foods, detect food spoilage, and safety, packaging, and wrapping of foods [7] Nanotechnology has a major impact on the lives of consumers, as is evident from the increased availability of nanoproducts. Food industry experts expect that nanotechnology will have a significant impact in various ways

on food products, whether directly or indirectly [2]

Food companies seek to apply nanotechnology in order to produce the best agricultural crops free of preservatives and harmful chemicals, which can be considered one of the most promising technologies in the food industries, which enables manufacturers to improve and develop the food composition, as well as the quality and safety of food products, as nanotechnology affects in Nanotechnology the functional structural properties of foods [4]. At the global

level, various nanoparticles have been applied in many foods, through which they have improved sensory qualities such as (improving flavour, color and texture) of many food products, increased absorption, as well as the stability of the structures of active compounds in foods, as well as the possibility of evaluating the safety of packaging of products. Also, adding nanoparticles can help increase the shelf life of foods by using them as antimicrobials that cause pathogens in foods [20]

Food is the basic foundation of life and health throughout a person's lifespan, as it is considered an important matter for protecting against malnutrition diseases and ensuring Functional foods normal growth. emerged as an effective means of preventing diseases, as this change encourages consumers to reconsider the food industry as well as. Applying modern manufacturing technology in food fortification [3]. Food wastes are end products of food processing industries that are not recycled or used for other purposes, so they are disposed of as waste. These wastes can be considered valuable by-products, if there are appropriate technological means [12] Fruit and vegetable peels are by-products that accumulate in large quantities in processing of various fruits and vegetables. The peel manufacturing processes represent a serious problem, as they have a harmful effect on the environment. They are an important part of a good diet and contain large quantities of life-active compounds. Thursday, 2022). Fruit and vegetable wastes and by-products obtained from the food industry consist of seeds, peels, and pomace, and the effective use of these components can lead to the extraction of many bioactive compounds including essential components such as sugars, proteins, dietary fibers, etc. This, and other secondary

components such antioxidants, as antimicrobials. and pigments, including natural pigments because they are natural (plant-based) and safe (non-toxic) can often find potential applications as well as they can enhance the aesthetic value of processed food goods. [22] The current research aims to study the effect of methanolic and nano-extracts of pumpkin peels on the chemical properties of ground beef during cold storage by estimating the peroxide value, thiobarbituric value, free fatty acids, pH, and total volatile nitrogen, which were prepared in a study. A precedent for us.

# MATERIALS AND METHODS Ground beef

Cut beef was purchased from the thigh area from one of the butcher shops in the city of Baghdad, and it was minced in a meat machine, then concentrations of methanolic pumpkin peel extract and nano extract were added to it in different proportions, which were prepared in a previous study by us, where the methanolic extract of raw pumpkin peels was prepared at concentrations of 100 and 200 mg/ ml and nano-extract of pumpkin peels loaded with nano chitosan using the ionic gelation method at concentrations (16 and 32 mg/ml). After estimating the total phenolic compounds and the antioxidant activity of the extracts, a concentration of 200 mg/ml for the methanolic pumpkin peel extract and a concentration of 32 mg/ml for the nanopumpkin peel extract was adopted in this research.

The treatments were A0 treatment (control), A1 treatment (3% methanolic pumpkin peel extract), A2 treatment (6% methanolic pumpkin peel extract), A3 treatment (0.3% nano extract), and A4 treatment (0.6% nano extract). Each treatment was mixed separately to homogenize the distribution of the extract

and stored in the refrigerator at a temperature of  $(5\pm1)^{\circ}$ C for 1, 3, 7, 9 and 12 days to conduct the following tests:

### - Estimating the peroxide value (P.V).

The estimate was based on Egan, et al (1981), where 2 g of the extracted fat was weighed using a Soxhlet device, and 30 ml of a mixture containing (3 parts of glacial acetic acid + 2 parts of chloroform) was added to it, with an increase of 5 0 ml of saturated potassium iodide, 30 ml of distilled water, and 1 ml of starch index (1%), then the mixture is flushed with a 0.01 M sodium thiosulfate solution until the blue color disappears, after which it is estimated based on the following equation:

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

# - Estimating the value of Thiobarbituric Acid (TBA)

Fat oxidation in the sample was measured by determining thiobarbituric acid according to the method of [24] Absorbance was measured at a wavelength (530 nm) using a spectrophotometer. The TBA value was calculated by multiplying the absorbance value by the factor 5.2, and the value was expressed on a mg basis. Malone dehyde (MDA) per kg according to the following equation:

TBA value mg/MDA kg =  $A_{530} \times 5.2$ 

# - Estimating the concentration of free fatty acids

Free fatty acids were estimated according to the method of [22] where the fat was extracted by the cold method. 10 g of fat was taken and 25 ml of ether and 25 ml of ethanol were added to it at a neutral concentration of 95%, in addition to 1 ml of phenonphthalein reagent and it was treated with Sodium hydroxide (0.1 M) until the solution became

pink in color, and the percentage of free fatty acids was calculated based on oleic acid, according to the following equation:

Acidity%=

Volume of base consumed (ml) x standard of base (0.1) x milli

Sample weight (g)

### - Measuring pH

The pH was measured according to the method presented by [21] which stipulates taking 10 g of the sample, adding 100 ml of distilled water to it, homogenizing it for 15 minutes, then filtering the sample and measuring its pH using a pH meter.

