MICROBIAL AND CHEMICAL QUALITY OF RETAILED SAUSAGE AND ANTIMICROBIAL EFFECT OF ESSENTIAL OILS OR LACTIC ACID BACTERIA AGAINST FOODBORNE PATHOGENS

ABSTRACT

Forty five of retailed sausage samples (including fresh, frozen and fermented sausage) were collected from local and central markets of Qalubia, Cairo and Giza Governorates and analyzed for some microbial groups enumeration, pathogens detection and chemical characteristics. Data showed that, all sausage types contained coliform microorganisms where frozen sausage had coliform counts over the permissible limits followed by fresh and fermented sausage. On the other hand, fermented sausage had the highest records of lactic acid bacteria, proteolytic and lipolytic bacteria. All samples were positive for presence of *Staph. aureus* especially fresh sausage which recorded the highest mean counts. *Listeria monocytogenes*, *E. coli* O157:H7 and *Salmonella spp.* were also presented at higher percentage in fresh sausage followed by frozen and fermented ones. Concerning chemical analysis, frozen sausage had the highest values of sodium nitrite and total volatile nitrogen while, the highest mean values of thiobarbaturic acid was observed in fermented one. Furthermore, antimicrobial activity of eight essential oils of spices and two strains of lactic acid bacteria against four strains of foodborne pathogens isolated and identified from the previous three types of sausage was studied. Maximum mean values of inhibition zones of spices against the tested pathogens were obtained by marjoram followed by cumin and mint essential oils. In addition, all tested pathogens were inhibited by either *Lactococcus lactis* or *Lactobacillus plantarum* and their mixture which gave the highest mean values of inhibition zones.

Key words: Sausage, coliform bacteria, *Staph. aureus*, *E.coli* O157:H7, *Salmonella sp*, *L. monocytogenes*, sodium nitrite, spices, lactic acid bacteria.

INTRODUCTON

The need for hygienic meat products has gained importance due to awareness among consumers about health risks associated with contaminated meat. Microbiological quality of either spoilage or food poisoning microorganisms in sausage depends on the meat used for mincing, sanitary conditions and practices in preparation time and temperature of storage (**Mantis *et al.*, 2005**). These factors may cause a major risk for subsequent foodborne infection in human (**Reid *et al.*, 2002**). Many foodborne diseases are associated with sausage consumption that attributed to the presence of pathogenic bacteria such as *Staph. aureus*, *Listeria monocytogenes*, *E.coli* O157:H7 and *Salmonella sp*. which makes sausage had human health hazard. In this respect, **Indu *et al.* (2006)** mentioned that there are more than 1.3 billion cases of human salmonellosis annually, with three million deaths. Also, enterohemorrhagic *E.coli* O157:H7 is implicated in large number of foodborne outbreaks in many parts of the world including developed countries. Moreover, *Listeria monocytogenes* has been isolated from various environments and is reported to cause 25% of all the deaths resulting from foodborne outbreaks in the United States annually. Recent investigations concentrated on finding out means to eliminate the pathogenic bacteria naturally contaminate meat and meat products (**Rafael and Martinis, 2005**). Many

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spices, herbs, their extracts and their essential oils are known for their antimicrobial and antioxidant activity (**Ani *et al.*, 2006**). On the other hand, many species of lactic acid bacteria are known as probiotic organisms those produce antimicrobial substances (**Salim *et al.*, 2006**).

This work concentrated on (I) evaluation of the microbiological and chemical quality of the Egyptian retailed commercial sausages and sausage safety by detection of some pathogenic bacteria that may occur in retailed sausage. (II) evaluation of the antimicrobial activity of both spices essential oils and lactic acid bacteria against contaminant pathogens isolated from the examined sausages.

MATERIAL AND METHODS

Sausage samples

Forty five of retailed sausage samples those collected from supermarkets and butcher shops of three Governorates, namely Qalubia, Cairo and Giza (15 samples from each of them). The samples included three types of sausages namely fresh, frozen and fermented sausage that produced by butcher shops, small factories and main factories in Egypt. Each type of these sausages contained 15 samples derived on three Governorates (5 of each type).

