SINAI Journal of Applied Sciences (ISSN: 2314-6079) Vol. (6) Is. (2), Aug. 2017 141-156

SINAI Journal of Applied Sciences

ARU-EGYPT SJAS Trust Science | It Creates

QUALITY EVALUATION AND ANTIMICROBIAL PROPERTIES OF BEEF BURGER PREPARED WITH CRUDE ISOFLAVONES EXTRACT, BHA AND ROSEMARY EXTRACT

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ABSTRACT

The current study was carried out to evaluate the use of crude isoflavones (CIE), crude rosemary extract (CRE), butylated hydroxy anisol (BHA) and CIE plus CRE, as antioxidant and antimicrobial agents in manufacture of beef burgers during frozen storage at -18ºC for three monthes. The antioxidant and antimicrobial effects of the CIE, CRE, BHA and CIE plus CRE, were evaluated in beef burger by measuring pH, tiobarbituric Acid (TBARS) values, unsaponifiable matter, texture profile, sensory and microbiological examinations. The results showed that extracts derived from soybean (CIE) and rosmary (CRE) leaves had the potential to reduce the oxidation of beef burgers and extend their shelf life. The combination composed of 0.03% of CIE and 0.06% of CRE provided the most effective antioxidative activity in terms of TBARS values until the latter stages of storage. The combined extracts showed good antibacterial activity againist total bacterial count, coliform, *staphylococous* and *E. coil* that led also to enhance extension of beef burger shelf life. The availability of these natural antioxidants and their possible synergistic effects suggests an interesting way of improving beef burger stability to prevent the degenerative diseases caused by fat oxidation products.

Key words: Beef burger; soy isoflavone; antioxidant activity; lipid oxidation; peroxide value; tba; rosemary; bha; antimicrobial.

INTRODUCTION

Utilization of plant extracts as an alternative to chemical or synthetic antimicrobials and antioxidants to control the food-borne diseases, lipid oxidation and accordingly extend the shelf life and quality of food products is an increasing trend in food industry **(Bjelakovic *et al.,* 2007)**. Based on the source of origin, antioxidants can be divided into two types; natural antioxidants (rosemary, isoflavones) and synthetic antioxidants (BHA, BHT, TBHQ). The synthetic antioxidants increase the risk of mortality in adult due to rigorous toxicity and the increase risk of cancer that they may cause in comparison to natural antioxidants. On the other hands, antioxidant activity of plant extracts such as rosemary, isoflavones were superior to the synthetic antioxidants and their larger intake in our diet improves the overall antioxidant capacity (**Bjelakovic *et al.,* 2007**).

The lack of inherent antioxidant and the availablity of high quality nutrients lead to the problem of perishability of the meat products (**Gupta and Savalia 2012; Das *et al*., 2013)**. Meat and meat products provide excellent growth media for a variety of microorganisms (bacteria, yeasts and melds) some of which are pathogenic **(Jay *et al.,* 2005)**. The most common genera of bacteria found in meat before spoilage is *Staphylococcus, Bacillus, Campylobacter, Clostridium, Listeria, Salmonella etc.*

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(Garcia-Lopez *et al.*, 1998). The storage conditions affect the type of microbes found in meat and meat products. The favourable pH for the growth of spoilage bacteria for meat is in the range of 5.5-7.0. Slime formation, structural components degradation, off odours and appearance change were found in meat as a result of microbial growth within this pH range **(Russell *et al.*, 1996)**.

The inherent anti-oxidants capacity of meat products is very low leading to concern about the quality and shelf life of meat and meat products. The antioxidants play a major role in reducing the oxidation of fat as well reducing the harmful free radicals that can damage the cells **(Russell *et al.*, 1996)**. By improving the antioxidant capacity of meat by adding natural antioxidants such as rosemary, isoflavones, rosemary and isoflavones, the overall quality as well as acceptability of meat and fish products can be further enhanced.

The meat products prepared from low value cuts and offal have poor cooking yield and emulsion stability due to higher collagen content leads to poor emulsifying and water binding capacity. These cuts by adding soy protein, the binding as well as functional value of meat products increased (**Ruban *et al.*, 2009)**. This study aimed to study the effect of natural crude soybean, rosemary, BHA and rosemary plus natural crude soybean extract as antioxidant on beef burger quality during storage under freezing storage.

MATERIALS AND METHODS

Collection of Samples

Soybean (*Glycine max*) was obtained from the Agriculture Research Centre, Giza, Egypt during season 2014. Rosemary was purchased from a local herb shop (Harraz, Cairo, Egypt), and BHA (Butylated Hydroxy Anisole) was obtained from Morgan Specialty Chemicals Company, El- Oboure city. Twenty kilo grams of freshly beef chuck, 24 hours postmortem were purchased from local butcher shop at Giza market-Egypt.

Preparation of Rosemary Crude Extract

Plant materials (rosemary) were used to prepare crude rosemary extract that contain the polar active compounds as following 10 g of dry powdered rosemary leaves (2 % W/V) were dissolved in distilled water. After maceration for 48 hours, the extract was filtered through filter paper. This filtrate was then frozen and kept at -18 °C until used **(Georgantelis *et al.*, 2007).**

Preparation of Crude Isoflavones Extract from Soybean

Crude isoflavones extract (CIE) from soybean was prepared to be used with beef burger preparation to evaluate its antioxidant and antimicrobial effect as follow: soybean seeds were milled for 3 minutes at a medium speed in a coffee bean blender. Then, the particles of the ground whole soy flour were ground to pass through a 1 mm sieve.The soy flour (150 g) was weighed into a thimble to extract the oil by Soxhlet apparatus for 8 hours (Fig. 1). The defatted soy flour (100g) was weighed then transferred to round bottom flask, then 400 ml of distilled water was added at ratio 1:4 (weight: volume, soy flour: water), then transferred to a flask and incubated at 45ºC for 5 hr. The mixture was transferred to high speed centrifuge to be clarified for 30 min at 20ºC and 5000 rpm speed (RCF g force, 5470). The solid phase was thrown away (discarded). The clarified solution was transferred to 2L round bottom flask to be concentrated under vacuum using rotary evaporator at 35 rpm speed and 55ºC up till almost reaching the volume of 50ml of concentrated soybean extract according to **Abd Allah (2011)**.

