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(Original Article)

Effect of Incorporating Grape Seeds as a Natural Preservative on the Properties of Beef Burgers During Freeze Preservation

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Abstract

Grape seeds are a rich source of phenolic substances, which are known for their potent antioxidants and antimicrobial properties. This study aims to estimate the total phenolic content and antioxidant activity in the aqueous extract of red and white grape seeds. Additionally, the effect of adding red and white grape seeds in the form of powder or aqueous extract as preservative films by incorporating carboxymethyl cellulose (CMC) on the quality characteristics of beef burgers during freezing storage at -18±1°C for 90 days. The results showed that grape seeds contain phenolic substances at a high rate in addition to high antioxidant activity in both varieties. Free phenolic contents and the antioxidant activity were 557.83 - 411.70 mg GAE/100g and 66.37 - 72.71% in the aqueous extract of white and red grape seeds, respectively. Addition of grape seeds led to improving the characteristics of the beef burger during freezing storage. The values of pH, peroxide, and TBA decreased compared to the control sample. The same treatments caused an improvement in the microbial quality of beef burger; the total counts of bacteria, fungi, and yeasts were significantly decreased in the treated samples during storage compared with the control sample. In general, addition of grape seeds powder or their extracts as a natural source of antioxidants and antimicrobials instead of the synthetic antioxidants such as butylated hydroxytoluene (BHT) can improve the quality characteristics of beef burger and increase its acceptability among the consumers.

Keywords: *Antioxidant activity, Carboxymethyl cellulose, Grape seeds, Natural antioxidant, Phenolic content.*

Abbreviation list

Carboxymethyl cellulose	(CMC)
Butylated hydroxytoluene	(BHT)
Tertiary butylhydroquinone	(TBHQ)
Butylated hydroxyanisole	(BHA)
Food and Drug Administration	(FDA)
Everything Added to Food in the United States	(EAFUS)

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Generally recognized as safe	(GRAS)
Egyptian organization for standardization	(EOS)
Gallic acid equivalents	(GAE)
2, 2-Diphenyl-1-picrylhydrazyl	(DPPH)
Thiobarbituric acid	(TBA)

Introduction

Grape seeds comprise about 5% of the fruit weight (Choi and Lee, 2009) and more than 3 million tons of them are discarded annually worldwide Fernandes *et al*. (2013). Grape seeds have a high nutritional value as they contain 6.71% moisture, 9.07% protein, 10.52% oil, 31.22% fiber, 2.83% ash and 39.65 % Carbohydrates (Aly and Mohamed, 2022).

Grape seeds are a rich source of phenolic substances and flavonoids known for their antioxidant and antimicrobial activity (Ghouila *et al*., 2017). The total phenolic content of white and red grape seeds varieties ranged from 1974.9 to 3884.4 mg/100g and from 1037.0 to 5759.1 mg/100g, respectively. The degree of maturity and environmental conditions influence the content of phenolic compounds. Moreover, the fruit parts, such as skin, pulp or seeds, exhibit different polyphenol concentrations (Samoticha *et al*., 2017).

On the other hand, lipid peroxidation is one of the major reasons for the deterioration of food products during processing and storage. Therefore, antioxidants are added to food products to extend their shelf life. Consequently, in industrial processing of meat, several synthetic antioxidants such as tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) have successfully been added. Because it delays lipid oxidation, prevents undesirable reactions, and prolongs shelf-life. However, the increasing awareness of consumers over the rigorous toxicity and the potential health hazards of synthetic antioxidants has recently emphasized nutritional value of natural antioxidants (Bjelakovic *et al*., 2007).

The extracts from plants are mostly utilized against anti-inflammatory effects, anti-cancer, anti-bacterial, antifungal, cardiovascular effects, and for various other activities as well (Ali *et al*., 2017). Extracts of grape seeds has been approved as GRAS by Food and Drug Administration (FDA) and is sold as a dietary supplement listed on the Everything Added to Food in the United States (EAFUS) database (Guo *et al*., 2007).

The antioxidant activity of grape seeds extract is demonstrated through the following mechanisms: inhibition of the malonaldehyde formation (El-Zainy *et al*., 2016), primary and secondary products of lipid oxidation (Brannan and Mah, 2007), scavenging initiating free radicals, decomposing peroxides so preventing their conversion into initiating radicals, and chain-breaking to prevent continued hydrogen abstraction by active radicals (Adedapo *et al*., 2008). Li *et al*. (2008) reported that the free radical scavenging activity of grape seeds was determined. The results indicated that the rate of DPPH scavenging activity depends on the phenolic content of the grape seed extracts.

Food spoilage means that its original nutritional value, flavor, and/or texture of the food being damaged, thus the food become unsuitable to be consumed (Mahmoudzadeh *et al*., 2010). Grape seeds extract acts as an excellent natural antioxidant compared to synthetic antioxidants (BHA). Addition of grape seeds extract enhanced the shelf-life of mutton slices, to at least 28 days compared to control and BHA during refrigerated storage (Reddy *et al*., 2013).

The ability of grape seeds extracts to delay lipid oxidation in ground beef during storage is most likely because grape seeds extract is rich in phenolic substances which have high antioxidant activity (Amin and Edris, 2017, and Padilla-González *et al*., 2022).

The present study aimed to evaluate the effect of adding grape seeds powder or extract as a natural preservative on quality characteristics of beef burger during freezing storage at -18±1ºC for 90 days.

Materials and Methods

Materials

Grape seed samples

Red and white grapefruits were obtained from the local market in Assiut city. Grape seeds were separated carefully from fresh fruits. Then, they were washed 3 times by distilled water and dried in a forced air oven. Dried grape seeds of the two varieties were grinded in a laboratory mill (using Moulinex mill model LM-240) and sieved by fine flour sieve to obtain a very fine powder.

