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Impact of Sodium Nitrite and Sodium Lactate as Chemical Preservatives on the Shelf Life of locally manufactured Frozen Beef Sausages as compared with Thyme and Cinnamon as Natural Preservatives

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ABSTRACT

Sausages are common traditional food that served in every Egyptian house. Nine groups of beef sausages were prepared using either natural preservatives (cinnamon 1% and thyme 1%) or chemical preservatives (sodium lactate 1.5%, 3% and sodium nitrite 0.01%) and control samples (without the addition of any preservatives). Samples were stuffed into natural and artificial casing, then kept at freezing condition (-18 °C) until exceeding the permissible limits (became unacceptable). Sensory properties and microbial loads decreased gradually with freezing time in all groups, followed by gradual increase in aerobic plate count (APC) on day 14 for cinnamon, thyme, nitrite and control groups in natural casing (4.54, 4.56 4.47 and 4.45 $\log_{10}$ CFU/g, respectively), while was on day 42 for lactate 1.5% and 3% groups (3.48 and 3.41 $\log_{10}$ CFU/g, respectively). APC of all sausage samples exceeded the permissible limits on 97th day of storage except for lactate 1.5% and 3% groups which were unfit on 104th (14 days after the end of shelf life) and on 118th day (28 days after the end of shelf life), respectively. The same manner was observed in total psychrotrophic count which decreased gradually with freezing time in all groups, followed by a gradual increase on day 14 for cinnamon, thyme, nitrite and control groups in natural casing (3.66, 3.72, 3.48 and 3.53 $\log_{10}$ CFU/g, respectively), while was on day 42 for lactate 1.5% and 3% groups (2.85 and 2.71 $\log_{10}$ CFU/g, respectively).

Chemical analysis revealed gradual increase in total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) in all sausage samples until exceeded the permissible limits on the 97th day of freezing, except samples treated with 1.5% and 3% SL. Acceptable values were clear for 3% SL even on 111th day of freezing. Samples treated with 1.5% SL exceeded the permissible

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limits on day 104 (21.07 mg/100 g and 0.96 mg malonaldehyde/kg, respectively) which went on the same way with APC. Samples treated with cinnamon, thyme and nitrite had the best sensory scores among other groups at the beginning of storage, but declined quickly from the 14th day of freezing. Samples packed in artificial (collagen) casing had lower bacterial load but less overall acceptability than those in natural sheep casing throughout the storage period. SL 3% improved the shelf life of frozen sausage. Thus, using sodium lactate works better as a food additive and preservative for beef sausages than other chemicals as nitrite and natural preservatives as cinnamon and thyme especially when stored under freezing

INTRODUCTION

The need to conserve meat soundness motivated the production of processed meat and meat products at first. As the oldest of these ways mainly include fermentation, drying, and salting **(Bosse *et al.* 2018)**. Beef sausage is one of Egyptian popular meat products, made primarily from beef, fat, salt, and spices. Sausage is a highly perishable food since it is made from fresh meat **(Shahin 2016)**. Sausages are processed in oxygen quasi-permeable wrapping and held at refrigeration temperatures due to their high fat content, which encourages lipid oxidation **(Hugo & Hugo 2015)**. Spices and herbs have been applied for the prevention of food decay and pathogenic organisms due to their antimicrobial properties. Ground mustard, clove, and cinnamon, as well as their oils, are considered to help prevent food spoilage due to microbial growth **(Al-Wabel 2007)**. **Smith-Palmer *et al.* (2001)** found that 1% of cinnamon or thyme was the most powerful concentration when comparing the anti-microbial effect of cinnamon and thyme at various concentrations (0.1%, 0.5%, and 1%). Because of their beneficial effect on meat quality and microbiological protection. Nitrates are among the most essential additives in meat industry. Nitrite was often used to preserve meat products like sausages **(Parthasarathy & Bryan 2012)** it has a potent bacteriostatic and bactericidal properties against a variety of spoilage bacteria and pathogenic microorganisms found in meat products **(Sindelar & Milkowski 2012)**. The antimicrobial effect of nitrites is most likely due to reactions involving the production of nitrous acid or nitric oxide **(Møller & Skibsted 2002)**. Nitrite has been reported to be more effective against Gram positive bacteria than Gram negative bacteria **(Pichner *et al.* 2006)**. Permissible limits for residual nitrites in meat products have been developed around the world, ranging from 40 to 100 ppm depending on the meat products' manufacturing condition **(ES/3597, 2005 ES/3598, 2005)**. However, when consumed in large quantities with food, nitrite and indirectly nitrate can pose a health risk to humans. Some of these cases could be handled, while others were fatal. Death can result from a sodium nitrite (SN) intake of 33-250 mg per kg of body weight **(Honikel 2004)**. Concerns that the use of nitrite may result in the formation of carcinogenic nitrosamines when it reacts with secondary amines found in meat has prompted the search for alternatives **(Houtsma 1996)**.

