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Effect of Natural Antioxidant Extracts on Oxidative and Microbiological Stability of Beef Burger

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ABSTRACT

In recent years, the demand for natural antioxidants has been increased mainly because of the adverse effects of synthetic antioxidants, which have been confirmed for their toxicological and carcinogenic effects. Thus, most of the recent investigations have been directed towards the identification of natural antioxidants from various plant sources, which will offer increased consumer acceptability, decreased potential health risks, and can often achieve the same degree of oxidation prevention as Synthetic antioxidants. Therefore, the aim of this study was to evaluate the antioxidant and antimicrobial effect of three plants aqueous extract mix (clove, sage and kiwifruit peel) to be used as natural preservative in meat product (burger patties), So the aqueous extract mix was applied as natural preservative in beef burger patties at three concentrations (0.1%, 0.25% and 0.5%) and compared with negative control and positive control (BHT) and the TBARS, DNPH, pH value, WHC and microbial load was evaluated through a storage period of 30 days and at 4 °C, during storage the treatment with aqueous extract mix showed a results superior to that of the synthetic antioxidant with a lower TBARS value, carbonyl content and microbial load at 30 days of storage with mean values of (0.84, 0.73, 0.73, 0.67 and 0.55 Mg MDA\ Kg), (14.05, 13.46, 13.25, 11.21 and 9.92 Nmol carbonyl content/mg protein) and ($5.2x10^6$, $2.9x10^5$, $3.1x10^5$, $2x10^5$and $2.8x10^4$ Cfu/g) respectively for (negative control, positive control (BHT), mix 0.1%, mix 0.25% and 0.5%). These findings show that this plant extract mix at the level of 0.5% is very effective against lipid and protein oxidation, and a powerful antimicrobial in beef burger patties and is a promising natural antioxidant and antimicrobial replacing the synthetic preservatives in meat processing

1. INTRODUCTION

One of the most significant challenges facing processors of meat products is oxidation (Oswell et al., 2018), these oxidation occur more quickly in minced meats than in intact meat, this is because the surface area of meat to air increases substantially after grinding (Naveena et al., 2008). Also products with high fat content, such as processed meat products, are more susceptible to oxidation which can result in undesirable odours and flavours as well as nutrient loss. Lipid oxidation is a major cause of muscle food deterioration (Ladikos and Lougovois, 1990), it affects the quality of the product through loss of desirable colour, odour and flavour and reduces shelf life (Faustman and Cassens, 1990), It has also been found that lipid oxidation can cause pathological changes in the mucous membrane of the alimentary tract, inhibit the activity of enzymes and increase the content of cholesterol and peroxides in blood serum, thus potentially leading to atherosclerosis. Moreover, lipid oxidation can also lead to the production of malondialdehyde, a potent mutagen and/or carcinogen (Frankel, 1991; Shan et al., 2009).

Also muscle foods may be more susceptible to protein oxidation because of their reduced pH post-rigor, which intensifies the autoxidation of heme proteins, protein oxidation reduces digestibility and can yield genotoxic and cytotoxic derivatives of amino acids. Consequently, it is often necessary to rely on antioxidants to prolong shelf life and preserve product quality(Das et al., 2012).

So in order to reduce oxidative changes and to prevent bacterial growth several synthetic food additives are regularly used by meat processors. However, in recent years due to increasing consumer awareness about potentially toxic effects and health issues the use of nitrites and synthetic antioxidants (BHT, BHA, TBHQ) decreased, while demand for natural additives rapidly increase (Šojić et al., 2018). Many of natural antioxidants also exhibit antimicrobial activity and thus have the advantage of being readily accepted by both consumers and meat processors (Jayawardana et al., 2015). In this regard, plants and spices are a valuable source of bioactive compounds, thus raising great interest as natural preservatives in order to improve the overall quality of meat and meat products.

However, natural agents are often more expensive and less effective than synthetic ones. Consequently, increasing attention is recently paid to the extraction of antioxidants from agro-food industry by-products (Lorenzo et al., 2014).

Clove (Syzygium aromaticum) is a widely used spice in meat and meat products, it is used as powder or extract of whole clove buds. Clove bud extract contains high levels of eugenol (32.15%) and caryophyllene (22.43%). Due to its high level of total phenolic content, its antioxidant effects have been evaluated in cooked ground beef, mutton and meat patties. Clove also has antimicrobial activity (Jayathilakan et al., 2007; Shi et al., 2014; Jin et al., 2016).

Sage (Salvia officinalis L.) represents medicinal plant from Lamiaceae family, which has been recognized for various biological activities due to its interesting chemical profile. Besides widely used application in pharmaceutical industry, sage has found application as flavouring agent in food products(Gali-Muhtasib et al., 2000).

Kiwi fruit (Actinidia deliciosa) originated in Asia and became popular worldwide due to its sensory and nutritional properties, such as a high level of fiber, minerals and bioactive compounds with antioxidant activity. These phytochemicals slow the speed of lipid oxidation reactions, which are responsible for the deterioration of food. They act as scavengers of free radicals, or prevent their formation, Flours obtained from the kiwi fruit skin and bagasse of both green and ripe fruit can be considered as sources of dietary fiber and bioactive compounds with antioxidant activity. The use of flours made from by-products of kiwi fruit can contribute to the reduction of agro-industrial waste and they are also promising ingredients for the enrichment of products with dietary fiber and bioactive compounds with antioxidant action(Soquetta et al., 2016).

Therefore the objectives of the present study was to investigate the potential role of natural antioxidant combination (clove, sage and kiwifruit peel) to be used as natural preservative in meat product (burger patties) and monitoring physic-chemical, microbiological and organoleptic changes of beef burger stored at 4^{o}C during 30 days of storage.

2. MATERIAL AND METHODS

2.1. Preparation of plants extracts (Water extraction):

The extracts of tested plants were prepared according to Kim et al. (2013) with some modifications, The leaves, buds and peels samples were dried at (40^{o}C/ 24hr) dried plants were ground using grinder, 50 grams of each plant powder were separately stirred in deionized water (1:20 W/V) at 45°C for 1hr then it was soaked for 24 h at room temperature using magnetic stirrer model F20500010; made in Europe. Mixture was centrifuged at 3000Xg for 10 min at 20°C then filtered through Whatman No. 1 filter paper. The extract was lyophilized by (Vacuum freeze dryer model: FDF 0350; Korea).The lyophilized powder of plant extract was then stored at -20^{o}C until analysis. (Vongsak et al., 2013).

Table 1. Description of herbal plants.

Parameter	Clove	Sage	Kiwifruit
Botanical name	Syzygium aromaticum	Salvia officinalis	Actinidia deliciosa,
Family	Myrtaceae	Lamiaceae	Actinidia
English name	Clove	Sage	Kiwifruit, Chinese gooseberry
Part used	Flower buds	leaves	The fruit peel

This plant extracts was evaluated for their safety by (Vijayasteltar et al., 2016) who studied the safety

evaluation of a polyphenol rich extract of clove buds (Clovinol) as shown by the acute (14 days) and subchronic oral gavage at 1 g/kg b. wt. for 90 days and mutagenicity studies and reported that clove extract is very safe. Clove and Sage is generally recognized as safe by the (Food and Drug Administration, 2018) and is approved for food use as a spice or seasoning.