Fresh beef meat

Ten kg of beef meat were purchased from a local market at Assiut city, Egypt for preparation of beef burger samples of this study.

Food additives

Other ingredients of the beef burger (salt, dry onion, dry garlic, spices, and natural flavors) were obtained from the local market, Assiut city, Egypt.

Chemicals

Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluenee (BHT) and Folin Ciocalteu's phenol reagent were obtained from Sigma-Aldrich (St. Louis, USA). Meanwhile, CMC and other reagents were purchased from EL-Gomhouria for Trading Chemicals and Drugs Company, Assiut City, Egypt.

Methods

Preparation of aqueous extract of grape seeds

Samples of grape seeds were extracted according to the method described by Ignat *et al*. (2011) with slight modifications.

Preparation of CMC film with grape seeds

CMC film with grape seeds powder was prepared as mentioned by Gupta *et al*. (2022).

Preparation of beef burger

Burgers have been prepared according to Egyptian standard of beef burger (ES: 1688/2005 ICS: 67.120.10) Egyptian Organization for Standardization (EOS), Arab Republic of Egypt with some modification. The fresh beef meat was minced (using Mienta meat mincer model ch-174Q). The minced meat and the other ingredients being used for preparing of burger as shown in Table (1).

Table1. Basal beef burger formula (100g).

Ingredients	%
lean meat	65
Animal Fat	15
Soybean powder	10
Dry onion	3.5
Bread crust	3.5
NaCl	1.70
Spices mixture	1.30
Total	100

Different treatments were employed to produce five groups of burgers as follows:

1-Control beef burger (basal formula without additional treatments).

2-Beef burger samples with BHT added at concentration of 100 ppm.

3-Beef burger samples with grape seeds powder added at concentrations of 1 and 2% (Per 100 grams of burger).

4-Beef burger samples with grape seeds aqueous extract added at concentrations of 0.1 and 0.2% (Per 100 grams of burger).

5-Beef burger samples covered with carboxymethyl cellulose (CMC) films containing grape seeds powder at concentrations of 1 and 2%.

The beef burger samples were treated with grape seeds and BHT as above mentioned and stored immediately at -18 ±1˚C for 90 days to study the effect of added grape seeds or their extracts as natural antioxidants in comparison to BHT as synthetic antioxidants during the storage period.

Determination of free phenolic content of grape seeds extract

The contents of free phenolic in the aqueous extracts were determined spectrophotometrically at (765 nm) according to the Folin-Ciocalteau method of Kang *et al*. (2010). The absorbance was recorded using a UV–Vis spectrophotometer (C7200, USA). Free phenolic content was expressed as mg gallic acid equivalents (GAE) per 100 grams.

Determination of antioxidant activity of grape seeds extract

According to Lee *et al*. (2003), antioxidant activity of extracts measurement was conducted in terms of radical-scavenging ability against stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm in a UV–Vis spectrophotometer (C7200, USA). Scavenging activity was calculated as follows:

DPPH radical scavenging activity (%) = [(Ac – As)/ Ac] × 100

Where Ac= absorbance of control, AS= absorbance of tested sample.

Analysis of prepared beef burger

pH value

The pH values of prepared beef burger were measured using a pH-meter (HANNA Instrument pH-211, Microprocessor pH meter, Romania) according to the method described by Fernández-López *et al*. (2006).

Peroxide value

The peroxide values of beef burger samples were determined according to method of AOAC (2016).

Thiobarbituric acid (TBA)

TBA values were determined in beef burger samples according to the method of Lemon (1975). The absorbance was recorded at 538 nm (C-7200, Peak Instruments, USA, UV-Visible spectrophotometer). The results were represented as mg of malonaldehyde/kg sample.

Microbiological evaluation

Sample preparation

The sample prepared according to the method described by Difco-Manual (1984).

Total bacterial counts of beef burger samples

The total bacterial counts were determined by the plate count technique on nutrient agar medium according to procedures by APHA (1976) and Difco-Manual (1984). The plates were incubated at 37°C for 48 hours.

Total fungi and yeast count of beef burger sample

Fungi and yeast counts were determined using Bacto yeast malt agar medium according to the methods described by Difco-Manual (1998). The plates were incubated at 28°C for 5-7 days.

Statistical analysis

The differences between the results were measured by analysis of variance by complete randomized design in triplicate. The values were averaged and reported along with the standard error (±SE) according to Fisher (1936). The mean values were compared by Duncan's multiple range test according to Duncan (1955), with the significance level of $P < 0.05$.

Results and Discussion

Plant-derived phenolic compounds are well known to exhibit antioxidant activity. The antioxidant effect of phenolic compounds is mainly due to the ease with which a hydrogen atom from an aromatic hydroxyl (OH) group can be donated to a free radical (Duthie and Crozier, 2000). The free phenolic contents and antioxidant activity were determined in aqueous extracts of two varieties of grape seeds. The amount of free phenolic varied among the two varieties under

investigation. Generally, the aqueous extract of red grape seeds showed higher values in the free phenolic content and antioxidant activity compared with the white grape seeds extract.

The data in Table (2) indicated that the values of the free phenolic content were 557.83 and 411.70 mg GAE/100g for red and white grape seeds extract, respectively. The highest antioxidant activities with an inhibition rate are found in red grape seeds extracts (72.71%) followed by white grape seeds extracts (66.37%), this is due to the higher content of free phenolics in the aqueous extracts of red grape seeds compared with white grape seeds. There was a significant difference between red and white aqueous extract of grape seeds. These results are in agreement with Hanaa *et al*. (2015) they reported that the content of phenolic compounds in the aqueous extract of grape seeds contains was large, which led to a high antioxidant activity.

