



EVALUATION OF THE EFFECT OF NISIN, POTASSIUM SORBATE AND SODIUM LACTATE ON FAT OXIDATION AND SOME QUALITATIVE AND MICROBIAL PROPERTIES OF CHILLED GROUND BEEF MEAT

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ABSTRACT

This study was conducted to analyze the effects of nisin, potassium sorbate and sodium lactate and their interactions in fat oxidation and some quality and microbial characteristics of chilled ground beef meat stored at 4°C for 0, 4, 8 and 12 d. All nisin, potassium sorbate and sodium lactate treatments significantly increased pH. Total volatile nitrogen (TVN), peroxide value (PV) and free fatty acids (FFA) were decreased (P<0.05) in nisin, potassium sorbate and sodium lactate treatments when compared with the control sample at all refrigerated storage time. Nisin, potassium sorbate and sodium lactate treatments reduced (P<0.01) the psychrophilic bacteria count during refrigerated storage times.

Keywords: Nisin, potassium sorbate, sodium lactate, BHA, bacteriocin, beef, psychrophilic.

تقييم تأثير النيسين وسوربات البوتاسيوم ولاكتات الصوديوم في أكسدة الدهون وبعض الخصائص النوعية والميكروبية
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الخلاصة

أجريت هذه الدراسة لغرض تقييم تأثير النيسين وسوربات البوتاسيوم ولاكتات الصوديوم في أكسدة الدهون وبعض الخصائص النوعية والميكروبية للحم البقر المفروم المبرد المخزن عند درجة حرارة 4 م لمدة 0، 4، 8 و 12 يوم، إذ سجلت المعاملات التي اضيف إليها النيسين وسوربات البوتاسيوم ولاكتات الصوديوم زيادة معنوية (P<0.05) في الاس الهيدروجيني وانخفاضاً معنوياً (P<0.05) في تركيز النيتروجين الكلي المتطاير (TVN) وقيمة البيروكسيد (PV) والأحماض الدهنية بالمقارنة مع معاملة السيطرة خلال جميع فترات الخزن، كذلك خفضت معاملات الاضافة من العدد الكلي للبكتريا المحبة للبرودة.

الكلمات المفتاحية: النيسين، سوربات البوتاسيوم، لاكتات الصوديوم، البكتريوسينات، العدد الكلي للبكتريا المحبة للبرودة.



INTRODUCTION

Meat and meat products provide essential components in the diets of human beings (Ali, 2018). The biological and chemical nature of meat and its products makes it vulnerable to damage and spoilage when stored as a result of chemical and bacterial deterioration, which exposes the consumer to so-called foodborne diseases that are sometimes very dangerous, especially in societies that lack health care. Meat has appropriate nutritional requirements for the growth of microorganisms such as high humidity, as their activity increases, causing damage and spoilage of meat and the secretion of many compounds that cause food poisoning for humans consuming this meat and its products, so the need for new methods to control pathogens has taken a large part of the attention of researchers recently (Amaral *et al.*, 2018).

Fat oxidation and color instability are other serious problems facing the meat industry, which affect consumer acceptance (Akamittath *et al.*, 1990). Antioxidants are often added to processed meats to counteract the negative effects of fat oxidation, and due to concerns about the toxic/carcinogenic effect of synthetic antioxidants (Juntachote *et al.*, 2006), researchers have turned to manufacturers using natural antioxidants to promote health and reduce the risk of the incidence of heart disease, cancer, cataracts, and other degenerative diseases associated with aging resulting from the use of artificial preservatives (Gadekar *et al.*, 2014).

Today, the application of chemical preservatives has been questioned because of the potentially toxic and carcinogenic effects (Woraprayote *et al.*, 2016).

Natural preservatives have emerged as alternatives to synthetic preservatives (Marrone *et al.*, 2021). Natural preservatives have shown the potential to provide effective antimicrobial activity while reducing negative health effects (Woraprayote *et al.*, 2016).

One of the oldest and the most extensively characterized bacteriocins is nisin A, which was discovered by Rogers (1928). Nisin A is produced by many strains of *Lactococcus lactis*. It has a broad antimicrobial spectrum against a wide range of Gram-positive genera, including staphylococci, streptococci, *Listeria* spp., bacilli, and enterococci. Nisin A has been used in the food industry as a biopreservative for more than 50 years without inducing microbial resistance (Cotter *et al.*, 2005). Therefore, the objective of this study was to evaluate the effect of nisin, potassium sorbate and sodium lactate on fat oxidation and some qualitative and microbial properties of chilled ground beef meat.

