ELEVATING CONTROL OF PATHOGENIC BACTERIA IN FERMENTED AND NON-FERMENTED SAUSAGE USING LACTIC ACID BACTERIA OR ESSENTIAL OILS

ABSTRACT

In this study, inocula of identified strains of *Staph. aureus*, *Listeria monocytogenes*, *E. coli* O157:H7 and *Salmonella sp* were added individually to manufactured sausage to evaluate the effect of different treatments on behavior of pathogens in fermented and non-fermented sausage. Fermented sausage was inoculated with activated starter containing *Lactococcus lactis* and *Lactobacillus plantarum* while, non-fermented sausage was treated by adding the minimum inhibitory concentration (MIC) of either cumin or marjoram essential oils. Survival of pathogens and microbiological, chemical and organoleptic evaluations were carried out at different intervals during 60 days. Coliform bacteria completely disappeared after 7 days for all starter treatments while it disappeared for all cumin or marjoram treatments after 15 days. Starter treatments had a slight higher proteolytic bacterial count than control while essential oils treatments were lower than control treatments. Both starter and essential oils treatments containing various pathogens recorded the rapid decrement in nitrite content and the lowest increment level of total volatile nitrogen (TVN) as compared to negative or positive control. On the other hand, using lactic acid bacteria or essential oils of cumin and marjoram showed the highest reduction level and the lowest counts of *Staph. aureus*, *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella sp* than positive control. In addition, decreasing of the pathogens counts began on the 3rd day of storage, while positive control of all pathogens showed a slight decrease in number of pathogens and then increased by increasing the storage period. Moreover, starter treatments were more constant treatments and showed maximum organoleptic scores as compared to negative and positive controls while cumin followed by marjoram treatments recorded the lowest decrement rate in overall scores than that of negative and positive controls.

Key words: Pathogenic bacteria, lactic acid bacteria, essential oil, cumin, marjoram, survival of pathogens, organoleptic evaluation

INTRODUCTION

Foodborne pathogens such as diarrheagenic serotypes of *Escherichia coli*, *Staph. aureus*, *Salmonella* and *Listeria monocytogenes* are widely distributed in nature, causing considerable mortality and morbidity in the population (**Indu *et al.*, 2006**). Therefore, there has been increasing concern of the consumers about foods free or with lower level of chemical preservatives because these could be toxic for humans. Also, they have demanded for foods with long shelf-life and absence of risk of causing foodborne diseases. This perspective has put pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternatives to obtain its goals concerning microbial safety (**Souza *et al.*, 2005).**

As a result, there has been great interest and research on naturally produced antimicrobials, such as bacteriocin-producing lactic acid bacteria (LAB). These natural antimicrobials present high potential to be applied in hurdle technology, which utilizes synergy of combined treatments to more effectively preserve foods (**Cleveland *et al.*, 2001**). Furthermore, the antimicrobial compounds produced by LAB play an important role in ensuring the safety and extending shelf-life of sausage (**Rafael and Martinis, 2005**). In addition, many spices, herbs, their extracts and their essential oils are known for their antioxidant and antimicrobial activity against certain foodborne pathogens. Spices are recognized to stabilize the foods front the microbial deterioration. Also,

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when spices show initially high microbial charge and as time progresses, the microbial growth become progressively slower or it is eventually totally suppressed (**Kizil and Sogut, 2003**). Antimicrobial activity of spices depend on several factors, which includes; kind of spice, composition and concentration of spice, microbial species and their occurrence level, substrate composition and processing conditions and storage (**Farag *et al*., 1989**).

Thus, in an effort to improve control of food-related pathogens, this study investigated the use of lactic acid bacteria and addition of spices essential oil to eliminate the sausage health hazard and studying the pathogens behavior in sausage in the presence of essential oils or lactic acid starter.

MATERIALS AND METHODS

Preparation of the pathogenic inocula

Identified strains of *Staph. aureus, Listeria monocytogenes, E. coli* O157:H7 and *Salmonella sp* (isolated from commercial sausage samples and previously identified) were cultivated in nutrient broth medium and incubated at 37°C for 18-24hrs to obtain cells density of about 10^9 cfu/ml. Each inoculum was added to the sausage mixture by insertion of the tip of sterile pipette and well mixed to achieve approximately 10^4 cfu/g **(Uyttendaele *et al*., 2001).**

Preparation of sausage

Sausage was prepared according to the method as described by **Lingnert and Lundgren (1980).** Frozen beef meat was cut into pieces and minced, then spices were powdered, weighed and mixed with minced meat. Fat tissues were also grounded and added to the mixture. Curing agents were dissolved in a small portion of water and added to sausage mixture. Afterwards, the mixture was grounded in order to get a homogenous sausage mixture.

