

## Effects of Adding Edible Bird Microparticulates on the Oxidation of Chicken Burger

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### Abstract

This study was carried out to determine the effect of adding micro-particulates of Malaysia's edible bird nests (EBN) on the shelf life and nutritional quality of chicken burger. Four treatments of chicken burger were prepared for this study and all samples were stored at 4°C for 12 d. The EBN of various sizes was evaluated for their antioxidant activities through 2, 2-diphenyl-1-picrylhydrazyl (DPPH), superoxide radical scavenging activity and metal chelating methods. Analyses of physiochemical and thiobarbituric acid reactive substance (TBARS) were conducted on the samples to study the shelf life quality of chicken burger. TBARS analysis showed that samples added with EBN 300 µm and 38 µm size had lower ( $p < 0.05$ ) TBARS values at the end of storage period. The addition of smaller sized EBN lowered ( $p < 0.05$ ) the cooking loss of chicken burger. The addition of EBN of varying sizes into chicken burger did not affect the sensory acceptance of chicken burger ( $p > 0.05$ ). EBN is a potential natural antioxidant and it increases the antioxidant activities with the reduction in particle size of EBN. It is effective in reducing the rate of lipid peroxidation without affecting most of the physicochemical characteristics and sensory attributes of chicken burger.

**Keywords:** edible bird nest, particle size, shelf life, nutritional quality, antioxidant activity, chicken burger.

### Introduction

There are more than 24 species of swiftlet distributed throughout the world, but only a few produce edible bird's nests (Goh *et al.*, 2001; Marcone, 2005). *Aerodramus fuciphagus* or white bird species of swiftlets that produce edible bird nests (EBN) are found only in Southeast Asia. These nests contain as much as 90-95% of edible bird nest and 5-10% wool and dirt (Ibrahim *et al.*, 2009). These nests are built by the male swiftlets within the weight range of 7-20 g in a period of about 35 d.

According to Macrone (2005), the composition of white birds' nests is 62.00% protein, 27.26% carbohydrate, 7.50%

moisture, 0.14% ash and 2.10% fat. Birds' nests are also rich in minerals that are needed by the human body. Numerous studies have been conducted to investigate the advantages and benefits of bird nests on human health (Chan, 2009)

In general, the bulk composition of the birds' nests are proteins such as amino acids. Therefore, it has the potential to be a natural antioxidant. In addition, the use of EBN is becoming increasingly popular for health and pharmaceutical purposes. Therefore, it opens up an opportunity for evaluation of antioxidant capacity of birds' nests and examines the effect of bird nests on the storage quality of the meat. A study was therefore carried out to determine the effect

of adding micro-particulates of Malaysia's EBN on the shelf life and nutritional quality of chicken burger.

## Materials and Methods

Edible Bird's nests were sourced from Aerodramus Bird Nest Industry Co., Ltd. Samples were supplied in the form of dry cleaned solids. The study was performed on the birds' nests that had not been processed. Chicken meat used for the processing of chicken burgers was obtained from a market in Bangi Selangor Malaysia. Chicken breast meat was used to make the chicken burgers.

### Preparation of Bird's Nest

The bird's nests were soaked in distilled water overnight so that the nests became soft and resolved the laminate strips. The nests were then placed in an oven at 50°C for 24 h and then ground to get three different sizes of 710, 300 and 38µm using Buchi Mixer Homogenizer (B-400, Switzerland).

### Formulation of Chicken Burger

Chicken burgers were prepared according to the formulation by Aleson-Carbonell *et al.* (2005) and Ibrahim *et al.* (2011) with some modifications. Four samples of chicken burgers were formulated: C: chicken burger + EBN original size, S1: chicken burger + EBN (710µm), S2: chicken burger + EBN (300µm) and S3: chicken burger + EBN (38µm). An antioxidant test was conducted to determine the EC50. EC50 is the broad parameter for the assessment of antioxidant activity where EC50 is the concentration required to reduce the original concentration of 1,1-diphenyl-2-picrylhydrazyl (DPPH) to 50%. With this, the minimum amount required for bird's nests to show antioxidant activity in chicken burgers can be identified.

### Solubility

The solubility of proteins is determined using the method described by Klompong *et al.* (2007) with some modifications. An amount of 0.125g of EBN samples was dissolved in 25 ml of deionized water and the mixture was stirred at room temperature for 30 min and autoclaved at 15000 rpm for 15 min. The protein content in the supernatant was determined using Kjeldhal method (AOAC, 1990).

$$\text{Protein solubility (\%)} = \frac{(\text{Protein content in the supernatant})}{(\text{Protein content in the sample})} \times 100$$

### Testing Free Radical Trapping Activity of DPPH

This method is based on free radical trapping activity of DPPH according to Shimada *et al.* (1992) with some modifications. An amount of 0.1ml of distilled water (control) / sample / reference antioxidant was mixed with 1.5 ml of distilled water. Next, coupled with 1.4 mL 0.15mm DPPH was dissolved in methanol. All samples were vortexed and soaked in water at room temperature for 30 min. Supernatant was removed and the absorption read at a wavelength of 517 nm by UV-vis spectrophotometer. Finally, the blank solution of 1.6ml distilled water was added to 1.6ml of methanol. The percentage entrapment evaluated was calculated using the following formula:

$$\text{Encapsulation percentage (\%)} = \frac{\text{OD (control)} - \text{OD (sample)}}{\text{OD (control)}} \times 100$$

### Testing Superoxide Anion Radical Trapping Activity

Superoxide anion scavenging activity was determined by measuring the inhibition of auto-oxidation of pyrogallol using the method of Marklund and Marklund (1974) with slight modification with 0.1mL and 2.8mL samples of 50 mM phosphate buffer (pH 8.24) added to 90µl 3mm pyrogallol (10 mM HCl). The rate of inhibition of auto-oxidation of pyrogallol was measured at 325 nm. The absorption of each sample at every 1 min for 10 min was recorded.