MATERIALS AND METHODS

Samples

The experiment of this study was conducted in the Meat Science and Technology Laboratory in the department of animal production and the animal nutrition laboratory for postgraduate students at University of Baghdad, college of agricultural engineering sciences. Eighteen kilograms of meat from the leg of the calf were taken immediately after the slaughter process, minced with an electric mincing machine and left in the refrigerator to remove the rigor mortis state. Then the meat was divided into 9 parts of 2 kg for each. After that, each part was treated with the additives (Nisin, Potassium Sorbate, Sodium Lactate, BHA)

Treatments

The experiment included nine treatments as follows: T1 (control), T2 (nisin 150mg/kg), T3 (potassium sorbate 0.075 g/kg), T4 (sodium lactate 3%), T5 (nisin 150mg/kg + potassium sorbate 0.075g/kg), T6 (nisin 150mg/kg + sodium lactate 3%), T7 (sodium lactate 3% + potassium sorbate 0.075g/kg), T8 (nisin 150mg/kg + sodium lactate 3% + potassium sorbate 0.075g/kg), T9 (addition of BHT), each treatment was homogenized separately to



obtain a separated homogeneous sample. The samples were placed in polyethylene bags and stored in the refrigerator for different periods (0, 4, 8, and 12 d) at 4°C to analyze the effect of the mentioned additives on the quality and microbial characteristics of the chilled ground beef meat.

Quality characteristics

pH and Peroxide value was evaluated according to **A.O.A.C. (2000)**, free fatty acids percentage was evaluated according to **Duston & Pearson (1985)**, total volatile nitrogen was measured according to the method described by **Egan et al. (1981)**, and Psychrophilic bacteria was counted according to **Andrew (1992)**.

Statistical analysis:

Data were statistically analyzed using the Completely Randomized Design Model (CRD) as a factorial experiment (4×9). 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 different treatments and storage periods on the pH value of chilled ground beef, as there was a significant increase ($P<0.01$) in the pH value of T8 (nisin 150 mg/kg + sodium lactate 3% + potassium sorbate 0.075 g/kg), in the 12d refrigeration storage period, which amounted to 6.10 compared with T1 (control treatment) which recorded the lowest value in pH (5.42) in the 0d refrigeration storage period, and there were significant differences between different treatments and different storage periods.

It is noted from (Table 1) that there are significant differences ($P<0.01$) between the rates of the different treatments, whereby T8 recorded the highest rate in the pH value, followed by T3, T5, T6 and T7, which was similar in effect, then followed by T4, T2, T9 and finally T1 which recorded the lowest rate in the pH value, and these results agreed with the results of **Hussein et al. (2012)**, where the addition of potassium sorbate salts led to an increase in the pH in camel meat as compared to the control treatment. The salts add new negative charges for the interaction of water with the protein composition resulting from the presence of the ion of these salts, which raises the percentage of moisture and the fact that the water molecules are not electrically neutral (they have ended with positive and other negative charges), which enables the salt ions to bind with them and thus the continuation of the high value of pH with the progression of the storage period (**Sebranek, 2009**).

The results of the statistical analysis indicated that there was a significant difference ($P<0.01$) between the different periods, where the pH value was at its lowest level in the 0d period (5.54), then it began to rise with the progression of the cold storage period, all the way to its highest level during the 12d refrigeration storage period, which amounted to (6.00), and the reason may be due to the decomposition of meat proteins with the progression of the cold storage periods as a result of the activity of some bacteria that work to decompose meat proteins and thus the rise of nitrogen compounds that lead to raising the pH (**Al-Salmay, 2020**), **Holmer et al. (2009)** indicated that the high pH is mainly caused by the degradation of proteins and the production of amines in meat, and this will lead to an increase in the spread of microbes and a decrease in the shelf life of meat.

Table (1): Effect of nisin, potassium sorbate and sodium lactate treatments and storage (or refrigeration) period in pH value of chilled ground beef meat (mean±std error).

