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Utilization of Bioactive Components Produced from Fermented Soybean (Natto) in Beef Burger

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***Abstract*—**Soybean Natto powder was added to the burger in order to enhance the oxidative stability as well as decreases the microbial spoilage. The soybean bioactives compound (soybean Natto) as antioxidant and antimicrobial were added at level of 1, 2 and 3%. Chemical analysis and physical properties were affected by soybean Natto addition. All the tested soybean Natto additives showed strong antioxidant properties. The microbiological indicators were significantly ($P < 0.05$) affected by the addition of the soybean Natto. 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 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 soybean Natto. Sensory attributes were also performed, added soybean Natto exhibits beany flavor which was clear about samples of 3% soybean Natto.

***Keywords*—**Antioxidant, antimicrobial, bioactive peptide, antioxidant peptides.

I. Introduction

FERMENTATION is one of the major processes used in the production of food from the soybean. The fermentation of these soybean products changes the bioactive components, such as isoflavonoids and peptides in ways which may alter their efficacy in the treatment of many chronic diseases [1].

Fermentation is an excellent processing method of improving nutritional and functional properties of soybean due to the increased content of small bioactives compound. The large protein, lipid and carbohydrate molecules in raw soybean are broken down by enzymatic hydrolysis during fermentation to small molecules such as peptides, amino acids, fatty acids and sugars, which are responsible for the unique sensory and functional properties of the final products [2].

Bioactive oligpeptides from fermented soybeans are an emerging area of research with great promise peptides from the soybean is currently the subject of investigation into new drugs and functional food ingredients for gut health and modulating the intestinal absorption of nutrients [3].

There is doubt that the lipid oxidation and microbial contamination is the main problems faced meat industry. These factors affect 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 [4]. 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 [5].

Consumers increasingly demand healthy meat products, if possible free of chemical additives. The use of natural preservatives to increase the shelf life of meat products is a promising technology since many vegetatives substances have antioxidant and antimicrobial properties. The aims at the current study are (1) preparation of fermented soybean Natto to improve its bioactives compounds, isoflavones and peptides, and (2) incorporate the produced soybean Natto to burger in different concentration in order 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. The strain of *Bacillus natto* (NBRC 13169) was obtained from National Institute of Technology and Evaluation Biotechnology Center (NITE), Japan. All other reagents were of highest analytical grades available.

B. Preparation of Natto

The method described by Wei et al. [6] was applied for Natto processing as follows: Soybean was soaked in tap water at 1:3 w/v ratios for 24h to avoid any fermentative acidification. The soaked beans were cooked at 121°C for 3 min using autoclaves. Fifty grams of cooked soybeans were cooled to 38°C inculcated with *Bacillus* strain and incubated at 38°C for 24hs. Natto product was obtained and stored until analysis.

Drying of Natto: Natto was dried using three methods: solar drying (60°C), under vacuum drying (60°C) and oven drying (60°C).

C. Burger Manufacture

Independent replicates of burger formula were processed on the same day containing additives as follows: Formula 1, 2, and 3 contain Natto in percentage of 1, 2 and 3%; respectively, formula 4 contains ascorbic acid as positive control of antioxidant and formula 5 contains no additive as negative control. All the products were prepared for a pilot plant

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according to commercial processings and were prepared in one of meat processing factory according to its commercial processing formula.

The meat was ground through a 5mm 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 (80rpm) for 5min. This mixture was shaped using a commercial burger maker (9 cm internal diam) to obtain the burger of approximately 50g. Plastic packaging was used to help maintaining the shape of the burger prior to storage [7].

D. Microbiological Analysis

The determination of 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 37ºC for 24 hrs. or 25ºC for 3 days for molds and yeast. After the incubation, coagulase tests were done according to the method described earlier [8].

E. Physicochemical Analysis

1. Proximate Analysis

Moisture, ash, protein and fat content were determined by the AOAC methods [9]. All parameters were tested for triplicate.