### - Total Volatile Nitrogen (T.V.N)

Total volatile nitrogen was estimated based on the method mentioned by [22] and the amount of volatile nitrogen was calculated according to the following equation:

Amount of volatile nitrogen (mg nitrogen / 100 g) = 14

Normal value of T.V.N = 19 mg / 100 gm.

#### RESULTS AND DISCUSSION

# - Estimating the value of the peroxide number (PV) in beef samples

The peroxide number is one of the important indicators that gives an idea about the amount of oxidation of oils and fats in foods. Peroxides are also primary products produced by the reaction that occurs between atmospheric oxygen and unsaturated fatty acids in fat. The amount of peroxides in fat is usually expressed by the peroxide number, which represents the amount of millimoles. The oxygen bound to the form of peroxide in one kilogram of fat [13]

By observing the results in Table (1), we find that the value of the peroxide number on the first day for the treatment (A0 control) was 3.25, and for the other treatments it was 3.20, 3.18, and 3.20 mmEq/kg fat, respectively. From the results of the statistical analysis, it is clear that there are no significant differences

between the different treatments. On the first day of storage, at a probability level (P≤.0.05), while during storage, the value of the peroxide number increased after 12 days for treatment A0, reaching 8.20 mEq /1 kg of fat. This development is considered significant in the peroxide number, while the values of the peroxide number for the auctioned treatments reached Methanol of pumpkin peels, and nano extract (A1 'A2' A3 'A4)) 7.00, 6.12, 5.88, 5.12 mEq/1 kg fat, respectively.

The results of the statistical analysis also showed that there were significant differences between the different Burger meat treatments, all with the control treatment (A0) and after 12 days of storage at the probability level  $(P \le 0.05)$ .

It is also noted that treatment A3 and A4, to which the nano extract was added at a concentration of 0.3 and 0.6%, respectively, outperformed all the different meat treatments in preserving the peroxide value.

The reason is due to the nano-extract containing phenolic compounds, which have the ability to absorb free radicals and thus preserved the meat samples.

These results encourage the use of nanoplant extracts in meat storage, because they possess highly effective antioxidants, where they maintained the cold-stored meat samples within the standard specifications.

These results are similar to what [18] found when she used the alcoholic extract of pumpkin seeds to preserve camel meat by cooling, as it was observed a significant decrease in the peroxide values of meat samples treated with the alcoholic extract of pumpkin seeds compared to the control treatment. It also agreed with [15] where The peroxide value in beef fat decreased when flavonoids extracted from Sidr leaves were added in comparison with the control treatment.

As [8] mentioned, when adding 300 to 500 ppm of carotenoids extracted from pumpkin to sunflower oil, the stability of the oil containing 200 ppm of the synthetic antioxidant BHT increased.

These results are also encouraging for the use of plant extracts in prolonging the storage life of meat because they possess highly effective antioxidants that preserved the preservation of cold-preserved meat samples, and this was confirmed by [10]

Table 1: Effect of methanolic extract of pumpkin peels and nano-extract on peroxide values mEq/kg of beef samples at different Storage periods

			Storage pe	eriods			
12day	9 <b>days</b>	7days	5 <b>days</b>	3days	1 <b>day</b>	treatments	
8.20	7.98	7.15	6.22	4.15	3.25	A0	
7.00	6.25	5.66	4.18	3.80	3.20	A1	
6.12	5.44	4.88	3.95	3.60	3.18	A2	
5.88	5.05	4.11	3.80	3.40	3.20	A3	
5.12	4.88	4.00	3.65	3.33	3.20	A4	
1.347*	1.294*	1.572*	1.45*	0.752*	0.177*NS	LsD	
NS * (P (0.05≥non significant							

Determination of the value of thiobarbituric acid (TBA) in beef samples

Table (2) indicates the values of thiobarbituric acid (TBA) for the different treatments, and the results showed that the

value of (TBA) for the control treatment A0 was 0.032, and for the treatments A1, A2, A3, and A4, it was 0.032 and 0.032. 0.033 and 0.33 mg malondehyde/kg, respectively. From the results of the statistical analysis, it is clear that there are no significant differences between the treatments on the first day of storage at the probability level (P≤.0.05). However, during storage, an increase in the values of thiobarbituric acid was observed, it reached After 3 days, the control treatment (A0) received 0.042 mg malondehyde/kg, while the other treatments A1, A2, A3, and A4 were (0.039, 0.037, 0.035, 0.034) mg malondehyde/kg, respectively.