Fermented sausage treatments

Pure culture of both *Lactococcus lactis* subsp. *lactis* (obtained from Food Science Dept., Fac. of Agric., Moshtohor) and *Lactobacillus plantarum* (obtained from Dairy Research Department, National Research Center) were grown in Man Rogosa Sharpe broth at 32°C for 24hrs. Each strain was not effective against the other. Broth activated culture (5%) was inoculated in sterile skim-milk and incubated at 32°C till the skim milk is clotted. Through sausage manufacture, starter culture containing *Lc. lactis* and *L. plantarum* was inoculated to starter treatments at a level of 0.1% (approximately 10^6/g), also 2% glucose was added. Sausage batter was grounded and divided into three portions. The first one was left without either pathogens or starter culture (negative control), while the second was divided into four groups, each one was inoculated with individual inoculum of *Staph. aureus, L. monocytogenes, E. coli* O157:H7 or *Salmonella sp.* (positive control), also, the third portion was inoculated with pathogens as previous and inoculated with starter inoculum. All treatments stuffed into natural mutton casings. Afterwards, sausage was incubated for 3 days at 37°C for fermentation process according to **Zaika *et al*. (1976).**

Essential oils treatments (non-fermented)

The sausage batter was divided into four portions, the first one was left without pathogens or essential oils (negative control), while the second was divided into four groups, each one was inoculated with individual inoculum of *Staph. aureus, L. monocytogenes, E. coli* O157:H7 or *Salmonella sp.* (positive control). The third and the fourth portions were inoculated with pathogens as previous and treated with the minimum inhibitory concentration of cumin or marjoram essential oils, respectively.

The non-fermented and fermented sausage (after fermentation) was cold stored (4-5°C) for 60 days. Samples were taken for analyses from fresh manufactured non-fermented and fermented sausage at initially and at 3, 7, 15, 30 and 60 days after the manufacture.

Microbiological determinations

Coliform group count was determined on violet red bile agar medium according to **British Standards Institution (1991)**. Also, skim milk agar medium was used to determine the proteolytic microorganisms (**Lee and Kraft, 1992)**.

Pathogens enumerations

Baird-Parker agar medium supplemented with Egg Yolk Tellurite was used for direct enumeration of coagulase-positive Staphylococci (*Staph. aureus*) according to **British Standards Institution (1983)**. For enumeration of *L. monocytogenes*, 0.1ml of decimal dilutions plated spread onto oxford agar supplemented with Oxford antimicrobic supplement **(Curtis *et al.*, 1989)**. After incubation at 30°C for 2 days, typical colonies were counted **(Juven *et al.*, 1998).** *Escherichia coli* O157: H7 was determined using MacConkey sorbitol agar supplemented with MUG (4-methylumbellifery-β-D-glucuronide **(Hinkens *et al.*, 1996)**. Typical colonies (which sorbitol negative white colonies) were counted. *Salmonella sp*. was determined on Xylose Lysine Dexolate at 37°C for 24hrs. Typical colonies (colonies that are the same color as the culture medium, translucent, sometimes with black center) were counted **(Skandamis *et al.*, 2002).**

Chemical analysis

Sodium nitrite content and total volatile nitrogen were determined in sausage according to **Deutsche Einheitsverfahren (1960)** and **Winton and Winton (1958)**.

Organoleptic evaluation

Experimental sausage samples were tested for color, flavor, texture and appearance initially and periodically during cold storage. Judges scored flavor and texture on a 7-points hedonic scale, while color and appearance were scored on 5-points, according to the procedure of **Tandler and Rodel (1983)**. The organoleptic parameter scale was divided into five ranges as well as their total scores. These categories were very good (<20-24), good (<16-20), accepted (<10-16), slightly undesirable (<6-10) and spoiled (4-6). Data of organoleptic properties were exposed to proper statistical analysis according to **Snedecor and Cochran (1989).**

RESULTS AND DISCUSSION

Microbiological quality

Coliform counts in fermented (Table, 1) and non-fermented sausage (Table, 2) showed that, in fermented sausage a marked decrease in coliform was shown in all treatments after fermentation. The faster decrement was noticed in starter treatments than that of both negative and positive controls. This may be due to the high acid production and other antimicrobial substances which produced by lactic acid bacteria **(Mukherjee *et al.*, 2006)**. Coliform group disappeared at 7 days in all starter treatments and at 30 days for negative and positive controls of *Staph. aureus* and *L. monocytogenes* treatments. On the other hand, coliform disappeared at 60 days for *E. coli* O157: H7 positive control, but not for positive control of *Salmonella sp.* This may be due to the high initial populations of both *E. coli* and *Salmonella sp* which can grow on coliform medium and fermented lactose, also due to resistant of *E. coli* O157:H7 for low pH, as reported by **Incze (1998)**.

