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RESEARCH ARTICLE

EFFECT OF VOLATILE OILS MIXTURE AND LASER RADIATION ON LIPID OXIDATION AND SHELF LIFE OF BEEF SAUSAGE DURING CRYOPRESERVATION

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Abstract

The aim of this research is to study the chemical components of cinnamon, rosemary, and clove volatile oils by GC/Mass as well as the antioxidant activity of these oils and their mixture by DPPH scavenging to select the best volatile oil or mixture for preparing beef sausage treated with laser rays (Helium-Neon laser wavelength 632.8 nm for 9 min for each side). Also, to study the effect of volatile oils mixture at 500 ppm and laser radiation on lipid oxidation and shelf-life of beef sausage during cold storage. Results indicated that 23, 10, and 18 compounds were identified from cinnamon, rosemary, and clove which represented 97.26, 98.50 and 98.80 %, respectively. Cinnamaldehyde, 1, 8 Cineole, and Eugenol were the most abundant chemical compounds in cinnamon, of cinnamon, rosemary, and clove volatile, respectively. Volatile oils mixture had significantly higher antioxidant activity than each volatile oil individual but significantly lowers than BHT as a synthetic antioxidant substance at all studied concentrations. Lipid oxidation (peroxide value, thiobarbituric acid, and acid value) of sausage treatments were not affected significantly by laser irradiation or volatile oils mixture immediately after processing (at zero time) but affected ($p<0.5$) during cold storage. Sausage treated with both laser irradiation and volatile oils mixture had significantly lower peroxide value, thiobarbituric acid and acid value than other sausage treatments at any time of cold storage. Also, laser irradiation plus the addition of volatile oils mixture caused high storage stability which increase the shelf-life of sausage to 20 days at 4±1ºC compared with sausage treated with volatile oils mixture only (15 days), sausage treated with laser rays only (10 days) and control sample (5 days).

Introduction:-

Lipid oxidation is a major cause of deterioration in meat and meat products due to their high fat content and low water activity leading to loss of nutritional value, unpleasant flavor and texture, and water-holding capacity **(Garcia-Lomillo et al., 2017; Ding et al., 2015).**

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In recent years, in order to control the spoilage of meat products, the formulation and production of novel processed meat, with functional properties and without chemical preservatives, has been one of the main priorities for the research and development section of meat industries to respond to the green marketing and consumerism demand **(Alirezalu et al., 2021; Pereira et al., 2019).**

Meat and meat products are an important source of essential amino acids, proteins, fat, and mineral. There are a wide variety of meat products including cured meats, patties, nuggets, meatballs, sausage and etc.(**Aminzare et al., 2016).**

Fresh sausage is one of the world's most popular processed meat products that are cheap, delicious, easily prepared, and can solve the problem of shortage of fresh meat. **(Dinstel, 2014, Sharma et al., 2017).**Meat and meat products, such as sausages, considered important food products have a lot of socioeconomic impacts. Regrettably, meat products are classified as perishable foods due to their rapid spoilage and safety challenges. The main factors causing the spoilage of sausage and reducing its shelf life are microbial growth and lipid oxidation **(Luong et al., 2020; Zehi et al., 2020).**

Essential oils (EOs) are natural compounds and have been widely used in processed meats due to their antibacterial, antifungal, and antioxidant properties. The addition of Eos to sausages formulation improves their microbial and chemical stability and safety **(Šojić et al., 2021; Tomović et al., 2020 Mohajer et al., 2021).**

EOs and plant extracts are commonly used as natural antioxidant, antimicrobial, and flavoring agents to enhance product quality in addition to extending shelf life by delaying microbial and oxidative reactions **Ghanbari et al. (2020).** However, they may have undesirable influences on the sensorial properties of meat products in relatively high concentrations **Rezaeifar et al. (2020).**

A promising approach to overcome this restriction is the application of packaging materials as carriers of these agents **Hashemi et al. (2020).**

Recently, the incorporation of EOs and plant extracts into the edible coating andfilms, named active packaging, have been investigated in several studies for the preservation of food products, **Langroodi et al. (2018).**

Du and Sun, (2005) reported that the world has been looking for development in technology. LASER is one of these technologies, and there are many applications in several fields such as agriculture, communication, and medicine.

