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Research Article

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Effects of Photochemical Extracted From Some Plants on Shelf Life and Quality of Beef Burger

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Abstract

This study investigated some aspects to enhance the quality of beef burger. Some plants extracts of green tea (GTE), roselle (RE), lemon grass (LGE) and olive leaf (OLE) were added individually during the preparation of beef burger. These extracts were tested against some pathogenic bacteria. Some chemical attributes and sensory evaluation of the product were investigated during storage of the product at 4 ºC for 18 days. These plants extracts showed high antioxidant activity for the tested sample due to their content of natural compounds such as phenolic compounds during cold storage. Phenolic content in green tea and olive leaf extracts were significantly higher than in roselle and lemon grass extracts. Sensory evaluation showed high scores for the tested samples. Bacteriological analysis revealed that the mean total bacterial count was ranged from 5.09 to 6.0 $\log_{10}$ (cfu/g). While mould and yeast mean count ranged from 1.87 to 3.46 $\log_{10}$ (cfu/g) and coliform bacterial count ranged from less than 10^2cfu/g. Bacillus cereus, Staphylococcus aureus and Listeria monocytogenes were not detected in all treatments of beef burger. This study showed that the addition of natural antioxidant extracts for beef burger could improve quality and provide safe product during storage by reducing microbial growth and lipid oxidation.

Keywords: Green tea, Olive leaf, Roselle, Lemon grass, Antioxidant, Refrigeration storage.

Introduction

Degradation of lipids and proteins lead to deterioration and shortening the shelf life in meat products due to lipids oxidation [1,2]. The quality of food products can be preserved especially during cold storage, through the use of some natural substances that have antioxidant properties and antimicrobial effects to maintain the quality properties of processed meat during storage [3]. The prevention of fat from rancidity and deterioration is through the use of synthetic antioxidants and recently the application of the addition of natural extracts from plants that have anti-oxidant properties and prevent the growth of microbes in food products [4,5]. However, using synthetic antioxidants, such as butylated hydroxyl anisole (BHA) or butylated hydroxyl toluene (BHT) have been related to human's health risks resulting in strict regulations over their use in foods [6].

On the other hand, natural substance) rosemary, sage, and green tea extracts (that have antioxidant properties and antimicrobial activity and beneficial to health are acceptable by consumers instead of synthetic antioxidants due to concerns about toxicological safety [7-9].

Plant extracts (Olive, cinnamon, roselle, green tea, Oregano) containing high levels of phenolic compounds with strong H• donating activity effectively scavenge free radical and reactive oxygen species in food products [10-12].

Green tea has substantial anti-oxidative activity, much of which appears to be due to natural flavonoids, tannins and some vitamins [13,14]. Adding tea catechins (from 200 to 400 mg/kg) to minced meat inhibited lipid oxidation in both raw and cooked beef much better than did Ascorbic acid [15,16]. This concentration of tea catechins were more effective than rosemary to prevent the oxidation process from occurring in cooked patties based on TBARS [17].

The addition of 0.5-2% of green tea leave extracts used as natural antioxidants have been reported to significantly decrease the levels of *Clostridium perfringens* in cooked ground beef, chicken and pork during abusive cooling [18]. The antioxidative property of green tea extract is due to some phenolic compounds such as catechins, epicatechins, epicatechin gallate, epigallocatechin, and epigallocatechin gallate are free radical scavengers and metal chelators [15,19,20].

This Research Was Intended To Determine:

1. Total phenolic compounds, the total antioxidant activity and the microbial inhibition of some plant extracts such as green tea, roselle, lemon grass and olive leaves.
2. Effect of using green tea, roselle, lemon grass and olive leaves extracts, applied individually, on the quality characteristics and assessment for bacteriological quality with special reference to food poisoning microorganisms of beef burger during storage at 4 ºC for 18 days.

Materials and Methods

Materials

Raw beef and all ingredients required for burger preparation were purchased from a local market (Dokki, Giza, Egypt). Soy protein was obtained from the Agricultural Research Center in Cairo, Egypt. Sodium tri-polyphosphate ($Na_5P_3O_{10}$) and potato starch was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Roselle; grape, red cabbage; green tea and lemon grass leaves were obtained from local market.

Preparation of Plant Extract

The plants leaves were washed and then dried in an air oven for 3 days at 38°C. The air-dried plant materials were ground in a blender with a particular size to ensure the plant powders in identical size. 10 g powder of each plant was extracted for 2 hrs. With 200 ml of 70% (v/v) aqueous ethanol at 38 ºC by a thermo-shaker which is fixed to 180 rpm. Then the samples were centrifuged at 5000 rpm for 15 minutes and the supernatant was carried to a rotary evaporator to remove ethanol under reduced pressure at 38 ºC, 120 rpm. The remaining aqueous solutions were lyophilized at–50ºC and 0.028 mbar. The percent extraction yields of plant materials were calculated. The crude extracts powders were packaged in a tight glass bottles and kept in refrigerator until further experiments. All plants leaves were collected during May, 2018.