Table (1): Coliform group and proteolytic bacterial counts changes of experimental fermented sausage during 60 days of cold storage (4-5°C).

Treatments	Coliform group ($\times 10^2$ cfu/g)						Proteolytic bacteria ($\log_{10}$ cfu/g)					
	Initial time	3 days	7 days	15 days	30 days	60 days	Initial time	3 days	7 days	15 days	30 days	60 days
Control (negative)	3.57	1.46	0.76	0.23	ND	ND	4.58	5.10	5.51	5.84	6.10	6.27
Control + *Staph. aureus*	2.93	1.52	1.12	0.28	ND	ND	4.62	5.23	5.70	5.88	6.13	6.31
Starter + *Staph. aureus*	2.15	0.34	ND	ND	ND	ND	4.54	5.26	5.77	6.07	6.24	6.10
Control + *L. monocytogenes*	3.76	1.78	0.94	0.31	ND	ND	4.48	5.28	5.77	6.02	6.08	6.23
Starter + *L. monocytogenes*	4.32	0.14	ND	ND	ND	ND	4.57	5.33	5.85	6.09	6.36	6.07
Control + *E. coli* O157:H7	12.18	2.85	1.06	0.62	0.09	ND	4.54	5.29	5.79	5.98	6.08	6.33
Starter + *E. coli* O157:H7	11.96	0.12	ND	ND	ND	ND	4.53	5.38	5.92	6.12	6.34	6.08
Control + *Salmonella sp.*	12.50	3.08	1.97	0.88	0.13	0.041	4.46	5.13	5.47	5.80	6.07	6.26
Starter + *Salmonella sp.*	12.40	0.26	ND	ND	ND	ND	4.49	5.21	5.89	6.12	6.41	6.08

These results are in agreement with those obtained by **Gonulalan *et al.* (2004)** who found that coliforms became undetectable after 3-7 days in experimental sausages made with starter.

Results in Table (2) indicated that, a fair decrease was noticed in coliform counts of all non-fermented sausage treatments with increasing of storage period. Coliform completely disappeared after 15 days for all cumin or marjoram treatments, except for both *E. coli* O157:H7 and *Salmonella sp* treatments as well as negative control, while disappeared after 60 days for positive control treatments except for both *E. coli* O157: H7 and *Salmonella sp* positive control. This may be due to the ability of *E. coli* and *Salmonella sp* to grow onto coliform medium. In spite of the high counts of coliform at initial time, cumin and marjoram had the ability to reduce this group after 30 days; this may be due to their antimicrobial activity. These results are confirmed with those obtained by **Shetty *et al.* (1994)** and **Deans and Svoboda (2006**) who reported that cumin and marjoram oils have strong antimicrobial activities against organisms that frequently occur in food.

Regarding the proteolytic bacteria in fermented sausages, data presented in Table (1) indicated that, proteolytic bacteria were increased by prolonged the storage period up to 60 days. Starter treatments had slight higher proteolytic bacteria than both negative and positive controls up to the 7th day. The end of storage starter treatments recorded sharp decreases than both negative and positive controls with all pathogenic treatments. The increasing of proteolytic group by starter may be due to the ability of starter strains to produce proteases as reported by **Fadda *et al*. (1998)** On the other hand, proteolytic bacteria in non-fermented sausage (Table, 2) increased in all treatments by increasing the storage period. Also, proteolytic bacteria in either cumin or marjoram treatments were lower than that of both negative and positive controls of all pathogenic treatments at most interval periods. Cumin and marjoram treatments had the same trend in the reduction of proteolytic bacterial counts; this may be due to sensitivity of proteolytic microorganisms for essential oils. Although cumin treatments may had lower numbers at all stages. These results are in compatible with those of **Hew *et al*. (2006)**.

Furthermore, fermented sausages had higher proteolytic bacteria than that of essential oils treated one, this may be due to incubation period which increase the initial microbial growth, also, lactic acid bacteria represent the major part of microorganisms in fermented sausage to produce proteolysis.

Chemical changes

Sodium nitrite

Obtained results In Table (3) indicated that, nitrite content in fermented sausage decreased by increasing the cold storage period at 4-5°C up to 60 days. In addition, all starter treatments with various pathogens recorded the rapid decrement and the lowest nitrite levels than both negative control and positive controls. These results are in agreement with those obtained by **Chia and Chin (1995)** who found that residual sodium nitrite in the fermented sausages significantly declined as storage period increased at 3°C. In addition, sausages inoculated with the starter cultures promoted the decomposition of residual sodium nitrite as reported by **Stahnke (1995).**

Non-fermented treatments in Table (4) revealed that, there are no differences in nitrite reduction of both negative and positive controls. While, both cumin or marjoram treatments showed lower nitrite content and maximum nitrite reduction with all pathogenic bacteria during all storage periods and at the end of storage. This may be due to essential oils support of the useful microflora especially lactic acid bacteria which helped in nitrite reduction.