Lactate at 1.5 – 3 g/100 g of meat weight is widely used in the meat industry to increase a variety of meat quality characteristics. Adding sodium lactate to ground beef and other meat products has been seen in several studies to enhance taste, color, tenderness, juiciness, and cooking yields **(Vote *et al.* 2000)**. According to **Koos (1992)** lactates' antimicrobial properties are because of the tendency to reduce moisture content and the lactate ion's inhibitory impact. Adding 2-3% of the 60 percent solution to meat and poultry products is generally recommended **(Houtsma 1996)**. According to **Sallam & Samejima (2004)**, sodium lactate, either by itself or in conjunction with sodium chloride, can be used to preserve chemical characters, minimize microbial growth, and extend the shelf life of ground beef in the re-

frigerator. This study aimed to compare between the implications of specific sodium nitrite and lactate concentrations as chemical preservatives with thyme and cinnamon as natural ones, on the consistency and shelf life of beef sausage in natural and artificial casing. This would be helpful in improving meat technology, specifically in terms of limiting the use of hazardous preservatives.

MATERIAL and METHODS

Fresh Egyptian sausage were prepared according to the quality criteria described by **ES/1972 (2005)**. Sodium nitrite (SN) and sodium lactate (SL; $C_3H_5NaO_3$: LOBA CHEMIE PVT. LTD Batch # L312122002) were obtained from El-Gomhouria Co., Egypt.

Sausage preparation:

In a bowl chopper, meat and fat were minced then mixed. Other spices and additives were applied before the starch was added. The paste obtained was divided into nine equal groups, each group weighed about 1000 g. G1 (control group) was stuffed in natural casing without adding any preservatives. G2 was treated with SL 1.5 g/100 g meat. G3 was treated with SL 3 g/100 g meat, which is the maximum limits as permitted by the USDA-Food Safety and Inspection Service (USDA-FSIS) at a level of up to 3 g/100 g meat **(Bedie *et al.* 2001)**. G4 and G5 were given 1% ground cinnamon and 1% ground thyme respectively, as recommended by **Smith-Palmer *et al.* (2001).** G6 was treated with SN at a concentration 0.01% (100 ppm), which is the maximum allowable amounts in meat products according to the **FAO/WHO (1991)** standard and the **ES/3597 (2005)**. G1 to G6 groups were stuffed in natural casing. G7 (control group) was stuffed in artificial casing without adding any preservatives. G8 was treated with SL 1.5 g/100 g meat. G9 was treated with SL 3 g/100 g meat. Groups G7, G8 and G9 were stuffed in synthetic collagen casing.

Cinnamon and thyme were added to the sausage formulations as ingredients, while SL solutions and SN powder were added after mixing the sausage ingredients. The final mixture was transferred to a commercial sausage filling machine and stuffed into the natural and the permeable synthetic casing with 21 mm calibre. Beef sausages of each group was then packed separately in polyethylene bags, labelled and stored at -18 °C.

Sausage Examination:

The treated and the control groups were inspected for physical, chemical, and microbial deteriorative parameters during freezing storage until spoilage occurred and each experiment was repeated three times, as stated below:

2.2.1. Sensory evaluation

Sensory tests were performed on sausage samples right after they were made, then periodically every 14 days as defined by **Chabela & Mateo-Oyague (2005)**. After frying and slicing the samples, an experienced nine-member committee evaluated the efficacy and sensory attributes features of the sausages as described by **Watts *et al.* (1989)** and focused to compare between natural and artificial casing. After the panel members had carried out the sensory parameters, they recorded the color, taste, odor, juiciness, springiness, and overall acceptability, depending on a nine-point rating scale, with 9 being extremely good and 1 being incredibly bad **(Jin *et al.* 2016)**. On the days of the microbiological study, the sausages were visually inspected for spoilage.

2.2.2. Bacteriological examination

The sausage sample groups were microbiologically evaluated immediately after preparation, then every fourteen days as the sensory evaluation. Sausage in each group was cut into a sterile bag and mixed. The 25 g samples from these mixtures were mixed in a stomacher for 2 minutes in 225 mL of 0.1 % peptone water in an aseptic stomacher bag.

Duplicate 1 mL inoculum of sufficient dilutions is plated into the following selected culture medium using serial decimal dilutions made of same diluent. Inoculated plate count agar was incubated for 72 hours at 30°C for the estimation of aerobic plate count (APC) according to **ISO 4833-2 (2013)**. While the enumeration of psychrotrophic count was calculated out in accordance with **APHA, (2001)** in which plates were incubated for 10 days at 7°C.