Beef Burger Preparation

The beef meat (500 g) was manually cut using a band saw (JG-210) and minced through a 4 mm–diameter grinder plate. Salt (2%Nacl) was added to minced beef (49.0 g) and mixed with a Hobart mixer for 3 min. Soy protein (50 g) was blended with water and fat at a ratio of 1:5:5 (w/v/w) using a mixer. The beef kept at 2–5°C. Sodium tri-polyphosphate, spices (black pepper, garlic powder, onion powder, and ground cayenne pepper), 3% potato starch, and plant extracts of the high content of both extracts of green tea, roselle, olive leaves and lemon grass of phenolic compounds and antioxidants for both grapes and red cabbage leaves. Therefore, research was conducted on those species in processing beef burger at ratio 1%, 2% and 3%. Beef burgers (70 g each) were prepared from the finished meat grounded. The cooked burgers were prepared by heating on a hot plate for 7–8 min, until the internal temperature reached 74 °C ± 1°Caccording to the protocol described by Zhanc et al [21].

Samples Preparation

Beef burger was divided into thirteen equal portions for a different treatment as shown in table 1. All treatments were packed in plastic bags and stored in refrigerator at 4±2 ◦C then at intervals of 0, 5, 10, 15 and 18 days

Table 1: Beef burger preparation with different plants extracts:

Samples	Treatments	Samples	Treatments
1	Control	8	1% roselle
2	1% lemon grass	9	2% roselle
3	2% lemon grass	10	3% roselle
4	3% lemon grass	11	1%olive leaf
5	1% green tea	12	2% olive leaf
6	2% green tea	13	3% olive leaf
7	3% green tea		

Chemical Analyses

Total Phenolic Compounds Content

Total phenolic compounds were estimated according to the method described by Meda et al [22]. The content of total phenolic compounds was determined using a standard curve prepared with Gallic acid.

Total Antioxidant Activities (DPPH Radical Scavenging Activity)

Fine ground powder (150 mg) of each sample was taken in 250 ml conical flasks and 50 ml water was added then kept on a shaker at 150 rpm for an hour. The flask contents were filtered using filter paper (Whitman No 1). Similarly, methanol or ethanol extracts were also prepared. The filtrate was used directly for 1.1-dipheny-2-picrythydrazyl (DPPH) assay described by Sharma and Bhat [23]. The absorbance of the sample was measured at 517nm using the UV Spectrophotometer (model T80 x UV-VIS Spectrometer PG Instruments Ltd). Gallic acid and BHA were used as standard references. DPPH radical scavenging effect was calculated as "inhibition of percentage" according to the following formula:

Inhibition of percentage (%) = [Ac (0)–Aa (t)/Ac (0)] x100
Where: Ac (0) is an absorbance of control DPPH solution at 0 min, and Aa (t) is absorbance of test sample after 20 min.

Proximate Composition of Beef Burger

The proximate composition of beef burger was determined using procedures described by AOAC for moisture, protein, fat and ash contents [24].

Lipid Oxidation

The 2-thiobarbituric acid (TBARS) assay was carried out according to the procedure of Schmedes and Holmer [25]. The absorbance was measured at 538 nm by using UV–VIS spectrophotometer. TBA value was expressed as mg malonaldehyde per kg of burger. The analyses were made in duplicates for all the treatments.

Color Measurement

Color measurement was performed according to previously reported methods [26,27]. Using a colorimeter (Lab. Scan XE, Hunter Lab., Murnau, Germany), and standardized with a white tile of Hunter Lab color standard (LX No. 16379): X = 77.26, Y = –81.94, and Z = 88.14 (L* = 92.46, a* = –0.86, b* = –0.16). The measured color parameters were L* (lightness), a* (redness), and b* (yellowness).

Determination of Antibacterial Activity

The antimicrobial activity of four different types of extracts (olive leaf, lemon grass, green tea and roselle) was determined by the agar well diffusion [28]. The seven pathogenic indicator bacteria strains were obtained from the stock cultures of the Dairy Microbiological Lab, National Research Centre: *Escherichia coli* 0157: H7 ATCC 6933, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 20231, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Listeria monocytogenes* ATCC 7644 and *Yersinia enterocolitica* ATCC 9610. Each strain was activated by in tryptone soy broth at 37 ºC for 24 h. One ml culture of the activated indicator strain (10^5 cells /ml) was inoculated into 20 ml of Mueller-Hinton agar (Becton Dickinson, USA) and poured in Petri dishes. After solidification of the agar, wells of 5 mm in diameter were cut from the agar with a sterile borer and 50µL of different plants extracts in each well.

The zone diameter of wells cut in Mueller-Hinton agar was 5.0 mm and the diameter of inhibition zone (DIZ) of negative control for each bacterium was also 5.0mm. If the DIZ value is 5.0 mm, which means the sample has no inhibitory activity against that bacterium. The plates were incubated at 37°C for 24 h. Zones of inhibition were measured at the end of the incubation period.

