Abstract

This investigation was carried out to study the influence of adding Melissa spice at concentrations of 0.5%, 1.5%, 2.0% and its oil at 0.05%, 0.1%, 0.2% in comparison with adding BHA at 0.01% as a chemical antioxidant compound on the quality of frozen beef burger. During storage prepared beef burger samples were chemically and microbiologically evaluated during storage at – 18^{o}C for 6 months. The obtained results indicated that all samples showed an increasing fat content and decrease moisture, protein and ash contents during storage, while the chemical composition of the control sample was more affected than the other samples. Melissa oil showed the highest antioxidant and antimicrobial compared with other samples and this activity was increased by increasing the concentration of Melissa oil. Beef burger samples treated with different concentrations of Melissa oil was concerning treatments for keeping quality of investigated beef burger.

.Kewords: Melissa, Beef burger, Antioxidant, Antimicrobial. BHA.

Introduction

Herbal remedies have been used for thousands of years. Early in human history, people practiced herbal medicine as a magical or religious healing art (Baker, 1965). Today, a lot of people use herbal medicine or rely on them.

There are many various medicinal. One of them is lemon balm (*Melissa officinalis* L.), in other words bee herb or sweet balm. Lemon balm, member of the family *Lamiaceae* (formerly *Labiatae*) in scientific classification is a perennial herb that is, one that lives at least three years (Anonymous, 2003).

Lemon balm (*Melissa officinalis L*.), also known as common balm or sweet balm, is a perennial lem- on-scented herb in the mint family native to the Mediterranean and to Southern Europe (Small 2006).

The parts mostly used are dried leaves often with flowering tops (Leung & Foster 2003).

There are many various drug plants. One of them is lemon balm (*Melissa officinalis* L.), in other words bee herb or sweet balm. Lemon balm, member of the family *Lamiaceae* (formerly *Labiatae*) in scientific classification is a perennial herb that is, one that lives at least three years (Anonymous, 2003

Meat is a perishable food, due to high moisture and fat contents, almost neutral in pH so, meat is a good medium to be contaminated with microorganisms and susceptible to oxidation. Also, meat processing and storage prior to consumption can have a significant effect on meat quality. Synthetic antioxidants are added to foods to delay

the oxidation process in meat and meat products Post (1996).

Natural substances such as spices powder or spice oils can be used to prolong the shelf life of food because they have antioxidant and antimicrobial agents, such as Melissa spice and its oil which are considered a good antioxidant and antimicrobial agent due to it's phenolic components , so we can use spices and extraction their oils as natural preservatives to avoid the bad effect of synthetic preservatives on public health. Burt, (2004), Abd El-Kader, et al (2005), and El-Bastawesy, et al (2011).

Therefore, this work was appling using Melissa spice and its extracted oil as antioxidant and antimicrobial agents in addition to maintain quality of beef Burger through processing and freezing of beef burger.

MATERIALS AND METHODS

Materials:

Melissa spice (*Melissa Officinalis.*) was was purchased from the Egyptian Aromatic Company, Cairo, Egypt during summer 2014, has been working at the Faculty of Agriculture, Cairo University labs

Beef Meat

A sample weighted 5 kg of beef meat was purchased from local markets in Cairo, Egypt. The meat transported immediately in icebox to the laboratory.

Butylated hydroxy anisole (BHA) was obtained from El-Gomheria company, Cairo, Egypt.

Methods

Extracted of Melissa volatile oil:

Melissa volatile oil was obtained by steam distillation Faculty of Specific Education, Ain Shams University labs of Melissa spice for 3 hours(Soxhlet extractor) as described by Guenther (1961).

Determination of Chemical composition of Melissa spice:

Moisture, protein, fat, ash and fiber contents were determined according to the method described by (AOAC 2005). Meanwhile nitrogen free extract was calculated by difference as follows: NFE = 100- % of (moisture + protein + fat + ash + fiber)

Physical and chemical properties of Melissa volatile oil:

Specific gravity, refractive index, solubility in alcohol, acid value, ester value and peroxide value of the volatile oil under study were determined according to the methods described by Guenther (1961).