Retailed sausages survey

Microbial groups enumeration, pathogens detection and chemical analyses were carried out of retailed sausages samples that purchased from butcher shops (for fresh type), retailer and supermarkets (for frozen and fermented types). The samples were immediately microbiologically analyzed for microbial populations included coliform group, lactic acid bacteria and counts of proteolytic and lipolytic bacteria. Also, pathogens detection, isolation and identification included *Staphylococcus aureus, Listeria sp*, *E. coli* O157:H7 and *Salmonella spp* were carried out immediately after samples collections. On the other hand, samples were stored under frozen till chemically analyzed. The chemical analysis included pH, residual nitrite content total volatile nitrogen (TVN) and thiobarbaturic acid (TBA).

Microbiological determinations

Enumeration of microbial groups

Coliform group count was determined on Violet Red Bile Agar medium according to **British Standards Institution (1991)**. Lactic acid bacteria were determined on Man Rogosa Sharpe Agar medium. Also, skim milk agar medium was used to determine the proteolytic microorganisms (**Lee and Kraft, 1992)**. While, Lipolytic bacterial counts were determined on Butter oil nutrient agar medium according to **Harrigan and MaCanc (1976).**

Pathogens detection and identification

***Staphylococcus aureus*.**

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 identification, five typical colonies were confirmed their identity using the appropriate biochemical tests according to the method described in **Egyptian standard No. 2364 (1993)** using coagulation test and D-Nase production test.

Listeria monocytogenes

Listeria monocytogenes was detected using pre-enrichment and enrichment (Frtaser broth) method according to **Lovett *et al*. (1987)** on Oxford agar supplemented with Oxford antimicrobic supplement **(Curtis *et al.*, 1989)**. Identification of *Listeria monocytogens* was confirmed according to the method described in **British Standard Institution (1993)**.

***Escherichia coli* O157: H7**

Escherichia coli O157:H7 was detected using pre-enrichment and enrichment broth [EC supplemented with MUG (4-methylumbellifery-β-D-glucuronide)] then spread on MacConkey sorbitol agar supplemented with MUG **(Hinkens *et al*., 1996)**. The typical sorbitol non-fermenting (sorbitol negative) (i.e. white) *E. coli* O157:H7 colonies from MacConkey sorbitol agar plates were biochemically confirmed according to the method described by **Szabo *et al.* (1986**). This strain showed sorbitol non-fermenting and beta glucuronidase negative.

Salmonella spp.

Salmonella spp were detected using pre-enrichment and enrichment (Rappaport Vasiliadis) culture method for the examination of processed animal proteins that described by **Great Britain (1989)**. Suspect colonies of *Salmonella sp.* from Xylose Lysine Dexolate were confirmed according to the method described in **Egyptian Standard No. 2233 (1992)** using biochemical tests.

Chemical analysis

The pH value, nitrite content, total volatile nitrogen and thiobarbituric acid were determined in sausage extract according to **Deutsche Einheitsverfahren (1960)**, **Winton and Winton (1958)** and **Pearson *et al*. (1981)**, respectively.

Determinations of antimicrobial activity

Effect of essential oils (clove, black cumin, garlic, onion, anise, cumin, mint and marjoram) on growth of the isolated pathogenic bacteria (*Staph. aureus, Listeria monocytogenes*, *E. coli* O157: H7 and *Salmonella sp.*) was studied by determination of inhibition zone and minimum inhibitory concentration as described by **Sleigh and Timburg (1981)**.

Lactic acid strains used as antimicrobial agent involved *Lactococcus lactis* sub. *lactis* obtained from Food Science Department, Faculty of Agriculture, Moshtohor. While, *Lactobacillus plantarum* obtained from Dairy Research Department, National Research Center. Each strain was not effective against the other. Both strains were grown in Man Rogosa Sharpe broth, then the cultures were centrifuged at 3000 rpm for 20 min. The clear supernatant of each culture was sterilized by filtration via micro pores filter (pore size 0.22μm). The resultant sterilized filtrate of each culture was tested for its inhibitory effect against isolated pathogens using the diffusion disc assay method (**Hassan *et al.*, 1994**).