Arthur et al. (2005) cleared that the low-dose, low-penetration electron beam (E-beam) irradiation has great potential as an antimicrobial intervention in the beef slaughter process. Because contamination of beef carcasses by pathogenic bacteria occurs on the external surface, a broadspectrum antimicrobial intervention that produces large reductions in pathogen load while minimally affecting the carcass would be ideal.

Antioxidant effects of essential oils from rosemary (*Rosmarinus officinalis*), clove (*Syzygium aromaticum*), and cinnamon (*Cinnamomum zeylanicum*) were determined on hazelnut and poppy oils. These essential oils were added to the oils at concentrations of 0.25% and 0.5%. Butylated hydroxyanisole (BHA) at 0.02% level served as standard besides the control groups for comparison. The samples were stored at 50 °C in darkness for 14 days. The antioxidant activity of the essential oils was determined by measuring peroxide values (meq O2/kg oil) at regular intervals. Based on the peroxide value assay, the essential oils showed a stronger antioxidant effect when compared to the control groups. BHA was more effective than the essential oils, whilst it exhibited no antioxidative effect on the first few days of storage. Amongst the investigated essential oils, cinnamon oil was the most effective on retarding lipid oxidation of crude oils, which was followed by clove and rosemary oils **(Özcan and Arslan, 2011).**

Therefore, the present study was carried out to identify the chemical components of cinnamon, rosemary, and clove volatile oils as well as the antioxidant activity of these oils and their mixture to investigate the utilization of their mixture in the shelf life extension of beef sausage treated with or without laser rays during cold storage at 4±1C°.

Materials and Methods:-

Materials

Meat and fat tissues:

Frozen beef and fat tissues (sheep tail) were purchased from the private sector shop in the local market at Giza, Egypt and immediately transported in an ice box to the laboratory, then carefully cut into fillets and finally weighed until use. Packaging materials natural casings of 20 ± 2 mm diameter were purchased at the neighborhood market for Attaba in Cairo, Egypt. Spice mixture, was purchased at the neighborhood market for Cairo, Egypt. Other ingredients such as texturized soy, salt, bread crust, ground onion, and polyethylene bags were purchased from the local market, in Giza, Egypt. Three different volatile oils (cinnamon bark, clove, and rosemary) were purchased from Kato Flavors & Fragrance Company at Shooting Club Street, Mohandessn, Giza, Egypt.

Chemicals:

2, 2-Diphenyl-1-Picryl-Hydrazyl (DPPH), Tween 20, and 2-thiobarbituric acid all were obtained from Sigma-Aldrich Chime, Steinheim, Germany. Hydrochloric acid, ethanol, sulfuric acid, sodium hydroxide, chloroform, glacial acetic acid, potassium iodide, starch, sodium thiosulfate, and phenolphthalein all were obtained from El-Nasser pharmaceutical Chemical Co., Egypt.

Methods:

Tenchnological methods

Preparation of beef sausage treatments :

Beef sausages were processed as described by **Osheba *et al.*, (2013)** with some modifications. Two batches of beef sausage were prepared. The first batch was prepared according to the following formula: Minced beef meat (60%), minced fat tissue (17.01%), water (7.0%), rehydrated texturized soy (8.0%), bread crust (1.40%), skimmed milk (1.70%), fresh onion (1.50%) salt (1.90%) and spices mixture (1.50%). Minced meat and fat tissues were ground once with the ice flakes by using mincer. The other ingredients were added and mixed together. The mixture was ground twice using a laboratory emulsifier (Hobbart Kneading machine) for 10 minutes. The obtained emulsion was then stuffed into a nature casings which were hand linked at about 10 cm intervals. The second batch was prepared using the same formula plus adding 500 ppm volatile oils mixture (cinnamon, clove and rosemary at ratio 1:1:1, v: v: v). Each batch was divided into two groups, the first was untreated and the second group was exposed to Helium-Neon laser for 18 minutes at wavelength 632.8 nm . All beef sausage samples were packaged in a foam plates and stored at 4±1˚C up to 25 days. The samples were taken for analysis every 5 days periodically.