2. Determination of pH

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

3. Total Volatile Basic Nitrogen (TVBN)

A sample (10g) was minced with 100ml distilled water and washed into a distillation flask with 100ml distilled water; then 2g of magnesium oxide and an antifoaming agent were added. The mixture was distilled using the micro Kjeldahl distillation apparatus. Distillate was collected for 25min into 25ml 4% boric acid and five drops of Tashero indicator. The solution was titrated using 0.1M HCl to calculate the total volatile basic nitrogen in the sample of mg VBN/100g sample [11].

4. Determination of TBARS (2-thiobarbituric acid reactive substances)

TBARS of samples was determined by the spectrophotometric method [12]. Two grams of homogenized the sample was taken and TBARS were extracted twice with 10ml of 0.4M perchloric acid. Extracts were collected and made up to 25ml with 0.4M perchloric acid then centrifuged for 5min at 1790rpm. After centrifugation, 1ml of supernatant was pipetted into glass stoppered test tube. TBA reagent (5 ml) was added and the mixture was heated in a boiling water bath for 35min. After cooling the absorbance of samples was read against the appropriate blank at 538nm. A standard curve was prepared to use 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, 8mm aperture of the instrument for illumination, and 8mm 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 [13].

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 1/10mm in triplicate for each peace for 30 s. A lower depth of penetration indicates a harder texture [14].

7. Cooking Properties

Burger: Samples were grilled in microwaves for about 5min and cooking properties were made by the method described by Aleson-Carbonella et al. [15] according to the following formula:

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

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

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

F. 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 to order (least to most) for the following attributes: Color = 8, tenderness = 8, taste = 8, residual taste = 5, texture = 8 and overall acceptance = 10. Results were decodified and rank sums were calculated and analyzed [15].

G. Statistical Analysis

Data was subjected to statistical analysis using the General

Linear Models Procedure of the Statistical Analysis System [16]. The significance of the differences between treatment groups was determined by Waller-Duncan k-ratio [17]. All statements of significance were based on probability of $P < 0.05$. The correlation calculation was carried out using ToolPack to determine whether two ranges of data move together.

III. Results and Discussion

A. Proximate Composition

Addition of soybean Natto significantly affected the proximate composition of restructured the burger in comparison with control. The addition of soybean Natto significantly increased moisture and protein content and reduced fat values (Table I).

TABLE I
Proximate Composition of Beef Burger Fortified with Soybean Natto Additives

Treatment	Moisture %	Protein %	Fat %	Ash %
1% N	58.71±0.03	13.65±0.01	21.37±0.05	2.88±0.01
2% N	59.04±0.03	14.09±0.05	20.97±0.03	2.97±0.04
3% N	59.77±0.03	14.20±0.01	19.68±0.04	2.94±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

N: Natto
All values is mean of triplicate determinations ± standard deviation (SD)

However, ash content did not change when fermented soybean Natto was added. The proximate composition of samples was consistent with meat product formulations of soybean Natto (Table I). Similar to these observations, Ho et al. [18] 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.

B. Physical Properties

1. Color

Consumers use color as an indicator of beef freshness and decide not to purchase when MetMb reaches 30-40% of total pigments on the surface of fresh beef. There is a closes a link between color preference and the decision to purchase beef and that consumers prefer to purchase bright red beef rather than purple or brown beef [19].

Color parameters of different burger additives are shown in Table II. For all samples, lightness was significantly higher of the added soybean Natto, while redness and yellowness were significantly lower compared to the control samples. This increase in b* is related to the transformation of yellow pigments to brown in soybean Natto added samples of fermentation. All color coordinates to show significant differences between treatments and storage days, except yellowness which only showed differences between treatments (Table II). In all samples, lightness increased to storage time and the highest values of L* was obtained in the control samples in the end of storage periods.

