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Quality Characterization of Burger Affected by Soybean Additives (Natto & Protein Hydrolysate) and Ascorbic Acid

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***Abstract*—**Soy protein is a common ingredient added to processed meats to enhance its functional characteristics. In our study, soybean products (fermented soy Natto and protein hydrolysate) containing hydrolyzed peptides and amino acids, with or without ascorbic acid were added to burger in order to improve its quality characteristics. Results showed that soy additives significantly increased moisture and protein content and reduced ($P < 0.05$) fat values. Ash content did not affect with Natto additive. Color tools, lightness and yellowness were higher ($P<0.05$) for the samples with added soybean products (with or without ascorbic acid), while redness decreased. Both of protein hydrolysate and ascorbic acid increased the softiness while, Natto additive increased the hardness of samples. Natto & protein hydrolysate additives increased the total volatile basic nitrogen while, samples with ascorbic acid decreased TVBN values at significant levels. Also, soy additives were improved both of cooking quality and sensory evaluation of the burger in order to prove that soy products actually affect the quality characteristics of meat products.

***Keywords*—**Burger, protein hydrolysate, fermented soy Natto, quality characterization.

I. Introduction

DURING the past decade, a rapidly growing number of foods with a functionality claim have entered the market place. The importance of the link between nutrition and health becomes more and more a hot topic which reflects the importance of eating healthy foods. There are a variety of reasons for this increase, the most important being consumer demand for health foods, the needs of the food industry for value-added products, and the scientific and technological developments that allow for the production of new categories of foods with increased functionality. In addition to the endogenous defenses, consumption of dietary antioxidants could be an important aspect of body's defense mechanism to protect against free radicals, such as superoxide anion radical, the hydroxyl radical and other ROS, and also many antioxidants are being identified as anticarcinogens [1], so that the higher dietary intake of fruits and vegetables the lower risk of most types of cancer [2]. Meat products or processed meats which are the basic meal of young people and infant are the result of the need to preserve meat; it's the main component of fast food which is contributed to salt and fat intake in the diet. The epic study [3] suggests a relation between colon cancer and the consumption of processed meat products. For the latter nitrosamines as cancerogenic substances remain actually. Which mean that consumption of processed meat may pose a significant risk on our body.

Soybean, which is a good source of antioxidants, is used in human foods in many forms such as, infant formulas, flours, protein isolates and concentrates which are essential components in processed meat, and textured fibers. On the other hand there are fermented and un-fermented soybean foods, such like, tempeh, soya sauce, miso, and natto. The consumption of soybean increasing day after day, this is due to the health benefits of soybean, and its nutritional value, in addition to the improving functional values of products and considering a fat replacer [4]. Our study is to add fermented soybean Natto to burger for improving its quality characterizations.

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II. Material and Methods

A. Material

Soybean (Glycine max) was obtained from The Agriculture Research Center-Giza-Egypt. The source of applied enzymes was crud Papain obtained from Technolab, Chemical-Scientific Equipments. The strain of Bacillus natto (NBRC 13169) which was obtained from National Institute of Technology and Evaluation Biotechnology Center (NITE), Japan. All other reagents were of highest analytical grade available.

B. Methods of Analysis

1. Processing

Soy Protein isolates hydrolysate (SPIH): was prepared in our laboratory as described by [5] and the enzymatic hydrolysis was carried out according to [6]. Natto: was prepared according to the method of [7].

Burger manufacture: Independent replicates of burger formula were processed on the same day containing additives as following: formula 1 contains natto + ascorbic acid; formula 2 contains soy protein hydrolyzate + ascorbic acid; formula 3 contains only natto; formula 4 contains soy protein hydrolyzate; formula 5 contains ascorbic acid as positive control; formula 6 contains no additives as negative control. Products were prepared in a pilot plant according to commercial processing.

2. Proximate Composition

Proximate analysis of meat: All parameters were tested in triplicate. Moisture, ash, protein and fat content were determined by AOAC methods [8].

3. Physicochemical Analysis

Determination of pH: The measurement of pH was carried out according to [9].

Total volatile basic nitrogen (TVBN) was determined using microKjeldahl according to [10].

Water holding capacity was measured according to [11].

Color determination: Color was evaluated using a colorimeter (Mod. CR-200, Minolta Camera Co., Osaka, Japan) Color was described by coordinates: lightness (L*), redness (a*, §redgreen) and yellowness (b*, §yellow-blue). Nine replicate measurements were taken for each sample [12].

Texture profile (Penetrometer values): Sur penetrometer (PNR 6, Berlin, Germany) equipped with a total 100 g load was used to evaluate samples for 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 [13].