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Table (2): Coliform group and proteolytic bacterial counts changes of experimental non-fermented sausage during 60 days of cold storage (4-5°C).

Treatments	Coliform group ($\times 10^2$ cfu/g)						Proteolytic bacteria ($\log_{10}$ cfu/g)					
	Initial time	3 days	7 days	15 days	30 days	60 days	Initial time	3 days	7 days	15 days	30 days	60 days
Control (negative)	2.76	2.10	0.42	0.25	ND	ND	3.36	4.09	4.29	4.47	4.89	5.10
Control + *Staph. aureus*	2.80	2.50	1.41	0.32	0.17	ND	3.63	4.27	4.52	4.75	5.09	5.44
Cumin + *Staph. aureus*	2.95	0.64	0.12	ND	ND	ND	3.46	3.87	4.15	4.35	4.76	4.95
Marjoram + *Staph. aureus*	3.10	1.45	0.37	ND	ND	ND	3.52	3.90	4.27	4.42	4.84	5.05
Control + *L. monocytogenes*	3.10	2.84	1.74	0.55	0.23	ND	3.52	4.14	4.42	4.64	5.05	5.33
Cumin + *L. monocytogenes*	2.16	0.56	0.43	ND	ND	ND	3.53	4.04	4.18	4.42	4.84	4.97
Marjoram + *L. monocytogenes*	2.54	1.25	0.67	ND	ND	ND	3.51	4.07	4.25	4.46	4.85	5.00
Control + *E. coli* O157:H7	13.85	9.31	3.52	1.13	0.92	0.21	3.54	4.18	4.32	4.62	5.03	5.27
Cumin + *E. coli* O157:H7	12.80	2.67	1.04	0.14	ND	ND	3.51	3.98	4.16	4.44	4.69	4.86
Marjoram + *E. coli* O157:H7	12.68	2.35	0.73	0.15	ND	ND	3.61	4.08	4.26	4.59	4.78	5.01
Control + *Salmonella sp.*	13.04	8.16	3.01	1.12	0.74	0.27	3.57	4.23	4.29	4.87	5.11	5.16
Cumin + *Salmonella sp.*	11.95	1.90	0.64	0.10	ND	ND	3.48	3.94	4.16	4.44	4.70	4.95
Marjoram + *Salmonella sp.*	12.73	1.10	0.52	0.075	ND	ND	3.46	3.97	4.20	4.47	4.74	4.96

Table (3): Sodium nitrite and total volatile nitrogen values changes of experimental fermented sausage during 60days of cold storage at 4-5°C.

Treatments	Sodium nitrite (ppm)						Total volatile nitrogen (TVN) (mg/100g)					
	Initial time	3 days	7 days	15 days	30 days	60 days	Initial time	3 days	7 days	15 days	30 days	60 days
Control (negative)	97.34	54.12	47.36	41.85	38.31	26.20	10.8	15.9	25.9	27.3	36.6	50.1
Control + *Staph. aureus*	96.55	57.75	53.10	42.45	40.14	24.25	10.6	17.1	34.5	39.6	42.1	59.3
Starter + *Staph. aureus*	96.11	45.89	38.84	35.44	24.35	18.25	9.4	13.3	21.5	25.8	29.7	38.5
Control + *L. monocytogenes*	97.36	56.54	49.20	43.77	32.63	22.53	9.8	18.7	34.1	38.5	50.6	61.6
Starter + *L. monocytogenes*	96.19	50.28	41.77	37.23	30.16	20.37	8.5	10.5	20.3	24.4	31.5	37.9
Control + *E. coli* O157:H7	95.93	53.25	52.82	43.27	35.18	25.78	8.1	18.3	33.4	36.8	56.8	67.9
Starter + *E. coli* O157:H7	95.78	43.65	35.75	31.16	22.50	19.50	8.3	9.8	16.1	21.0	29.2	32.4
Control + *Salmonella sp.*	96.21	51.98	50.30	41.36	34.20	27.69	10.6	16.4	28.5	33.7	42.5	52.4
Starter + *Salmonella sp.*	97.48	48.64	42.89	33.55	23.78	17.64	9.7	12.2	18.3	25.5	34.3	43.6

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Table(4): Sodium nitrite and total volatile nitrogen values changes of experimental non-fermented sausage during 60days of cold storage at 4-5°C.

