

Grape Seed Extract as Natural Antioxidant and Antibacterial in Minced Beef

Reham, A. Amin*, Shimaa, N. Edris

Food Control Department, Faculty of Veterinary Medicine, Benha University, Egypt.

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*Corresponding author: Reham, A. Amin; Email: rehamnour2007@yahoo.com



Abstract

This study aimed to investigate the effect of grape seed extract (GSE) on sensory attributes, delaying lipid oxidation and bacterial growth in raw fresh minced beef during refrigerated storage at 4°C for 10 days. In addition, a synthetic antioxidant (BHT 0.01%) and a control group were used, thus there were five groups of minced beef: Control, BHT 0.01%, GSE₅₀, GSE₂₀₀ and GSE₁₀₀₀. Overall acceptability, pH and total bacterial counts (TBC) of the control and treated minced beef samples were determined every 48 hrs during refrigerated storage. Development in lipid oxidation was measured by thiobarbituric acid-reactive substances (TBARs) and antioxidant activity (AOA %). Scores of overall acceptability of all treated minced beef samples were more acceptable than untreated controls. GSE reduced pH values significantly ($p < 0.05$). Supplementation of GSE significantly retarded the oxidative rancidity of minced beef during refrigerated storage. TBARs values significantly reduced in all treated minced beef samples during storage compared to controls. Moreover, GSE suppressed total bacterial counts (TBC) significantly ($p < 0.05$) relative to the control samples. The antioxidant and antibacterial efficiency of GSE was concentration-dependent. After 10 days of refrigerated storage, the lowest pH, TBARs values and total bacterial counts (TBC) were recorded in minced beef groups treated with GSE₁₀₀₀. Antioxidant and antibacterial activities of the tested minced beef groups were in the order GSE₁₀₀₀ > GSE₂₀₀ > GSE₅₀ > BHT > Control. Our findings suggest that grape seeds, as a natural agro-waste, could be very effective in extending shelf life and delaying lipid oxidation of minced beef during chilling without affecting sensory attributes, providing the consumer with more healthful food containing a natural antioxidant and antibacterial additive, as an alternative to synthetic additives.

Keywords: Natural Antioxidant, Antioxidant Activity, Lipid Oxidation, Antibacterial Activity, BHT, Grape Seed Extract.

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INTRODUCTION

The ground meat production and consumption have been increased as a result of trending toward convenience foods. When ground meat stored at refrigeration, microbial growth as well as oxidative stress will cause lipid oxidation, damage in structure of protein, change sensory attributes, lowering nutritive value of ground meat and decrease its shelf life thus affect quality and acceptability of meat products (Dave and Ghaly, 2011). The complex process of lipid oxidation is affected by numerous factors, as chemical structure of meat, entrance of oxygen and light, storage temperature as well as some technological procedures during processing. Lipid oxidation lowers nutritional value, leading to loss of vitamins, changes in essential fatty acids and production of toxic compounds. Meat is naturally low in antioxidants (Ansorena and Astiasaran, 2004).

One of the major strategies for preventing lipid and protein oxidation during storage and retail display is using

antioxidants (Shirahigue *et al.*, 2011). The antioxidants have a great ability to prevent or even reduce lipid oxidation, counteract the harmful free radicals in tissues, protect cells from damage, protect the cellular components, as DNA, proteins, and membrane lipids, from attacks of reactive oxygen species, thus protect the consumer from cancer, arteriosclerosis and several heart diseases (Su *et al.*, 2007). Consequently, in industrial processing, several synthetic antioxidants, such as butylated hydroxyl toluene (BHT), have successfully been added to delay lipid oxidation, to prevent undesirable reactions, to prolong shelf-life of meat. However, the increasing awareness of consumers over the rigorous toxicity, carcinogenicity and the potential health hazards of synthetic antioxidants has recently emphasized nutritional value of natural antioxidants and increased their demand in food industry (Bjelakovic *et al.*, 2007). The plant extracts are mostly utilized against anti-inflammatory effect, anti-cancer, anti-bacterial, antifungal, cardiovascular effects and for various other activities as well (Ali *et al.*, 2017).

So, in the last few years, interest in natural antioxidants and the search on natural plant extracts possessing both antioxidant and antimicrobial activities has dramatically increased in the meat industry (Lorenzo *et al.*, 2013). Among the advantages of natural antioxidants are improving overall quality and nutritional value of meat through retardation or inhibition of lipid oxidation without harming the desirable sensory characteristics for the consumer, extending shelf life by inhibiting the growth of food borne pathogens and preventing economic loss (Barbosa-Pereira *et al.*, 2015).

