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التخطيط الإستراتيجي للتعليم النوعي في ضوء التحديات والتحول العائبة "آفاق وحلول"

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The effectiveness of using lemon and orange essential oils as natural antimicrobials and antioxidants in beef burger

ABSTRACT

" The effectiveness of using lemon and orange essential oils a natural antimicrobials and antioxidants in beef burger "

Healthier food products have become a key target for the food industry. Consumer's demands for healthier meat and meat products are rapidly increasing world-wide.

In this study, we assessed chemical composition and antimicrobial activities of Sweet Orange (*Citrus sinensis*) and Lemon (*Citrus limon*) essential oils (OE and LE) with its preservative effect against pathogenic bacteria by the determination of agar well diffusion method and the minimum inhibitory concentrations (MICs).

In order to improve the functional value of meat product, application of orange or lemon essential oil (OE or LE) as promising cheap natural antimicrobial and antioxidant in beef burger was carried out. Chemical composition, quality properties and microbial analysis of the suggested beef burger as affected by adding OE or LE during freezing storage at -18°C for 90 days were studied. The addition of 0.5, 1 and 1.5 % of OE and LE to the beef burger caused a reduction in thiobarbituric acid reactive substances, peroxide value, total volatile basic nitrogen and microbial count.

Sensory evaluation of beef burger treated with OE and LE showed that they were organoleptically acceptable in terms of color, taste, odour and tenderness, that in concentrations 0.5 and 1 %, while the high concentration 1.5% was less acceptable, but it was not rejected at all.

Key Words: orange, lemon essential oil, Antimicrobial, Antioxidant, Beef burger.

Introduction

Method of preservation Meat is a necessary to transport meat without spoiling and loss of nutritional value for long distances. The addition of sodium nitrite, potassium nitrite, can be used to preserve the cured meat, but such chemical compounds have bad effects on human health (Nychas, et al ٢٠٠٨).

Consumers have become aware of the health problems (diseases related to oxidative stress) in recent years, which are caused by artificial chemical additives to processed foods. Natural additives as essential oils and their extracts can be used to reduce the use of chemical preservatives and their risks. Such materials can extend the shelf life of meat and their products and control or inhibit the microbial growth (Saricaoglu, and Turhan ٢٠١٩). Orange (*Citrus sinensis*) and lemon (*Citrus limon*) oils are an essential oil produced by cells within the rind of an orange and lemon fruit. It is composed of mostly (greater than ٩٠٪) d-limonene, and is often used in place of pure d-limonene. D-limonene can be extracted from the oil by distillation. (Verzera, ٢٠٠٤).

The essential oils of citrus are excellent sources of natural antioxidants such as phenolic, flavonoids, alkaloids, tannins and phenolic acids. The TBA value is commonly used as an indicator of fat oxidation in meat products (Domínguez, ٢٠١٩). Also, citrus essential oils have an antimicrobial activity. The most important component that have an antimicrobial effect were; Geraniol, Menthol, Cinnamil Alcohol, Linalool, Citronellol, Carvacrol, Cinnamaldehyde, Eugenol, Thymol, Estragol, Carvone and Chavicol (Sharma, et al., ٢٠١٩).

Material and Methods

Material:

١- oils.

The essential oil of Sweet Orange (*Citrus sinensis*) and Lemon (*Citrus limon*) was purchased from Cato Aromatic for aromatic, medicinal and food products.

٢- Ingredients used in the preparation of experimental samples of burger:

Six kilos of fresh meat, Soy protein concentrate, Salt and spices were purchased from the local market in Fayuom city.

Table (I) Suggested formula for beef burger:

Ingredients	%
Beef lean	٨٠
Fat	١٥
Soy protein concentrate	١٠
Salt	١,٥
Onion	١,٠
Powdered spices mixture	٠,٢
Sodium pyrophosphate	٠,٣
Ice	١٠

*Powdered spices mixture contained; fennel, coriander, paprika, rosemary, black pepper, cloves and laurel leaves.

٣- Beef burger preparation:

Fresh meat was mixed with the ingredients given in Table (I) minced beef meat was subdivided into eight equal parts and beef patties were prepared to provide eight treatment samples. control sample was formulated without any treatment. The samples were kept in a foam white trays covered with polyethylene sheets and stored at -18°C for further analysis at a periodicals of 10 days up to three-month storage.

