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# EVALUATION THE EFFECT OF USING RESVERATROL SUPPLEMENT TO THE CHILLED GROUND BEEF ON FAT OXIDATION AND SOME QUALITATIVE AND MICROBIAL CHARACTERISTICS

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## ABSTRACT

**This study was conducted to evaluate the effect of adding resveratrol on fat oxidation and some qualitative and microbial characteristics of ground beef cold storage at 2 C° for 1, 3, 6, 9 and 12 d. All resveratrol treatments significantly increased myoglobin concentration as compared with control treatment during cold storage periods. There was a significant decrease in peroxide value (PV) (P<0.05), the percentage of the drip loss and cooking loss between the different treatments as compared with control treatment during cold storage periods. Resveratrol treatments also significantly (P<0.05) reduced the numbers of psychrophilic bacteria during cold storage periods.**

**Keywords:** polyphenol, myoglobin, BHA, psychrophilic bacteria.

# تقييم تاثير اضافة الريسفيراترول الى اللحم البقري المفروم المبرد في اكسدة الدهون وبعض الخصائص النوعية والميكروبية

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## الخلاصة

**أجريت هذه الدراسة لتقييم تأثير اضافة الريسفيراترول في أكسدة الدهون وبعض الصفات النوعية والميكروبية للحم البقر المفروم والمخزن بالتبريد عند درجة حرارة 2 م° للمدد 1، 3، 6، 9، 12 يوم. أدت جميع معاملات اضافة الريسفيراترول الى ارتفاع ملحوظ في تركيز المايوغلوبين مقارنة مع معاملة السيطرة خلال فترات التخزين المبرد وانخفاض معنوي في قيمة البيروكسيد (PV) والفقد بالسائل الناضح والفقد عند الطبخ بين المعاملات المختلفة بالمقارنة مع معاملة السيطرة وفي جميع أوقات التخزين المبردة، كذلك ادت اضافة الريسفيراترول إلى انخفاض معنوي (P<0.05) في اعداد البكتيريا المحبة للبرودة خلال فترات التخزين المبردة بالمقارنة مع معاملة السيطرة.**

**الكلمات المفتاحية:** البوليفينول، المايوغلوبين، BHA، البكتريا المحبة للبرودة.

[*] **This article is taken from the first researcher's master's thesis.**

## INTRODUCTION

The global meat industry is constantly evolving due to changes in consumer desires, interests and lifestyles in addition to many other factors. Part of this development has become the introduction of natural antioxidants to increase the shelf life of meat and their products, to reduce meat spoilage due to the oxidation of fats and proteins, as they are safer, healthier, and derived from plants. In meat systems **(Manessis *et al.*, 2020)**, phenolic compounds are the main natural antioxidants, the most important of which are Resveratrol these compounds have a strong ability to donate hydrogen and the ability to scavenge free radicals **(Nikmaram *et al.*, 2018)**. Resveratrol is a non-flavonoid polyphenol that possesses many of biological properties **(Saad *et al.*, 2020)**. This natural polyphenol has been discovered in more than 70 plant species, especially in grape skins and its seeds. It has also been found in separate quantities in red wine, many studies have proven that resveratrol has very high potential as an antioxidant in goat fattening. A recent study indicated **(Shen *et al.*, 2022)** that 150 mg/kg dietary resveratrol improves meat quality it causes increased muscle fat content, redness, and improved tenderness, **Li *et al.*, (2022)** provided the first evidence that dietary resveratrol supplementation (5 g/cattle/day) improves beef quality by enhancing the conversion of type II muscle fibers to type I muscle fibers, also increased the activities and expressions of antioxidant enzymes in the serum and muscles of cows. As a result, adding resveratrol to the feed additive for beef cattle improved the stability of meat color, WHC and tenderness **(Cui *et al.*, 2023)**, **(Jin *et al.*, 2021)** also noted that dietary resveratrol improves the quality of duck meat, as evidenced by increased post-slaughter pH, increased tenderness, and decreased of cooking loss, which It may be due to the enhanced antioxidant capacity and inhibition of fat and protein oxidation in broiler chickens. Resveratrol also causes a decrease in pH and Malondialdehyde (MDA) content, in addition, dietary resveratrol improves the meat quality of broiler chickens exposed to heat stress **(Zhang *et al.*, 2018)**. Therefore, the objective of this study was designed to evaluate the effect of adding resveratrol directly to meat on fat oxidation and some qualitative, physical and microbial characteristics of chilled ground beef.