2.2.3. Chemical analysis

Total volatile basic nitrogen value (TVB-N) was measured according to the method of the Association of Official Analytical Chemists **(AOAC, 2000)**. Thiobarbituric acid (TBA) value was determined using the distillation method applied according to a technique described by **AOAC, (2000)**.

2.2.4. Statistical analysis

The experiment was conducted in triplicate for microbiological and chemical examinations. After logarithmic transformation of the bacterial count, the data was analyzed statistically using SPSS software (IBM, co). ANOVA was employed to find significance (α = 0.05) at the same day. To determine whether there was a significant difference between groups, the least significant difference test (LSD) was utilized. Fisher's least significant difference test (LSD) was used to separate the means, and significance was determined at 0.05. The values obtained were then defined as mean ± standard deviation (SD), with $p \leq 0.05$. Differences were considered to be statistically significant with values of $P<0.05$.

RESULTS

Table (1): Scores of sensory characters in sausage samples with natural casing during freezing storage.

Sensory trait	Treatment	Freezing Time (Days)										
		0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 90	Day 97	Day 104	Day 118
Taste	G1	7.8	7.3	6.6	6.1	5.7	5.2	4.8	4.2	3.7	2.8	2.2
	G2	6.5	6.1	5.9	5.4	4.8	4.4	3.9	3.3	2.6	2.2	1.7
	G3	6.7	5.9	5.3	5.1	4.5	4.0	3.5	2.9	2.3	1.8	1.4
	G4	8.5	7.8	6.2	5.4	4.6	4.2	3.3	2.7	2.2	1.9	1.5
	G5	8.3	7.3	5.8	4.9	4.4	4.1	3.5	2.4	2.0	1.6	1.3
	G6	7.3	6.8	5.5	4.5	4.1	3.8	3.4	2.6	2.1	1.7	1.2
Odor	G1	7.2	6.5	5.9	5.3	5.1	4.8	4.4	3.4	2.8	2.4	1.8
	G2	7.1	6.8	6.0	5.6	5.3	4.9	4.1	3.6	3.2	2.6	1.9
	G3	6.7	6.1	5.4	5.4	5.0	4.6	4.2	3.5	3.0	2.6	2.0
	G4	8.2	7.6	6.3	5.7	5.2	4.5	4.0	3.3	2.7	2.3	1.7
	G5	7.7	7.4	5.8	5.1	4.7	4.1	3.5	2.9	2.4	2.0	1.5
	G6	7.0	6.6	5.9	5.0	4.3	4.0	3.2	2.5	1.9	1.6	1.2
Color	G1	7.6	6.3	5.5	5.3	5.0	4.7	4.2	3.5	2.9	2.4	1.8
	G2	6.8	6.6	5.8	5.5	5.1	4.4	3.9	3.2	2.6	2.1	1.5
	G3	7.2	6.4	5.3	4.8	4.5	4.1	3.4	2.8	2.3	1.9	1.4
	G4	7.1	6.7	5.5	4.3	3.9	3.5	3.1	2.3	1.8	1.4	1.1
	G5	7.6	6.9	6.1	5.6	5.1	4.7	4.4	2.5	1.9	1.5	1.0
	G6	8.4	8.1	7.2	6.2	5.5	4.9	4.6	4.2	3.6	2.8	2.2
Flavor	G1	7.3	6.9	6.0	5.4	5.0	4.2	3.4	2.8	2.5	2.0	1.4
	G2	6.7	6.5	5.7	5.1	4.6	4.1	3.5	2.7	2.2	1.7	1.2
	G3	6.4	6.3	5.9	4.8	4.3	3.8	3.2	2.9	2.4	1.9	1.3
	G4	8.6	7.5	6.5	4.9	4.2	3.7	3.3	2.6	2.1	1.7	1.2
	G5	8.3	7.2	6.1	4.5	3.8	3.5	2.9	2.3	1.8	1.5	1.1
	G6	7.2	6.7	5.8	5.1	4.7	4.2	3.6	3.0	2.6	2.2	1.7
Juiciness	G1	6.8	6.3	5.6	5.0	4.4	3.8	3.2	2.7	2.4	1.9	1.4
	G2	6.4	6.1	5.2	4.8	4.2	3.5	2.8	2.4	1.9	1.4	0.9
	G3	6.3	6.0	5.5	4.7	4.0	3.3	2.6	2.0	1.5	1.1	0.6
	G4	7.3	6.8	5.7	5.4	4.7	3.6	3.4	2.6	2.3	1.8	1.1
	G5	6.9	6.4	5.5	4.5	3.9	3.3	2.9	2.4	2.0	1.6	0.9
	G6	6.5	6.1	5.1	4.8	4.1	3.7	3.0	2.6	2.1	1.5	0.8
Springiness	G1	5.8	5.3	4.7	4.0	3.6	3.1	2.6	2.0	1.6	1.4	0.8
	G2	5.6	5.1	4.5	3.7	3.2	2.7	2.2	1.8	1.4	1.1	0.5
	G3	5.2	4.9	4.2	3.4	3.0	2.5	2.1	1.6	1.3	0.9	0.5
	G4	5.5	4.9	4.5	4.1	3.3	2.5	1.9	1.3	1.0	0.7	0.4
	G5	5.7	4.7	4.3	3.8	3.0	2.7	1.5	1.0	0.8	0.5	0.3
	G6	5.5	4.6	4.0	3.4	2.8	2.3	1.2	1.0	0.7	0.4	0.1
Overall acceptability	G1	78.7 %	71.5 %	56.6%	51.6%	46.3%	40.8%	33.7%	29.4%	23.4%	19.9%	13.6%
	G2	72.4 %	68.9 %	61.3%	55.7%	50.4%	41.4%	35.8%	31.7%	25.8%	20.4%	14.2%
	G3	71.3 %	65.9 %	63.9%	57.1%	52.8%	44.3%	37.2%	35.1%	26.2%	21.7%	15.5%
	G4	83.7 %	76.5 %	59.2%	52.2%	47.9%	40.7%	35.2%	27.4%	25.1%	21.3%	16.3%
	G5	82.4 %	73.9 %	58.4%	52.6%	46.1%	41.5%	35.9%	25%	21.5%	17.8%	12.4%
	G6	77.6 %	72.0 %	60.0%	53.7%	47.2%	42.4%	35.2%	29.4%	23.7%	18.4%	13.9%