Beef burger formulated was divided into eight groups as follow: -

Samples treatment ١ (T_1): treated with orange essential oil at a concentration of ٠,٥% Per ١٠٠ g of beef burger

Samples treatment ٢ (T_2): treated with orange essential oil at a concentration of ١% Per ١٠٠ g beef burger

Samples treatment ٣ (T_3): treated with orange essential oil at a concentration of ١,٥% Per ١٠٠ g beef burger

Samples treatment ٤ (T_4): treated with lemon essential oil at a concentration of ٠,٥% Per ١٠٠ g of beef burger

Samples treatment ٥ (T_5): treated with lemon essential oil at a concentration of ١% Per ١٠٠ g beef burger

Samples treatment ٦ (T_6): treated with lemon essential oil at a concentration of ١,٥% Per ١٠٠ g beef burger

Samples treatment ٧ (T_7): treated with sodium nitrite at a concentration of ٢٠٠ Ppm (part per million) of beef burger.

Control: be treated without lemon and orange oil, (as a control group)

All treatments are kept frozen. All chemical and microbiological tests are carried out on the treated samples at the beginning of the experiment (zero time) and every two weeks, for ٩٠ days of frozen storage, taking into consideration the acceptable sensory test.

Analytical Methods:

A- Gross Chemical composition:

Moisture content, crude Protein content, Fat content. Salt content and Ash were determination according to by A.O.A.C. (٢٠١٢).

B- Quality indices

١-The PH value and Total volatile nitrogen content were measured according to by A.O.A.C. (٢٠١٢).

٢- Thiobarbituric acid (TBA) as a fat oxidation index was calculated directly from the sample as defined by the Pearson, (١٩٧٦).

C- Microbiological examination:

Aerobic plate counts, *Staphylococcus aureus* and *Coliforms* group. in accordance with Difco manual (١٩٩٨).

D- Sensory evaluation.

Sensory attributes i.e., after cooking, colour, taste, odour and tenderness of the beef burger samples were evaluated using a ١٠-point numerical scale; where ten corresponded to "the highest quality", a score less than ٤ indicate that the sample is "rejected" according to the method defined by Golk et al. (٢٠٠٨).

E-Statistical analysis.

All obtained data were subjected to the statistical analysis using SPSS version ١٩,٠ software (SPSS. ٢٠٠٢. Statistical Package for Social Sciences. SPSS Inc., Chicago, IL, USA.), and Sigma plot ١٢,٠ software programs.

RESULTS AND DISCUSSION

١- Chemical Composition Beef burger treated with different concentration of lemon and orange essential oils.

The contents of moisture, protein, fat, salt and ash were determined in beef burger treated with different concentration of lemon and orange essential oils, and the results are shown in Table ١. The moisture content of all samples is in agreement with the Egyptian standard specifications (E.S.S) (١٦٨٨/٢٠٠٥) that stated the moisture content doesn't exceed ٦٠%. All of the treatments had significant differences in moisture content, with the exception of T٧ (Beef burger treated with nitrite ٢٠٠ ppm) which are consistent with each T١ (Beef burger treated with ٠,٥% orange essential oils) and control samples. These results are lower than those had been recorded by Maky, et al. (٢٠٢٠) who demonstrated that the mean value of moisture content in beef burger was ٦١,٠٢ %.

The mean values of protein % in the beef burger were ١٨^a ± ٢,٠, ١٩,٥^a ± ٢,٥, ٢١,٥^a ± ٢,١, ٢١^a ± ٢,٠, ٢١,١^a ± ٢,٠, ٢١,٥^a ± ٢,١, ٢١^a ± ٢,٠ and ٢٠^a ± ٢,٠ for T١, T٢, T٣, T٤, T٥, T٦, T٧ and control, respectively (Table, ١). No significant differences between them, and all samples is in agreement with E.S.S (١٦٨٨/٢٠٠٥), which limited protein was >١٥% in beef burger. These results are much higher than those found by Edris, et al. (٢٠١٢) and Maky, et al. (٢٠٢٠) who denoted that the mean value of protein in beef burger were ١٥,٢٢ and ١١,٥٤ %, respectively. The fat contents in beef burger treated with different concentration of lemon and orange essential oils were ranged from ١٧:١٨%, no significant differences between them (Table, ١). All samples are in compliance with the E.S.S. (١٦٨٨/٢٠٠٥), the labeled limit was >٢٠%. These results are, to some

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extent, in agreement with the data obtained by Edris, et al. (٢٠١٢) and Maky, et al. (٢٠٢٠).