2.2. Preparation of different mixes of the three tested plant extracts:

To figure out the activity of different extracts combinations in preservation of meat products three different mixes of 3 plant extract were prepared and tested as shown in Table 2. Each individual lyophilized extract was weighed in grams and mixed in a ratio of 1:1:1 clove, sage and kiwifruit peel extract.

Table 2. Preparation of different plants extracts Mixture

Mixes of plant extract	Weight of extract per gm/kg meat		
	Clove	Sage	Kiwifruit peel
Mix 0.1%	0.33	0.33	0.33
Mix 0.25%	0.83	0.83	0.83
Mix 0.5%	1.67	1.67	1.67

2.3. Preparation of meat product:

The processing of beef burger was carried out according to Egyptian Standards (2005) for frozen beef burger as shown in Table (3).Three plant extracts; water extracts of Clove (CE), Sage (SE) and Kiwifruit peel (KE). Mixture of plant extracts at same amount (CE, SE and KE) was added to the samples at three different levels; 0.1, 0.25 and 0.5% = 1000, 2500 and 5000 ppm.

The ground beef meat was mixed until a homogenous distribution was obtained, then it was divided into five equal portions. The first portion was remained without any additions, negative control (NC); the second portion was manufactured using 0.02% BHT (200 ppm), Positive control (PC), and other portions at three concentrations; 0.1, 0.25 and 0.5% from plant extract mixture. The beef meat mixture of each treatment was shaped to burger (12 cm diameter, 0.5 cm thickness with average weight 70 g). Each 5 burgers were emplaced into foam plates, wrapped with polyethylene sheets and stored at 4 ± 1 °C for 30 days for further analysis.

Table 3. Recipe of manufactured beef burger samples

Ingredients %	Treatments				
	Control	BHT	PEM1000	PEM2500	PEM5000
Lean meat	65	65	65	65	65
Fat	20	20	20	20	20
Salt	1.5	1.5	1.5	1.5	1.5
Spices mixture	1	1	1	1	1
Ice water	7.5	7.5	7.5	7.5	7.5
Fresh onion	5	5	5	5	5
BHT%	-	0.02	-	-	-
PEM%	-	-	0.1	0.25	0.5

Spices mixture was prepared from black pepper (25%), Cardamom (25%), Chinese cubeb (20%), Cinnamon (10%), red pepper (10%), and laurel leaf (10%). Each type spice was powdered before mixing. (PEM) plant extracts Mixture.

2.4. Chemical composition of raw meat and meat product:

After preparing the samples, proximate composition (Moisture, protein, fat, ash and carbohydrate contents) of samples were carried out according to AOAC (2000). For determination of moisture contents, 3 g of sample were dried at 105 °C until constant weight. Protein content was determined according to the Kjeldahl method. For conversion of nitrogen into crude protein, a factor 6.25 was used. Fat was determined by fat extraction system with petroleum ether and calculating the weight loss. Ash content was determined by aching the sample at 600°C for 2 h and calculating the weight loss. The total carbohydrate (%) was calculated by differences for different samples. All determinations were conducted in Food Technology Lab, Arid Land Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

2.5. Thiobarbituric acid reactive substances (TBARS) value:

Meat samples were analyzed for thiobarbituric acid reactive substances (TBARS), the method described with (Radha Krishnan *et al*., 2014). Five gram of meat was homogenized with 15 ml of deionized distilled water. 1 ml of the meat homogenate was transferred to a test tube and 50 µL of BHT (7.2%) and 2 ml of thiobarbituric acid (TBA) - trichloroacetic acid (TCA) (15 mM TBA–15% TCA) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min to develop color. Then samples were subjected to cooling for 10 min, vortexed again, and centrifuged for 15 min at 2500 ×g. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 ml of deionized water and 2 ml of TBA–TCA solution. The amount of TBARS was expressed as milligrams of malonaldehyde per kilogram of meat.

2.6. Protein oxidation (Modified DNPH carbonyl assay):

The method applied was based on the traditional spectrophotometric DNPH assay described by Levine *et al*. (1990).The novel method was as follows: One g (in triplicates) of meat sample was homogenized in 10 ml of ice-cold 0.15 M KCl solution. Three aliquots 100 µl of the homogenate were mixed with 1 ml of 10% TCA and centrifuged at 1000 rpm for 5 min (Eppendorf Centrifuge 5424). After removing the supernatant, 400 µl of 5% SDS were added to the pellet that was subsequently heated at 100º C for 10 min. The samples (3 replicates) were then treated with 0.8 mlof 0.3% (w/v) DNPH in 3 M HCl while 0.8 ml of 3 M HCl were added to the blank (2 replicates). After 30 min incubation, 400 µl of 40%TCA were added to precipitate the proteins and the supernatant was separated by centrifugation at 10000 rpm for 5 min.

After removing the supernatant, the pellet was washed three times with 1 ml of ethanol–ethyl acetate (1:1, v:v) solution by centrifugation at 15000 rpm for 5 min. These washing steps aimed to remove any free DNPH that could interfere with the spectrophotometric measurement giving an overestimation of the carbonyl content. After the final wash, the resulting pellets were dried with nitrogen and subsequently dissolved in 1.5 ml of 6 M guanidine hydrochloride in 20 mM NaH_2PO_4 (pH 6.5). After incubation overnight at 4º C, the ml aliquots from 10^{-1}to10^{-6}dilutions). The prepared samples were subjected to the following examination.

absorbance at 280 and 370 nm was measured with a UV–VIS 1800 spectrophotometer in order to quantify protein concentration and carbonyl content, respectively. Carbonyl content, expressed as nmol/mg of protein, was calculated according to the following equation (Levine *et al*., 1994) with slight modifications aimed at considering the potential interference given by the hydrazone at 280 nm:

Carbonyl group contents [n mol/mg protein]

$$\text{Carbonyl group content} = \frac{\text{Abs 370 s} - \text{Abs 370 b}}{2200 \times [\text{Abs 280 s} - (\text{Abs 370 s} - \text{Abs 370 b}) \times 0.43]} \times 10^6$$

70 s = absorbance of samples at 370 nm
Abs 370 b = absorbance of blank at 370 nm
Abs 280 s = absorbance of samples at 280 nm
Blank = 800 µl HCl 3M + 1.5 ml Guanidine. HCl in NaH2PO4 Solution

2.7. PH values:

PH values were determined according to Lemay et *al*. (2002) 10 g of meat sample was homogenized in 100 ml distilled water, and the mixture was filtered. The pH of the filtrate was measured using a pH meter (Adwa, AD1030 PH/ mv. temperature meter).