## MATERIALS AND METHODS

### Samples

This experiment was carried out in the Meat Science and Technology Laboratory of animal production department and in the animal nutrition laboratory for postgraduate students at College of Agricultural Engineering Sciences, University of Baghdad. Fourteen kg of meat took from the leg of the calf at age of 1.5 years immediately after the slaughter process from the local market and it was minced with an electric mincing machine, then it was left at the refrigerator for 12 hours to remove the rigor mortis state. The minced meat was divided into 5 parts of 2 kg for each part. After that, each part was treated with the additives (Resveratrol) and control (no addition) and BHA (Beta hydroxycarboxylic acid) treatments.

**Treatments**

The experiment included seven different treatments: T1 (control treatment without addition), T2 (Resveratrol at a concentration of 0.10% g/kg), T3 (Resveratrol at a concentration of 0.20% g/kg), T4 (Resveratrol at a concentration of 0.30% g/kg) T5 (Resveratrol at a concentration of 0.40% g/kg), T6 (Resveratrol at a concentration of 0.50% g/kg) and T7 (0.01% Beta hydroxycarboxylic acid (BHA)).

Each treatment was separately homogenized by hand using medical gloves to obtain a separate homogenized sample. The samples were placed in polyethylene bags and stored in the refrigerator for different periods of ds (1, 3, 6, 9 and 12 ds) at a temperature of 2 C° to determine the effect of the additive on quality and microbial properties of chilled ground beef.

**Quality characteristics**

The peroxide value was evaluated according to **A.O.A.C. (2000)**, the myoglobin concentration was calculated based on the method of **Zessin *et al.*, (1961)**, and drip loss percentage was estimated according to the method of **Young & Lyon (1997)**, and the percentage of cooking loss was estimated according to the method of **Barton & Purchas (1976)**, and cold-loving bacteria were counted according to **Andrew (1992)**.

**Statistical analysis:**

Data were statistically analyzed using the Completely Randomized Design Model (CRD) as a factorial experiment (5×7). Duncan's **(Duncan, 1955)**, multiple range test was used to determine the significant differences among treatment's means and periods using **SAS (2018)**.

## RESULTS AND DISCUSSION

The results in Table 1 refer to the effect of the interaction between Resveratrol treatments and storage periods on myoglobin concentration of chilled ground beef, as there was a significant increase ($P<0.05$) in myoglobin concentration of all Resveratrol treatments compared with T1 (control treatment) which recorded the lowest myoglobin concentration in all storage periods (1, 3, 6, 9 and 12 d). As T6 (resveratrol 0.50% g/Kg) recorded the highest myoglobin concentration (4.65 mg/g of meat) at 1 d of cold storage compared with T1 which recorded the lowest myoglobin concentration at 12 d of cold storage (3.2 mg/g of meat).

It is noted from Table 1 that there are significant differences ($P<0.05$) between the rates of the different treatments. whereby T6 recorded the highest concentration of myoglobin followed by T5, T4, T3 and T7 then followed by T2 and finally T1 which recorded the lowest concentration of myoglobin. The reason for this may be that adding dietary resveratrol prevents oxidative stress and enhances antioxidant capacity and meat quality, which improves the color and tenderness of meat **(Jin *et al.*, 2021)**.

The results of the statistical analysis indicated that there was a significant difference ($P<0.05$) between the different periods, where myoglobin was at its highest concentration in

the 1d period (4.78 mg/g of meat), then it began to decrease with the progression of the cold storage period, all the way to its lowest concentration during the 12 d refrigeration storage period, which amounted to (3.77 mg/g of meat). The reason for the bright red color of chilled ground beef during the period of 1 d of storage is due to the presence of the pigment oxymyoglobin (Oxymb) on the surface of the meat. The process of storing the meat by cooling led to the change of the red color to brown due to lack of oxygen and then the formation of metmyoglobin (Metmb) pigment on the surface of the meat **(Pogorzelska *et al.*, 2018)**.