Quantitative determination and identification of the constituents of Melissa volatile oil:

Gas liquid chromatography – Mass spectrometry (GC-Ms) was used to separate and identify the components of Melissa volatile oil. Analytical method determined in

Laboratory of Kato Aromatic, Co., described by Nath, (1996).

Preparation of beef burger:

Beef burger contained lean beef meat, cow fat and dried onion, salt at percentages of 87.35, 11, 0.4 and 1.25, respectively according to the method described by Karpinska *et al*. (2001) was prepared with some modification. For preparing beef burger, meat and fat were mixed well with the other ingredients and adding Melissa as a natural additive and BHA as a synthetic additive to the prepared samples at different concentrations as follows, Melissa spice (0.5%, 1.5%, 2%), Melissa oil (0.05%, 0.1%, 0.2%) and BHA (0.01%). The concentration of natural additives used according to the threshold value. But for synthetic additives according to Egyptian Standards (1991). All samples were frozen at – 18c^{o} for 6 months.

Chemical analysis of beef burger:

Moisture, protein, fat and ash contents were determined by the methods described by AOAC (2005). Peroxide, acid and thiobarbituric acid (TBA) values were determined according to the methods described by AOCS (1997).

Physical characteristics of beef burger:

Cooking loss was determined according to the method described by lin and Zayas (1987). Also, water holding capacity (WHC) and plasticity were determined according to Solovier (1966).

Microbiological analysis:

Total bacterial count was determined according to the method described by NMKL. (1999). Total fungal count was determined according to NMKL (1995). Salmonella was determined according to Ellis et al. (1979) and E. coli was determined according to method described by Difco (1989).

RESULTS AND DISCUSSION

Chemical compositions of Melissa spice:

Chemical composition of Melissa spice were : moisture 12.00% protein 10.81%. Fat 4.80% ash 11.70%, fiber 11.40% and nitrogen free extract 49.29%. These results agree with the data reported by Nutritional Food Group (2004).

Table (1):Percentage of Chemical composition percentage of Melissa spice

Items	moisture	protein	Fat	ash	fiber
Melissa spice	12.00	10.81	4.80	11.70	11.40

Physical and chemical characteristics of Melissa volatile oil:

Physical and chemical characteristics of majoram oil were determined and the obtained results are shown in Table (2). The results regarding the specific gravity at 22 ± 2 ^{o}C (0.9091), refractive index at 22 ± 2 ^{o}C (1.3496), acid value (1.3), ester value (10.2) and peroxide value (0.002). These results are in agreement with some investigators such as Guenther (1961) and Food Chemicals Codex (1981)

Table (2): Physical and chemical properties of Melissa volatile oil

Characteristics	Melissa volatile oil	Reference range
Specific gravity at 20oC	0.9091	0.899 – 0.913
Refractive index at 20oC	1.3496	1.260 – 1.439
Acid value	1.3	Up to 1.4
Ester value	10.2	10.0-38.0
Peroxide value	0.002	Up to 0.004

Chemical constituents of Melissa (*Melissa Officinalis.*) volatile oil:

The chemical constituents of Melissa volatile oil were identified in order to define their importance as antioxidant and antimicrobial agents. The obtained results are shown in Table (3) which revealed that there were 14 identified components in Melissa volatile oil, presented (57.06%). While unknown component was 42.94% The chemical compounds identified classified according to the quantity to three groups. The first group (>10%) were found namely : linalool 10.92%. The second group (< 2 to 10%) were Globulol 5.55%, B-pinine 4.92% and Terpineolene 2.56%. While, other component were recorded (<2-0%) as descript in table 3. These results are agree with Guenther (1961), Farrell (1990), Baser et al (1993) and Jorge et al (1997).

Table (3) Relative percentage of chemical constituents of Melissa volatile oil.