Microbiological Analysis

About 25 g of each sample was mixed and homogenized in sterile mixer, and diluted with buffered peptone water to make the sufficient dilutions for the microbiological analysis. Ten-fold dilutions of homogenates samples were prepared and inoculated onto plates of selective media.

Total bacterial count was determined by aerobic plate count method on plate count agar. Plates were incubated at 37º C for 24-48 h. (Oxoid) [29]. Coliform group was determined using solid medium method onto plates of violet red bile agar medium (Oxoid) plates were incubated for 24 hrs., at 35ºC [29]. Enumeration of *Staphylococcus aureus* determined by Baird Parker media (Oxoid) supplemented with egg yolk and potassium tellurite solution [29]. *Listeria monocytogenes* was determined using selective oxford agar base (Oxoid) incubated at 35ºC for 48 hrs., [29]. *Bacillus cereus* was determined by the surface plating technique onto the *Bacillus cereus* agar medium (Oxoid), supplemented with polymyxin B and egg yolk [29]. Mould and Yeast count was determined by potato dextrose agar medium (Oxoid). Plates were incubated at 22-25º C for 3-5 daysc [29]. The results expressed as colony forming unit cfu/g.

Sensory Evaluation

Sensory evaluation was performed by 10 non-trained panelists consisting of staff in the Food Technology Department, National Research Center (Dokki, Giza, Egypt) according to a previously reported method [30]. The evaluation of samples of burger for color, odor, taste, and texture was conducted for overall acceptance 7-point hedonic scale (1 = strongly dislike, 4 = neither like nor dislike, and 7 = strongly like). Significance was established at $P < 0.05$ unless otherwise indicated.

Statistical Analysis

The data generated from the study were subjected to one-way analysis of variance (ANOVA) and significant differences ($P<0.05$) between means were determined by Scheffe multiple comparison test using SPSS [31].

Results

Total Phenolic Compounds

The contents of phenolic compounds for green tea, roselle, lemon grass and olive leaves are presented in (Figure1). It was (34.12 mg/g) in green tea leaves which was significantly higher than its found in roselle (17.1 mg/g), lemon grass (12.26 mg/g) and olive leaves (12.1 mg/g).

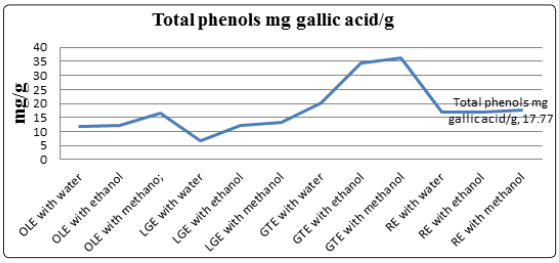

Figure 1: Total phenolic compounds of olive (OLE), lemon grass (LGE), green tea (GTE) and roselle (RE)

Antioxidant Activity

Water, ethanol and methanol extracts from green tea, roselle, lemon grass and olive leaves were evaluated their antioxidant activity and illustrated in (Figure 2). Methanolic extract of green tea appeared high antioxidant activity (35.58mg) > lemon grass (18.8mg) > olive (15.91mg)> roselle (14.02mg). Meanwhile, water extracts of green tea, roselle, lemon grass, olive leaves did not show significant differences in antioxidant activity. Roselle extracts characterize by higher antioxidant properties compared to BHA and vitamin E due to its high polyphenol components (Rhee et al. [32]. Polyphenols of tea extract and olive leaves extract have a stronger anti-oxidative activity than butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and DL-α-tocopheroldue to the phenolic compounds like soleuropein, tyrosol and hydroxyltyrosol [33].

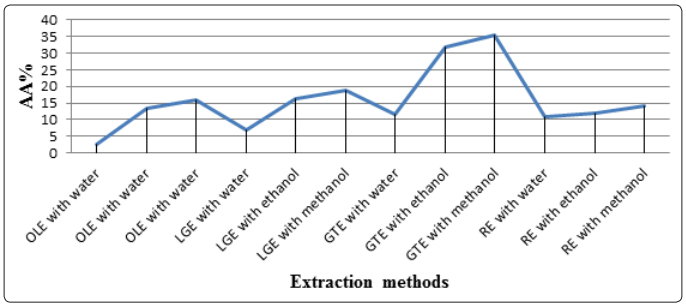

Figure 2: Antioxidant activity DPPH radical content of olive (OLE), lemon grass (LGE), green tea (GTE), and roselle (RE)

Antibacterial Activity of Different Plants Extracts

Results recorded in Table 2, revealed that all four different types of leaf olive extract, Lemon extract, Green tea extract and roselle extract examined have variable antibacterial activity against all of the tested pathogenic bacteria.

Roselle extract was the most effective extract among all extracts tested against pathogenic bacteria. From table 2, roselle extract showed strongly inhibitory activity towards *L. monocytogenes, Bacillus cereus, Staphylococcus aureus, E. coli* 0157: H7, *Salmonella typhimurium, Yersinia enterocolitica* and *P. aeruginosa*as the most sensitive indicators with the diameter zones of 45, 40, 38, 37, 32, 31 and 30 mm, respectively.

