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Antioxidant and Antimicrobial Properties of Peptides as Bioactive Components in Beef Burger

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***Abstract*—**Dried soy protein hydrolysate powder was added to the burger in order to enhance the oxidative stability as well as decreases the microbial spoilage. The soybean bioactive compounds (soy protein hydrolysate) as antioxidant and antimicrobial were added at level of 1, 2 and 3 %.Chemical analysis and physical properties were affected by protein hydrolysate addition. The TBA values were significantly affected ($P < 0.05$) by the storage period and the level of soy protein hydrolysate. All the tested soybean protein hydrolysate additives showed strong antioxidant properties. Samples of soybean protein hydrolysate showed the lowest ($P < 0.05$) TBA values at each time of storage.

The counts of all determined microbiological indicators were significantly ($P < 0.05$) affected by the addition of the soybean protein hydrolysate. Decreasing trends of different extent were also observed in samples of the treatments for total viable counts, Coliform, *Staphylococcus aureus*, yeast and molds. Storage period was being significantly ($P < 0.05$) affected on microbial counts in all samples *Staphylococcus aureus* were the most sensitive microbe followed by Coliform group of the sample containing protein hydrolysate, while molds and yeast count showed a decreasing trend but not significant ($P < 0.05$) until the end of the storage period compared with control sample. Sensory attributes were also performed, added protein hydrolysate exhibits beany flavor which was clear about samples of 3% protein hydrolysate.

***Keywords*—**Antioxidant, antimicrobial, isoflavones, bioactive peptide, antioxidant peptides, soybean protein hydrolysate.

I. Introduction

SOY proteins are widely used in meat products of the forms of soy flour, and soy protein concentrate or isolate to improve water and fat binding ability, enhance emulsion stability, improves nutritional content, and increase yields [1].

Soy protein isolates are very hydrophilic and thus can be incorporated into meat products to reduce cooking loss. In frankfurters and fish frankfurter analogs, incorporated soy protein hydrolysates reduced bacterial counts and extended their shelf-life stored at 25°C without influencing the flavor and texture properties of the products [2]. However, soy flour produced some beany flavor and soy protein concentrates and isolates provided some undesirable palatability in soy-added meat products [3]. To overcome these disadvantages, dried soy tofu powder was added in frankfurters and pork sausage patties. Incorporation of tofu powder resulted in lower fat and higher protein and moisture content, but did not affect sensory parameters in lean pork sausages. Lean frankfurters added with tofu powder had lower moisture content, but their texture and overall acceptability was better than control [4].

There is no doubt that the lipid oxidation and microbial contamination are the main problems of meat industry. These factors affected on the safety, shelf life and appeal to consumers and consequently, sales of the product. Lipids oxidation is responsible for reduction in nutritional quality as well as changes in flavor [5].

Oxidative processes are also associated with discolouration of meat products, as lipid oxidation results in the formation of pro-oxidants capable of reacting with oxymyoglobin, which lead to metmyoglobin formation [6]; so it caused to drip loss, off-odor and off-flavor development, and the production of potentially toxic compounds [7].

For cooked meat, thermal processes can promote lipid oxidation by disrupting cell membranes and releasing prooxidants, thereby inducing ‘‘warmed-over flavor’’ (WOF) during refrigerated storage and subsequent reheating [8]. Nowadays, it has been paying much attention to protein oxidation also. Proteins are damaged by the action of free radicals resulting in loss of their functions. From the nutritional point of view, meat is an ideal source of proteins that can barely be substituted for other protein sources, especially in the infant age. In addition to their nutritional properties, functional dipeptides were described in meat [9]. Both carnosine (b-alanyl-L-histidine) and anserine (N-b-alanyl-1-methyl-L-histidine) are antioxidative histidyl dipeptides and the most abundant antioxidants in meats. The concentrations of carnosine in meat ranges of 500 mg/kg of chicken thighs to 2700 mg/kg of pork shoulder. On the other hand, anserine is especially abundant in chicken muscle. Their antioxidant activities may result from their ability to chelate transition metals such as copper and iron [10]. Free radicals generated during lipid oxidation, in addition to the presence of transition metals, promote the accumulation of oxidized proteins and it is probable that meat active peptides could loss their functionality due to this process. As show by Insani et al. [11] dietary differences between cattle had minor influence on protein oxidation in fresh meat. Oxidation has to be enhanced by ageing or storage of meat (PM) under commercial conditions. This study aim are (1) To make soybean protein isolates hydrolysates to improve its bioactive compounds, isoflavones and peptides; (2) To incorporate soybean protein hydrolysates to the burger in different concentration in order

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to inhibit spoilage, lipid oxidation and microorganisms growth.

II. Materials and Methods

A. Materials

Plant materials: soybean (*Glycine max*) was obtained from the Agriculture Research Center, Giza, Egypt during season 2007. The source of applied enzymes was crude Papain obtained from Technolab, Chemical-Scientific Equipments. All other reagents were of highest analytical grade available.

B. Preparation of Soy Protein Isolates (SPI)

Soybean was grounding then defatted with hexane using Sohxlet apparatus. The method described by [12] for the preparation of SPI was adapted.

C. Enzymatic Hydrolysis (Soy Protein Isolates Hydrolysate SPIH)

The enzymatic hydrolysis of SPI was carried out according to [13]. One hundred mg SPI was dissolved in 33 ml of distilled water. Protease (30 mg) was added to the protein solution after the pH was properly adjusted. Enzymatic hydrolysis with Papain was performed at pH 8.0, and 38°C. After digestion, hydrolysates were heated in boiling water for 3 mins to inactivate proteases, neutralized and the centrifuged (4000Xg for 20 min). The supernatants were stored at –18°C until use.