TABLE II
Changes in Instrumental Color and Texture Quality of Beef Burger with Different Soybean Natto Additions during Frozen Storage

Treatment	Color			Texture
Zero	L*	a*	b*	Penetration (mm)
1% N	59.35	6.88	20.83	19.89±1.50
2% N	60.28	6.15	20.48	19.67±1.76
3% N	59.99	5.86	20.36	19.56±1.41
Control	59.89	7.17	21.43	22.50±2.33
Ascorbic	57.89	8.00	20.24	14.50±0.92
First				
1% N	60.79	9.37	22.72	16.86±1.49
2% N	59.96	9.15	22.57	16.63±1.61
3% N	59.56	7.99	22.00	15.52±1.37
Control	59.72	10.51	22.26	19.13±2.53
Ascorbic	60.79	10.68	22.40	11.47±0.95
2 months				
1% N	61.54	6.33	20.67	13.33±1.66
2% N	64.15	5.54	20.13	6.86±0.91
3% N	63.10	5.40	20.35	7.36±1.69
Control	61.43	6.22	21.05	14.29±1.39
Ascorbic	59.11	6.90	19.74	13.29±1.65
3 months				
1% N	60.03	6.49	20.57	8.50±1.54
2% N	61.42	4.58	18.83	5.90±1.38
3% N	60.30	4.82	18.81	5.63±1.65
Control	60.77	5.79	19.53	14.75±1.44
Ascorbic	58.39	6.54	19.98	8.20±0.68

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

Some authors reported that this increase could be related to the increase in MMb formation. These results suggest that the presence of antioxidant compounds in the natural extracts could retard metmioglobin formation of meat and so L* values decreased [20]. For this reason, it could be expected that treatments for fermented soybean Natto would have the lowest lightness values because they had the highest antioxidant capacity (Table II), but at zero time, treatments with soybean Natto showed the highest L* values. This could be explained by the increased water retention and because these extracts were prepared for a dry powder and needed much water additives to formula. This relation between water content and lightness in meat and meat products has been reported [20].

In all samples, a yellowness value was modified by storage time. Therefore, the differences in b* values observed between storage periods and treatments incorporating soybean Natto which can be attributed to the presence of pigments in the beans and not to the oxidation processes. In all samples redness were decreased to the storage time progressed 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 hemoglobin, 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 was lower than zero time or those treated with antioxidant. Several reports 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 treated with high antioxidant activity (Table II) would have the highest a* values at the end of storage period [20]. Several authors have related the evolution of redness with lipid oxidation in meat products while others report that the development of lipid oxidation provokes a decrease in redness [7].

2. Texture (Penetration)

A penetration value is seen in Table II. The burger with the soybean Natto additive 3% had the lowest (the hardest in the texture) penetration values comparing to other soybean additives. Binders or extenders may be used without added water or with added water, which was reported to cause a softening effect on the texture [21].

C. Chemical Analysis

1. Lipid Oxidation (TBA)

Zhang et al. [22] suggested 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, the burger was prepared in order to determine whether the fermented soybean Natto with strong antioxidant properties (as determined by DPPH) can effectively inhibit meat lipid peroxidation. Our results clearly showed that the meat lipid peroxidation was gradually preceded against the storage time for reflected on the dramatic increase in TBARS values (Fig. 1). The analysis of variance between the TBARS data indicates that the TBA value was significantly affected ($P < 0.05$) by both the storage period and the soybean Natto additives. Initial (zero time) was TBA values for all samples significantly lower than those for the control ($P < 0.05$). Results showed that, increasing soybean Natto levels resulting in decreased TBA values, which emphasis the lipid peroxidation supration of soybean Natto additives. These results suggest that these antioxidants retarded lipid oxidation during and immediately after formulation of burger (Fig. 1) and agree with those reported elsewhere [20] for other natural antioxidants applied to meatballs. At the end of storage time (month three), all treatments resulted in significant decreases ($P < 0.05$) in TBA values when compared to the control, which indicated that all the tested soybean bioactives compounds showed strong antioxidant properties. Moreover, fermented soybean Natto has also been detected by using the DPPH. These results provide clear evidence that fermented soybean Natto can effectively inhibit food lipid peroxidation.