Preparation of Beef Burger Samples

Beef burger was prepared in agreement with the Egyptian Standard Specifications for burger (ESS 1688/2005) as follows: twenty kilo grams of freshly beef chuck 24 hours postmortem were transported to the laboratory in an ice box to be minced in electrical mincer which was sterilizated with ethanol 70%. Minced meat 70%, fat 20%, black pepper 0.3%, salt 2% and water 8% were thoroughly mixed for five minutes and divided into ten portions.

First portion was used as control, while the other portions were either mixed with Butylated Hydroxy-Anisole (BHA) at concentrations of 0.01 and 0.02, or crude isoflavones extract (CIE) at concentrations of 0.01, 0.02 and 0.03%. Crude rosemary extract (CRE) at concentrations of 0.02, 0.04 and 0.06%, or (CIE) 0.02% plus (CRE) 0.02% were also tested. The obtained mixtures were formed into 50g beef burger using cardboard meat box, packed in foam plates and stored in a freezer at -18ºC until tested. Five samples for each treatment were examined for the following characteristics every month for three monthes.

Cooking of Beef Burger

The beef burger samples were grilled for 2 minutes on each side at (75ºC) for measuring cooking yild, cooking loss and shrinkage. The sensory evaluation was also performed according to **Mittal (2006).**

$$\textbf{Cooking yield (\%)} = \frac{\text{Cooked weight X 100}}{\text{Raw weight}}$$

$$\textbf{Cooking loss (\%)} = \frac{\text{Raw weight} - \text{Cooked weight X 100}}{\text{Raw weight}}$$

$$\textbf{Shrinkage (\%)} = \frac{\text{Raw diameter} - \text{Cooked diameter X100}}{\text{Raw diameter}}$$

Analytical Methods

Chemical properties

Water content, pH values, total protin, fat and ash content were determind according to methods described by **AOAC (1995)**. Thiobarbituric acid reactive substances (TBARS) thiobarbituric acid value was determined as described by **Siu and Draper (1978).**

Bacteriological Examinations

To determine the microbial counts of total bacterial count, pathogenic bacteria, including *Staphylococcus aureus*, *E. coli* and Coliform bacteria were carried out as, follow: ten g of burger samples were aseptically transferred to sterile plastic bags containing 90 ml peptone water (Oxoid CM 9, UK). The samples were homogenized for 1-2 min (Interscience Bag Mixer 400), then 10-fold serial dilutions were made in sterile peptone salt water up to 10^{-7} and inoculated onto specific culture media for bacterial count (nutrient agar), *Staphylococcus aureus*, were determined according to FAO and Oxoid. Coliform bacteria were applied using Violet Red Bile (VRB) agar medium. The plates were incubated at 34ºC for 24 hours, coagulase tests were done according to the method described by **Siriken, *et al.*, (2006).**

Colour Determination for Beef Burger

Colour was evaluated using a colorimeter (Mod. CR-200, Minolta Camera Co., Osaka, Japan). Nine replicate measurements were taken for each sample, following the guidelines on colour measurements of the American Meat Science Association **(Hunt *et al.*, 1991).**

Texture Profile

Sur penetrometer (PNR 6, Berlin, Germany) equipped with a total 100g load was used to evaluate samples of hardness. Depth puncture was determined to 1/10 cm in triplicate for each piece for 30 sec. A lower depth of penetration indicates a harder texture **(Yildiz-Turp *et al.*, 2005)**.

Sensory Evaluation of Cooked Beef Burger

Overall acceptability was evaluated by ten panellists and statistically analyzed according to **Basker, (1988).** The procedure recommended was adopted as follows:

A special score sheet was designed to evaluate each sample by panelists, during storage period. Quality assessment scheme was used to identify the quality index demerit score **Basker, (1988)**.

Statistical analysis

All measurement were done in triplicate and data was reported as means ± standard deviation (SD) using SPSS software (version 16.0 for windows, SPSS Inc., Chicago).

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of control and treated samples. The control beef burger contained 13.56% protein, 58.57% moisture, 23.11% fat, 0.39% unsaponifiable and 2.85% ash. In comparison with control, the addition of the crude isoflavones extract (CIE) affected the proximate composition of burger. The CIE increased moisture, ash and protein content and reduced fat content. Ash content was increased by increasing CIE concentration. These results are in agreement with those obtained by **Ho *et al.* (1997) and Abu-Salem *et al.* (2014**).