Table 2. Free phenolic content and antioxidant activity of grape seed extracts.

Samples		Free phenolic content of grape seeds	Antioxidant activity* (%)
Aqueous extract	**Red**	557.83±2.77[a]	72.71± 1.326[a]
	White	411.70±3.20[b]	66.37± 1.079[b]

*(% Inhibition of DPPH radical Scavenging). Mean ± SE (standard error). The different small letter in the table means significantly difference ($p<0.05$) between varieties.

Changes in the characteristics of beef burger treated with grape seeds during freezing storage at -18±1ºC for 90 days

The changes that occur in beef burgers during frozen storage at (-18±1°C) for 90 days were evaluated by studying the pH, peroxide value, TBA value, and the total count of bacteria, fungi, and yeasts.

Changes in pH values of the studied beef burger during freezing storage (-18±1ºC) for 90 days

The pH value is an important factor that determines the quality, juiciness, flavor, shelf life, safety and nutritional value of meat and meat products (Clarke *et al*., 1988). The changes in pH value of beef burger treated with grape seeds during freezing storage (-18±1°C) for 90 days are presented in Table (3).

The obtained data showed that the pH value decreased in all beef burger samples until the second month and then returned to increase again until the end of freezing storage.

The addition of grape seeds powder, aqueous extract and CMC films caused a slight change in pH values of beef burger compared to control sample (untreated) at the end of storage period. Also, statistical analysis of the mean treatments showed that the rate change in pH value of control sample was high ($p<0.05$), while addition of grape seeds led to a low rate change in pH values of beef burger. Data of the pH values in current study are consistent with the data of Abdelhakam *et al*. (2019)

The decrease and increase in pH values during freezing storage are related to the growth and activity of microorganisms. The decrease in pH values of beef

burger during freezing storage may be due to the psychrophilic bacteria activity at the beginning of the freezing process which deteriorates the carbohydrate producing lactic acid. While the increasing of pH values may be due to breakdown of protein by microorganisms and enzymes which resulting in protein deamination and producing alkali compound such as ammonia, dimethyl amine, and trimethyl amine (Leygonie *et al*., 2012).

Table 3. Changes in pH values of the studied beef burger during freezing storage (-18±1ºC) for 90 days

		Storage period (days)				
Treatments		**Zero time**	**30 days**	**60 days**	**90 days**	**Mean treatments**
Control		6.80±0.014^{lmnopqr}	6.65±0.017^{y}	6.89±0.011^{hi}	7.25±0.014^{a}	**6.90^{A}**
BHT		6.83±0.01^{jklmnopq}	6.77±0.008^{rstuv}	6.84±0.017^{ijklmn}	6.95±0.011^{efg}	**6.85^{CDE}**
pH values for beef burger treatments						
Grape seeds powder	Red 1%	6.84±0.008^{ijklmno}	6.74±0.008^{uvw}	6.84±0.018^{ijklmno}	7.05±0.020^{c}	**6.87^{BC}**
	Red 2%	6.85±0.012^{ijkl}	6.80±0.01^{nopqrst}	6.85±0.008^{ijklm}	6.94±0.011^{fg}	**6.86^{BC}**
	White 1%	6.81±0.008^{klmnopqr}	6.72±0.020^{wx}	6.86±0.017^{ijk}	7.11±0.015^{b}	**6.87^{B}**
	White 2%	6.85±0.011^{ijklmn}	6.77±0.011^{rstuv}	6.85±0.020^{ijklm}	6.96±0.012^{defg}	**6.86^{BC}**
Aqueous extract of grape seeds	Red 0.1%	6.84±0.011^{jklmno}	6.75±0.017^{tuvw}	6.84±0.015^{jklmno}	7.00±0.021^{d}	**6.86^{BC}**
	Red 0.2%	6.87±0.005^{ij}	6.79±0.008^{opqrstu}	6.83±0.008^{jklmnop}	6.92±0.012^{gh}	**6.85^{BCD}**
	White 0.1%	6.82±0.008^{jklmnopq}	6.73±0.014^{vwx}	6.85±0.011^{ijklmn}	7.08±0.014^{bc}	**6.87^{BC}**
	White 0.2%	6.83±0.017^{jklmno}	6.75±0.014^{tuvw}	6.82±0.008^{jklmnopq}	6.93±0.011^{gh}	**6.83^{DE}**
CMC films with grape seeds	Red 1%	6.78±0.003^{pqrstu}	6.64±0.005^{y}	6.77±0.008^{rstuv}	6.98±0.025^{def}	**6.79^{GH}**
	Red 2%	6.80±0.015^{nopqrst}	6.71±0.006^{wx}	6.81±0.003^{klmnopqr}	6.99±0.023^{de}	**6.83^{EF}**
	White 1%	6.78±0.003^{qrstu}	6.63±0.011^{y}	6.75±0.017^{stuvw}	6.99±0.030^{de}	**6.79^{H}**
	White 2%	6.80±0.020^{mnopqrs}	6.69±0.008^{x}	6.80±0.015^{nopqrst}	6.96±0.024^{defg}	**6.81^{FG}**
Mean days		**6.82^{B}**	**6.72^{C}**	**6.83^{B}**	**7.01^{A}**	

Mean ± SE (standard error). Different capital bold letters in the same column means significant difference ($p<0.05$) between treatments. Different capital letters in the same row means significant difference ($p<0.05$) between storage periods. Different small letters in the table means significantly difference ($p<0.05$) between treatments and storage periods (interactions).

