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Microbiological Changes in Chicken Burger Formulated With Some Spices and Herbs During Frozen Storage.

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Abstract:

This study was conducted to test the effect of microbial inhibitor of spices and herbs commonly added to the foods. Spices and herbs were used in this study namely: thyme, rosemary, sage, marjoram and black seeds to study the effect of these spices on the growth of aerobic plate counts, psychrotrophic bacteria, proteolytic bacteria, lipolytic bacteria, yeasts and molds as well as one type of bacteria causing food poisoning, (*Staphylococcus aureus*) as well as coliform group in order to preserve and improve the microbial quality of chicken burger. Two concentrations of these spices (0.5 and 1%) were employed in chicken burger stored at -18˚C for 6 months. Study indicated that the number of microorganisms decreased with increasing duration of frozen storage in all treated samples compared to that of control. The study revealed that the addition of spices and herbs at level 1% led to reduce the number of microorganisms as much more than the addition at 0.5%. Likewise, the results showed that thyme was the most effective herb on reduction of microorganism followed by sage, rosemary and then marjoram, while black seeds gave less inhibitory effect. Generally, all treatments of chicken burger as well as control were acceptable microbiologically by the end of storage period recording less than 10^5 cell/gm.

Keywords: microbial inhibitor, chicken burger, thyme, sage, rosemary, marjoram, black seeds.

Introduction:

Chicken meat and its products had experienced increasing popularity and became widely spread all over the world (Barbut, 2001). However during storage, quality attributes of the product deteriorated due to lipid oxidation and microbial growth. Microbial contamination can precipitate major public health hazards and economic loss in terms of food poisoning and meat spoilage. Thus, application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss (Yin & Cheng, 2003). Strong consumer demand for safe and high-quality

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foods can be attributed in part to the wide spread availability and accessibility of quality health data and information. There are also new concerns about food safety due to increasing occurrence of new food-borne disease outbreaks caused by pathogenic micro-organisms. This raises considerable challenges, particularly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit growth of spoilage and pathogenic micro-organisms (Brandi *et al.*, 2006; Aslim & Yucel 2007 and Arques *et al.*, 2008). Food processing technologies such as chemical preservatives cannot eliminate food pathogens or delay microbial spoilage totally (Gutierrez *et al.*, 2009).

However, consumers are concerned about the safety of synthetic food additives. This concern had led to arouse a great interest in natural additives (Pokorny, 1991). Natural agents possessing antioxidant and antimicrobial properties had the advantage of being readily accepted by consumers, as they are considered natural. As a consequence, natural antimicrobials were receiving a good deal of attention for a number of microorganism-control issues. Reducing the need for antibiotics, controlling microbial contamination in food, improving shelf-life extension technologies to eliminate undesirable pathogens and/or delay microbial spoilage, decreasing the development of antibiotic resistance by pathogenic microorganisms or strengthening immune cells in humans are some of the benefits (Gaysinsky & Weiss, 2007; Abou-taleb & Kawai, 2008 and Fisher & Phillips, 2008).

Antimicrobial agents are used in food for two main reasons: (1) to control natural spoilage processes (food preservation), and (2) to prevent/control growth of microorganisms, including pathogenic micro-organisms (food safety) (Davidson, 2006; Gaysinsky & Weiss, 2007; Patrignani *et al.*, 2008). Spices, herbs and their essential oils (EOs) are used in the food industry as natural agents for extending the shelf life of foods. A variety of plant- and spice-based antimicrobials is used for reducing or eliminating pathogenic bacteria, and increasing the overall quality of food products (Holley & Patel, 2005; Silva et al., 2007 and Arques et al., 2008). The objective of the present study was to investigate the effect of the antimicrobial activity of some spices and herbs in increasing the shelf-life of chicken burger freeze stored at -18ºC up to 6 months.