D. Burger Manufacture

Independent replicates of burger formula were processed on the same day containing additives as follows, in experimental formula 1, 2, and 3 contain soy protein hydrolyzate in percentage of 1, 2 and 3%, respectively; formula 4 contains ascorbic acid as positive control of antioxidant; formula 5 contains no additive as negative control. All products were prepared for one of meat processing factories according to its commercial processing formula.

The meat was ground through a 5-mm plate (Olotinox, Olox, Spain) in a mincer attached to a mixer (CATO 114, Sabadell, Spain). Afterwards water, additives and spices were added into the bowl and mixed with the spiral dough hook at medium speed (80 rpm) during 5 mins. This mixture was shaped using a commercial burger maker (9 cm internal diam) to obtain the burger of approximately 50 g. Plastic packaging was used to help maintaining the shape of the burger prior to storage [14].

E. Microbiological Analysis

To determine the microbial counts of pathogenic flora analysis of the, total viable bacterial count including *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, Coliform bacteria, and yeast as well as fungal count were carried out as follow: ten g burger or minced meat samples were aseptically taken and 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 total aerobic plate count (nutrient agar), coagulase positive *staphylococci*, coliforms, sulphite-reducing anaerobic bacterial counts, *Salmonella*, *B. cereus* and moulds/yeasts. For the isolation from coagulase positive *Staphylococci*, up to five typical colonies (black or grey colonies) grown on BP agar were selected and, transferred to tubes contained Brain Heart Infusion Broth. The tubes were incubated at 37°C for 24 hs. or 25°C for 3 days for the mold and yeast. After the incubation, coagulase tests were done according to the method described by [15].

F. Physicochemical Analysis

1. Proximate Analysis of Meat

Moisture, ash, protein and fat content were determined by the AOAC methods [16]. All parameters were tested for triplicate.

2. Determination of pH

The measurement of pH was carried out on 10 g of the sample homogenized in distilled water (1/10 sample/water) using a pH meter according to [17].

3. Total Volatile Basic Nitrogen (TVBN)

A sample (10 g) was minced with 100 ml distilled water and washed into a distillation flask with 100 ml distilled water; then 2 g of magnesium oxide and an antifoaming agent were added. The mixture was distilled using the micro Kjeldahl distillation apparatus. Distillate was collected for 25 min into 25 ml 4% boric acid and five drops of Tashero indicator. The solution was titrated using (0.1 M) HCl to calculate the total volatile basic nitrogen in the sample of terms of mg VBN/100g sample [18].

4. Determination of TBARS (2-Thiobarbituric Acid Reactive Substances)

TBARS of samples was determined by the spectrophotometric method [19]. Two gram of homogenized samples were taken and TBARS were extracted twice with 10 ml of 0.4 M perchloric acid. Extracts were collected and made up to 25 ml with 0.4 M perchloric acid then centrifuged for 5 mins at 1790 Xg. After centrifugation, 1 ml of supernatant was pipetted into glass stoppered test tubes. TBA reagent (5 ml) was added and the mixture was heated in a boiling water bath for 35 min. After cooling the absorbance of the sample was read against the appropriate blank at 538 nm. A standard curve was prepared using 1, 1, 3, 3-tetraethoxypropane (TEP).

5. Color Determination

Color was evaluated using a colorimeter (Mod. CR-200, Minolta Camera Co., Osaka, Japan) with illuminant D65, 2° observer, Diffuse/O mode, 8 mm aperture of the instrument for illumination and 8 mm for measurement. The colorimeter was standardized with a white tile (L*D98, *a**D0.23 and b*D1.89). Color was described by coordinates lightness (L*), redness (a*, red green) and yellowness (b*, yellow-blue). Nine replicate measurements were taken for each sample, following the guidelines on color measurements of the American Meat

Science Association [20].

6. Texture Profile (Penetrometer Values)

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 mm in triplicate for each peace for 30s. A lower depth of penetration indicates a harder texture [21].

7. Cooking Properties

Burger: Samples was grilled in microwaves for about 5 mins and cooking properties were made by the method described by [22].

$$\% \text{ Cooking yield} = \frac{\text{Cooking weight}}{\text{Raw weight}} \times 100$$

$$\% \text{ Cooking loss} = \frac{\text{Raw weight} - \text{Cooking weight}}{\text{Raw weight}} \times 100$$

$$\% \text{ Shrinkage} = \frac{\text{Raw diameter} - \text{Cooking diameter}}{\text{Raw diameter}} \times 100$$

G. Sensory Analysis

A panel of 10 assessors was selected to evaluate the product. The sensory ballots prompted panelists to order a series of 10 randomly placed samples of increasing order (least to most) for the following attributes: Colour = 8, tenderness = 8, taste = 8, residual taste = 5, texture = 8 and overall acceptance = 10. Results were decodified and rank sums were calculated and analyzed by SAS [22].

H. Statistical Analysis

Data was subjected to statistical analysis using the General Linear Models Procedure of the Statistical Analysis System [23]. The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio [24]. All statements of significance were based on probability of $P \leq 0.05$. The correlation calculation was carried out using ToolPack to determine whether two range from data moves together.

III. Results and Discussion

Whey and soy proteins are common ingredients added to processed meats to enhance the products functional characteristics, e.g. to reduce cooking weight loss and to improve sliceability. However, soy flour produced some beany flavor and soy protein concentrates and isolates provided some undesirable palatability in soy-added meat products [3]. To overcome these disadvantages, dried protein hydrolysate powder was added to the burger and minced meat in order to enhance the oxidative stability and decrease the microbial spoilage by soybean bioactive compounds (antioxidant and antimicrobial) additives at level of 1, 2 and 3%.