Analytical methods:-

Gas chromatography–mass spectrometry analysis (GCMS)

The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Abd El-Motaleb et al., (2021). Samples were diluted with hexane (1:19, v/v). The GC was equipped with an HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:10, injection volume of 1 µL and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 5 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 40-550 and solvent delay of 3 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Antioxidant activity of volatile oil (DPPH radical scavenging assay):

The free-radical scavenging activity of volatile oilswas measured as a decrease in the absorbance of methanolsolution of DPPH as reported by **Sreejayan and Rao (1996)**.Scavenging activity was expressed as the percentageinhibition calculated using the following formula:

$$\%\text{Anti-radical activity} = \frac{\text{Control Absorbance} - \text{Sample absorbance}}{\text{Control Absorbance}} \times 100$$

Thiobarbituric acid (TBA):

The TBA as an indication for lipid oxidation was determined according to the method described by **Kirk and Sawyer (1991)**.Thiobarbituric an acid value sample was determined calorimetrically method. The TBA values were expressed as mg malonaldehyde/kg of sample.

Peroxidevalue:
Lipid was extracted from the mixed sausage treatments with a mixture of chloroform / methanol (2: 1 v/v) according to the method described by Folch et al. (1957). Peroxide value (PV) was expressed in units meq /kg lipid was determined by the titration method (Kirk and Sawyer, 1991).

Acid value (A.V)
Acid value of the fat extracted from beef sausage was determined according to the **A. O. A. C. (2016)**.The extraction of lipid from beef sausage was carried out according to the method described by **Folch et al. (1957).**

Statistical analysis:
The obtained results were analyzed using comparison of variance (ANOVA) and least significant different (L.S.D) at the 5% level of probability; as reported by **Snedecor and Cochran (1994).**

Results and Discussion:-

Fractionation and identification of cinnamonvolatile oil byGC/MS:
From Table (1), it could be noticed that 23 compounds were fractionated from cinnamon volatile oil all of them were identified. The identified components represented (97.26 %) of the cinnamon volatile oil. Cinnamaldehyde was the major component of cinnamon volatile oil which represented 72.11% of the total identified chemical compounds followed by Benzaldehyde, 2- hydroxyl (4.37%).

Table (1):- Fractionation and identification of cinnamon volatile oil byGC/MS.

No.	Volatile oil components	Cinnamon volatile oil	
		RT*	Area %
1	Styrene (cymene)	8.32	2.37
2	Linalyl acetate	8.53	0.62
3	α-pinene	9.53	0.79
4	Benzaldehyde phenyl menthanol	10.91	2.87
5	Benzaldehyde	11.52	0.79
6	1, 8 Cineole	12.78	0.52
7	Benzaldehyde, 2- hydroxyl	13.51	4.37
8	2-H-1- benzopyran	16.48	0.64
9	Benzenepropanal	17.18	0.84
10	Phenyl acetate	19.00	0.86
11	Cinnamaldehyde	30.32	72.11
12	Δ- cadinene	32.14	0.97
13	Para- methoxy cinnamic aldehyde	33.95	1.04
14	Para-3- methoxy cinnamic aldehyde	34.45	0.95
15	Cinnamaldehyde –O-methoxy	34.53	1.21
16	Nerolidol	34.80	1.44
17	Spathulenol	35.59	0.98
18	Phellandrene, 7 ethenyl- 1	51.15	0.76
19	Naphthalene, decylhydro, 1 trimethyl	51.67	0.56
20	Azulene	54.60	0.97
21	N- acety – 8,13- imino	55.28	0.37
22	1-O-methoxy benzyl-naphthalene	61.98	0.64
23	Di- (2-ethylhexy) phthalate	64.37	0.59
	Total known		97.26
	Total unknown		2.74

*** RT: Retention time.**

Among the most abundant chemical components in the volatile oil of cinnamon, styrene (cymene) was the least prevalent (2.37% of all identified chemical compounds). These findings are consistent with those made by **Li et al. (2013); Lv et al. (2011); Radi et al. (2022) and Filho et al. (2022),** who found that cinnamaldehyde was the main compound in cinnamon essential oil with the highest content (71.3%) with ultrasound-enhanced subcritical water extraction and the lowest with ultrasoudal ultrasound.