RESULTS AND DISCUSSION

Microbiological quality of retailed sausage samples

It is clear from the obtained results (Table, 1) that coliform group counts greatly varied among samples in each type of sausages, also there were great variations from type to another. Fermented sausage had the minimum mean count of coliform bacteria, followed by fresh then the frozen one, which contained the higher count of coliform group. Except fermented type, most of sausage samples had coliform bacterial counts above the permissible limits of Egyptian standard specifications. These results agreed with those obtained by **Rosangela *et al.* (2003)** who reported that frozen sausage contain high counts of coliform. Concerning lactic acid bacteria, it could be noticed from Table (1) that, the highest count of lactic acid bacteria was observed in fermented sausage followed by frozen, while fresh one recorded the minimum average count. This may be attributed to inoculation with lactic acid bacteria starter. **Abd El-Khalek (1990)** observed similar trend of counts of lactic acid bacteria in commercial Egyptian sausage samples.

Regarding the count of proteolytic bacteria, data presented in Table (1) showed that fermented sausage recorded the highest mean count of proteolytic bacteria, while the frozen one showed the lowest mean count. This may be due to incubation period in fermented sausage, which supports the bacterial growth, also due to ability of lactic acid starter to protein degradation as reported by **Fadda *et al.* (2002)**.

Data given in Table (1) also showed that, samples that had the minimum lipolytic counts had also minimum proteolytic bacterial counts. In the same trend, samples with the highest lipolytic bacteria had also the highest counts of proteolytic bacteria. This may be due to the ability of many aerobic proteolytic bacteria to produce lipase as reported by **Pietra *et al.* (2001)**. Generally, fermented sausage had the highest mean count, while the fresh had the lowest one. This may be due to ability of many strains of lactic acid bacteria those used as sausage starter or those developed during incubation period to produce lipase as reported by **Pyrcz *et al.* (2005)**.

Pathogens detection in retailed sausage samples

Staphylococcus aureus

Data presented in Table (2) showed the detection and counts of *Staph. aureus* in retailed sausage samples. It is clear that all retailed sausage samples from three types were positive for the presence of *Staph. aureus*, but the numbers greatly varied from type to another, also among samples in each type. Fresh sausage had the highest mean count, while fermented and frozen sausage had the lowest mean counts. This may be due to the effect of fermentation and high acidity in fermented sausage and may be due to the action of freezing and the added preservatives, probably in high levels to sausage mixture such as nitrite and sodium chloride salt (**Meisel *et al.*, 1989**).

It could be concluded that, occurrence of *Staph. aureus* in all tested samples (100%) may be attributed to the absence of hygienic conditions during manufacturing, processing, storage and retailing of sausage. In addition, sausage can provide a highly nutritious menstruum for the growth of Staphylococci (**Mantis *et al.*, 2005**).

After the identification using the appropriate biochemical tests according to the method described in **Egyptian standard No. 2364 (1993)**, the isolate was *Staphylococcus aureus.*

Table (1): Coliform group, lactic acid bacteria, proteolytic bacteria and lipolytic bacteria counts in retailed sausage samples.