Table (2): Scores of sensory characters in sausage samples with synthetic casing during freezing storage.

Sensory trait	Treatment	Freezing Time (Days) 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 90	Day 97	Day 104	Day 118
Taste	G7	6.3	5.8	5.5	4.9	4.5	3.8	3.3	2.7	2.4	2.1	1.6
	G8	6.0	5.6	5.1	4.7	4.2	3.4	3.1	2.5	2.1	1.6	1.2
	G9	6.1	5.3	5.0	4.5	4.1	3.0	2.6	2.1	1.7	1.4	1.0
Odor	G7	6.9	6.5	6.1	5.7	5.1	4.6	4.3	3.9	3.5	3.0	2.6
	G8	6.6	6.4	5.8	5.2	4.7	4.5	4.0	3.5	3.1	2.7	2.3
	G9	6.5	6.0	5.6	5.3	4.5	4.1	3.8	3.4	2.9	2.5	2.2
Color	G7	6.7	6.4	6.0	5.5	5.0	4.5	4.1	3.6	3.3	2.9	2.3
	G8	6.5	6.1	5.7	5.3	4.6	4.1	3.9	3.5	2.8	2.5	2.0
	G9	6.4	6.1	5.6	5.1	4.8	4.3	3.7	3.3	2.6	2.1	1.7
Flavor	G7	6.4	6.0	5.4	4.9	4.6	4.0	3.5	2.9	2.6	2.3	1.6
	G8	6.1	5.8	5.3	4.7	4.3	3.7	3.3	2.7	2.2	1.6	1.2
	G9	6.2	5.6	5.1	4.5	4.1	3.8	3.1	2.8	2.4	1.9	1.5
Juiciness	G7	6.3	5.8	5.3	4.9	4.4	4.1	3.6	3.0	2.6	2.1	1.7
	G8	6.1	5.6	5.4	4.8	4.3	3.7	3.4	2.8	2.4	1.7	1.3
	G9	6.3	5.4	5.1	4.5	4.0	3.7	3.2	2.9	2.2	1.6	1.2
Springiness	G7	7.6	7.5	7.0	6.4	6.1	5.7	5.4	5.1	4.5	3.9	3.4
	G8	7.5	7.3	6.9	6.3	6.0	5.8	5.3	5.1	4.3	3.6	3.1
	G9	7.6	7.1	6.7	6.0	5.8	5.5	5.0	4.7	4.0	3.4	2.8
Overall acceptability	G7	74.4%	70.4%	55.3%	52.8%	49.6%	39.4%	36.8%	32.3%	27.1%	21.6%	16.7%
	G8	71.8%	68.1%	60.3%	57.4%	50%	45.7%	42.9%	37.2%	32.7%	27.5%	22.2%
	G9	72.4%	65.7%	62.3%	59.3%	51.6%	46.2%	44.6%	39.4%	34.8%	30.9%	25.3%

Table (3): Aerobic plate count (log_{10} CFU/g) in sausage samples with natural and synthetic casing during freezing storage