Treatments	Sodium nitrite (ppm)						Total volatile nitrogen (TVN) (mg/100g)					
	Initial time	3 days	7 days	15 days	30 days	60 days	Initial time	3 days	7 days	15 days	30 days	60 days
Control (negative)	95.92	90.34	85.25	74.92	68.41	53.67	8.6	10.3	17.2	22.5	31.7	54.3
Control + *Staph. aureus*	98.16	91.82	78.35	71.67	65.53	52.29	8.4	19.4	25.5	39.1	57.6	81.7
Cumin + *Staph. aureus*	95.66	88.13	73.64	67.66	44.34	34.58	9.3	10.3	17.2	17.1	23.7	35.2
Marjoram + *Staph. aureus*	95.95	80.50	73.41	61.17	38.37	30.67	10.2	11.5	16.7	14.3	21.2	32.6
Control + *L. monocytogenes*	96.34	87.25	71.40	63.27	56.78	31.19	8.5	18.9	23.7	30.4	52.8	78.5
Cumin + *L. monocytogenes*	95.18	75.38	68.54	57.38	37.98	22.25	9.7	11.8	15.4	18.6	23.5	38.6
Marjoram + *L. monocytogenes*	96.79	80.56	69.34	62.54	49.63	26.81	8.8	10.9	16.3	19.7	26.5	41.1
Control + *E. coli* O157:H7	96.84	84.72	76.77	68.84	62.54	47.98	8.1	18.4	25.5	37.7	54.3	107.5
Cumin + *E. coli* O157:H7	97.21	73.58	63.94	55.21	39.75	32.66	11.9	13.3	14.1	14.0	16.9	41.7
Marjoram + *E. coli* O157:H7	96.15	75.25	67.13	61.15	42.36	37.21	9.8	11.6	16.8	21.1	28.5	53.6
Control + *Salmonella sp.*	95.48	91.07	78.30	66.48	61.17	43.65	10.4	20.3	25.4	31.5	62.7	114.1
Cumin + *Salmonella sp.*	95.78	88.23	72.74	65.78	45.73	28.33	9.6	11.7	13.3	16.0	21.5	50.4
Marjoram + *Salmonella sp.*	97.25	90.18	73.21	65.25	46.21	28.51	10.1	12.5	13.6	18.5	30.6	52.7

Also, antioxidant activity of essential oils may support the nitrite reduction to nitrogen oxides. These results are in agreement with those obtained by **Lin *et al.* (1992)**.

Obtained data indicated that nitrite rapidly decreased in fermented sausages and recorded lower levels than that of fresh sausages at all storage periods (except initial time) up to 60 days. This may be due to fermentation period which support the growth of useful microflora especially lactic acid bacteria that promoted the decomposition of nitrite. **(Ck *et al*., 2004)**.

Total volatile nitrogen (TVN)

Results presented in Table (3) showed that TVN of both negative and positive controls of fermented treatments gradually increased with increasing the storage period. Starter treatments also showed an increase in TVN by increasing the storage period, but the increment rate was lower than that of negative and positive controls. This may be due to addition of starter which suppressed the increment of TVN. These results are confirmed by those obtained by **Chia and Chin (1995)** who found that, inoculation with lactic acid bacteria suppressed TVN values.

Concerning the non-fermented sausage, data in Table (4) indicated that negative control treatment showed gradual increase in TVN by increasing the storage period, but the increment rate was lower than that of all positive control treatments which recorded faster increase in TVN content than all treatments. This may be due to high initial microbial load of these treatments which led to the bacterial breakdown associated with the formation of some volatile substances such as ammonia, which caused the rapid development of TVN as mentioned by **El-Kholie (1994).** On the other hand, both cumin or marjoram treatments showed the lowest increment ratio and lowest levels of TVN up to the end of storage. This may be due to its antimicrobial activity that suppressed the microbial growth, subsequently the TVN increment. These results are confirmed by those obtained by **El-Harery (1997)**.

On the other side, TVN of fermented sausage treatments (Table 3) appeared lower levels than which of essential oils treated sausages (Table 4). This may be due to pH decrement by fermentation process, which subsequently inhibited proteolytic enzymes present in meat tissues and developed during growth of microflora.

