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Evaluate the antioxidant and antimicrobial effect of marjoram, purslane and sowthistle leaves extracts to be used as natural preservative in meat product

Amany M. Basuny [1], Hossam E. F. Abdel-Raheam[2] and Maha A. Zayed[2]
[1]Biochemistry Department; Faculty of Agriculture, Beni –Suef University, Egypt.
[2]Food Science Department, Faculty of Agriculture, Beni –Suef University, Egypt.

Corresponding Author: Amany Mohamed Basuny; E-mail:dramany_basuny@yahoo.com

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ABSTRACT
Lipid oxidation is one of the most important problems that decrease the shelf life of meat and meat products. Therefore, the aim of this study was to evaluate the antioxidant and antimicrobial effect of three plants Marjoram (Origanum majorana L.), Purslane (Portulaca oleracea L.) and Sowthistle (Sonchus oleraceus) leaves extracts to be used as natural preservative in meat product (burger patties). Powders of Marjoram, Purslane and Sowthistle were incorporated into freshly minced beef meat at concentration (1%) and compared with negative control and positive control (BHT). antimicrobial activity of the leaves was studied. Chemical composition, physical characteristics, and sensory evaluation were evaluated for cooked burgers. The physical characteristics included (water holding capacity), cooking characteristics (shrinkage, cooking loss and cooking yield), shelf life limiting parameters (TBA, TVN and total plate count) and sensory evaluation test were all investigated, while the chemical characteristics included moisture, protein, fat contents and pH values of chilled prepared beef burgers stored at freezer (-18°C) for three months. Moisture, protein and water holding capacity increased in both additives (Marjoram, Purslane and Sowthistle leaves extracts) added to the burger recipe and these parameters decreased at the end of storage period while fat increased with additives and after storage. All cooking parameters improved as shrinkage and cooking loss decreased with marjoram addition while cooking yield increased. TBA, TVN and total plate count at all added marjoram Purslane and Sowthistle samples were showed less increase rate comparing to that of control. The sensory evaluation showed no significant differences ($p < 0.05$) between the control samples and the prepared beef burger samples with the added extracts. The findings of the current study recommend possible use of Marjoram, Purslane, and Sowthistle extracts as natural sources of antioxidants and preservatives to extend the burgers shelf life under chilling storage to provide consumers with save healthy beef burger.

1. Introduction

Meat and meat products are an excellent source of essential nutrients with high-quality proteins, fat and mineral. There are a wide variety of meat products including cured meats, patties, nuggets, meatballs and etc [10]. 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 [76].Cooking of meat involves the formation of hydro-peroxides that can be easily broken down to various volatile organic compounds such as alkanes, alkenes, aldehydes, ketones, alcohols, esters and acids that are responsible for reducing the sensorial quality and leading to oxidative flavors, loss of pigments and vitamins in meat and meat products [30]. Antioxidants can be added to meat and meat products during processing to delay lipid oxidation. Plant polyphenols and essential oils (EOs) are considered as major natural source of bioactive compounds to increase the shelf life of meat and meat [68]. Different Synthetic antioxidants such as butylatedhydroxy anisole (BHA), butylatedhydroxy toluene (BHT) and tert-butyl hydroquinone (TBHQ), are widely used in food industry to retard or minimize oxidative deterioration of foods, thus extending the shelf life of meat products [58, 74] In contrast, synthetic compounds have significant disadvantages, such as the risks of manipulation and increase of chemical residues disposed in the food and in the environment. In addition, these preservatives might have negative consequences for the health of the consumers, being associated with possible carcinogenic effects [16]. The plant kingdom is one of the most abundant source of natural antioxidants, which are abundantly present in spices (seeds), herbs and essential oils used in meat products for sensory enhancement [67]. In recent years, the use of natural plant preservatives to increase the shelf-life of food products is promising technology since they derived substances have antioxidant and antimicrobial properties. Consumers have increasingly favored meat products that contain natural additives due to concerns over adverse health effects of synthetic substances particularly some synthetic antioxidants. Many herbs, spices, and their extracts have been added in a variety of foods to improve their sensory characteristics and extend shelf-life [38].

Marjoram (genus Origanum L., family: Lamiaceae) is one of the most beneficial plant used for decades by wide range of consumers as spice as well as a medicinal plant because of its pharmaceutical benefits. Marjoram was reported to contain high amounts of bioactive polyphenolic compounds that is very useful for health and have its therapeutic effect. Traditionally, marjoram has been used as a folk remedy [18]. For centuries, marjoram oil have been used for curing various diseases [3]. Marjoram is considered among the main crops for increasing Egypt income from foreign currency [23]. Purslane (Portulaca oleracea L.) is a warm season plant which has a lot of medicinal properties [51]. It contain high content of phenolic compounds [19]. They are known by their antioxidant capacity due to being able to provide protection against neurodegenerative, oncologic and cardiovascular diseases, besides lipid oxidation on food [53]. Sowthistle (Sonchus oleraceus L.) represents medicinal plant from asteraceae family, which has been recognized for various biological activities due to its interesting chemical profile. Besides widely used application in pharmaceutical industry, Sowthistle has found application as flavouring agent in food products [35, 48]. Therefore, the aim of this study was to evaluate the antioxidant and antimicrobial effect of three plants Marjoram (Origanum majorana L.), Purslane (Portulaca oleracea L.) and Sowthistle (Sonchus oleraceus) leaves extracts to be used as natural preservative in meat product (burger patties).

2. Methodology

2. Materials

2.1. Source of plants:

Each of Fresh leaves of Marjoram (Origanum majorana L.), Purslane (Portulaca oleracea L.), and Sowthistle (Sonchus oleraceus) were collected from various cultivated fields in Beni – seuf governorate.

2.2. Chemicals and reagents

All chemicals and reagents used in the analytical methods (analytical grade) were produced by sigma chemical co. (St. Louis, M., USA) and purchased from EL. gamhouria trading chemicals and drugs co., Egypt

2.3. Ingredients of beef burger

Boneless grounded beef meat was purchased from a local butcher shop at Beni- seuf city, Egypt in the day before the experiment was done and stored in a refrigerator at 5±1°C overnight.

2.4. Spices and additives

Spices (black pepper, nutmeg, clove, thyme, celery, white pepper and rosemary) were obtained from a local market (Beni- seuf, Egypt). All spices were fresh ground directly before used and sieved through mesh 60 (0.25 mm).

2.2. Methods

2.2.1. Preparation of Plants extracts

Experimental plants were used for both quantitative and qualitative screening was washed with distilled water, cut into smaller parts (for easy drying), shade-dried for two weeks and then ground into fine powder using a high-speed blender mill (25000/min), (WK-1000A; Qing Zhou Machinery Co., Ltd.), and then stored in polyethylene bags at 4°C until analysis. Air dried plants were extracted in 5 gms in 50 ml of distilled water, kept in a shaker. After 48hrs Brown extract obtained was 2 Cooled and filtered using Whatman filter paper.

2.2.2. Aqueous extraction

Ten grams of each plant powder Marjoram (Origanum majorana L.), Purslane (Portulaca oleracea L.), and Sowthistle (Sonchus oleraceus) were taken separately and soaked in 50ml cold water in a conical flask stoppered with sterilized cotton and left undisturbed for 48 hours in shaker's incubator. Then the crude extract was filtered out using a sterile filter paper (Whatman No.1) into a sterile conical flask and was evaporated at 100°C and centrifuged at 5000 rpm for 10 minutes and the supernatant was stored at 4°C for further use [62].

2.2.3. Preparation of beef burger patties

Table 1 shows the different ingredients used in the beef burger patties formulation, and they were prepared using Lean meat. Meat samples were ground twice at 12- and 5-mm plates respectively, by using a pilot mincer, two kilograms of Lean meat were prepared to make beef burger patties. Beef burger formulation samples were treated as follows (Table 2): T1 − no extract was added (Control), T2 − 1% of Marjoram extract powder (MAREP), T3 − 1% of Purslane extract powder (PUREP), and T4 − 1% of Sowthistle extract powder (SOWEP). From the meat mixture, beef burgers weighing approximately fifty grams were shaped using a machine for making burger patties. Beef burgers were put in polyethylene bags and stored at -18 °C for 3 months. Fresh and stored samples were subjected to physical, chemical, and microbiological analysis.

