

The antioxidant effects of *Cosmos caudatus* and *Polygonum minus* in refrigerated duck meatballs

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Abstract: This study evaluated lipid oxidation in refrigerated ($\pm 4^{\circ}\text{C}$) duck meatballs treated with novel antioxidants over 21 days of storage. The duck meatballs were treated with a control substance, *Cosmos caudatus* (ulam raja) or *Polygonum minus* (kesum) extract, or BHT (butylated hydroxytoluene), and data was collected every three days. These results showed that *Cosmos caudatus* and *Polygonum minus* had better antioxidant effects on duck meatballs than BHT or the control. Folding and microbial potency test results were not significantly different among the three antioxidants tested but were better in antioxidant-treated samples than in control samples. However, *Cosmos caudatus* and *Polygonum minus* were slightly more effective in preventing microbial growth. This result suggests that *Cosmos caudatus* and *Polygonum minus* may be potentially useful natural resources for enhancing the shelf life of duck meatballs.

Keywords: duck meat, meatballs, lipid oxidation, antioxidant, refrigerated temperature, shelf life

Introduction

A number of researchers have focused on the application of natural antioxidants to various meat products. For example, rosemary oil extract, rosemary water extract, rosemary oil and water extract, garlic, lemon fiber, and orange fiber extract have all been tested in meatballs (Fernández-López *et al.*, 2005); fresh garlic, garlic powder, and garlic oil have been tested in chicken sausages (Sallam *et al.*, 2004); and rosemary has been tested in goat sausages (Nassu *et al.*, 2003). In addition, grape antioxidant dietary fiber has been tested in chicken hamburgers (Sáyago-Ayerdi *et al.*, 2009); rosemary extract has been tested in beef burgers (Georgantelis *et al.*, 2007); grapeseed extract and pine bark extract have been tested in cooked ground beef (Ahn *et al.*, 2002); and rosemary and green tea extract have been tested in surimi (Pérez-Mateos *et al.*, 2006).

Cosmos caudatus is a popular herb in Malaysia and is locally known as ulam raja (Ong and Norzalina, 1999). This plant is native to tropical regions of the Americas and has been naturalized in Java, where it is often cultivated as an ornamental plant (Fuzzati *et*

al., 1995). *Polygonum minus*, which is locally known as kesum, has some similarities to the synthetic antioxidant BHT and thus may be a potential source of natural antioxidants (Huda-Fauzan *et al.*, 2007).

This study investigated the potency of *Cosmos caudatus* and *Polygonum minus* as antioxidants in duck meatballs. The objective of this study was to determine whether natural antioxidants derived from *Cosmos caudatus* and *Polygonum minus* are sufficiently potent to replace the synthetic antioxidants currently used in meatballs. BHT was used to represent synthetic antioxidants.

Materials and Methods

Cosmos caudatus and *Polygonum minus* leaves were bought at a local wet market in Penang, Malaysia. Each leaf was soaked in distilled water (9:1) and homogenized using an IKA®T25 digital homogenizer (Ultra-Turrax, Germany). The homogenate was then filtered with Whatman number 1 filter paper. The filtrate was frozen in a blast freezer (Rhinox, Italy) at -20°C for 30 minutes and subsequently dried in a freeze dryer at -46°C . Commercially prepared

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food-grade BHT (Euro Chemo-Pharma Sdn. Bhd., Malaysia) was used for comparisons with the natural extracts.

Duck meatballs were manufactured according to the following formula: 70% Peking duck meat (deboned meat from live duck obtained in Southern Malaysia), 8% cassava flour, 2.3% garlic, 1.50% fried onion, 0.2% pepper, 2.5% salt, and 15.5% cool water. The 150 ppm antioxidant per kg duck meatballs formulation was dissolved in the water. The flour and the spices were mixed together with the water and then mixed with the meat for 5 minutes using a mixer (Robot Coupe, France). The duck meatball was shaped by hand, preheated (40°C for 20 min) and then heated (95°C for 20 min). Meatballs with relatively similar diameters (± 3 cm) and masses (± 15 g) were chosen for evaluation.