Table 2: Antimicrobial activity of different types of extracts measured as zone of inhibition (mm)

Strains	RE	LGE	OLE	GTE
Staphylococcus aureus	38	18	15	40
Bacillus cereus	40	33	25	30
Listeria monocytogenes	45	20	18	35
E. coli 0157: H7	37	19	0	0
Yersinia enterocolitica	31	30	20	0
Salmonella typhimurium	32	27	15	0
Pseudomonas aeruginosa	30	20	20	0

List: *Listeria monocytogenes* ATCC 7644
Staph: *Staphylococcus aureus* ATCC 20231
B. c: *Bacillus cereus* ATCC 33018

E. c: *Escherichia coli* 0157: H7 ATCC 6933
Yerse: *Yersinia enterocolitica* ATCC 9610
Sal: *Salmonella typhimurium* ATCC 14028
Pse: *Pseudomonas aeruginosa* ATCC 9027
1: Roselle extract
2: Lemonextract
3: leaves olive extract
4: Green tea extract

Chemical Composition of Beef Burger with Plants Extracts Samples

Proximate analysis of beef burger among different treatments is shown in table 3.

Table 3: Proximate composition of beef burger treated with different a concentration of olive, green tea, roselle and lemon grass leaves extract

Treatments	Proximate analysis			
	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control	69.10	21.10	14.67	2.26
olive leaves 1%	69.22	21.36	14.77	2.18
olive leaves 2%	70.11	21.40	14.79	2.01
olive leaves 3%	71.25	21.88	14.80	1.70
Green tea1%	62.60	15.15	14.67	1.11
Green tea2%	63.12	15.43	15.00	1.00
Green tea3%	64.22	15.98	15.12	0.89
Roselle1%	63.70	20.78	14.58	3.01
Roselle2%	64.12	21.10	14.34	2.97
Roselle3%	65.26	21.57	14.22	2.22
Lemon grass1	70.12	17.99	15.06	2.22
Lemon grass2	71.34	18.15	14.98	1.70
Lemongrass3%	71.87	18.21	14.86	1.11

Chemical composition of beef burger was in agreement with previous studies performed by Aytul [34].

Lipid Oxidation

Changes in TBA values of the control and treated samples during storage are given in Table 4. The TBA values of control and treated samples were 0.156-0.630 mg malonaldehyde /kg at zero time. Higher level of oxidation in 1% olive leaves extracts (OLE) samples may be explained by the considerably lower concentration of antioxidant material within samples.

Table 4: TBA values as mg malonaldehyde equiv/kg for beef burger treated with different concentrations of olive, lemon grass, green tea and roselle leaves extracts during 18 days of storage at 4ºC

	Storage Time (days)				
Treatments	0	3	6	9	18
control	0.630±0.19cx	0.830±0.23b	1.470±0.3bx	4.470±0.13a	6.230±0.13ax
olive leaves 1%	0.172±0.27cwx	0.172±0.16bw	0.174±0.17bw	0.181±0.10ay	0.192±0.20ay
olive leaves 2%	0.170±0.09bwx	0.170±0.01by	0.172±0.18by	0.177±0.08az	0.184±0.06 a, x
olive leaves 3%	0.169±0.02dw	0.169±0.03bw	0.170±0.02cxy	0.175±0.16Ax	0.180±0.17aw
lemon grass 1%	0.187±0.003e	0.216±0.004d	0.242±0.003c	0.284±0.004b	0.313±0.005a
lemon grass 2%	0.186±0.003e	0.226±0.004d	0.231±0.003c	0.242±0.003c	0.285±0.004d
lemon grass 3%	0.184±0.003e	0.228±0.003e	0.230±0.004b	0.254±0.003c	0.295±0.004d
green tea 1%	0.161 ±0.0283xa	0.164 ±0.00141xc	0.187 ±0.0109yb	0.249 ±0.0990zc	0.268 ±0.0134zc
green tea 2%	0.158±0.0113xa	0.155±0.00354xbc	0.178±0.0141yab	0.268±0.0148zbc	0.257±0.00495zbc
green tea 3%	0.156±0.00778xa	0.153 ±0.0184ya	0.172±0.00212ya	0.250 ±0.0197za	0.235 ±0.00141za
Rosselle 1%	0.197±0.003e	0.221±0.004d	0.286±0.004c	0.314±0.005b	0.370±0.002a
Rosselle 2%	0.195±0.003e	0.201±0.004d	0.275±0.004c	0.302±0.004b	0.348±0.oo3a
Rosselle 3%	0.193±0.003e	0.200±0.004d	0.272±0.003d	0.289±0.004c	0.316±0.006b

A-d: Means having different letters within each treatment denote significant difference at $p<0.05$.
w-z: Means having different letters within each storage time denote significant difference at $p<0.05$.
Data are mean values ± S.D. (n=3)