2.8. Water Holding Capacity (WHC):

Water holding capacity (WHC) of meat samples was measured by centrifugation assay according to Mehri *et al*. (2015). The samples were cut into cubes 50 mg and then centrifuged at 1000 ×g at 4◦C for 15 min and WHC was calculated using the following formula:

WHC (%) = (weight before centrifugation/ weight after centrifugation) × 100

2.9. Microbiological analysis (American Public Health Association APHA, 1992):

2.9.1. Preparation of samples for bacteriological examination (ICMSF, 1978):

Meat samples were microbiologically analyzed at days; 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 of cold storage at 4◦C.

Then 1 gram of each sample was taken aseptically using sterile forceps and scissors. The removed portion was placed in sterile test tube containing 9 ml of sterilized peptone water (0.1%). The contents were homogenized at 14000 rpm for 2.5 minutes and allowed to stand for about 6 minutes at room temperature. After that serial dilution was prepared (1

2.9.2. Determination of total aerobic bacterial count (Mailoa *et al*., 2017)(Cruickshank *et al*., 1975):

Carried out by using standard plate count agar medium (PCA) (Conda, Spain), One ml from each of the previously prepared serial dilutions was aseptically transferred into duplicate plate of sterile petri-dishes, and then about 15 ml of sterile standard plate count agar previously melted and cooled at 45 c were poured and thoroughly mixed in a horizontal position. After solidification sample plates as well as control plates were incubated at an inverted position at 37 c for 24 hours. Only plates contain 30-300 colonies were counted and recorded as a total aerobic bacterial count CFU/g.

2.9.3. Determination of total coliforms count (ICMSF, 1996) (Ray and Speck, 1978):

The same technique of the previous pour plate method was applied using Violet Red Bile agar medium (VRB) (Conda, Spain). The plates were incubated at 37^{o}C for 24 hrs. All dark red colonies were then counted and the average colonies were determined. The Coliforms count CFU/g was calculated.

2.9.4. Determination of total yeast and molds count (Copetti et al., 2009):

Pour plate method was applied using Potato dextrose agar (PDA) medium (Conda, Spain), incubated at 28◦C for 72 h for yeast and 5 days for mould. The yeast and mould count CFU/g was calculated

2.10. Sensory analysis:

Sensory analysis of beef burger patties was performed immediately after processing. For sensory evaluation, 20 experienced panelists (from both sexes in the age range of 30 to 45 years) were chosen from the staff members of the Food Technology Department, Arid Land Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

Moreover, they received a preparatory session related to descriptive profile of sensory attributes (appearance, flavor, taste, color, tenderness and overall acceptability) prior to testing so that each panelist could thoroughly discuss and clarify each attribute to be evaluated. All testing was carried out under controlled conditions. Tap water was provided between samples to cleanse the palate. Ten beef patties from each formula were cooked at 180 °C in a forced draught oven (Heraeus D-63,450 Hanau, Germany) to a core temperature 75 °C and maintained warming the oven until testing within 3–8 min (Fernández-López *et al*., 2006). Burger patties where cut in equal pieces of approximately 1.5 cm–2 cm and served at room temperature. Each panelist evaluated three replicates of all formulas in a randomized order and asked to assign a numerical value between 1 and 10 for following attributes: appearance, flavor, taste, color, tenderness and overall acceptability where 10denotes extremely acceptable and 1 denotes extremely unacceptable. At the end of evaluation of the given sample, each panelist was asked to give a score for overall acceptability from 1 (dislike very much) to 10 (like very much).

The scale points were as follows: excellent, 9; very good, 8; good, 7; acceptable, 6; poor (first off-odor, off-taste development), 6; a score of 6 was considered the lower limit of acceptability. A product was defined as unacceptable after the development of the first off-odor or off-taste. Sensory evaluation was held a day after processing.

2.11. Statistical Analysis:

Data collected were analyzed using one-way analysis of variance (ANOVA) with Duncan by SPSS® version 16.0. A statistical probability (p value) less than 0.05 indicated a statistically significant difference between groups (Steel and Torrie, 1980).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of raw meat and meat product:

Proximate composition of raw meat and meat products (on dry basis) recorded in table (4). Data showed that the highest moisture content found in raw meat recorded 65.16±1.32% and 57.14±0.96% for burger samples, Protein content showed difference between raw meat and burger product (40.42±0.86 and 51.70±0.35 % respectively). Ash content recorded 0.96±0.41 % in raw meat, while ash content in burger product was 1.62±0.23 %. Fat content ranged between 10.15±0.62% and 16.02±0.54% in raw meat and burger samples respectively. Carbohydrate content was recorded 1.94±0.31% in raw meat and 5.67±0.51% for burger samples. The obtained results in agreement with Ramadan *et al*. (2011) which reported that fat content in processed meat products should not exceed than 30%, but it was slightly different from the results presented by (Cobos and Díaz, 2015) who reported that Meat is composed of approximately 72–75 % water, 21 % nitrogenous compounds, 1 % non-nitrogenous compounds (vitamins) and carbohydrates, and 1 % ash. The most variable compounds are lipids, with values that can vary between 1 % and 15 % based on wet basis. Meat composition is variable due to the influence of several factors: animal species, breed, sex, feeding, muscle, etc. Results for beef burger were in agreement with Egyptian Standards (2005) for frozen beef burger.

The variance between data of raw meat and meat product is due to the difference between the additions to raw meat.

3.2. Thiobarbituric acid bioactive substances (TBARS) of beef burger samples during storage at 4 ◦C for 30 days

TBARS' assay is one of the most widely used methods for measuring secondary oxidation products mainly malonaldehyde which are known as the cause of oxidative rancidity which may contribute to the off-flavor of oxidized fat (Zhang *et al*., 2016), TBARS are produced through second stage autoxidation during which peroxides are oxidized to aldehydes and ketones (e.g., MDA) (Turgut *et al*., 2016).

The result is showed in Table (5) shows the effect of the three concentrations of the plant extract mix on lipid oxidation in different burger samples during the 30 days of storage at 4 °C, Thiobarbituric acid reactive substances (TBARS) as an indicator of lipid oxidation were measured based on mg of malonaldehyde per kg of the sample, permissible limit should not exceed 0.9 Mg MDA\ Kg according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005), at zero day there was no significant difference ($p<0.05$) in TBARS value between the burger samples but through the 30 days of storage the TBARS value increased significantly in the negative control, positive control and mix 0.1% the results changed from (0.17, 0.15 and 0.13 Mg MDA\ Kg) respectively at zero day to (0.84, 0.73 and 0.73 Mg MDA\ Kg) respectively at 30 days of storage, while in mix 0.25 and 0.5% the results changed from (0.14 and 0.13 Mg MDA\ Kg) respectively at zero day to (0.67 and 0.55 Mg MDA\ Kg) respectively at 30 days of storage, so we conclude that the negative control group with very close to cross the permissible limits at 30 days with a result of 0.84 Mg MDA\ Kg compared with the mix 0.5% group which doesn't changed significantly at 30 day with a result of 0.55 Mg MDA\ Kg which show a strong antioxidant activity superior to such synthetic antioxidant compounds as BHT which showed the result of 0.73 Mg MDA\ Kg at 30 days which is very close to the permissible limit.