4. Cooking Properties

Burger cooking: Samples were grilled in microwaves for about 5 min.

Cooking properties was made by the method described by [14].

Sensory analysis: A panel of 10 assessors was selected to evaluate the product for the following attributes: colour = 8, tenderness = 8, taste = 8, residual taste = 8, texture = 8 and overall acceptance = 10. Results were decodified and rank sums were calculated [14].

5. Statistical Analysis

Data were subjected to statistical analysis using the General Linear Models Procedure of the Statistical Analysis System [15].

III. Results and Discussion

A. Proximate Composition

Addition of soybean products (natto and protein hydrolysate) significantly affected the proximate composition of burger. It was increased ($P < 0.05$) in moisture and protein content and reduced ($P < 0.05$) fat values (Table I).

TABLE I
EFFECT OF SOYBEAN BIOACTIVE COMPOUNDS ADDITIVES ON PROXIMATE COMPOSITION (GM/100GM)

Treatments	Moisture	Protein	Fat	Ash
NA	56.98±0.02	10.54±0.05	13.65±0.17	3.13±0.05
HA	57.68±0.07	10.70±0.02	14.06±0.08	3.34±0.02
N	56.45±0.14	10.40±0.13	14.31±0.23	2.88±0.02
H	56.63±0.01	10.57±0.01	14.26±0.03	3.20±0.02
A	56.64±0.09	9.58±0.13	15.07±0.06	3.11±0.04
C	56.42±0.07	9.52±0.18	14.76±0.09	2.98±0.03

The additives were as following: NA=natto+ascorbic acid, HA= protein hydrolysate+ascorbic acid, N= natto, H= protein hydrolysate, A= ascorbic acid, C= control (no additives).

Ash content did not affected ($P > 0.05$) with natto addition, in contrary, Ash content increased significantly with increasing the soybean protein hydrolysates concentration.

Reference [16] found that Incorporation of tofu powder resulted in lower fat and higher protein and moisture content of lean pork sausages.

While refrigerated storage affected on proximate composition, in which protein decreased significantly ($P < 0.05$), it may be attributed to the protein denaturation also bacterial and enzymatic hydrolysis increasing the loss of protein.

Moisture also had decreased significantly ($P < 0.05$) this is due to evaporation effect from meat. Ash content were significantly ($P < 0.05$)decreased as a result of releasing out with drip, while fat content was increased during storage as a result of moisture decreased.

B. Physicochemical Properties

1. Color Determination

Color intensity of different burger additives is shown in (Table II); there was a significant ($P < 0.05$) decreasing trend in color with time. For all samples, lightness and yellowness were higher ($P<0.05$) for the samples with added soybean products (with or without ascorbic acid), while redness was lower ($P<0.05$) compared to control samples. This increment in b* is related to the incorporation of yellow pigments present in soybean. Refrigerated storage affected significantly on lightness (decreased with storage time ($P< 0.05$), and the highest values of L* were obtained in control samples in the end of storage period.

TABLE II
EFFECT OF REFRIGERATED STORAGE ON THE COLOR OF BURGER WITH DIFFERENT BIOACTIVE COMPOUNDS ADDITIVES

	Zero			4Days			8Days			12Days		
	*L**	a*	b*	*L*	a*	b*	*L*	a*	b*	*L*	a*	b*
NA	60.6	10.87	19.83	56.86	8.87	17.43	60.18	7.51	17.71	59.31	7.72	17.69
HA	58.73	11.02	20.03	57.75	9.51	18.55	57.59	8.54	18.06	57.3	9.76	18.79
N	59.1	9.62	20.22	59.73	7.97	18.31	58.2	6.82	17.82	57.58	6.85	16.6
H	60.45	9.66	19.62	58.91	8.39	18.07	56.9	7.23	17.82	57.44	7.29	17.95
A	60.64	11.08	19.05	56.31	9.75	17.66	59.9	8.46	16.1	60.06	9.12	16.36
C	60.08	10.68	19.63	59.15	8.76	17.85	60.85	7.55	16.43	59.58	7.93	16.36

The additives were as following: NA=natto+ascorbic acid, HA= protein hydrolysate+ascorbic acid, N= natto, H= protein hydrolysate, A= ascorbic acid, C= control (no additives).