Reports on the effect of frozen storage on properties of meat products are contradictory. The role of freezing and frozen storage in causing chemical and structural changes in product, essentially the result is a reduction in functionality which negatively affects the quality (texture, water and fat binding properties, sensory characteristics and others) of the final processed products. As such protein damage increased, water holding capacity was reduced, favoring increased cooking loss; this adversely affected shear strength of burger, which increased with storage time [25].

A. Proximate Composition

In comparison with control, addition of the soybean significantly affected the proximate composition of restructured the burger. The soybean increased significantly ($P < 0.05$) moisture and protein content and reduced ($P < 0.05$) fat values (Table I). Ash content was increased significantly to increasing the soybean protein hydrolysates concentration. The proximate composition of samples was consistent with meat product formulations of protein hydrolysate different concentration (Table I). Ho et al. [4] found that incorporation of tofu powder resulted in lower fat and higher protein and moisture content, but did not affect sensory parameters in lean pork sausages.

TABLE I
Proximate Composition of Burger Fortified with Soybean Protein Hydrolysate Additives

Treatment	Moisture %	Protein %	Fat %	Ash %
1% H	58.86±0.02	13.73±0.02	21.91±0.03	3.13±0.01
2% H	59.83±0.07	14.19±0.03	21.05±0.04	3.29±0.06
3% H	60.26±0.01	14.59±0.06	19.16±0.03	3.40±0.04
Control	58.57±0.09	13.56±0.01	23.11±0.05	2.85±0.06
Ascorbic	58.53±0.15	13.54±0.01	23.15±0.04	2.86±0.03

H: Protein hydrolysate
All values are mean of triplicate determinations ± standard deviation (SD)

B. Physical Properties

1. Color

Consumers use color as an indicator of beef freshness, and decide not to purchase when metmioglobin reaches 30–40% of total pigments on the surface of fresh beef. The natural nutritional antioxidant has been reported to prolong color stability of beef. This color stabilizing effect is indirectly due to delayed oxidation of metmioglobin via direct inhibition of lipid oxidation. Tea catchin and vitamin E have greater antioxidant activity in meat, they react readily with iron in meat to produce brown discoloration, in particular, under alkaline condition [26].

Hunter color parameters of different burger additives are shown in Table II. For all samples, lightness and yellowness were higher ($P < 0.05$) of the added soybean protein hydrolysate concentrations, while redness was lower ($P < 0.05$) compared to control samples. This increase may be due to incorporation of yellow pigments present in the soybean, which occur to higher amounts of the concentrated than low concentration of the soybean. All color coordinates to show differences ($P < 0.05$) among treatments and storage days, except yellowness which only showed differences between treatments (Table II). In all samples, lightness increased to storage time ($P < 0.05$), and the highest values of L* were obtained in control samples of the end of storage periods.

Some authors reported that this increase could be related to the increase in metmioglobin formation. These result suggested that the presence of antioxidant compounds in the natural extracts could retard metmioglobin formation of meat and so L* values decreased.

TABLE II
CHANGES IN INSTRUMENTAL COLOR AND TEXTURE QUALITY OF BEEF BURGER WITH DIFFERENT SOYBEAN PROTEIN HYDROLYSATE ADDITIONS DURING FROZEN STORAGE

Treatment	Color			Texture
Zero	L*	a*	b*	Penetration (mm)
1% H	60.02	6.39	20.96	18.44±2.11
2% H	59.57	6.34	21.16	18.28±1.71
3% H	59.79	6.52	21.68	20.11±1.68
Control	59.89	7.17	21.43	22.50±2.33
Ascorbic	57.89	8	20.24	14.50±0.92
First				
1% H	58.05	9.09	22.06	15.41±2.08
2% H	59.99	8.15	22.32	15.24±1.77
3% H	59.91	8.01	22.91	17.08±1.61
Control	59.72	10.51	22.26	19.13±2.53
Ascorbic	60.79	10.68	22.4	11.47±0.95
2 months				
1% H	59.33	6.05	20.66	11.75±0.89
2% H	61.55	5.31	21	13.75±2.70
3% H	58.86	6.58	21.69	18.70±1.56
Control	61.43	6.22	21.05	14.29±1.39
Ascorbic	59.11	6.9	19.74	13.29±1.65
3 months				
1% H	59.66	5.69	20.21	6.50±1.62
2% H	58.84	5.72	20.52	9.10±0.93
3% H	58.9	5.95	21.14	10.63±1.21
Control	60.77	5.79	19.53	14.75±1.44
Ascorbic	58.39	6.54	19.98	8.20±0.68

H: Protein hydrolysate
a*= redness, b*=yellowness, L*=lightness
(n=10) Nine replicate measurements were taken for each sample

For this reason, it could be expected that treatments of soybean protein hydrolysate would have lowest lightness values because they had the highest antioxidant capacity (Table II), but at zero time, treatments with soybean protein hydrolysate showed the highest L* values. This could be explained by the increased water retention and because these extracts were prepared for a dry powder they needed many water additives to formula. This relation between water content and lightness in meat and meat products has been reported by [27].

In all samples yellowness values were modified ($P < 0.05$) by storage time. Therefore, the differences in b* values observed between storage periods and treatments incorporating soybean protein hydrolysate, which can be attributed to the presence of pigments in the beans and not to the oxidation processes. In all samples redness decreased to the storage time progressed ($P < 0.05$) but red color (a* values) of the control sample faded very rapidly.