Materials and Methods

1-Materials

1-Chicken meat:

10 kg of fresh chicken meat from broiler carcasses (7-8 weeks age with an average weight 1.5-2 kg) were obtained from El-Borssa Company for Poultry at February

2010. On receipt at the laboratory, they were washed carefully then deboned within two hours of slaughtering, the chicken meat was minced using a meat mincer and then chilled at 4±1ºC for 24hours before using in processing of chicken burgers.

2-Selection of spices and herbs:

Selected spices and herbs were used in chicken burger formula namely thyme (*thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), black seeds (*Nigella sativa* L.), sage (*Salvia officinalis*), and marjoram (*Origanum majoranum)*, were obtain from the Agricultural Research Center, Giza, Egypt.

2-Methods

1-Preparation of chicken burger:

Fresh chicken burger samples were prepared as described by Mikkelsen (1993). All ingredients were minced twice, after mincing, the chicken mixture was shaped manually using a patty marker (stainless steel model "Form") to obtain round discs 10 cm diameter and 0.5 cm thickness. Burgers were packaged in polyethylene bags (in foam dishes).

The Basal constituents of chicken burger were prepared as follows:

The chilled minced chicken meat formula included fat 71.5%, fresh onion (finely ground) 7.0%, whole egg (blended) 5.0%, bread crust powder 5.0%, rehydrated extruded soy 10.0% and sodium chloride 1.50%. These ingredients were mixed together, divided to six equal portions, the first portion was remained without any addition (control) and the five reminder portions were individually mixed with two concentrations of each spices and herbs (0.5% and 1%) to give five treatments . All burgers treatments and control were freeze stored at -18±2ºC up to 6 months.

2-Microbiological examinations:

a-Sample preparation:

Ten grams of representative chicken burger sample were mixed with 90 ml of sterile saline solution (9 gm NaCl/1L distilled water) in a blender, under sterile conditions, to give 1/10 dilution. Serial dilutions were prepared for counting total aerobic bacteria, psychrotrophic counts, coliform group, lipolytic bacteria, proteolytic bacteria, *Staphylococcus aureus*, yeast and molds (Osheba, 1998). The tested samples were conducted at zero time, 1, 2, 3, 4, 5 and 6 months of frozen storage at -18˚C±2.

b- Microbiological methods:

1- Aerobic plate counts and psychrotrophic bacteria:

The aerobic plate counts was determined using nutrient agar medium according to the procedure as described by A.P.H.A (1976) and Osheba (1998). The plates were incubated at 30ºC for 3 days, while psychrotrophic bacteria was determined as recommended by A.P.H.A (1976) and Difco Manual (1998). The plates were incubated at 8ºC for 5 days.

2-Proteolytic bacterial counts:

The proteolytic bacterial was determined using milk agar medium according to the method described by Difco Manual (1998). The plates were incubated at 30ºC for 72 hrs.

3-Lipolytic bacterial counts:

The lipolytic bacterial was determined using butter fat agar medium according to the method described by Marshall (1992). The plates were incubated at 25-30ºC for 10 days.

4-Yeast and mold counts:

Yeasts and molds was determined using YM agar medium according to the method described by Difco Manual (1998). The plates were incubated at 30±2ºC for 18-72 hrs.

5- Coliform bacteria counts:

The coilform bacteria were determined using Machonkey agar medium according to the method described by Difco Manual (1998). The plates were incubated at 37ºC for 24-48 hrs.

6-*Staphylococcus aureus* counts:

Staphylococcus aureus bacteria was determined using Mannitol salt agar medium according to the method described by Difco Manual (1998). The plates were incubated at 35±2ºC for 18-24 to 48 hrs.

Resutls and Discussion

1-Aerobic plate counts (A.P.C):

The result given in Table (1) showed aerobic plate counts (A.P.C) in different chicken burger treatments, compared with control during frozen storage at -18ºC up to 6 months. Data indicated that, aerobic plate counts gradually decreased after and throughout the storage period in all spiced samples but with variable degrees.