Recently, daily consumption of fruits and vegetables proved to reduce risks of certain diseases, including cancer and cardio and cerebro-vascular diseases (Liu *et al.*, 2000; Iqbal *et al.*, 2016). In addition, the use of grape extract, as a waste product from processing of fruits, could effectively valorize such waste and offer a practical and economic source of potent natural antioxidants instead of synthetic antioxidants (El-Zainy *et al.*, 2016). Scientific studies have shown that grape seed extract (GSE), obtained from wine or grape juice, is known to be astringent, a more potent scavenger of reactive oxygen species (Monteleone *et al.*, 2004), rich in proanthocyanidins, flavonoids, and polyphenolic compounds (Vaithyanathan *et al.*, 2011), which are more powerful antioxidants than vitamins C and E (Shi *et al.*, 2003).

Grape seed contains significant levels of minerals (calcium, potassium, sodium, iron) and vitamins (A, B1, B2, C and niacin) (Konar, 2010). In addition, grape seed oil contains high amount of vitamin E which prevents liver damage (Maheswari and Rao, 2005). Also, β -glucan and antioxidant in grape seed oil provides defense against cardiovascular disease (Lutterodt *et al.*, 2011) and colorectal cancer (Bloom, 2009). Grape seed oil is rich in oleic acid and linoleic acid, which are essential fatty acids for human metabolism, but human lacks the enzymes required for their synthesis. So, these fatty acids should be provided daily (Baydar and Akkurt, 2001). However, the disadvantage of using GSE is that it may change the sensory attributes of meat products (Moure *et al.*, 2001), so investigating the dose effect in food is necessary for better characterization of GSE.

Therefore, the goal of the present study was to investigate both the antioxidant and antimicrobial efficiencies of commercial GSE, in varying concentrations (50, 200 and 1000 mg GSE/kg of meat) as a natural preservative, and BHT (0.01%) as a synthetic antioxidant on sensory properties, lipid oxidation and bacterial growth in raw fresh minced beef stored at 4°C. The development of lipid oxidation was monitored by values of thiobarbituric acid reactive substances (TBARS). Antioxidant activity (AOA %) was calculated. pH was determined.

MATERIALS AND METHODS

Materials and chemicals

Grape seed extract was obtained from local market, Cairo, Egypt. Testing chemicals, including BHT and 1,1,3,3-

tetraethoxypropane, were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents and chemicals used in the study were of analytical grade.

Preparation of raw minced beef samples

A total of 1250 g of fresh raw minced beef were obtained from local market in Tanta, Gharbia governorate, Egypt, and minced in an industrial meat grinder. The samples were transferred directly and aseptically to the laboratory in an ice box. Then, the samples were divided into five equal groups (250 g each) as follows:

- 1) Control (without antioxidant addition);
- 2) BHT (0.01%, in accordance with Decree No. 1004 of the Secretariat of Health Surveillance, Brazil, was dissolved in 5 ml of soybean oil without antioxidant) (Shirahigue *et al.*, 2011);
- 3) GSE₅₀ (50 mg GSE/kg of meat);
- 4) GSE₂₀₀ (200 mg GSE / kg of meat);
- 5) GSE₁₀₀₀ (1000 mg GSE/kg of meat) (Pateiro *et al.*, 2015).

Immediately, the five meat batches were separately well-mixed. Finally, the batches were aerobically packaged in sterile polyethylene bags, labeled and stored at 4±1°C. Sensory analysis, pH, TBARS, TBC and antioxidant activity (AOA %) were determined in the examined batches at zero day and every 48 hrs of refrigerated storage. The experiment was applied in triplicate.

Sensory analysis

Overall acceptability of the minced beef batches was evaluated every 48 hrs of refrigerated storage using a ten - point numerical scale, where, ten corresponds to 'the highest quality'. The panel system consists of 10 staff members (Ibrahim *et al.*, 2012).

pH determination

pH values of all minced beef groups were measured with a digital pH meter (HAANA, HI902 meter, Germany) every 48 hrs using 10 g of the examined sample, homogenized in a blender with 50 ml distilled for one minute. The pH value was measured in the minced beef batches to evaluate if the addition of BHT and GSE would alter their pH values and these values can also monitor the quality of the groups relating to their bacteriological quality (Shirahigue *et al.*, 2011; Ibrahim *et al.*, 2012).

Determination of lipid oxidation

The extent of lipid oxidation was determined by the formation of thiobarbituric acid-reactive substances (TBARS) during storage, based on the fact that the products of primary oxidation mainly consist of hydro peroxides, which are quickly degraded into several substances reactive to the thiobarbituric acid, particularly carbonyls, with malondialdehyde the most important element (Bernardi *et al.*, 2016).