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Table (1): Recipe of manufactured beef burger samples

Ingredient type, %	Treatments			
	T1	T2	T3	T4
Lean meat	80	80	80	80
Fat	10	10	10	10
Ice water	8.9	7.9	7.9	7.9
Spices mixture	0.1	0.1	0.1	0.1
Salt	1	1	1	1
MAREP		1		
PUREP			1	
SOWEP				1
Total	100	100	100	100

T1 − no extract was added (Control), T2 − 1% of Marjoram extract powder (MAREP), T3 − 1% of Purslane extract powder (PUREP), and T4 − 1% of Sowthistle extract powder (SOWEP).

2.2.4. Cooking method of beef burger

All the prepared burger samples were grilled, with no added fat, on a pan- fried electric skillet (Heraeus D-63,450 Hanau, Germany) for 3-8 min at 180 ºC on each side. The cooked burgers were prepared just prior to the sensory evaluation.

2.3. Analytical methods

2.3.1. Proximate chemical analysis

Moisture, ash, protein ($N \times 6.25$), fat, and crude fibers contents were tested according to the methods described by the A.O.A.C. (2016). Total carbohydrates content were calculated by differences according to Aly et al. [2]. All determinations were conducted in Food Technology Lab, faculty of agriculture, beni-seuf University, Egypt.

2.3.2. Determination of total phenol contents (TPC)

The total phenol compound contents were carried out using the Folin-Ciocalteu reagent, following the method of Dewanto et al. [21]. One mg extract was dissolved in 1ml deionized water and 500 µl of dissolved sample was taken and added to 0.5 ml of distilled water and 0.125 ml of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 minutes before addition of 1.25 ml of 7% Na2CO3. The solution was adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark for 30 min, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of Gallic acid (standard, from 10-100 µg/ml). Total phenol contents (TPC) were expressed as Gallic acid equivalent (GAE)/mg of dry weight and calculated using the following liner equation based on the calibration curve:

$y = 0.0099x - 0.0616$ $R^2 = 0.997$

Where: (y) is absorbance (x) is the concentration (GAE mg) / g sample (R^2) is correlation coefficient. All determinations were performed in triplicates.

2.3.3. Determination of total flavonoid contents (TFC):

Total flavonoid contents of the plant extracts were determined by a modified colorimetric method described by Sakanaka et al. [64], using catechol as a standard at concentrations of (20 – 200 µg/ ml). Extracts or standard solutions (250 µl) were mixed with distilled water (1.25 ml) and 75 µl of 5% sodium nitrite (NaNO2) solution followed by the addition of 150 µl of 10% aluminum chloride (AlCl3) solution after 5 min. After 6 min, 0.5 ml of 1 M sodium hydroxide (NaOH) and 0.6 ml distilled water were added. The mixture was then mixed and absorbance was measured at 510 nm. Total flavonoids content was expressed as catechol equivalent (CE) and calculated using the following liner equation based on the calibration curve:

$y=0.0059$ x - 0.0356, $R^2 = 0.982$

Where: (y) Is absorbance, (X) is the concentration (mg CE /g extract). (R^2) = correlation coefficient. All determinations were performed in triplicate.

2.4. Antioxidant activity assays

2.4.1. DPPH radical scavenging activity

The free radical scavenging activity of plant extracts was measured by the DPPH method as proposed by Brand-Williams et al. [13], with some modifications. A solution of 0.2 Mm DPPH in methanol (0.0078 g/100 ml) was prepared and 1 ml of this radical solution was added to 1 ml of sample or standard solution at different concentrations (1:1 V/V). The mixture was incubated for 30 Min in the dark at room temperature and then the absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid solutions as standards in the concentration range of (5-500 µg/ml) were used to establish a standard curve. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalent (AAE)/g dried sample. The percentage DPPH radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (% inhibition) = $(AbsControl - AbsSample)\ /AbsControl \times 100$

For control, all reagents were added except plant extract and all determinations were performed in triplicate.

2.4.2. Separation and identification of phenolic compounds by using HPLC

Phenolic compounds of GA ethanol extract was separated and identified by HPLC apparatus (Type: Shimadzu LC-6A model) in Central Laboratory of Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt according to the method of Goupy et al. [31] under the following conditions: Column: Water-Bondapack C18 column (250 × 4.6 mm) and as SCL-6A system controller; the solvent system used was a gradient of A (CH3COOH 2.5%), B (CH3COOH8%) and C (acetonitrile). The solvent flow rate was 0.7 ml/ min and separation was performed at 35°C; injection volume: 20 µl; detector: UV-visible spectrophotometer SPD- 6 AV (Leicestershine LE17 5BH, UK); phenolic compounds were assayed by external standard calibration at 280 nm and expressed in µg/ L-1 equivalent (+) - catechin.

2.5. Chemical quality attributes

2.5.1. Total volatile nitrogen (T.V.N.)

Total volatile nitrogen (TVN) was determined according to the method described by [45]. In a blender, a mix formed of 100 grams of the beef burger sample, 200 mL of trichloroacetic acid (TCA) (7.5%), the mix was passes through a filter paper. 25 of the filterate was applied to macro-kjeldahl apparatus distillation unit, spiked with 5mL NaOH (10%) and distillate was received in 15mL of boric acid (4%), then titrated by H2SO4 (0.05N) and the end point was known using methylene red - bromocresol green. A 25 mL of trichloroacetic acid (7.5%) instead of the sample was used as blank. TVN was calculated as mg/100g using the following equation:

T.V.N (MGN/100) =ml of 0.05 H2 SO4× 14 × (200+/ Moisture content/100-100)/25×100

2.5.2. Thiobarbituric acid reactive substances (TBARS) value

TBARS number was assessed in triplicates by the TBA method of Abdulla et al. [5]. Briefly, ten grams of beef burger sample was well homogenized with 25ml of distilled water for 2 minutes, mixed with 25ml of 10% trichloroacetic acid (TCA). Sample was filtrated (through Whatman filter paper No. 1), one ml of thiobarbituric acid (0.06 M) in 90% acetic acid (TBA reagent) was added to 4 ml of the filtrate in vial and mixed well. Vials were capped and heated in a boiling water bath for 10 min to develop the chromogen, cooled to room temperature. Absorbance at 538 nm was recorded, against a blank prepared with 4ml distilled water and 1ml TBA-reagent, using a spectrophotometer. The TBA numbers were calculated as mg malondialdehyde/kg sample according to the following equation:

TBARS number (kg) = Absorbance × 7.8

2.6. Physical analysis

2.6.1. PH values

pH values of the prepared burger samples was determined by homogenizing 10g of sample with 90 ml of distilled water representing 1:9 (meat : water) ration for 1minute, then pH value of the slurry is measured [24].

2.6.2. Water Holding Capacity (WHC)

The water-holding capacity (WHC) of burgers was measured according to the method adapted by Mehri et al., [46].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

2.7. Cooking properties of burgers:

2.7.1. Cooking loss

The cooking loss of the prepared burgers was measured using the formula of Akwetey and Knipe, [11] as follows:

% cooking loss = [Raw sample weight (g) – Cooked sample weight (g)]/ Raw sample weight x100

2.7.2. Cooking yield

-The cooking yield was determined as reported by Naveena et al., [46] as follows:

% cooking yield = Weight of cooked burger x 100 / Weight of raw burger

2.7.3. Shrinkage

The percentage of shrinkage was calculated as described by Serdaroğlu and Değırmencioğlu (2004) by using the following equation:
%Shrinkage= (Raw thickness – Cooked thickness) + (Raw diameter- Cooked diameter)/ (Raw thickness + Cooked diameter) x 100

2.8. Bacteriological methods

Total Microbial Count in the Prepared Beef Burger Microbiological contamination and growth in the prepared beef burger was analysed using the total plate count (TPC) following the method described by (Abdulla et al., 2016). Briefly, during the storage period at zero time and at 1, 2 and 3 months, 10 g of the beef burger samples were taken blended thoroughly with 90 ml of sterilized peptone water using a lab dancer. A serial dilutions were made and 100µl of each dilution were transferred on a prepared plate count agar (Difco Laboratories, Detroit, MI, USA). After incubation for 48 hours at 35°C, number of colonies were count and reported as log10 CFU/g [5].