Component	Retention Time	Relative percentage
Thujene	4.143	0.36
α - pinine	4.175	0.93
B-pinine	5.667	4.92
Limonene	7.346	1.33
B- myrcene	9.290	0.57
Terpineolene	9.725	2.56
Beta ocimene Z	10.207	0.03
Alfa-humulene	13.28	0.07
Beta-caryophyllene	14.24	13.25
Caryophyllene oxide	15.8	0.03
Globulol	15.81	5.55
Humulene epoxide	16.18	0.10
5-cedranone	16.29	0.19
Linalool	19.463	10.92
Total		57.06
unknown		42.94

Chemical composition of beef burger treated with different doses of Melissa and its oil during frozen storage:

Table (4) showed the chemical composition of prepared beef burger treated with Melissa spice at concentrations of (0.5%, 1.5 and 2%) and Melissa oil at (0.05%, 0.1% and 0.2%) and BHA at 0.01% then frozen at – 18^{o}C for 6 months. The untreated beef burger at zero time reveled that moisture was 60.12%; protein 18. 12%; fat, 16.02% and ash, 2.10%. These results were within the permissible limits of (Egyptian Standards 1973 – 1991). Generally, it could be noticed that, the moisture content was slightly decreased during frozen storage, due to defrosting and drip loss and decreasing also protein content. This might be explained by the escape of some nitrogen with the separation drip. Similar results were also reported by Dawood (1995). After 6 months of storage, samples with Melissa spice and its oil had slightly higher moisture, protein and ash content compared with the control and BHA treated samples and also had the lowest fat content. This indicated that Melissa spice and its oil improved the chemical composition of beef burger during frozen at – 18^{o}C for 6 months. Shady, (1999).

Table (4): Effect of Melissa spice and its oil and BHA agent on the chemical compositions (%) of beef burger during storage at – 18oC for 6 months.

Storage periods		0				6			
component		Moisture	Protein	Fat	Ash	Moisture	Protein	Fat	Ash
control		60.12	18.12	16.02	2.10	56.02	16.05	19.66	1.56
BHA		59.20	19.42	15.61	2.61	56.00	17.70	19.41	1.56
Melissa spice	0.5%	59.71	18.81	16.66	1.43	55.25	16.76	17.16	1.44
	1.5%	57.43	19.52	16.12	1.49	56.63	17.81	17.40	1.40
	2%	59.44	19.11	16.20	1.48	55.20	17.30	14.20	1.44
Melissa oil	0.05 %	60.20	18.20	16.20	1.52	56.24	17.88	17.61	1.33
	0.1%	60.63	18.61	16.05	1.78	56.40	16.50	17.45	1.23
	0.2%	60.31	18.20	16.15	1.76	56.16	16.50	17.31	1.22

Changes in acid value during storage :

Table (4) showed the acid value of freshly prepared beef burger treated with Melissa (specie or oil) and BHA during frozen at 18 Cº for 6 months. It could be observed that the A.V. of control sample was 2.96 (mg koH/g sample) which increased to 4.85 at the end of storage. The control sample had the highest value compared to the other beef burger samples at all storage periods. Addition of BHA at 0.01% decreased the A.V. to 2.85 at zero time which increased to 4.49 at the end of storage. On the other hand, addition Melissa spice at (0.5%, 1.5% and 2%) decreased the A.V. at zero time compared to control sample. At zero time the lowest acid value obtained at 2% which was 2.7. This value increased to 4.25 at the end of storage. Besides, the addition of Melissa oil at (0.05%, 0.1% and 0.2%) decreased the (A.V.) at zero time compared to the control sample. At zero time the lowest acid value obtained at 0.2% which was 2.31, this value increased to 4.06 at the end of storage. From above results it could be concluded that all treatments showed increasing in acid value of the fat extracted from prepared beef burger during frozen storage. On the other hand Melissa oil was more effective to reduce the acid value than spice, control and BHA. Also the acid value decreased as spice and its oil concentration increased. In this connection it could be mentioned that frozen storage besides addition of spice or it oil it may be retared lipase activity which hydrolyze the triglycerides to glycerol and

free fatty acids and consequently improved extent beef burger. This data were in the line by those reported by Economou et al(1991) and Karpinska et al (2001).