Treatments	pH				Range
	Storage periods (d)*				
	0	4	8	12	
T1	5.42±0.06 N	5.61±0.07 JKLM	5.72±0.06 HIJK	5.80±0.05 EFGHI	5.63±0.05d
T2	5.51±0.05 LMN	5.70±0.05 HIJK	5.83±0.04 EFGH	5.96±0.03 ABCDE	5.75±0.05c
T3	5.60±0.06 JKLM	5.87±0.05 DEFGH	5.92±0.04 BCDEF	6.09±0.03 AB	5.87±0.06 ab
T4	5.56±0.06 KLMN	5.75±0.05 FGHIJ	5.90±0.04 DEFG	6.03±0.05 ABCD	5.81±0.06bc
T5	5.58±0.06 JKLMN	5.85±0.05 EFGH	5.94±0.05 ABCDE	6.10±0.04 A	5.86±0.06ab
T6	5.59±0.05 JKLMN	5.83±0.05 EFGH	5.95±0.05 ABCDE	6.08±0.04 ABC	5.86±0.06ab
T7	5.58±0.05 JKLMN	5.83±0.05 EFGH	5.91±0.09 CDEFG	6.09±0.04 AB	5.85±0.06ab
T8	5.61±0.06 JKLM	5.85±0.07 EFGH	6.04±0.05 ABCD	6.10±0.04 A	5.90±0.06a
T9	5.44±0.08 MN	5.65±0.04 IJKL	5.74±0.07 GHIJ	5.82±0.04 EFGH	5.66±0.04d
Range	5.54±0.02d	5.77±0.02c	5.88±0.02b	6.00±0.02a	

*The averages that bear different letters, differ significantly ($P<0.01$) among them (lowercase letters are the main effect of treatments and storage times and large letters are the effect of the intraction between treatments and storage d).

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 ($P<0.01$) in the peroxide value (9.38 mEq/kg fat) for T1 (control treatment) and (7.42 mEq/kg fat) for T2 (nisin 150 mg/kg), followed by T4 (sodium lactate 3%) (7.38 mEq/kg fat), then T6 (nisin 150 mg/kg + sodium lactate 3%) which did not differ significantly with it which amounted to (7.35 mEq/kg fat) in the 12d storage period, while T8 (nisin 150 mg/kg + sodium lactate 3% + potassium sorbate 0.075 g/kg) was recorded the lowest peroxide value (0.59 mEq/kg fat) in the storage period of 0 d.

It is noted from (Table 2) that there were no significant differences ($P<0.01$) in the rates of T2, T4 and T6 adding treatments, which amounted to 4.55, 4.56, and 4.50 (mEq/kg fat) respectively, and then T8 (nisin 150 mg/kg + sodium lactate 3% + potassium sorbate 0.075 g/kg) had the lowest rate (2.62 mEq/kg fat) as compared to T1 (control treatment) which recorded the highest rate (5.82 mEq/kg fat), **Al-Ghanimi (2019)** indicated a decrease in the peroxide value in ground beef when it was treated with natural antioxidants such as Astaxanthin and Allyl isothiocyanate compared to the control treatment.

The results of the statistical analysis indicated that there were significant differences ($P<0.01$) in the value of the peroxide between the periods, as the value of the peroxide was at its lowest level (0.92 mEq/kg fat) in the period of 0 d and then began to rise during the periods to reach Its highest level (6.84 mEq/kg fat) in the 12 d period, and it is noted that all of the

adding treatments did not exceed the upper limit allowed (10 mEq/kg fat) by the **IQS 2688: Iraqi Standard Specification (1987)**.

Table (2): Effect of nisin, potassium sorbate and sodium lactate treatments and storage (or refrigeration) period on Peroxide value of chilled ground beef meat (mean±std error).