Freezing time	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Zero time	4.37±0.01	4.33^{c} ±0.01	4.27^{b}±0.01	4.48±0.03	4.56±0.02	4.40±0.01	4.31^{c} ±0.01	4.29^{a}±0.01	4.25^{b}±0.01
Day 1	4.32±0.01	4.28±0.02	4.18±0.02	4.37±0.02	4.42±0.02	4.35±0.01	4.19±0.01	4.12^{a}±0.02	4.13^{a}±0.02
Day 14	4.45^{a} ±0.02	4.16±0.03	4.03±0.02	4.54±0.01	4.56±0.01	4.47^{a} ±0.02	4.33±0.01	4.02±0.00	3.88±0.02
Day 28	4.52±0.02	3.56±0.01	3.53±0.01	4.61^{a} ±0.01	4.63^{a} ±0.01	4.54±0.01	4.47±0.01	3.85±0.01	3.67±0.02
Day 42	4.73±0.01	3.48±0.01	3.41±0.00	4.89±0.01	4.92±0.01	4.70±0.02	4.62±0.02	3.97±0.01	3.55±0.01
Day 56	4.89±0.01	4.03±0.01	3.85±0.00	4.94±0.01	5.01±0.01	4.81±0.01	4.79±0.01	4.21±0.01	4.26±0.01
Day 70	5.45 ±0.01	4.14±0.01	4.10±0.00	5.18±0.01	5.25±0.01	5.16^{a}±0.01	5.15^{a}±0.01	4.40^{b}±0.01	4.39^{b}±0.01
Day 84	5.50±0.02	4.36±0.01	4.28±0.02	5.29±0.01	5.34±0.00	5.22±0.01	5.26±0.01	4.72±0.00	4.61±0.02
Day 90	5.66±0.01	4.85±0.02	4.33±0.02	5.37±0.01	5.40±0.01	5.31±0.02	5.33±0.01	5.08±0.01	5.03±0.02
Day 97	6.99±0.01	5.47±0.01	4.71±0.00	6.82±0.02	6.85±0.01	6.71±0.01	6.87±0.02	5.41±0.01	5.15±0.00
Day 104	U	6.93±0.01	5.18±0.02	U	U	U	U	6.58±0.01	5.29±0.01
Day 111	U	U	5.63±0.01	U	U	U	U	U	5.40±0.02
Day 118	U	U	6.30±0.01	U	U	U	U	U	6.24±0.01

Values are expressed as mean ± SD. There are significance differences at ($P<0.05$) between all the groups in all examination times except those means having the same alphabetical letters superscript along row.
U= means Unacceptable according to the **ES/1972 (2005).**

Table (4) Psychrotrophic count (log_{10} CFU/g) in sausage samples with natural and synthetic casing during freezing storage.

Freezing time	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Zero time	3.11[a] ±0.01	3.05±0.01	3.02[b] ±0.02	3.26±0.01	3.28±0.01	3.09±0.03	3.10[a] ±0.01	3.03±0.01	3.00[b] ±0.02
Day 1	3.07±0.01	2.94±0.02	2.90±0.01	3.12[a] ±0.02	3.15[a] ±0.01	3.04±0.01	3.01±0.01	2.82±0.01	2.75±0.01
Day 14	3.53±0.03	2.80±0.01	2.76±0.02	3.66±0.02	3.72±0.01	3.48±0.01	3.39±0.03	2.63±0.03	2.60±0.03
Day 28	3.86±0.01	2.73±0.01	2.64[a] ±0.01	3.95±0.01	3.98±0.02	3.80±0.03	3.52±0.01	2.50[a] ±0.01	2.43±0.01
Day 42	4.61[a] ±0.02	2.85±0.01	2.71±0.01	4.77±0.01	4.88±0.03	4.52[a] ±0.02	4.44±0.01	2.61±0.01	2.56±0.02
Day 56	4.90±0.01	3.16±0.02	3.05[a] ±0.01	4.98±0.03	5.00±0.02	4.84±0.02	4.74±0.01	3.01[a] ±0.02	2.82±0.01
Day 70	5.37±0.02	3.91±0.01	3.58±0.02	5.40[a]±0.01	5.56±0.01	5.39[a] ±0.01	5.15±0.03	3.42±0.02	3.20±0.01
Day 84	5.47±0.02	4.18±0.00	4.10±0.01	5.51±0.02	5.72±0.01	5.42±0.00	5.65±0.01	4.03 ±0.02	4.39±0.00
Day 90	5.58±0.01	4.57±0.03	4.32±0.01	5.64±0.02	5.83±0.01	5.55±0.01	5.81±0.02	4.16±0.03	4.44±0.01
Day 97	6.81[a] ±0.01	5.63±0.02	4.89±0.03	6.76±0.01	6.80[a] ±0.01	6.71±0.02	6.73±0.01	5.39±0.01	4.93±0.02
Day 104	NP	6.67±0.01	5.25±0.02	NP	NP	NP	NP	6.51±0.01	5.14±0.01
Day 111	NP	NP	5.74±0.01	NP	NP	NP	NP	NP	5.67±0.02
Day 118	NP	NP	6.23±0.01	NP	NP	NP	NP	NP	6.18±0.01

Values are expressed as mean ± SD. There are significance differences at (P<0.05) between all the groups in all examination times except those means having the same alphabetical letters superscript along row.
NP = means "Not Performed" as their corresponding aerobic plate counts were unacceptable

Table (5): Total volatile nitrogen (mg /100g) in sausage samples with natural and artificial casing during freezing storage.