Sample source	Coliform group ($\times 10^4$ cfu/g)			Lactic acid bacteria ($\times 10^4$ cfu/g)			Proteolytic bacteria ($\times 10^3$ cfu/g)			Lipolytic bacteria ($\times 10^3$ cfu/g)		
	Type of sausage											
	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented
Qalubia 1	5.91	8.36	4.08	136.70	46.05	16.60	3.20	3.20	9.40	118.00	116.00	7.15
Qalubia 2	5.29	0.87	7.03	92.30	20.80	2.40	19.10	2.35	4.35	99.30	43.30	48.00
Qalubia 3	2.32	1.73	4.30	22.80	99.20	3.20	11.65	13.15	1.20	54.30	26.65	2.70
Qalubia 4	1.95	252.80	8.40	13.70	130.40	3536.00	20.60	5.25	10.65	100.30	93.00	27.35
Qalubia 5	3.81	374.40	13.20	96.10	78.00	1776.00	20.30	14.15	9.30	69.40	90.65	224.00
Cairo 1	5.49	1.31	6.01	75.40	92.65	189.05	23.95	15.85	14.20	37.80	63.00	114.55
Cairo 2	6.30	5.22	0.99	95.20	36.25	40.10	9.50	12.65	11.10	11.90	32.85	11.25
Cairo 3	5.24	1.99	0.71	6.95	52.55	2197.00	4.20	14.95	9.25	7.30	83.00	5.40
Cairo 4	9.15	173.80	5.08	3.40	26.80	26.70	5.60	21.45	4.15	22.80	184.00	27.35
Cairo 5	7.41	72.10	9.10	17.85	110.30	1554.00	18.90	5.40	31.15	34.90	119.00	8.10
Giza 1	5.03	3.12	0.36	51.75	49.60	1413.00	60.30	8.70	29.15	189.00	72.00	131.00
Giza 2	6.86	182.10	1.17	51.90	4.05	306.65	23.60	14.65	59.00	59.10	31.20	214.00
Giza 3	9.77	155.80	0.15	4.05	12.15	983.00	19.75	1.10	39.35	97.40	7.95	297.00
Giza 4	13.97	0.57	5.02	6.10	20.30	15.75	36.85	7.35	10.65	11.05	99.00	102.05
Giza 5	6.79	1.25	2.28	11.50	103.30	31.95	2.55	9.00	126.00	5.80	114.00	385.00
Mean	6.35	82.36	4.52	45.71	58.83	806.09	18.67	9.95	24.59	61.22	78.37	106.99

Table (2): Detection of *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella spp.* in retailed sausage samples.

Sample source	*Staphylococcus aureus* ($\times 10^3$ cfu/g)			*Listeria monocytogenes*			*Escherichia coli* O157:H7			*Salmonella spp.*		
	Type of sausage											
	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented
Qalubia 1	10.27	1.93	0.05	+	+	+	+	+	ND	ND	ND	ND
Qalubia 2	0.86	0.14	1.12	+	ND	+	+	ND	ND	+	+	ND
Qalubia 3	3.46	0.53	2.94	+	+	+	ND	+	+	ND	ND	ND
Qalubia 4	2.28	0.42	0.58	+	+	+	ND	ND	ND	+	+	ND
Qalubia 5	4.66	1.76	1.34	+	+	+	+	+	+	ND	+	ND
Cairo 1	1.86	3.41	1.24	+	+	+	ND	+	ND	+	ND	+
Cairo 2	4.66	0.10	0.11	+	ND	ND	+	ND	+	+	+	ND
Cairo 3	5.64	0.56	0.14	+	+	+	+	+	+	ND	ND	ND
Cairo 4	8.24	1.21	1.12	+	+	ND	+	ND	ND	ND	ND	+
Cairo 5	11.28	0.47	1.36	ND	+	+	+	ND	+	+	+	ND
Giza 1	0.56	0.71	0.23	ND	+	+	ND	+	ND	ND	ND	ND
Giza 2	4.26	1.36	0.14	+	+	ND	+	+	ND	+	+	ND
Giza 3	1.14	0.93	0.39	+	+	+	ND	ND	+	ND	ND	+
Giza 4	0.30	0.27	0.64	+	ND	+	ND	ND	+	+	ND	ND
Giza 5	0.70	0.44	0.03	+	+	ND	+	+	ND	ND	ND	ND
Mean	4.01	0.95	0.76	86.6%	80.0%	73.33%	60.0%	53.3%	46.7%	46.7%	40.0%	20.0%

ND: Not detected

Listeria sp.

Detection of *Listeria sp* in retailed sausage samples is presented in Table (2). Results showed that presence of *Listeria sp* was high in the three types; the fresh sausage had the highest level, while the fermented sausage had the lowest one. Occurrence of *Listeria sp* in about 80% of 45 retailed sausage samples (from three types) indicated the absence of hygienic conditions during manufacturing and absence of exacting quality control in many factories. These results are in the same trend of **Metaxopoulos *et al.* (2001)**. On the other hand, fermented sausage had the lowest percentage of *Listeria sp*. This may be attributed to inoculation with starter culture, which produced high acidity and bacteriocin and consequently inhibited the bacterial growth.