Changes in peroxide values (meq. O_2/kg) of the studied beef burger during freezing storage (-18±1ºC) for 90 days

Lipid peroxidation is a primary cause of quality deterioration in meat and meat products while the peroxide values indicate the degree of oxidative rancidity of lipids (Min and Ahn, 2005). The results in Table (4) show the effect of added grape seeds as natural antioxidant compared with BHT as synthetic antioxidants on peroxide value of the beef burger samples during freezing storage at -18±1ºC for 90 days. Both antioxidants effectively reduced the oxidation rate in the beef burger samples.

Table 4. Changes in peroxide values (meq. O_2/kg) of the studied beef burger during freezing storage (-18±1ºC) for 90 days.

Peroxide values (meq. O_2/kg) for beef burger treatments	Storage Period (days)					
	Treatments		Zero time	30 days	60 days	90 days	Mean treatments
	Control		2.24±0.018^{v}	3.19±0.020^{no}	4.19±0.031^{b}	6.16±0.021^{a}	**3.95^{A}**
	BHT		2.20±0.012^{v}	2.73±0.018^{pqrs}	3.39±0.026^{jklm}	3.96±0.015^{de}	**3.07^{DE}**
	Grape seeds powder	Red 1%	2.25±0.024^{v}	2.79±0.010^{pq}	3.43±0.014^{jkl}	4.12±0.012^{bc}	**3.15^{BC}**
		Red 2%	2.16±0.012^{v}	2.63±0.014^{rst}	3.25±0.017^{mno}	3.67±0.017^{ghi}	**2.93^{GH}**
		White 1%	2.24±0.010^{v}	2.84±0.008^{p}	3.53±0.014^{hij}	4.17±0.003^{b}	**3.19^{B}**
		White 2%	2.20±0.012^{v}	2.67±0.017^{qrst}	3.31±0.014^{lmn}	3.85±0.023^{ef}	**3.01^{EF}**
	Aqueous extract of grape seeds	Red 0.1%	2.18±0.008^{v}	2.59±0.020^{stu}	3.31±0.011^{lmn}	3.72±0.020^{gh}	**2.95^{GH}**
		Red 0.2%	2.18±0.015^{v}	2.46±0.015^{u}	3.12±0.013^{o}	3.48±0.017^{ijk}	**2.81^{I}**
		White 0.1%	2.21±0.003^{v}	2.70±0.014^{pqrst}	3.34±0.018^{klmn}	3.78±0.017^{fg}	**3.01^{EF}**
		White 0.2%	2.19±0.008^{v}	2.58±0.020^{stu}	3.22±0.020^{no}	3.63±0.017^{hi}	**2.90^{H}**
	CMC films with grape seeds	Red 1%	2.29±0.011^{v}	2.76±0.021^{pqr}	3.40±0.014^{jklm}	4.01±0.014^{cd}	**3.11^{CD}**
		Red 2%	2.22±0.005^{v}	2.55±0.017^{tu}	3.21±0.014^{no}	3.60±0.012^{hi}	**2.89^{H}**
		White 1%	2.29±0.018^{v}	2.82±0.008^{pq}	3.48±0.012^{ijk}	4.11±0.011^{bc}	**3.18^{BC}**
		White 2%	2.23±0.015^{v}	2.63±0.023^{rst}	3.29±0.008^{lmn}	3.79±0.017^{fg}	**2.98^{FG}**
	Mean days		**2.22^{D}**	**2.71^{C}**	**3.39^{B}**	**4.00^{A}**	

Mean ± SE (standard error). Different capital bold letters in the same column means significant difference ($p<0.05$) between treatments. Different capital letters in the same row means significant difference ($p<0.05$) between storage periods. Different small letters in the table means significantly difference ($p<0.05$) between treatments and storage periods (interactions).

Data observed that the peroxide values (meq. O_2/kg) gradually increased with increment of freezing time reaching to the highest value after 90 days of storage for all samples. The peroxide values at zero time of storage were 2.16-2.29 meq. O_2/kg, while at the end of storage (90 days) it was 3.48- 4.17 meq. O_2/kg for all treatments. The peroxide value of control sample was 2.24 meq. O_2/kg at zero time of storage then increased to 6.16 meq.O_2/kg at the end of storage. On the other hand, the beef burger sample containing 0.2% of red grape seeds aqueous extract showed the lowest peroxide value (3.48 meq. O_2/kg) among all beef burger treatments at the end of storage period. Generally, at the end of freezing storage for 90 days -18±1ºC, peroxide values of the beef burger samples containing concentrations of grape seeds aqueous extract recorded the lowest value (3.48-3.78 meq. O_2/kg) followed by samples containing grape seeds films (3.60-4.11 meq.O_2/kg) then samples containing grape seeds powder (3.67-4.17 meq.O_2/kg). These results agreed with the results obtained by Brannan and Mah (2007) and Cagdas and Kumcuoglu (2015). The acceptable limit for human consumption is 10 meq. O_2/kg with reference to the European Pharmacopoeia and Norwegian

Medicinal Standard. Thus, peroxide values for all treatments were in the acceptable range at the end of storage. All samples treated with grape seeds (powder, extract, or films) had significantly ($p<0.05$) lower peroxide values than the control sample during freezing storage as an indication of retarding peroxidation, probably because antioxidant constituents of grape seeds terminated free radical chain reaction (Cagdas and Kumcuoglu, 2015). The peroxide values generally decreased by increasing the amount of grape seeds extract or powder in beef burger.

Changes in TBA values (mg malonaldehyde/kg) of the studied beef burger during freezing storage (-18±1ºC) for 90 days:

Thiobarbituric acid (TBA) value (malonaldehyde/kg) is used as an index for measuring oxidative rancidity which takes place in meat products during storage (Tsikas, 2017). Malonaldehyde is generated from the peroxidation of fatty acids in foods containing high amounts of unsaturated fats. Thus, malonaldehyde is a robust biomarker in terms of measuring lipid peroxidation (Kanner *et al*., 2012).