#### Encapsulation rate (%)

$$= [1 - ((A_{10} - A_1) / A_0) \times 100]$$

where  $A_{10}$  = absorption of the sample at 10 min

$A_1$  = absorption of the sample at 1 min

$A_0$  = Absorption blank (10 min Absorption - Absorption at 0 min)

### Metal Chelating Method

The metal chelating activity of bird's nests was determined according to the method of Klompong *et al.* (2007). A total of 250µl sample was added to 4.45 ml of distilled water. Then 200µl 2mm iron (II) chloride, FeCl<sub>2</sub> and 100µl 5mm ferrozine, C<sub>20</sub>H<sub>13</sub>N<sub>4</sub>NaO<sub>6</sub>S<sub>2</sub> [acid 3 - (2-pyridyl) - 5,6-in (2-furyl) -1,2,4-triazin-5', 5''-disulfonic] were added to the samples and left for 10 min. Absorption was measured at 562 nm wavelength. Finally, for a blank solution, 250µl distilled water was replaced by the sample.

#### Chelation Activity (%)

$$= [1 - (\text{sample Absorption}) / (\text{blank Absorption})] \times 100$$

### Analysis of Thiobarbituric Acid Reactive Substance (TBARS)

The test was conducted based on the method described by Buege and Aust (1978) with slight modification. A total of 0.5 g ground chicken burger was added with 2.5ml of 0.25 N hydrochloric acid, 2.5 ml 15 % trichloroacetic acid and 2.5 ml 0.375 % thiobarbituric acid (TBA). The mixture was shaken and heated in hot water (100 °C) for 10 min for the formation of pink kromogen. For kromogen formation, the mixture was cooled under running tap water. Next, 1 ml of chloroform was added to the mixture to precipitate an oil suspension from reading spectrophotometer. Then, this mixture was centrifuged at 5500 rpm speed for 2 min. The absorption of the supernatant of the mixture was measured with a spectrophotometer at a wavelength of 532 nm with reference to the control sample (test solution without meat).

TBA value (mg malonaldehyd / kg sample) = value of the absorption of the sample x 2.77

### Determination of Cooking Loss

Chicken burgers were weighed before and after the grilling process to determine cooking loss (Aaslyng *et al.*, 2003). Grilling time for chicken burgers was 7-8 min until internal temperature reached  $72 \pm 1$  °C (Wan Rosli *et al.*, 2011). According Novakofski *et al.* (1989) determination of cooking loss for each treatment was evaluated using the following formula:

#### Cooking loss (%)

$$= [(\text{weight of meat before cooking} - \text{weight after cooking meat}) / (\text{weight of meat before cooking})] \times 100$$

### *Water Holding Capacity*

The ability of meat to retain water is known as water holding capacity (WHC, Bertram *et al.*, 2002). WHC in the present study was evaluated using the method of Katoh (1981) with some modifications. Filter paper was stuffed in a centrifuge tube (10 ml) to a depth of about two thirds of the length of the tube. Water that remained in the meat after centrifuge was used to determine WHC using the following formula:

$$\text{Water Holding Capacity (\%)} = \frac{(\text{weight before centrifuge} - \text{weight after centrifuge})}{(\text{weight before centrifuge})} \times 100$$

### *pH Test*

pH test performed on a burger chicken was done according to AOAC (1990). Five g samples of ground chicken burger were mixed with 50 ml of distilled water and homogenized using a homogenizer for 2 min. Then, the pH of chicken burgers was determined using a pH meter calibrated with standard buffer of pH 7.

### *Texture Profile Analysis*

The texture of chicken burgers was determined by using texture analyzer (Shimadzu AGS-J 500N, Japan). The sample was placed on the texture analyzer and a probe measuring cylinder ( $f = 1.0$  cm) was used. The samples were compressed to 75% of their original height with a speed of 100 mm / min (Bourne 1978).

### *Sensory Evaluation*

Sensory evaluation of chicken burger was conducted by the hedonic approach. A 7-point hedonic scale test was conducted on 30 untrained people comprising of students and staff of UKM. The sensory evaluation was conducted to determine the acceptance level of chicken burger which contained different sizes of EBN powder. Attributes that were measured included color, aroma, taste, juiciness, texture and overall acceptability.

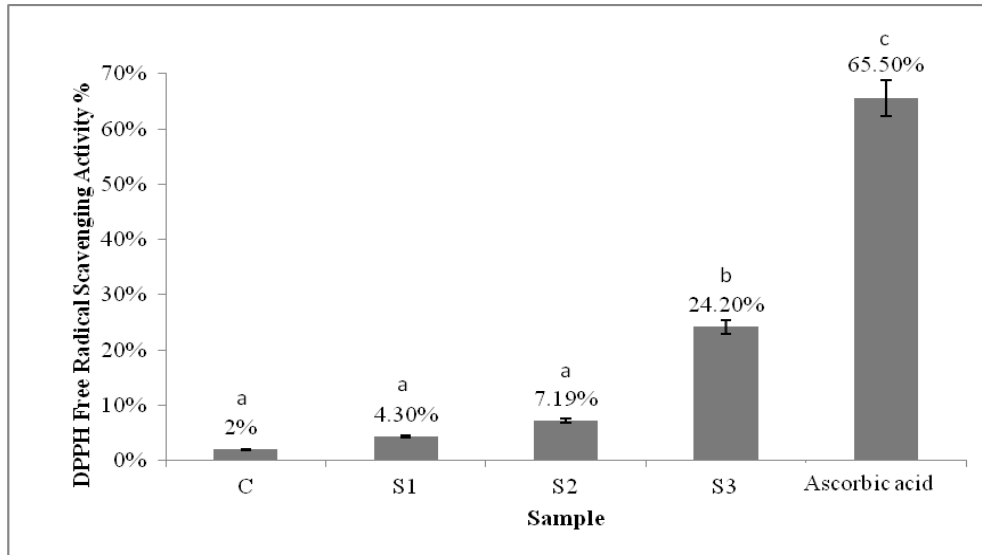
### *Statistical Analysis*

Data were analyzed using the Statistical Analysis System (SAS). ANOVA and Duncan test were performed to determine significant differences among the treatment groups studied. The significant differences were based on 95% confidence level.