**Table (1):** The effect of interaction between treatments and storage periods on the concentration of myoglobin pigment for ground beef and cold storage for different periods.

| Treatments No. | Myoglobin (mg/g meat) | | | | | Range |
|---|---|---|---|---|---|---|
| | Storage periods (d)* | | | | | |
| | 1 | 3 | 6 | 9 | 12 | |
| T1 | 4.45±0.05 efgh | 4.1±0.1 ijkl | 3.70±0.1 nop | 3.40±0.1 qr | 3.2±0.20 r | 3.77±0.15 G |
| T2 | 4.52±0.02 defg | 4.39±0.01 efgh | 3.9±0.1 klmn | 3.65±0.01 nopq | 3.45±0.05 pqr | 3.98±0.138 F |
| T3 | 4.8±0.1 bcd | 4.56±0.01 def | 4.18±0.02 hijk | 4.1±0.1 ijkl | 3.89±0.01 lmn | 4.30±0.11 D |
| T4 | 4.9±0.1 abc | 4.65±0.02 cde | 4.40±0.1 efgh | 4.22±0.02 hij | 4.0±0.2 jklm | 4.43±0.11 C |
| T5 | 5.05±0.05 ab | 4.85±0.01 abc | 4.52±0.02 defg | 4.30±0.1 eghi | 4.1±0.1 ijkl | 4.56±0.11 B |
| T6 | 5.12±0.01 a | 5.02±0.02 ab | 4.80±0.1 bcd | 4.55±0.05 def | 4.25±0.05 ghij | 4.74±0.10 A |
| T7 | 4.65±0.05 cde | 4.45±0.01 efgh | 4.1±0.1 ijkl | 3.8±0.2 mno | 3.55±0.05 opq | 4.11±0.13 E |
| Range | 4.78±0.06 A | 4.57±0.07 B | 4.22±0.09 C | 4.00±0.107 D | 3.777±0.10 E | --- |

***Means with different letters differ significantly (P<0.05) from each other (large letters are main effect of treatments and storage times and lowercase letters are the effect of the interaction between treatments and storage times). *T1 (control treatment without addition); T2 (Resveratrol 0.1 g/kg); T3 (Resveratrol 0.2 g/kg); T4 (Resveratrol 0.3 g/kg); T5 (Resveratrol 0.4 g/kg); T6 (Resveratrol 0.5 g/kg); T7 (BHA 0.01%).**

The results in Table 2 refer to the effect of the interaction between different treatments and storage periods on the peroxide value of chilled ground beef. A significant increase (P<0.05) in the peroxide value which T1 (control treatment) recorded the highest PV (13.1 mEq/kg fat) in the storage period of 12 d compared to T6 (3.39 mEq/kg fat) which recorded the lowest PV in the storage period of 1 d.

It is noted from Table 2 that there are significant differences (p<0.05) between the rates of the different treatments. Whereby the T2 was recorded the highest level of PV (7.56 mEq /kg fat) followed by T7 (7.19 mEq /kg fat) and T3 (6.93 mEq/kg fat) and T4 (6.57 mEq /kg fat) T5 and (6.13 mEq/kg fat) and finally T6 (4.88 mEq /kg fat) as compared to T1 (control

treatment) which recorded the highest rate (9.88 mEq /kg fat) this agrees with the findings of **Mula & AL-Rubeii (2023)** who indicated a decrease in the peroxide value.

The results of the statistical analysis indicated that there were significant differences (P<0.05) in the value of the peroxide between the periods, as the value of the peroxide was at its lowest level (4.93 mEq/kg fat) in the period of 1d and then began to rise during the periods to reach Its highest level (8.88 mEq/kg fat) in the 12 d period. Peroxide values increase as the storage period advances and decrease with increasing concentration of the antioxidant **(Zainy & Alrubeii, 2023; Mula & AL-Rubeii, 2023; Zangana, 2015)**.