This results in agreement with (Velasco and Williams, 2011) who showed the relation between Antioxidant capacity and total phenolic compound content of some methanolic plant extracts and their effects on meat quality during 7-d refrigeration, with sage plant extract the TBARS was less than 0.5 Mg MDA\ Kg at 7 days of storage according to Tanabe *et al*. (2002), and less than 0.3 Mg MDA\ Kg at 7 days of storage according to (Fasseas *et al*., 2008), and also it was in agreement with clove plant extract the TBARS was less than 0.5 Mg MDA\ Kg at 7 days of storage according to (Tanabe *et al*., 2002).

Nevertheless, these results clearly showed that the use of natural antioxidant sources could be effective in preventing meat products against lipid oxidation at chilled storage. The inhibitory effect of plant extract mix on lipid oxidation is attributed to its phenolic content showing antioxidant activity. The phenolic content with antioxidant activity inhibits lipid oxidation by blocking radical chain reaction in the oxidation process (Negi and Jayaprakasha, 2003). Natural antioxidants are believed to interrupt free radical chains by offering hydrogen from the phenolic groups, result in the formation of a stable end product. This reasoning is in line similarly to that illustrated by Zhang *et al*. (2016) that the results demonstrate the effectiveness of clove extract in reducing lipid oxidation.

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Table 4. Chemical composition of raw meat and meat product.

Sample	Moisture	Protein	Fat	Ash	Carbohydrate
Raw Meat	65.16 ± 1.32^{b}	40.42 ± 0.86^{e}	10.15 ± 0.62^{b}	0.96 ± 0.41^{c}	1.94 ± 0.31^{d}
Burger	57.14 ± 0.96^{f}	51.70 ± 0.35^{b}	16.02 ± 0.54^{a}	1.62 ± 0.23^{b}	5.67 ± 0.51^{c}

- Results are in g/100g sample (based on dry weight)
- Reported values are the mean ± SD of three replicates. Means in the same column followed by different lower case letters are significantly different ($p<0.05$).

Table 5. Effect of plant extract mix on lipid oxidation (TBARS) of beef burger samples during storage at 4◦C for 30 days

Mg MDA\ Kg Storage Time (Days)	Treatments				
	Control	**BHT**	**Mix 0.1%**	**Mix 0.25%**	**Mix 0.5%**
0	0.17 ± 0.02^{Aa}	0.15 ± 0.01^{Aa}	0.13 ± 0.04^{Aa}	0.14 ± 0.06^{Aa}	0.13 ± 0.03^{Aa}
3	0.23 ± 0.01^{ABa}	0.22 ± 0.05^{ABa}	0.21 ± 0.03^{ABa}	0.21 ± 0.02^{ABa}	0.20 ± 0.01^{ABa}
6	0.28 ± 0.02^{ABCa}	0.26 ± 0.01^{ABa}	0.25 ± 0.04^{ABa}	0.24 ± 0.04^{ABa}	0.23 ± 0.01^{ABa}
9	0.31 ± 0.05^{ABCDa}	0.29 ± 0.08^{ABa}	0.28 ± 0.07^{ABa}	0.28 ± 0.06^{ABa}	0.24 ± 0.07^{ABa}
12	0.41 ± 0.09^{ABCDEa}	0.33 ± 0.04^{ABCa}	0.33 ± 0.02^{ABCa}	0.29 ± 0.04^{ABCa}	0.26 ± 0.03^{ABa}
15	0.53 ± 0.03^{ABCDEa}	0.35 ± 0.04^{ABCa}	0.35 ± 0.06^{ABCDa}	0.32 ± 0.09^{ABCa}	0.31 ± 0.07^{ABa}
18	0.59 ± 0.15^{ABCDEFa}	0.43 ± 0.02^{ABCDa}	0.41 ± 0.16^{ABCDa}	0.40 ± 0.13^{ABCa}	0.37 ± 0.02^{ABa}
21	0.65 ± 0.19^{CDEFa}	0.54 ± 0.14^{BCDa}	0.52 ± 0.12^{BCDa}	0.50 ± 0.18^{ABCa}	0.40 ± 0.11^{ABa}
24	0.68 ± 0.27^{DEFa}	0.57 ± 0.15^{BCDa}	0.56 ± 0.17^{BCDa}	0.53 ± 0.21^{BCa}	0.47 ± 0.16^{ABa}
27	0.75 ± 0.01^{EFa}	0.69 ± 0.14^{CDa}	0.69 ± 0.13^{CDa}	0.59 ± 0.20^{BCa}	0.51 ± 0.05^{ABa}
30	0.84 ± 0.23^{Fa}	0.73 ± 0.19^{Da}	0.73 ± 0.15^{Da}	0.67 ± 0.10^{Ca}	0.55 ± 0.08^{Ba}

- Reported values are the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$). Means in the same raw followed by different letters are significantly different ($p<0.05$). Means with similar letters are not significantly different at ($P<0.05$).Upper case letters for columns and lower case letters for rows.
- Permissible limit should not exceed 0.9 Mg MDA\ Kg according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005)

3.3. Evaluation of protein oxidation (DNBH) of beef burger samples during storage at 4◦C for 30 days

The result is showed in Table (6) shows the effect of the three concentrations of the plant extract mix on protein oxidation in different burger samples during the 30 days of storage at 4 °C, (DNPH) values of carbonyl content Nmol carbonyl content/mg protein as an indicator of protein oxidation were measured, permissible limit should not exceed 14 Nmol carbonyl content/mg protein according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005), at zero day there was no significant difference ($p<0.05$) in carbonyl content between the burger samples but through the 30 days of storage the carbonyl content increased significantly in the negative control, positive control and mix 0.1% this change indicate increase in protein oxidation in this samples the results changed from (0.63, 0.62 and 0.63 Nmol carbonyl content/mg protein) respectively at zero day to (14.05, 13.46 and 13.25 Nmol carbonyl content/mg protein) respectively at 30 days of storage, while in mix 0.25 and 0.5% the results changed from (0.62 and 0.68 Nmol carbonyl content/mg protein) respectively at zero day to (11.21 and 9.92 Nmol carbonyl content/mg protein) respectively at 30 days of storage, so the present study conclude that the negative control group exceeded the permissible limits at 30 days with a result of 14.05 Nmol carbonyl content/mg protein compared with the mix 0.5% group which doesn't reach the permissible limits at 30day with a result of 9.92 Nmol carbonyl content/mg protein which show a strong antioxidant activity superior to such synthetic antioxidant compounds as BHT(butylated hydroxytoluene) which showed the result of 13.46 Nmol carbonyl content/mg protein at 30 days which is very close to the permissible limit. So we conclude that the plant extract mix has percent of inhibition against the formation of protein carbonyls in the burger patties. This results was in agreement with (Ganhão et al., 2010) who study the effect of fruit peels and pulp extract on burger patties at chilled storage and observed that storage had a significant effect on protein oxidation as the amount of carbonyl compounds increased significantly in all patties this increase was considerably more intense in the control samples at day one it showed the result of 3.68 and increased to reach 9.52 nmol carbonyls/mg protein at day 12 compared with the treated counterparts which showed the result of 2.58 at day one and it increased to 4.21 nmol carbonyls/mg protein at day 12.