## **Results and Discussion**

### *Solubility*

Figure 1 shows that there were significant differences ( $p < 0.05$ ) between all treated samples and control sample. S3 sample had the highest solubility compared to the others, meaning that particle size affected the sample solubility. According to Wu and Nancollas (1998), a smaller particle size contributed to a greater surface area per unit volume, thus helping to increase the solubility. However, there was no significant difference between S1 and S2 samples on solubility, perhaps because of the effect sizes were not much difference.



Note: Bars show the mean  $\pm$  standard deviations from the experimental replicates, n = 3, a-b Bars with different letters indicate significant differences at  $p < 0.05$ , Ascorbic acid shows antioxidant reference, C shows original size EBN, S1 shows 710 $\mu$ m sized EBN, S2 shows 300 $\mu$ m sized EBN, S3 shows 38 $\mu$ m sized EBN

Figure 1: Percentage Solubility % EBN different sample sizes

The effectiveness of antioxidants in food systems depends on the concentration and solubility. Solubility of antioxidants will determine whether the soluble antioxidant in the lipid phase or water and may affect the overall antioxidant activity, particularly in systems involving such particles as in ground beef (Naveena *et al.*, 2013). According to Ying *et al.* (2011), chitosan had solubility in water effectively which demonstrated the ability of higher DPPH radical scavenging.

*Antioxidant Activity - DPPH Radical Scavenging*

Ascorbic acid showed the highest anti-radical activity (65.5  $\pm$  0.06%) (Table 2). The rate of scavenging activity of DPPH was inversely proportional to the EBN particle size. There was significant difference ( $p < 0.05$ ) between S3 and C samples. S1 and S2 were not significantly different ( $p > 0.05$ ) with C. S3 showed the highest DPPH

scavenging activity (24.2  $\pm$  4.27%) compared to C (2.00  $\pm$  1.73%), S1 (4.30  $\pm$  0.65%) and S2 (7.19  $\pm$  3.86%). This meant that EBN with the smaller particle size gave higher antioxidant activity and higher scavenging activities of DPPH compared to larger size particles. DPPH scavenging activities for C, S1 and S2 were similar, perhaps caused by the small size difference and the non-differential antioxidant activity by DPPH test.

*Superoxide Anion Scavenging Activity*

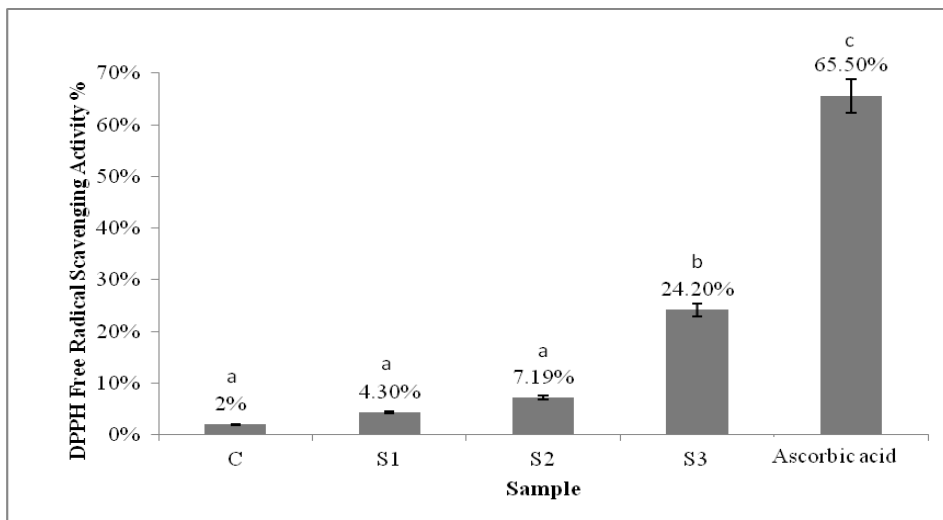
Figure 3 shows that there was no significant difference between all samples compared with sample C ( $p > 0.05$ ) except S3. The rate of superoxide radical trapping activity was proportional to the particle size inverting EBN. Samples S3 provided the highest entrapment (74.64  $\pm$  3.65%) compared to sample C (63.45  $\pm$  1:35%), S1 (65.35  $\pm$  2.12%) and S2 (67.71  $\pm$  1.56%).

This proves that EBN samples of smaller sizes provide the highest antioxidant activity. The rate of superoxide radical trapping activity is proportional to the particle size inverting EBN.

Many biological reactions generate superoxide radicals which are highly toxic species. Although it cannot start lipid peroxidation, superoxide anion radicals are precursors for highly reactive species such as hydroxyl radicals (Li *et al.*, 2008). Therefore, the study of radical scavenging activity is very important. Superoxide trapping method is a fast, sensitive and effective in measuring the activity of entrainment in biological and non-biological samples. However, this method has certain drawbacks which are limited and trapping activities can be affected by temperature, pH and extraction time (Xu *et al.*, 2013).

*Metal Chelating Activity*

The metal chelating activity of sample C was  $11.58 \pm 12.58\%$  and S1, S2 and S3 had metal chelating activity of  $12.43 \pm 0.85\%$ ,  $13.02 \pm 3.38\%$  and  $20.16 \pm 1.97\%$ , respectively (Figure 4). Samples S1 and S2 did not show significant differences ( $p > 0.05$ ) and only S3 sample was significantly different ( $p < 0.05$ ) compared with sample C. Metal chelation activity for smaller sized EBN was higher than EBN with bigger size which could be due to changes in protein structure during the process of size reduction, thus causing improvement in antioxidant capacity. Through these tests, it is proven that small size EBN has higher antioxidant activity than the EBN of larger size.



Note: Bars show the mean  $\pm$  standard deviations from the experimental replicates, n = 3, a-c Bars with different letters indicate significant differences at  $p < 0.05$ , Ascorbic acid shows antioxidant reference, C indicates EBN original size, S1 shows 710 $\mu$ m sized EBN, S2 shows 300 $\mu$ m sized EBN, S3 shows 38 $\mu$ m sized EBN.