Color analysis

The average L*, a*, b* values for all samples during 18 days of storage at 4ºCare shown in Table 5., The treatment and storage time had significant effects on all color attributes ($p<0.05$). Control samples had higher values of L*, a*, b* parameters than that of samples tested and with increased the concentration of olive leaf extract, the L*, a*, b* values of the samples (stored up to 9 days) decreased. Redness (a*) values decreased ($p< 0.05$) progressively during storage period. Redness of control samples was higher than that of treated samples during refrigerated storage. Yellowness of both control and samples tested decreased during storage. At the end of the 9 days of refrigerated storage, yellowness of beef burger treated with 3% olive leaves (OLE) was greater than that of control and samples treated with lower concentrations of OLE.

Table 5: Color analysis for beef burger treated with different concentrations of plants extracts during 18 days of storage at 4ºC

Storage Time (days)																
Color values treatments	0			3			6			9			18			
	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b	
control	55.355± 0.46e	8.8± 1.76c	19.38± 2.02c	52.31± 0.39bw	7.01± 0.23abw	19.42± 0.45b,y	50.35± 0.01aw	5.84± 0.06abw	18.24± 0.02aw	51.61± 0.16cw	5.33± 0.09aw	18.42± 0.40bw	53.63± 0.33dwy	4.91± 0.08bcw	18.44± 0.30cy	
olive leaves 1%	54.05± 0.46c	7.29± 1.76c	21.45± 2.02d	50.77± 0.16abw	5.24± 0.12bx	17.00± 0.01by	49.49± 2.51awy	4.45± 1.48aw	16.42± 1.34ay	51.14± 0.15by	4.37± 0.53dy	16.675± 0.51cy	52.26± 0.13bw	3.92± 0.37dy	14.73± 0.34ex	
olive leaves 2%	50.105± 0.46c	6.4± 1.76c	20.765± 2.02d	45.74± 0.91aw	6.03± 0.18by	18.63± 0.91bw	44.66± 0.04ayx	5.15± 0.05aw	19.46± 0.12ay	46.85± 0.07bx	5.53± 0.23ex	18.43± 0.01cy	48.89± 0.52byx	4.88± 0.16dx	18.73± 0.07ey	
olive leaves 3%	50.99± 0.46d	5.83± 1.76a	22.32± 2.02d	48.5± 0.38by	4.99± 0.13bz	19.84± 0..20bx	47.93± 0.49ax	4.42± 0.54by	20.15± 0.60ax	48.23± 0.08cz	4.40± 0.03cz	19.47± 0.08cx	49.55± 1.32cx	4.22± 0.85by	18.70± 0.84dw	
lemon grass 1%	50.22± 2.55c	8.47± 0.10by	20.35± 0.9bw	48.16± 0.05ay	6.54± 0.16bw	18.54± 0.73cy	48.21± 0.16bw	6.01± 0.04dz	18.29± 0.18dx	48.2± 0.04dz	6.49± 0.73cy	19.60± 0.07cw	48.88± 0.16dyx	5.81± 0.18ex	17.54± 1.17cy	
lemon grass 2%	52.10± 2.55c	8.36± 0.73bw	21.03± 0.06aw	46.14± 0.86c	6.51± 0.53bx	19.61± 0.27bz	49.93± 0.22ay	5.13± 0.31dx	17.3± 0.00cy	49.2± 0.28dw	5.24± 0.28dy	18.41± 0.18dx	50.64± 0.07cw	5.22± 0.07cx	16.23± 1.82ay	
lemon grass 3%	52.15± 2.55c	7.96± 0.22bx	19.56± 0.22ay	48.11± 0.86c	6.70± 0.53by	19.72± 0.00bx	50.23± 0.21aw	5.12± 0.31dx	17.36± 0.00cy	49.37± 0.18dw	5.29± 0.16dyx	18.50± 0.37dy	48.78± 0.06cy	5.01± 0.08cw	18.54± 0.88bwy	
green tea 1%	53.62± 0.71bw	6.84± 0.00awy	19.06± 0.05ay	51.47± 0.01ax	6.36± 0.00by	17.52± 0.02ay	48.88± 1.01aw	6.48± 0.00dy	18.92± 0.01aw	50.87± 0.88ax	6.24± 0.036cy	17.48± 0.88cx	51.27± 1.02xw	6.18± 0.00ax	16.86± 0.02xz	
green tea 2%	52.65± 0.24cw	6.91± 0.00awx	18.89± 0.05ax	50.59± 0.01ay	6.42± 0.00by	17.47± 0.03ax	50.43± 0.73ay	5.43± 0.01dx	16.29± 0.22ay	50.81± 0.76cw	4.48± 0.37cw	15.01± 0.77wy	49.31± 1.22xz	4.915± 0.01ay	16.755± 0.04xy	
green tea 3%	53.59± 0.00aw	6.86± 0.00awx	19.67± 0.05ay	49.03± 0.01ax	7.02± 0.00by	17.78± 0.01aw	47.97± 0.36ax	5.74± 0.02ay	16.83± 0.35aw	48.94± 0.03cy	5.37± 0.37cx	15.68± 1.02ax	48.975± 0.08xw	5.15± 0.00ay	15.285± 0.07aw	
rosselle 1%	50.28± 0.28dw	7.16± 0.21aw	15.91± 0.22ay	46.64± 0.27bz	5.60± 0.71bx	15.83± 0.24ay	45.80± 0.86c	5.51± 0.71bw	15.90± 0.31dw	45.93± 0.88ax	5.74± 0.33dy	15.76± 1.01wy	51.52± 1.22dx	6.01± 1.26aw	15.655± 0.77ay	
rosselle 2%	49.43± 0.28dx	6.865± 0.21awx	14.81± 0.22aw	46.02± 0.27bw	4.75± 0.71bw	13.58± 0.24aw	48.03± 0.86	4.61± 0.71by	13.01± 0.31dx	47.96± 0.77aw	5.41± 0.22cw	12.93± 1.01xw	48.83± 1.02dw	5.605± 1.22xy	12.915± 0.27dwx	
rosselle 3%	46.03± 0.28dy	6.74± 0.21aw	12.09± 0.22a	44.93± 0.27bx	4.66± 0.72aw	11.65± 0.24ax	46.37± 0.86c	4.83± 0.71bx	11.44± 0.31dy	47.79± 0.28cw	5.42± 0/32ae	12.15± 1.01ax	47.84± 0.88dy	5.745± 1.01ax	12.08± 0.55xy	