Fractionation and identification of rosemary volatile oil byGC/MS:

The fractionated and identified chemical components of rosemary volatile oil were presented in Table (2). From these data, it could be noticed that ten compounds were isolated and identified from rosemary volatile oil. The identified components represented (98.50%) of the rosemary volatile oil. 1, 8 Cineole, α-pinene, and camphor were the most abundant chemical compounds in rosemary volatile oil which represented (88.53%) of the total identified chemical compounds. 1, 8 Cineole (55.63%) was the main component of rosemary volatile oil followed by camphor (22.64%)and α-pinene (10.26%) of the total chemical compounds was the lowest one among the most permanent chemical compounds in rosemary volatile oil. These results are in agreement with those obtained by Puvaˇca et al. (2021) reported that the main component of rosemary essential oil was 1,8 cineole(64.02%), Limonene (11.86%)and α-pinene (8.38%) which have an anti-bacterial effect. Fadel et al. (2020) reported that 1, 8-Cineol was the predominant compound followed by camphor, α-terpineol, α-pinene, bornyl acetate, and borneol.

Table (2):- Fractionation and identification of rosemary volatile oil byGC/MS.

No.	Volatile oil components	Rosemary volatile oil	
		RT*	Area %
1	α-pinene	5.76	10.26
2	camphene	6.33	5.42
3	β-pinene	7.01	0.35
4	d-limonene	8.49	0.66
5	1, 8 Cineole	8.63	55.63
6	Camphor	13.21	22.64
7	Endo borynyl acetate	19.72	0.32
8	Benzene – 1- methyl	21.78	2.81
9	Trans -caryophyllene	25.30	0.30
10	1,4 pentadiene	32.41	0.11
	Total known		98.50
	Total unknown		1.50

*** RT: Retention time.**

Fractionation and identification of clove volatile oil byGC/MS:

The chemical constituents of clove bud volatile oil were fractionated and identified by GC/Mass technique. From the results tabulated in Table (3), it could be noticed that 18 compounds were isolated from clove volatile oil all of them were identified. The identified components represented (98.80%) of the clove volatile oil. Eugenol (80.51), β-Caryophellene(7.15%) , Caryophyllene oxide (3.58%), and α- Caryophyllene (0.87%) were the most abundant chemical compounds in clove volatile oil which represented (92.11%)of the total identified chemical compounds. Eugenol (80.51%) of the total chemical compounds) was the highest chemical compound in clove volatile oil. However, Caryophyllene oxide (3.58%) of the total chemical compounds was the lowest one among the most abundant chemical compounds in clove volatile oil. This result complies with those obtained by **Paiano et al. (2020); de Almeida et al. (2023)** stated that eugenol (86.25%) was the major compound of clove EO and in the same trend **(Boˇzik et al., 2017)** found that eugenol (81.74%) was identified as the major compound of clove EO. Also, Eycolyptol (0.86%) , α-cubebene (0.85%), Aromadendrene oxide(0.75%) , α-farnesene (0.69%) , Trans-Caryophellene (0.64%), Isoleden (0.60%) , Acetic acid- phenyl-methyl esters(0.58%), Naphthalene(0.56%) and other compounds were lessened than 1% in clove volatile oil which represented 7.56 % of the identified chemical compounds. These results are in accordance with those obtained by **Fadel et al. (2020)** reported that the seven volatile compounds identified in the hydrodistilled oil of clove buds were representing 99.9% of the total oil. Eugenol was the major compound (89.9%) followed by eugenol acetate (7.9%), b-caryophyllene (1.4%), and humulene (0.4%). Also, β-myrcene (1.34 %) , α-pinene (7.68%), 4-carene (1.25%) , α-cubebene (1.85%). trans-caryophyllene (3.64%), caryophyllene oxide (4.58%) , eycolyptol (2.86%). α-farnesene (3.69%).,benzoic acid (3.15%), nonanone (1.18%), isoledene (1.60%), and naphthalene (2.56%)

were present in moderate amounts in clove volatile oil which represented 15.81% of the identified chemical compounds.