This is not surprising as meat which has been stored longer would be expected to have predominantly either oximyglobin or metmyglobin, as opposed to deoxymyoglobin (DMb), which in turn would predispose the meat to a faster browning rate. At the end of storage (month 3), a* values of the control samples were lower ($P < 0.05$) than zero time, due to the antioxidant treatments. Several authors have studied the effect of different antioxidants on the color of meat and meat products and have reported that meat oxidation decreases a* values (redness). Therefore, samples of the soybean bioactive compound additives, which were the treatments of high antioxidant activity (Table II), would have the highest a* values at the end of storage period. Several authors have related the evolution of redness with lipid oxidation in meat products, while others reported that the development of lipid oxidation provokes a decrease in redness [14].

2. Texture (Penetration)

Factors responsible for textural properties in comminuted meat proteins are degree of extraction of myofibrillar proteins, stromal protein content, degree of comminuting and type and level of non-meat additives. Desired textural attributes of the burger are affected by many reasons including vegetable oil additives. Some additives like soy protein can resolve this problem [28].

Penetration values are seen in Table II and indicated that burger with 3% protein hydrolysate had the highest (the softest in the texture) penetration values comparing to other soybean additives. Binders or extender may be used with or without addition of water or with added water, which was reported to cause a softening effect on the texture [28].

In our study water were added to the burger formulation. The soybean with high protein content showed minimal performance and functionality and the resulting burger with soybean protein were tougher than the control burger formula. The binding water of hydrolyzed protein may cause a softer texture thus leading to an increase in penetrometer values because of the increased moisture content.

C. Chemical Analysis

1. Lipid Oxidation (TBA)

A number of food protein hydrolysates, specific peptides and phenolics have been shown to effectively inhibit lipid peroxidation in different food products [29], suggesting that phenolics and specific food-derived peptides can be utilized as natural antioxidants adding into food products to improve quality and stability.

In this study, burger was prepared in order to determine whether the soy protein hydrolysate with strong antioxidant properties (as determined by DPPH) can effectively inhibit meat lipid peroxidation.

The TBA test has been widely used to measure lipid oxidation in meat and meat products. Our results clearly showed that the meat lipid peroxidation was gradually preceded against the storage time for reflected by the dramatic increase in TBARS values (Fig. 1). The analysis of variance in the TBARS data indicated that the TBA values were significantly affected ($P < 0.05$) by both the storage period and the soybean protein hydrolysate. Initial (zero time) were TBA values for all samples significantly lower than those for the

control ($P < 0.05$). Results showed that increasing soybean protein hydrolysate levels resulting in decreased TBA values, which emphasis the lipid peroxidation suppression of soybean additives. These results agree with that reported by [27] for other natural antioxidants applied to meatballs.

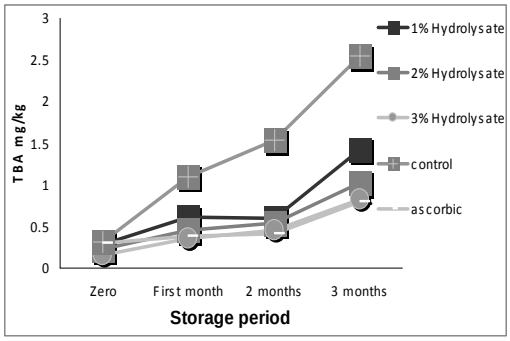

Fig. 1 Change in TBA of the burger with soybean protein hydrolysate additives during frozen storage periods.
All values are mean of triplicate determinations ± standard deviation (SD). Mean within row of different letters are significantly different ($P < 0.05$)

At the end of storage time (month three), all treatments showed significant lower ($P < 0.05$) TBA values when compared to the control, which indicates that all the tested soybean bioactive compounds show strong antioxidant properties (Fig. 1). The product samples with soybean protein hydrolysate showed the lowest ($P < 0.05$) TBA values at each time of storage (0.15 mg/kg in 1% hydrolysate compared to the control which was 0.317 mg/kg).

Consistently, this difference in antioxidant properties of soybean protein hydrolysate has also been detected by using the DPPH, as explained before. These results provide clear evidence that soy protein hydrolysates can effectively inhibit food lipid peroxidation. In this concern, Mc Carthy et al. [30], suggested that increasing of lipid oxidation during frozen storage may be due to the fact that freeze slows down oxidation while it does not inhibit it and that lipid-free radicals are more stable at low temperatures which allow them to diffuse to greater distances, thereby, increasing the reaction. Recently, Zhang and Zhou [29] studied the effect of soybean protein hydrolysate on lipid oxidation of fat-rich food and suggested that the commercial microbial proteases such as *B. subtilis* and *B. licheniformis* could be used to produce effective antioxidant hydrolysates from food proteins. Furthermore, the antioxidant peptides may exert strong synergistic effects on some other antioxidants, such as phenolic compounds. During hydrolysis, the soy protein structure is altered and more active amino acid R groups are exposed. Therefore, soybean peptides can exhibit higher antioxidant activity than intact protein [31].

2. pH Determination

The results of the current study showed that soybean additives caused slight and not significant ($P < 0.05$) increases in pH values of samples (Fig. 2). Frozen storage affected significantly ($P < 0.05$) pH of samples, and therefore simply shows the pH value over the storage period. Serrano et al. [25] studied the pH of restructured beef steak or ground pork and found slight increased with frozen storage.