In comparison with control, the addition of rosemary affected the proximate composition of burger. Rosemary increased moisture, ash and protein contents and reduced fat content. Ash content was increased by increasing the crude rosemary extract (CRE) concentration remained unchanged with different concentrations of CRE Table 1.These results are in agreement with those obtained by **Fernandez-Lopez *et al.* (2005) and Abu-Salem *et al.* (2014).**

PH Value

PH values increased from 6.00 to 6.33 in samples treated with various levels of crude rosemary extract (CRE) through out 1st and 2 nd monthes of storage, then PH values trended to decrease to 6.23

PH values gradually increased from 6.1 to 6.27 for control samples, and increased from 6.08 to 6.15 in sample treated with different levels of Butylated Hydroxy-Anisol (BHA) from 6.30 to 6.23 in samples treated with different levels of crude rosemary extract (CRE). On the other hand, it increased from 6.08 to 6.16 in samples treated with different levels of CIE while it decreased from 6.23 to 6.18 in samples treated with CIE plus CRE Table 2 after the 2 nd month of storage Similar results were obtained by **Fernandez-Lopez *et al,* (2005), Abdel-Hamied *et al.* (2009); Ahmed, *et al.* (2010) and Abu-Salem *et al.* (2014).**

According to **Khouraiba (1981**) the increase of pH value was due to the proteolysis process leading to the increase of free basic amino acids as well as the accumulation of ammonia, amines and other basic products of bacteria breakdown.

(CIE) Crude Isoflavones Extract, (BHA) Butylated Hydroxy-Anisol and (CRE) Crude Rosemary Extract

TBARS Value

TBA test has been widely used to measure lipid oxidation in meat and meat products. Results clearly in Table 3 show that TBA values increased in control sample during storage period. Results showed that increasing the CIE, CRE, BHA and CIE plus CRE levels resulted in decreasing the TBA values Table 3.

These results agree with those reported by **Fernandez-Lopez *et al.* (2005), Abdel-Hamied *et al.* (2009); Ahmed, *et al.* (2010) and Abu-Salem *et al.* (2014)** for other natural antioxidants applied to meatballs.

Table (1): Proximate composition (mean ± S.D) of burger prepared with CIE, BHA and CRE additives during storage at -18ºC ± 1.

Treatment	Moisture (%)	Protein (%)	Fat (%)	Unsaponifiable (%)	Ash (%)
Control	58.57±0.09	13.56±0.01	23.11±0.05	0.39±0.34	2.85±0.06
BHA 0.01%	58.95±0.02	13.75±0.04	22.21±0.06	0.38±0.16	2.95±0.04
BHA 0.02%	59.73±0.07	13.39±0.05	23.01±0.07	0.37±0.21	2.89±0.09
CIE 0.01%	58.86±0.02	13.73±0.05	21.91±0.03	0.39±0.36	3.13±0.01
CIE 0.02%	59.83±0.07	14.19±0.03	21.05±0.04	0.39±0.32	3.29±0.06
CIE 0.03%	60.26±0.01	14.59±0.06	19.16±0.03	0.38±0.33	3.40±0.04
CRE 0.02%	58.97±0.02	13.84±0.05	20.16±0.08	0.38±0.26	2.88±0.08
CRE 0.04%	59.93±0.17	13.37±0.07	19.91±0.13	0.39±0.25	3.19±0.03
CRE 0.06%	59.96±0.02	13.64±0.05	22.12±0.05	0.39±0.41	3.10±0.07
CIE 0.02% plus CRE 0.02%	60.17±0.31	13.89±0.25	20.16±0.08	0.39±0.24	3.22±0.14

(CIE) Crude Isoflavones Extract, (BHA) Butylated Hydroxy-Anisol and (CRE) Crude Rosemary Extract.

Table (2): The pH values (mean ± S.D) of burger prepared with CIE, BHA and CRE additives during storage at -18 ºC ± 1.

Treatment	Storage time (month)			
	Zero	**1**	**2**	**3**
Control	6.01 ± 0.07	6.20 ± 0.05	6.19 ± 0.07	6.27 ± 0.04
BHA 0.01%	6.09 ± 0.10	6.11 ± 0.02	6.12 ± 0.07	6.13 ± 0.22
BHA 0.02%	6.08 ± 0.05	6.09 ± 0.03	6.13 ± 0.03	6.15 ± 0.04
CIE 0.01%	6.09 ± 0.10	6.11 ± 0.02	6.12 ± 0.08	6.13 ± 0.02
CIE 0.02%	6.08 ± 0.05	6.09 ± 0.03	6.13 ± 0.03	6.15 ± 0.04
CIE 0.03%	6.09 ± 0.06	6.10 ± 0.04	6.13 ± 0.08	6.16 ± 0.07
CRE 0.02%	6.00 ± 0.05	6.27 ± 0.05	6.28 ± 0.08	6.26 ± 0.04
CRE 0.04%	6.09 ± 0.06	6.30 ± 0.04	6.33 ± 0.08	6.26 ± 0.07
CRE 0.06%	6.07 ± 0.10	6.24 ± 0.02	6.22 ± 0.07	6.23 ± 0.12
CIE 0.02% plus CRE 0.02%	6.03 ± 0.25	6.19 ± 0.03	6.23 ± 0.08	6.18 ± 0.14

Table (3): The TBARS values (mean ± S.D) of burger prepared with CIE, BHA and CRE additives during storage at -18ºC ± 1.