Table (3):- Fractionation and identification of clove volatile oil byGC/MS.

No.	Volatile oil components	Clove volatile oil	
		RT*	Area %
1	α-pinene	7.57	0.18
2	β -Myrcene	9.05	0.34
3	4-carene	9.69	0.15
4	Eycolyptol	10.07	0.86
5	Nonanone	11.65	0.18
6	Acetic acid- phenyl-methyl esters	13.63	0.58
7	Benzoic acid	14.41	0.15
8	α-cubebene	17.81	0.85
9	Eugenol	18.78	80.51
10	β-Caryophellene	20.29	7.15
11	α- Caryophellene	20.40	0.87
12	Trans-Caryophellene	20.50	0.64
13	Isoleden	21.00	0.60
14	Naphthalene	21.06	0.56
15	Aromadendrene oxide	21.42	0.75
16	α-farnesene	21.47	0.69
17	Caryophellene oxide	23.21	3.58
18	Eugenol acetate	30.29	0.16
	Total known		98.80
	Total unknown		1.20

*** RT: Retention time.**

Antioxidant activity of volatile oils:

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating the free radical-scavenging activity of antioxidants (Fenglin et al., 2004). Data presented in Table (4) showed the antioxidant activity of cinnamon, clove, and rosemary volatile oils and a mixture of them (1:1:1, v: v: v) ability to scavenge DPPH free radicals in comparison to BHT as a synthetic antioxidant. From these data, it could be noticed that all studied samples exhibited good radical scavenging activity with different degrees. The inhibition percentages of DPPH free radicals were increased by increasing concentrations of BHT and a mixture of individual volatile oils. The highest scavenging activity on DPPH radicals (% inhibition 44.66, 70.92, 85.34, and 92.43%) was recorded for BHT followed by volatile oils mixture (36.50, 61.00, 78.32, and 89.32 %) at concentrations (200, 400, 600 and 800 mg/ml, respectively) with significant differences ($p \leq 0.05$) between them. Also, volatile oils mixture had significantly higher antioxidant activity than each volatile oil individually, which might be due to volatile oils mixing led to synergistic or potentiating effect **(Burt, 2004and Sharma, 2020).** Moreover, cinnamon volatile oil had significantly higher antioxidant activity when compared with clove volatile oil and rosemary volatile oil at any studied concentration. These results are in agreement with those obtained by Wang et al. (2008) found that cinnamon volatile oil had the highest DPPH radical scavenging activity when compared with clove, ginger, cardamom, and coriander volatile oils. Finally, the DPPH free radical scavenging activities for all studied samples are sorted descending as follows: BHT > volatile oils mix >cinnamon >clove > rosemary .

Table (4):- Antioxidant activity of different volatile oils and their mixtures.

Concentrations (mg/ml)	DPPH -free radical scavenging activity (%)					L.S.D at 0,05%
	Cinnamon volatile oil	Rosemary volatile oil	Clove volatile oil	Volatile oils mix.	BHT	
200	$24.29^{C}\pm0.23$	$13.25^{E}\pm0.21$	$19.85^{D}\pm0.60$	$36.50^{B}\pm0.15$	$44.66^{A}\pm0.90$	1.963
400	$51.31^{C}\pm0.19$	$26.64^{E}\pm0.03$	$41.20^{D}\pm0.27$	$61.00^{B}\pm0.58$	$70.92^{A}\pm0.75$	1.824
600	$72.41^{C}\pm0.53$	$52.37^{E}\pm0.71$	$67.67^{D}\pm0.90$	$78.32^{B}\pm0.24$	$85.34^{A}\pm0.37$	1.430
800	$80.75^{C}\pm0.15$	$64.74^{E}\pm0.22$	$75.64^{D}\pm0.56$	$89.32^{B}\pm0.23$	$92.43^{A}\pm0.59$	1.429

Where: A, B, C, D in the same rows are not significantly different (p>0.05), (Mean ± standard error).
BHT: butylated hydroxy toluene. L.S.D: Least significant differences at 0.05 levels.