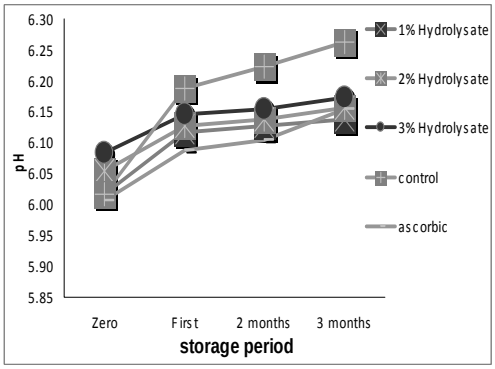

Fig. 2 Change in pH values of the burger with soybean protein hydrolysate additives during frozen storage periods.
All values are mean of triplicate determinations ± standard deviation (SD). Mean within row of different letters are significantly different ($P < 0.05$)

These results are agreement with [14] that found a significant difference ($P < 0.05$) between the pH value obtained from pork meat formula and this was explained on the base of the special characteristics of ostrich meat, which has an ultimate pH of 6.0. The presence of 30% beef and pork meat in the other formulation decreased the pH of products.

3. Total Volatile Basic Nitrogen (TVBN)

The TVBN analyses of the burger with soybean protein hydrolysate are shown in Fig. 3. Data showed that addition of soybean protein hydrolysate was significantly varied from samples of different concentrations. Soybean additives increased the total volatile basic nitrogen at significant level ($P < 0.05$) as compared to the control sample specially protein hydrolysate which contained high amounts of NH^4.

After storage periods, the total volatile basic nitrogen of soybean protein hydrolysate additives were lower than control samples, but was not significantly different (Fig 3). Similar to these observations; Al-Bachir and Mehiob [18] found that same effect with irradiated buffalo meat.

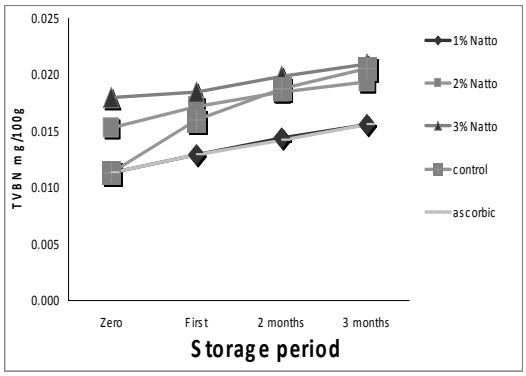

Fig. 3 Change in total volatile basic nitrogen (TVBN) of the burger with soybean protein hydrolysate additives during frozen storage periods. All values are mean of triplicate determinations ± standard deviation (SD). Mean within row of different letters are significantly different ($P < 0.05$)

D. Cooking Properties

Meat products usually used soy proteins to enhance the products functional characteristics, reducing cooking loss and improving sliceability. The current results show that the soybean protein hydrolysate additive treatments significantly ($P < 0.05$) reduced cooking loss (Fig. 4). Hydrolyzed soybean protein was particularly effective and reduced significantly the cooking loss of control samples from 19.64% to 12.19% in protein hydrolysate (Fig. 4). This reduction may be due to protein denaturation.

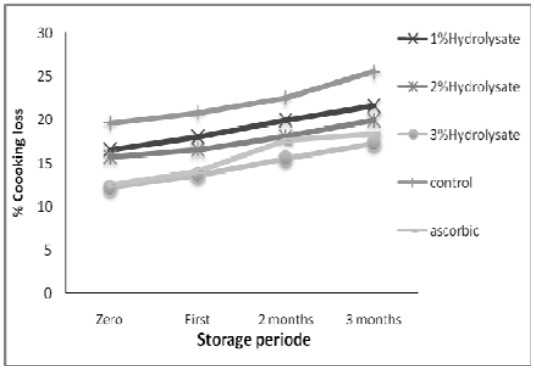

Fig. 4 Effect of frozen storage periods and soybean protein hydrolysate additives on the cooking loss of burger.
All values are mean of triplicate determinations ± standard deviation (SD). Mean within row of different letters are significantly different ($P < 0.05$)

The increments represented additional improvements in cooking to yield of the burger with 3% protein hydrolysate was 87.81% (Fig. 5). Froze storage affected in cooking yield significantly ($P < 0.05$), whereas it was decreased to increasing storage periods (Fig. 5). The higher cooking yield of protein hydrolysate 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 [32].

In this regard, Aleson et al. [22] reported that lemon albedo improved cooking performance due to albedo addition which appeared to be related with their fat and water holding capacity.

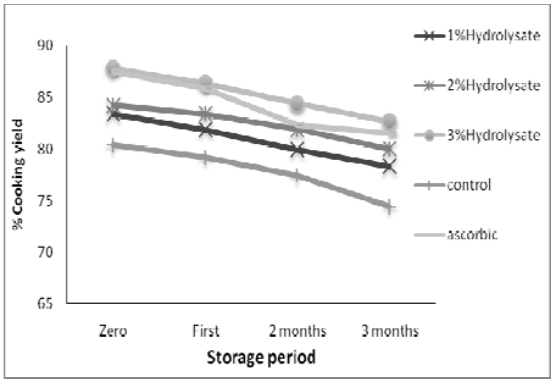

Fig. 5 Effect of frozen storage periods and soybean protein hydrolysate additives on the cooking yield of burger
All values are mean of triplicate determinations ± standard deviation (SD).Mean within row of different letters are significantly different ($P < 0.05$)

Surface shrinkage is important to maintaining quality standards of the burger. The surface of all samples decreased after cooking, from 20.87% to 14.59% (Fig. 6). There was significantly less surface shrinkage of the burger ($P < 0.05$) as the soybean protein hydrolysate additives content was increased (Fig. 6). Frozen storage affected significantly ($P < 0.05$) the burger samples. However, Serrano et al. [25] found that froze storage did not affect cooking loss for each sample and changes of cooking loss in restructured steak may be responsible for the differences in the dimensions of cooking changes.