Table (1): Aerobic plate counts in chicken burger formulated with some spices and herbs (0.5 and 1%) during freeze storage period at -18ºC. (x 10^4 CFU/g)*

Storage period (months)	Control	Thyme		Sage		Rosemary		Marjoram		Black seeds	
		0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
0	184.00	148.00	128.00	150.00	130.00	159.00	131.50	162.00	140.00	163.00	142.50
1	155.50	106.00	87.00	110.00	89.00	116.00	90.00	117.50	98.50	120.00	101.00
2	110.00	76.00	61.00	77.00	65.00	80.00	69.00	83.50	72.50	108.00	75.00
3	84.50	62.00	52.00	63.00	57.00	67.00	60.00	70.00	66.00	77.00	68.00
4	60.50	45.00	43.00	47.00	44.50	49.00	46.00	52.00	50.00	54.00	51.00
5	47.00	35.00	31.00	36.00	32.00	37.50	34.00	39.00	36.00	41.00	38.00
6	39.00	27.00	25.50	28.50	26.50	30.00	27.50	31.50	29.00	33.00	30.00

*CFU = Colony Form Unit.

Furthermore, addition of 0.5% of spices or herbs to chicken burger formula caused a decrease in aerobic plate counts after storage

for 6 months at -18ºC compared with the control sample. Moreover, addition of spices or herbs at level 1% caused more reduction in aerobic plate counts in fresh chicken burger compared with chicken burger formulated with spices and herbs at level 0.5% and the control sample. This might be attributed to the antimicrobial effect of more essential oils of spices and herbs at high levels. These results agreed with those reported by Sirnik and Gorisek (1983), Deans and Ritchie (1987), and Badei et al., (1991).

Generally, it could be noticed that thyme showed a stronger inhibitory effect among the used spices and herbs on microbial growth. The effectiveness of inhibitories followed the sequence:

Thyme > Sage > Rosemary > Marjoram >Black seeds.

These results are on line with Ting and Deibel (1992), Henfnawy et al., (1993) and Pandit and Shelef (1994), who studied the effect of spices and their essential oils for inhibiting the growth of *lesteria monocytogenes* in food. Jamaican pepper, cloves, cumin, garlic powder, cinnamon, oregano, sage, thyme, paprika, red and black pepper and rosemary in addition to the essential oils of fir and pine trees gave good results in terms of their capability of reducing the numbers of these microorganisms.

However, frozen storage had a strong effect on the destruction of microbial cells which led to death of some cells. Amar et al., (1988) studied the effect of freezing at -20ºC and -30ºC for 6 months on the microbial counts of cow meat and found that the number of microorganisms markedly and progressively decreased upon prolonged freezing storage. It is notable that, all studied samples by the end of freezing storage (after 6 months) were microbiologically acceptable according to E.O.S. (1991) as the total aerobic count (A.P.C) was less than 10^5CFU/gm.

2-Coliform group counts:

The most important organism of this group is *E.coli* which is basically related to gastroenteritis symptoms, especially diarrhea, beside its importance in affecting the sanitary quality of minced meat either raw or frozen Cruickshamk et al., (1975).

Data presented in Table (2) indicated coliform group counts in untreated and treated chicken burger. Results showed that the same trend of aerobic plate count (A.P.C) occurred in the coliform group count.

Table (2): Coliform group counts in chicken burger formulated with some spices and herbs (0.5 and 1%) during freeze storage period at -18ºC. (x 10^4 CFU/g)*

Storage period (months)	Control	Thyme		Sage		Rosemary		Marjoram		Black seeds	
		0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
0	7.000	4.000	3.500	5.000	4.000	5.500	4.500	6.000	5.500	6.500	6.000
1	5.500	2.500	1.500	3.000	2.000	3.500	2.500	4.000	3.000	4.500	3.500
2	4.000	1.000	0.500	1.500	1.000	2.000	1.500	2.500	2.000	3.500	2.500
3	2.500	0.150	0.100	0.500	0.150	1.000	0.200	1.500	0.500	2.000	1.000
4	2.000	0.100	0.050	0.150	0.100	0.500	0.150	0.500	0.200	1.000	0.500
5	1.500	0.100	0.015	0.150	0.050	0.200	0.100	0.300	0.150	1.000	0.250
6	1.500	0.050	—	0.100	0.010	0.150	0.050	0.200	0.100	0.500	0.200

*CFU = Colony Form Unit.