The results of the statistical analysis also indicate that there are no significant differences between the different Burger meat treatments, all of them with the control treatment, after 3 days of storage period. After 5 and 7 days, these values increased further and in a non-significant manner as well. After 12 days of storage period, the acid value reached Thiobarbituric for the treatment was 0.079 mg malondehyde/kg, while for treatments A1, A2, A3, and A4 it was (0.068, 0.060, 0.052, and 0.049) mg malondehyde/kg, respectively. The results of the statistical analysis indicated that there were significant differences between the meat treatments. All different types of beer with the control treatment A0 after 12 days of storage at the probability level ( $P \le 0.05$ ).

It was also observed that the treatments to which the nano extract was increased at concentrations of 0.3 and 0.6% were excelled on the control treatment (A0) and the treatments to which the methanolic extract of pumpkin peels was increased at concentrations of 3 and 6% in maintaining the thiobarbiturate value within acceptable limits until the end of the preservation period. This is due to the presence of phenolic compounds. In addition, chitosan possesses antioxidant activity, which is further enhanced by the loss of chitosan derivatives, as chitosan retains two hydroxyl groups and an amino group, which enables it to interact with free radicals, so it has the ability to suppress free radicals [23]

These results agreed with the findings of [9] in his study of fermented pastrami. He noted an increase in TBA values, but they were within permissible limits. [6] also confirmed that pomegranate peel extract led to a decrease in thiobarbiturate when the meat was stored at a temperature of 18°C.

Table 3: Effect of methanolic extract of pumpkin peels and nano-extract on the thiobarbituric values of Burger meat samples during a storage period of 12 days at a temperature of (1+5)°C.

			Storage 1	periods		
12day	9 <b>days</b>	7days	5 <b>days</b>	3days	1 day	treatments
0.079	0.074	0.062	0.058	0.042	0.032	A0
0.068	0.062	0.052	0.047	0.039	0.032	A1
0.060	0.052	0.049	0.044	0.037	0.032	A2
0.052	0.048	0.045	0.042	0.035	0.033	A3
0.049	0.046	0.043	0.040	0.034	0.033	A4
0.0271*	0.024*	0.022*	0.019*	0.017*	0.009 *NS	LSD
	•	NS:	* ( <b>P</b> (0.05≥ <b>n</b>	on significa	ınt	•

### - Free fatty acids A.F.F

Fatty decomposition is one of the dangerous spoilage factors that negatively

affects products, and the lipase enzyme secreted by cold-loving bacteria and sporeproducing bacteria is the main cause of this

decomposition because it is resistant to high temperatures [11]

From observing the results in Table (4), we find that the value of free fatty acids on the first day for treatment A0 (control) was 0.36, and for the other treatments (A1, A2, A3, A4) it was 0.35, 0.36, 0.35, and 0.36 mEq/kg, respectively. The results of the statistical analysis show that there are no significant differences between the different treatments on the first day of storage at the probability level ( $P \le 0.05$ ). However, during storage, the value of free fatty acids increased after 12 days for the treatment (A0), reaching 0.85 mEq/kg.

While the values of the free fatty acids auctioned for the methanolic extract of pumpkin peels and the nano-extract represented by (A1, A2, A3, A4) reached 0.69,

0.60, 0.53, 0.50 mEq/kg, respectively, as all treatments maintained the value of the free fatty acids, as the reason The percentage of fats and fatty acids remained within the limits in the samples treated with the nanocomposite due to the presence of phenolic compounds, which slowed the process of lipolysis, which reduces the lipase-enhancing bacteria that cause lipolytic rancidity [13], as well as nanochitosan. Which is considered an antimicrobial agent due to its unique physical and chemical traits, which led to maintaining the percentage of chemical components within acceptable limits (Marangon, et al, 2020).

As stated by [1] nano-extracts increase their effectiveness as antioxidants, balancing with extracts of the substance itself, and in a natural way.

Table 4: Effect of methanolic extract of pumpkin peels and nano-extract on free fatty acids (A.F.F) of beef samples during a storage period of 12 days at a temperature of  $(5\pm1)^{\circ}$ C.