The isolate was identified as *listeria monocytogenes* according to the method described in **British Standard Institution (1993)**.

***Escherichia coli* O157:H7.**

Data in Table (2) showed the *E. coli* O157:H7 detection in retailed sausage samples. Results indicated that, *E. coli* O157:H7 was presented in commercial sausage samples at rates of 60%, 53.33% and 46.66% for fresh, frozen and fermented sausage, respectively. In general, *E. coli* O157:H7 occurred in 24 of 45 samples from three types (53.33%). Presence of *E. coli* O157:H7 at these high percentages due to inadequate hygienic quality of raw materials and absence of exacting quality control during manufacturing and handling as reported by **Lopez *et al.* (2000)**. In addition, data revealed that fermented sausage had the lowest *E. coli* O157:H7 detection while fresh sausage had the highest one. Also, little percentage which differed between fermented sausage and other two types may be due to the resistance of enterohaemorrhagic *E. coli* including O157:H7 to low pH as reported by **Incze (1998)**. The obtained isolate was biochemically confirmed as *E. coli* O157:H7 according to the method described by **Szabo *et al.* (1986)**.

Salmonella spp.

Data in Table (2) also revealed that, 46.6% of fresh sausage and 40% of frozen sausage samples were contaminated with *Salmonella spp.* Results also indicated that, fermented sausage had the lowest contamination level, since 20% of the samples were positive for *Salmonella* detection. This may be due to the effect of reduction of pH by fermentation, which caused growth to be influence by the temperature of incubation and by the presence of sodium chloride and nitrite salts. These results are in compatible with those obtained by **Soultos *et al.* (2003**).

Suspect isolate was confirmed as *Salmonella sp.* according to the method described in **Egyptian Standard No. 2233 (1992)** using biochemical tests.

Chemical quality of retailed sausage samples

Data presented in Table (3) indicated that the fermented sausage recorded the lowest pH mean value (4.85), while both fresh and frozen sausage had pH mean values 6.52 and 6.51, respectively. This may be due to addition of starter and incubation period in fermented sausage, which increased acidity, and consequently decreased pH. There are a great of variations among fermented sausage pH values. This may be due to inoculum size, used strains, incubation period, longevity and available carbohydrate sources as reported by **Vignolo *et al.* (1989)**.

Table (3): Chemical quality of different types of retailed sausage samples collected from Egyptian markets.

Sample source	pH			Sodium nitrite (ppm)			Total volatile nitrogen (mg/100g)			Thiobarbaturic acid (mg/100g)		
	Type of sausage											
	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented	Fresh	Frozen	Fermented
Qalubia 1	7.33	7.13	5.43	0.0	159.2	112.3	7.00	19.60	16.80	0.366	0.678	1.120
Qalubia 2	6.40	6.22	4.97	0.0	205.3	97.3	13.10	20.15	31.30	0.387	0.858	0.970
Qalubia 3	6.37	6.14	4.78	0.0	156.4	53.3	8.72	19.75	20.40	0.482	1.053	0.817
Qalubia 4	6.54	6.38	4.32	280.9	231.5	30.2	9.19	25.38	25.31	0.501	0.575	0.765
Qalubia 5	6.23	6.21	4.07	0.0	336.1	43.7	10.65	30.40	12.55	0.321	0.798	0.983
Cairo 1	6.87	6.58	5.21	0.0	232.2	33.4	8.43	33.35	19.38	0.485	0.974	1.019
Cairo 2	6.14	6.44	4.83	0.0	219.2	18.4	7.25	34.70	19.53	0.433	0.745	1.130
Cairo 3	7.14	5.99	5.30	0.0	198.3	31.4	9.34	32.40	22.23	0.317	0.633	0.975
Cairo 4	5.97	6.28	5.65	331.4	225.6	56.5	10.13	31.70	17.41	0.335	0.515	0.675
Cairo 5	6.18	6.19	5.47	0.0	187.4	63.0	12.52	37.50	30.14	0.257	0.421	0.858
Giza 1	6.21	6.77	4.99	0.0	356.6	30.0	9.68	39.17	33.15	0.375	0.677	1.056
Giza 2	6.38	6.83	4.38	0.0	313.5	34.8	8.87	30.00	32.58	0.455	0.814	0.866
Giza 3	7.03	7.06	4.17	0.0	202.1	103.5	8.01	31.20	28.20	0.576	0.622	0.973
Giza 4	7.10	7.31	4.55	0.0	176.3	84.4	7.22	33.10	19.07	0.500	0.654	0.737
Giza 5	5.84	6.16	4.71	187.2	255.6	21.2	7.89	39.30	13.18	0.475	0.750	0.516
Mean	6.52	6.51	4.86	53.3	228.4	54.2	9.20	30.51	22.75	0.417	0.718	0.987