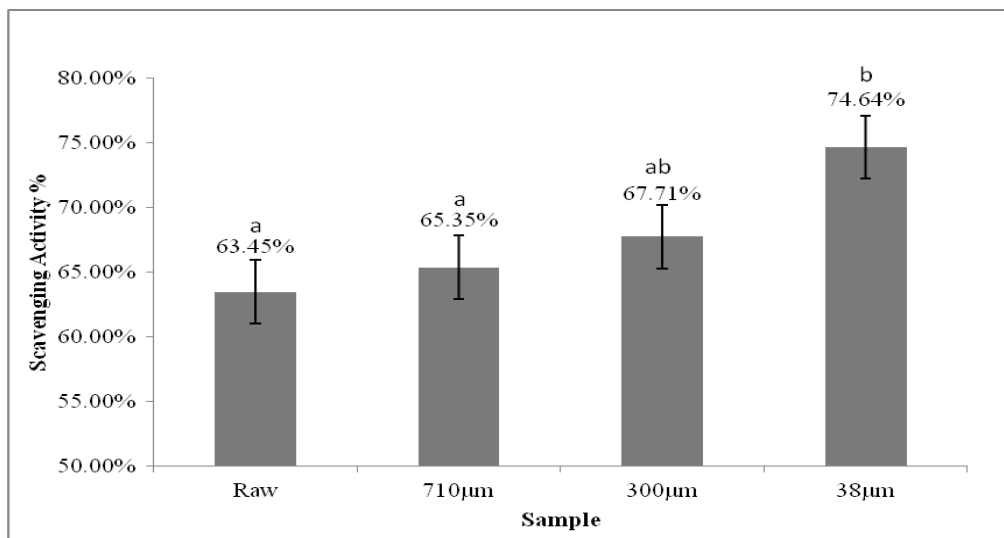
Figure 2. DPPH radical trapping activity % for four different size EBN samples

*Correlation Relation between Antioxidant Tests Results*

Table 1 shows the relationship between the results of antioxidant tests of DPPH radical scavenging activity test, superoxide anion radical scavenging method and metal chelation on the antioxidant activity of EBN. DPPH test results showed a positive and significant correlation with superoxide anion radical trapping activity test ( $r=0.612$ ,  $p<0.05$ ) and metal chelating methods ( $r=0.796$ ,  $p<0.01$ ). In this study, a strong correlation between these antioxidant tests showed that compounds in edible bird's nest could remove free radicals DPPH and

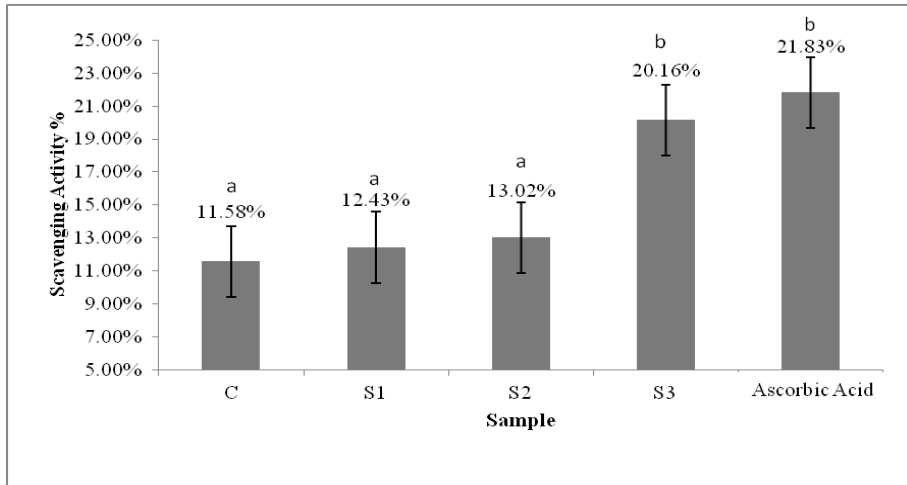
superoxide anion and also had the metal chelating ability (Wong *et al.*, 2006). EBN with small size had higher antioxidant activity and solubility than the other samples.

Antioxidant ability of edible birds' nests is not limited to the ability to trap free radicals but can be influenced by the redox properties of molecules, which allow them to act as reducing agent, hydrogen donor, oxygen radical scavenger and metal chelator (Javanmardi *et al.*, 2003). The positive relationship further supported the results of the antioxidant activity of all of the antioxidant tests which indicated that when the particle size decreased the antioxidant activity tended to increase.



Note: Bars show the mean ± standard deviations from the experimental replicates, n = 3, a-b Bars with different letters indicate significant differences at  $p < 0.05$ , C shows EBN original size, S1 shows 710µm sized EBN, S2 shows 300µm sized EBN, S3 shows 38µm sized EBN

Figure 3. Scavenging activity of superoxide anion radical % in different size EBN samples



Note: Bars show the mean ± standard deviations from the experimental replicates, n = 3, a-b Bars with different letters indicate significant differences at p < 0.05, Ascorbic acid shows antioxidant reference, C shows original size EBN, S1 shows 710µm sized EBN, S2 shows 300µm sized EBN, S3 shows 38µm sized EBN

Figure 4. Metal chelating activity % in different sample size EBN samples

Table 1: Correlation coefficient between antioxidant test results

Test <sup>1</sup>	PL	SOA
DPPH	0.796**	0.612*
PL		0.804**

<sup>1</sup>DPPH shows 2,2-difenil-1-pikrilhidrazil radical scavenging activity test, PL shows metal chelation method, SOA shows superoxide anion radical scavenging activity test

\*\* p<0.01, \*p<0.05

*Determination of Acid Value of TBARS Reactive Materials*

The oxidative stability of food especially in meat and meat products could be determined by TBARS (Robards *et al.*, 1988). According Pfalzgraf *et al.* (1995), malondialdehyde (MDA) formed during lipid oxidation and a red pigment was formed in the presence of thiobarbituric acid. Active methylene group of TBA which reacted with MDA was seen in the maximum value of 532-535 nm (Sinnhuber *et al.*, 1958). TBA threshold value before rancidity and identification flavors of 'warmed over' was

between 0.5-1.0 mg malonaldehyde/kg samples (Tarladgis *et al.*, 1960). This increase could probably be due to lipid peroxidation leading to oxidative rancidity. TBARS values of samples ranged from 0.08919 ± 0.022 mg MDA/kg sample on the first day of storage to 0.29267 ± 0:03 mg MDA/kg sample on the 12th day of the storage period (Figure 5).