Table 6. Effect of plant extract mix on protein oxidation (DNBH) of beef burger samples during storage at 4◦C for 30 days

Nmol carbonyl content/mg protein Storage Time (Days)	Treatments				
	Control	BHT	Mix 0.1%	Mix 0.25%	Mix 0.5%
0	0.63 ± 0.05^{Aa}	0.62 ± 0.04^{Aa}	0.63 ± 0.08^{Aa}	0.62 ± 0.04^{Aa}	0.68 ± 0.01^{Aa}
3	0.77 ± 0.12^{ABa}	0.78 ± 0.24^{Aa}	0.75 ± 0.15^{Aa}	0.78 ± 0.26^{ABa}	0.71 ± 0.02^{Aa}
6	1.00 ± 0.15^{Ba}	0.95 ± 0.28^{Aa}	0.97 ± 0.37^{Aa}	0.85 ± 0.37^{ABa}	0.83 ± 0.19^{Aa}
9	2.91 ± 0.34^{Ca}	1.99 ± 0.29^{Bb}	1.93 ± 0.48^{Bb}	0.99 ± 0.15^{Bc}	0.91 ± 0.23^{ABc}
12	3.39 ± 0.34^{Da}	3.23 ± 0.57^{Ca}	3.27 ± 0.65^{Ca}	2.26 ± 0.34^{Cb}	1.23 ± 0.45^{Bc}
15	4.57 ± 0.35^{Ea}	4.40 ± 0.16^{Da}	4.49 ± 0.85^{Da}	3.08 ± 0.47^{Db}	2.19 ± 0.38^{Cc}
18	6.29 ± 0.65^{Fa}	5.26 ± 0.57^{Eb}	5.21 ± 0.75^{Eb}	4.47 ± 0.65^{Ec}	3.24 ± 0.64^{Dd}
21	9.26 ± 0.25^{Ga}	7.18 ± 0.24^{Fb}	7.47 ± 0.34^{Fb}	5.43 ± 0.15^{Fc}	4.76 ± 0.24^{Ed}
24	10.21 ± 0.98^{Ha}	9.24 ± 0.45^{Gb}	9.35 ± 0.75^{Gb}	7.26 ± 0.65^{Gc}	5.53 ± 0.34^{Fd}
27	13.18 ± 0.34^{Ia}	11.27 ± 0.25^{Hb}	10.25 ± 0.62^{Hc}	9.38 ± 0.34^{Hd}	7.37 ± 0.48^{Ge}
30	14.05 ± 1.41^{Ja}	13.46 ± 0.85^{Ib}	13.25 ± 0.71^{Ib}	11.21 ± 0.37^{Ic}	9.92 ± 0.68^{Hd}

- Reported values are the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$). Means in the same raw followed by different letters are significantly different ($p<0.05$). Means with similar letters are not significantly different at ($P<0.05$).Upper case letters for columns and lower case letters for rows. Permissible limit should not exceed 14 Nmol carbonyl content/mg proteins according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005).

Also in agreement with (Lorenzo et al., 2018**)** who reported that burger treated with pitanga leaf extract and BHT had an effect on protein oxidation the carbonyl concentration gradually increased in all burgers over the refrigerated period, showing the highest values in control group after 18 days of storage (7 nmol/mg protein). Statistical analysis showed significant differences ($P < 0.001$) among treatments after 15 days of storage, presenting the lowest values in pitanga leaf extract samples (2.37 nmol/mg protein). It is conclude that antioxidants from a natural sources provides a good alternative to conventional antioxidants because of high phenolics and other active ingredients, which can effectively prevent initiation of protein oxidation reactions (Yogesh et al., 2015). These extracts inhibit protein oxidation and degradation of meat and meat products and thus help preserve their nutrition value, Application of these extracts improved the overall sensory and nutritional quality of meat and meat products and hence their shelf-life (Wang et al., 2013). Total or partial inhibition of protein oxidation could prevent an undesirable impact in meat related to tenderness, water holding capacity and nutritional quality (Jia et al. 2012).

3.4. PH Values for beef burger samples during storage at 4 °C for 30 days:

Table (7) shows the effect of the three concentrations of the plant extract mix on the pH values of burger meat samples during storage times at 4 °C for 30 days.

Table 7. Effect of plant extract mix on the pH values of beef burger samples during storage at 4 °C for 30 days.

Storage Time (Days)	Treatments				
	Control	BHT	Mix 0.1%	Mix 0.25%	Mix 0.5%
0	6.17 ± 0.02^{Aa}	6.02 ± 0.01^{Aa}	5.94 ± 0.06^{Aa}	5.89 ± 0.03^{Aa}	5.91 ± 0.04^{Aa}
3	5.81 ± 0.04^{Ba}	5.92 ± 0.02^{ABa}	5.84 ± 0.01^{ABa}	5.87 ± 0.04^{Aa}	5.88 ± 0.05^{Aa}
6	5.76 ± 0.07^{Ba}	5.82 ± 0.02^{ABa}	5.84 ± 0.01^{ABa}	5.81 ± 0.02^{Aa}	5.87 ± 0.01^{Aa}
9	5.72 ± 0.01^{Ba}	5.79 ± 0.02^{ABa}	5.82 ± 0.04^{ABa}	5.80 ± 0.03^{Aa}	5.84 ± 0.04^{Aa}
12	5.69 ± 0.01^{Ba}	5.77 ± 0.02^{ABa}	5.76 ± 0.01^{ABa}	5.79 ± 0.04^{Aa}	5.8 ± 0.01^{Aa}
15	5.64 ± 0.01^{Ba}	5.76 ± 0.02^{ABa}	5.73 ± 0.01^{ABa}	5.71 ± 0.01^{Aa}	5.78 ± 0.02^{Aa}
18	5.58 ± 0.02^{Ba}	5.74 ± 0.01^{ABa}	5.68 ± 0.02^{ABa}	5.7 ± 0.03^{Aa}	5.72 ± 0.04^{Aa}
21	5.55 ± 0.01^{Ba}	5.72 ± 0.03^{ABa}	5.67 ± 0.01^{ABa}	5.7 ± 0.02^{Aa}	5.71 ± 0.01^{Aa}
24	5.49 ± 0.02^{Ba}	5.62 ± 0.01^{Ba}	5.61 ± 0.02^{ABa}	5.69 ± 0.04^{Aa}	5.71 ± 0.03^{Aa}
27	5.46 ± 0.01^{Ba}	5.6 ± 0.02^{Ba}	5.59 ± 0.01^{ABa}	5.65 ± 0.01^{Aa}	5.69 ± 0.01^{Aa}
30	5.46 ± 0.02^{Ba}	5.59 ± 0.03^{Ba}	5.51 ± 0.02^{Ba}	5.65 ± 0.01^{Aa}	5.68 ± 0.01^{Aa}

- Reported values are the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$). Means in the same raw followed by different letters are significantly different ($p<0.05$). Means with similar letters are not significantly different at ($P<0.05$).Upper case letters for columns and lower case letters for rows.