Treatment	Storage time (month)			
	Zero	1	2	3
Control	0.31 ± 0.03	1.20 ± 0.05	2.12 ± 0.14	3.23 ± 0.15
BHA 0.01%	0.30 ± 0.11	0.52 ± 0.21	0.68 ± 0.18	0.85 ± 0.13
BHA 0.02%	0.31 ± 0.06	0.59 ± 0.03	0.93 ± 0.03	0.97 ± 0.24
CIE 0.01%	0.32 ± 0.14	0.31 ± 0.12	0.74 ± 0.17	1.32 ± 0.12
CIE 0.02%	0.30 ± 0.12	0.39 ± 0.03	0.63 ± 0.13	0.95 ± 0.14
CIE 0.03%	0.31 ± 0.06	0.33 ± 0.11	0.51 ± 0.08	0.71 ± 0.07
CRE 0.02%	0.29 ± 0.05	0.32 ± 0.14	1.08 ± 0.09	2.38 ± 0.22
CRE 0.04%	0.30 ± 0.13	0.35 ± 0.12	0.55 ± 0.17	1.75 ± 0.14
CRE 0.06%	0.31 ± 0.11	0.33 ± 0.04	0.38 ± 0.07	0.83 ± 0.13
CIE 0.02% plus CRE 0.02%	0.31 ± 0.32	0.36 ± 0.33	0.56 ± 0.23	0.96 ± 0.44

TBA values of beef burger samples increased gradually during frozen storage and this increase could be attributed to the oxidation of beef burger lipids and the formation of some TBA reactive compounds during the storage period as reported by **Stahnke (1995)**.

(CIE) Crude Isoflavones Extract, (BHA) Butylated Hydroxy-Anisol and (CRE) Crude Rosemary Extract

Cooking Properties

The cooking yield (%)

As shown in Table 4, cooking yield percentage of beef burger samples containing BHA at levels of 0.01 and 0.02% is slightly decreased with concentration increase. While samples contain CRE at levels of 0.02, 0.04 and 0.06% were slightly decreased in comparison to the control sample.

Similar trend was observed with the addition of CRE plus CIE, frozen storage decreased the cooking yield with increasing storage periods. The higher cooking yield of protein treated samples probably resulted from an increased number of charged and polar amino and carboxylic groups due to peptide cleavage which led to a stronger protein-water interaction **(Abu-Salem *et al.*, 2014)**.

The cooking loss (%)

As shown in Table 5, cooking loss percentage of beef burger samples containing 0.01 and 0.02% of BHA slightly increased with increased percentage in comparison with control. While samples contain CRE at levels of 0.02, 0.04 and 0.06% slightly increased with increasing the addition level of CRE than control.

Similar results were observed with the addition of CRE plus CIE**.** These results agree with those reported by **Ahmed *et al.* (2010), Hegazy (2011) and Abu-Salem *et al.* (2014).**

Table (4): The cooking yield % (mean ± S.D) of burger prepared with CIE, BHA and CRE additives during storage at – 18ºC ± 1.

Treatment	Storage (month)			
	Zero	1	2	3
Control	80.31 ± 0.13	78.20 ± 0.15	74.53 ± 0.24	73.23 ± 0.19
BHA 0.01%	81.51 ± 0.21	79.70 ± 0.25	75.53 ± 0.24	72.73 ± 0.16
BHA 0.02%	80.81 ± 0.11	78.90 ± 0.14	76.83 ± 0.24	74.89 ± 0.22
CIE 0.01%	84.52 ± 0.24	83.37 ± 0.17	79.74 ± 0.37	76.32 ± 0.32
CIE 0.02%	85.36 ± 0.17	84.89 ± 0.13	80.63 ± 0.13	78.95 ± 0.34
CIE 0.03%	88.67 ± 0.26	86.43 ± 0.31	83.51 ± 0.18	81.74 ± 0.17
CRE 0.02%	81.29 ± 0.15	80.32 ± 0.10	76.38 ± 0.09	73.38 ± 0.22
CRE 0.04%	80.93 ± 0.12	79.45 ± 0.12	75.55 ± 0.17	72.75 ± 0.14
CRE 0.06%	82.11 ± 0.21	81.33 ± 0.04	77.38 ± 0.07	76.83 ± 0.13
CIE 0.02% plus CRE 0.02%	83.46 ± 0.47	82.69 ± 0.53	79.94 ± 0.27	77.98 ± 0.44

Table (5): The cooking loss % (mean ± S.D) of burger prepared with CIE, BHA and CRE additives during storage at -18 ºC ± 1.

Treatment	Storage time (month)			
	Zero	1	2	3
Control	19.70 ± 0.15	21.80 ± 0.15	25.48 ± 0.14	26.78 ± 0.16
BHA 0.01%	18.88 ± 0.11	20.31 ± 0.15	24.47 ± 0.26	27.28 ± 0.26
BHA 0.02%	19.20 ± 0.21	21.02 ± 0.12	23.17 ± 0.21	28.12 ± 0.12
CIE 0.01%	16.48 ± 0.24	16.67 ± 0.15	20.26 ± 0.32	23.68 ± 0.22
CIE 0.02%	15.74 ± 0.17	15.11 ± 0.12	19.37 ± 0.13	21.05 ± 0.35
CIE 0.03%	12.43 ± 0.23	13.57 ± 0.21	16.49 ± 0.14	18.46 ± 0.18
CRE 0.02%	18.71 ± 0.25	19.68 ± 0.12	23.62 ± 0.17	26.62 ± 0.12
CRE 0.04%	19.07 ± 0.22	20.55 ± 0.16	24.45 ± 0.23	27.25 ± 0.18
CRE 0.06%	17.89 ± 0.23	18.67 ± 0.08	22.62 ± 0.17	23.17 ± 0.13
CIE 0.02% plus CRE0.02%	16.84 ± 0.17	15.81 ± 0.32	20.67 ± 0.13	23.45 ± 0.25