Treatments	PV (mEq/ kg fat)				Range
	Storage periods (d)*				
	0	4	8	12	
T1	1.93±0.04R	4.52±0.04J	7.45±0.04B	9.38±0.05A	5.82±0.85a
T2	0.90±0.04S	4.12±0.04K	5.76±0.04F	7.42±0.05B	4.55±0.72b
T3	0.85±0.05S	3.25±0.04O	5.11±0.05GH	6.75±0.05C	3.99±0.66c
T4	0.89±0.05S	4.05±0.05KL	5.95±0.04E	7.38±0.05B	4.56±0.73b
T5	0.78±0.05ST	2.97±0.04P	4.99±0.04HI	6.58±0.04D	3.83±0.65d
T6	0.88±0.04S	3.92±0.04LM	5.88±0.05EF	7.35±0.04B	4.50±0.73b
T7	0.68±0.05TU	2.64±0.05Q	3.86±0.05M	5.15±0.04G	3.08±0.49e
T8	0.59±0.05U	1.95±0.05R	3.05±0.05P	4.89±0.05I	2.62±0.47f
T9	0.86±0.05S	3.45±0.04N	5.10±0.04GH	6.67±0.04CD	4.02±0.64c
Range	0.92±0.07d	3.43±0.015c	5.23±0.23b	6.84±0.24a	

*The averages that bear different letters, differ significantly ($P<0.01$) among them (lowercase letters are the main effect of treatments and storage times and large letters are the effect of the interaction between treatments and storage d).

The results in (Table 3) refer to the effect of the interaction between different treatments and cold storage periods on the percentage of volatile fatty acids in chilled ground beef. It is noted from the table that there is a significant increase ($P<0.01$) in the percentage of volatile fatty acids (1.52%) for T1 (control treatment without addition) in the 12 d refrigeration storage period as compared to treatment T8 (nisin 150 mg/kg + 3% sodium lactate + 0.075 g/kg potassium sorbate) which recorded the lowest rate (0.20%) in the 0 d, and there were significant differences between different treatments and different storage periods.

It is noted from (Table 3) that there are significant differences ($P<0.01$) between the rates of the adding treatments, as T1 recorded the highest percentage (1.08%) compared to T8, which recorded the lowest percentage (0.67%), followed by T9, T4, T6, T2 and T3 which were similar in effect, and finally T5 and T7. These results agreed with the results of (AL-Rubeii *et al.*, 2009) who found that adding some antioxidants to the chilled ground beef led to a decrease in the percentage of free fatty acids for the adding treatments compared to the control. It also agreed with the results of (Khidhir, 2014) who found that sodium lactate reduced the percentage of free fatty acids in fish meat treated and stored in cold storage at 4° C.

The results of the statistical analysis indicated that there was a significant difference between the periods, as the percentage of free fatty acids was at its lowest level in the 0 d storage period (0.41%), and then began to rise during the periods of storage reaching its highest level in 12 d of refrigeration (1.35%), and it is noted that all addition treatments did not exceed the upper limit allowed by the (IQS 2688: Iraqi Standard Specification, 1987), which indicated that meat is rejected when the percentage of free fatty acids exceeds 1.5%. The percentage of free fatty acids is measured to study the progress of the hydrolysis of fats, which is considered an indication for determining the deterioration of food products (Khidhir, 2014). The reason for the increase in free fatty acids with the progression of storage periods may be due to the effect of lipolytic enzymes resulting from an increase in the total number of bacteria, which leads to an increase in free fatty acids, which contribute to the production and formation of an undesirable smell and flavor in meat (Al-Sherick, 2005).

Table (3): Effect of nisin, potassium sorbate and sodium lactate treatments and storage (or refrigeration) period on Free fatty acids of chilled ground beef meat (mean±std error).

Treatments	FFA (%)				Range
	Storage periods (d)*				
	0	4	8	12	
T1	0.59±0.06 KLMN	0.90±0.05GHI	1.32±0.09 ABCDE	1.52±0.05A	1.08±0.11a
T2	0.45±0.05 MNOP	0.71±0.09 IJKL	1.15±0.02 CDEF	1.36±0.11ABC	0.91±0.11 bcd
T3	0.40±0.10 NOPQ	0.70±0.06 IJKL	1.12±0.04DEFG	1.35±0.06 ABCD	0.89±0.11 bcd
T4	0.50±0.12 LMNO	0.80±0.07IJK	1.18±0.08 BCDEF	1.40±0.14AB	0.97±0.11bc
T5	0.35±0.03OPQ	0.65±0.05 JKLM	1.10±0.06EFG	1.35±0.06 ABCD	0.86±0.12cd
T6	0.45±0.04 MNOP	0.72±0.04 IJKL	1.15±0.03 CDEF	1.40±0.06AB	0.93±0.11bc
T7	0.25±0.04PQ	0.63±0.09 JKLMN	1.05±0.08FGH	1.32±0.08 ABCDE	0.81±0.12d
T8	0.20±0.06Q	0.55±0.04 LMNO	0.85±0.08HIJ	1.10±0.06EFG	0.67±0.10e
T9	0.50±0.08 LMNO	0.83±0.06IJ	1.20±0.08 BCDEF	1.40±0.05AB	0.98±0.11 ab
Range	0.41±0.03d	0.72±0.03c	1.12±0.03b	1.35±0.03a	