The effect of the addition of spices and herbs at concentrations of 1% with freezing on coliform group counts was more observable when compared to that treated with 0.5% of spices and herbs and the control sample. Moreover, it could be noticed the gradual decrease in coliform group counts might be attributed to the antimicrobial effect of spices volatile oils and freezing storage. These results agreed with Williams et al., (1980), who found that, coliform and *E.coli* recoveries of ground beef as affected by freezing storage recorded significant reduction after 11 days storage at -20˚C. On the same concept, Elliott and Michner, (1984) reported that, as the storage time extended counts of coliform tended to decline. This behavior may be attributed to the rapid death of *E.coli* during freezing storage. El-Shawaf (1990) reported that coliform group were in the range of $0.11x10^3$ to $16.00x10^3$ cell/gm and 2.5 to $3.30x10^4$ in fresh locally produced sausages and fresh prepared sausages; respectively. freezing storage of fresh sausages at -18±2˚C, decreased the coliform counts. Restaino et al., (2001) observed that the injury of *E.coli* O157:H7 was maximal after storage for 30 days in the beef infusion at -25˚C in a conventional freezer and most rapid cellular death and injury occurred within the first 10 days.

3-*Staphlococcus aureus* counts:

Data presented in Table (3) showed that the behavior of *Staphylococcus aureus* counts are basically similar to those obtained in total aerobic and coliform group counts in different chicken burger treatments.

Table (3): *Staphylococcus. .aureus* counts in chicken burger formulated with some spices and herbs (0.5 and 1%) during freeze storage period at -18ºC. (x 10^4 CUF/g)*

Storage period (months)	Control	Thyme		Sage		Rosemary		Marjoram		Black seeds	
		0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
0	1.500	0.210	0.060	0.300	0.200	0.400	0.250	0.800	0.450	0.900	0.750
1	1.350	0.035	—	0.100	0.060	0.150	0.100	0.200	0.150	0.500	0.200
2	0.350	—	—	0.025	—	0.035	—	0.070	0.035	0.150	0.100
3	0.350	—	—	—	—	0.015	—	0.050	0.020	0.100	0.050
4	0.300	—	—	—	—	0.010	—	0.045	0.030	0.050	0.035
5	0.250	—	—	—	—	0.010	—	0.040	0.025	0.050	0.030
6	0.200	—	—	—	—	0.005	—	0.030	0.015	0.040	0.025

*CFU = Colony Form Unit.

The data obtained in Table (3) revealed that *Staphylococcus aureus* were completely disappeared in samples treated with 1% thyme after one months and also disappeared after two months till the end of storage period in the two used concentrations. When samples treated with 1% sage *Staphylococcus aureus* also disappeared after 2 month and till the end of storage period by the two used concentrations. However, rosemary indicated less effect, and *Staphylococcus aureus* completely disappeared only in samples treated with 1% after two months and till the end of storage period.

However, the frozen storage of chicken burger sample showed a negative effect on the growth of *Staphylococcus aureus* counts which gradually decreased as storage period prolonged. These results were in agreement with Frazier and Westhoff (1979), Rashad (1990).

4-Psychrotrophic bacterial counts:

The data presented in Table (4) indicated that the behavior of psychrotrophic bacterial counts was almost similar to that obtained previously of total aerobic, coliform group and *Staphylococcus aureus* counts in different chicken burger treatments.