A: Means having different letters within each treatment denote significant difference at $p<0.05$.

w-z: Means having different letters within each storage time denote significant difference at $p<0.05$.

Data are mean values ± S.D. (n=3)

Sensory evaluation

Table (6) showed all the sensory characteristics for the samples of beef burger after processing. It was found that, all treated samples of burger had an acceptable taste with good score. The taste scores order of samples under investigation was presented as extract of green tea > extract of olive > extract of lemon grass > roselle > control sample. Lemon grass extract was found to be of highest score in texture and color, while roselle extract was of lowest score in the same last characteristics.

Odor score was the highest in beef burger compared to other studied samples; while control sample was of worst score. However, all the investigated samples (including the control) realized good color scores.

Table 6: Sensory evaluation for beef burger as influenced by antioxidant extracts

Samples	color	odor	taste	texture	Overall acceptability
control	7.2±0.918a	7.9±1.449a	7.0±1.141a	7.1±0.994a	7.1±0.994a
Roselle 1%	7.1±0.875bc	7.3±1.494a	6.8±0.788b	6.9±0.737b	6.6±0,843de
Roselle 2%	6.6±0.966c	7.1±1.1a	6.5±1.080b	6.8±0.632bc	6.6±0.966de
Roselle 3%	5.7±1.567d	6.8±1.316ab	6.5±0.971b	6.0±1.621c	5.9±1.370e
Green tea 1%	7.7±0.674ab	7.8±0.788a	7.5±0.707a	7.5±0.527ab	6.9±1.370cd
Green tea 2%	7.9±0.316a	7.9±0.737a	7.6±0.699a	7.6±0.699ab	7.4±0.843abcd
Green tea 3%	7.8±0.421ab	7.7±0.674a	7.6±1.054a	7.5±0.971ab	7.1±0.994bcd
Olive leaf 1%	7.9±0.737a	7.7±0.823a	7±0.948ab	7.6±0.516ab	7.3±0.823abcd
Olive leaf 2%	7.9±1.1a	7.4±0.843a	7.3±0.971ab	7.6±0.699ab	7.0±0.816cd
Olive leaf 3%	7.9±0.875a	7.6±0.843a	7.5±1.032a	7.8±0.632a	7.2±0.918bcd
Lemon grass 1%	7.9±0.737a	7.7±0.823a	7.2±1.059ab	7.5±0.971a	7.6±0.843abc
Lemon grass 2%	8±0.666a	7.8±0.788a	7.3±0.966ab	8.1±0.737a	7.9±0.737ab
Lemon grass 3%	7.9±0.736a	7.3±1.251a	7.8±0.979a	8.0±0.942a	7.5±0.527abc

Microbiological Analysis

Microbial analysis (total viable bacterial counts, coliform group, Mould & Yeast, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes*) of manufacturing beef burger.

Total Viable Bacterial

Figure 3 presents the total viable bacterial counts for 13 treatments of beef burger. The changes in total bacterial counts (log10 cfu/g) of beef burger manufactured with different types of extracts during storage at 2-7°C for 18 days are shown in Figure 3. In general, the total bacterial counts were increased in all treatments of beef burger manufactured through the storage period until 10th day's storage, and then the trend was decreased gradually till the end of storage. The results of manufactured beef burger the mean total bacterial count was ranged from 5.09 to 6.0 $\log_{10}$ (cfu/g) Figure 3. The results of control manufactured beef burger had increased in total viable bacterial counts from $\log_{10}$ cfu/g 5.36 in fresh to log10 cfu/g 6.0 after 10 days, then that decreased gradually till the end of storage was log10 cfu/g 5.82.