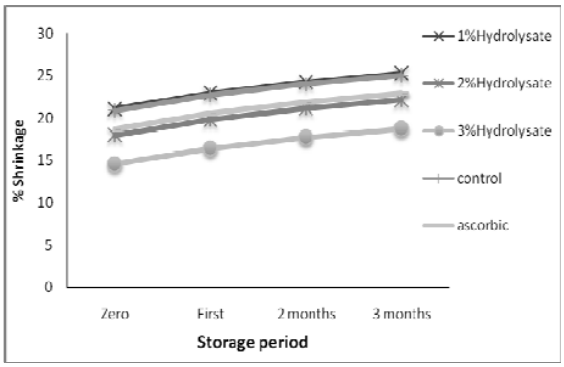

Fig. 6 Effect of frozen storage periods and soybean protein hydrolysate additives on the shrinkage of burger
All values are mean of triplicate determinations ± standard deviation (SD). Mean within row of different letters are significantly different ($P < 0.05$).

E. Microbiological Profile

Vallejo-Cordoba et al. [4] found that in frankfurters and fish frankfurter analogs, incorporated soy protein hydrolysates reduced bacterial counts and extended their shelf-life stored at

-18°C without influencing the flavor and texture properties of the products. The results presented in Tables III-VI show the microbiological analyses of the burger during the storage period. The data showed that the counts of all determined microbiological indicators were significantly ($P < 0.05$) affected by the addition of the protein hydrolysate (Table III). The same trend was also detected in burger samples which all the microbial groups (Coliform, *Staphylococcus aureus* and yeasts and moulds) decreased to additive protein hydrolysates as compared to the control sample. This antimicrobial effect of soybean protein hydrolysates additives could be due to the presence of phenolic compounds, and although the synergistic effect of phenolic and bioactive peptides could be participated in antimicrobial activities. The possible mechanisms for antimicrobial effect of phenolic compounds include: altering microbial cell permeability interfering with membrane function including electron transport, nutrient uptake, protein and nucleic acid synthesis, and enzyme activity [33] which interact with membrane proteins causing deformation in structure and functionality; and substituting alkyls into phenol nucleus [34].

TABLE III
EFFECT OF FROZEN STORAGE ON TOTAL COUNT MICROORGANISM (CFU/GM) OF BURGER WITH SOYBEAN PROTEIN HYDROLYSATE ADDITIVES

Treatment	Zero	First	2 months	3 months
1% H	$5.6 X 10^5$	$1.3X10^5$	$5.0X10^4$	$1.5X10^4$
2% H	$4.2 X 10^5$	$0.2X10^5$	$4.4X10^4$	$1.2X10^4$
3% H	$3.7 X 10^5$	$9.3X10^4$	$3.0X10^4$	$0.4X10^4$
Control	$7.2 X 10^5$	$5.0X10^5$	$7.1X10^4$	$2.6X10^4$
Ascorbic	$6.8 X 10^5$	$3.9X10^5$	$6.0X10^4$	$1.3X10^4$

H: Protein hydrolysate

Coliform came in the second order and it reduced from 1 x 10^3 to 8 x 10 cfu/gm compared to control the sample which was 2.4 x 10^3 cfu/gm, while molds and yeast counts were decreased significantly ($P < 0.05$) until the end of the storage period (Table IV).

Storage period was significantly ($P < 0.05$) affected the microbial count in all samples. *Staphylococcus aureus* had the most decreased microbes count (from 2x 10^2 to 1 x 10 cfu/gm) in the samples containing protein hydrolysates compared to the control sample which from 6 x 10^2 at zero time and the decreased to reach 4 x 10 cfu/gm at the end of storage (Table V), while mold and yeast counts show decreased trend significantly ($P < 0.05$) until the end of the storage period (Table VI). It is known that froze storage affected microorganisms, whereas it is destroys the bacterial cells as a result of ice crystals formation.

TABLE IV
EFFECT OF FROZEN STORAGE ON COLIFORM GROUP (CFU/GM) OF THE BURGER WITH SOYBEAN PROTEIN HYDROLYSATE ADDITIVES

Treatment	Zero	First	2 months	3 months
1% H	$1.4X10^3$	$8.0X10^2$	$6.0X10^2$	$1.1X10^2$
2% H	$1.2X10^3$	$6.8X10^2$	$2.2X10^2$	8.0X10
3% H	$1.0X10^3$	$4.0X10^2$	$0.2X10^2$	5.0X10
Control	$2.4X10^3$	$1.8X10^3$	$9.0X10^2$	$5.0X10^2$
Ascorbic	$1.2X10^3$	$0.6X10^3$	$8.0X10^2$	$4.0X10^2$

H: Protein hydrolysate

TABLE V
EFFECT OF FROZEN STORAGE ON *STAPHYLOCOCCUS AUREUS* (CFU/GM) OF THE BURGER WITH SOYBEAN PROTEIN HYDROLYSATE ADDITIVES

Treatment	Zero	First	2 months	3 months
1% H	$5.2 X10^2$	$1.9X10^2$	6.8X10	3.0X10
2% H	$3.2X10^2$	$0.3X10^2$	6.0X10	3.0X10
3% H	$2.0X10^2$	$8.0X10^2$	3.6X10	1.0X10
Control	$6.0X10^2$	$3.6X10^2$	8.4X10	4.0X10
Ascorbic	$4.9 X10^2$	$2.0X10^2$	7.0X10	3.0X10