Meat products usually used soy proteins to enhance the products functional characteristics, reducing cooking loss and improving slice ability. The current results showed that the CIE additive treatments increased the cooking yield and effectively reduced the cooking loss. These results agree with those reported previously by **Abu-Salem *et al.* (2014).**

The Shrinkage (%)

As shown in Table 6, the diameter of all samples decreased after cooking, from 25.44% to 14.89%. There was a less surface shrinkage of the burger made with CIE additive**,** surface shrinkage percentage of beef burger samples increased linearly for all beef burger samples during frozen storage, but it was more obvious in control sample than other treated samples containing CIE, BHA and CRE additives**.**

These results agree with the results reported by **Sharaf *et al.* (2009); Ahmed *et al.* (2010); Hegazy (2011) and Abu-Salem *et al.* (2014).**

(CIE) Crude Isoflavones Extract, (BHA) Butylated Hydroxy-Anisol and (CRE) Crude Rosemary Extract

The Texture

As shown in Table 7, texture values of beef burger samples decreased linearly for all beef burger samples during frozen storage, but it was more evident in control sample than other treated samples containing CIE. Slight differences were observed among treatments with BHA and CRE additives. However, the treatment with CRE had the lowest texture score, which may be due to the high amount of soluble fiber in the CRE (19.07 increased to 27.25g/100g sample). Soluble fibers bind to water and form gels, which may have given some elasticity and resistance to chewing, affecting negatively the texture of the burger, these results agree with previous results reported by **Sharaf *et al.* (2009), Ahmed *et al.* (2010), Hegazy (2011), Abu-Salem *et al.* (2014) and Subhani (2014).**

(CIE) Crude Isoflavones Extract, (BHA) Butylated Hydroxy-Anisol and (CRE) Crude Rosemary Extract

Microbiological Evaluation

The results presented in Table 8 show the microbiological examination of burgers during storage period. Generally, the addition of different levels of CIE, CRE, BHA and CIE plus CRE slowed a decrease in the bacterial growth during the storage period and this decrease has increased with the increase of CIE, CRE, BHA and CIE plus CRE concentrations. The microbiological quality of meat products as purchased by the consumer is relied on a number of factors, such as the quality of the raw materials, other materials used or added during processing operations to the products as extraneous contaminants, sanitation during processing and packaging. At concentration of 0.01, 0.02 and 0.03%, CIE and 0.02, 0.04 and 0.06 % of CR) and CIE plus CRE reduced all the microbial groups (Total bacterial count, coliform, *Staphylococcus aureus*,) counts in the samples, these results are in agreement with previous results reported by **(Dorman and Hiltunen, 2000; Ahmed, *et al.,* 2010 and Abu-Salem *et al.,* 2014).**

Total Bacterial Count (TBC)

Total bacterial count (TBC) was decreased from 5.58 to 3.53 $\log_{10}$ CFU/g in samples contain 0.03% of CIE in comparison to the control sample (Table 8). The TBC $\log_{10}$ CFU/g of samples that have 0.06% CRE showed a decrease in microbial counts from 5.65 to 4.12 $\log_{10}$ CFU/g by the end of storage period. On the other hand, BHA concentrations (0.01 and 0.02%) showed a slight decrease in bacterial count even with high concentrations by the end of storage time in comparison with the samples contain CIE, CRE and CIE plus CRE. These results agree with those reported by **Dorman and Hiltunen (2000), Ahmed *et al.* (2010) and Abu-Salem *et al.* (2014).**

Table (6): The shrinkage percentage (mean ± S.D) of burger prepared with CIE, BHA and CRE additives during storage at -18 ºC ±1.

Treatment	Storage time (month)			
	Zero	1	2	3
Control	20.20 ± 1.35	22.50 ± 2.15	23.43 ± 1.14	25.18 ± 2.16
BHA 0.01%	21.10 ± 1.34	22.60 ± 1.15	23.23 ± 2.13	24.84 ± 2.15
BHA 0.02%	20.40 ± 1.45	21.90 ± 2.35	23.63 ± 1.44	25.38 ± 2.32
CIE 0.01%	20.62 ± 1.22	22.20 ± 1.34	23.23 ± 1.14	25.28 ± 1.42
CIE 0.02%	18.50 ± 1.25	19.42 ± 2.23	20.23 ± 1.45	22.58 ± 1.36
CIE 0.03%	14.89 ± 1.31	15.84 ± 2.45	16.22 ± 2.21	17.85 ± 2.26
CRE 0.02%	20.56 ± 1.24	21.80 ± 2.15	24.20 ± 2.33	25.44 ± 2.35
CRE 0.04%	21.20 ± 1.15	22.30 ± 2.32	23.43 ± 1.44	24.94 ± 1.42
CRE 0.06%	20.32 ± 1.22	22.24 ± 1.75	23.22 ± 2.21	25.25 ± 1.19
CIE 0.02% plus CRE 0.02%	19.24 ± 1.37	20.83 ± 2.32	21.67 ± 2.18	23.55 ± 2.25

Table (7): The texture values (mean ± S.D) of burger prepared with CIE, BHA and CRE additives during storage at -18 ºC ± 1.