Table (4): Psychrotrophic bacterial counts in chicken burger formulated with some spices and herbs (0.5 and 1%) during freeze storage period at -18ºC. (x 10^4 CUF/g)

Storage period (months)	Control	Thyme		Sage		Rosemary		Marjoram		Black seeds	
		0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
0	25.000	15.000	11.000	16.000	11.500	17.000	12.000	19.500	13.000	21.000	14.500
1	19.000	11.000	8.000	12.000	8.500	13.500	10.000	15.500	11.000	17.000	11.500
2	14.000	5.500	4.000	6.500	4.500	7.000	5.000	9.000	7.000	11.000	8.000
3	4.000	0.150	0.100	0.500	0.300	1.000	0.500	2.000	1.500	2.500	2.000
4	3.500	0.100	0.050	0.150	0.100	0.500	0.250	2.000	1.000	2.500	1.500
5	3.000	0.050	0.025	0.100	0.050	0.500	0.100	1.500	0.500	2.000	1.500
6	2.000	0.015	0.005	0.050	0.020	0.150	0.100	1.000	0.300	1.500	1.000

*CFU = Colony Form Unit.

Generally, psychrotrophic bacterial counts gradually decreased after and throughout storage periods in all spiced sample but with variable degrees. Such results might be attributed to the effect of freezing storage on microbial cells which led to death of some cells, beside the antimicrobial effect of spices and herbs. However, it could be noticed that thyme showed strong inhibitory effect on microbial growth. The effectiveness of inhibitories followed the sequence: Thyme > Sage > Rosemary > Marjoram > Black seeds. Such findings are on line with Tabak et al., (1996), who tested extracts of several plants (marjoram, thyme, rosemary, chamomile, cinnamon, sage and garlic) for inhibitory activity against Helicobacter pyloi, and found among these plants thyme was the most effective.

5-Proteolytic bacterial counts:

Data given in Table (5) showed that proteolytic bacterial counts also took the same trend of total aerobic, coliform group, *staphylococcus aureus* and psychrotrophic bacteria counts in different chicken burger treatments.

Table (5): Proteolytic bacterial counts in chicken burger formulated with some spices and herbs (0.5 and 1%) during freeze storage period at -18ºC. (x 10^4 CUF/g)*

Storage period (months)	Control	Thyme		Sage		Rosemary		Marjoram		Black seeds	
		0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
0	89.00	68.00	56.00	70.00	57.50	71.50	60.00	73.00	63.50	75.00	64.50
1	77.50	48.00	39.50	50.00	40.00	53.00	42.00	55.00	42.50	57.00	44.50
2	54.00	39.00	31.00	41.00	32.00	44.50	34.50	46.00	38.00	49.00	40.00
3	45.00	29.00	28.50	31.00	29.00	33.00	31.00	34.50	32.00	39.00	33.00
4	37.00	25.50	23.00	27.00	24.00	29.00	26.50	32.00	28.00	35.00	30.00
5	28.00	18.50	16.00	19.00	17.00	22.00	18.50	24.00	21.00	25.00	23.00
6	21.00	13.00	11.50	15.00	13.50	16.00	14.50	17.50	15.50	19.00	16.00

*CFU = Colony Form Unit.

From these data it could be obvious that addition of 0.5% of spices and herbs to chicken burger formula caused a decrease in proteolytic bacterial counts after storage for 6 months at -18˚C compared with that of the control sample. Meanwhile, addition of spices and herbs at level 1% caused more reduction in proteolytic bacterial counts.

Generally, it could be noticed that thyme showed as strong inhibitory effect on microbial growth compared with the other used spices and herbs. The effectiveness of inhibitories followed the sequence:

Thyme > Sage > Rosemary > Marjoram > Black seed.

Such results are in a reasonable agreement with Shelef et al., (1980), Aureli et al., (1992), Outara et al., (1997), Bagamboula et al., (2003) and Kalemba and Kunicka (2003), who studied antimicrobial activity of many spices and classified their activities as strong, medium or weak and they reported that cinnamon, clove, pimento, thyme, oregano, sage and rosemary had strong and consistent inhibitory effect against several pathogen and spoilage bacteria.

6-Lipolytic bacterial counts:

Data presented in Table (6) showed that lipolytic bacterial counts also took the same trend of aerobic plate counts, coliform group, *staphylococcus aureus*, psychrotrophic and proteolytic bacterial counts in different chicken burger treatments.