Table (5) effect of Melissa spice and its oil and BHA on acid value of beef burger during frozen storage at -18ºC for 6 months.

Storage period/month	Control	BHA 0.01 %	Melissa spice			Melissa oil		
			0.5 %	1.5 %	2%	0.05 %	0.1 %	0.2 %
0	2.96	2.85	2.82	2.78	2.70	2.46	2.39	2.31
2	3.68	3.34	3.17	2.99	2.85	2.94	2.89	2.89
4	4.69	4.02	3.97	3.86	3.80	3.81	3.75	3.68
6	4.85	4.49	4.51	4.38	4.25	4.23	4.18	4.06

Changes in peroxide value during storage :

Table (5) showed the peroxide value of the control was 3.88 (m.equiv/kg) oil which increased to 7.91 at the end of storage. The control sample had the highest value (P.V.)

compared to the other beef burger samples at all storage periods. Addition of BHA at 0.01% decreased the (P.V.) to 3.06 at zero time which increased to 5.58 at the end of storage. On the other hand, addition Melissa spice at (0.5%, 1.5% and 2%) decreased the (PV) at zero time compared to the control sample. At zero time the lowest peroxide value obtained at 2% which was 3.52. This value increased to 5.33 at the end of storage. In besides, the addition of Melissa oil at 0.05%, 0.1% and 0.2% decreased the PV at zero time compared to the control sample. At zero time the lowest peroxide value obtained at 0.2% which was 2.13%. This value increased to 4.58 at the end of frozen storage. From above results it could be concluded that Melissa whatever spice or oil had the highest antioxidants activity compared to control. And the activity increased by increasing concentration. Also, the addition of oil was more effective than spice. Finally, storage at -18ºC for 6 months delays the peroxide formation due to retarding the peroxide enzymes and in parallel improved the beef burger. These results were agreement with Abd El-Hlim et al (1999), Karpinska et al (2001), and Misharina et al (2009).

Table (6) Effect of Melissa spice and its oil and BHA on peroxide value of beef burger during frozen storage at 18c° for 6 months.

Storage period/month	Control	BHA 0.01 %	Melissa spice			Melissa oil		
			0.5 %	1.5 %	2%	0.05 %	0.1 %	0.2 %
0	3.88	3.06	3.73	3.52	3.50	2.57	2.20	2.13
2	4.70	4.51	4.29	4.16	4.07	3.14	2.97	2.63
4	6.81	5.03	5.00	4.94	4.72	4.42	4.14	4.03
6	7.91	5.58	5.86	5.40	5.33	4.85	4.61	4.58

Changes in thiobarbituric acid value (TBA) during storage:

Data presented in Table (6) show the thiobarbituric acid values (TBA) of beef burger samples as affected by Melissa spices and its oil and BHA agent, during storage at – 18oC for 6 months. It could be observed that TBA values increased continuously with progress of storage time for all treatments, which indicated the development of both lipid oxidation and hydrolysis. The maximum changes in TBA

values during storage were recorded for the control sample, followed by the BHA then Melissa spice. Melissa oil was more efficient in decreasing the lipids changes, different constituents in Melissa oil containing terpenes and sesquiterpene alcohols and ketons. It may be maintance the quality of beef burger against oxidation the oil. Karpinska et al. (2001).

Table (7): Effect of Melissa spice and its oil and BHA on the thiobarbituric acid

value of beef burger during frozen storage at -18oc for 6 months.

Storage period/ month	Control	BHA 0.01%	Melissa spice			Melissa oil		
			0.5%	1.5%	2%	0.05%	0.1%	0.2%
0	0.80	0.39	0.53	0.47	0.40	0.49	0.42	0.31
2	1.06	0.48	0.62	0.51	0.49	0.58	0.50	0.42
4	1.28	0.52	0.70	0.63	0.54	0.62	0.54	0.47
6	1.43	0.86	0.80	0.72	0.62	0.74	0.66	0.60

Antimicrobial effect of Melissa spice and its oil and BHA on beef burger during frozen storage:

Table (7) shows that the untreated beef burger at zero time contained total bacterial count (TPC) of 24 x 103 cfu, Total fungal count (TFC) was 14 x 10 cfu, E- coli was 22 x

10 cfu and all samples were free from Salmonella. These results were within the permissible limits of Egyptian Standards, (1973 – 1991). All samples increased in TPC and Esherichia coli during storage. Melissa oil at 0.2% was the highest inhibitor for TPC and Esherichia coli during storage. Total fungi were not detected in all beef burger samples expect control samples which contain 14x10 c. f. u. at zero time which increased to 20x102 after 6 months of storage. This antimicrobial activity of Melissa due to chemical composition of the oils and the antimicrobial activities of these oils. Giamperi et al., (2002), Deans & Katerina (2006) and Lidiane et al (2009).

Physical characteristics of beef burger samples during frozen storage:-

Water holding capacity (WHC) Plasticity and cooking loss:

The obtained results in Table (8) appeared that addition of Melissa oil improved the WHC and plasticity during storage, This may be due to the more double bonds of the essential oils and also of the spice compounds of the powder which play an important role in preventing the formation of free radicals and therefore no more water molecules can form hydrogen bonds and in parallel affect beef burger plasticity. Also, the loss of WHC by frozen storage was attributed to protein denaturation. Abd El Sattar, (2001) and Shady, (1999).

Table (8): The effect of Melissa spice and its oil on the microbial quality (c. f. u) of beef Burger during frozen storage at – 18 oC for 6 months.

Storage period/ month	Micro biological Examination	Control	BHA 0.01%	Melissa spice			Melissa oil		
				0.50%	1.50%	2%	0.05%	0.1%	0.2%
0	TPC	$24x10^3$	$26x10^2$	$18x10^2$	$16x10^2$	$13x10^2$	$15x10^2$	$12x10^2$	$11x10^2$
	TFC	$14x10^1$	-	-	-	-	-	-	-
	E-coli	$22x10^1$	$19x10^1$	$17x10^1$	$14x10^1$	$12x10^1$	$14x10^1$	$12x10^1$	$10x10^1$
2	TPC	$32x10^2$	$24x10^1$	$19x10^1$	$18x10^1$	$7x10^1$	$9x10^1$	$8x10^1$	$36x10^1$
	TFC	$30x10^1$	-	-	-	-	-	-	-
	E.coli	$13x10^2$	$24x10^1$	$19x10^1$	$18x10^1$	$17x10^1$	$18x10^1$	$16x10^1$	$14x10^1$
4	TPC	$24x10^3$	$20x10^3$	$24x10^3$	$22x10^3$	$20x10^3$	$22x10^3$	$19x10^3$	$15x10^3$
	TFC	$40x10^1$	-	-	-	-	-	-	-
	E-coli	$20x10^2$	$14x10^2$	$15x10^2$	$13x10^2$	$12x10^2$	$12x10^2$	$10x10^2$	$6x10^2$
6	TPC	$21x10^5$	$17x10^4$	$16x10^4$	$15x10^4$	$13x10^4$	$16x10^4$	$13x10^4$	$10x10^4$
	TFC	$20x10^2$	-	-	-	-	-	-	-
	E. coli	$19x20^2$	$20x20^2$	16 $x20^2$	14 $x20^2$	12 $x20^2$	14 $x20^2$	$12x20^2$	8 $x10^2$

Also, data inTable (8) revealed that the addition of BHA, Melissa spice and its oil in beef burger samples reduce the

total cooking loss compared to the control sample. The lowest cooking loss, was recorded for beef burger with Melissa oil at concentration of 0.2%. Therefore, it could be concluded that using Melissa oil can decrease the cooking loss. This could be related to the effect of Melissa oil due to its effect in reducing the rendering rate of fat and juice during cooking. Similar findings were reported by Salama and El – Wakeil, (1994) and El – Harery (1997).

Finally, it could be concluded that, Melissa spice and its oil could be recommended for addition to beef burger. Such addition proved a potentiality as natural antioxidants, antimicrobial agents and enhancement the quality at zero time as well as after frozen storage up to six months.

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