Thiobarbituric acid (TBA) of beef sausage:

Data presented in Table (5) showed that the thiobarbituric acid (TBA) of different beef sausage samples was affected not only by the type of treatment, i.e. exposure to laser rays, the addition of volatile oils mixture and combination between them but also by cold storage period at 4±1°C up to 25 days. From the statistical analysis of these data, a significant difference ($p \leq 0.05$) in TBA values between different treatments either at zero time or throughout the storage periods were recorded. The thiobarbituric acid of values of different beef sausage treatments ranged from 0.349 to 0.387mg malonaldehyde/kg sample at zero time. These results are in agreement with those obtained by **Rab (2019)** mentioned that the TBA value of beef sausages was 0.450 malonaldehyde/Kg sample at zero time. Also, **Moghazy (2019)** reported that the TBA value of beef burgers was 0.385 malonaldehyde/kg sample. Sausage treated with laser rays had the highest TBA value (0.387mg malonaldehyde/kg sample) followed by the control sample with non-significant differences. On the contrary, the lowest TBA value (0.349 malonaldehyde/Kg sample) was recorded for sausage treated with an additional volatile oils mixture. This reflected the higher antioxidant effect of the volatile oils mixture **(Hassanen, 2005).** Furthermore, the thiobarbituric acid of all beef sausage treatments was significantly affected by cold storage time. By increasing the cold storage period, TBA values of all beef sausage treatments were increased significantly ($p \leq 0.05$). This increase in TBA value during cold storage could be indicating continuous oxidation of lipids and consequently the production of oxidative by-products **(Osheba et al., 2013).** These results are in agreement with those obtained by **Rab (2019) and Hashem et al. (2021)** stated that the TBARS values of all beef sausages samples increased significantly with the extension of the storage period. At any time of cold storage, the lowest increment rate of TBA values was recorded for sausage treated with both volatile oils mixture and laser rays followed by sausage treated with volatile oils mixture only. On the contrary, the highest increment rate of TBA value was recorded for the control sample. The addition of volatile oils mixture with or without exposure to laser rays in preparation of sausage led to a decrease in the increment rate in TBA value from 205.96% for untreated sample (control) to 92.76, 86.19 and 64.18 % for sausages treated with laser only, volatile oils mixture only and that treated with combination between them, respectively, at 10th day of cold storage. This might be due to the laser antimicrobial activity **(El-Adly et al., 2007 and Hassan et al., 2015)** and volatile oil mixture had strong antioxidant and antimicrobial activity **(Abd El-Qadr, 2014; Hassanen, 2005 ; Moghazy, 2019and Sharma 2020**) which led to retard oxidation and inhibition some microorganisms such as lipolytic bacteria (producing lipases) consequently prevent lipid hydrolysis.TBA values of different sausage treatments exceeded the permissible level (0.9 mgmalonaldehyde/kg) reported by Egyptian standard specifications (2005) on the 10thday of cold storage for the control sample, 15th for sausage treated with laser rays, 20th for sausage treatedwith volatile oils mixture only and 25th day for sausagetreated with both volatile oils mixture and laser rays.

Table (5):- Thiobarbituric acid values (mg malonaldehyde/kg) of sausage as affected by different treatments and cold storage periods at 4±1°C.

Storage time (days)	Beef sausage treatments				LSD at 0,05%
	Control	laser beam radition	Volatile oils mix.	Volatile oils mix with laser beam	
Zero time	0.379^{Ca} ±0.010	0.387^{Ca} ±0.004	0.349^{Db}±0.0012	0.355^{Db}±0.0011	0.016
5	0.780^{Ba} ±0.013	0.638^{BCb} ±0.012	0.495^{Cc} ±0.010	0.443^{CDd} ±0.0023	0.029
10	1.129^{Aa} ±0.12	0.746^{ABb} ±0.027	0.661^{Bbc}±0.012	0.573^{BC}±0.0035	0.175
15	ND	0.979^{Aa} ±0.205	0.788^{Bb}±0.04	0.651^{Bb}±0.021	0.171
20	ND	ND	0.921^{Aa} ±0.1	0.723^{Bb} ±0.043	0.161
25	ND	ND	ND	0.916^{A} ±0.156	
LSD at 0,05%	0.197	0.287	0.129	0.185	