2.9. Sensory Evaluation

Experiment Sensory evaluation test of the prepared beef burger samples were performed at zero time only. Beef burger samples were evaluated for sensory parameters including colour, taste, aroma, texture and over all acceptability (OAA) on a five point's hedonic scale as 1 is dislike extremely and 5 like extremely [40].

2.10. Statistical analysis

All treatments and determinations were carried out in triplicates and the data are presented as means ±SD. They were subjected to analysis of variance (ANOVA) accompanied with Duncan test using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago) to identify the significance ($p< 0.05$) among the treatments [37].

3. Results and Discussion

Lipid oxidation, microbial growth and color changes are important factors for shelf-life and consequently for consumer acceptance of fresh meat [33, 50]. Natural preservatives can protect the human body from free radicals and can retard the progress of many chronic diseases as well lipid oxidation and microbial growth in foods due to their phenolic compounds [15].

Proximate Chemical composition of powdered marjoram, purslane and sowthistle:-

The chemical composition of marjoram, purslane and sowthistle powder was shown in Table (2).The results Clarified that the total protein in marjoram, purslane and sowthistle are 9.30%, 19.83 % and 1.88 % respectively, moisture varied from 4.23%, 8.06 % and 91.83 % for marjoram, purslane and sowthistle respectively, The results in ash for marjoram, purslane and sowthistle were 5.18%, 5.16 %, and 1.06 % respectively, crude fat and crude fiber were 2.97%, 4.02%,7.0% and 25.70%,7.19%,3.97% in marjoram, purslane and sowthistle respectively, And for carbohydrates in marjoram, purslane and sowthistle it were found to contain 52.62, 25.10 and 0.17% (on dry weight basis), respectively. The marjoram powder (MP) followed by the purslane were recorded the highest content of total Carbohydrate, crude fiber and ash while purslane powder (PP) recorded the highest values of Crude protein. The sowthistle powder (SP) was recorded the highest content of moisture and followed by the purslane. The increase in fiber content of both marjoram, purslane and sowthistle powder have several healthy benefits as it will aid digestion in the colon and reduce constipation often associated with products this results agree with [64].

Table (2): Preliminary phytochemical analysis of Marjoram, Purslane, and Sowthistle leaves dried powder (dry weight).

	Treatments		
Chemical composition (g/100g sample)	**Marjoram**	**Purslane**	**Sowthistle**
Moisture	**4.23±0.02**	**8.06 ±0.088**	**91.83 ± 0.25**
Carbohydrate	**52.62±2.36**	**25.10 ±0.173**	**0.17 ± 0.04**
Crude protein	**9.30±0.79**	**19.83 ±0.145**	**1.88 ± 0.0**
Fat	**2.97±0.06**	**4.02 ±0.011**	**7.0 ± 0.23**
Crude fiber	**25.70±2.13**	**7.19 ±0.017**	**3.97 ±0.0**
Ash	**5.18±0.38**	**5.16 ±0.145**	**1.06 ± 0.02**

Data are expressed as mean ± SE. Each sample was analyzed three times

Total phenolic content (TPC)

Total Phenolic Compounds marjoram, purslane and sowthistle were analyzed for total phenols (Table 3). The results showed that purslane had the highest percentage of total phenols (623.00 mg/100 g), followed by marjoram (524.50mg/g) and finally sowthistle (455.00 mg/g).These results agree with those reported by Akyol et al. [6]. Therefore, marjoram, purslane and sowthistle could be a good source of bioactive compounds, which have high antioxidative propertie.

Table (3): Total phenolic content of herbal infusions

Herbal extracts	Total phenolic content (mg/g)
Marjoram	**524.50**
Purslane	**623.00**
Sowthistle	**455.00**

Data are expressed as mean ± SE. Each sample was analyzed three times

Total Flavonoids:

Total Phenolic Compounds marjoram, purslane and sowthistle were analyzed for total phenols (Table 4). The results showed that purslane had the highest percentage of total phenols (198.00 mg/100 g), followed by marjoram (186.00mg/g) and finally sowthistle (103.00 mg/g). Such results are in accordance with those reported by Kim et al., [37].

Table (4): Total Flavonoids of herbal infusions:

Herbal extracts	Total Flavonoids content (mg/g)
Marjoram	**186.00**
Purslane	**198.00**
Sowthistle	**103.00**

Data are expressed as mean ± SE. Each sample was analyzed three times

DPPH radical scavenging activity:

There are different methods for estimation of antioxidant activity but the most widely methods are those that involve generation of free radical species which are then neutralized by antioxidant compounds. DPPH radical is commonly used as substrate to evaluate antioxidant activity; it is useful and stable free radical that can accept on electron or hydrogen radical to become a stable molecule. The reduction of DPPH free radical was determined by the decrease in its absorbance at 517 nm induced by different antioxidants. DPPH free radical reacts with antioxidant, consequentially, absorbance decreases and the DPPH free radical is converted into the DPPH form. The degree of discoloration indicates the scavenging potential of antioxidant compounds of extracts in terms of hydrogen donating ability. Table 5 Marjoram, Purslane and Sowthistle leaves compared with ascorbic acid were used with a very high scavenging capacity of 40.00 after only 10 min. in all cases the scavenging capacity did not increase after the first 10 min of incubation. The reactions of ascorbic acid with DPPH were similar to Marjoram, Purslane and Sowthistle leaves with DPPH; the scavenging capacities were similar. A significant increase ($p \leq 0.05$) was observed in the antioxidant activity of (DPPH) in burger samples as compared with control sample. Also, the gradient increase in the amounts of all added powders caused a proportional increase in the antioxidant activity. Thus, the highest levels were observed in purslane exhibited higher antioxidant activities 26.30%. These results were related to the composition of the purslane powders which had high levels of antioxidant activity 26.30%. The antioxidant properties of phenolic compounds were very well documented and a significant relation between phenolic content and antioxidant activity. Thus, the high level of antioxidant activity in beef burgers containing purslane powder was attributed to the high level of phenolic compounds found in these powders. These results are confirmed by [36].

Table (5): Antioxidant activity (%) of selected herbs powder

Samples	Scavenging capacity (%)
control	**15.00±2.00**
Ascorbic acid	**25.20±4.34**
marjoram	**25.50±3.32**
purslane	**26.30±4.14**
sowthistle	**23.00±6.50**

Data are expressed as mean ± SE. Each sample was analyzed three times

Identification of Phenolic Compounds by HPLC

Polyphenols are secondary metabolites that are widespread throughout the plant kingdom with more than 8000 phenolic compounds, and it is known for their pharmacological properties, for example as antioxidants, anti-inflammatory, anti-mutagenic, anti-

carcinogenic, and antimicrobial [32]. Fractionation, identification and content of phenolic compound, extraction from Marjoram, Purslane and Sowthistle are presented in Table (6) from these table it could be stated that, 14 compounds were found for all tested powder. Purslane powder contained higher amount of phenolic compound than other sample (except compound Vanillic acid, ρ-Hydroxybenzoic acid, Gallic acid, Neochlorogenic acid and Hydroquinone which were higher in Marjoram). Compounds Syringic acid, Coumarinic acid, Protocatechuic acid, Arbutin, Vitexin and Lithospermic acid had the higher values it were 1.05, 2.87, 0.34, 3.12, 2.11 and 0.27ppm/g respectively. On the other side, sowthistle had the lower numbers of phenolic compound especially Vitexin, Vanillic acid and Ferulic acid. These results agree with that reported by [34].