Sodium nitrite was not detected in fresh sausage except for 3 of 15 samples (20%). While frozen sausage had higher sodium nitrite content, this may be due to using of low quality raw materials by the manufacturers. In addition, fermented sausage had the lowest average level of nitrite. This may be due to nitrite reduction during fermentation as reported by **Chia and Chin (1995)** who found that residual sodium nitrite content of sausage inoculated with lactic acid bacteria significantly declined. In addition, they stated that starter cultures promoted the decomposition of residual sodium nitrite. Sodium nitrite was used at high levels, which exceeded those of Egyptian standard specifications in 100% of frozen sausage samples. Furthermore, only fermented sausage contained sodium nitrite at permissible limits. These results are in agreement with those observed by **Habbal (2000)**.

Concerning total volatile nitrogen, the lowest values were observed in fresh sausage, this might be due to freshness of used animal materials, while frozen samples had the highest TVN values than other two types. In addition, fermented sausage had the moderate values between fresh and frozen. This may described as low values, although fermented sausage had the higher proteolytic bacterial count as shown in Table (1). This may be as a result of inoculation with starter, which formed organic acid that decreased the pH value of sausage and subsequently inhibited the proteolytic enzymes present in meat tissues as mentioned by **El-Deep (1987)**.

On the other hand, fresh sausage had the lowest values of TBA, followed by frozen sausage, while the highest mean value of TBA was observed in fermented sausage samples. This may be attributed to high load of lipolytic bacterial counts as shown in Table (1). Moreover, the lowest nitrite content in fermented sausage which support the lipid oxidation as reported by **Salem *et al.* (1983)** who found that the higher level of added nitrate and residual nitrite, the lower TBA values.

Antimicrobial activity of essential oils and lactic acid bacteria

Isolated pathogenic bacteria, which identified as *Staph. aureus, Listeria monocytogenes, E. coli* O157:H7 and *Salmonella sp*. were used in this part. The antimicrobial activity of essential oils of some spices and lactic acid bacteria were assessed to use them as natural preservatives.

Antimicrobial activity of spices essential oils

Data of preliminary screening of antimicrobial activity of the eight essential oils against isolated pathogenic bacteria were presented in Table (4).

Obtained results revealed that all tested essential oils inhibited *Staph. aureus* and gave different values of inhibition zone. The minimum inhibition zone recorded by garlic oil, while the maximum one was recorded by mint and marjoram followed by cumin oil. On the other hand, cumin oil showed the highest antimicrobial activity against *Listeria monocytogenes*, followed by mint oil, then marjoram while both garlic and onion oils showed the lowest ones.

Table (4): Antimicrobial activity of spices and herbs essential oils against some pathogenic bacteria isolated from retailed sausage samples.