Freezing time	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Zero time	9.13±0.21	8.57±0.25	8.90±0.36	9.33±0.31	8.87±0.21	9.07±0.25	7.37±0.25	8.17±0.42	7.80±0.50
Day 14	11.53±0.15	10.53±0.25	10.50±0.36	11.87±0.31	12.33±0.40	10.73±0.35	9.50±0.20	9.13±0.31	8.67±0.21
Day 28	12.83±0.47	11.90±0.30	11.83±0.35	13.34±0.21	13.71±0.50	11.99±0.10	11.27±0.45	10.97±0.60	9.50±0.20
Day 42	14.17±0.32	13.00±0.30	12.05±0.20	14.83±0.30	14.17±0.35	12.60±0.30	12.68±0.10	12.50±0.20	11.67±0.15
Day 56	15.73±0.25	14.83±0.25	13.53±0.15	15.26±0.20	15.84±0.25	13.74±0.10	14.09±0.45	13.93±0.25	12.50±0.46
Day 70	16.52±0.40	15.12±0.35	14.90±0.15	16.73±0.61	16.29±0.10	15.83±0.45	14.83±0.35	14.07±0.35	13.11±0.30
Day 84	18.61±0.15	16.74±0.40	15.87±0.31	17.55±0.31	17.37±0.31	16.04±0.20	16.12±0.25	15.80±0.15	14.73±0.45
Day 90	19.84± 0.30	17.67±0.10	16.83±0.31	18.37±0.45	19.47±0.35	18.73±0.25	18.23±0.40	16.29±0.40	15.22±0.35
Day 97	21.70±0.20	18.39±0.20	17.16±0.15	24.43±0.31	25.17±0.35	23.90±0.36	25.23±0.55	17.17±0.60	16.57±0.25
Day 104	U	21.07±0.31	18.57±0.25	U	U	U	U	23.77±0.35	17.86±0.10
Day 111	U	U	19.67±0.15	U	U	U	U	U	18.24±0.15
Day 118	U	U	21.29±0.20	U	U	U	U	U	23.10±0.00

Values are expressed as mean ± SD. There are significance differences at (P<0.05) between all the groups in all examination times
U= Unacceptable according to the **ES/1972 (2005)**

Table (6): Thiobarbituric acid (mg-malondialdehyde/kg) in sausage samples with natural and artificial casing during freezing storage.

Freezing time	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Zero time	0.25±0.02	0.25±0.03	0.21±0.03	0.24±0.05	0.29±0.03	0.25±0.05	0.28±0.03	0.23±0.03	0.26±0.03
Day 14	0.29±0.01	0.27±0.02	0.25±0.03	0.31±0.04	0.36±0.02	0.33±0.03	0.37±0.03	0.29±0.04	0.30±0.04
Day 28	0.37±0.02	0.30±0.01	0.29±0.02	0.39±0.02	0.40±0.03	0.38±0.01	0.41±0.01	0.36±0.01	0.36±0.00
Day 42	0.41±0.03	0.38±0.02	0.37±0.03	0.43±0.01	0.46±0.04	0.44±0.01	0.49±0.04	0.43±0.03	0.39±0.00
Day 56	0.53±0.03	0.42±0.03	0.40±0.01	0.58±0.03	0.55±0.04	0.47±0.02	0.55±0.03	0.47±0.01	0.41±0.02
Day 70	0.60±0.02	0.51±0.03	0.49±0.03	0.65±0.02	0.68±0.05	0.62±0.02	0.68±0.03	0.51±0.05	0.44±0.01
Day 84	0.78±0.03	0.59±0.02	0.53±0.03	0.74±0.02	0.76±0.01	0.72±0.00	0.70±0.03	0.59±0.02	0.49±0.00
Day 90	0.85±0.01	0.65±0.03	0.58±0.01	0.80±0.06	0.86±0.02	0.88±0.03	0.81±0.05	0.63±0.03	0.55±0.02
Day 97	0.93±0.02	0.74±0.03	0.63±0.02	1.27±0.03	1.24±0.04	0.96±0.03	1.03±0.05	0.74±0.02	0.65±0.04
Day 104	U	0.96±0.03	0.67±0.05	U	U	U	U	0.91±0.01	0.75±0.02
Day 111	U	U	0.75±0.04	U	U	U	U	U	0.83±0.02
Day 118	U	U	0.90±0.02	U	U	U	U	U	0.93±0.03