Table (6): Phenolic compounds concentration (mg/100g) in herbs powder

Phenolic Compounds	The tested powders		
	Marjoram	Purslane	Sowthistle
Vanillic acid	0.22	0.11	0.09
Ferulic acid	0.07	ND	0.12
Caffeic acid	1.02	0.23	1.90
Syringic acid	0.13	1.05	ND
ρ-Hydroxybenzoic acid	2.22	0.18	0.56
Coumarinic acid	1.15	2.87	0.21
Gallic acid	2.13*	ND	0.71
Neochlorogenic acid	2.45	0.54	0.41
Protocatechuic acid	0.06	0.34	ND
Coumaric acid	ND	0.42	0.32
Hydroquinone	1.05	ND	0.41
Arbutin	0.19	3.12	3.01
Vitexin	0.56	2.11	0.02
Lithospermic acid	ND	0.27	0.19

ND = Not Detected

Data are expressed as mean ± SE. Each sample was analyzed three times

Moisture contents of burger:

In meat products moisture is a very critical quality parameter that affects its juiciness as less moisture indicates a less juicy meat product [65]. Moisture contents of beef burger at zero time and after storage period for 3 months are presented in Figure 1, while the moisture contents of burger through frozen storage period (1st month and 2nd month in addition to zero time and the 3rd month) at -18°C. Fig.1. shows the differences between moisture contents in different burger formulas that some of it contained marjoram extract and the other contained Purslane and the other contained Sowthistle and compared with control samples. From Figure 1 it could be merged that minimal moisture contents were scored by the control beef burger samples (59.00%). A significant increase ($P<0.05$) in moisture contents was scored by the burger with an added purslane extract, with a maximal moisture contents scored by the burger sample with of purslane powder with a score of 59.25% with an increase of comparing to control burger. Same trend of changes was also obtained after the storage period (at frozen conditions) between different burger formula and additives. On the other hand, storing the beef burger for three months decreased moisture contents but the decrease was not significant ($P<0.05$). Maximum moisture contents scored by the higher amount of purslane powders might be because of the high dietary fibre in the powder, fibers could retain moisture more strongly and hold it within the food system. Same trends of effects were reported at the application of buckwheat (high dietary fibre additive) in cookies as it caused an increased moisture contents [9].

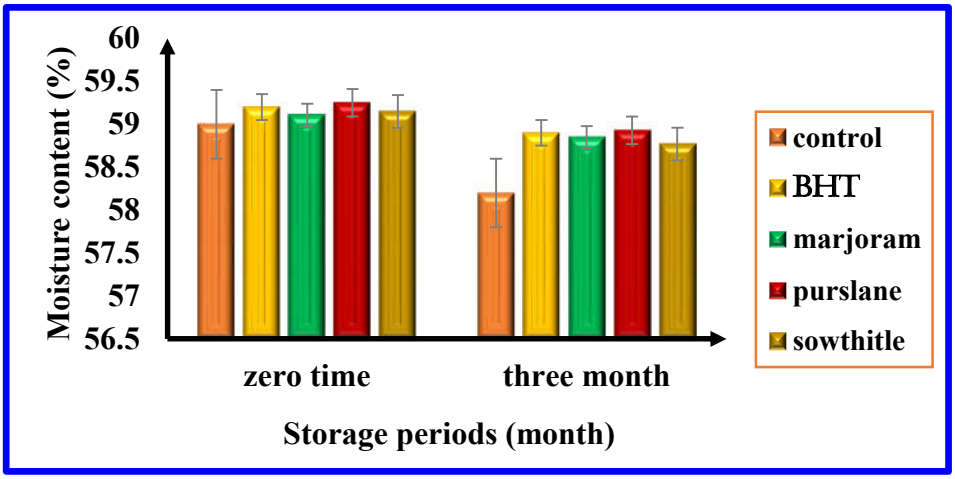

Fig. (1). Effects of powder on the moisture of beef burger at the zero time and after the storage for 3 months. Data are expressed as mean ± SE. Each sample was analyzed three times

Protein contents of burger:

Animal proteins is the only known source of essential amino acids and the body could not form it which give meat protein its importance and biological value [44]. In Figure 2 protein contents of the prepared beef burger without any addition (control sample), with the addition of marjoram, Purslane and Sowthistle was presented. Protein contents in the used sowthistle powder was high, that is why its addition to the beef burger as powder scored significantly ($p < 0.05$) higher protein contents comparing to negative control and positive control. Maximal protein percentage was 23.85% and it was scored by the beef burger sample with 1% sowthistle powder addition. And minimal protein contents was scored in the control sample as it scored 21.00 %. A sharp decrease in protein contents was obtained after storage period (for 3months) as it decreased to become15.0, 15.01, 15.04 and 15.06% in control. Highest sowthistle powder added treatments respectively, which comes in line with the results of Abdel-Salam et al. (2014) who reported a decrease in protein contents of the frozen beef burger samples after storage for 60 days [8].

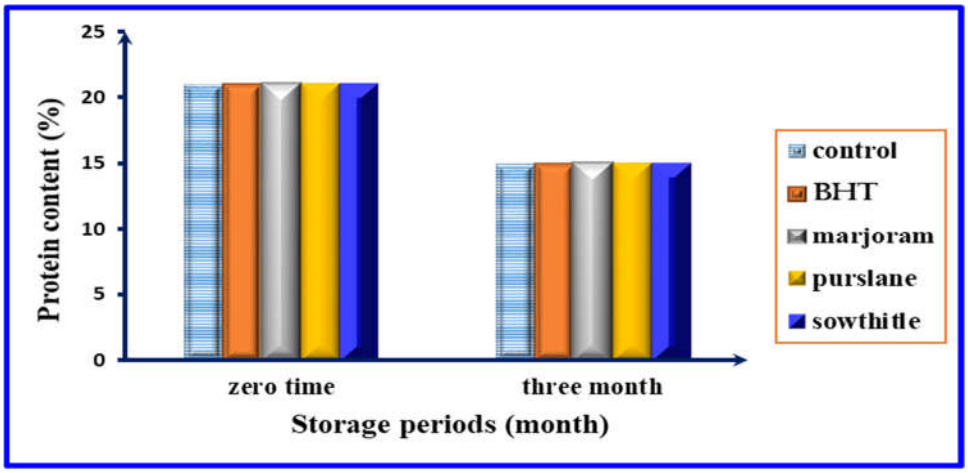

Fig. (2). Effects of powder on the protein of beef burger at the zero time and after the storage for 3 months. Data are expressed as mean ± SE. Each sample was analyzed three times

Fat contents of burger:

Data in the Figure 3 showed that, after storage for three months, fat contents increased significantly ($p < 0.05$) and that might be due to the decreased protein and moisture contents which caused a relative increase of fat to the total weight. Fat contents in marjoram, purslane and sowthistle powders is lower than that of meat itself that is why its addition to burger recipe showed decreased fat content13.90, 13.85 and 13.88 % comparing to 13.90 and13.89 % in control samples. Same trends of changes was obtained by Abdel-Salam et al. [8] who reported an increase in fat contents from through storage period which was 2 months [8]. Higher increase rat in fat contents in the marjoram added samples might also be ascribed to the fat holding capacity of the marjoram dietary fiber which was in accordance to what was found by AlJuhaimi et al. [4] who obtained an increased fat contents at the application of baobab seeds poder which is rich in fibers [4].

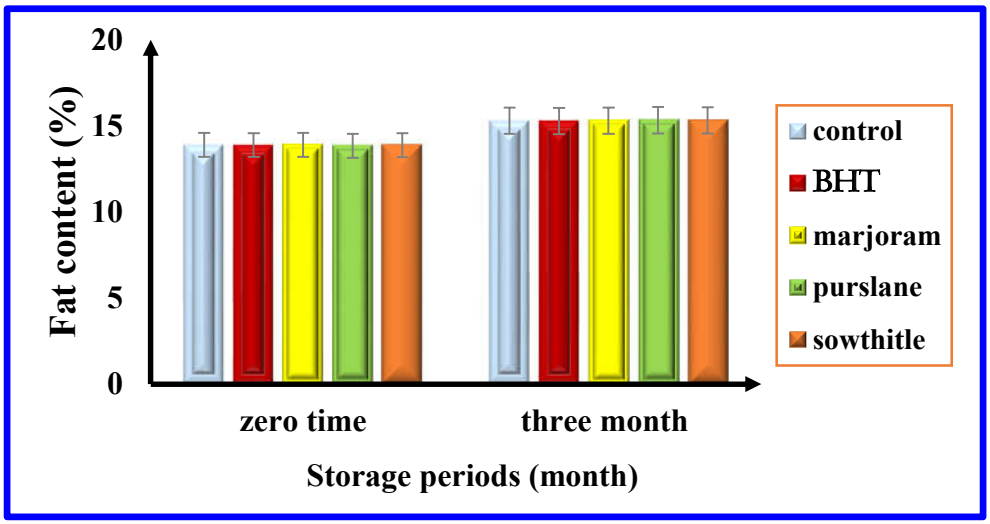

Fig. (3). Effects of powder on the fat content changes of beef burger at the zero time and after the storage for 3 months. Data are expressed as mean ± SE. Each sample was analyzed three times

Effects of powder on the cooking characteristics of beef burger:

-Physical properties

Cooking loss

In meat products specially beef burger, cooking parameters are fundamental because it affects the consumers' acceptability through affecting quality and juiciness and furthermore, it affects nutritional value such as losing soluble vitamins and amino acids [12]. Cooking characteristics of the prepared beef burger with the addition of Marjoram (Origanum majorana L.), Purslane (Portulaca oleracea L.), and Sowthistle (Sonchus oleraceus) leaves extracts significantly ($p < 0.05$) improved as could be seen in Figer 4.