Induction period (IP) in chemical kinetics is the early stages of slow chemical reaction and after the IP, the reaction speeds up (IUPAC, 1997). In the early stages of oxidation, the IP is the period in which rancid smell cannot be detected and the food



quality is acceptable to consumers. This is followed by the propagation phase where drastic chemical changes occur to produce foreign odors and flavors. Thus, the IP has been the standard guide for shelf life study that a longer IP gives a longer food shelf life and effort is often taken to maintain the food system in the IP (Labuza and Dugan Jr.,

1971; Lundberg, 1962; Zhu *et al.*, 2012). C, S2 and S3 samples showed the termination of the induction period on the third day with a significant increase in TBARS value on day 6 ( $p < 0.05$ ). The S1 sample showed expiry induction on the first day and a significant increase in TBARS value at day 3 ( $p < 0.05$ ).

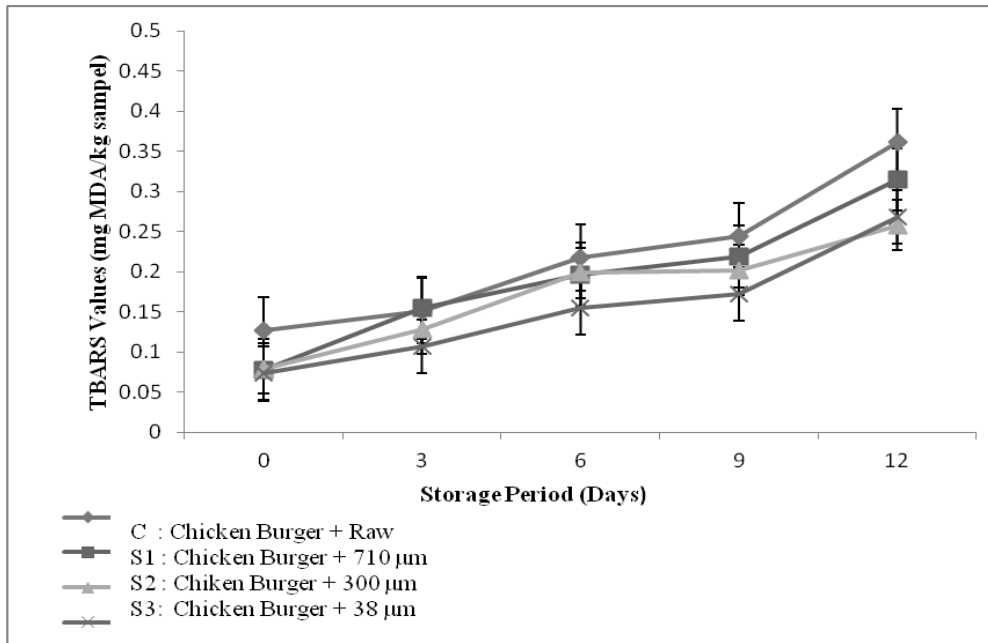


Figure 5. TBARS values (mg MDA/kg sample) for four chicken burgers in cold storage ( $4 \pm 1^\circ\text{C}$ ) for 12 d

TBARS values for all samples showed no significant difference ( $p > 0.05$ ) on the first day of storage. At the end of storage, TBARS values of C samples remained higher than other treatment samples. C and S1 samples did not show a significant difference ( $p > 0.05$ ), while S2 and S3 samples showed significant differences with sample C ( $p < 0.05$ ). This means that EBN of smaller size act more efficiently as a natural antioxidant that can enhance oxidative stability of chicken burgers during

refrigerated storage. In addition, samples C and S1 showed higher TBARS values than the others. This meant that the rate of lipid oxidation occurred at a faster rate than samples of other treatments. High acceleration rate of lipid oxidation may also be caused by the effects of cooking at high temperatures because cooking is one of the major pro-oxidation processes in the preparation of meats (Chan, 2008; Jo *et al.*, 2003).

*Cooking Loss*

Figure 6 shows the cooking loss between treatments throughout the storage period from the first day to the 12<sup>th</sup> day. There were significant differences in cooking loss of chicken burgers for all treatments throughout the storage period ( $p < 0.05$ ). Chicken burger incorporated with EBN sized 38 $\mu\text{m}$  (S3) had a lower cooking loss during storage and this may be due to the high protein interaction. Cooking loss is due to loss of fluid and solutes from the meat while cooking. Increase in temperature will cause the liquid which is the main ingredient shows a decrease in the level (Heymann *et al.* 1990). According to Aaslyng *et al.* (2003), the loss of fluid from the flesh caused by heat and protein denaturation will cause a low

level of liquid trapped between protein structures.

According to Foegeding (1988), the process of cooking will cause the protein to denature and aggregate to form a three-dimensional cross linked gel matrix and water and fat are trapped physically and chemically between 3-dimensional matrixes. Therefore, with the same protein content, the higher interaction caused by higher surface area of protein can bind more fat and water and reduce cooking loss. Range of cooking loss was less than 15% of all available treatments and this percentage is commonly accepted for processed meat products (Ambrosiadis *et al.*, 1996). The different cooking loss may be due to different rates of protein denaturation resulting in different rates of structural changes in meat (Aaslyng *et al.*, 2003).