Essential oils	Tested pathogens				
	Staph. aureus	*Listeria*	*E. coli* O157:H7	*Salmonella*	Mean
	Inhibition zone (mm)*				
Control	--	--	--	--	--
Clove	10.00	9.07	10.67	10.13	9.97
Black Cumin	13.67	17.93	10.83	12.67	13.78
Garlic	9.67	8.20	8.50	12.33	9.68
Onion	16.66	8.20	13.50	12.20	12.64
Cumin	19.50	31.50	13.00	17.67	20.42
Anise	13.27	14.67	9.00	7.80	11.18
Mint	32.00	23.34	11.50	13.00	19.96
Marjoram	31.66	19.33	19.33	14.67	21.25

* Paper disc diameter = 6mm

Regarding the effect of tested essential oils on *E. coli* O157:H7, data presented in Table (4) showed that, all essential oils were active against *E. coli* O157:H7. Garlic oil showed the lowest inhibition zone, while marjoram oil showed the highest one, followed by onion and cumin. Moreover, *Salmonella sp* was inhibited with all essential oils. The maximum inhibition zone was recorded by cumin oil followed by marjoram.

From overall activity, data presented in Table (4) emphasized that, maximum values of inhibition zones against all tested pathogenic bacteria were obtained by marjoram, cumin and mint. Therefore, these essential oils were tested for their minimum inhibitory concentration. These results are in agreement with those obtained by **Ani *et al.* (2006)** who found that cumin and various spices exhibited antimicrobial activity against various species of Gram positive and Gram negative bacteria.

Minimum inhibitory concentration of most potent essential oils

Most potent essential oils (marjoram, cumin and mint), that exhibited the maximum antimicrobial activity against pathogenic strains were tested to their minimum inhibitory concentration (MIC). Data presented in Table (5) showed that both *Staph. aureus* and *Listeria monocytogenes* were inhibited at 500ppm with all most potent essential oils. While *E. coli* O157:H7 was inhibited at 3000ppm with all essential oils. Furthermore, *Salmonella sp* was inhibited at 1500, 2000 and 1500ppm of cumin, mint and marjoram oils, respectively.

It could be concluded that, *Staph. aureus* and *L. monocytogenes* had minimum inhibitory concentration less than that of *E. coli* O157:H7 and *Salmonella sp*. These results are agreed with those obtained by **Farag *et al.* (1989)** who reported that Gram positive bacteria were more sensitive to the antimicrobial compounds in spices than Gram negative. In addition, they reported that very low concentration (0.25-12 mg/ml) of the various essential oils were sufficient to prevent microbial growth.

Table (5): **Minimum inhibitory concentration (ppm) of selected essential oils of spices and herbs against some pathogenic bacteria.**

Essential oils	Tested pathogens			
	Staph. aureus	***L. mono-cytogenes***	***E. coli*** O157:H7	***Salmonella sp.***
	Minimum inhibitory concentration (ppm)			
Marjoram	500	500	3000	1500
Cumin	500	500	3000	1500
Mint	500	500	3000	2000

Antimicrobial activity of lactic acid bacteria

Many strains of lactic acid bacteria had the ability to produce bacteriocins and had an antimicrobial activity against many species of bacteria as reported by many investigators (**Rafael and Martinis, 2005** and **Salim *et al.*, 2006)**. Therefore, the ability of lactic acid bacteria strains (*Lactococcus lactis* and *Lactobacillus plantarum*) which used as starter culture to inhibit the pathogenic bacteria was studied.

Data recorded in Table (6) showed that both *Lactococcus lactis* and/or *Lactobacillus plantarum* inhibited all tested pathogenic bacteria. *L. plantarum* strain recorded higher inhibition effect compared to *Lactococcus lactis*. In addition, mixed culture gave the highest inhibition effect on *Staph. aureus* and *E. coli* O157:H7 than individually strain, while *L. plantarum* gave the highest inhibition zone values on *L. monocytogenes* and *Salmonella sp* than mixed culture. In addition, *L. plantarum* had the strongest antimicrobial activity than *Lc. lactis*. While, the mixed culture recorded the maximum mean values of inhibition zone.