Cooking loss refers to the reduction in weight of meat during the cooking process. Cooking loss is an important data that are used by the meat industry to predict the behavior of their products during processing [69]. Major components of cooking losses are thawing, dripping and evaporation. Cooking loss contents of beef burger at zero time and after storage period for 3 months are presented in Figure 4, while the Cooking loss contents of burger through frozen storage period (1st month and 2nd month in addition to zero time and the 3rd month) at -18°C.Table 4. shows the differences between Cooking loss contents in different burger formulas that some of it contained marjoram extract and the other contained Purslane and the other contained Sowthistle and compared with control samples.at zero time there was no significant difference ($p<0.05$) in Cooking loss between the burger samples but through the 3 months of storage the Cooking loss increased significantly in the negative control, positive control and marjoram extract, the results changed from (22.60, 21.70 and 21.50%) respectively at zero time to (21.50, 24.90, 24.90) respectively at 3 months of storage, while in Purslane and sowthistle the results changed from (21.33 and 21.20%) respectively at zero time to (24.85 and 24.70%) respectively at 3 months of storage. Decreased cooking loss might be because of the higher fiber contents in the sowthistle dried powder which could retain more water and the antioxidant activity in leaves extract Sowthistle. After storage period for three months, same trend of changes among treatments was obtained and all samples showed an increase in cooking loss, which may be due to the decreased protein, moisture contents and the decrease in WHC as a result. These findings comes in accordance to what was found by Darwish et al. [20] who found that cooking loss decreased when thyme, rosemary marjoram and sage was added to chicken burger and author also reported an increased cooking loss after storage [20].

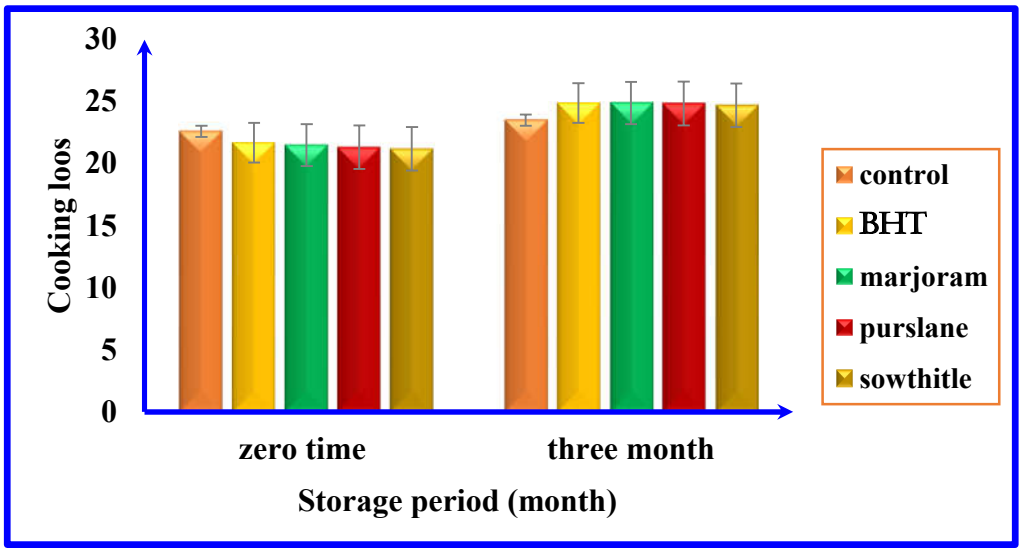

Fig. (4). Effects of powder on the cooking loss of beef burger at the zero time and after the storage for 3 months. Data are expressed as mean ± SE. Each sample was analyzed three times

Cooking yield:

Cooking yield is one of the quality parameters which determine the product behaviour during cooking. Cooking yield contents of beef burger at zero time and after storage period for 3 months are presented in Figure 5, while the Cooking yield contents of burger through frozen storage period (1st month and 2nd month in addition to zero time and the 3rd month) at -18°C. The results showed that the positive influence in cooking yield was obtained by the addition leaves extracts of Marjoram, Purslane, and Sowthistle as it increased from 77.96 and 78.90 % in control and BHT samples to reach 79.01, 79.1, 79.0 in the purslane, sowthistle, marjoram leaves extracts addition samples, respectively. All samples of beef burger showed a remarkable significant decrease ($p \leq 0.05$) in cooking yield during cold storage at -18°C °C for 3rd month). The increase in cooking yield might be due to the existence of high amounts of fibre which is a hydrophilic constituents that adsorb water and form gels resulting in its retention in food system [14]. In addition to the higher protein contents in the additives. After storage for three months decrease in protein, moisture and WHC caused a decrease in cooking yield with the same patterns of different samples

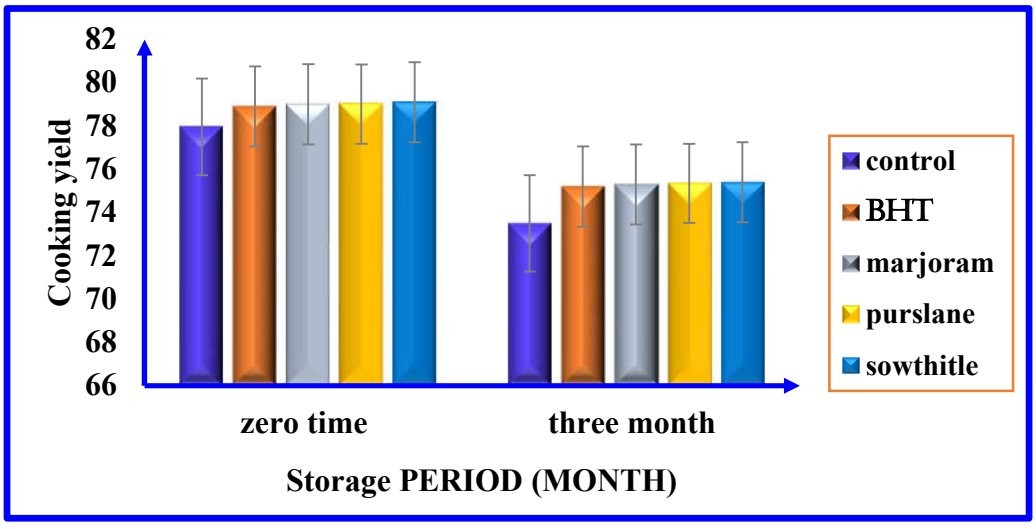

Fig. (5). Effects of powder on the cooking yield of beef burger at the zero time and after the storage for 3 months. Data are expressed as mean ± SE. Each sample was analyzed three times

Shrinkage:

Shrinkage after beef burger cooking measures the differences between the burger diameter before and after cooking and it reflects the amount of water and fat separated from the burger. It can be a clue on the quality of protein and on the ability of burger matrix to hold

fat and water [20]. For consumers' thinking and believes, shrinkage of burger might be linked to the addition of water to the burger recipe which is un-preferred [26]. Shrinkage contents of beef burger at zero time and after storage period for 3 months are presented in Figure 6, while the Shrinkage contents of burger through frozen storage period (1st month and 2nd month in addition to zero time and the 3rd month) at -18°C. Fig.6. at zero time there was no significant difference ($p<0.05$) in Shrinkage between the burger samples but through the 3 months of storage the Cooking loss increased significantly in the negative control, positive control and marjoram, sowthistle extracts, the results changed from (24.50, 23.90, 23.75 and 23.75) respectively at zero time to (26.70, 25.60, 25.55 and 25.40) respectively at 3 months of storage, while Further decrease in shrinkage was the result of the addition of purslane dried powder gradually to reach minimal shrinkage score (23.70%) respectively at zero time to (25.45%) respectively at 3 months of storage. Decreased shrinkage might be due to the higher protein contents, higher fiber contents and may also be attributed to the antioxidant activity of the additives which all pour in the sake of improving water holding capacity and water retention in the burger system through cooking. Decreased protein contents, moisture contents and the fall of pH which might decrease the bioavailability of protein were all the reason behind decreasing in WHC and that was clearly reflected to an increased shrinkage scores through storage of beef burger samples. That was the same finding of Darwish et al. [20] who reported a decreased shrinkage when some medicinal plants was added to chicken burger and shrinkage also increased through storage period. The results agreed with those of Ragab et al. [52] an increase in the percentage of weight loss after cooking and the percentage of shrinkage in diameter of beef Burger tablets that were stored for 3 months by freezing.