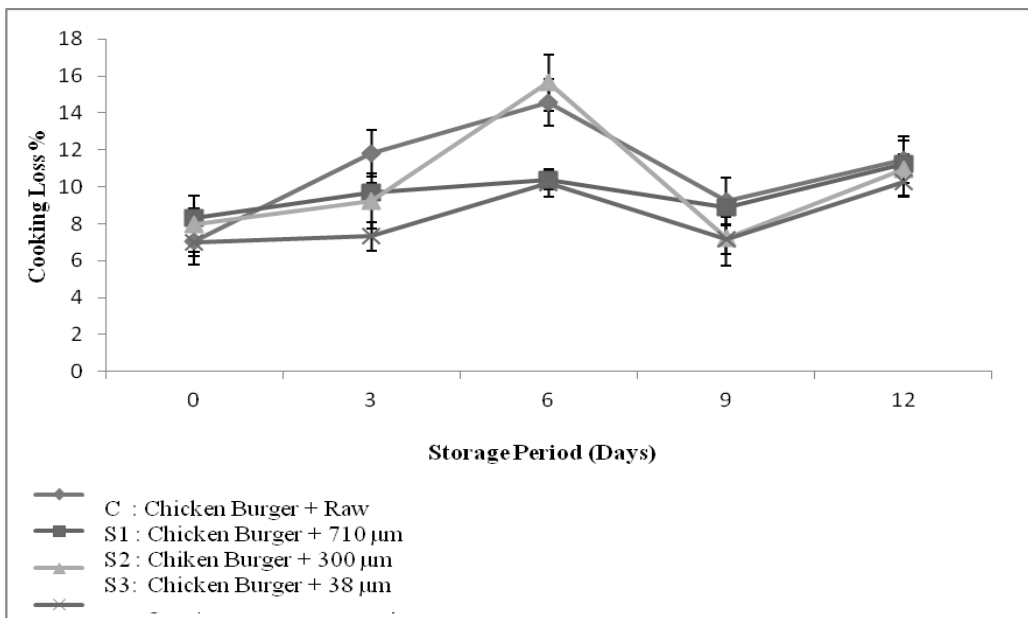


Figure 6. Cooking loss % for 4 treatment samples of chicken burger in cold storage ( $4 \pm 1^\circ\text{C}$ ) for 12 d

*Water Holding Capacity*

Water holding capacity is important and it is a key aspect in determining the quality of meat and meat products (Huff-Lonergan, 2002). WHC values for all samples ranged from  $2.96 \pm 0.29\%$  to  $0.18 \pm 3.53\%$  at the first day of storage and  $2.2 \pm 0.14\%$  to  $2.88 \pm 0.18\%$  on the last day of storage (Figure 7). All samples did not show significant difference in the first and last day ( $p > 0.05$ ). S1, S2 and S3 samples did not show significant differences ( $p > 0.05$ ) throughout the storage period. However, only sample C showed a decrease ( $p < 0.05$ ) in the WHC value after day 6. This shows that the

addition of EBN of varying size as antioxidant in chicken burger has no effect on the water holding capacity in chicken burger.

WHC is extremely sensitive to pH value changes of meat. Increase in pH will increase the WHC value of the meat (Alina, 2000). According to Wiklund and Johansson (2011) pH value plays an important role in influencing WHC, color and shelf life of venison meat. Thomsen and Zeuthen (1988) stated that the pH of meat was an important aspect in determining the properties of meat products. An increase in pH would increase the WHC in mechanically deboned pig meat.

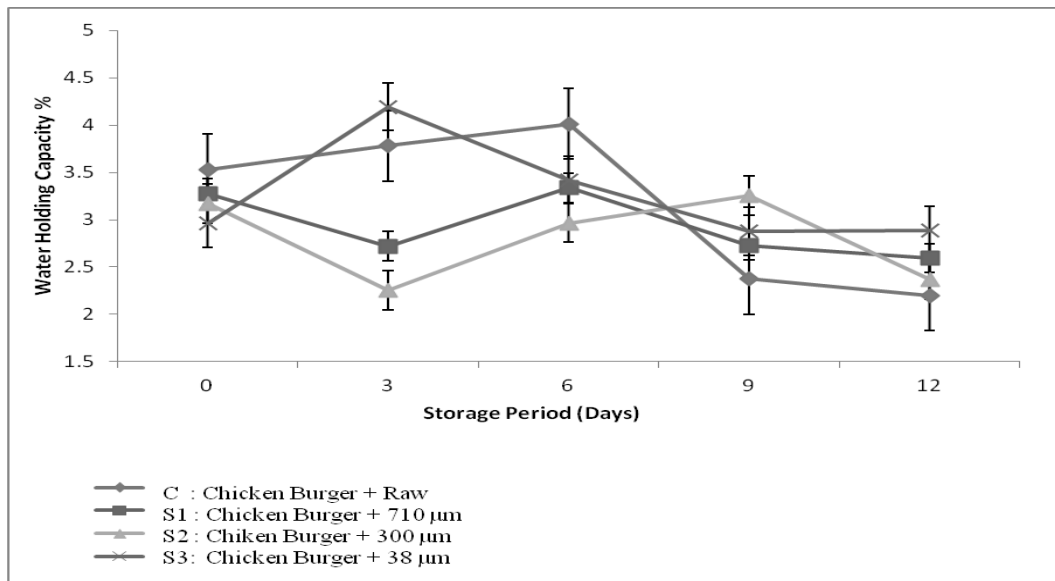


Figure 7. Water Holding Capacity (%) for 4 chicken burger samples in cold storage ( $4 \pm 1^\circ\text{C}$ ) for 12 d

*pH Value*

The pH values of all samples ranged from  $5.65 \pm 0.04$  to  $5.86 \pm 0.05$  on the first day and  $5.59 \pm 0.15$  to  $5.9 \pm 0.08$  on the last day of storage (Figure 8). The last day storage the pH values were lower than the first day of storage. However, it was found

that the pH value of the sample did not influence the WHC of the sample.

WHC value depends not only on the pH value only but also by the ingredients in the formulation, processing techniques, applied temperature and processing time. WHC

functional properties are also affected by the ability of the gel matrix meat protein complex to trap excess water and fat (Alina, 2000; Foegeding and Lanier, 1987).