From Table (١), it was clear that the mean values of salt content in beef burger samples were range from ١,٢ to ١,٤ %, there are no significant differences between samples. The mean values of ash % in the examined samples were ٢,٧٤, ٣,٤٦, ٢,٩٠, ٢,٨٣, ٢,٩٠, ٢,٩٥, ٢,٤٤ and ٢,٧٠ % for T١, T٢, T٣, T٤, T٥, T٦, T٧ and control, respectively. There are significant differences between treatment T٢ and other treatment samples. These results are lower than those recorded by Edris, et al. (٢٠١٢) and Maky, et al. (٢٠٢٠), who denoted that the mean values of ash % in the examined beef burger samples were ٤,٠١ and ٣,٣٦%. Ashes are the complete minerals present in foods such as sodium, phosphorus and iron that can be added to meat as raw materials, salt and spices (Fernández-López et al., ٢٠٠٦).

Table (١) Chemical composition of beef burger treated with different concentration of lemon and orange essential oils.

Treatment	Component %				
	Moisture	Protein	Fat	Salt	Ash
T١	٥٨,٨٦ b ±٠,٨٠	١٨ a ±٢,٠	١٧ a ±٢,٦٤	١,٤ a ±٠,٢٦	٢,٧٤ ab ±١,٥٢
T٢	٥٨,٢٢ c ±٠,١١	١٩,٥ a ±٢,٥	١٧,٥ a ±١,٥٢	١,٣ a ±٠,٣٥	٣,٤٦ a ±٠,١٩
T٣	٥٥,٩٠ f ±٠,٢١	٢١,٥ a ±٢,١	١٨ a ±٣,٠٠	١,٢ a ±٠,٢١	٢,٩٠ ab ±٠,٢٦
T٤	٥٧,٣٩ d ±٠,١٠	٢١ a ±٢,٠	١٧,٥ a ±١,٥٢	١,٢ a ±٠,٢١	٢,٨٣ ab ±٠,٢٠
T٥	٥٦,٦٤ e ±٠,٠٣	٢١,١ a ±٢,٠	١٨ a ±٣,٠٠	١,٤ a ±٠,٢٦	٢,٩٠ ab ±٠,٢٦
T٦	٥٥,٠٨ g ±٠,٠١	٢١,٥ a ±٢,١	١٧,٥ a ±٢,٥	١,٣ a ±٠,٣٥	٢,٩٥ ab ±٠,٢٥
T٧	٥٨,٣٧ ab ±٠,٠٢	٢١ a ±٢,٠	١٧ a ±٣,٠٠	١,٢ a ±٠,٢١	٢,٤٤ ab ±٠,٢٦
Control	٥٨,٣٣ a ±٠,٠١	٢٠ a ±٢,٠	١٧,٥ a ±١,٥٢	١,٣ a ±٠,٣٥	٢,٧٠ ab ±٠,٢٠
E.S.S	≥٦٠	≤١٥	≥٢٠	-	-

T١: Beef burger treated with ٠,٥% orange essential oils, T٢: Beef burger treated with ١,٠ % orange essential oils, T٣: Beef burger treated with ١,٥% orange essential oils, T٤: Beef burger treated with ٠,٥% lemon

essential oils, T^٥: Beef burger treated with ١,٠% lemon essential oils, T^٦: Beef burger treated with ١,٥% lemon essential oils, T^٧: Beef burger treated with nitrite ٢٠٠ppm, C: Control beef burger without any addition. Values with different superscript letters in a column are significantly different ($P \leq 0,05$).

Keeping quality indices:

١-Hydrogen ion concentration (pH):

Changes in pH values in beef burger samples treated with different concentration of orange and lemon essential oils during freeze storage at $-18 \pm 1^\circ\text{C}$ for ٩٠ days are presented in Figure (١). As shown from the Figure (١), the control sample has the lowest PH value ($6,47 \pm 0,10$) at zero day of storage, followed by the sample treated with lemon oil ١,٥ % and the value was $6,62 \pm 0,10$. A slight increase occurred during the storage period by freezing, and it rose at the end of the ٩٠ days to its maximum value, which is $6,98 \pm 0,3$, for the sample treated with orange essential oil ١%, followed by the samples treated with lemon oil essential, the value was $6,89 \pm 0,10$.

Figure (١) Hydrogen ion concentration (pH).

Total volatile basic nitrogen (TVBN).