Thiobarbituric acid-reactive substances (TBARS)

Two grams of each homogenized minced beef group were taken and TBARS were extracted twice with 10 ml of 0.4M perchloric acid. Extracts were made up to 25ml with 0.4M perchloric acid and then centrifuged for 5 min at 1790g. After centrifugation, 1ml of extract was poured into a glass stoppered test -tube. A TBARS reagent (5ml) was added and the extract was heated for 35 min in a boiling water bath. After cooling under tap-water, the absorbance of the sample was measured against the blank solution at 538nm. A standard curve was drawn using 1,1,3,3-tetraethoxypropane (TEP). TBARS value was expressed as mg of malonaldehyde/kg of the minced beef sample (Ibrahim *et al.*, 2012).

Antioxidant activity (AOA %)

The antioxidant potential expressed in terms of percentage of antioxidant activity (AOA %) was determined by the following equation (Ibrahim *et al.*, 2012).

$$\text{AOA \%} = \frac{[\text{TBARS value of the control} - \text{TBARS of the test sample}]}{\text{TBARS value of the control}} \times 100$$

Bacteriological analysis

Minced beef samples (10 g) were aseptically homogenized with 90 ml sterile peptone water (0.1%) for 2 minutes using a stomacher (Stomacher 400 Circulator; Seward Medical Ltd., London, UK). Then, 10-fold serial dilutions (with 0.1% sterile peptone water) were prepared. Subsequently, 1 ml from each of the previously prepared serial dilutions was separately plated onto standard plate count agar (PCA) to determine total bacterial count (TBC) (Qin *et al.*, 2013). The inoculated plates were incubated at 37°C for 24 hrs. After incubation, the colonies were counted and transformed as log colony forming units (CFU)/g of sample (Jeong *et al.*, 2015).

Statistical analysis

All the obtained data were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS package (SPSS 19.0, Chicago, IL, USA). Significant ($P < 0.05$) differences between treatments were determined using Duncan's post hoc test. Data were expressed as means \pm standard deviation (SD). All experiments were performed in triplicate.

RESULTS

Sensory analysis

Mean sensory scores for overall acceptability of raw minced beef with BHT and GSE during refrigerated storage were shown in Table 1. Although GSE altered the color and odor of minced beef samples, the scores of overall acceptability of treated samples were significantly higher ($p < 0.05$) than those of control samples and were acceptable by the panelists. Moreover, the control samples spoiled and

showed significant difference ($p < 0.05$) in color and rancid odor after two days of refrigerated storage.

pH values

The recorded pH values for the different groups were shown in Table 2. The control group showed the highest pH value compared to other tested groups. Groups with antioxidants displayed lower pH values than that of the control group. Significant differences in pH values ($p < 0.05$) were found between the examined groups during refrigerated storage, indicating that pH of GSE affected pH of minced beef. Regarding the used dose of GSE, it was observed that pH reduces with increasing the concentration of GSE (Table 2). The pH value of GSE₁₀₀₀ treated samples after 8 days of refrigeration storage was 5.33.

TBARS

Table 2 presented the effect of BHT (0.01%) and GSE (50, 200 and 1000) on values of TBARS during refrigerated storage of minced beef samples. TBARS values significantly ($P < 0.05$) reduced in all treated groups compared to those of control. GSE showed a significant protective effect against lipid oxidation during refrigerated storage, but to a different extent. The highest inhibition ($P < 0.05$) of lipid oxidation was in GSE₁₀₀₀ treated samples compared to those treated with GSE₅₀ and GSE₂₀₀ during all storage times. The significant increase ($P < 0.05$) of TBARS values in control samples was the highest in relation to all other treated samples. TBARS values for the control and treated groups can be arranged in descending order as follows: Control > BHT > GSE₅₀ > GSE₂₀₀ > GSE₁₀₀₀, indicating reduction in TBARS values with increasing the concentration of GSE. It was also, observed that GSE₁₀₀₀ treated samples remained the lowest TBARS values until the 10th day.

Antioxidant activity (AOA %)

Antioxidant activity of minced beef groups treated with BHT, GSE₅₀, GSE₁₀₀ and GSE₂₀₀ as antioxidants and stored at 4°C, were declared in Figure 1. A significant difference was observed between AOA% of the examined groups, as a result of adding BHT and GSE (50, 200 and 1000) during storage for 10 days. The antioxidant activity was arranged as: GSE₁₀₀₀ > GSE₂₀₀ > GSE₅₀ > BHT > control group. So, the lowest AOA% was in BHT treated groups. This observed difference may be due to different phenolic contents of GSE₅₀, GSE₂₀₀ and GSE₁₀₀₀.