The pH values for all samples were found to increase significantly ( $p < 0.05$ ) quickly in the first 3 days but decreased following that. The increase of the pH in the first 3 days may be due to lactic acid content in chicken burger that decreases during storage at 4°C (Chin, 1995) and also may be

due to the antioxidant effects which retard the formation of free fatty acids (Kenawi *et al.*, 2011). However, the pH value decreased after 3 days of storage may have been caused by increased fatty acid oxidation of lipids and decreased antioxidant efficiency. The antioxidant system remains efficient in the first stage of storage and efficiency tends to decrease after the first stage with an increase in the rate of lipid oxidation.

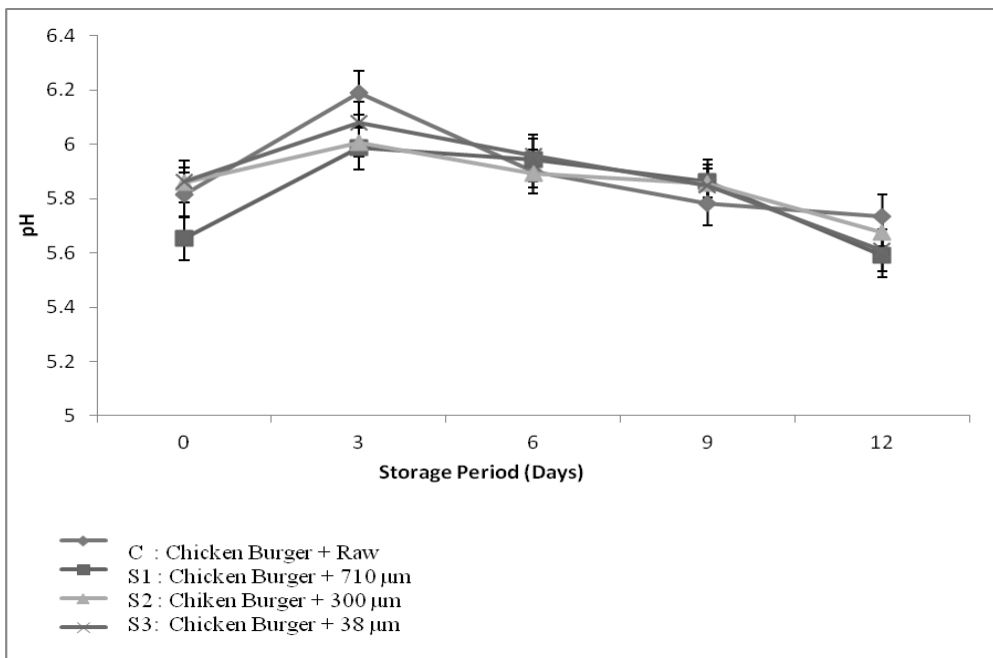


Figure 8. pH value for 4 chicken burger samples in cold storage ( $4 \pm 1^\circ\text{C}$ ) for 12 d

*Texture Profile Analysis*

The levels of sample hardness were higher on the last day of storage ranging from  $483.347 \pm 1.02$  to  $488.068 \pm 0.13$  compared to the first day of storage with a range from  $480.445 \pm 0.16$  to  $482.936 \pm 1.18$  (Figure 9). On the last day of storage, all samples showed a significant difference ( $p < 0.05$ ) with sample C. The hardness of sample C was quite high on the last day of storage which might be due to the high cooking loss where more fat and water had been removed. However, apart from S1 samples, all samples showed no significant difference ( $p > 0.05$ ) when compared to the duration of storage. This might be due to the quantity of EBN added to formulations was low which only

2% per 100 g sample and had no significant effect on the texture of chicken burgers.

Various rheological properties, physical and chemical properties will determine the texture of meat. Specifically aging meat is characterized as physical and chemical reactions that will cause a change in texture (De Huidobro *et al.*, 2005). Hardness is the main factor that determines the commercial value of meat (Shi *et al.*, 2013). In addition, the protein also has a great influence on the texture characteristics of meat (Karlović *et al.*, 2009). Texture analysis was performed on samples of chicken burgers using the texture tool Shimadzu AGS -J 500N. The shear stress was not tested on the samples as the pieces of meat were not uniform in size and thus could provide great variation in sample size reading.

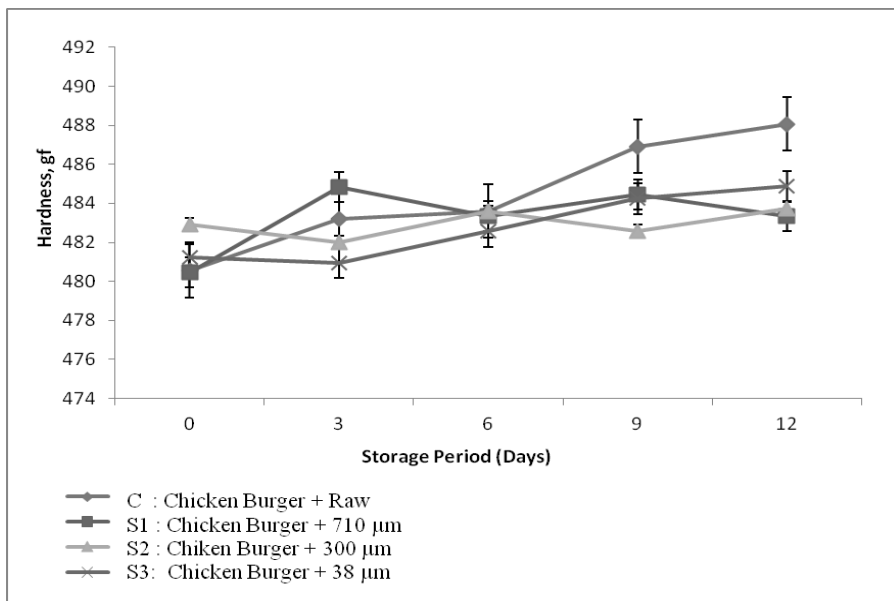


Figure 9: Hardness value (gf) for 4 chicken burger sample in cold storage ( $4 \pm 1^\circ\text{C}$ ) for 12 d