2. pH Determination

Addition of soybean Natto caused insignificant increases in pH values of samples. Frozen storage affected significantly ($P < 0.05$) pH values in samples and therefore data presented in Fig. 2 simply shows the mean pH value of the storage period. Fernandez- Lopez et al. [7] found a significant different ($P< 0.05$) between the pH value obtained from pork meat formula and suggested that this difference may be due to the special characteristics of Ostrich meat, which has an ultimate pH of 6.0. The presence of 30 % beefs and Pork meat in the other formulations decreased the pH of products. Serrano et al. [23] studied the pH of restructured beef steak or ground pork and found that it has been increased slightly with frozen storage.

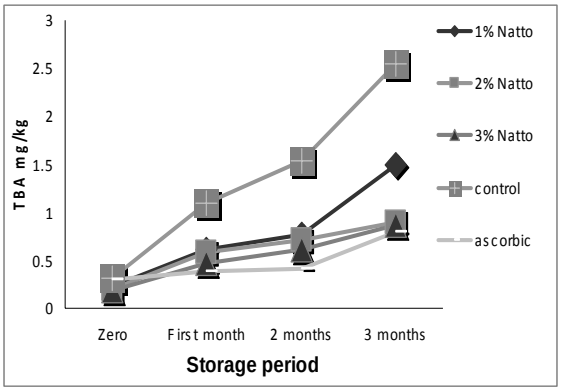

Fig. 1 Change in TBA of the burger with soybean Natto additives during frozen storage periods (All values are mean of triplicate determinations ± (SD). Mean within row with different letters are significantly different ($P<0.05$)

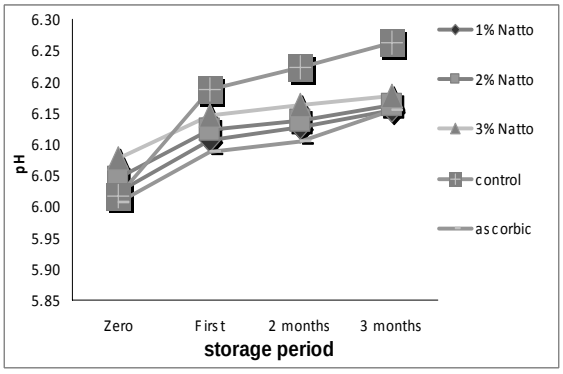

Fig. 2 Change in pH values of the burger with soybean Natto additives during frozen storage periods (All values are mean of triplicate determinations ± (SD). Mean within row with different letters are significantly different ($P<0.05$)

3. Total Volatile Basic Nitrogen (TVBN)

The TVBN analysis of burger showed significant differences between samples of soybean Natto at different concentrations. It is clear that these additives increased the total volatile basic nitrogen at significant level ($P < 0.05$) as compared to the control sample (Fig. 3). After storage periods, the total volatile basic nitrogen of soybean Natto additives was insignificant decreases compared to the control (Fig. 3). Al-Bachir & Mehiob [11] found that fresh buffalo meat, irradiated with a 2.5 kGy dose and stored at 0–3ºC had a shelf-life up to 4 weeks with low total volatile basic nitrogen values and acceptable sensory score.

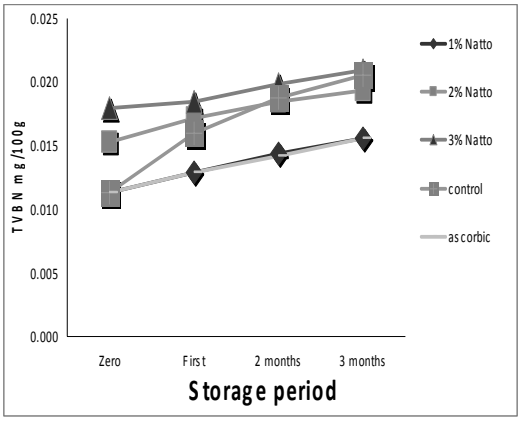

Fig. 3 Change in total volatile basic nitrogen (TVBN) of the burger with soybean Natto additives during frozen storage periods (All values are mean of triplicate determinations ± (SD). Mean within row with different letters are significantly different (P<0.05).