H: Protein hydrolysate

TABLE VI
EFFECT OF FROZEN STORAGE ON MOLD AND YEAST (CFU/GM) OF THE BURGER WITH SOYBEAN PROTEIN HYDROLYSATE ADDITIVES

Treatment	Zero	First	2 months	3 months
1% H	$4.0X10^3$	$1.1X10^3$	$8.0X10^2$	$4.0X10^2$
2% H	$3.0X10^3$	$0.4X10^3$	$6.6X10^2$	$3.0X10^2$
3% H	$2.0X10^3$	$8.9X10^2$	$5.0X10^2$	$2..0X10^2$
Control	$4.0X10^3$	$1.9X10^3$	$7.0X10^2$	$4.0X10^2$
Ascorbic	$4.0X10^3$	$2.0X10^3$	$7.8X10^2$	$4.0X10^2$

H: Protein hydrolysate

F. Sensory Evaluation

Sensory evaluation scores of burger samples treated with soybean protein hydrolysate at zero time are presented in Table VII. Color is among the most important attributes influencing customer choice, and texture also plays a relevant role on the perception of quality of meat products. Color shows no differences ($P < 0.05$) between treatments, which could be related to lightness results obtained by instrumental analysis (Table VIII). Texture showed slight differences ($P < 0.05$) among treatments (Table IX).

TABLE VII
PANEL TEST OF BURGER AS AFFECTED BY SOYBEAN PROTEIN HYDROLYSATE ADDITIVES AT ZERO TIME

Treatment	Color	Texture	Taste	Tenderness	Residual taste	Overall acceptability
1% H	$6.60^{a}\pm0.34$	$6.20^{b}\pm0.25$	$6.90^{b}\pm0.23$	$6.70^{c}\pm0.26$	$4.33^{a}\pm0.29$	$7.80^{bc}\pm0.25$
2% H	$6.70^{a}\pm0.26$	$6.30^{b}\pm0.30$	$6.50^{c}\pm0.17$	$7.20^{b}\pm0.20$	$3.44^{b}\pm0.18$	$7.70^{bc}\pm0.33$
3% H	$6.40^{a}\pm0.34$	$6.80^{b}\pm0.29$	$5.50^{d}\pm0.22$	$7.60^{a}\pm0.16$	$2.67^{c}\pm0.33$	$6.50^{d}\pm0.27$
Control	$6.70^{a}\pm0.21$	$6.40^{b}\pm0.27$	$6.50^{c}\pm0.17$	$6.00^{d}\pm0.26$	$3.89^{ab}\pm0.35$	$6.50^{d}\pm0.17$
Ascorbic	$6.60^{a}\pm0.27$	$6.60^{b}\pm0.27$	$6.50^{c}\pm0.17$	$6.10^{d}\pm0.23$	$4.00^{ab}\pm0.33$	$7.10^{cd}\pm0.23$

H: Protein hydrolysate
Means ± SD within the same column with the same letter are not significantly different ($p < 0.05$), using 10 panelists

TABLE VIII
EFFECT OF FROZEN STORAGE AND SOYBEAN PROTEIN HYDROLYSATE ADDITIVES ON THE COLOR OF BURGE

Treatment	Zero	First	2 months	3 months
1% H	6.60^{Aa}±0.34	6.40^{Aa}±0.31	6.10^{Aa}±0.31	5.90^{ABa}±0.28
2% H	6.70^{Aa}±0.26	6.40^{Aab}±0.22	6.20^{Ab}±0.20	6.00^{ABb}±0.21
3% H	6.40^{Aa}±0.34	5.90^{Aa}±0.38	5.50^{Ab}±0.31	5.30^{Bb}±0.30
Control	6.70^{Aa}±0.21	6.50^{Aab}±0.27	6.20^{Ab}±0.25	6.00^{ABb}±0.21
Ascorbic	6.60^{Aa}±0.27	6.50^{Aa}±0.27	6.20^{Aa}±0.25	6.00^{ABa}±0.21

H: Protein hydrolysate
Means ± SD within the same column with the same capital letter are not significant; data within the same row with the same small letter are not significantly differ ($p < 0.05$), using 10 panelists.

TABLE IX
EFFECT OF FROZEN STORAGE AND SOYBEAN PROTEIN HYDROLYSATE ADDITIVES ON THE TEXTURE OF BURGER

Treatment	Zero	First	2 months	3 months
1% H	6.20^{Ba}±0.25	5.70^{Cab}±0.21	5.40^{BCb}±0.22	5.00^{Bb}±0.26
2% H	6.30^{Ba}±0.30	5.80^{BCab}±0.25	5.50^{BCab}±0.27	5.10^{Bb}±0.35
3% H	6.80^{ABa}±0.29	6.30^{BCab}±0.26	6.00^{ABab}±0.26	5.60^{ABb}±0.34
Control	6.40^{Ba}±0.27	5.90^{BCab}±0.28	5.60^{BCab}±0.27	5.20^{Bb}±0.29
Ascorbic	6.60^{Ba}±0.27	6.10^{BCab}±0.28	5.80^{BCab}±0.29	5.40^{Bb}±0.31

H: Protein hydrolysate
Means ± SD within the same column with the same capital letter are not significant; data within the same row with the same small letter are not significantly different ($p < 0.05$), using 10 panelists

About the attributes used for taste evaluation, there was a significant difference between treatments ($P < 0.05$), 2%, 3% protein hydrolysate that had the lowest score of taste than control sample, this may be due to a bitter taste of hydroloysate as a result of free amino acids existence (Table X).