The samples were placed into plastic containers, sealed with one layer of a semi-permeable film vacuum packaging (PE bag, 150mm x 250mm, 70 μ m thick, moisture and oxygen proof), and stored at $\pm 4^\circ\text{C}$. Sampling and storage conditions were recorded at 0, 3, 6, 9, 12, 15, 18, and 21 days of storage time. Every sample was analyzed promptly as follows:

pH

The pH value was determined by balancing 5 gr sample, then put it in a breaker glass and then dissolved with 45 ml aquadest using a homogenizer (IKA®T25 digital Ultra-Turrax, Germany) and then measured the pH value by using a pH meter (Mettler Toledo delta 320, Switzerland).

Thiobarbituric acid reactive substances (TBARS) levels

TBARS content was measured with Pikul's aqueous extraction method as modified by Ulu (2004). A 10-g sample was homogenized with 35 ml of cold (4°C) extraction solution containing 4% percholic acid and 1 ml of BHA in homogenizer (IKA®T25 digital Ultra-Turrax, Germany) at 13,800 rpm for 1 min. The blended sample was filtered through Whatman number 4 paper into a 50-ml Erlenmeyer flask and washed with 5ml of distilled water. The filtrate was adjusted to 50 ml with 4% perchloric acid, 5ml of the filtrate was added to 5 ml of 0.02 M TBA. Test tube was heated in thermostatically controlled water bath for 40 min at 80°C to develop the malonaldehyde-TBA complex and then cooled for 10 min with cold tap water. The absorbance was determined by a UV-visible recording the spectrophotometer (model UV-160A, Shimadzu, Japan); UV scanning at 532 nm against a blank containing 5ml of distilled water and 5 ml of 0.02 M TBA solution.

Peroxide value (POV)

POV was determined according to AOAC International method as described by Sallam *et al.* (2004). The sample (3 gr) was weighed in a 250-ml glass stoppered Erlenmeyer flask and heated bath at 60°C for 3 min to melt the fat, then thoroughly agitated for 3 min with 30 ml acetic acid-chloroform solution (3:2v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman filter paper number 1 to remove meat particles. Saturated potassium iodide solution (0.5 ml) was added to filtrate and continued by adding starch solution. The titration was allowed to run against standard solution of sodium thiosulfate (25g/l). POV was calculated and expressed as milliequivalent peroxide per kg of sample:

$$\text{POV (meq/Kg)} = \frac{S \times N}{W} \times 100$$

Where S is the volume of titration (ml) and N is the normality of sodium thiosulfate solution (N=0.01), and W is the sample weight (g).

Color determination

Color measurement was done based on CIE (1978) system color profile of lightness (L^*), redness (a^*), yellowness (b^*), chroma (c^*), and hue angle (H°) value was measured by a reflectance of colorimeter (Minolta Spectrophotometer CM-3500d, Japan). The colorimeter was calibrated throughout the study using a standard white ceramic tile.

Folding test

Folding test was determined according to Yu (1994). The test piece was held between thumb and forefinger. The specimen was cut into a round shape with 3 mm thick. Specimen was folded to observe the way it broke. Six replicates were tested for each sample evaluated by five-stage method as follows: (1) Breaks by finger pressure, (2) Cracks immediately when folded into half, (3) Cracks gradually when folded into half, (4) No crack showing after folding in half, and (5) No crack showing after folding twice.

Aerobic plate counts

Aerobic plate counts were performed according to the guide described by Sallam *et al.* (2004). Duck meatball sample (25g) was homogenized with 225 ml of steril peptone water (1g/l) in a laboratory homogenizer (Bag Mixer, Interscience, France) and serial dilution were prepared, then 0.1 of each dilution was spread with disposal spreader (SPL Labware) on triplicate plates of Merck plate count of agar. After

48-h incubation at 25°C, colonies were counted and the result was expressed as \log_{10} CFU/g of duck meatball sample.