*The averages that bear different letters, differ significantly ($P<0.01$) among them (lowercase letters are the main effect of treatments and storage times and large letters are the effect of the intraction between treatments and storage d).

The results in (Table 4) refer to the effect of the interaction between different treatments and refrigeration periods on the total volatile nitrogen concentration of chilled ground beef. A significant increase ($P<0.01$) was observed in the total volatile nitrogen concentration (15.22 mg N/100 g meat) in T1 (Control treatment) in the 12 d storage period in refrigeration, while the T8 (Nisin 150 mg/kg + 3% sodium lactate + 0.075 g/kg potassium sorbate) was recorded the lowest concentration (2.90 mg N/100 g meat) of total volatile nitrogen in the 0 d storage period and there were significant differences ($P<0.01$) between different treatments during different storage periods.

It is noted from (Table 4) that there are significant differences ($P<0.01$) between the rates of the different treatments, where T1 (control treatment) recorded the highest concentration, followed by T9 (adding of BHT), T2 (nisin 150 mg/kg), T7 (sodium lactate 3% + potassium sorbate 0.075 g/kg), T5 (niacin 150 mg/kg + potassium sorbate 0.075 g/kg), T3 (Potassium sorbate 0.075 g/kg), T4 (sodium lactate 3%), T6 (nisin 150 mg/kg + sodium lactate 3%), and finally T8 (nisin 150 mg/kg + sodium lactate 3% + potassium sorbate 0.075 g/kg). The reason for this may be attributed to the fact that these salts act as chelating compounds that prevent the formation of free radicals, as they maintain the stability of the protein, and this why the percentage of total volatile nitrogen decreases As for the storage periods, it is noticeable that the concentration of total volatile nitrogen increased with the progression of storage, as it recorded its lowest concentration (3.80 mg N/100 g of meat) in the 0 d storage period, reaching its highest rate in the 12 d, which amounted to (11.17) mg N/100 g of meat, that the cause of

the increase in the concentration of total volatile nitrogen is due to the change and decomposition of nitrogenous substances during cold storage, as well as to the increase in the activity of proteolytic enzymes. It is noted that all the adding treatments within the acceptable limits for total volatile nitrogen which are 20 mg N/100 g of meat and the meat is considered spoiled if the value of total volatile nitrogen increases more than that limit according to the standards allowed by the (IQS 2688: Iraqi Standard Specification, 1987).

Table (4): Effect of nisin, potassium sorbate and sodium lactate treatments and storage (or refrigeration) period on Total Volatile Nitrogen of chilled ground beef meat (mean±std error).

Treatments	TVN				Range
	Storage periods (d)*				
	0	4	8	12	
T1	4.85±0.04U	8.11±0.05L	10.39±0.04F	15.22±0.03A	9.64±1.13a
T2	3.92±0.03W	7.22±0.05O	9.91±0.05G	12.10±0.04C	8.28±0.92c
T3	3.65±0.1X	5.88±0.04R	8.46±0.04K	10.35±0.05F	7.08±0.76f
T4	3.50±0.04Y	5.49±0.04S	7.95±0.04M	9.61±0.03H	6.63±0.70g
T5	3.70±0.05X	6.11±0.05Q	8.65±0.04J	10.57±0.04E	7.25±0.78e
T6	3.45±0.06Y	5.18±0.04T	7.61±0.03N	9.25±0.07I	6.37±0.67h
T7	3.75±0.03X	6.74±0.04P	9.67±0.04H	11.37±0.04D	7.88±0.87d
T8	2.90±0.04Z	3.79±0.04WX	5.25±0.04T	8.44±0.04K	5.09±0.63i
T9	4.53±0.04V	7.85±0.05M	9.95±0.04G	13.65±0.04B	8.99±0.99b
Range	3.80±0.10d	6.26±0.25c	8.64±0.29b	11.17±0.40a	

*The averages that bear different letters, differ significantly ($P<0.01$) among them (lowercase letters are the main effect of treatments and storage times and large letters are the effect of the intraction between treatments and storage d).