The data obtained in Table (5) indicated that the TBA value increased with progressive freezing storage period in control as well as all treatments. The results declared that there were significant differences ($p <0.05$) in TBA values between the treated and untreated sample at zero time.

During storage periods, TBA values of control sample showed continuous progressive significant increases to reach the highest value (0.616 mg malonaldehyde/kg sample) at the end of storage period. Although, the other treatments showed a slight significant increment in TBA values (0.293-0.379 mg malonaldehyde/kg sample) during frozen storage at -18±1ºC for 90 days. These increments were much lower than those of the control. Also, data showed that the beef burger sample treated by 0.2% of red grape seeds extract had lowest TBA value (0.293 mg malonaldehyde/kg) at the end of storage (90 days) among all treated beef burger samples. The TBA values of the treated beef burger samples after 90 days of freezing storage were below the permissible limit (< 0.9 mg malonaldehyde/kg) set by Egyptian Standards (2005).

The reduced increment rate of the TBA values in treated samples might be due to the preservative effect of grape seeds (powder, extracts, and films) which slowed down or reduced the autolysis in meat and accordingly reduced the breakdown of protein, while the high TBA value of control sample may be attributed to the auto oxidation of meat lipids in addition to the bacteriological and oxidative rancidity (Nalini *et al*., 1998). This effect of natural antioxidants, in particular phenolic substances, has been attributed to their scavenging the free radical species or through activation of antioxidant enzymes (Du *et al*., 2010). From the obtained results, it could be observed that addition of grape seeds (powder, extracts, or films) to beef burger may decline the increment rate of TBA values compared with control sample during freezing storage. This may be due to its phenolic contents and antioxidant activity as recorded in Table (2). These

results are in agreement with those obtained by Brannan and Mah (2007) and Abdelhakam *et al*. (2019).

Table 5. Changes in TBA values (mg malonaldehyde/kg) of the studied beef burger during freezing storage (-18±1°C) for 90 days.

TBA values (mg malonaldehyde/kg) for beef burger treatments	Storage Period (days)				
Treatments	**Zero time**	**30 days**	**60 days**	**90 days**	**Mean treatments**
Control	0.196±0.000^{a}	0.295±0.001^{m}	0.398±0.001^{b}	0.616±0.001^{a}	**0.376^{A}**
BHT	0.193±0.001^{a}	0.246±0.000^{t}	0.303±0.002^{l}	0.349±0.000^{f}	**0.273^{F}**
Grape seeds powder Red 1%	0.196±0.000^{a}	0.253±0.00 ^{s}	0.319±0.001^{i}	0.366±0.001^{d}	**0.283^{D}**
Grape seeds powder Red 2%	0.191±0.001^{a}	0.233±0.001^{v}	0.290±0.001^{no}	0.335±0.002^{h}	**0.262^{IJ}**
Grape seeds powder White 1%	0.193±0.000^{a}	0.261±0.001^{r}	0.332±0.001^{h}	0.379±0.001^{c}	**0.291^{B}**
Grape seeds powder White 2%	0.194±0.001^{a}	0.243±0.001^{tu}	0.293±0.001^{mn}	0.342±0.001^{g}	**0.268^{G}**
Aqueous extract of grape seeds Red 0.1%	0.194±0.001^{a}	0.234±0.000^{v}	0.277±0.001^{p}	0.313±0.002 ^{j}	**0.254^{K}**
Aqueous extract of grape seeds Red 0.2%	0.193±0.001^{a}	0.216±0.001^{x}	0.265±0.002^{q}	0.293±0.001^{mn}	**0.242^{M}**
Aqueous extract of grape seeds White 0.1%	0.192±0.001^{a}	0.241±0.000^{u}	0.289±0.000^{no}	0.332±0.002^{h}	**0.263^{HI}**
Aqueous extract of grape seeds White 0.2%	0.194±0.001^{a}	0.221±0.000^{w}	0.274±0.001^{p}	0.305±0.001^{kl}	**0.249^{L}**
CMC films with grape seeds Red 1%	0.209±0.001^{y}	0.243±0.001^{tu}	0.309±0.000^{jk}	0.357±0.000^{e}	**0.279^{E}**
CMC films with grape seeds Red 2%	0.200±0.000^{z}	0.225±0.001^{w}	0.286±0.002^{o}	0.331±0.001^{h}	**0.260^{J}**
CMC films with grape seeds White 1%	0.212±0.002^{xy}	0.252±0.000^{s}	0.322±0.001^{i}	0.365±0.002^{d}	**0.288^{C}**
CMC films with grape seeds **White 2%**	0.203±0.001^{z}	0.230±0.001^{v}	0.291±0.000^{mn}	0.333±0.001^{h}	**0.264^{H}**
Mean days	**0.197^{D}**	**0.242^{C}**	**0.303^{B}**	**0.358^{A}**	

Mean ± SE (standard error). Different capital bold letters in the same column means significant difference ($p<0.05$) between treatments. Different capital letters in the same row means significant difference ($p<0.05$) between storage periods. Different small letters in the table means significantly difference ($p<0.05$) between treatments and storage periods (interactions).

Changes in total bacterial counts (cfu/g×10^4) of the studied beef burger during freezing storage (-18±1°C) for 90 days

Total bacterial counts have been used to assess sanitary quality organoleptic ability, safety and utility of various meat products (Fliss *et al*., 1991).