D. Cooking Properties

In the present study, all the soybean Natto treatments significantly reduced burgers cooking loss (Fig. 4). Fermented soybean Natto at 3% was particularly effective and induces a significant decrease in the cooking loss compared to the control burger (19.64 % to 11.51%) as shown in Fig. 4. Moreover, cooking loss was increased to the increase storage period which may be due to protein denaturation. The increments represented additional improvements in cooking to yield with 3% fermented soybean Natto additives were 88.49% (Fig. 5). Freeze storage affected cooking to yield significantly whereas: it was decreased to encreasing storage periods (Fig. 5). The higher cooking yield of soybean 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 [24]. Surface shrinkage is important to maintaining quality standards of the burger. The surface of all samples was decreased to cooking from 20.87% in control samples to 11.89% in 3% soybean Natto (Fig. 6). There was significantly less surface shrinkage of the burger as the soybean Natto additives content was increased (Fig. 6). Frozen storage was affected significantly on burger samples. It could be noted that burger samples treated with fermented soybean Natto had better cooking properties. Aleson-Carbonell et al. [15] reported that lemon albedo improved cooking performance due to albedo addition appears to be related with their fat and water holding capacity. However, Serrano et al. [23] found that froze storage did not affect cooking loss for each sample.

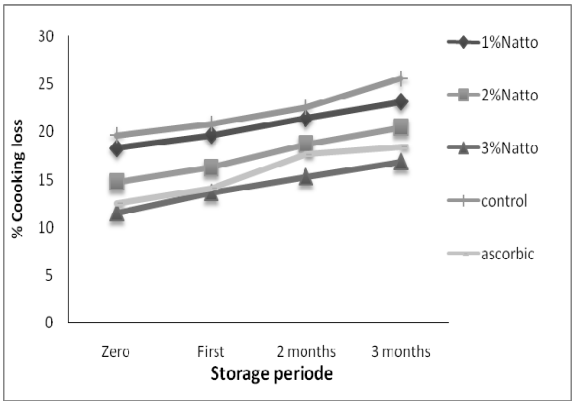

Fig. 4 Effect of frozen storage periods and soybean Natto additives on the cooking loss of burger (All values are mean of triplicate determinations ± (SD). Mean within row with different letters are significantly different (P<0.05)

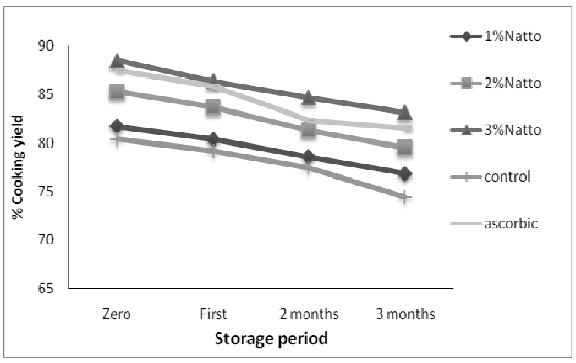

Fig. 5 Effect of frozen storage periods and soybean Natto additives on the cooking yield of burger (All values are mean of triplicate determinations ± (SD). Mean within row with different letters are significantly different (P<0.05)

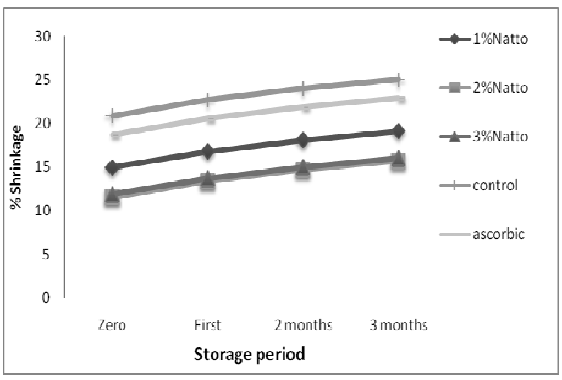

Fig. 6 Effect of frozen storage periods and soybean Natto additives on the shrinkage of burger (All values are mean of triplicate determinations ± (SD). Mean within row with different letters are significantly different (P<0.05))

E. Microbiological Profile

Results of the burger microbiological analyses during the storage period are presented in (Tables III-VI). The counts of all determined microbiological indicators were significantly (P

< 0.05) affected by the addition of the natural soybean bioactive compounds (Table III).