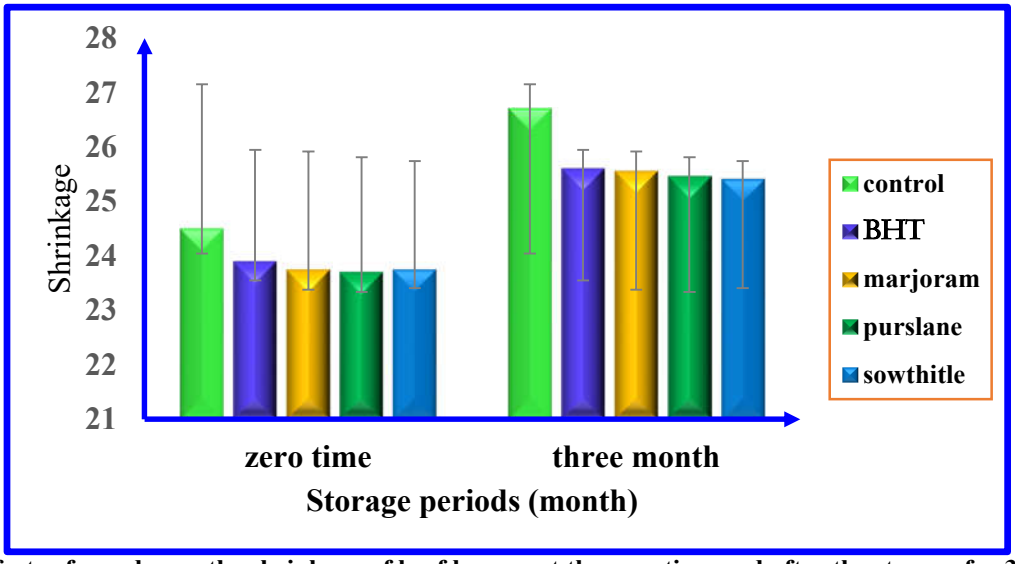

Fig. (6). Effects of powder on the shrinkage of beef burger at the zero time and after the storage for 3 months. Data are expressed as mean ± SE. Each sample was analyzed three times

Chemical quality attributes

Thiobarbituric acid reactive substances (TBARS)

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 [73]**,** TBARS are produced through second stage autoxidation during which peroxides are oxidized to aldehydes and ketones (e.g., MDA) [66]. The result in Figure (7) shows the effect of the three concentrations of the plant extract mix on lipid oxidation in different burger samples during the 3 months of storage at -18 °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 [22]**,** at zero time there was no significant difference ($p<0.05$) in TBARS value between the burger samples but through the 3 months of storage the TBARS value increased significantly in the negative control, positive control and marjoram1% the results changed from (0.42, 0.36 and 0.31Mg MDA\ Kg) respectively at zero time to (0.77, 0.60 and 0.51Mg MDA\ Kg) respectively at 3months of storage, while in Purslane and sowthistle the results changed from (0.30, 0.28 Mg MDA\ Kg) respectively at zero time to (0.52 and 0.52 Mg MDA\ Kg) respectively at 3months of storage, so we conclude that the negative control group with very close to cross the permissible limits at 3months with a result of 0.77 Mg MDA\ Kg compared with the Marjoram group which doesn't changed significantly at 3 months with a result of 0.51 Mg MDA\ Kg which show a strong antioxidant activity superior to such synthetic antioxidant compounds as BHT which showed the result of 0.60 Mg MDA\ Kg at 3 months which is very close to the permissible limit. These findings was same to what was found by Darwish et al. [20] who found that, the spiking of chicken burger using rosemary, thyme and marjoram decreased the TBA and decreased the rate of its increase through storage [20]. Same findings and recommendation of stopping storage at 2months period was noted by Sharaf et al. [54] because the TBA increased dramatically through storage. 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 [47]. 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. [73] that the results demonstrate the effectiveness of purslane and sowthistle extracts in reducing lipid oxidation.

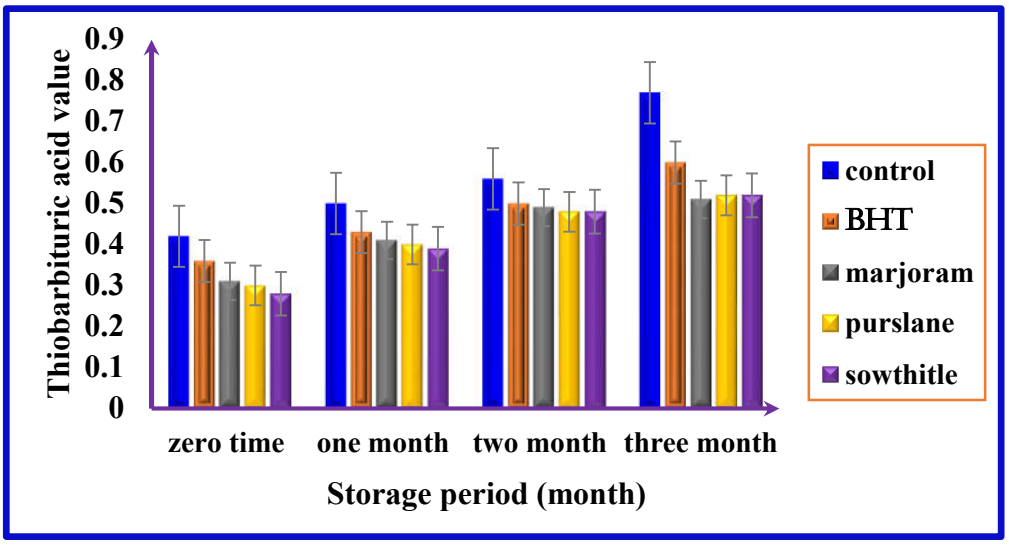

Fig. (7). Effects of powder on TBA through the storage period for three months. Data are expressed as mean ± SE. Each sample was analyzed three times

Functional and physical properties

Changes of total volatile nitrogen (TVN) in prepared beef burger

Degradation of protein in preserved meat products produces volatile nitrogen compounds, amines and hydrogen sulphites which all cause a loss of the quality and bioavailability of proteins and of which ability to hold water decreases, nevertheless the loss of nutritional value because protein is the most important nutrient in meat products [39].The degradation of protein and nitrogenous substances to volatile nitrogen might also be caused by the microbiological activity that could increase TVN values through storage of meat products [41]. The obtained data in Figure (8) indicate that, TVN content was gradually increased ($p \leq 0.05$) during storage of different formulated beef burger samples. Results also revealed that, the control sample had highest TVN content at all storage period, being 5.50 mg TVN/100 g sample at the beginning of the storage period, and increased to 8.77 mg TVN/100 g sample after 3 months. None of the prepared beef burger samples exceeded the quality standard reported by the EOSQC (2005) as the maximum TVN value was 8.77 mg/100 g (obtained by control at the third month time).On the other hand, the corresponding TVN value for prepared beef burger samples containing concentrations1% of marjoram, purslane and sowthistle powder had lower TVN content at all of storage times especially marjoram at the initial of storage period (4.40mg/100 g,), and at the end of the storage period after 3 months (7.51 mg/100 g).