Mold counts

Mold counts were made according to the guide described by Abdullah and Ahmad (2004). Duck meatball sample (25g) was homogenized with 225 ml of steril peptone water (1g/l) in a laboratory homogenizer (Bag Mixer, Interscience, France) and serial dilution were prepared, then 0.1 of each dilution was spread with disposal spreader (SPL Labware) on triplicate plates of Merck potatoes dextrose of agar. After 4-5 days incubation at 25°C, colonies were counted and the result was expressed as \log_{10} CFU/g of duck meatball sample.

Statistic analysis

The data collected was analyzed by using the statistic package for social science (SPSS) version 11.5. The means of treatments showing significant differenced ($P < 0.05$) were subjective to anova test.

Results and Discussion

pH value

The initial pH range of the meatballs was from 6.18-6.20 in all treatments, as shown in Figure 1. The pH was slightly decreased after 3 days of storage time but increased thereafter. The type of antioxidant had no significant effect on pH, indicating that the pH value was not affected by antioxidant treatments, but showed significant changes with increased storage time. Similarly, Sallam *et al.* (2004) reported that the pH values of sausages tended to increase significantly ($P > 0.05$) with increased storage time. A similar result was also reported by Biswas *et al.* (2004) in a study of precooked pork patties treated with a mixture of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); in that study, the pH values increased significantly ($P < 0.05$) during storage regardless of the antioxidant treatment used.

The increase in pH may be due to the presence of microorganisms in the duck meatballs. Both bacteria and mold can secrete substances that increase pH; therefore, their presence will increase pH over longer storage times. As noted by Jay & Shelef (1976), the increase in the pH of meat can be caused by the microbial growth and the release of NH_3 from amino acids. This study showed an initial decrease in pH in the earliest days of storage but an increase at longer storage times. A decrease and subsequent increase in pH is also found in pork patties, as reported by Mc Carthy *et al.* (2001). They found variable pHs for fresh

patties stored for 0, 3, 6, and 9 days and treated with rosemary (5.81, 5.77, 5.79, and 5.92, respectively), aloe vera (5.81, 5.71, 5.60, and 5.67, respectively), or fenugreek (5.81, 5.77, 5.67, and 5.73, respectively). On the other hand, ginseng-treated (5.91, 5.76, 5.91, 6.24, respectively) and mustard-treated (5.91, 5.90, 5.76, 6.24, respectively) patties that were processed from previously frozen meat and stored under chilled (4°C) display conditions also exhibited decreasing and increasing pH during storage.

TBARS levels

The effect of different antioxidant treatments on TBARS levels is shown in Figure 2. The control sample (no antioxidant addition) had a higher TBARS level than did samples that were subjected to treatment with *Cosmos caudatus*, *Polygonum minus*, or BHT. The TBARS levels of the *Cosmos caudatus*- and *Polygonum minus*-treated samples were significantly ($P > 0.05$) lower than that of the BHT-treated sample. Generally, the TBARS level significantly ($P > 0.05$) increased with storage time, indicating a decrease in quality. However, the increase in TBARS appeared to taper off or decrease at the last day of analysis in some of the treatments.

The results of this study confirm that *Cosmos caudatus* and *Polygonum minus* can significantly delay lipid oxidation and reduce the risk presented by lipid oxidation products. In this capacity, they outperform BHT, a synthetic antioxidant that is currently used in the commercial food industry.

Other researchers have reported similar trends, and other studies of natural antioxidants also showed their potential as substitutes for synthetic antioxidants. For example, Racanicci *et al.* (2004) also found an increase in TBARS in stored more than 8 days. Lipid oxidation was observed in meatballs subjected to each of five different treatments (control, 0.05% dittany, 0.10% dittany, 0.05% rosemary, 0.10% rosemary), as demonstrated by an increase in TBARS value; however, some samples showed a decrease in TBARS level at 10 days. These authors also found that dittany and rosemary at a concentration of 0.10% protected the product significantly.