Antibacterial Activity:

Total bacterial counts (TBC) of minced beef samples treated with BHT 0.01% and GSE (50, 200 and 1000 ppm) were summarized in Table 3. Significant differences were observed among treatments. TBC of control samples significantly increased ($p < 0.05$). While, TBC of BHT and GSE-treated samples were significantly lower ($p < 0.05$) than those of the control samples. Generally, GSE reduced the

Table 3. Effect of BHT (0.01%) and GSE (50, 200, 1000 ppm) on TBC (log CFU/g) in minced beef during refrigerated storage (n=3)

Groups	Zero day	2 nd day	4 th day	6 th day	8 th day	10 th day
Control	8.62 ± 0.01 ^a	8.78 ± 0.01 ^a	Spoiled	Spoiled	Spoiled	Spoiled
BHT (0.01%)	8.56 ± 0.01 ^b	8.31 ± 0.02 ^b	7.85 ± 0.02 ^a	Spoiled	Spoiled	Spoiled
GSE ₅₀	8.45 ± 0.01 ^c	8.09 ± 0.45 ^c	7.62 ± 0.07 ^b	6.81 ± 0.30 ^a	Spoiled	Spoiled
GSE ₂₀₀	8.30 ± 0.01 ^d	7.47 ± 0.01 ^d	6.88 ± 0.62 ^c	6.47 ± 0.57 ^b	6.07 ± 0.01	Spoiled
GSE ₁₀₀₀	8.24 ± 0.01 ^e	7.06 ± 1.03 ^e	6.85 ± 0.34 ^d	5.90 ± 1.06 ^c	5.72 ± 0.01	5.60 ± 0.02

The values represent Mean ± SD of three experiments.

Means within a column followed by different letters are significantly different ($P < 0.05$).

Therefore, delaying lipid oxidation and preventing bacterial growth are significant for extension of shelf life as well as keeping the good quality and sensory characteristics of minced beef. In the past few decades, several synthetic food additives with antioxidant and antimicrobial properties, such as BHT, have been used to achieve these goals (El-Zainy *et al.*, 2016).

The overall acceptability of meat products containing natural additives is an important factor in the development of functional meat products (Hayes *et al.*, 2010). The addition of natural antioxidant such as GSE, did not significantly alter effect the sensory attributes of raw and cooked pork (Rojas and Brewer, 2007), irradiated and non-irradiated chicken breasts (Rababah *et al.*, 2005) or other meat products (Siripatrawan and Noipha, 2012). Moreover, GSE reduced the rancid flavor without affecting the color of raw beef throughout 6 days of chilling storage (Banon *et al.*, 2007). Meat color in particular is assumed to be an indicator of meat quality and freshness which greatly affect the consumer purchasing decision (Jeong *et al.*, 2015). It is known that protein oxidation in meat is directly affected by lipid oxidation, primarily causing changes in aroma and color (Estévez *et al.*, 2006). Also, oxidation of red oxymyoglobin to metmyoglobin (MMG), leads to the development of undesirable brown color of meat (Nerín *et al.*, 2006). However, GSE can delay loss of meat color by intensification of red color and retarding the formation of MMG (Monteleone *et al.*, 2004).

GSE leads to slight variation in pH (0.15 and 0.32 in cooked and raw chicken products, respectively) during frozen storage (Shirahigue *et al.*, 2011). GSE treated minced beef samples displayed pH values lower than those of control group due to the acidic pH of GSE (4.29) (Pateiro *et al.*, 2015). Several factors such as fruit variety, maturity status, and post-harvest handling can also contribute to the variations in pH values, affecting meat quality and water-holding capacity (Ibrahim *et al.*, 2012).

Oxidative changes serve as indicator for the efficiency of meat preservation. The concentrations of GSE used in the present study were sufficient to maintain oxidative stability which was measured based on TBARS index, the frequent marker of lipid oxidation. In general, lipid oxidation considerably depends on a complex interaction between several factors as type and concentration of active

compound(s) and the nature of the food system (Jeong *et al.*, 2015). Our present result agrees with those of Sammet *et al.* (2006), Ganhão *et al.* (2011) and Pateiro *et al.* (2015) who reported gradual increase in TBARS values in dry - cured sausages during ripening. The rise in TBARS values in control group occurs during refrigerated storage due to lipid oxidation (Ganhão *et al.*, 2011). Oxidative rancidity of minced beef, even during refrigerated storage, occurs because while microbiological and enzymatic deterioration is inhibited by low temperature, lipid oxidation still occurs normally, although at low rates (Grau *et al.*, 2000). Lipid oxidation requires oxygen as an oxidizing agent to gain access to lipids. Thus, the increase in TBARS values of samples packed aerobically certainly occurred because the film covering the samples being permeable to oxygen, allowing initiation of lipid oxidation and its occurrence at higher rates (Shirahigue *et al.*, 2011). In this regard, Shirahigue *et al.* (2011) indicated that the restricted access of oxygen limited lipid oxidation in chicken meat.