			Stora	ige periods		
12day	9 <b>days</b>	7days	5 <b>days</b>	3days	1 <b>day</b>	treatments
0 .85	0.80	0.75	0.68	0.50	0,36	A0
0.69	0.63	0.59	0.50	0.41	0,35	A1
0.60	0.57	0.50	0.47	0.39	0,36	A2
0.53	0.50	0.45	0.41	0.37	0,35	A3
0.50	0.46	0.41	0.39	0.37	0,36	A4
0.289*	0.259*	0.278*	0.128*	0.115*	0,088 *NS	LSD
	1	NS	S * (P (0.0)	5≥ non sign	ificant	1

- pH

To follow up on the changes occurring in the acidity of the treated and studied samples during their cryopreservation, the pH was estimated. Table (5) shows the results of determining the pH of the burger meat samples during the storage period of 12 days and at a temperature of (1 + 5) C, as the value reached The pH of the samples was (5.89-6.58) on the first day of storage. The results of the statistical analysis indicated that there

were no statistically significant differences at the level ( $P \le 0.05$ ).

During the storage period, a decrease in pH values was observed for all treatments, where the pH value after 12 days for the control treatment A0 reached (4.84), while it was (5.74, 5.80, 5.33, and 5.48) for the treatments (A1, A2, A3, A4). ) respectively, and the reason for the decrease in pH is due to the activity of enzymes that hydrolyze glycogen into lactic acid [19] and the results show that the pH values of the auctioned

treatments to which the methanolic extract and nano extract were lower than the control treatment and this is due to containing The extracts contain phenolic compounds and flavonoids, which act as antioxidants. The results also show the superiority of the nanoextract over the methanolic extract of pumpkin peels in lowering the pH. The reason for the lack of development in acidity for the

samples treated with the nanocomposite is attributed to the efficiency of the nanocomposite that contains the antimicrobial chitosan compound, which helps It reduces the number of contaminated bacteria that cause high **acidity** [5,14also indicated the inhibitory effectiveness of chitosan against Grampositive and Gram-negative bacteria as well as fungi.

Table 5: Effect of methanolic extract of pumpkin peels and nano-extract on the pH of beef samples during a storage period of 12 days at a temperature of  $(5\pm1)^{\circ}$ C.

			Stor	age period	S	
12day	9 <b>days</b>	7days	5 <b>days</b>	3days	1 <b>day</b>	treatments
4 .84	5.00	5.25	5.60	5.72	5.89	<b>A0</b>
5.74	5.89	6.00	6.10	6.24	6.33	A1
5.80	5.97	6.09	6 .25	6.44	6.58	A2
5.33	5.45	5.59	5.70	5.87	6.00	A3
5.48	5.62	5.71	5.85	5.92	6.09	A4
0.788*	0.842*	0.692*	0.546*	0.613*	*0.0622NS	LSD
	1	ľ	NS * (P (0.0	)5≥ non sig	gnificant	

## - Total volatile nitrogen (T.V.N)

The results in Table (3) indicated that there was no change or difference in the values of total volatile nitrogen (T.V.N) when treated with the nanocomposite and the methanolic extract balanced with the control treatment on the first day of storage, while the value of total volatile nitrogen increased after 12 days of storage balanced. On the first day.

It was observed that there was a decrease in the value of total volatile nitrogen when treated with the nanocomposite at a concentration of 0.3 and 0.6% (3A4.A) to reach 7.11 and 6.55 mg/100 g, respectively, while it reached 8.62 and 8.02 mg/100 g when treated with the methanolic extract at a concentration 3 and 6%, respectively, in comparison with the control treatment, which

amounted to 10.89 mg/100 g. From the results of the statistical analysis, it was noted that there were significant differences between the various treatments, all with the control treatment (A0) after 12 days of storage at the probability level ( $P \le 0.05$ ).

This increase in TVN value is attributed to protein breakdown as a result of the activity of microbial strains and proteolytic enzymes [16].

as the value (N.V.T) increased in a sample of chicken meat pies during cold storage. Al-Tamimi and Abu Maali (2011) indicated a decrease in the value of total volatile nitrogen for treatments of Burger meat auctioned with ginger when balanced. With control treatment.

Storage periods 12day 9days 7days 5days 3days 1**day** treatments 10.89 9.11 8.48 7.11 5.98 4.22  $\mathbf{A0}$ 8.62 7.85 7.15 6.22 5.33 4.21 **A1** 8.02 7.08 6.74 6.00 5.12 4.22 **A2** 7.11 6.77 6.00 5.75 5.00 4.21 **A3** 6.55 5.85 3.36 4.21 6.00 4.85 **A4** 0.702\* 2.065\* 1.853\* 2.091\* 1.055\* 0.317 \*NS LSD NS \* (P (0.05 > non significant)

Table 3: Effect of methanolic extract of pumpkin peels and nano-extract on the values of total volatile nitrogen (T.V.N) of Burger meat samples during a storage period of 12 days at a temperature of (1+5)°C.

#### **CONCLUSION**

According to the results of the current study, it can be concluded that the prepared Cu/Cs-NPs nanocomposite has an effect in preserving the qualitative properties of beef and prolonging the preservation period, as this study found that the antioxidant activity of the methanolic extract increased by loading it with nano chitosan.

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