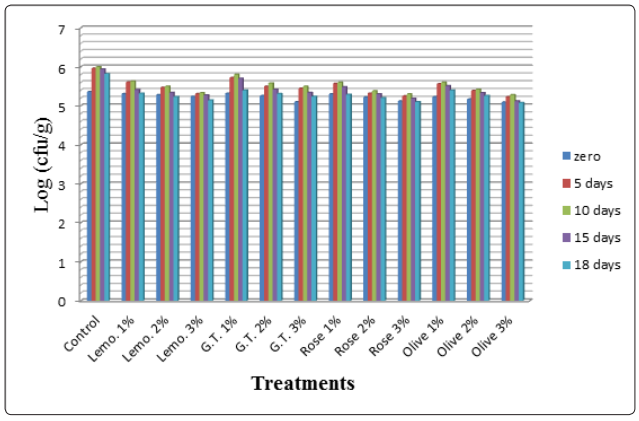

Figure 3: Log of total bacterial counts (cfu/g) in beef burger manufactured with different extracts during cold storage period for 18 days

Figure 4 shows the changes in coliform bacterial counts (log10 cfu/g) of beef burger manufactured with different types of extracts during storage at 2-7°C for 18 days. Coliform bacterial count was undetected in all treatment until 5 days from storage period, after 5 days from storage period some beef burger treatments as control were found coliform bacterial count. Storage of beef burger manufactured lead to a significant increase in the coliform bacterial count in control. Coliform bacterial count ranged from less than 10^2 cfu/g, but coliform bacterial count was undetected in beef burger with 3% of lemon grass extract.

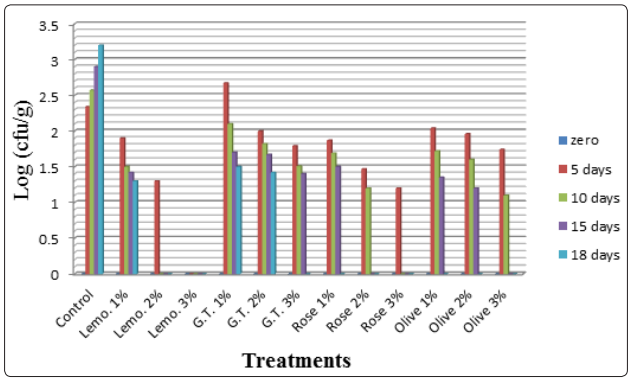

Figure 4: Log of coliform counts (cfu/g) in beef burger manufactured with different extract during cold storage period for 18 days

Figure 5 shows the changes in Mould and yeast counts ($\log_{10}$ cfu/g) of beef burger manufactured with different types of extracts during storage at 2-7°C for 18 days. Mould and yeast mean count ranged from 1.87 to 3.46 log10 (cfu/g), Mould and yeast counts undetected in all treatment until 5 days from storage period, after 5 days from storage period some beef burger treatments as control were found Mould and yeast counts, but undetected in green tea and olive leaves extracts through storage period.

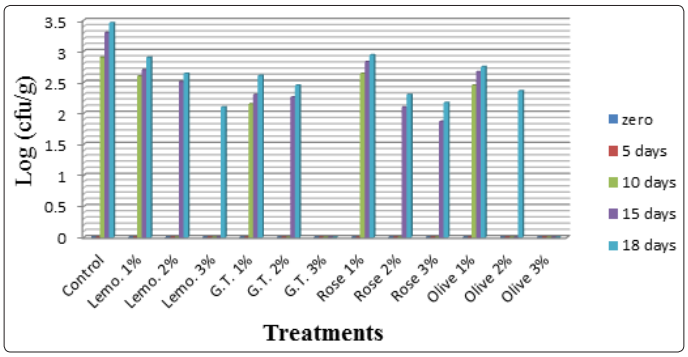

Figure 5: Log of mould and yeas counts (cfu/g) in beef burger manufactured with different extract during cold storage period for 18 days

Discussion

The total phenolic compounds are consistent with Ferial, et al., who said that, the content of total phenolic compounds for green tea leaves was 34.3 mg/g [35]. Wanasundara and Shahidi mentioned that the content of polyphenols in green tea reached to 36% (dry basis) [36]. Bouaziz, et al., showed that olive leaf extract has antioxidative and antimicrobial activates inhibit lipid oxidation and protect low-density lipoprotein from oxidation due to the content of phenolic compounds [37]. Altiok, et al., found that olive leaves extract has highest phenolic compounds and contained 10.3 mg /g leaf by using 70% ethanol as extraction solution obtained 26.5 mg oleuropein /g lyophilized olive leaves extract by using water as solvent [38].