Survival of pathogenic bacteria

Data presented in Table (5) showed the changes of pathogenic bacteria of fermented sausage treatments. Negative control of fermented sausage contained *Staph. aureus* only while the other tested pathogens were not detected. In addition, the counts of *Staph. aureus* of negative control decreased by fermentation process at 3 days, followed by gradual increase up to the end of storage of fermented sausages. In all positive controls, slight reduction of *Staph. aureus*, *L. monocytogenes*, *E. coli* O157: H7 or *Salmonella sp*. counts was observed immediately after fermentation, this may be due to low pH after fermentation process and cold storage **(Chikthimmah *et al*., 2001)**, then the counts of pathogens increased by increasing the storage period. Furthermore, sharp reduction of all pathogens counts was observed in starter treatment immediately after fermentation by mixed culture of *L. plantarum* and *Lc. lactis* (at 3 days), then a gradual decrease was shown by increasing the storage period up to 60 days. Also, at the end of storage, the counts were very low. This may be due to the presence of bacteriocin and antimicrobial activity of lactic acid bacteria against the tested pathogens. These results are in agreement with those obtained by **Meisel *et al*. (1989), Incze (1998), Tyopponen *et al*. (2003)** and **Rafael and Martinis, (2005)** who found that foodborne pathogens grew to a maximum level in absence of any starter, while starter organisms significantly reduced the growth of pathogens bacteria.

Table (5): Pathogenic bacteria (log_{10} cfu/g) changes of fermented sausage during 60 days of cold storage at 4-5°C.

Treatments	Storage period (days)					
	Initial time	3	7	15	30	60
	Staphylococcus aureus					
Control (negative)	2.72	2.32	3.43	4.90	5.17	5.35
Control + *Staph. aureus*	4.85	3.14	3.92	4.59	5.76	6.14
Starter + *Staph. aureus*	4.95	2.94	2.70	1.48	1.48	0.71
	Listeria monocytogenes					
Control + *L. monocytogenes*	4.60	3.26	4.11	5.40	5.51	5.81
Starter + *L. monocytogenes*	4.86	2.20	2.15	2.08	1.95	1.00
	***Escherichia coli* O157:H7**					
Control + *E. coli* O157:H7	4.70	3.90	4.34	5.08	6.13	6.44
Starter + *E. coli* O157:H7	4.79	2.30	2.10	1.87	1.81	1.56
	Salmonella sp.					
Control + *Salmonella sp.*	4.71	3.57	4.58	5.80	5.87	6.22
Starter + *Salmonella sp.*	4.89	2.72	2.61	2.48	2.34	1.26

**L. monocytogenes, E. coli* O157:H7 and *Salmonella spp*. were not detected in negative control treatment.

Moreover, pathogenic bacteria didn't completely disappear during 60 days of cold storage from both positive control and starter treatments. This may be due to the high initial level occurred. These results are confirmed by those obtained by **Cosanu and Ayhan (2000)** who found that number of *Escherichia coli* O157:H7 in 5.51 log cfu/g decreased to 2.10 log cfu/g at the end of storage period.

Data presented in Table (6) showed the changes in pathogens count of sausage treated with essential oils during 60 days. The obtained results indicated that *L. monocytogenes, E. coli* O157:H7 and *Salmonella sp*. were not detected in negative control treatment (not inoculated), while *Staph. aureus* decreased on the 3rd day of cold storage, then increased by increasing storage period. The same trend was observed in positive controls (that inoculated by *Staph. aureus*, *L. monocytogenes*, *E. coli* O157:H7 or *Salmonella sp*. without essential oils). Decrement of all tested pathogens count on the 3rd day may be due to cold storage effect. On the other hand, essential oils (cumin and marjoram) treatments that contained the tested pathogens showed that, viable counts of all pathogens decreased rapidly after cumin or marjoram oils added up to 3 days, then a gradual decrease up to the end of storage period that recorded the lowest counts of all the tested pathogens. The high decrease in both cumin and marjoram treatments may be due to the antimicrobial effect of essential oils **(Hew *et al.*, 2006).** It could be also, noticed that either cumin or marjoram essential oils reduced the counts of pathogenic bacteria, but the pathogens were not completely inhibited, this may be due to the high initial level of pathogens.

On the other hand, essential oil of cumin had the majority of antibacterial activity against *Staph. aureus* and *L. monocytogenes* than marjoram. On the other hand, marjoram gave maximum reduction of *E. coli* and *Salmonella* treatments than cumin. Also, both *Staph. aureus* and *Listeria monocytogenes* treatments recorded higher bacterial reduction and lower pathogen counts than that of *E. coli* O157: H7 and *Salmonella* treatments. This may be due to high sensitivity of Gram positive bacteria to essential oils than that of Gram negative one as reported by **Kedzia and Ostrowski**

(2003). Also, **Hew *et al*. (2006)** reported that cumin oils possessed very strong antimicrobial activity against *L. monocytogenes, E. coli* O157: H7 and *Salmonella sp*.

Table (6): Pathogenic bacteria (log_{10} cfu/g) changes of non-fermented sausage during 60 days of cold storage at 4-5°C.