**Table (2):** The effect of interaction between treatments and storage periods on peroxide value (mEq/kg fat) for ground beef and cold storage for different periods.

| Treatments* No. | Peroxide Value (mEq/kg fat) Storage periods (d)* | | | | | Range |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 9 | 12 | |
| T1 | 6.39±0.04 kl | 8.58±0.02 ef | 10.05±0.05 c | 11.29±0.01 B | 13.1±0.1 a | 9.88±0.76 A |
| T2 | 5.61±0.01 no | 6.87±0.03 jk | 7.53±0.03 hi | 8.6±0.1 Ef | 9.22±0.02 d | 7.56±0.42 B |
| T3 | 5.07±0.03 pq | 6.18±0.02 lm | 6.92±0.02 jk | 7.95±0.05 Gh | 8.57±0.03 ef | 6.93±0.41 D |
| T4 | 4.65±0.05 q | 5.89±0.01 lmn | 6.44±0.04 kl | 7.76±0.01 Gh | 8.15±0.05 fg | 6.57±0.42 E |
| T5 | 4.15±0.05 r | 5.67±0.02 mno | 6.12±0.02 lmn | 6.94±0.04 Jk | 7.78±0.98 gh | 6.13±0.43 F |
| T6 | 3.39±0.02 s | 4.1±0.1 r | 4.82±0.02 pq | 5.66±0.02 Mno | 6.44±0.01 kl | 4.88±0.36 G |
| T7 | 5.25±0.05 op | 6.45±0.05 kl | 7.10±0.1 ij | 8.25±0.05 Fg | 8.92±0.02 de | 7.19±0.43 C |
| Range | 4.93±0.25 E | 6.24±0.34 D | 6.99±0.4 C | 8.06±0.44 B | 8.88±0.54 A | --- |

***Means with different letters differ significantly (P<0.05) from each other (large letters are main effect of treatments and storage times and lowercase letters are the effect of the interaction between treatments and storage times). *T1 (control treatment without addition); T2 (Resveratrol 0.1 g/kg); T3 (Resveratrol 0.2 g/kg); T4 (Resveratrol 0.3 g/kg); T5 (Resveratrol 0.4 g/kg); T6 (Resveratrol 0.5 g/kg); T7 (BHA 0.01%).**

The results in Table 3 refer to the effect of the interaction between Resveratrol treatments and storage periods on drip loss percentage of chilled ground beef, as there was a significant decrease (P<0.05) in drip loss of all Resveratrol treatments compared with T1 (control treatment) which recorded the highest drip loss percentage in all storage periods (1, 3, 6, 9 and 12 days). As T6 (resveratrol 0.50% g/Kg) recorded the lowest drip loss percentage (0.9%) at 1 d of cold storage compared with T1 which recorded the highest drip loss percentage at 12 d of cold storage (3.9%).

It is noted from Table 3 that there are significant differences (P<0.05) between the rates of the adding treatments, as T1 recorded the highest percentage (2.61%) compared to T6, which recorded the lowest percentage (1.46%), followed by T5, T3 which did not differ significantly with T7 and which were similar in effect to T4 and T2. This is due to the fact that the addition of resveratrol works to increase the ability to retain water and reduce only the loss of the exudate liquid during storage period **(Li *et al.*, 2022)**, which may be due to an increase in type I muscle fibers and a decrease in type II muscle fibers **(Meng *et al.*, 2020)**, or this result may be due to enzyme activity by the phenols present in natural extracts **(Cao *et al.*, 2022)**.

The results of the statistical analysis indicated that there was a significant difference between the periods, as the percentage of drip loss was at its lowest level in the 1 d storage period (1.19%), and then began to rise during the periods of storage reaching its highest level in 12 d of refrigeration (3.03%), the reason for this may be due to the decomposition of meat proteins due to hydrolytic enzymes, which are responsible for some subtle changes in the permeability of the cell membrane or the structural structure of the protein in the cell, which is then followed by a decrease in the ability of the meat to retain water **(AL-Rubeii & Muhammad, 2018)**.

**Table (3):** The effect of interaction between treatments and storage periods on the percentage of drip loss for ground beef and cold storage for different periods.