Treatment	Storage time (month)			
	Zero	1	2	3
Control	22.60 ± 1.45	19.70 ± 2.35	15.48 ± 1.44	15.78 ± 2.16
BHA 0.01%	23.34 ± 2.32	18.80 ± 2.25	14.88 ± 2.45	16.18 ± 2.46
BHA 0.02%	22.63 ± 1.47	19.25 ± 2.33	15.33 ± 1.37	14.88 ± 2.14
CIE 0.01%	18.48 ± 2.24	15.67 ± 2.15	12.26 ± 2.32	07.68 ± 2.22
CIE 0.02%	18.64 ± 2.17	16.31 ± 1.62	13.37 ± 2.13	09.05 ± 2.35
CIE 0.03%	20.43 ± 2.23	17.57 ± 2.21	18.49 ± 2.14	10.46 ± 2.18
CRE 0.02%	18.71 ± 1.25	19.68 ± 1.12	23.62 ± 1.17	26.62 ± 2.12
CRE 0.04%	19.07 ± 2.22	20.55 ± 2.16	24.45 ± 1.23	27.25 ± 2.18
CRE 0.06%	17.89 ± 1.23	18.67 ± 2.18	22.62 ± 2.17	23.17 ± 1.13
CIE 0.02% plus CRE 0.02%	18.78 ± 2.17	21.55 ± 2.26	19.75 ± 1.23	16.71 ± 1.42

Table (8): Effect of CIE, BHA and CRE additives on total bacterial count (log_{10} CFU/gm) of burger during frozen storage period (mean ± S.D).

Treatment	Storage time (month)			
	Zero	1	2	3
Control	5.86 ± 0.25	5.72 ± 0.15	4.57 ± 0.35	4.54 ± 0.22
BHA 0.01%	5.87 ± 0.32	5.78 ± 0.23	4.86 ± 0.32	4.56 ± 0.23
BHA 0.02%	5.86 ± 0.14	5.73 ± 0.15	4.87 ± 0.21	4.57 ± 0.12
CIE 0.01%	5.75 ± 0.31	5.64 ± 0.13	4.70 ± 0.41	4.23 ± 0.07
CIE 0.02%	5.63 ± 0.23	5.66 ± 0.22	4.55 ± 0.13	4.13 ± 0.25
CIE 0.03%	5.58 ± 0.31	4.75 ± 0.32	4.39 ± 0.15	3.53 ± 0.17
CRE 0.02%	5.67 ± 0.25	5.56 ± 0.12	4.75 ± 0.17	4.42 ± 0.12
CRE 0.04%	5.68 ± 0.21	5.56 ± 0.21	4.64 ± 0.23	4.32 ± 0.32
CRE 0.06%	5.65 ± 0.25	4.69 ± 0.25	4.56 ± 0.24	4.12 ± 0.22
CIE 0.02% plus CRE 0.02%	5.75 ± 0.33	5.65 ± 0.31	4.72 ± 0.15	4.24 ± 0.32

Coliform Group

Generally, the addition of different levels of CIE, CRE, BHA and CIE plus CRE showed a decrease in the bacterial growth over the storage period and this reduction was increased with the increase of used concentrations of additives (Table 9). Results agree with those reported by previous studies **(Dorman and Hiltunen, 2000; Ahmed *et al.*, 2010; Abu-Salem *et al.*, 2014).**

E. coli

As previously described, high concentrations of CIE, CRE, BHA and CIE plus CRE reduced the numbers of *E. coli* in treated samples during the storage period. The numbers of *E. coli* were reduced from 1.49 to 0.74 log_{10} CFU/g by the end of storage time in control sample. While storage period affected the microbial count in all samples and the highest reduction rate of *E. coli* (from 1.29 to Nil log_{10} CFU/g was observed in samples treated with 0.03% of CIE. Similar results were observed with CRE which decreased the *E. coli* count from 1.36 log_{10} CFU/g to undetectable limit during the first month and the same was observed with samples treated with CIE plus CRE (from 1.27 to Nil log_{10} CFU/g) by the second month of storage followed by samples treated with BHA from 1.45 log_{10} CFU/g to undetectable limit at the third month only after three months of storage (Table 10). These results agree with those reported by **(Dorman and Hiltunen (2000); Ahmed *et al.* (2010); Osama A. and Kassem (2011) and Abu-Salem *et al.*, (2014).**

Staphylococcus Aureus

The results in Table 11 presented the numbers of *S. aureus* in all treatments including control. It was clear that, the addition of different concentrations of CIE, CRE, BHA and CIE plus CRE reduced the numbers of *S. aureus* during storage period and this reduction was increased with the use of high concentrations of additives. *Staphylococcus aureus* is reduced from 2.28 to 1.54 log_{10} CFU/g at the end of storage in control sample and this may be due to the effect of storing temperature while samples treated with BHA showed a

Table (9): Effect of CIE, BHA and CRE additives on coliform count (log_{10} CFU/gm) of burger during frozen storage period at – 18°C (mean ± S.D).

Treatment	Storage time at – 18°C			
	Zero	1	2	3
Control	3.39 ± 0.12	3.35 ± 0.15	2.36 ± 0.37	2.39 ± 0.32
BHA 0.01%	3.35 ± 0.32	3.34 ± 0.14	2.26 ± 0.35	2.44 ± 0.08
BHA 0.02%	3.33 ± 0.12	3.32 ± 0.45	2.27 ± 0.32	2.24 ± 0.32
CIE 0.01%	3.15 ± 0.21	3.24 ± 0.14	2.27 ± 0.31	2.17± 0.12
CIE 0.02%	3.13 ± 0.11	2.23 ± 0.22	2.14 ± 0.24	1.05± 0.23
CIE 0.03%	3.16 ± 0.31	2.14 ± 0.12	2.07 ± 0.15	1.02± 0.17
CRE 0.02%	3.37 ± 0.25	2.47 ± 0.21	2.27 ± 0.31	2.14 ± 0.12
CRE 0.04%	3.27 ± 0.21	2.38 ± 0.16	2.25 ± 0.24	1.20 ± 0.23
CRE 0.06%	3.34 ± 0.23	2.27 ± 0.31	2.23 ± 0.35	1.07 ± 0.18
CIE 0.02% plus CRE 0.02%	3.26 ± 0.41	2.37 ± 0.42	2.29 ± 0.34	1.18 ± 0.43

Table (10): Effect of CIE, BHA and CRE additives on *E. coli* (log_{10} CFU/g) of burger during frozen storage period at – 18°C (mean ± S.D).