Treatments	Storage period (days)					
	Initial time	3	7	15	30	60
	Staphylococcus aureus					
Control (negative)	2.36	2.32	4.76	5.23	5.33	5.67
Control + *Staph. aureus*	4.93	4.38	4.40	5.44	5.62	6.65
Cumin + *Staph. aureus*	4.92	2.94	2.83	1.77	1.15	1.09
Marjoram + *Staph. aureus*	4.95	2.99	2.93	1.84	1.56	1.33
	Listeria monocytogenes					
Control + *L. monocytogenes*	4.95	4.55	4.56	5.62	6.64	6.73
Cumin + *L. monocytogenes*	4.90	2.12	2.11	1.69	1.48	1.18
Marjoram + *L. monocytogenes*	4.85	2.16	2.10	1.86	1.78	1.38
	***Escherichia coli* O157:H7**					
Control + *E. coli* O157:H7	4.32	4.27	4.28	5.32	5.38	6.55
Cumin + *E. coli* O157:H7	4.70	3.57	3.32	3.11	2.95	2.82
Marjoram + *E. coli* O157:H7	4.95	3.26	3.05	2.96	2.79	2.76
	Salmonella sp.					
Control + *Salmonella sp.*	4.81	4.59	4.60	5.62	5.75	6.78
Cumin + *Salmonella sp.*	4.90	3.67	3.49	3.42	3.13	2.94
Marjoram + *Salmonella sp.*	4.88	3.42	3.30	3.27	2.99	2.81

* *L. monocytogenes, E. coli* O157:H7 and *Salmonella spp*. were not detected in negative control treatment.

Organoleptic properties

Data presented in Table (7) showed that, overall scores of the quality characteristics of fermented sausages treatments increased with increasing the storage time, to reach grade of being in "very good" quality grade after 3, 7 and 15 days except of *Staph. aureus* positive control and *L. monocytogens* positive control which showed faster decrease of overall scores than other treatments. However, overall scores of starter treatments were significantly higher than those of other treatments. At the end of storage, starter treatments being in "good" grade, while other treatments were in "accepted" quality grade. These results explain the role of lactic acid bacteria which improve the sensory properties of fermented sausage as reported by **Naes *et al*. (1995)** who found that sensory analysis after 14 days of ripening showed a significant increase in flavor maturity, acidity, better taste and hardness in the presence of starter.

Concerning the overall sensory values of experimental sausages treated with essential oils, Table (8) indicated that, all essential oils treatments were in the "very good" grade (20-24) at the beginning of storage. These scores remained constant after 3 days of storage especially for cumin or marjoram treatments, then slightly decrease was observed after 7 days of cold storage. The scores of all treatments declined to "good" grade (16-20) after 7 days; however cumin or marjoram treatments recorded higher overall scores than other treatments and were significantly differed than other treatments.

Table (7): Sensory evaluation scores changes of experimental fermented sausage during 60 days of cold storage at 4-5°C.

Treatments	Storage period (days)					
	Initial time	3	7	15	30	60
Control (negative)	20	21.90	22.00	22.60	17.00	14.20
Control + *Staph. aureus*	20	21.50	21.00	19.75	15.75	12.00
Starter + *Staph. aureus*	20	24.00	23.75	23.70	20.00	16.25
Control + *L. monocytogenes*	20	21.00	21.40	19.20	15.50	11.25
Starter + *L. monocytogenes*	20	23.20	23.00	22.95	19.00	16.50
Control + *E. coli* O157:H7	20	21.30	21.95	21.70	16.50	13.25
Starter + *E. coli* O157:H7	20	24.00	24.00	23.20	20.75	16.95
Control + *Salmonella sp.*	20	20.80	20.90	21.20	18.00	13.50
Starter + *Salmonella sp.*	20	23.80	23.40	24.00	20.35	15.75
LSD at 5%	0.817	0.794	0.717	0.649	0.877	0.707

The organoleptic parameter scale was very good (<20-24), good (<16-20), accepted (<10-16), slightly undesirable (<6-10) and spoiled (4-6).

In addition, after 15 days of storage overall scores of all pathogenic treatments without essential oils gradually declined but still remained accepted up to 30 days except for both *Staph. aureus* and *Salmonella sp* those were "slightly undesirable" and 'spoiled", respectively. Moreover, after 60 days of storage, all essential oils treatments as well as negative control remained accepted except for marjoram with *Staph. aureus* treatment that became "slightly undesirable". On the other hand, the other treatments (positive controls) were being in "slightly undesirable" and "spoiled" grade. These results are confirmed with the obtained data in TVN values (Table, 4).

Table (8): Sensory evaluation scores changes of experimental non-fermented sausage during 60 days of cold storage at 4-5°C.