These results indicated the significant ($p \geq 0.05$) positive effect of addition of marjoram powder on the inhibition of microbial growth especially proteolytic microorganisms that cause the breakdown of protein resulting in volatile nitrogen compounds [41]. Lower TVN values through storage in the marjoram added beef burger samples might refer to its protective effects against microorganisms which fasten the degradation of protein to volatile nitrogen. That was in accordance with what was reported by Ozogul et al. [49] when oregano, green tea and laurel extracts was added to a fish burger samples at frozen storage.

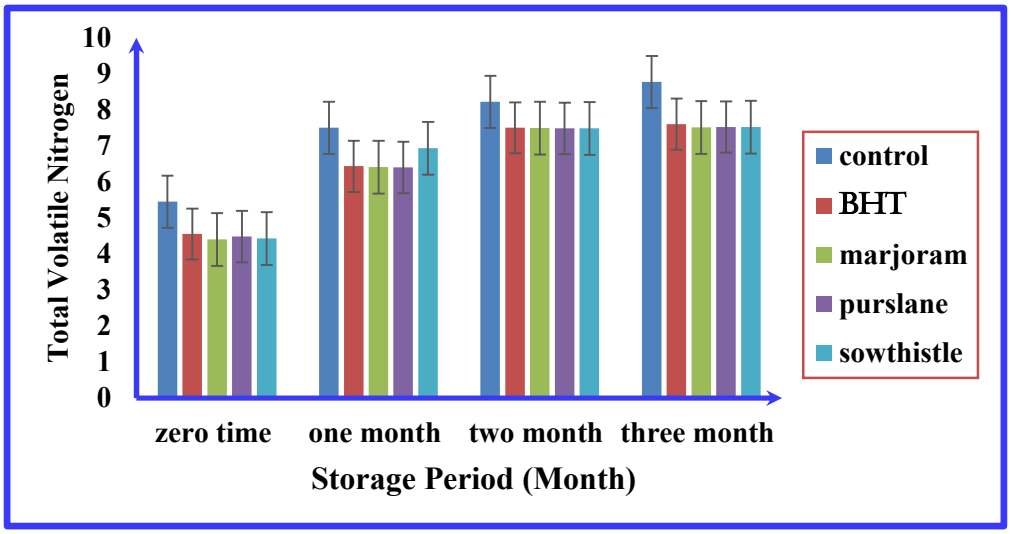

Fig. (8). Effects of powder on TVN through the storage period for three months. Data are expressed as mean ± SE. Each sample was analyzed three times

PH values of burger

One of the main quality parameters of meat and meat quality is pH values which gives an indication of acid and alkalinity and pH is linked to all other quality parameters including colour changes, water holding capacity, texture and of course shelf life [7] . Figure 9 shows the effect of the three plant extracts on the pH values compared with control groups during chilling temperature at -18 °C for 3 months. The pH of negative control was found to be 6.00 while the pH value of positive control was found to be 6.00 and the PH value for treatments mix marjoram, purslane and Sowthistle was (6.01, 6.03 and 6.00) 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 [60], but during the 3 months of storage there was a significant differences found through the storage periods which reached final pH values of 5.80 for negative control and 5.81for positive control and the PH value for treatments mix marjoram 1% which reached a value of 5.83 while there was no significant difference in mix purslane and Sowthistle which showed the result of (5.80, 5.80) 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 purslane and Sowthistle 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 [62] who reported that the burger samples treated with plant extract doesn't experience decrease in pH value as the untreated burger samples.

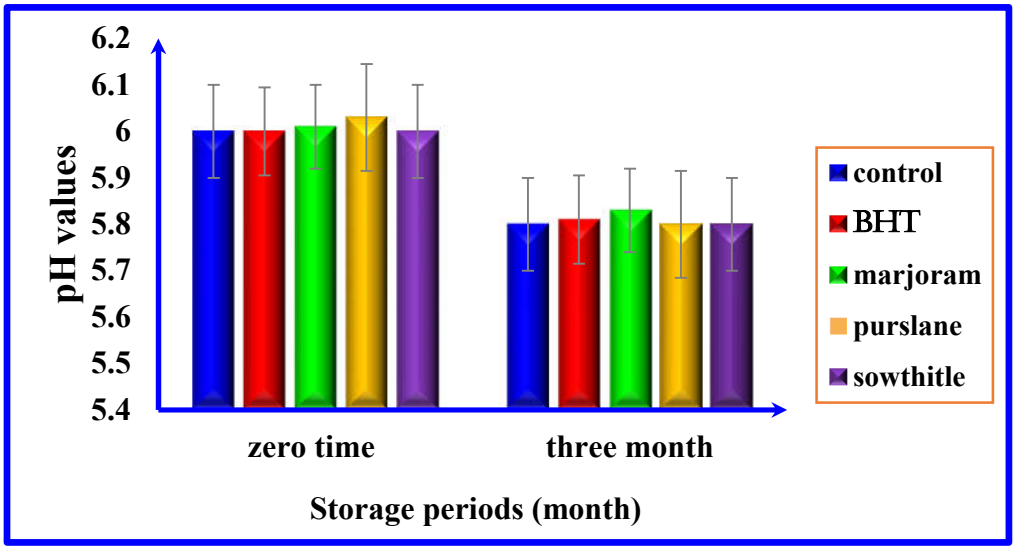

Fig. (9). Effects of powder on the PH of beef burger at the zero time and after the storage for 3 months. Data are expressed as mean ± SE. Each sample was analyzed three times

Water holding capacity:

Water holding capacity is defined as the ability of meat and meat products to retain moisture and it is one of the most quality characteristics that decide the juiciness and quality of meat and meat products. Visual acceptability, weight loss, cooking characteristics and sensory traits depends on WHC of meat and meat products. WHC capacity mechanisms is centered in structures of proteins especially myofibrillar that bind and entrap water which is strongly altered by the decline in pH, ionic strength and oxidation which affect the efficiency of myofibrillar protein to retain water [72].Eating quality, tenderness, juiciness, thawing drip and cooking loss in meat and meat products are associated with the decrease of WHC [42]. It was reported that fibers of plant sources is strongly associated with the WHC and water swelling activity [75]. Figure (10) 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 -18 °C for 3 months, at zero time there was no significant difference ($p<0.05$) in WHC between the burger samples but through the 3 months of storage the WHC decreased significantly in the negative control, positive control and marjoram the results changed from (65.00, 65.50 and 66.10 %) respectively at zero time to (58.50, 60.50 and 60.11%) respectively at 3 months of storage, while in Purslane and sowthistle the results changed from (66.80, 66.55%) respectively at zero time to (60.00 and 60.09%) respectively at 3months 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 [61] who reported that the burger samples treated with plant extract doesn't experience decrease in WHC value as the untreated burger samples.

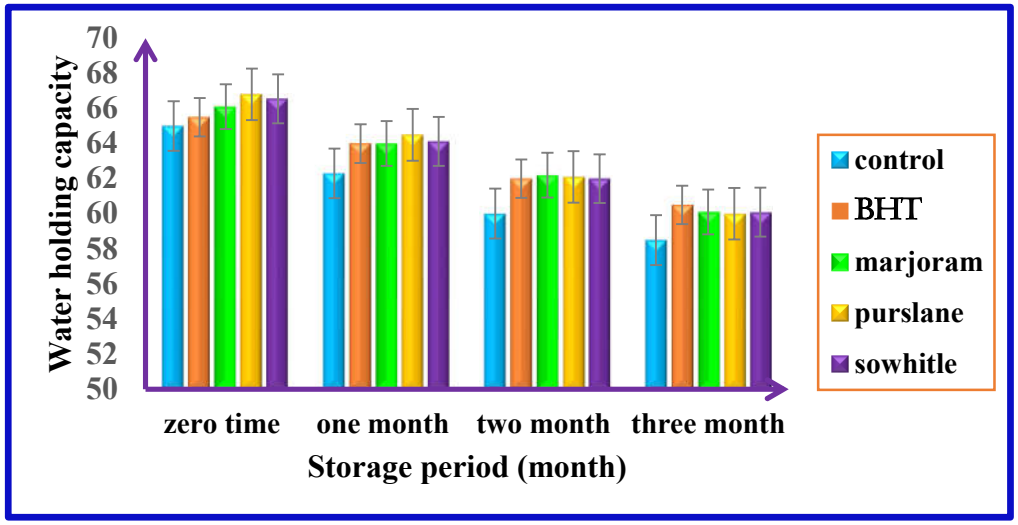

Fig. (12). Effects of powder on water holding capacity of beef burger for three months. Data are expressed as mean ± SE. Each sample was analyzed three times