Treatment	Storage time (month)			
	Zero	1	2	3
Control	1.49 ± 0.42	1.48 ± 0.23	1.00 ± 0.35	0.74 ± 0.15
BHA 0.01%	1.52 ± 0.55	1.49 ± 0.23	1.02 ± 0.27	0.75 ± 0.15
BHA 0.02%	1.44 ± 0.52	1.35 ± 0.23	1.00 ± 0.55	0.72 ± 0.15
CIE 0.01%	1.34 ± 0.31	1.29 ± 0.15	1.01 ± 0.21	Nil
CIE 0.02%	1.32 ± 0.31	0.77 ± 0.12	Nil	Nil
CIE 0.03%	1.29 ± 0.41	Nil	Nil	Nil
CRE 0.02%	1.45 ± 0.31	0.82 ± 0.15	Nil	Nil
CRE 0.04%	1.34 ± 0.31	0.63 ± 0.12	Nil	Nil
CRE 0.06%	1.36 ± 0.41	Nil	Nil	Nil
CIE 0.02% plus CRE 0.02%	1.27 ± 0.21	0.57 ± 0.32	Nil	Nil

Table (11): Effect of CIE, BHA and CRE additives on *Staphylococcus aureus* (log_{10} CFU/g) of burger during frozen storage period at – 18°C (mean ± S.D).

Treatment	Storage time (month)			
	Zero	1	2	3
Control	2.28 ± 0.12	2.52 ± 0.23	1.79 ± 0.25	1.54 ± 0.42
BHA 0.01%	2.35 ± 0.15	2.41 ± 0.23	1.68 ± 0.27	1.57 ± 0.33
BHA 0.02%	2.23 ± 0.52	2.51 ± 0.23	1.71 ± 0.35	1.48 ± 0.42
CIE 0.01%	2.20 ± 0.21	2.12 ± 0.15	1.12 ± 0.21	1.04 ± 0.32
CIE 0.02%	2.16 ± 0.31	1.84 ± 0.12	1.07 ± 0.23	1.02 ± 0.23
CIE 0.03%	2.11 ± 0.41	1.32 ± 0.12	1.02 ± 0.15	1.00 ±0.13
CRE 0.02%	2.27 ± 0.31	2.31 ± 0.13	1.54 ± 0.16	1.43 ± 0.33
CRE 0.04%	2.18 ± 0.23	2.14 ± 0.13	1.47 ± 0.33	1.28 ± 0.15
CRE 0.06%	2.09 ± 0.21	1.89 ± 0.16	1.42 ± 0.13	1.27 ± 0.22
CIE 0.02% plus CRE 0.02%	2.20 ± 0.45	1.97 ± 0.22	1.23 ± 0.35	1.04 ± 0.33

decrease by the end of storage period. The highest reduction rate (from 2.11 to 1.00 log10 CFU/g) was observed in the samples treated with 0.03% CIE in comparison to samples treated with CRE which showed a decrease (from 2.09 to 1.27 log10 CFU/g followed by samples treated with BHA from 2.21 to 1.56 log10 CFU/g.

The control sample decreased from 2.28 at zero time to reach 1.54 log10 CFU/g at the end of storage period (Table 11). These results agree with those reported by others **Dorman and Hiltunen (2000); Ahmed *et al.* (2010); Hać-Szymańczuk (2011); Abu-Salem *et al.* (2014).**

(CIE) Crude Isoflavones Extract, (BHA) Butylated Hydroxy-Anisol and (CRE) Crude Rosemary Extract

Color measurements

The redness color decreased for samples treated with CIE, CRE, BHA and CIE plus CRE during the storage period in comparison with control samples. All the treatments had effect on decreasing the red color of the beef burger except CRE. This reduction in a* values and L* values might be due to oxygenation of meat myoglobin Table 12. These results agree with those reported by **Hać-Szymańczuk (2011), and Abu-Salem *et al.* (2014).**

Overall Acceptability

As show in table 13 the overall acceptability decreased from 6.57 to 5.78 for control samples (without any additives), while it decreased from 6.77 to 5.88 in samples treated with BHA. It is also decreased from 7.77 to 5.28 in samples treated with CIE and from 7.52 to 6.28 in samples treated with CRE. Overall acceptability also decreased from 7.78to 6.78 in samples treated with CIE plus CRE (Table 13). These results agree with those reported by **Ahmed *et al.*, (2010), Hać-Szymańczuk (2011), Kenawi *et al.* (2011), Hussein *et al.* (2012), Sahari *et al.* (2013) and Abu-Salem *et al.* (2014).**

Conclusion

The results showed that all samples treated with crude isoflavones extract (CIE), showed strong antioxidant and antimicrobial properties. The results showed that extracts derived from crude rosemary leaves extract (CRE) had the potential to reduce the oxidation of beef burger and extend their shelf life. The combined extracts between (CIE) and (CRE)

Table (12): The effect of CIE, BHA and CRE additives on L*, a*and b*values (color) of burger during frozen storage period at -18°C.