Table 8. Effect of plant extract mix on Water Holding Capacity (WHC) of beef burger samples during storage at 4 ◦C for 30 days

WHC% Storage Time (Days)	Treatments				
	Control	**BHT**	**Mix 0.1%**	**Mix 0.25%**	**Mix 0.5%**
0	95.36±0.15^{Aa}	95.45±0.18^{Aa}	95.62±0.24^{Aa}	95.34±0.15^{Aa}	95.54±0.26^{Aa}
3	95.23±0.21^{Aa}	95.25±0.31^{ABa}	95.45±0.25^{ABa}	95.28±0.14^{ABa}	95.48±0.25^{Aa}
6	94.85±0.14^{Bb}	95.14±0.24^{ABCab}	95.38±0.35^{ABa}	95.13±0.24^{ABCab}	95.39±0.14^{Aa}
9	94.62±0.24^{Bb}	95.03±0.34^{BCa}	95.24±0.29^{BCa}	95.02±0.17^{ABCa}	95.26±0.18^{ABa}
12	94.13±0.15^{Cb}	94.85±0.31^{CDa}	95.14±0.21^{BCa}	94.95±0.17^{BCa}	95.18±0.15^{ABa}
15	94.02±0.01^{Cc}	94.56±0.03^{DEb}	94.95±0.05^{CDa}	94.82±0.02^{CDab}	94.96±0.04^{BCa}
18	93.95±0.02^{Cb}	94.48±0.04^{Ea}	94.76±0.02^{DEa}	94.77±0.30^{CDa}	94.72±0.12^{CDa}
21	93.84±0.15^{Cb}	94.36±0.24^{EFa}	94.52±0.29^{EFa}	94.56±0.34^{DEa}	94.68±015^{CDa}
24	92.65±0.18^{Db}	94.12±0.16^{FGa}	94.38±0.24^{Fa}	94.24±0.18^{EFa}	94.38±0.19^{DEa}
27	92.31±0.25^{Eb}	93.98±0.23^{Ga}	94.19±0.24^{FGa}	94.12±0.19^{Fa}	94.28±0.16^{Ea}
30	92.12±0.21^{Ec}	93.64±0.23^{Hb}	93.99±0.14^{Gab}	94.08±0.24^{Fa}	94.15±0.24^{Ea}

- Reported values are the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$). Means in the same raw followed by different letters are significantly different ($p<0.05$). Means with similar letters are not significantly different at ($P<0.05$).Upper case letters for columns and lower case letters for rows.

The pH of negative control was found to be 6.17±0.02 while the pH value of positive control was found to be 6.02±0.01 and the PH value for treatments mix (0.1, 0.25 and 0.5 %) was (5.94, 5.89, 5.91) respectively at zero time with no significant different ($p<0.05$) the reduced acidity of the treated burger patties could be due to the acidic nature of the mix due to the presence of kiwifruit peels which is lower than 4.5 as reported by (Soquetta et al., 2016) , but during the 30 days of storage there was a significant differences found through the storage periods which reached final pH values of 5.46±0.02 for negative control and 5.59±0.03 for positive control and the PH value for treatments mix 0.1% which reached a value of 5.51 while there was no significant difference in mix (0.25 and 0.5 %) which showed the result of (5.65, 5.68) respectively. The reduced pH during storage has been attributed to the microbial growth. It seems that acid producing bacteria grow in both beef burgers without the plant extract mix and those containing 0.1% plant extract mix. Acid production was higher in burgers lacking the plant extract that may be due to the higher growth rate of lactic acid bacteria. The antimicrobial effect of the extract mix may be the cause of the lower acid production and less pH reduction with increasing the extract mix concentration. This data greed with the result presented by (Soltanizadeh and Ghiasi-Esfahani, 2015) who reported that the burger samples treated with plant extract doesn't experience decrease in pH value as the untreated burger samples.

3.5. Water Holding Capacity (WHC) of beef burger samples during storage at 4 ◦C for 30 days

Table (8) shows the effect of the three concentrations of the plant extract mix on the Water Holding Capacity (WHC**)** of burger meat samples during storage times at 4 °C for 30 days, at zero day there was no significant difference ($p<0.05$) in WHC between the burger samples but through the 30 days of storage the WHC decreased significantly in the negative control, positive control and mix 0.1% the results changed from (95.36, 95.45 and 95.62%) respectively at zero day to (92.12, 93.64 and 93.99 %) respectively at 30 days of storage, while in mix 0.25 and 0.5% the results changed between day zero and day 30 of storage they changed from (95.34 and 95.54%) respectively at zero day to (94.08 and 94.15%) respectively at 30 days of storage.

This indicate that the plant extract mix at all its concentrations, increased the water holding capacity of the burger samples, this result was in agreement with (Soltanizadeh and Ghiasi-Esfahani, 2015) who reported that the burger samples treated with plant extract doesn't experience decrease in WHC value as the untreated burger samples.

4.6. Total bacterial count (cfu/g) of beef burger samples during storage at 4◦C for 30 days

Results presented in table (9) shows the effect of the three concentrations of the plant extract mix on the total bacterial count in burger samples during the 30 days of storage at 4 °C, total bacterial count is expressed as (cfu/g) colony forming unit/gram of sample,

permissible limit should not exceed 10^5 Cfu/g according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005) and 10^6 Cfu/g according to (Food administration, 1995), at zero day there was no significant difference ($p<0.05$) in bacterial count between the burger samples but through the 30 days of storage the bacterial count increased significantly in the negative control, positive control, mix 0.1% and mix 0.25 results changed from (6x10, 2x10, 1x10 and 4x10 Cfu/g) respectively at zero day to ($5.2x10^6$, $2.9x10^5$, $3.1x10^5$ and $2x10^5$Cfu/g) respectively at 30 days of storage which all have exceeded the permissible limits, while mix 0.5% showed the most remarkable results which started from 8x10 Cfu/g at zero day and reached $2.8x10^4$ Cfu/g at 30 days of storage, so the negative control group was unfit between day 21 and 24 of storage while BHT group and mix 0.1% was unfit by day 24 of storage and mix 0.2% become unfit between day 27 and 30 of storage, but the mix 0.5% group didn't reach the permissible limit at day 30 of storage.

Table 9. Effect of plant extract mix on total bacterial count (cfu/g) of beef burger samples during storage at 4◦C for 30 days.