On the other hand, increasing protein hydrolysate level result of decreasing overall acceptability ($P < 0.05$). No difference between control and 3% hydroloysate could be traced (Table XIII).

TABLE X
EFFECT OF FROZEN STORAGE AND SOYBEAN PROTEIN HYDROLYSATE ADDITIVES ON THE TASTE OF BURGER

Treatment	Zero	First	2 months	3 months
1% H	6.90^{BCa}±0.23	6.60^{BCab}±0.16	6.30^{BCab}±0.26	5.90^{B}b±0.31
2% H	6.50^{Ca}±0.17	6.20^{Cab}±0.13	5.90^{BCDbc}±0.18	5.50^{BCc}±0.27
3% H	5.50^{Da}±0.22	5.20^{Dab}±0.13	4.90^{Ebc}±0.18	4.40^{BC}±0.22
Control	6.50^{Ca}±0.17	6.20^{Ca}±0.20	5.50^{DEb}±0.17	4.70^{DEc}±0.15
Ascorbic	6.50^{Ca}±0.17	6.20^{Cab}±0.20	5.80^{CDb}±0.13	5.20^{CDc}±0.20

H: Protein hydrolysate
Means ± SD within the same column with the same capital letter are not significant; data within the same row with the same small letter are not significantly different ($p < 0.05$), using 10 panelists

These results were agreed with those reported by [14]. Storage affected significantly the entire sensory attribute (Tables VII, IX-XIII), however, the deterioration in samples of protein hydrolysate was less than that happened to the control sample.

TABLE XI
EFFECT OF FROZEN STORAGE AND SOYBEAN PROTEIN HYDROLYSATE ADDITIVES ON THE TENDERNESS OF BURGER

Treatment	Zero	First	2 months	3 months
1% H	6.70^{BCa}±0.26	6.40^{BCab}±0.16	6.00^{BCab}±0.26	5.70^{BCb}±0.26
2% H	7.20^{ABa}±0.20	6.70^{ABab}±0.15	6.50^{ABb}±0.22	6.20^{ABb}±0.20
3% H	7.60^{Aa}±0.16	7.10^{Ab}±0.10	6.90^{Abc}±0.18	6.60^{Ac}±0.16
Control	6.00^{Da}±0.23	5.50^{Eab}±0.12	5.30^{CDb}±0.21	5.00^{Db}±0.26
Ascorbic	6.10^{CDa}±0.33	5.60^{DEab}±0.11	5.40^{CDb}±0.22	5.10^{CDb}±0.23

H: Protein hydrolysate
Means ± SD within the same column with the same capital letter are not significant; data within the same row with the same small letter are not significantly different ($p < 0.05$), using 10 panelists

TABLE XII
EFFECT OF FROZEN STORAGE AND SOYBEAN PROTEIN HYDROLYSATE ADDITIVES ON THE RESIDUAL TASTE OF BURGER

Treatment	Zero	First	2 months	3 months
1% H	4.33^{Aa}±0.29	4.00^{Aab}±0.33	3.67^{ABab}±0.24	3.44^{ABb}±0.18
2% H	3.44^{Ba}±0.18	3.00^{BCab}±0.29	2.67^{BCb}±0.24	2.44^{Cb}±0.18
3% H	2.67^{Ca}±0.33	2.33^{Ca}±0.41	2.22^{CDa}±0.32	2.11^{Ca}±0.31
Control	3.89^{ABa}±0.35	3.56^{ABab}±0.34	3.22^{ABab}±0.28	3.00^{Bb}±0.17
Ascorbic	4.00^{ABa}±0.33	3.56^{ABab}±0.34	3.22^{ABb}±0.28	3.00^{Bb}±0.17

H: Protein hydrolysate
Means ± SD within the same column with the same capital letter are not significant; data within the same row with the same small letter are not significantly different ($p < 0.05$ using 10 panelists

TABLE XIII
EFFECT OF FROZEN STORAGE AND SOYBEAN PROTEIN HYDROLYSATE ADDITIVES ON THE OVERALL ACCEPTABILITY OF BURGER

Treatment	Zero	First	2 months	3 months
1% H	7.80^{BCa}±0.25	7.30^{Ba}±0.26	7.00^{BCa}±0.30	6.40^{BCa}±0.40
2% H	7.70^{BCa}±0.33	7.20^{Ba}±0.36	6.90^{Ba}±0.31	6.30^{BCa}±0.40
3% H	6.50^{Da}±0.27	5.90^{Dab}±0.23	5.60^{Db}±0.22	5.20^{Db}±0.20
Control	6.50^{Da}±0.17	6.30^{CDa}±0.21	6.00^{CDa}±0.30	5.60^{CDa}±0.27
Ascorbic	7.10^{CDa}±0.23	6.90^{BCa}±0.23	6.60^{BCDa}±0.31	6.20^{BCa}±0.33

H: Protein hydrolysate
Means ± SD within the same column with the same capital letter are not significant; data within the same row with the same small letter are not significantly different ($p<0.05$), using 10 panelists

IV. CONCLUSION

A dried soy protein hydrolysate powder was added to the burger in order to enhance the oxidative stability as well as decreased the microbial spoilage. The soybean bioactive compounds (soy protein hydrolysate) as antioxidant and antimicrobial were added at level of 1, 2 and 3%. These results suggest that these antioxidants retarded the lipid oxidation during storage burger. All the tested soybean bioactive compounds (soy protein hydrolysate) showed strong antioxidant and antimicrobial properties.

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