TVBN changes of beef burger samples treated with different concentration of orange and lemon essential oils are illustrated in (Figure ٢). At the beginning of storage period (zero day), there were no significant differences between the samples, as the value of TVN was

approximately ٧ mg N/١٠٠gm this is an indication of good quality raw material used in this study, and it gradually increased during the storage period, so the highest value was 36.58 ± 0.20 mg N/١٠٠gm for the control sample, followed by the samples treated with ٠,٥% lemon essential oil, ٠,٥% orange essential oil, ١,٠% lemon essential oil, ١% orange essential oil and ١,٥% lemon essential oil and the values were 27.56 ± 0.30 , 26.66 ± 0.25 , 26.50 ± 0.30 , 24.65 ± 0.25 and 19.34 ± 0.15 , respectively. The lowest value was 18.92 ± 0.25 for samples treated with ١,٥% orange essential oil. A level above ٢٠ mg N/١٠٠ g of the sample is commonly considered spoiled minced meat (ESS ١٦٩٤/٢٠٠٥) with respect to TVBN values as a spoilage index for meat products. At the end of the storage period, most of the samples did not conform to the ESS ١٦٩٤/٢٠٠٥, with the exception of samples treated with both ١,٥% orange and lemon essential oil. These results are, to some extent, in agreement with the data obtained by Ibrahim, (٢٠١٨) who demonstrated that the addition of ١ or ٢% orange or lemon peels to the beef burger caused a reduction in total volatile basic nitrogen TVBN values compared to the control samples during refrigeration storage at $\pm 1^{\circ}\text{C}$ for ١٥ days.

Figure (٢) Total volatile basic nitrogen (TVBN). Thiobarbituric acid reactive substances (TBARS).

The TBARS method has been commonly used to assess the degree of lipid oxidation used during storage as a lipid oxidation index in meat products (Klangpetch et al., ٢٠١٦). Data in Figure (٣) show the changes

in TBARS values of the prepared beef burger samples treated with different concentration of orange and lemon essential oils during freeze storage at $-1 \pm 1^{\circ}\text{C}$ for 90 days. Results indicated that TBARS values for all treatment samples increased over the storage period. The values of samples on the zero day were approximately 0.157 (mg MDA kg⁻¹ meat) and they gradually increased during the storage period by freezing, and after 60 days of storage, the control sample became unfit for consumption due to the increase in the value of TBARS to 1.32 (mg MDA kg⁻¹ meat), followed by samples treated with sodium nitrite after 90 days of storage the value was 1.23 (mg MDA kg⁻¹ meat), and at the end of the storage period, the value of TBARS increased to 0.938 (mg MDA kg⁻¹ meat) in samples treated with 0.5% lemon oil.

All samples treated with orange and lemon essential oil in different concentrations at the end of the storage period were in compliance with the (ESS 1694/2005) for meat products, which recommended that the value of TBARS is less than 0.9 mg malonaldehyde/kg meat (MDA kg⁻¹ meat).

It should be noted that samples with high concentration (1.5%) of orange and lemon essential oils showed good quality till 90 day freeze storage. The high efficiencies found in orange and lemon essential oil have verified the main function of these phenolic compounds as free radical scavengers and as primary, chain-breaking antioxidants (Hanan, et al., 2013 and Trabelsi, 2014). These findings are similar to that obtained by Klangpetch et al. (2016) who reported that TBARS values increased significantly in control sample (2.5 mg MDA/kg) but remained relatively

low in lime peel treated samples with no substantial variations at all concentrations (sample of ١,٥-١,٨ mg MDA/kg).

٢-Microbial Assessment of beef burger samples treated with different concentration of orange and lemon essential oils.

Total Aerobic bacterial count of beef burger samples treated with different concentration of orange and lemon essential oils.

Table (٢) Total Aerobic bacterial count (cfu/g) of beef burger samples treated with different concentration of orange and lemon essential oils during storage period at $-1 \pm 1^{\circ}\text{C}$ for ٩٠ days.