Data in Table (6) shows that the changes in total bacterial counts (cfu/g x10^4) of the prepared beef burger during frozen storage (-18±1°C) for 90 days. With progressive freezing storage, the total bacterial counts decreased in control as well as treated products, and that might be due to low temperature and no free water as well as destruction of bacterial cells. However, the decrement rate of total bacterial count was more evident in the treated samples (significant difference <0.05) compared to control sample (untreated).

Results in Table (6) show that total bacterial counts of the treated beef burger samples ranged between 70.33×10^4 to 79.66×10^4 cfu/g at zero time. At the end of

freezing storage, (-18±1°C) for 90 days, the total bacterial counts in beef burger samples contained different concentration of grape seeds powder were between 17.66×10^4 to 27×10^4 cfu/g. Meanwhile, the samples contained grape seeds extract counts were between 13×10^4 to 22×10^4 cfu/g while the samples contained grape seeds films were between 17.00×10^4 to 25.33×10^4 cfu/g in both varieties (red and white grape seeds).

Table 6. Changes in total bacterial counts (cfu/g×10^4) of the studied beef burger during freezing storage (-18±1°C) for 90 days

Total bacterial counts (cfu/g×10^4) for beef burger treatments	Treatments		Storage Period (days) Zero time	30 days	60 days	90 days	Mean treatments
	Control		87.66±0.881^{a}	67.66±0.881^{g}	58.33±0.881^{h}	35.66±1.452^{op}	**62.32^{A}**
	BHT		74.66±0.881^{cde}	50.00±1.154^{i}	39.00±1.154^{no}	23.66±0.666^{tuvw}	**46.83^{D}**
	Grape seeds powder	Red 1%	78.66±0.881^{b}	49.66±0.881^{i}	41.33±0.881^{klmn}	24.33±0.881^{tuv}	**48.50^{CD}**
		Red 2%	72.66±1.452^{defg}	43.00±1.154^{jklm}	33.33±0.881^{pq}	17.66±1.201^{yz}	**41.66^{F}**
		White 1%	79.66±1.452^{b}	52.66±0.881^{i}	43.33±1.201^{jkl}	27.00±1.154st	**50.66^{B}**
		White 2%	76.00±0.577^{bcd}	44.66±0.881^{jk}	35.00±1.00^{p}	20.33±0.88^{wxy}	**44.00^{E}**
	Aqueous extract of grape seeds	Red 0.1%	75.00±1.527^{cde}	45.66±2.333^{j}	35.66±1.201^{op}	21.33±1.201^{vwx}	**44.41^{E}**
		Red 0.2%	70.33±0.881^{fg}	40.33±0.881^{lmn}	29.00±1.154^{rs}	13.00±0.577^{a}	**38.16^{G}**
		White 0.1%	78.66±0.881^{b}	50.00±0.577^{i}	38.66±0.881^{no}	22.00±1.527^{uvw}	**47.33^{D}**
		White 0.2%	71.33±0.881^{efg}	41.66±0.881^{klmn}	30.66±1.201^{qr}	14.33±0.88^{za}	**39.50^{G}**
	CMC films with grape seeds	Red 1%	78.00±1.00^{bc}	49.33±1.201^{i}	39.33±0.881^{mn}	24.00±0.577^{tuv}	**47.66^{D}**
		Red 2%	73.66±1.763^{def}	42.33±0.881^{jklmn}	32.66±1.201^{pq}	17.00±0.577^{yz}	**41.41^{F}**
		White 1%	79.33±0.333^{b}	51.00±0.577^{i}	43±1.154^{jklm}	25.33±0.333^{tu}	**49.66^{BC}**
		White 2%	74±1.00^{de}	43.66±0.881^{jkl}	33.66±0.881^{pq}	18.00±1.154^{xy}	**42.33^{F}**
	Mean days		**76.40^{A}**	**47.97^{B}**	**38.07^{C}**	**21.69^{D}**	

Mean ± SE (standard error). Different capital bold letters in the same column means significant difference ($p<0.05$) between treatments. Different capital letters in the same row means significant difference ($p<0.05$) between storage periods. Different small letters in the table means significantly difference ($p<0.05$) between treatments and storage periods (interactions).

On the other hand, the total bacterial counts of the treated beef burger samples were lower (13×10^4 - 27×10^4 cfu/g) than the control sample (35.66×10^4 cfu/g) at the end of storage (-18±1°C) for 90 days. Also, the sample treated by 0.2% of red grape seeds extract were significantly lower (<0.05) in total bacterial counts (13×10^4 cfu/g) among all treated beef burger samples at the end of storage (90 days). From these results, it could be observed that the incorporation of grape seeds (powder, extracts, or films) in beef burger samples caused a notable decrease in total bacterial count. This decrease corresponds with the grape seeds' incorporation level increase. This decrease in the total bacterial count may be attributed to the higher phenolic content in grape seeds as recorded in Table (2),

which acts a natural antibacterial. Such findings agreed with those of Abdelhakam *et al*. (2019) and Memar *et al*. (2019) who reported that the grape seed extracts have an inhibitory effect against a broad spectrum of Gram-negative and Gram-positive bacteria. The mechanisms suggested that the antibacterial effect of phenolic compounds have been mainly related to cytoplasmic membrane damage, making it more permeable (Cushnie and Lamb, 2011).

Changes in fungal count (cfu/g×10^4) of the studied beef burger during freezing storage (-18±1ºC) for 90 days:

The growth of fungi in food may cause spoilage and result in a reduction in food quality and quantity, some *Aspergillus* species are xerophilic fungi and responsible for many of food and feed contamination (Soliman and Badeaa, 2002).