CFU/gm Storage Time (Days)	Treatments				
	Control	**BHT**	**Mix 0.1%**	**Mix 0.25%**	**Mix 0.5%**
0	$6x10^{Aa}$	$2x10^{Abc}$	$1x10^{Ac}$	$4x10^{Aab}$	$8x10^{Aa}$
3	$1.5x10^{2Ba}$	$1.3x10^{2Ba}$	$1.4x10^{2Ba}$	$1.2x10^{2Ba}$	$1.1x10^{2ABa}$
6	$2.6x10^{2Ba}$	$2.9x10^{2Ca}$	$2.9x10^{2BCa}$	$2x10^{2BCa}$	$1.7x10^{2ABCa}$
9	$6.5x10^{2Ca}$	$3.5x10^{2Cab}$	$4.2x10^{2Cab}$	$2.8x10^{2Cab}$	$2.1x10^{2BCb}$
12	$8.6x10^{2Ca}$	$4.9x10^{2Cabc}$	$5.9x10^{2Cab}$	$2.8x10^{2BCbc}$	$2.4x10^{2BCc}$
15	$5.5x10^{3Da}$	$3.7x10^{3Da}$	$4.5x10^{3Da}$	$3.4x10^{2Cb}$	$2.7x10^{2Cb}$
18	$2.8x10^{4Ea}$	$6.7x10^{3DEb}$	$5.7x10^{3Db}$	$2x10^{3DDc}$	$1.8x10^{3Dc}$
21	$5.4x10^{4Fa}$	$1.3x10^{4Eb}$	$2.4x10^{4Eab}$	$3.5x10^{3DEc}$	$2.9x10^{3Dc}$
24	$6.4x10^{5Fa}$	$5.3x10^{4Fb}$	$5.9x10^{4Fb}$	$4.6x10^{3Ec}$	$3.9x10^{3Dc}$
27	$1.3x10^{6Fa}$	$2.7x10^{5Gb}$	$2.3x10^{5Gb}$	$2.3x10^{4Fc}$	$1.4x10^{4Ec}$
30	$5.2x10^{6Ga}$	$2.9x10^{5Gb}$	$3.1x10^{5Gb}$	$2x10^{5Gb}$	$2.8x10^{4Ec}$

- Reported values are the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$). Means in the same raw followed by different letters are significantly different ($p<0.05$). Means with similar letters are not significantly different at ($P<0.05$).Upper case letters for columns and lower case letters for rows.
- Permissible limit should not exceed 10^5 Cfu/g according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005).

This results was in agreement with (Shan et al., 2009) who study the effect of 5 spice and herb extracts as natural preservative in meat and reported that between this 5 extracts the clove show the most remarkable results during 9 days of storage against L. monocytogenes, S. aureus and S. enterica (5.99, 6.87 and 7.42 log CFU g−1respectively) which with lower than the control by 1.36 and 0.96 log CFU g−1 for L. monocytogenes and S. aureus respectively.

It was also in agreement with (Šojić et al., 2018) who study the effect of sage extract on the microbiological stability of sausage during preservation to 8 days and reported that sage reduce the total bacterial count during storage in comparison with the control which reach to 7.66 log Cfu/g and treatment reach to 6.50 log Cfu/g at day 8 of storage. Phenolic compounds might predominantly contribute to the antibacterial activities of the plant extract mix. The partial hydrophobic nature of phenolic compounds may degrade the cell wall, interact with the composition of and disrupt the cytoplasmic membrane, damage membrane proteins and interfere with membrane-integrated enzymes, which may eventually lead to cell death(Shan et al., 2007).

4.7. Coliform count (cfu/g) of beef burger samples during storage at 4◦C for 30 days

Results presented in table (10) shows the effect of the three concentrations of the plant extract mix on the coliform count in burger samples during the 30 days of storage at 4 °C, coliform count is expressed as (cfu/g) colony forming unit/gram of sample, permissible limit should not exceed 10^2 Cfu/g according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005), at zero day there was no significant

difference ($p<0.05$) in coliform count between the burger samples but through the 30 days of storage the coliform count increased significantly in the negative control, positive control, mix 0.1% and mix 0.25 results changed from (1x10, 3x10, 4x10 and 4x10 Cfu/g) respectively at zero day to ($1.7x10^2$, $1.5x10^2$, $1.3x10^2$and $1.2x10^2$Cfu/g) respectively at 6 days of storage which all have exceeded the permissible limits, while mix 0.5% showed the most remarkable results which started from 3x10 Cfu/g at zero day and reached $1.2x10^2$Cfu/g at 15 days of storage which exceeded the permissible limits, the raw meat had high initial count of coliform but the plant extract mix was apple to stabilize it till day30 of storage reaching $2.8x10^3$ Cfu/g.

This results was in agreement with (Šojić et al., 2018) who study the effect of sage extract on the microbiological stability of sausage during preservation to 8 days and reported that sage was able to stabilize the coliform count during the 8days of storage.

Table 10. Effect of plant extract mix on coliform count (cfu/g) of beef burger samples during storage at 4◦C for 30 days

CFU/gm Storage Time (Days)	Treatments				
	Control	**BHT**	**Mix 0.1%**	**Mix 0.25%**	**Mix 0.5%**
0	$1x10^{Ab}$	$3x10^{Aa}$	$4x10^{Aa}$	$4x10^{Aa}$	$3x10^{Aa}$
3	$7x10^{Ba}$	$5x10^{Aa}$	$6x10^{ABa}$	$6x10^{ABa}$	$4x10^{ABa}$
6	$1.7x10^{2Ca}$	$1.5x10^{2Ba}$	$1.3x10^{2BCa}$	$1.2x10^{2BCa}$	$4x10^{ABb}$
9	$2.8x10^{2CDa}$	$1.9x10^{2BCab}$	$1.7x10^{2CDab}$	$1.6x10^{2CDab}$	$9x10^{BCb}$
12	$3.1x10^{2CDa}$	$2x10^{2BCab}$	$2.1x10^{2CDEab}$	$1.7x10^{2CDab}$	$1x10^{2Cb}$
15	$5.9x10^{2Da}$	$3.6x10^{2CDab}$	$3.4x10^{2DEab}$	$2.3x10^{2CDEbc}$	$1.2x10^{2Cc}$
18	$1.5x10^{3Ea}$	$4.9x10^{2Db}$	$4.1x10^{2Ebc}$	$3.1x10^{2DEbc}$	$1.9x10^{2CDc}$
21	$5.6x10^{3Fa}$	$1.2x10^{3Eb}$	$1x103^{Fbc}$	$4.4x10^{2Ecd}$	$3.2x10^{2DEd}$
24	$2.4x10^{4Ga}$	$3.9x10^{3Fb}$	$3.1x10^{3Gb}$	$1.2x10^{3Ec}$	$5.3x10^{2EFc}$
27	$5.4x10^{4Ha}$	$4.7x10^{3Fb}$	$4.3x10^{3Gb}$	$3.7x10^{3Gb}$	$1.1x10^{3Fc}$
30	$2.7x10^{5Ia}$	$1.3x10^{5Gab}$	$1.1x10^{5Hb}$	$4.2x10^{3Gc}$	$2.8x10^{3Gc}$

- Reported values are the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$). Means in the same raw followed by different letters are significantly different ($p<0.05$). Means with similar letters are not significantly different at ($P<0.05$).Upper case letters for columns and lower case letters for rows. Permissible limit should not exceed 10^2 Cfu/g according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005).