TABLE III
EFFECT OF FROZEN STORAGE ON TOTAL COUNT MICROORGANISM (CFU/G) OF BURGER WITH SOYBEAN NATTO ADDITIVES

Treatment	Zero	First	2 months	3 months
1% N	$5.6 X 10^5$	$1.7X10^5$	$5.8X10^4$	$0.4X10^4$
2% N	$7.6 X 10^5$	$2.1X10^5$	$6.8X10^4$	$1.4X10^4$
3% N	$8.8 X 10^5$	$4.0X10^5$	$8.0X10^4$	$2.6X10^4$
Control	$7.2 X 10^5$	$5.0X10^5$	$7.1X10^4$	$2.6X10^4$
Ascorbic	$6.8 X 10^5$	$3.9X10^5$	$6.0X10^4$	$1.3X10^4$

N: Natto

All microbial groups were increased to the control burger but in samples containing 1, 2, and 3% soybean Natto, total to count were increased (8.8×10^5 cfu/g) from soybean Natto additives (containing *Bacillus subtilis*) as compared to the control sample (7.2×10^5 cfu/g). Decreasing trends of different extent were also observed in samples of the remaining treatments for total viable counts, coliform group, *Staphylococcus aureus*, yeasts and moulds. Coliform came in the second order reduced from 1.1×10^3 to 8 x10 cfu/g in samples containing soybean Natto compared to the control sample which was 2.4×10^3 cfu/g (Table IV).

TABLE IV
EFFECT OF FROZEN STORAGE ON COLIFORM GROUP (CFU/G) OF BURGER WITH SOYBEAN NATTO ADDITIVES

Treatment	Zero	First	2 months	3 months
1% N	$2.1X10^3$	$1.3X10^3$	$8.0X10^2$	$3.4X10^2$
2% N	$1.2X10^3$	$5.0X10^2$	$1.3X10^2$	9.3X10
3% N	$1.1X10^3$	$3.0X10^2$	$0.9X10^2$	8.0X10
Control	$2.4X10^3$	$1.8X10^3$	$9.0X10^2$	$5.0X10^2$
Ascorbic	$1.2X10^3$	$0.6X10^3$	$8.0X10^2$	$4.0X10^2$

N: Natto

Storage period significantly affected the microbial count in all samples. *Staphylococcus aureus* had the most decreased microbes count from (2.0×10^2 to 1.0×10^2 cfu/g) in samples containing soybean Natto as compared to the control sample which was from 6.0×10^2 cfu/g at zero time then decreased to 4.0 x 10 cfu/g at the end of storage (Table V). While mold and yeast counts showed significant decrease until the end of the storage period (Table VI). It is known that froze storage affected microorganisms since it destroys the bacterial cells as a result of ice crystals formation.

TABLE V
EFFECT OF FROZEN STORAGE ON *STAPHYLOCOCCUS AUREUS* (CFU/G) OF BURGER WITH SOYBEAN NATTO ADDITIVES

Treatment	Zero	First	2 months	3 months
1% N	$3.6 X10^2$	$0.3X10^2$	4.7X10	3.0X10
2% N	$2.4X10^2$	9.0X10	3.7X10	1.0X10
3% N	$2.0 X10^2$	7.0X10	3.0X10	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