| Treatments No. | Drip Loss (%) Storage periods (d)* | | | | | Range |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 9 | 12 | |
| T1 | 1.5 ±0.2 ijkl | 1.75 ±0.05 hi | 2.65 ±0.03 e | 3.25 ±0.02 Bc | 3.9 ±0.1 a | 2.61±0.3 A |
| T2 | 1.33±0.33 jklmn | 1.55±0.05 ijk | 2.25 ±0.05 f | 2.9±0.1 De | 3.45±0.05 b | 2.29±0.2 B |
| T3 | 1.18 ±0.02 klmno | 1.45 ±0.01 ijklm | 1.95 ±0.02 fgh | 2.6 ±0.1 E | 3.05 ±0.05 cd | 2.04±0.2 C |
| T4 | 1.15 ±0.05 lmno | 1.35 ±0.01 jklmn | 1.8 ±0.2 ghi | 2.25 ±0.05 F | 2.77 ±0.03 de | 1.86±0.1 B |
| T5 | 1.05±0.05 no | 1.25 ±0.02 klmno | 1.7 ±0.3 hij | 2.1 ±0.1 F | 2.7 ±0.1 de | 1.76 ±0.2 D |
| T6 | 0.9±0.1 o | 1.10 ±0.1 mno | 1.5 ±0.1 ijkl | 1.72 ±0.02 Hi | 2.1 ±0.1 fg | 1.46±0.1 E |
| T7 | 1.25 ±0.05 klmno | 1.5 ±0.1 ijkl | 2.1 ±0.1 fg | 2.70 ±0.1 De | 3.30 ±0.1 bc | 2.17±0.2 BC |
| Range | 1.19±0.06 E | 1.42±0.05 D | 1.99 ±0.1 C | 2.50 ±0.13 B | 3.03 ±0.15 A | --- |

***Means with different letters differ significantly (P<0.05) from each other (large letters are main effect of treatments and storage times and lowercase letters are the effect of the interaction between treatments and storage times). *T1 (control treatment without addition); T2 (Resveratrol 0.1 g/kg); T3 (Resveratrol 0.2 g/kg); T4 (Resveratrol 0.3 g/kg); T5 (Resveratrol 0.4 g/kg); T6 (Resveratrol 0.5 g/kg); T7 (BHA 0.01%).**

The results in Table 4 refer to the effect of the interaction between Resveratrol different treatments and storage periods on the percentage of cooking loss of chilled ground beef. A significant decrease ($P<0.05$) in the cooking loss percentage which T1 (control treatment) recorded the highest cooking loss percentage (31.1%) in the storage period of 1 d compared to T6 (19.15%) which recorded the lowest value in the storage period of 12 d.

It is noted from Table 4 that there are significant differences ($P<0.05$) between the rates of the adding treatments, as T1 recorded the highest percentage (28.58%) compared to T6, which recorded the lowest percentage (22.58%), followed by T5, T4, T3 T2 and T7 respectively, the decrease in the percentage of loss during cooking is due to the decrease in the percentage of moisture as the cold storage period progresses as a result of the evaporation of water occurring on the surface of the meat as well as the decomposition of meat proteins in the presence of proteolytic enzymes that work to break the bonds that bind the protein with water, which is susceptible to evaporation **(Pang *et al.*, 2021)**.

The results of the statistical analysis indicated that there was a significant difference between the periods, as the percentage of cooking loss was at its highest level in the 1 d storage period (28.62%), and then going to its lowest level during the periods of storage reaching its lowest level in 12 d of refrigeration (22.138%), The reason for this is the decrease in the percentage of humidity due to the evaporation of the water present on the surface of the meat and the decomposition of meat proteins by hydrolytic enzymes that lead to the breaking of the bonds that connect the protein with water and thus the ability of the meat to bind to water is reduced it is susceptible to evaporation **(Juárez *et al.*, 2010)**, and this result may be due to the inhibition of microbial reproduction and enzyme activity by the phenols present in natural extracts, which led to a slowdown in the deterioration of the microstructure of the muscle fibers and an increase in the ability to retain water and thus a decrease in the rate of loss during cooking as well as a decrease in oxidation **(Cao *et al.*, 2022)**.

**Table (4):** The effect of interaction between treatments and storage periods on the percentage of cooking loss for ground beef and cold storage for different periods.