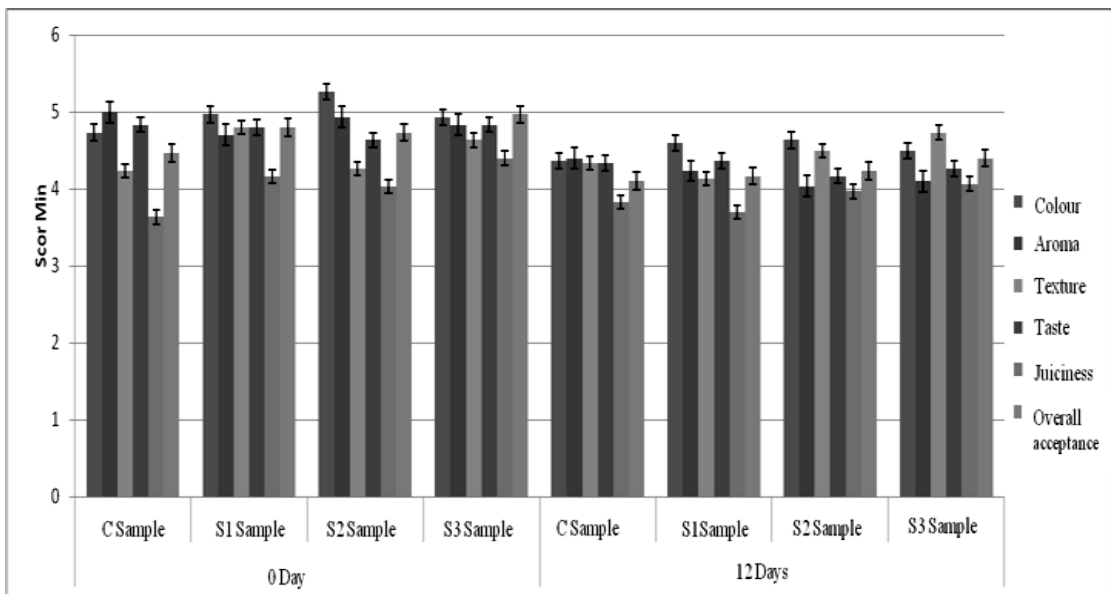
Texture profile analysis (TPA) was tested in 2-time cycle probe which was lowered carefully so as to not cause total deformation of the samples. TPA results showed acts of sample hardness along the cold storage.

*Sensory Evaluation*

Sensory attributes of a food is a determinant affecting consumer acceptance of the food. Added antioxidant in food products should be suitable, non-toxic and does not contribute to negative effects such as odor, taste and color surprises. In addition, the antioxidant must continuously provide effective antioxidant effects before and after processing (Chan 2008; Schuler 1990). Lipid oxidation in chicken burger after cold storage for 12 d can cause rancid odor or "warmed-over" flavor and could affect the sensory

attributes of the other. Thus, the sensory evaluation was conducted to assess the impact of EBN incorporation in chicken burger to its sensory acceptance.

Figure 10 shows the mean scores of sensory attributes for the four chicken burger samples before and after storage. Mean scores for the attributes of color, aroma, texture, flavor, juiciness and overall acceptance ranged from 4.7-5.3, 4.7-5.0, 4.2-4.8, 4.6-4.8, 3.6-4.4 and 4.5-5.0, respectively, at the first day of storage. All samples showed no difference in the mean scores for all attributes studied ( $p > 0.05$ ) and no significant difference ( $p > 0.05$ ) between the first day and the last day of storage. This means the addition of EBN into a chicken burger does not affect the color, aroma, taste, texture, and overall acceptability of juiciness chicken burger review.



Notes: Bars show mean ± standard deviation value from the replicates experiment, n=30, C shows Chicken Burger + raw EBN, S1 shows Chicken Burger + EBN sized 710µm, S2 shows Chicken Burger + EBN sized 300µm, S3 shows Chicken Burger + EBN sized 38µm

Figure 10: Mean scores of sensory attributes of chicken burgers 4 x sample survey on the first and last days of storage

As for the last day of storage, the mean scores were 4367-4633, 4033-4400, 4133-4733, 4167-4367, 3700-4067 and 4100-4233 for the attributes color, aroma, texture, flavor, juiciness and overall acceptance, respectively. All samples showed no significant difference in mean scores for all attributes studied ( $p > 0.05$ ) and also no significant difference ( $p > 0.05$ ) with the first day of storage. This may be due to the antioxidant power of EBN slowing down the process of lipid oxidation. In addition, the storage temperature was not uniform and the temperature sometimes dropped below 4°C (< 4°C) which might have also slowed the process of lipid peroxidation. According to Boles (1990), the taste threshold for rancid odor or "warmed -over" flavor in meat products is between 0.5 - 2.0 mg MDA/kg sample. TBARS value range for all chicken burger samples studied was between 0258-0362 mg MDA/kg sample. Therefore, samples of chicken burgers on the last day of storage did not affect the sensory acceptance by the panel. In addition, the results of chemical analysis showed a decrease in the pH, WHC and improvement in the texture of the chicken burger at the end of the storage period, but it did not affect the panel's perception of flavor, juiciness and texture of chicken burger studied.

### Conclusion

Results showed that EBN of smaller size exhibited higher antioxidant activity than the larger size based on radical scavenging activity test, superoxide anion radical scavenging method and metal chelation activity test. The results showed that an increase in oxidative stability during storage of chicken burger with EBN sized 300 µm and 38 µm compared to chicken burger with raw EBN. In addition, physicochemical analysis showed that addition of EBN microparticulates reduced cooking loss of

chicken burger while the texture, water holding capacity and pH were not affected. All samples showed no significant differences in mean scores for all attributes during the storage period. Edible bird nest is a highly potential natural antioxidant and its antioxidant activity is increased with the size reduction. Edible bird nest is also capable of replacing synthetic antioxidants in meat products as it is effective in reducing lipid peroxidation without affecting most of the physicochemical and sensory attributes.

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