Table (6): **Antimicrobial activity of lactic acid bacteria against some pathogens.**

Lactic acid bacteria	Tested pathogens				
	Staph. aureus	***L. mono-cytogens***	***E. coli* O157:H7**	***Salmonella sp***	**Mean**
	Inhibition zone (mm)*				
Control	--	--	--	--	--
Lactococcus lactis	8.50	10.00	9.50	9.50	9.375
Lactobacillus plantarum	9.50	12.25	12.00	10.50	11.06
Mixed culture	10.50	11.50	14.00	9.00	11.25

* Paper disc diameter = 6mm

The antimicrobial activity of lactic acid bacteria may be attributed to various factors including the production of bacteriocins and bacteriocin-like substances. Furthermore, *Lactobacillus plantarum* exhibited the highest acid production. The combination of organic acids with bacteriocin enhanced its activity against pathogenic bacteria (**Scannell *et al.*, 2000**). These results are in harmony with those obtained by **Rafael and Martinis (2005)** and **Salim *et al.* (2006)** who found that the *Lactobacillus plantarum* and *Lactococcus lactis* were very potent against some pathogenic bacteria.

In conclusion, results of this study indicated that high percentage of foodborne pathogens in all samples may attributed to absence of hygienic conditions, absence of

quality control and multi contamination sources during sausage manufacturing and sale. Furthermore, retailed Egyptian sausage might pose a potential health hazard, making it imperative to institute sanitary measures during its production and sale. Therefore, the natural products such as spices essential oils and lactic acid bacteria could be recommended, since they play an important role in control of foodborne pathogens.

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الجودة الميكروبية والكيماوية للسجق المعروض بالأسواق والأثر المضاد للزيوت العطرية وبكتريا حمض اللاكتيك على الميكروبات المرضية الموجودة فى السجق

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تم تجميع ٤٥ عينة سجق (طازج – مجمد – متخمر) من المعروض بالأسواق فى محافظات القليوبية والقاهرة والجيزة وذلك لتحليلها ميكروبيولوجيا وكيماويا والكشف عن وجود أو غياب بعض الميكروبات المرضية. وقد أظهرت النتائج أن كل أنواع السجق احتوت على بكتريا القولون إلا أن السجق المجمد زاد محتواه من بكتريا القولون عن المسموح به تلاه فى ذلك السجق الطازج. وقد وجد أيضا أن السجق المتخمر احتوى على أعلى قيم لمتوسط أعداد بكتريا حمض اللاكتيك وكذلك البكتريا المحللة للبروتين والمحللة للدهون.

أما بالنسبة للميكروبات المرضية فقد أوضحت النتائج أنه لم تخلو أى عينة من الميكروبات العنقودية الذهبية *Staph. aureus* وقد سجل السجق الطازج أعلى قيمة لمتوسط أعداد هذه الميكروبات ، كما احتوى السجق الطازج أيضا على أعلى معدلات من بكتريا *Listeria monocytogenes, E. coli* O157:H7 , *Salmonella spp.* تلاه السجق المجمد أما السجق المتخمرفقد سجل أقل أعداد للبكتريا المرضية. على الجانب الآخر، كان السجق المجمد أعلى الأنواع فى محتواه من النيتريت والنتروجين الكلى المتطاير، بينما سجل السجق المتخمر أعلى محتوى من حمض الثيوباربتيوريك.

ولقد تم اختبار النشاط المضاد لثمانية أنواع من الزيوت العطرية وسلالتين من بكتريا حمض اللاكتيك ضد البكتريا المرضية المعزولة من عينات السجق المختبر. وقد اتضح من النتائج أن زيت البردقوش سجل أعلى متوسط لقيم النشاط التضادى للزيوت العطرية ضد الميكروبات المرضية المختبرة تلاه فى ذلك الكمون ثم النعناع، ثم اختبرت هذه الأنواع الثلاثة للحصول على أقل تركيز مثبط منها. وقد أظهرت النتائج أيضا أن كل الميكروبات المرضية المختبرة قد تم تثبيطها سواء باستخدام بكتريا *Lactococcus lactis* أو *Lactobacillus plantarum* وكذلك المزرعة المختلطة منهما والتى سجلت أعلى نشاط تضادى ضد الميكروبات المختبرة.