| Treatments No. | Cooking Loss (%) | | | | | Range |
|---|---|---|---|---|---|---|
| | Storage periods (d)* | | | | | |
| | 1 | 3 | 6 | 9 | 12 | |
| T1 | 31.1±0.1 a | 30.95±0.01 ab | 28.5±0.1 f | 27.15±0.01 i | 25.20±0.1 o | 28.58 ±0.75 A |
| T2 | 29.40±0.1 c | 28.75±0.01 e | 27.85±0.01 h | 26.19±0.01 l | 22.91±0.01 s | 27.02 ±0.773 C |
| T3 | 28.90±0.1 e | 28.1 ±0.1 g | 26.47±0.01 k | 23.3±0.1 r | 22.18±0.01 u | 25.79 ±0.878 D |
| T4 | 28.10±0.1 g | 27.15±0.01 i | 25.60±0.1 n | 22.1±0.1 u | 20.95±0.01 w | 24.78 ±0.93 E |
| T5 | 26.80±0.1 j | 25.9±0.1 m | 23.97±0.01 q | 22.70±0.1 t | 20.22±0.01 x | 23.918 ±0.78 F |
| T6 | 25.3±0.1 o | 24.15±0.01 q | 23.1±0.01 rs | 21.2±0.1 v | 19.15±0.01 y | 22.58 ±0.72 G |
| T7 | 30.8±0.1 b | 29.15±0.01 d | 28.11±0.01 g | 26.8±0.1 j | 24.36±0.01 p | 27.84 ±0.72 B |
| Range | 28.62±0.5 A | 27.73±0.57 B | 26.22±0.54 C | 24.20±0.6 D | 22.13±0.5 E | --- |

***Means with different letters differ significantly (P<0.05) from each other (large letters are main effect of treatments and storage times and lowercase letters are the effect of the interaction between treatments and storage times). *T1 (control treatment without addition); T2 (Resveratrol 0.1 g/kg); T3 (Resveratrol 0.2 g/kg); T4 (Resveratrol 0.3 g/kg); T5 (Resveratrol 0.4 g/kg); T6 (Resveratrol 0.5 g/kg); T7 (BHA 0.01%).**

Figure 1 shows the effect of the interaction between treatments and storage periods on the number of psychrophilic bacteria in chilled ground beef. It is noted that the logarithm of psychrophilic bacteria number decreased significantly (P<0.05) in T6 which amounted to 0.16 colony-forming units/gm of meat in the 1 d of cold storage, while the logarithm of the total number of bacteria increased in the T1 (control) in the 12 d of cold storage, as it recorded 5.8 colony-forming units/gm of meat, and significant (P<0.05) differences between treatments and for different storage periods.

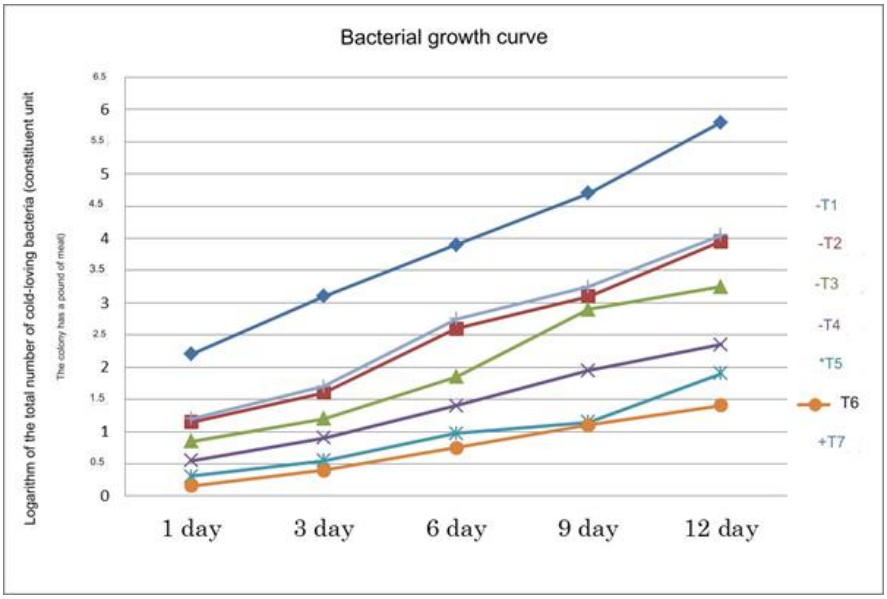


**Figure (1):** The effect of the interaction between different treatments and cold storage periods on the logarithm of the total number of psychrophilic bacteria (colony-forming units/g of meat) in ground beef stored in cold storage for periods (1-3-6-9-12) d.

## CONCLUSION

We conclude that the addition of the antioxidant resveratrol can prevent or reduce fat oxidation, improve the physical properties of chilled ground beef, and increase the microbial stability of ground beef stored under refrigeration at 2 C° for approximately 12 d.

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