Treatmet	Storage period (day)						
	٠	١٥	٣٠	٤٥	٦٠	٧٥	٩٠
T١	$3,0 \times 10^4 \pm 1,1 \times 10^4$ ghij	$2,0 \times 10^4 \pm 2 \times 10^3$ a	$2,0 \times 10^4 \pm 3,6 \times 10^3$ hij	$1,6 \times 10^4 \pm 4 \times 10^3$ ij	$1,4 \times 10^4 \pm 7 \times 10^3$ ij	$1,2 \times 10^4 \pm 3,6 \times 10^3$ ij	$1,0 \times 10^4 \pm 2,6 \times 10^3$ j
T٢	$2,0 \times 10^4 \pm 7,2 \times 10^3$ a	$2,0 \times 10^4 \pm 3,6 \times 10^3$ ahij	$1,0 \times 10^4 \pm 6,0 \times 10^3$ ij	$1,0 \times 10^4 \pm 0,1 \times 10^4$ ij	$8,0 \times 10^3 \pm 3,6 \times 10^3$ ij	$5,0 \times 10^3 \pm 2 \times 10^3$ ij	$2,1 \times 10^3 \pm 1 \times 10^3$ j
T٣	$2,0 \times 10^4 \pm 1 \times 10^4$ hij	$1,0 \times 10^4 \pm 3,2 \times 10^3$ b	$1,4 \times 10^3 \pm 0 \times 10^3$ ij	$1,0 \times 10^3 \pm 0 \times 10^3$ j	$6,0 \times 10^3 \pm 2,0 \times 10^3$ ij	$3,0 \times 10^3 \pm 2 \times 10^3$ j	$1,0 \times 10^3 \pm 0,7 \times 10^3$ j
T٤	$1,0 \times 10^4 \pm 2 \times 10^3$ ij	$1,3 \times 10^4 \pm 2,6 \times 10^3$ i	$8,4 \times 10^3 \pm 3,0 \times 10^3$ ij	$7,2 \times 10^3 \pm 2 \times 10^3$ i	$6,4 \times 10^3 \pm 4,0 \times 10^3$ cdef	$5,3 \times 10^3 \pm 2,6 \times 10^3$ ij	$4,0 \times 10^3 \pm 2 \times 10^3$ j
T٥	$1,3 \times 10^4 \pm 4,0 \times 10^3$ ij	$1,0 \times 10^4 \pm 3 \times 10^3$ ij	$8,0 \times 10^3 \pm 4,0 \times 10^3$ ij	$6,6 \times 10^3 \pm 2,0 \times 10^3$ cdef	$5,0 \times 10^3 \pm 0,7 \times 10^3$ ij	$4,2 \times 10^3 \pm 2,6 \times 10^3$ j	$3,2 \times 10^3 \pm 2 \times 10^3$ j
T٦	$1,1 \times 10^4 \pm 3,6 \times 10^3$ ij	$9,1 \times 10^3 \pm 2,6 \times 10^3$ i	$7,0 \times 10^3 \pm 4,0 \times 10^3$ ij	$6,2 \times 10^3 \pm 2,0 \times 10^3$ cde	$5,0 \times 10^3 \pm 2,0 \times 10^3$ j	$3,7 \times 10^3 \pm 2,6 \times 10^3$ defghij	$2,1 \times 10^3 \pm 1,7 \times 10^3$ j
T٧	$8,0 \times 10^3 \pm 2,0 \times 10^3$ c	$7 \times 10^3 \pm 2 \times 10^3$ cd	$4,4 \times 10^3 \pm 2,0 \times 10^3$ defghi	$3,3 \times 10^3 \pm 2,1 \times 10^3$ efghij	$2,1 \times 10^3 \pm 7 \times 10^3$ hij	$8,0 \times 10^3 \pm 3,6 \times 10^3$ ij	$2,7 \times 10^3 \pm 1,1 \times 10^3$ j
Control	$8,1 \times 10^3 \pm 3,1 \times 10^3$ d	$7,3 \times 10^3 \pm 2,0 \times 10^3$ c	$6,1 \times 10^3 \pm 2,6 \times 10^3$ cdefg	$5,6 \times 10^3 \pm 3,0 \times 10^3$ cdefgh	$3,6 \times 10^3 \pm 6,1 \times 10^3$ ghi	$2,1 \times 10^3 \pm 6,0 \times 10^3$ ij	$1,3 \times 10^3 \pm 1,6 \times 10^3$ i

E.S.S

١٠٥

T^١: Beef burger treated with ٠,٥% orange essential oils, T^٢: Beef burger treated with ١,٠ % orange essential oils, T^٣: Beef burger treated with ١,٥% orange essential oils, T^٤: Beef burger treated with ٠,٥% lemon essential oils, T^٥: Beef burger treated with ١,٠% lemon essential oils, T^٦: Beef burger treated with ١,٥% lemon essential oils, T^٧: Beef burger treated with nitrite ٢٠٠ppm, C: Control beef burger without any addition. Values with different superscript letters in a column are significantly different ($P \leq ٠,٠٥$).

The results illustrated in Table (٢) proved that the total Aerobic bacterial count (cfu/g) of beef burger samples treated with different concentration of orange and lemon essential oils decreased during freezing storage period (٩٠ days). As for the beef burger samples treated with orange essential oils, the samples treated with the highest concentration of ١,٥% had the lowest microbial counts, whether at zero days or at the end of the storage period the values were $٢,٠ \times ١٠^٤ \pm ١ \times ١٠^٤$ and $١,٠ \times ١٠^٣ \pm ٥,٧ \times ١٠^٢$ cfu/g respectively, compared to the other concentrations ٠,٥ and ١%, which the counts in the end of ٩٠ days of storage were $١,٠ \times ١٠^٤ \pm ٢,٦ \times ١٠^٣$ and $٢,١ \times ١٠^٣ \pm ١ \times ١٠^٣$ cfu/g respectively. Organ essential oils has strong antimicrobial activity against the most Gram-positive and Gram-negative bacteria (Trabelsi, ٢٠١٤)