Microbiological quality attributes of beef burger samples during storage at 4◦C for 3months:

Total bacterial count (TPC)

Microbiological infections and growth in food systems cost a huge lost in food because it causes a serios of effects represents quality deterioration such as changing pH values, loss of protein and increasing TVN, secretion of toxins, and ends by the loose of food or might also cause food poisoning. Contamination and infections of microorganisms to the meat and meat products might be during slaughtering, processing, packaging, transportation or storage. Extensive use of synthetic and chemical antibiotics has led to resistant microorganisms which increased the preferability of natural alternative preservatives against microorganisms' especially pathogenic ones[59]. The use of Marjoram, purslane and sowthistle leaves extracts in this study was to inhibit the growth of microorganisms and that was measured through total bacterial count and the results was presented as Log10CFU/g (Figer 11).Results presented in Figer (11) shows the effect of marjoram, purslane and sowthistle leaves extracts on the total bacterial count in burger samples during the 3 months of storage at -18 °C, total bacterial count is expressed as (cfu/g) colony forming unit/gram of sample, permissible limit should not exceed 105 Cfu/g according to Egyptian Organization for Standardization and Quality for frozen beef burger [20] and 106 Cfu/g according to [27], at zero day there was no significant difference ($p<0.05$) in bacterial count between the burger samples but through the 3months of storage the bacterial count increased significantly in the negative control, positive control, marjoram and purslane results changed from (2.60, 2.53, 2.50 and 2.21Cfu/g) respectively at zero day to (3.55, 3.31, 3.09 and 2.93Cfu/g) respectively at 3months of storage which all have exceeded the permissible limits, while sowthistle extract showed the most remarkable results which started from 2.15 Cfu/g at zero day and reached 2.74 Cfu/g at 3months of storage, so the negative control group was unfit between month one and two of storage while BHT group was unfit by month two of storage and marjoram extract become unfit between month two and three of storage, but the sowthistle group didn't reach the permissible limit at month three of storage. This results was in agreement with [57] 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 [58] 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 [56].

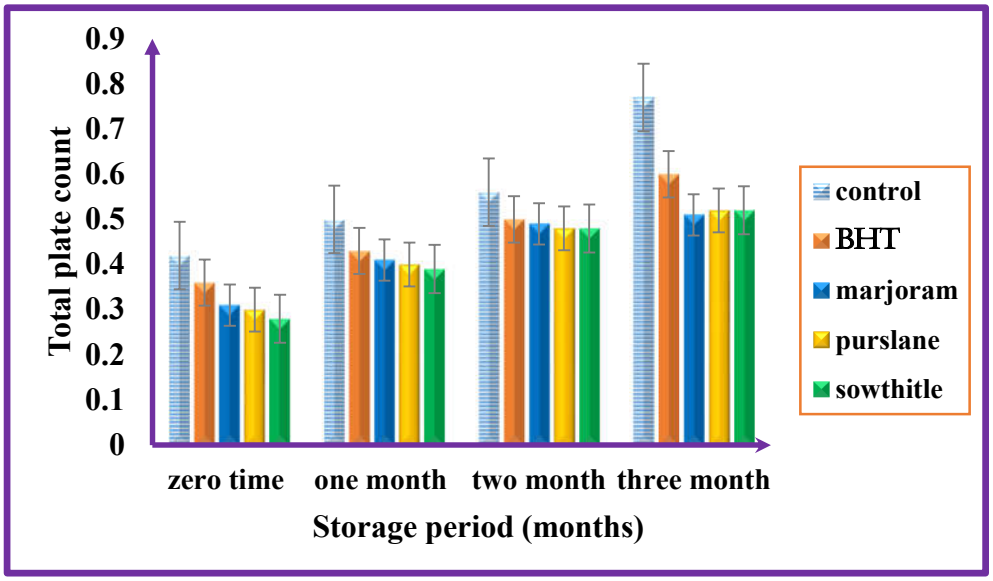

Fig. (11).Effects of powder on Total plate count of beef burger during storage period. Data are expressed as mean ± SE. Each sample was analyzed three times

Sensory Evaluation

Of all parameters that could be analyzed, sensory analysis is the most useful test that reflect the real consumers' opinion about the product that has been prepared or developed especially in case of modified recipes [29]. Table 7 shows the sensory characteristics of control and treated beef steak with marjoram, purslane, and sowthistle at a concentration of 1% stored at 4°C. According to sensory analysis, No significant differences between groups in flavor, tenderness and taste compared with color and appearance. No changes appeared in the overall acceptability, which considered acceptable to the consumer. This results is in agreement with [58] 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 [55]. Fernandes, et al., [28] 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.

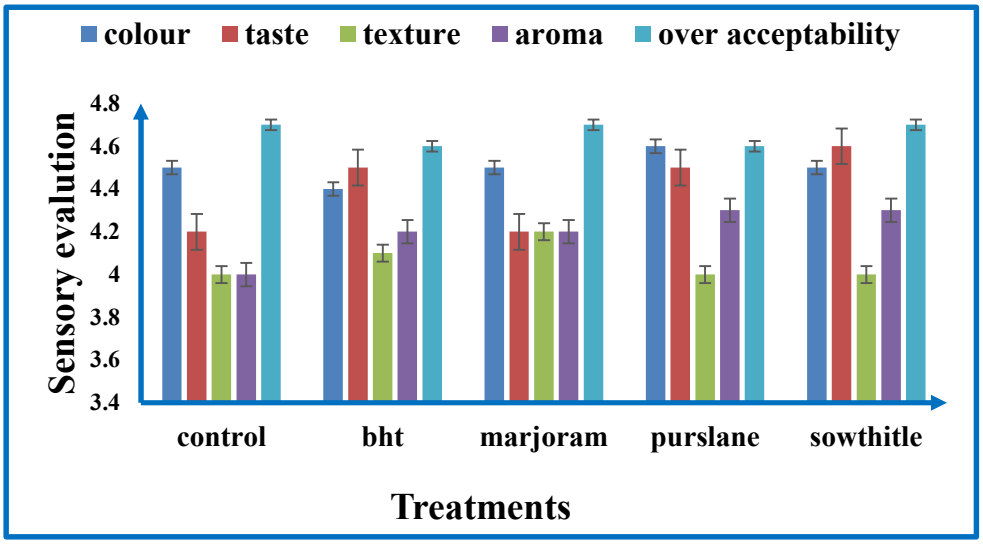

Fig. (12). Effects of powder on sensory evaluation parameters; color, taste, texture, aroma, and over-all acceptability of beef burger. Data are expressed as mean ± SE. Each sample was analyzed three times

The study demonstrated the antioxidant effect of marjoram, purslane and sowthistle leaves extracts on burger meat during 3 months frozen the result confirmed that: Chemical and physical analysis indicated that marjoram, purslane and sowthistle powder had high antioxidant activity and retarded the lipid oxidation, which is very important for human health benefit. Improvement of Flavor and aroma, Tenderness, Juiciness, Color and Overall acceptability were observed after Powders of Marjoram, Purslane and Sowthistle were incorporated into freshly minced beef meat .Nutritional value of burger meat was increased after adding Marjoram, Purslane and Sowthistle to meat samples which lead to decrease of moisture contents, protein contents, and water holding capacity of beef burger while it caused a decrease in fat contents, TBA and TVN values. Cooking parameters were improved, microbiological growth was limited and sensory evaluation was not 3.4 3.6 3.8 4 4.2 4.4 4.6 4.8 control BHT marjoram purslane sowthistle Sensory evalution Treatments colour taste texture aroma over acceptability affected by the additives. Therefore, it is possible to use them in food as natural preservatives instead of the chemicals without compromising the sensory attributes in addition to keeping them in good microbiological and chemical quality.

3. References

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