Similarly, several previously published studies showed higher capacity of GSE to reduce lipid oxidation by reducing TBARS values supporting the possibility of using GSE as a natural antioxidant instead of BHT as a commercially used synthetic antioxidant in meat products (Jayawardana *et al.*, 2011). In raw meat, GSE was effective in reducing the level of primary products (e.g. lipid hydroperoxides and hexanal) and secondary products (e.g. TBARS) of lipid oxidation in beef (Vaithyanathan *et al.*, 2009), chicken (Shirahigue *et al.*, 2010), turkey (Mielnik *et al.*, 2006), fish (Pazos *et al.*, 2005) and pork (Sasse *et al.*, 2009). As in the present study, many previous studies have shown that the antioxidant effect of GSE in meat is concentration dependent (Brannan, 2009), descending the production of TBARS at increasing levels of GSE. While, others speculate that higher concentrations of GSE adversely affect the color of meat (Brannan, 2009). Meanwhile, decrease in TBARS index occurs when the reaction rate of the carbonyls in proteins becomes higher than the rate of TBARS formation (Racanicci *et al.*, 2004).

Antioxidant activity could be used to predict oxidative stability of meat. GSE possess the ability to work more effectively at the interface of the lipid -and water - compatible portions of meat due to its partially hydrophobic nature. This physicochemical property allows using of GSE as a suitable

ingredient in meat products (Ibrahim *et al.*, 2012). The ability of GSE to delay lipid oxidation in minced beef during refrigerated storage, is most likely due to the fact that GSE is rich in polyphenolic and phenolic compounds, especially benzoic acid, protocatechuic acid, gallic acid, catechin, epicatechin, resveratrol, flavonoids, monomer flavan-3-ols, dimeric, trimeric, tetrameric and oligomeric procyanidins and proanthocyanidins, which have high antioxidant activity (Reddy *et al.*, 2013). The antioxidant activity is mainly due to the high reactivity of their hydroxyl groups especially at 3 positions resulting in 10 times more antioxidant capacity, in addition to the double bond at C2-C3 (Kumar *et al.*, 2013). Therefore, the higher number of hydroxyl groups, the greater expected antioxidant activity (El-Hadary and Tahoon, 2013).

The antioxidant activity of GSE can be explained by multiple mechanisms: inhibition of the formation of malonaldehyde (El-Zainy *et al.*, 2016) and primary and secondary products of lipid oxidation (Brannan and Mah, 2007), scavenging initiating free radicals, thereby preventing initiation or propagation of further lipid oxidation (Nam and Ahn, 2002), decomposing peroxides so preventing their conversion into initiating radicals; chain-breaking to prevent continued hydrogen abstraction by active radicals (Adedapo *et al.*, 2008), decreasing localized oxygen concentrations, transition-metal chelation to prevent generation of initiating radicals (Mukai *et al.*, 2005).

In this aspect, GSE exhibited antibacterial activity (Perumalla *et al.*, 2013; Widsten *et al.*, 2014; El-Zainy *et al.*, 2016). Antibacterial activity of GSE could be due to the presence of phenols and polyphenols which act as bactericidal through disruption of the bacterial cell wall (El-Zainy *et al.*, 2016). However, BHT did not exhibit high antibacterial activity due to lack of antimicrobial functional group (Qin *et al.*, 2013).

CONCLUSION

In conclusion, GSE treated minced beef samples showed significantly lower values of pH, TBARS, AOA% and TBC than those of control and BHT treated samples during refrigerated storage. GSE provided satisfactory protection against lipid oxidation and microbial spoilage, and may be used as an alternative to the synthetic antioxidant BHT, without altering the sensory attributes. The efficiency of GSE is concentration dependent. Therefore, GSE could be used as both natural antioxidant and antibacterial during refrigerated storage of meat. Natural antioxidants may encourage the meat industry to develop novel meat products with enhanced nutritional value and health benefits, prolonged shelf life, improved safety and quality. In the future, GSE would be molecules for use in treatment of certain diseases and as natural additives for food preservation.

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CONFLICT OF INTEREST

The authors declare that they don't have any conflicts of interest and are also not interested in competing with anyone.

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