Table (6): Lipolytic bacterial counts in chicken burger formulated with some spices and herbs (0.5 and 1%) during freeze storage period at -18ºC. (x 10^4 CUF/g)*

Storage period (months)	Control	Thyme		Sage		Rosemary		Marjoram		Black seeds	
		0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
0	49.00	40.00	28.00	41.00	29.00	43.50	30.00	44.00	34.00	44.50	39.50
1	38.00	25.00	21.00	26.00	22.00	26.50	23.50	27.50	25.50	28.50	27.00
2	28.00	13.50	10.00	14.00	11.00	17.00	13.00	18.50	14.00	19.00	16.00
3	19.00	7.50	6.00	9.00	6.50	11.00	8.00	12.00	9.00	14.00	10.00
4	11.00	5.50	4.50	6.50	5.00	7.50	6.00	8.50	7.00	9.00	8.00
5	9.00	5.00	3.50	5.50	4.00	6.00	4.50	7.50	6.00	8.00	7.00
6	7.50	3.50	3.00	4.50	3.50	5.00	4.00	6.00	5.50	6.50	5.50

*CFU = Colony Form Unit.

From the same table it could be noticed that, lipolytic bacterial counts gradually decreased after and throughout the storage periods in all spiced samples. However, thyme showed a strong inhibitory effect on this microbial growth. The effectiveness of inhibitiories followed the sequence:

Thyme > Sage > Rosemary > Marjoram > Black seeds.

Such results are in agreement with Bara and Vanetti (1995) who examined the antibacterial effects of ground spices on the growth of *yersinia enterocolitica* and found that all spices showed bacteriostatic effect, the most effective spices were cloves, thyme, sage, cinnamon, rosemary and oregano of which ethanol extracts reduced bacterial cell counts.

7-Yeast counts:

The results given in Table (7) showed that yeast counts recorded the similar trend of aerobic plate counts, coliform group,*Staphylococcus aureus,* psychrotrophic, lipolytic and proteolytic bacterial counts in different chicken burger treatments.

Table (7): Yeasts counts in chicken burger formulated with some spices and herbs (0.5 and 1%) during freeze storage period at -18ºC. (x 10^4 CUF/g)*

Storage period (months)	Control	Thyme		Sage		Rosemary		Marjoram		Black seeds	
		0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
0	15.000	10.000	7.500	11.000	8.000	12.000	9.000	13.000	10.000	14.000	12.000
1	13.000	8.000	6.500	9.000	7.000	10.000	7.500	11.000	8.000	11.500	9.000
2	10.000	5.500	3.500	6.000	4.000	6.500	5.000	7.500	6.000	9.000	7.000
3	4.000	1.000	0.100	1.500	0.150	2.000	0.500	2.500	1.000	3.000	1.500
4	3.500	0.500	0.050	1.000	0.100	1.500	0.150	2.000	0.500	2.500	1.000
5	3.000	0.050	0.025	0.100	0.050	0.500	0.100	1.500	0.150	2.500	1.000
6	2.000	0.015	0.010	0.030	0.020	0.100	0.050	0.100	0.050	1.500	0.150

*CFU = Colony Form Unit.

Generally, yeast counts gradually decreased during the storage periods in all spiced samples but with variable degrees. In addition, thyme showed the strongest inhibitory effect on yeasts growth. The effectiveness of inhibitories followed as the sequence:

Thyme > Sage > Rosemary > Marjoram > Black seeds.

These results are in agreement with Conner and Beuchat (1984) who investigated thirty-two essential oil for their inhibitory effect against 13 food spoilage and industrial yeasts and they found that, cinnamon, clove, garlic, onion, oregano, savory and thyme were the most inhibitory. Many of the essential oils tested were non inhibitory or only slightly inhibitory to the growth of the test yeasts.

8-Mold counts:

The presence and growth of fungi in food may cause spoilage and result in a reduction in food quality. Some Aspergillus species are zerophilic fungi and responsible for many of food and feed contamination Soliman and Badeaa, (2002).