In all samples yellowness values were modified (P < 0.05) by storage time, also, redness decreased as the storage time progressed (P < 0.05). Soybean phenolics, isoflavones may have role in the color of samples, where the increment in metmyglobin formation was the main reason of these results, in which the presence of natural antioxidant could retard MMb formation in meat and so L* values decreased, also, phenolic acids oxidize to quinones that promotes myoglobin polymerization, exposing the heme moiety [17]. Addition of A (ascorbic acid) alone gave the highest redness (a*) value, while, A + protein hydrolysate and A + Natto also gave significantly higher a* values (P<0.05) than the control which mean that ascorbic acid is very effective in maintaining redness of beef burger, whereas at the end of the storage days, A + protein hydrolysate kept the high redness value than control sample and other additions (Table II). As a result of antioxidant activity of ascorbic acid, it's improving the color stability of meat because of declining the myoglobin oxidation. The pigment oxidation may catalyze lipid oxidation and free radicals produced during oxidation may oxidize the iron atoms or denature the myoglobin molecules, negatively changing the color of the products [18].

Desired textural attributes of burger are affected by many reasons including vegetable oil additives; some additives like soy protein can resolve this problem [19]. Penetration values are seen in Fig. 1. Burger with protein hydrolysate had the highest (the softest in the texture) penetration values comparing to other soybean additives, while, natto additive had less penetration value (the hardest in the texture). Ascorbic acid increasing softness were natto+A and protein hydrolysate+A had the less softness than others.

2. Texture

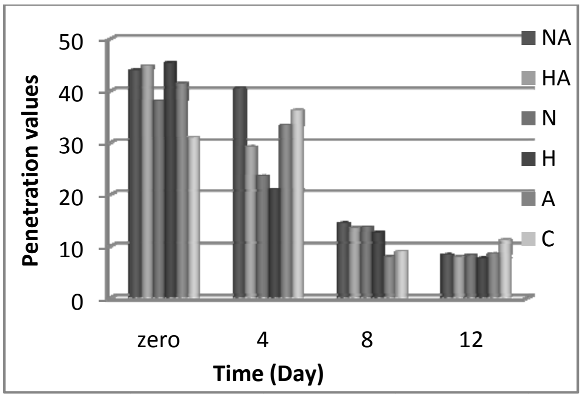

Fig. 1 Effect of refrigerated storage on the texture of burger with different bioactive compounds additives

The pending water of hydrolyzed protein may cause a softer texture thus leading to an increase in penetrometer values because of the increased moisture content. Refrigerated storage affected significantly (p<0.05) the texture of samples, whereas increasing the storage period resulting in increasing the softness (Fig. 1). Substitution of muscle proteins leads to less soluble myofibrillar protein, thus, less protein interactions occurred resulting in a softer texture. Reference [20] found the same effect on refrigerated frankfurter.

3. WHC

Water-holding capacity, determined initially and over the storage period, is shown in Fig. 2. Results showed that addition of soybean protein hydrolysate led to the increment of WHC, while natto decreased WHC comparing to the control samples. The mechanisms of co-precipitation, hydrogen binding and hydrophobic interactions are reported as the major binding modes. Hydrogen binding between the phenolic group of polyphenols and the amide group of protein has been emphasised by the fact that the phenol can be absorbed on the protein surface and may interact with protein in reversible and irreversible ways leading to conformational changes. If a polyphenol–protein complex is formed an alteration of charge distribution would occur which may have resulted in increased WHC following the addition of soybean [21]. In general, values showed an increase (P<0.05) towards the end of storage period. However, this increment was probably due to free water released from the product over time. With the decrease in pH, water holding capacity of proteins might have decreased resulting in water exudates in the packages.