Treatment	Storage time (month)											
	Zero			1			2			3		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Control	60.12	6.39	20.96	59.74	10.52	22.36	61.45	6.42	21.25	60.57	5.85	18.83
BHA 0.01%	56.12	7.76	20.95	58.96	8.78	22.14	60.16	6.82	20.74	59.69	5.86	18.55
BHA 0.02%	57.89	7.89	20.24	60.79	10.67	21.13	59.81	6.52	20.76	58.39	6.97	19.63
CIE 0.01%	60.12	6.39	20.96	57.95	9.19	21.36	59.43	6.15	21.06	59.56	5.75	20.31
CIE 0.02%	59.72	6.34	21.26	58.65	8.29	22.24	61.54	5.33	21.16	58.74	5.70	20.11
CIE 0.03%	59.68	6.53	21.72	59.91	8.13	22.18	58.86	6.53	21.76	58.9	5.90	21.14
CRE 0.02%	59.15	7.86	20.32	57.62	8.69	22.46	59.83	9.05	21.75	59.66	7.76	19.61
CRE 0.04%	58.76	8.25	20.96	59.67	9.29	22.84	61.54	8.63	22.36	60.64	8.80	19.15
CRE 0.06%	56.87	8.38	21.32	60.41	9.73	21.78	60.46	8.73	22.42	61.93	8.50	20.24
CIE 0.02% plus CRE 0.02%	57.49	7.48	20.42	58.96	8.75	22.52	61.34	7.52	21.53	59.65	7.67	20.34

The L* value was a measure of darkness on a scale from 0 (lightest) to 100 (darkest). The * value measures red to green color, and the b* value measures yellow to blue color. The data was collected, transferred to an Excel file, and analyzed.

Table (13). The effect of CIE, BHA and CRE additives on sensory evaluation (the overall acceptability) of burger during frozen storage period at – 18°C (mean ± S.D).

Treatment	Storage time (month)			
	Zero	1	2	3
Control	6.57 ± 0.32	6.24± 0.43	6.04± 0.31	5.78± 0.52
BHA 0.01%	6.32 ± 0.22	6.27± 0.31	6.14± 0.52	5.98± 0.22
BHA 0.02%	6.77± 0.36	6.34± 0.43	6.22± 0.51	5.88± 0.15
CIE 0.01%	7.82± 0.26	7.34± 0.45	6.78± 0.34	6.38± 0.22
CIE 0.02%	7.77 ± 0.32	7.20± 0.23	6.33 ± 0.21	6.20± 0.52
CIE 0.03%	6.52± 0.32	5.93± 0.43	5.74± 0.26	5.28± 0.14
CRE 0.02%	7.52± 0.27	7.14 ± 0.45	6.45± 0.34	6.30± 0.22
CRE 0.04%	7.57± 0.22	7.24± 0.23	6.53± 0.21	6.40 ± 0.25
CRE 0.06%	7.52± 0.42	6.63± 0.43	6.44± 0.23	6.28± 0.14
CIE 0.02% plus CRE 0.02%	7.55± 0.38	6.32± 0.43	6.31± 0.51	6.28 ± 0.56

showed in addition some antibacterial activity that led also to a significant extension of beef burger shelf life. The availability of these natural antioxidants and their possible co antioxidant or synergistic effects suggests an interesting way of improving beef burger stability and preventing degenerative diseases caused by fat oxidation products.

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الملخص العربي

تقييم الجودة والخصائص الميكروبية لبرجر اللحم (البيف برجر) المعد من مستخلص الأيزوفلانيور و BHA والحصالبان

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تهدف هذه الدراسة إلى تقييم أثر إضافة كل من مستخلص الأيسوفلافون وإكليل الجبل والمقارنة مع مضاد الأكسدة الصناعي BHA من حيث قدرتها على إطالة فترة التخزين للبرجر أثناء الحفظ على -١٨م° لمدة ٣ شهور، حيث تم إضافتهم بنسبة ٠,٠١ % و٠,٠٢% و٠,٠٣% للأيسوفلافون و ٠,٠٢% و٠,٠٤% و٠,٠٦% لإكليل الجبل و٠,٠١% و٠,٠٢% من BHA ومخلوط كل من الأيسوفلافون ٠,٠٢% وإكليل الجبل بنسبة ٠,٠٢%. وخلال فترة الحفظ تم إجراء مجموعة من الاختبارات الحسية والكيميائية والفيزيائية مثل حامض الثيوباربتيوريك والأس الهيدروجيني وقيم المواد غير المتصبنة وقيم العائد من الطبخ وقيم الفقد خلال الطبخ والانكماش. والاختبارات الميكروبيولوجية مثل العدد الكلي للبكتيريا ومجموعة الكوليفورم وذلك للوقوف على تأثير كل منهم على زيادة فترة حفظ البرجر. وقد أظهرت النتائج أن مستخلص الأيسوفلافون وأكليل الجبل بتركيزات ٠,٠٣% و٠,٠٦% على التوالي كان له أثر واضح في الحد من أكسدة البرجر وبالتالي زيادة مدة الحفظ، كما كان لها تأثير واضح الحد من النشاط الميكروبي لكل من العدد الكلي للبكتريا ومجموعة الكوليفورم، كما أظهرت النتائج أن مستخلص الأيسوفلافون كان الأفضل من حيث الخواص الحسية مثل قيم العائد من الطبخ والانكماش.

الكلمات الاسترشادية: جودة برجر اللحم، الخصائص الميكروبية لبرجر اللحم، مستخلص الأيزوفلانيور و BHA والحصالبان.