N: Natto

TABLE VI
EFFECT OF FROZEN STORAGE ON MOLD AND YEAST (CFU/G) OF BURGER WITH SOYBEAN NATTO ADDITIVES

Treatment	Zero	First	2 months	3 months
1% N	$3.6X10^3$	$7.2X10^2$	$5.0X10^2$	$2.0X10^2$
2% N	$3.0X10^3$	$6.1X10^2$	$3.0X10^2$	$1.0X10^2$
3% N	$2.0X10^3$	$4.0X10^2$	$1.0X10^2$	8.0X10
Control	$4.0X10^3$	$1.9X10^3$	$7.0X10^2$	$4.0X10^2$
Ascorbic	$4.0X10^3$	$2.0X10^3$	$7.8X10^2$	$4.0X10^2$

N: Natto

This antimicrobial effect of soybean Natto additives could be due to the presence of phenolic compounds and although a synergistic effect of phenolic and bioactive peptides could be participated in antimicrobial activities. According to Bajpai et al. [25], the possible mechanisms for antimicrobial effect of phenolic compounds include; altering microbial cell permeability interfering with membrane functions including electron transport, nutrient uptake, protein and nucleic acid synthesis, and enzyme activity which interact with membrane proteins causing deformation in structure and functionality and substituting alkyls into phenol nucleus [26].

Fernandez-Lopez et al. [20] reported that citrus bioflavonoid also had antimicrobial properties. These compounds have reportedly wide-ranging antimicrobial properties effective against a broad range of human pathogens, fungi and food spoilage organisms.

F. Sensory Evaluation

Sensory evaluation at zero time for soybean Natto addition is presented in Table VII. No significant differences were observed in color between treatments, which could be related to lightness results obtained by instrumental analysis (Table VIII).

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

Treatment	Color	Texture	Taste	Tenderness	Residual taste	Overall acceptability
1% N	$6.80^{a} \pm 0.29$	$6.60^{b} \pm 0.34$	$6.50^{c} \pm 0.17$	$5.90^{d} \pm 0.18$	$4.56^{a} \pm 0.24$	$7.40^{bc} \pm 0.16$
2% N	$6.60^{a} \pm 0.31$	$7.00^{b} \pm 0.26$	$7.10^{b} \pm 0.18$	$6.50^{cd} \pm 0.22$	$4.33^{a} \pm 0.17$	$7.90^{b} \pm 0.23$
3% N	$6.40^{a} \pm 0.40$	$7.60^{a} \pm 0.16$	$7.80^{a} \pm 0.13$	$7.30^{ab} \pm 0.21$	$3.78^{b} \pm 0.22$	$8.80^{a} \pm 0.29$
Control	$6.70^{a} \pm 0.21$	$6.40^{b} \pm 0.27$	$6.50^{c} \pm 0.17$	$6.00^{d} \pm 0.26$	$3.89^{ab} \pm 0.35$	$6.50^{d} \pm 0.17$
Ascorbic	$6.60^{a} \pm 0.27$	$6.60^{b} \pm 0.27$	$6.50^{c} \pm 0.17$	$6.10^{cd} \pm 0.23$	$4.00^{ab} \pm 0.33$	$7.10^{cd} \pm 0.23$

N: Natto
Mean ± 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 NATTO ADDITIVES ON THE COLOR OF BURGER

Treatment	Zero	First	2 months	3 months
1% N	6.80^{Aa}±0.29	6.70 ^{Aa}±0.21	6.40 ^{Aa}±0.22	6.20 ^{Aa}±0.20
2% N	6.60^{Aa}±0.31	6.20 ^{Aa}±0.33	5.90 ^{Aa}±0.31	5.70^{AB}±0.26
3% N	6.40^{Aa}±0.40	6.00 ^{Aa}±0.42	5.80 ^{Aa}±0.47	5.60^{ABa}±0.40
Control	6.70^{Aa}±0.21	6.50 ^{Aa}±0.27	6.20 ^{Ab}±0.25	6.00^{ABa}±0.21
Ascorbic	6.60^{Aa}±0.27	6.50 ^{Aa}±0.27	6.20 ^{Aa}±0.25	6.00^{ABa}±0.21