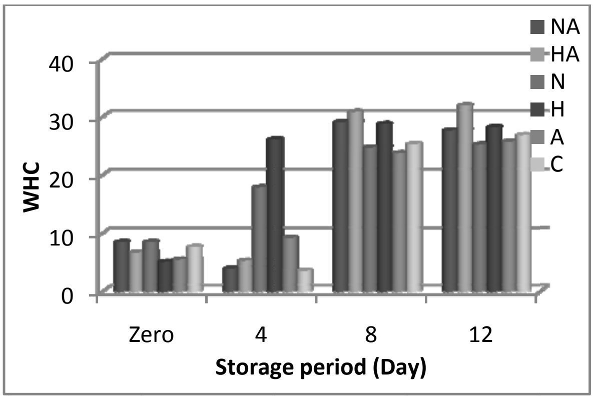

Fig. 2 Effect of soybean additives on WHC of burger

4. pH

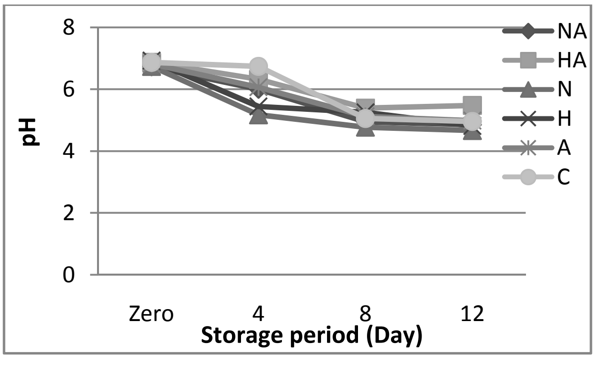

Fig. 3 Effect of bioactive compounds on the pH of refrigerated burger

pH, a reliable indicator of food stability is associated with microbial and chemical reactions that cause food deterioration. Changes of pH values during the 12-days storage period are presented in Fig. 3. Addition of soybean caused slight but not significant increases in pH values of samples. Storage at 4°C affected significantly ($P < 0.05$) in pH values over the storage period. Ascorbic acid didn't affect significantly on the pH of samples. Reference [17] found that pH in pork batter samples were decreased, probable due to acid production by contaminated microflora.

5. Total Volatile basic nitrogen (TVBN)

The TVBN analysis of burger (Fig. 4) showed significant differences between samples with soybean additives (natto & protein hydrolysate) with or without ascorbic acid.

It is clear that Natto & protein hydrolysate additives increased the total volatile basic nitrogen at significant level ($p<0.05$) due to amount of free nitrogen of hydrolysed protein in fermented soybean products. Samples with ascorbic acid (with or without soybean additives) decreased TVBN values at significant levels, comparing to the control sample. During storage period, increasing level in TVBN of soybean additives was lower than control sample Fig. 4.

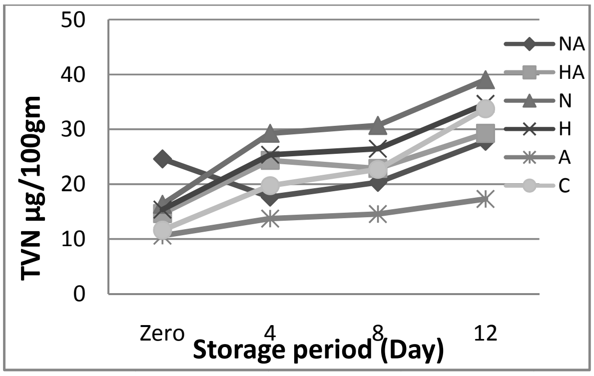

Fig. 4 Effect of bioactive compounds on the TVBN of refrigerated burger

C. Cooking Properties

Soy proteins is a common ingredient added to processed meats to enhance the products' functional characteristics, e.g. to reduce cooking weight loss and to improve slicability. In the present study, all the soybean additives significantly reduced burgers' cooking loss than control burger ($P<0.05$; Fig. 5). The reductions represented additional improvements in cooking yield (Fig. 6) in hydrolyzed soybean protein and fermented soy natto additives, while ascorbic acid addition increased the cooking loss and decreased the yield (Fig. 6) comparing to other sample, but still better than control sample (Fig. 6). The higher cooking yield of protein hydrolysate-treated and natto burger 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 [22].

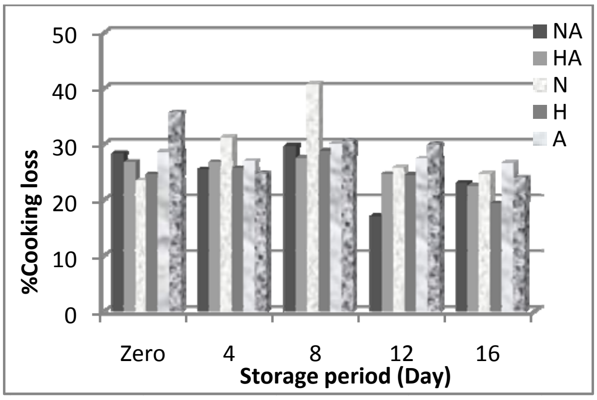

Fig. 5 Effect of refrigerated storage on the cooking loss of burger with different bioactive compounds additives

Surface shrinkage is important in maintaining quality standards of burger. The surface of all samples decreased after cooking. There was less surface shrinkage of treated burger ($P < 0.05$, Fig. 7) as the soybean additives content was increased. Ascorbic acid addition decreased shrinkage values compared to control. [14] reported that lemon albedo improved cooking performance due to albedo addition appears to be related with their fat and water holding capacity.