Treatments	Storage period (days)					
	Initial time	3	7	15	30	60
Control (negative)	20	19.30	18.00	17.75	14.00	10.90
Control + *Staph. aureus*	20	18.25	17.00	15.75	9.00	6.00
Cumin + *Staph. aureus*	20	20.00	19.00	18.00	14.40	10.60
Marjoram + *Staph. aureus*	20	20.00	19.00	17.00	13.00	9.50
Control + *L. monocytogenes*	20	19.00	17.25	16.00	10.00	6.60
Cumin + *L. monocytogenes*	20	20.00	19.50	18.00	14.50	12.90
Marjoram + *L. monocytogenes*	20	20.00	18.50	17.75	12.50	10.50
Control + *E. coli* O157:H7	20	19.25	18.00	15.00	10.55	7.50
Cumin + *E. coli* O157:H7	20	20.00	18.95	17.00	16.80	11.00
Marjoram + *E. coli* O157:H7	20	20.00	18.95	16.25	14.20	12.00
Control + *Salmonella sp.*	20	18.50	18.25	14.25	4.00	4.00
Cumin + *Salmonella sp.*	20	20.00	19.00	17.25	15.50	12.00
Marjoram + *Salmonella sp.*	20	20.00	18.75	16.50	15.00	10.00
LSD at 5%	0.877	0.538	0.707	0.751	0.825	0.766

The organoleptic parameter scale was very good (<20-24), good (<16-20), accepted (<10-16), slightly undesirable (<6-10) and spoiled (4-6).

In conclusion, results in this study indicated that, nature products such as lactic acid bacteria or effective essential oils of spices play an important role in microbial and chemical quality of sausage. Furthermore, raising control of any pathogen that contaminated sausage is more available by using either lactic acid bacteria or essential oils of spices which led to longer shelf-life of sausage. Starter cause higher overall acceptability than essential oils treatments. Also, there are no "spoiled" or 'slightly undesirable" fermented sausage samples even at the end of cold storage. Therefore, spices essential oils and lactic acid bacteria could be recommended for elevating control of pathogens and betterment sausage quality.

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زيادة التحكم فى البكتريا المرضية الموجودة فى السجق المتخمر وغير المتخمر باستخدام بكتريا حمض اللاكتيك أو الزيوت العطرية

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تم اضافة أربع سلالات من البكتريا المرضية *Staph. aureus, L. monocytogenes, E. coli* O157:H7 و *Salmonella sp.* إلى السجق لدراسة سلوك هذه البكتريا فى السجق المتخمر فى وجود بكتريا حمض اللاكتيك *Lc. Lactis* و *L. plantarum* وكذلك السجق غير المتخمر فى وجود أقل تركيز مثبط من الزيت العطرى للكمون أو البردقوش. وقد تم تقدير الجودة الميكروبية والكيماوية للسجق ولكذلك تتبع أعداد الميكروبات المرضية خلال فترة تخزين السجق، أيضا تم دراسة الصفات الحسية لنوعى السجق.

وقد أظهرت النتائج أن بكتريا القولون قد اختفت كلية بعد ٧ أيام نتيجة المعاملة ببكتريا حمض اللاكتيك ، بينما اختفت بكتريا القولون نتيجة إضافة الزيت العطرى للكمون أو البردقوش بعد ١٥ يوما. أيضا نتيجة إضافة البادئ فى معاملات السجق أدى إلى زيادة أعداد البكتريا المحللة للبروتين عن معاملة المقارنة بينما انخفضت هذه البكتريا نتيجة إضافة الزيت العطرى عن تجربة المقارنة . أما من الناحية الكيماوية فقد أدت المعاملة بكل من البادئ أو الزيوت العطرية إلى انخفاض فى محتوى السجق من النيتريت وكذلك أقل زيادة فى نسبة النيتروجين الكلى المتطاير عن المعاملات المقارنة. وقد اتضح من النتائج أيضا أن استخدام بكتريا حمض اللاكتيك أو الزيوت العطرية للكمون أو البردقوش قد أدى إلى الحصول على أقصى انخفاض فى محتوى السجق من البكتريا المرضية المختبرة . وقد بدأ الإنخفاض بعد ٣ أيام من التخزين واستمر الانخفاض إلى نهاية فترة التخزين ، بينما زادت أعداد البكتريا المرضية فى المعاملات بدون إضافة البادئ أو الزيت العطرى بزيادة مدة التخزين. علاوة على ذلك، كانت معاملات البادئ أكثر ثباتا فى صفات السجق الحسية مقارنة بتجارب المقارنة ، بينما سجلت معاملات الكمون يليه البردقوش أقل انخفاض فى الصفات الحسية عن تلك التى خلت من الزيوت العطرية.