From Table (٢), it was clear that as well as beef burger samples treated with lemon essential oils, samples treated with the highest concentration of ١,٥% had the lowest microbial counts, whether at zero days ($١,١ \times ١٠^٤ \pm ٣,٦ \times ١٠^٣$) or at the end of the storage period ($٢,١ \times ١٠^٣ \pm ١,٧ \times ١٠^٣$), compared to the other of the concentrations ٠,٥ and ١%, which the counts in the end of storage period were $٤,٠ \times ١٠^٣ \pm ٢ \times ١٠^٣$ and $٣,٢ \times ١٠^٣ \pm ٢ \times ١٠^٣$ cfu/g respectively. There is antibacterial activity of the lemon essential oil against both Gram-positive and Gram-negative bacterial (Ali, ٢٠١٨).

While the beef burger samples treated with nitrite salts (٢٠٠ ppm) and the control sample (without any addition), the microbial counts were $٨,٥ \times ١٠^٤ \pm ٢,٥ \times ١٠^٤$ and $٨,١ \times ١٠^٤ \pm ٣,١ \times ١٠^٤$ cfu/g respectively, at zero day of storage and decreased during storage until it reached $٢,٧ \times ١٠^٣ \pm ١,١ \times ١٠^٣$ and $١,٣ \times ١٠^٤ \pm ١,٦ \times ١٠^٣$ cfu/g respectively, at the end of the ٩٠ days of storage. It is understood that microbial growth declines at around -١٨°C , but even at the lowest cold storage temperature, most bacteria are not killed (Ranken ٢٠٠٠). All samples were in compliance with the

Egyptian specifications (١٦٨٨/٢٠٠٥), which allow a total Aerobic bacterial count of beef burger was 10^6 cfu/g. The results in the presented study are consistent with that recorded by Mousa, et al., (٢٠١٤) who noticed that the total Aerobic bacterial count in beef burger samples, ranged from 9.0×10^1 to 4.39×10^2 cfu/g.

Total Coliforms count of beef burger samples treated with different concentration of orange and lemon essential oils.

Table (٣) Total Coliforms count (cfu/g) of beef burger samples treated with different concentration of orange and lemon essential oils during storage period at $-18 \pm 1^\circ\text{C}$ for ٩٠ days.

Treatment	Storage period (day)						
	٠	١٥	٣٠	٤٥	٦٠	٧٥	٩٠
T١	4.5 ± 1.5^{bc}	2.4 ± 1.4^{fghij}	1.4 ± 0.9^{ijkl}	.	.	.	.
T٢	3.5 ± 1.5^{cdef}	2.0 ± 1.2^{ghijk}	1.3 ± 0.6^{jkl}	.	.	.	.
T٣	2.0 ± 1.0^{ghijk}	1.3 ± 0.2^{ghijk}	.	.	.	.	.
T٤	4.5 ± 1.5^{bc}	2.7 ± 1.2^{efghi}	1.5 ± 0.7^{hijk}	.	.	.	.
T٥	4.2 ± 1.2^{cd}	2.3 ± 1.4^{fghijk}	1.0 ± 0.4^{kl}	.	.	.	.
T٦	3.0 ± 0.5^{defg}	1.1 ± 0.6^{jkl}	.	.	.	.	.
T٧	4.4 ± 1.4^{bc}	2.8 ± 1.8^{efgh}	$1.5, 1.6 \pm 0.5^{hijk}$	.	.	.	.
Control	9.0 ± 3.0^a	5.5 ± 0.5^b	3.9 ± 1.4^{cde}	.	.	.	.
E.S.S	١.٢						

T١: Beef burger treated with ٠.٥% orange essential oils, T٢: Beef burger treated with ١.٠ % orange essential oils, T٣: Beef burger treated with ١.٥% orange essential oils, T٤: Beef burger treated with ٠.٥% lemon essential oils, T٥: Beef burger treated with ١.٠% lemon essential oils, T٦: Beef burger treated with ١.٥% lemon essential oils, T٧: Beef burger treated with nitrite ٢٠٠ ppm, C: Control beef burger without any addition. Values with different superscript letters in a column are significantly different ($P \leq 0.05$).

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Coliforms is a group of organisms is often used as hygiene indicator organisms. Table (٣) showed the total Coliforms count of beef burger samples treated with different concentration of orange and lemon essential oils during freezing storage period which decreased until it reached to zero before the end of the storage period.