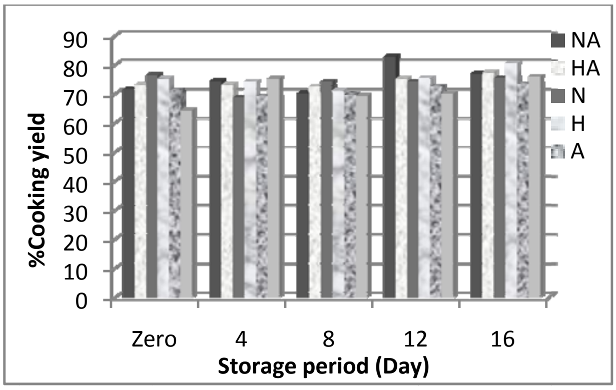

Fig. 6 Effect of refrigerated storage on the cooking yield of burger with different bioactive compounds additives

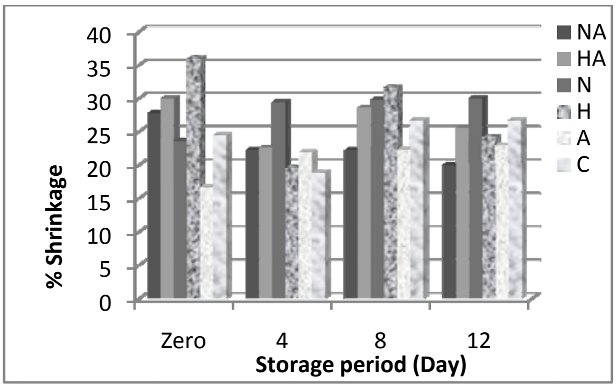

Fig. 7 Effect of refrigerated storage on the shrinkage of burger with different bioactive compounds additives

D. Panel test

Sensory evaluation at zero time with soybean additives (natto and protein hydrolysate) with or without ascorbic acid is presented in Table III. 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 showed a slit differences (P<0.05) between treatments, which could be related with lightness results obtained by instrumental analysis (Table II) and texture (Table III).

About the attributes used for taste evaluation, soybean additives enrich the taste of burger. There was a significant differences between treatments (P<0.05), natto + AA had the highest score comparing with control sample. From the attributes selected for overall acceptability evaluation, in general, the overall acceptability of samples with soybean additives had a significant difference comparing with control samples which mean that soybean additives enrich acceptability and quality attributs of burger.

TABLE III
PANEL TEST OF REFRIGERATED BURGER AT ZERO TIME AS AFFECTED BY SOYBEAN BIOACTIVE COMPOUNDS ADDITIVES

Sample	Color	juiciness	tenderness	taste	texture	residual taste	overall acceptability
NA	7.60a±0.16	6.90b±0.10	7.00ab±0.15	7.60a±0.16	7.50a±0.17	4.80a±0.13	9.00a±0.21
HA	7.30ab±0.15	6.80b±0.13	6.80b±0.13	7.30ab±0.15	7.10ab±0.23	4.30bc±0.15	8.60a±0.31
N	7.20ab±0.20	7.60a±0.16	7.40a±0.16	7.40ab±0.16	7.20ab±0.20	4.40abc±0.16	8.70a±0.26
H	7.00b±0.15	7.40a±0.16	7.10ab±0.10	7.10ab±0.18	6.80bc±0.13	4.10c±0.10	8.30ab±0.21
A	7.40ab±0.16	6.30c±0.15	6.20c±0.13	6.90b±0.18	6.80bc±0.13	4.70ab±0.15	8.30ab±0.15
C	6.90b±0.23	7.20ab±0.13	7.20ab±0.13	6.40c±0.16	6.40c±0.16	4.50abc±0.17	7.90b±0.18

*Means in the same column with different letters are significantly (p < 0.05) different.
The additives were as following: NA=natto+ascorbic acid, HA= protein hydrolysate+ascorbic acid, N= natto, H= protein hydrolysate, A= ascorbic acid, C= control (no additives).

IV. CONCLUSION

Improving of quality characterization of burger with addition of soybean products (protein hydrolysate and fermented soy Natto) with or without ascorbic acid has been estimated. Soy additives affected significantly on proximate composition of burger. Color attribute was also affected by soy additives in which, lightness and yellowness were increased (with or without ascorbic acid), while redness decreased. Both of protein hydrolysate and ascorbic acid increased the softiness while, Natto additive increased the hardness of samples. Soy additives (Natto & protein hydrolysate) increased the total volatile basic nitrogen while, samples with ascorbic acid decreased TVBN values at significant levels. Soy additives were improved both of cooking quality and sensory evaluation of the burger.

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