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

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

Treatment	Zero	First	2 months	3 months
1% N	6.60^{Ba}±0.34	6.10^{BCab}±0.23	5.80^{BCb}±0.20	5.40^{Bb}±0.22
2% N	7.00^{ABa}±0.26	6.50^{ABab}±0.17	6.20^{ABb}±0.20	5.80^{ABc}±0.25
3% N	7.60 ^{Aa}±0.16	7.10^{Ab}±0.10	6.80 ^{Abc}±0.13	6.40^{Ac}±0.22
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

N: Natto
Mean ± 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

Texture showed that 3 % soybean Natto had highest scored (Table IX). About the attributes used for taste evaluation, there was a significant difference between treatments and 3 % soybean Natto that had the lowest score of taste than control sample (Table X).

It is important to observe that 1 % soybean Natto had the highest TBA value (Fig. 1) and increased rancidity was not detected by the panelists (Tables X & XIII). From the attributes selected for overall acceptability evaluation increasing soybean Natto level results of increasing overall acceptability.

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

Tenderness	Zero	First	2 months	3 months
1% N	5.50^{Ca}±0.17	6.20^{Cab}±0.13	5.90^{BCDbc}±0.10	5.50^{BCc}±0.22
2% N	7.10^{Ba}±0.18	6.80^{Bab}±0.13	6.50^{Bb}±0.17	5.90^{Bc}±0.18
3% N	7.80^{Aa}±0.13	7.50 ^{Aa}±0.117	7.20^{Ab}±0.13	6.70^{Ac}±0.15
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	5.8 0^{CDb}±0.13	5.20^{CDc}±0.20

N: Natto
Mean ± 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 earlier [7]. Storage affected significantly the entire sensory attribute (Tables VIII-XIII); however, the deterioration in samples of soybean Natto was less than that happened to the control sample.

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

Treatment	Zero	First	2 months	3 months
1% N	5.90^{Da}±0.18	5.40^{Eb}±0.16	5.20^{Dbc}±0.13	4.90^{Dc}±0.18
2% N	6.50^{CD}a±0.22	6.00 ^{CDa}±0.15	5.80^{CDb}±0.20	5.50 ^{CDb}±0.22
3% N	7.30^{ABa}±0.21	6.80^{ABab}±0.13	6.60^{ABb}±0.16	6.30^{ABb}±0.20
Control	6.00^{Da}±0.23	5.50^{Eab}±0.12	5.30 ^{CDb}±0.12	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

N: Natto
Mean ± 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 NATTO ADDITIVES ON THE RESIDUAL TASTE OF BURGER

Treatment	Zero	First	2 months	3 months
1% N	4.56^{Aa}±0.24	4.22^{Aab}±0.28	3.89 ^{Ab}±0.20	3.67 ^{Ab}±0.17
2% N	4.33 ^{Aa}±0.17	4.11^{Ab}±0.26	3.78^{ABbc}±0.15	3.56 ^{ABc}±0.18
3% N	3.78 ^{Ba}±0.22	3.67^{ABab}±0.24	3.33^{ABab}±0.17	3.22 ^{ABb}±0.1 5
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

N: Natto
Mean ± 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 NATTO ADDITIVES ON THE OVERALL ACCEPTABILITY OF BURGER

Treatment	Zero	First	2 months	3 months
1% N	7.40^{BCa}±0.16	6.90^{Cab}±0.23	6.60^{BCDbc}±0.27	6.2 0 ^{BCa}±0.25
2% N	7.90 ^{Ba}±0.23	7.40^{Ba}±0.27	7.10 ^{Ba}±0.28	6.70 ^{B}±0.21
3% N	8.80 ^{Aa}±0.27	8.50 ^{Aa}±0.27	8.20 ^{Aa}±0.25	7.80 ^{Aa}±0.25
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

N: Natto
Mean ± 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 soybean Natto powder was added to the burger in order to enhance the oxidative stability as well as decreases the microbial spoilage. The soybean bioactives compound (soybean Natto) 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 bioactives compound (soybean Natto) to show strong antioxidant and antimicrobial properties.

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