Samples of beef burger treated with ١,٥% orange essential oils had the lowest total Coliforms counts at zero days ٢٠ ± ١٠ cfu/g, followed by beef burger samples treated with ١,٥% lemon essential oils ٣٠ ± ٥ cfu/g. The counts decreased after ٣٠ days of freezing storage to zero, while in the other treatments it reached zero after ٤٥ days of storage by freezing. All samples were in agreement with the Egyptian specifications (١٦٨٨/٢٠٠٥), which allow a total coliform group count of beef burger was $١٠^٢$ cfu/g. There is a clear significant difference between the control sample and the other treatments, while there are no clear significant differences between the other treatments, and each other. These results are much lower than those recorded by Mousa, et al. (٢٠١٤) who denoted that the total Coliforms count of beef burger ranged from $٩,٠ \times ١٠$ to $١,٩٨ \times ١٠^٣$ with a mean value of $٢,٩٢ \times ١٠^٢$ cfu/g.

Total *Staphylococcus aureus* count of beef burger samples treated with different concentration of orange and lemon essential oils.

Table (٤) Total *Staphylococcus aureus* count (cfu/g) of beef burger samples treated with different concentration of orange and lemon essential oils during storage period at $-١٨ \pm ١^\circ\text{C}$ for ٩٠ days.

Treatment	Storage period (day)						
	٠	١٥	٣٠	٤٥	٦٠	٧٥	٩٠
T١	٩٠ ± ٢^{ab}	٧٤ ± ٢^{bcde}	٣٤ ± ١^{ghij}	١٨ ± ٣^{ijkl}	٠	٠	٠
T٢	$٨٤ \pm ٣,٦^{abc}$	٦٧ ± ٢^{cde}	٤٢ ± ١^{efgh}	١٦ ± ٦^{ijkl}	٠	٠	٠
T٣	$٦٥ \pm ٥,٥^{cde}$	٤١ ± ١^{fgh}	٢٨ ± ٨^{hijk}	٩ ± ٤^{lk}	٠	٠	٠
T٤	$٧٣ \pm ٣,٦^{bcde}$	٥٧ ± ١^{ef}	٣١ ± ١^{ghij}	١٨ ± ٣^{ijkl}	٠	٠	٠
T٥	٦٥ ± ٥^{cde}	٤٣ ± ١^{fgh}	٢٤ ± ٩^{hijk}	١٥ ± ٥^{ijkl}	٠	٠	٠

T١	٤٤,٣±٤,١ ٦ fg	٢٧±٧ ghijk	١١±٢ kl	٠± ^l	٠	٠	٠
T٧	٨٤±٣,٦ abc	٦٣±١٣ de	٤٢±١٢ fgh	٢١±٦ ijkl	٠	٠	٠
Control	٩٨±٣,٦ a	٨١±٤٧,٤ ٦ abcd	٦٥±١٥ cde	٣٥±١٥ ghi	٠	٠	٠

T١: Beef burger treated with ٠,٥% orange essential oils, T٧: Beef burger treated with ١,٠ % orange essential oils, T٣: Beef burger treated with ١,٥% orange essential oils, T٤: Beef burger treated with ٠,٥% lemon essential oils, T٥: Beef burger treated with ١,٠% lemon essential oils, T٦: Beef burger treated with ١,٥% lemon essential oils, T٧: Beef burger treated with nitrite ٢٠٠ppm, C: Control beef burger without any addition. Values with different superscript letters in a column are significantly different ($P \leq ٠,٠٥$).

Staphylococcus aureus can be responsible for food poisoning. And if it develops in large numbers, toxins may be left in the product that can withstand the heating process. Table (٤) showed the total *Staphylococcus aureus* of beef burger samples treated with different concentration of orange and lemon essential oils during ٩٠ days of freezing storage. As it is evident from the figure that the lowest count of *Staphylococcus aureus* was for samples of beef burger treated with lemon essential oil ١,٥%, the count was ٤٤,٣±٤,١٦ cfu/g in zero day of storage. Followed by samples treated with ١,٥% orange essential oil, the count was ٦٥±٥,٥٦ cfu/g during the same storage period. This may be due to the antimicrobial effect of orange and lemon essential oil (Ali, (٢٠١٨); Edogbanya, et al., (٢٠١٩) and Teneva, et al., (٢٠١٩). The control samples had the highest count of st. aureus (٩٨±٣,٦ cfu/g), followed by the samples treated with orange oil ٠,٥% (٩٠±٢ cfu/g) at zero days. During storage by freezing, the counts decreased to zero before the end of the period. All samples are in compliance with the E.S.S. (١٦٨٨/٢٠٠٥), which allow up to ١٠٠ cfu/g.

Sensory evaluation.