The results in (Table 5) refer to the effect of the interaction between different treatments and refrigeration periods on the total count of psychrophilic bacteria in chilled ground beef. It is noted that the logarithm of the total number of psychrophilic bacteria decreased significantly ($P<0.01$) in T6 (nisin 150 mg/kg + 3% sodium lactate), which amounted to 0.45 colony-forming units/g meat during the 0 d storage period, while it increased in T1 (control treatment) in the 12 d period of cold storage which amounted 7.85 colony-forming units/g of meat, and there were significant differences ($P<0.01$) between the treatments and for the different storage periods and this agreed with (AL-Mamoori, 2018).

The significant effect ($P<0.01$) of the treatments on the total number of psychrophilic bacteria is shown in (Table 5), as the number of bacteria decreased in T6, T2, T4, T5, T7, T8, T3 and T9, respectively, compared to T1. In a study conducted by (Teng *et al.*, 2012), it was concluded that potassium sorbate inhibits Gram-negative bacteria more effectively than Gram-positive bacteria, and since nisin has a narrow spectrum of affecting bacteria in meat and meat products, it only affects Gram-positive bacteria. As well as the study conducted by (Lin *et al.*, 2018) compare the effect of nisin, potassium sorbate and sodium lactate on *Staphylococcus aureus* growth and the production of *Staphylococcus enterotoxins*, which concluded that sodium lactate was the best among the treatments, This may why T6 (nisin 150 mg/kg + 3% sodium lactate) recorded the lowest total number of psychrophilic bacteria.

As for the periods of refrigeration, (Table 5) shows a significant increase ($P<0.01$) in the logarithm of the total number of psychrophilic bacteria in 12, 8, and 4 d periods compared to the period of 0 d of cold storage and this agreed with (Zangana,2016) ; Zangana & AL-Shamery, 2016).

Table (5): Effect of nisin, potassium sorbate and sodium lactate treatments and storage (or refrigeration) period on psychrophilic bacterial count of chilled ground beef meat (mean±std error).

Treatments	Psychrophilic Bacteria (colony-forming unit/g)				Range
	Storage periods (d)*				
	0	4	8	12	
T1	1.60±0.300.Q	3.20±0.15GH	6.01±0.02C	7.85±0.07A	4.66±0.73a
T2	0.58±0.04VW	1.10±0.06ST	2.11±0.05NO	2.83±0.01IJK	1.65±0.26f
T3	0.98±0.04STU	1.75±0.03PQ	2.74±0.03JK	3.80±0.05E	2.31±0.31c
T4	0.65±0.04VW	1.20±0.11RS	2.28±0.04MN	2.95±0.02HIJ	1.77±0.27ef
T5	0.77±0.03UV	1.45±0.07QR	2.10±0.05NO	3.05±0.07HI	1.84±0.25e
T6	0.45±0.02W	0.86±0.07TUV	1.95±0.02OP	2.75±0.02IJK	1.50±0.27g
T7	0.76±0.03UV	1.56±0.03Q	2.45±0.02LM	3.40±0.20FG	2.04±0.30d
T8	0.85±0.02TUV	1.70±0.15PQ	2.60±0.21KL	3.50±0.05F	2.16±0.30d
T9	1.52±0.09Q	2.95±0.02HIJ	5.70±0.15D	6.95±0.02B	4.28±0.65b
Range	0.90±0.08d	1.75±0.15c	3.10±0.29b	4.12±0.35a	

*The averages that bear different letters, differ significantly ($P<0.01$) among them (lowercase letters are the main effect of treatments and storage times and large letters are the effect of the intraction between treatments and storage d).

CONCLUSION

It can be concluded that the addition of salts (potassium sorbate and sodium lactate) with nisin could improve the quality characteristics of the chilled ground beef meat, and extend the microbial stability of the ground beef stored under refrigeration for about two weeks.

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