Data presented in Table (7) show that the fungal count (cfu/g ×10^4) decreased during storage time progress (-18±1°C for 90 days) of the treated and untreated beef burger samples. Data showed that, at zero time of freezing storage, the fungal count in beef burger samples formulated with grape seeds (powder, extracts, or films) at different concentrations ranged between 0.33x10^4 to 2x10^4 cfu/g, fungal counts in these samples were lower than the control sample (3x10^4 cfu/g). On the other hand, fungal counts gradually decreased during storage periods in all treated samples but with variable degrees. The decrement rate in total fungal count of the beef burger samples treated with grape seeds (powder, extracts, or films) was higher than the control sample during freezing storage for 90 days.

Furthermore, fungi completely disappeared in the treated beef burger samples containing a different concentration of grape seeds (powder, extracts, or films) after storage for 60 days, except for the fungi found in the samples treated by 0.2% of red and white grape seeds extract disappeared after 30 days of freezing storage. On the other hand, from the same table it could be noticed that the fungi disappeared in the control sample (untreated) after storage for 90 days. These results are in agreement with those obtained by Han (2007) and Memar *et al*. (2019), they reported that the grape seed extracts have an inhibitory effect against fungi and yeasts such as *C. albicans*, *Candida albicans*, *Penicillium expansum*, and *Aspergillus niger*. They reported a relationship between the chemical compounds in the grape seed extracts and their antimicrobial activity. The absent of fungal counts during freezing storage might be attributed to effect of freezing on the destruction of microbial cell which led to death of some cells, beside the antimicrobial effect of grape seeds according to Al-Otibi *et al*. (2021) who showed that grape seeds extract had significant reduction in the growth of fungi (strong antifungal activity).

Table 7. Changes in fungal counts (cfu/g×10^4) of the studied beef burger during freezing storage (-18±1ºC) for 90 days

Total fungal counts (cfu/g×10^4) for beef burger treatments	Treatments		Storage Period (days) Zero time	30 days	60 days	90 days	Mean treatments
	Control		2.66±0.333^{a}	2.00±0.577^{ab}	0.66±0.333^{b}	ND^{b}	1.33^{A}
	BHT		1.33±0.333^{ab}	1.00±0.577^{ab}	ND^{b}	ND^{b}	0.58^{AB}
	grape seeds powder	Red 1%	1.66±0.333^{ab}	1.00±0.577^{ab}	ND^{b}	ND^{b}	0.67^{AB}
		Red 2%	1.00±0.00^{ab}	0.33±0.333^{b}	ND^{b}	ND^{b}	0.33^{BC}
		White 1%	2.00±0.00^{ab}	1.33±0.333^{ab}	ND^{b}	ND^{b}	0.83^{AB}
		White 2%	1.33±0.333^{ab}	0.33±0.333^{b}	ND^{b}	ND^{b}	0.41^{AB}
	Aqueous extract of grape seeds	Red 0.1%	1.33±0.333^{ab}	0.33±0.333^{b}	ND^{b}	ND^{b}	0.41^{AB}
		Red 0.2%	0.33±0.333^{b}	ND^{b}	ND^{b}	ND^{b}	0.08^{C}
		White 0.1%	1.66±0.333^{ab}	0.66±0.333^{b}	ND^{b}	ND^{b}	0.58^{AB}
		White 0.2%	0.66±0.333^{b}	ND^{b}	ND^{b}	ND^{b}	0.16^{C}
	CMC films with grape seeds	Red 1%	1.66±0.333^{ab}	0.66±0.333^{b}	ND^{b}	ND^{b}	0.58^{AB}
		Red 2%	1.33±0.333^{ab}	0.33±0.333^{b}	ND^{b}	ND^{b}	0.41^{AB}
		White 1%	2.00±0.00^{ab}	1.00±0.00^{ab}	ND^{b}	ND^{b}	0.75^{ABC}
		White 2%	1.33±0.333^{ab}	0.33±0.333^{b}	ND^{b}	ND^{b}	0.41^{ABC}
	Mean days		1.448^{A}	0.666^{B}	0.047^{C}	0.00^{D}	

ND= not detected. Mean ± SE (standard error). Different capital bold letters in the same column means significant difference ($p<0.05$) between treatments. Different capital letters in the same row means significant difference ($p<0.05$) between storage periods. Different small letters in the table means significantly difference ($p<0.05$) between treatments and storage periods (interactions).

Changes in yeast counts (cfu/g×10^4) of the studied beef burger during freezing storage (-18±1ºC) for 90 days

Data in Table (8) showed that the yeast counts in beef burger samples treated with grape seeds (powder, extracts, or films) at zero time of storage ranged between 1×10^4 and 3×10^4 cfu/g, while the control sample were 3.66×10^4 cfu/g. Furthermore, after storage for 60 days, yeast counts disappeared in the beef burger samples treated with grape seeds (powder, extracts, or films) except yeasts in the samples containing 0.1% of red and white grape seeds powder, which disappeared after 90 days of freezing storage (-18±1ºC), However, yeasts were not detected in the samples treated by 0.2% of red grape seeds extract after 30 days of freezing storage.

On the other hand, after storage for 90 days at -18±1ºC, yeast counts disappeared in all treated samples except control sample which had 0.33×10^4 cfu/g. From the data obtained, it is observed that control sample had higher yeast counts compared with all samples treated by grape seeds (powder, extracts, or films). However, the gradual decrease in yeast counts may be attributed to the effect of

freezing on the destruction of microbial cells, besides the antimicrobial activity of grape seeds. These results agree with those obtained by Han (2007) and Memar *et al*. (2019).