3.8. Yeast and mould count (cfu/g) of beef burger samples during storage at 4◦C for 30 days

Results presented in table (11) shows the effect of the three concentrations of the plant extract mix on the yeast and mould count in burger samples during the 30 days of storage at 4 °C, yeast and mould count is expressed as (cfu/g) colony forming unit/gram of sample, sample Should be free from visible fungal growth according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005), at zero day there was no significant difference ($p<0.05$) in yeast and mould count between the burger samples but through the 30 days of storage the count increased significantly in all burger samples at zero day there was no yeast or mould count but at day 15 in negative control group the count had a result of 8x10 Cfu/g, while the BHT, mix 0.1%,mix 0.25% and 0.5% show the results of (6x10, 4x10, 4x10 and 2x10 Cfu/g) respectively at 18 days of storage, but the plant extract mix 0.25% and 0.5% was able to stabilize this count till day 30 of storage to ($2.8x10^2$ and $2.4x10^2$ Cfu/g) respectively, which show considerable antifungal effect. There was visible fungal growth in negative control group starting from day 24 of storage while the rest of the treated samples and positive control doesn't show any visible mould growth till day 30 of storage.

3.9. Sensory evaluation of beef burger samples

The sensory scores of treated burger patties and controls are presented in table (12). The beef burger patties treated with plant extract mix or without extract (PC and NC) gained no a significant difference ($P>0.05$) in all sensory criteria including appearance, taste, color, flavor, taste and overall acceptability after processing, but there was a significant difference in the overall grade with the highest score for the BHT group 48.14 followed by the mix 0.5% group 47.85 then the mix 0.25% group 47.64 and the lowest scores were for the control group followed by the mix 0.1% group (47.14, 46.21) respectively, which considered appositive results as the treatment were acceptable to the consumer. This results is in agreement with (Šojić

et al., 2018) who reported that the addition of sage extract to sausage cause no significant difference (P >0.05) on sensory proprieties of sausage. For centuries, dietary herbs and spices have been traditionally used as food additives throughout the world, especially in China and India, not only to improve the sensory characteristics of foods but also to extend their shelf life(Shahidi et al., 1992).

Fernandes, et al., (2016) reported that maintaining the sensory stability was limited to15 days of storage in addition of oregano extract, the changes in off-odour are consistent with the microbial counts that indicated spoilage over time, from 10 days, with no burgers being microbiologically acceptable after 15 days of refrigerated storage.

Table 11. Effect of plant extract mix on yeast and mould count (cfu/g) of beef burger samples during storage at 4◦C for 30 days

CFU/gm Storage Time (Days)	Treatments				
	Control	**BHT**	**Mix 0.1%**	**Mix 0.25%**	**Mix 0.5%**
0	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}
3	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}
6	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}
9	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}
12	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}	ND^{Aa}
15	$8x10^{Ba}$	ND^{Ab}	ND^{Ab}	ND^{Ab}	ND^{Ab}
18	$1.9x10^{2Ca}$	$6x10^{Bb}$	$4x10^{Bbc}$	$4x10^{Bbc}$	$2x10^{Bc}$
21	$2.1x10^{2Ca}$	$1x10^{2Bab}$	$7x10^{Bbc}$	$7x10^{Bbc}$	$4x10^{BCc}$
24	$3.5x10^{2Ca}$	$2.5x10^{2Ca}$	$2.1x10^{2Ca}$	$9x10^{BCb}$	$8x10^{CDb}$
27	$1.8x10^{3Da}$	$3.6\ x10^{2Cb}$	$2.4\ x10^{2Cb}$	$1.9x10^{2CDb}$	$1.5\ x10^{2DEb}$
30	$3.3x10^{3Da}$	$1.4x10^{3Db}$	$1x10^{3Db}$	$2.8x10^{2Dc}$	$2.4x10^{2Ec}$

- Reported values are the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different (p<0.05). Means in the same raw followed by different letters are significantly different (p<0.05). Means with similar letters are not significantly different at (P<0.05).Upper case letters for columns and lower case letters for rows. Should be free from visible fungal growth according to Egyptian Organization for Standardization and Quality for frozen beef burger (2005).

Table 12. The sensory scores of treated burger patties with plant extract mix at different concentrations and controls

Samples	Appearance	Flavor	Taste	Color	Tenderness	Overall acceptability	Overall grade
Control	$7.79±1.25^{a}$	$7.86±1.17^{a}$	$7.86±1.29^{a}$	$8.07±1.33^{a}$	$7.50±1.22^{a}$	$8.07±0.92^{a}$	$47.14±7.18^{c}$
BHT	$7.93±1.21^{a}$	$8.29±1.27^{a}$	$8.29±1.38^{a}$	$8.07±1.44^{a}$	$7.50±1.56^{a}$	$8.07±1.14^{a}$	$48.14±7.99^{a}$
Mix 0.1%	$7.57±1.28^{a}$	$7.79±1.42^{a}$	$7.79±1.48^{a}$	$8.07±1.54^{a}$	$7.29±1.54^{a}$	$7.71±1.38^{a}$	$46.21±8.65^{d}$
Mix 0.25%	$8.21±1.19^{a}$	$7.93±1.21^{a}$	$7.86±1.41^{a}$	$8.21±1.31^{a}$	$7.57±1.45^{a}$	$7.86±1.35^{a}$	$47.64±7.92^{b}$
Mix 0.5%	$8.14±1.29^{a}$	$7.86±1.35^{a}$	$8.00±1.36^{a}$	$8.14±1.23^{a}$	$7.64±1.50^{a}$	$8.07±1.27^{a}$	$47.85±8.00^{ab}$

- Reported values are the mean ± SD of three replicates. Means in the same column followed by different lower case letters are significantly different (p<0.05). Means with similar letters are not significantly different at (P<0.05).

4. CONCLUSION

In this study, the results confirmed the anti-oxidative and antimicrobial effect of clove, sage and kiwifruit peel extract mix as natural preservative when added to beef burger patties at the level of 0.5% increased the shelf life of beef burger in the storage period during refrigeration at 4 °C exceeding that of the negative and positive control. The addition of natural antioxidants on burger patties improved the oxidative stability and inhibited the microbial growth, reducing the degradation showing results superior to that of the synthetic antioxidant and resulted in a product with a great acceptability. So the best concentration level of extract mix that can be recommended is 0.5%.

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