Where: Mean values (Mean ± standard error).in the same row (small letter) or column (capital letter) with the same letter are not significantly different (at p> 0.05) **.** LSD: Least significant differences at 0.05 levels.ND: Not determined

Peroxide value of beef sausage:

Data given in Table (6) clarified that peroxide values of different beef sausage samples as affected not only by the type of treatment i.e., exposure to laser rays, the addition ofvolatile oils mixture, and combination between them but also by cold storage period at 4°C for 25th day. From these data, it could be observed that there were no significant differences ($p>0.05$) in peroxide values between all beef sausages treatments whether untreated (control sample) or other different beef sausage treatments at zero time, while significant differences were recorded between different

beef sausage treatments along cold storage periods. The peroxide value ranged from 1.92 to 2.12 m. equiv. / kg fat immediately after processing. These results are in agreement with those obtained by **Rab (2019)** stated that the peroxide value of fresh beef sausages ranged between 1.8 and 4.4 equiv. / kg fat during cold storage for 21 days . Also, the peroxide values of different beef sausage treatments were affected significantly by the cold storage period. During the cold storage at 4+1ºC, the peroxide values progressively increased ($p \leq 0.05$) as the period of storage increased for all beef sausage treatments. These results in the line with those obtained by **Rab (2019)** mentioned that cold storage raised the peroxide value of beef sausage. The peroxide value of the control sample increased from 2.12 m. equiv.O2 /kg fat at zero to 7.83 m. equiv.O2 /kg fat after 10 days of cold storage which had the highest increment rate of peroxide value (269.34%). While, treatment of sausage with laser rays only, volatile oils mixture only, and combination between them led to a decrease increment in the rate of peroxide value from 269.34 for control to 136.13, 123.5, and 74.48% respectively at 10th of cold storage. Generally, at any time of cold storage, sausage prepared with an additional volatile oils mixture and exposure to laser rays had the lowest increment rate of peroxide when compared with other sausage treatments. This might be due to the laser had antimicrobial activity (El-Adly et al., 2007 and Hassan et al., 2015) which led to the inhibition of some microorganisms like lipolytic bacteria consequently preventing lipid hydrolysis and volatile oil mixture had strong antioxidant activity **(Abd El-Qadr, 2014, Nashi et al., 2015, Abdl Fattah et al., 2016 and Moghazy, 2019)** which led to retard oxidation. Finally, peroxide values of the control sample, laser radiation sample, and volatile oils mixture sample were not evaluated at fifteen, twenty, and twenty-five days of storage, respectively because they were spoiled.

Table (6):- Peroxide values of sausage as affected by different treatments and cold storage periods at 4±1°C.

Storage time(days)	Beef sausage treatments				LSD at 0,05%
	Control	laser beam radition	Volatile oils mixture.	Volatile oils mix with laser beam	
Zero time	2.12^{Ca}±0.09	2.13^{Ca}±0.16	1.95^{Da}±0.13	1.92^{Ea}±0.24	0.32
5	4.65^{Ba}±0.05	3.45^{Cb}±0.07	3.17^{Cc}±0.08	2.97^{Dc}±0.09	0.21
10	7.83^{Aa}±0.17	5.03^{Bb}±0.95	4.36^{Bc}±0.65	3.35^{Dd}±0.21	0.51
15	ND	7.11^{Aa}±0.16	6.72^{Ab}±0.12	5.18^{Cc}±0.17	0.32
20	ND	ND	7.38^{Aa}±0.21	6.56^{Bb}±0.13	0.48
25	ND	ND	ND	7.95^{A}±0.08	
LSD at 0,05%	0.33	1.35	0.88	0.45	

Where: Mean values (Mean ± standard error).in the same row (small letter) or column (capital letter) with the same letter are not significantly different (at p> 0.05) **.**LSD: Least significant differences at 0.05 levels.ND: determined

Acid valueof beef sausage:

Acid values of different beef sausage samples as affected not only by the type of treatment (exposure to laser rays, addition of volatile mixture, and combination between them) but also by cold storage period during cold storage (4 ± 1°C) for 25 days were presented in Table (7). According to statistical analysis of these data, it could be observed that no significant differences ($p>0.05$) were recorded in acid values between all beef sausage treatments (ranged between 0.58 and 0.61 mg KOH/g fat) at zero time but showed significant differences ($p \leq 0.05$) during different cold storage periods. Moreover, the acid values of all beef sausage treatments were significantly affected ($p \leq 0.05$) by cold storage time. Acid values significantly increased ($p \leq 0.05$) by cold storage period increment for all beef sausage treatments. The acid value of the control sample significantly increased from 0.58 mg KOH/g fat at zero time to 3.67 mg KOH/g fat after 10 days of cold storage being the highest increment rate (532.75 %) when compared with (165.57 %) for sausage prepared with exposure to laser rays, (100%) for sausage prepared with addition volatile oils mixture and (50.84%) for sausage prepared with both volatile oils mixture and laser rays. Similar results were obtained by **Abd El-Qadr (2014)** stated that acid values of all dried chicken meat treatments significantly increased by increasing the storage time. Also, **Moghazy (2019)** reported that beef burgers prepared with volatile oils had lower acid values than the untreated sample (control) during storage. After 15 days of cold storage acid value of the control sample was not evaluated because it spoiled (showed off an odor), while acid values for other treatments ranged from 1.24 to 2.21 mg KOH/g fat. The highest acid value was recorded for sausage prepared by exposure to laser rays. While the lowest acid value was recorded for sausage prepared with both a volatile oils mixture and laser rays.

Generally, at any time of cold storage, sausage prepared with both volatile oils mixture and laser beam had the lowest acid value followed by sausage prepared with volatile oils mixture only. These results reflect the strong antimicrobial effect of both volatile oil mix and laser beam rays which led to the inhibition of some microorganisms

such as lipolytic bacteria (producing lipases) consequently preventing lipid hydrolysis as well as decreasing the level of free fatty acids (**Davies and Board, 1998; El-Adly et al., 2007; Hassan et al., 2015 and Moghazy (2019).**

Table (7):- Acid values of sausage as affected by different treatments and cold storage periods at 4±1°C.

Storage time (days)	Beef sausage treatments				LSD at 0,05%
	Control	laser beam radition	Volatile oils mix.	Volatile oils mix with laser beam	
Zero time	0.58^{Ca} ±0.05	0.61^{Ca}±0.12	0.58^{Ca}±0.08	0.59^{Ca}±0.09	0.16
5	1.92^{Ba}±0.178	0.93^{BCb}±0.04	0.77^{BCc}±0.06	0.73^{Cc}±0.09	0.13
10	3.67^{Aa}±0.086®	1.62^{Bb}±0.05	1.12^{BCc}±0.13	0.89^{Cbd}±0.10	0.21
15	ND	2.21^{Aa}±0.06	1.55^{Ab}±0.13	1.24^{BCc}±0.17	0.27
20	ND	ND	2.02^{Aa}±0.16®	1.62^{Bb}±0.25	0.38
25	ND	ND	ND	2.14^{A}±0.22	
LSD at 0,05%	0.33	0.22	0.33	0.48	

Where: Mean values (Mean ± standard error).in the same row (small letter) or column (capital letter) with the same letter are not significantly different (at p> 0.05) **.** LSD: Least significant differences at 0.05 levels.ND: determined

Conclusions:-

In conclusion, results reported in the present paper show that the use of laser exposure with volatile oil mix500ppm techniques provides new and fascinating possibilities for detecting the onset of spoilage in beef sausage during refrigerated storage for 25 days at 4±1°C. In the present study, four samples of beef sausage were control, laser beam exposure, volatile oils mix and laser beam exposure with volatile oils mix samples. Determination of peroxide values, acid value, thiobarbituric acid (TBA), and pH value. The results obtained via laser beam exposure with volatile oils mix and volatile oils mix techniques concerning fat degradation with storage time have been validated by analyzing beef sausage samples with a conventional meat analyzer. Compared with other previously published works in this field, this work presents successful laser beam with volatile oils mix techniques, as cost-effective that can be controlled to follow up spoilage of beef sausage during storage for 25 days at 4±1°C.

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