Table (٩) Sensory evaluation of beef burger samples treated with different concentration of orange and lemon essential oils during storage period at -١٨±١°C for ٩٠ days.

Treatment	Component %			
	Color	Taste	Odour	tenderness
T١	٨,٤±١,٢٢ ab	٧,٦±١,٢٦ b	٧,٧±٠,٦٧ ab	٨,٢±١,١٣ a

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T٢	٨,٢±٠,٩٦ab	٧,٥±١,٢٦b	٧,٤±٠,٩٦b	٧,٨±٠,٩١a
T٣	٧,٣±١,١٥bc	٦,٠±١,١٥c	٧,١±٠,٧٣bc	٧,٥±١,٠٨ab
T٤	٦,٧±١,٧٦c	٥,٤±١,٣٤c	٦,١±١,٢٨cd	٦,٣±١,٨٨bc
T٥	٦,٩±١,٥٢c	٥,٤±١,٥٧c	٥,٤±١,٧١d	٥,٨±٢,٠٤c
T٦	٦,٨±٢,٠٩c	٤,٩±٠,٧٣c	٥,٢±٢,٠٤d	٥,٨±١,٦١c
T٧	٩,٢٥±٠,٦٣a	٨,٨±٠,٦٣a	٨,١±٠,٨٧ab	٨,٢±١,٢٢a
Control	٩,٣٥±٠,٧١a	٨,٨±٠,٧٨a	٨,٧±٠,٩٤a	٨,٢±١,٦١a

T١: Beef burger treated with ٠,٥% orange essential oils, T٢: Beef burger treated with ١,٠ % orange essential oils, T٣: Beef burger treated with ١,٥% orange essential oils, T٤: Beef burger treated with ٠,٥% lemon essential oils, T٥: Beef burger treated with ١,٠% lemon essential oils, T٦: Beef burger treated with ١,٥% lemon essential oils, T٧: Beef burger treated with nitrite ٢٠٠ppm, C: Control beef burger without any addition. Values with different superscript letters in a column are significantly different ($P \leq 0,05$).

The results of the sensory properties analysis are presented in Table ٩. The mean values of color, texture, odour and tenderness were evaluated. The values of samples treatment T١, T٢ and T٧ were ٨,٢, ٨,٤ and ٩,٢٥ respectively, where it was close to control sample (٩,٣٥) in color attribute, there was no significant difference ($p \geq 0,05$) among the samples. While there was significant difference ($p \geq 0,05$) between these treatment and other treatment i.e. T٣, T٤, T٥ and T٦, which the values were ٧,٣, ٦,٧, ٦,٩ and ٦,٨ respectively. In taste attribute there was no significant difference ($p \geq 0,05$) among control and samples treatment T٧, while there was significant difference ($p \geq 0,05$) among samples treatments T١ and T٢ with T٣, T٤, T٥ and T٦. All Samples treatment have an acceptable taste because they have values higher than ٤. Control and samples treatments T٧, T١, T٢ and T٣ had the highest values of taste attribute (٨,٨, ٨,٨, ٧,٦, ٧,٥ and ٦,٠ respectively), while the lowest values were ٥,٤, ٥,٤ and ٤,٩ for samples treatments T٤, T٥ and T٦ respectively.

In odour attribute, there was significant difference ($p \geq 0,05$) between samples treatments T١, T٧ and control with other treatments. The highest values of odour attribute were ٨,٧, ٨,١, ٧,٧, ٧,٤, ٧,١ and ٦,١ for control, T٧, T١, T٢, T٣ and T٤, While the lowest values for samples treatment T٥ and T٦ (٥,٤ and ٥,٢).

Tenderness, there was significant difference ($p \geq 0,05$) between samples treatments T١, T٢, T٧ and control with other treatments. Control and samples treatments T٧, T١, T٢, T٣ and T٤ had the maximum values of ٨,٢, ٨,٢, ٨,٢, ٧,٨ and ٦,٣, while the minimum values of ٥,٨ and ٥,٨

for sample treatments T^٥ and T^٦. The values of sensory attributes obtained were significantly similar with that reported by Ibrahim, et al. (٢٠١٨).

Conclusion

Adding the essential oil of orange and lemon to the beef burger at concentrations of ٠,٥, ١, and ١,٥ % cause a decrease in the values of TVBN and TBA during the storage period for ٩٠ days in freezing, compared to the high control values of these estimates due to the effect of these essential oils as a natural antioxidant.

Also, the addition of these essential oils in the same proportions led to a decrease in the microbial numbers of total aerobic count, coliform group and staphylococcus aureus count compared to the control, which had high values for these estimates due to the action of these essential oils as natural antimicrobials.

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