Data given in Table (8) showed that mould counts recorded similar trend to that obtained and previously mentioned for microbiological tests in different chicken burger treatments. The obtained results revealed that, molds disappeared in the sample of chicken burger formula containing thyme at level 1% after storage for 2 months and after 3 months by using 0.5% only.

Table (8): Mould counts in chicken burger formulated with some spices and herbs (0.5 and 1%) during freeze storage period at -18ºC. (x 10^4 CUF/g)*

Storage period (months)	Control	Thyme		Sage		Rosemary		Marjoram		Black seeds	
		0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%	0.5%	1%
0	0.550	0.250	0.150	0.300	0.200	0.350	0.250	0.450	0.300	0.500	0.400
1	0.400	0.150	0.100	0.200	0.150	0.250	0.200	0.300	0.250	0.350	0.300
2	0.300	0.005	—	0.050	0.010	0.100	0.050	0.200	0.100	0.250	0.150
3	0.150	—	—	0.005	—	0.010	0.005	0.015	0.010	0.050	0.015
4	0.100	—	—	—	—	0.005	—	0.015	0.005	0.050	0.010
5	0.050	—	—	—	—	—	—	0.010	—	0.015	0.005
6	0.010	—	—	—	—	—	—	—	—	0.005	—

*CFU = Colony Form Unit.

By the end of frozen storage molds disappeared in all different chicken burger treatments except in the sample of chicken burger formula containing black seeds at level 0.5% which recorded $0.005x10^4$ CFU/gm, and it was also lower than the control sample which had reached by the end of storage periods to $0.01x10^4$ CFU/gm.

In conclusion thyme showed a stronger inhibitory effect on microbial growth among the studied spices and herbs. The effectiveness of inhibitories followed the sequence:

Thyme > Sage > Rosemary > Marjoram > Black seeds.

Such findings are in good agreement with those previously reported by Ozcan (1998); Giamperi et al., (2002); Soliman and Badeaa (2002) and Ozcan (2005).

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التغيرات الميكروبيولوجيه لبرجر الدجاج المحضرباضافة بعض التوابل والاعشاب اثناء فترة التخزين بالتجميد

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اجريت هذه الدراسة لاختبار التاثير المثبط الميكروبى لبعض انواع من التوابل والاعشاب الشائع اضافتها الى الاغذية. و كانت التوابل والاعشاب المستخدمة فى هذه الدراسة على وجه التحديد: الزعتر وحصى البان (الروزمارى) والمريمية والبردقوش وحبة البركه لدراسة تاثيرها على العدد الكلى للبكتريا- البكتريا المحبه للبروده- البكتريا المحلله للبروتين- البكتريا المحلله للدهون- الخمائر والفطريات واحد انواع من البكتريا المسببه للتسمم الغذائى (*Staphylococcus aureus*) وكذلك مجموعة بكتريا القولون وذلك لحفظ وتحسين الجودة الميكروبية لبرجر الدجاج. تم استخدام تركيزان (0,5، 1%) لكل نوع من التوابل والاعشاب المدروسه فى عمل برجر الدجاج والذى خزن على – 18±2°م لمدة ستة شهور.واشارت الدراسه ان اعداد الميكروبات تتناقص مع زيادة مدة التخزين بالتجميد فى كل العينات المعامله مقارنة بالكنترول. كما اوضحت الدراسه ان اضافة التوابل والاعشاب بتركيز 1% ادى الى خفض اعداد الميكروبات بدرجه اكبر من اضافتها بتركيز 0,5%. كما اظهرت النتائج ان الزعتر هو اكثر الاعشاب قوة فى تاثيره على خفض اعداد الميكروبات يليه المريمية، الروزمارى ثم البردقوش بينما سجلت حبة البركة اقل تأثيرا مثبطا للميكروبات. عموما فان كل معاملات برجر الدجاج سواء المعاملة اوغير المعاملة (الكنترول) كانت مقبوله ميكروبيولوجيا فى نهاية مدة التخزين حيث كان الحمل الميكروبى اقل من 10^5 خلية/جم.