Table 8. Changes in yeast counts (cfu/g×10^4) of the studied beef burger during freezing storage (-18±1ºC) for 90 days

Yeast counts (cfu/g×10^4) for beef burger treatments	Treatments		storage Period (days) Zero time	30 days	60 days	90 days	Mean treatments
	Control		3.66±0.333^{a}	2.66±0.333^{bc}	1.33±0.333^{efg}	0.33±0.333 ^{hi}	**2.00^{A}**
	BHT		2.33±0.333^{bcd}	1.00±0.577^{fgh}	0.33±0.333^{hi}	ND^{i}	**0.91^{BCD}**
	grape seeds powder	Red 1%	2.66±0.333^{bc}	1.33±0.333^{efg}	0.66±0.333^{ghi}	ND^{i}	**1.16^{B}**
		Red 2%	2.33±0.333^{bcd}	0.66±0.333^{ghi}	ND^{i}	ND^{i}	**0.75^{BCD}**
		White 1%	2.66±0.333^{bc}	1.33±0.333^{efg}	0.66±0.333^{ghi}	ND^{i}	**1.16^{B}**
		White 2%	2.33±0.333^{bcd}	0.66±0.333^{ghi}	ND^{i}	ND^{i}	**0.75^{BCD}**
	Aqueous extract of grape seeds	Red 0.1%	2.33±0.333^{bcd}	0.66±0.333^{ghi}	ND^{i}	ND^{i}	**0.75^{BCD}**
		Red 0.2%	1.00±0.577^{fgh}	ND^{i}	ND^{i}	ND^{i}	**0.25^{E}**
		White 0.1%	2.33±0.333^{bcd}	1.00±0.00^{fgh}	ND^{i}	ND^{i}	**0.83^{BCD}**
		White 0.2%	1.66±0.333^{def}	0.33±0.333^{hi}	ND^{i}	ND^{i}	**0.50^{DE}**
	CMC films with grape seeds	Red 1%	2.33±0.333^{bcd}	1.00±0.00^{fgh}	ND^{i}	ND^{i}	**0.83^{BCD}**
		Red 2%	1.66±0.333^{def}	0.33±0.333^{hi}	ND^{i}	ND^{i}	**0.50^{DE}**
		White 1%	3.00±0.577^{ab}	1.33±0.333^{efg}	ND^{i}	ND^{i}	**1.08^{BC}**
		White 2%	2.00±0.577^{cde}	0.66±0.333^{ghi}	ND^{i}	ND^{i}	**0.66^{CDE}**
	Mean days		**2.30^{A}**	**0.92^{B}**	**0.21^{C}**	**0.02^{C}**	

ND= not detected. Mean ± SE (standard error). Different capital bold letters in the same column means significant difference ($p<0.05$) between treatments. Different capital letters in the same row means significant difference ($p<0.05$) between storage periods. Different small letters in the table means significantly difference ($p<0.05$) between treatments and storage periods (interactions).

Conclusion

Today, many consumers are paying more attention to healthy products that are processed with natural and healthy ingredients. The addition of natural antioxidants instead of chemical or synthetic antioxidants can reduce lipid oxidation, improve product quality, and maintain the nutritional value of foods. The current study may conclude that Grape seeds are rich in phenolic substances and have antioxidant and antimicrobial activity. Consequently, it could be used as natural preservatives to improve the quality characteristics of beef burger during freezing storage.

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تأثير دمج بذور العنب كمواد حافظة طبيعية على خصـــائص برجر اللحم البقري اثناء الحفظ بالتجميد

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الملخص

بذور العنب هي مصدر غني بالمواد الفينولية والفلافونويدات، المعروفة بخصائصها القوية كمضادات للأكسدة ومضادات للميكروبات .تهدف هذه الدراسة الي تقدير الفينولات الكلية والنشاط المضاد للأكسدة في المستخلص المائي لبذور العنب الأحمر والأبيض، وكذلك دراسة تأثير اضافة بذور العنب في صورة مسحوق ومستخلص مائي وأستخدامها كأغشية حافظة من خلال دمجها مع مادة الـــ CMC علي خصائص جودة برجر اللحم البقري أثناء الحفظ بالتجميد علي -18±1م لمدة 90 يوم، أوضحت النتائج أن بذور العنب لها نشاط عالي مضاد للأكسدة في كلا الصنفين حيث يتراوح النشاط المضاد للأكسدة في المستخلص المائي للبذور من 66.37% إلي 72.71%، كما أنها تحتوي علي كمية عالية من المواد الفينولية تتراوح في المستخلص المائي من 411.70 ملجم/ 100 جم الي 557.83 ملجم/100 جم في كلا الصنفين، كما انها عند إضافتها في صورة مسحوق أو مستخلص مائي أو أغشية مع مادة الـــ CMC أدت الي تحسين خصائص البرجر حيث كان معدل التغير في قيم الـpH منخفض وكذلك كانت قيم البيروكسيد والـTBA منخفضة مقارنة بالعينة الضـــابطة ، وكذلك إضـــافة بذور العنب الي البرجر أدت الي تحســـين الجودة الميكروبية له حيث أنخفضت أعداد الميكروبات بشكل كبير في العينات المعاملة ببذور العنب (مسحوق أو مستخلص مائي أو أغشـــية) مقارنة بالعينة الضـــابطة. بشـــكل عام يمكن أن تؤدي إضـــافة بذور العنب أو مستخلصاتها أو في صورة أغشية مدمجة مع مادة الـــ CMC كمصدر طبيعي لمضادات الأكسدة والميكروبات إلى قبول كبير لدي المستهلكين حيث لا يوجد تأثير صحي ضار لأستخدام المكونات الطبيعية في الطعام مقارنة بمضادات الأكسدة الاصطناعية مثل الـBHT.

الكلمات المفتاحية: *النشاط المضاد للأكسدة، كربوكسي ميثيل سيليلوز، بذور العنب، مضادات أكسدة طبيعية، المحتوى الفينولي*