From the results of antioxidants activity it could be seen that methanol, ethanol and water extracts of green tea, roselle, olive leaves and lemon grass has high phenolic compounds and antioxidants activity than that of grapes and red cabbage leaves. Therefore, research was conducted on those extracts as antioxidants to improve the quality of beef burger during cold storage.

According to the analyses of chemical composition of beef burger, fresh beef burger contains14.67% crude fat, 21.10% crude protein, 2.26% ash and 69.10% moisture content. Burger with olive leaf extracts contains high level of moisture (69.22% to 71.25%) than burger with roselle extract (63.12 to 65.26%) while, high level of protein (20.78%) in roselle burger than green tea (15.15).

The TBA values increased during storage due to the lipid oxidation. TBARS values burger samples were reduced with addition of the natural antioxidant extracts compared to control sample where the TBARS values were reached to the highest in control sample at the end of storage. Tea catechin (300 mg/kg meat) which contains high levels of phenolic compounds were also found to be more effective in retardation of lipid oxidation in raw and cooked beef and chicken meat, poultry and fish muscle [39]. The Phenolic compounds interrupt the propagation of the free radical auto oxidation chain with the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes [40,41]. In the current study, since the phenolic compounds in natural extracts used in preparing beef burger, could cause an inhibition of lipid oxidation [42].

The changes in color attributes may be attributed to the natural yellow-brown color of the olive leaf extract [43]. Antioxidant treatments significantly ($p<0.05$) slowed the burgers discoloration. Treatment with green tea extract and carnosine significantly retarded discoloration ($p< 0.05$) compared with other treatments. The effectiveness of the antioxidants in preserving the redness value was green tea>olive leaf extract >roselle> lemon grass. The lightness of beef burger increased during storage while increasing concentration of olive leaf (OLE) extract caused a decrease in lightness of beef samples.

Thus, using natural antioxidants is important to preserve the quality of meat products and prevent their oxidation [44]. Therefore, it is suggested that roselle extract as a natural herb, could be used to extend the shelf -life of chicken patties and provide the consumer with food containing natural additives, which might be more healthful [45]. Yanishlleva et al., showed that the catechins of green tea does not affect color, overall sensory quality and reduce the production of putrescine and tyramine of sausages [46].

Antibacterial Activity of Different Plants Extracts

These results agree with Chao and Yin 2009 who mentioned that the roselle calyx aqueous and ethanol extracts and protocatechuic acid effectively and dose-dependently inhibited the growth of *S. typhimurium* DT104, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *B. cereus* in ground beef. Lemon extract showed inhibitory activity of 33mm towards *Bacillus cereus*. These results agree with Nascimento et al., who mentioned that the extracts from basil, clove, guava, jambolan, lemon balm, pomegranate, rosemary and thyme presented antimicrobial activity to at least one of the tested microorganisms [47]. Green tea extract showed inhibitory activity towards *Staphylococcus aureus* was less sensitive with the diameter zone 40mm. These results are in agreement with Chan et al., who mentioned that all the extracts showed inhibitory effects on Gram-positive but not on Gram-negative bacteria [48].

Microbiological Analysis

The results of all manufactured beef burger had increased in total viable bacterial counts, then that decreased gradually till the end of storage. These results are in agreement with who mentioned that the quality of burger products. Elkhatim et al., was assessed using microbiological analyses as well as the total bacterial counts [49]. Storage of meat and meat products lead to a significant increase in the total viable bacterial count. Musa reported an average aerobic plate count of $1.2x10^6$ cfu/g in fresh beef before processing whereas; during processing the average was $9.4x10^5$cfu/g, $1.1x10^7$ cfu/g and $2.6x10^8$ cfu/g for minced meat, sausage and beef burger, respectively [50].

Coliform bacterial count was detected after 5 days from storage period some beef burger treatments as control were found coliform bacterial count. These results are in agreement with Abdelhai et al., who mentioned that raw meat used for the processing of Shawarma is a low-quality meat [51]. Shawarma showed poor microbiological quality since the load in many cases exceed the allowed standard level. The cooking temperature (heat treatment) to which Shawarma products are exposed is not sufficient to eliminate harmful microorganisms such as *E. coli*.

Mould and yeast counts undetected in all treatment until 5 days from storage period, These results are in agreement with Abdelhai et al., who mentioned that the conditions of the environment in the manufacturing rooms, stores, refrigerators and shops are very suitable for the development of moulds inside the products, but

more frequently on the surface of various sorts of meat and meat products [51].

Conclusion

Extracts from green tea, roselle, lemon grass and olive leaf could be used as a natural antioxidant to extend the shelf-life of beef burger, polyphenols had the potential to reduce the oxidation, provided the most effective antioxidant activity in terms of lowest TBARS values until the latter stages of storage and that improve quality and provide safe product during storage by reducing microbial growth and lipid oxidation. Green tea extracts had longer antioxidant effect than olive leaf, lemon grass and roselle. The ethanol extracts had healthy effects on the sensory profile of beef burger improving meat products stability.

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