Values are expressed as mean ± SD. There are significance differences at ($P<0.05$) between all the groups in all examination times

U= Unacceptable according to the **ES for frozen sausage (2005).**

DISCUSSION

4.1. Effect of preservatives on sensory properties of sausage samples

Data in tables 1 and 2 showed that sensory traits of frozen meat sausages (taste, odor and color as well as flavor, juiciness and springiness) decreased gradually with storage time in all group samples. This could be due to freezing that causes massive ice crystals formation, leading to loss of nutrients, loss of juice and unfavorable appearance upon thawing **(Marapana *et al* .2018)**. G4 and G5 had the highest sensory scores and overall acceptability among other groups at the first 14 days of storage as shown in table 1. This is due to the familiar flavor of cinnamon and thyme to the Egyptian consumers who used to add these herbs during sausage preparation. This agreed with **Batiha *et al.* (2021)**; **Mendonca *et al.* (2018)**; and **Shan *et al.* (2009)** who mentioned that those herbs had an immediate effect on sensory parameters when applied to food. On the other hand, **Jin *et al.* (2016)** concluded that adding thyme to sausages at the start of storage does not enhance sensory evaluation, but it could promote anti-oxidative and antimicrobial activity because thyme is good natural sources of dietary bioactive components.

G6 had the best color scores during the first 14 days of freezing as the bright red color of sausage is one of characteristic effects of nitrite on meat product which agreed with **Wójciak *et al.* (2019)**. Taste ratings were poorer in case of synthetic casing (G7, G8 and G9) than natural ones as illustrated in table 2. This could be referred to the fact that sausages stuffed in artificial casing are unfamiliar to Egyptian customers (edible but not preferable) due to its high elasticity and since they are not common in butchers and beef retails markets. These findings disagree with **Marapana *et al.* (2018)** who found that the flavor scores were high in Devro -Collagen and cellulose casing as it allows water to pass through during cooking giving the good flavor and taste of sausages. However, collagen casing maintained uniform shape and

length of sausage samples throughout the freezing storage giving good overall acceptability. Flavor scores of G2, G3, G8 and G9 were lower than all other groups from day zero to day 14. This disagreed with **Houtsma (1996)** who reported that SL enhances the meat flavor. However, G2 and G3 had higher sensory scores than other groups near the end of the storage time, meaning that SL had the ability to delay the deterioration in sensory characters of beef sausages during freezing. Even more, SL had not change the taste or odor of sausages even when examined directly after preparation and on the 90th day of storage.

On 90th day of freezing storage at - 18°C, no signs of spoilage appeared on beef sausage even the control groups which could be attributed to freezing process that delay spoilage process. But all sausage groups had very bad sensory evaluations and became not accepted by all committee members on day 97 of storage except G2 and G8 became unacceptable on the 104th day of storage while G3 and G9 on the day 118th. These findings go in the same way with **Massart *et al.* (1986)** who found that sodium lactate inhibit the formation of off-flavors in fresh beef due to its indirect inhibition of autoxidation. Moreover, in the present research yellowish discolorations of the external surface of beef sausage samples in G3 were noticed on the 84th day. However, the current study disagreed with **Brewer *et al.* (1991)** who reported that SL up to 3% does not affect the color of the meat products otherwise, it tended to maintain reddish color, and to enhance tastes in sausage. Besides, the yellowish discolorations in the current study disagreed with **Papadopoulos *et al.* (1991)** who reported that the increase in SL levels from 0-4% in cooked beef roasts resulted in a darker red color with less gray surface of this meat product. Juiciness of all sausage samples reduced throughout freezing storage, due to the surface dehydration **FAO (1986)**. So far, there are no reliable studies on the side effects or cumulative impact of using sodium lactate as a chemical preservative in food.

Effect of chemical and natural preservatives on microbiological properties of sausage samples

Data in table 3 showed that microbial loads of aerobic plate count (APC) decreased gradually with freezing time in all groups, followed a gradual increase in APC on day 14 for G1, G4, G5, G6 and G7 (4.45, 4.54, 4.56, 4.47 and 4.33 $\log_{10}$ CFU/g, respectively), while was on day 42 for G2, G3, G8 and G9 (3.48, 3.41, 3.97 and 3.55 $\log_{10}$ CFU/g, respectively). Table 4 showed that total psychrotrophic count declined steadily with freezing time in all groups in the same way followed by a progressive rise in psychrotrophic count on day 14 for G1, G4, G5, G6 and G7 (3.53, 3.66, 3.72, 3.48 and 3.39 $\log_{10}$ CFU/g, respectively), while was on day 42 for G2, G3, G8 and G9 (2.85, 2.71, 2.61 and 2.56 $\log_{10}$ CFU/g, respectively), meaning that adding SL would delay the rise of microbial load during freezing up to 28 days than other groups with natural casing without treatment. On 90 days of storage in the freezer, the APC of all groups remained within the permissible limits according to the **ES/1972 (2005)** (less than 10^6) but G3 had the lowest TAC (4.33 $\log_{10}$ CFU/g) and psychrotrophic count (4.32 $\log_{10}$ CFU/g). However, G2 and G8 exceeded the permissible limits at 104th day (6.93 and 6.58 $\log_{10}$ CFU/g respectively) of storage (14 days after the end of shelf life), while was on 118th day (28 days after the end of shelf life) for G3 and G9 (6.30 and 6.24 $\log_{10}$ CFU/g, respectively). These results supported those of **Maca *et al.* (1997)** who found that SL (3%) reduced the mesophilic bacteria count and enhanced the cooked roast beef color for beef rounds. In addition, **Lamkey *et al.* (1991)** concluded that the shelf life of sausage was extended by more than two weeks when 3% sodium lactate was added. Moreover, these results were consistent with those of **Marapana *et al.* (2018)** who investigated the impact of freezing conditions on quality of sausages and reported that APC decreased within 3 weeks of freezing and started to increase after 7th week and remain within the accepted level even after 3 months period (Mean value of the slow frozen samples 3.37 x10^4 cfu/g). Furthermore, **Shelef (1994)** found that lactic acid reduced the APC. Our findings disagreed with **Álvarez-Astorga *et al.* (2002)** who recorded high microbial loads in sausages in Spain markets which had total bacterial counts of mesophiles and psychrotrophs from 7.14 to 7.28 and 7.72 to 7.87

log CFU/g, respectively and about 80% of those sausages were deemed unsuitable for consumption. G6 showed lower TAC than G4 and G5 throughout the freezing storage and even on the 90th day (G6=5.55, G4= 5.64 and G5= 5.83 log_{10} CFU/g). This agreed with **Houtsma (1996)** who assumed that the microbial safety of meat products is improved by the addition of nitrite. In addition, **Smith-Palmer *et al.* (2001)** mentioned that 1% thyme or cinnamon had a strong antimicrobial effect.

APC of G9 on 90th day of storage was lower than that of G7 and G8 (5.03, 5.33 and 5.08 log_{10} CFU/g, respectively), but higher than that of G3 (4.33 log_{10} CFU/g) as illustrated in table 3. These observations could be clarified according to **Marapana *et al.* (2018)** who mentioned that temperature fluctuations and frequent freezing/thawing of sausage samples during storage may act as stress factors that are harmful to microorganisms.

Effect of preservatives on chemical properties of sausage samples

The permissible limits of TVB-N and TBA are 20 mg/100g and 0.9 mg malonaldehyde/kg respectively according to the **ES/1972 (2005).** TVB-N and TBA values of G2 became unacceptable on day 104 (21.07 mg/100g and 0.96 mg malonaldehyde/kg respectively) as shown in tables 5 and 6 and the same for G8 (23.77 mg/100g and 0.91 mg malonaldehyde/kg respectively). While those of G3 and G9 become unacceptable on 118th day of storage. These results agreed with **Choi & Chin (2003)** who reported that sausage containing 3.3% SL itself had lower ($P<0.05$) thiobarbituric acid amounts than the control. In addition, **Abd El-Salam (1978)** added that the TVB-N content of frozen meat increased during storage, minimizing the shelf life of sausage made from frozen meat. However, **Brewer *et al.* (1991)** mentioned that TBA was unaffected by SL level.

The results of the current research indicated that natural preservatives as cinnamon and thyme didn't differ significantly than the control sausage samples throughout the storage time. The current study backed up **Sallam & Samejima (2004)** findings, which show that sodium lactate alone can be used to preserve chemical properties, minimize microbial growth, and extend the shelf life.

CONCLUSION

From the current study it is clear that using sodium lactate at 3% as a preservative extends the shelf life of beef sausages better than other chemicals such as nitrite and natural preservatives such as cinnamon and thyme even for 3 weeks after its original shelf life when stored at -18 °C which could be positively reflected on the customer health. The addition of sodium lactate 3% did not change the natural flavor of sausages and those packed in synthetic casing had reasonable microbial quality and chemical values but lower sensory attributes. Thus, it is recommended using sodium lactate 3% as a reliable preservative in the sausage industry. While the addition of cinnamon or thyme improved the sensory traits of sausage for a couple of weeks after freezing and it had antibacterial effect. Eventually, freezing sausage might be a reliable option to hold its microbial quality for up to ninety days allowing a better health significance.

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Authors Contributions

The two authors contributed equally to this work.

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