Similar results were noted by Juntachote *et al.* (2007), who reported increasing TBARS levels over time (0, 7, and 14 days) in cooked ground pork subjected to various treatments. The TBARS levels were 1.40, 5.61, and 6.00 mg MDA/kg meat in the control sample, 0.73, 3.90, and 4.44 mg MDA/kg meat in a sample treated with ethanolic galangal extracts (0.15%), and 1.06, 4.95, 5.12 mg MDA/kg meat in a sample treated with dried galangal powder

(0.17%).

Thomas *et al.* (2006) reported that TBARS in emulsified and restructured buffalo meat nuggets showed significantly ($P < 0.05$) higher TBARS values after 10, 25 and 30 days of storage compared with the TBARS value measured at the preceding time point.

Peroxide value

The effect of different antioxidant treatments on peroxide value is shown in Figure 3. At 0 days, the peroxide values ranged from 0.71-0.92 (mEq/kg sample). Overall, peroxide values were higher in the control sample than in the other samples at all time points, although peroxide values increased with increasing storage time in all samples. The most potent antioxidant (i.e., the treatment that produced the lowest overall peroxide values) was *Cosmos caudatus*, followed by *Polygonum minus*, and finally BHT.

Others have also reported increased peroxide values over time in products with or without antioxidants added. Sallam *et al.* (2004) reported an initial POV of chicken sausage is 6.32 and increased values after 21 days of storage: 4.92-6.22 (fresh-garlic formulation), 6.68-6.91 (garlic-powder formulation), 7.74-8.88 (garlic-oil formulation), and 7.21 (BHA formulation). These values were significantly lower than that of the control (15.61). Some of the treated samples also showed higher POVs at earlier time points, the levels declining toward the end of storage. Georgantelis *et al.* (2007) found that the peroxide values of frozen (18°C) beef burgers treated with rosemary were 0.24, 0.45, 0.66, 1.05, 1.27, 1.46, and 1.59 (mEq peroxides/kg fat) at 0, 30, 60, 90, 120, 150, and 180 days of storage time, respectively.

POV measurements in cooked plain meat loaf made with ground goat meat increased over time, as reported by Rhee & Myers (2003); the POVs of plain meat loaf were 0.38, 1.33, and 2.40 at 0, 3, and 6 days of aerobic storage at 4°C. Meanwhile, Waszkowiak *et al.* (2007) found that the peroxide values in sausages subjected to one of three treatments (rosemary extract, collagen fiber preparation impregnated with rosemary extract, and collagen hydrolysate impregnated with rosemary extract) were lower than those of untreated sausages. The POVs (in mEq O₂/kg lipids) of control sausages were 2.84, 2.88, and 3.09 at 1, 3, and 21 days of storage time, whereas those of sausages treated with rosemary extract (1.87, 1.92, and 2.36), collagen fiber preparation impregnated with rosemary extract (1.87, 1.55, and 1.84), or collagen hydrolysate impregnated with rosemary extract (2.40, 2.25, and 2.88) were lower.

Color

Changes in the color of duck meatballs over time are shown in Figure 4. Antioxidant treatment is an attractive strategy for slowing myoglobin oxidation and preventing color change in meat (Connolly and Decker, 2004). Furthermore, Sánchez-Escalante *et al.* (2001) explained that color protection against metmyoglobin formation can be achieved through antioxidant application.

A similar trend in L* (lightness), a* (redness), and b* (yellowness) values was observed by Brannan (2009), who showed an initial decrease in color values and an increase at later days of storage in ground chicken treated with grapeseed extract and refrigerated. For storage periods of 0, 4, 8, and 12 days, they reported L* values of 62.6, 61.7, 63.0, and 64.0; a* values of 5.1, 4.6, 3.7, and 4.2; and b* values of 9.7, 9.7, 9.2, and 9.4, respectively.

Similar results showing that L* values decreased in the early storage period but increased continuously toward the end of storage was reported in restructured steaks treated with propyl gallate and stored at -29°C (Reverte *et al.*, 2003). These authors reported L* values of 33.3, 30.2, 31.1, and 32.9 at 0, 1, 3, and 6 months of storage in forage-finished steer meat. Restructured steaks from grain-supplemented steers also showed a similar trend, with L* values of 31.9, 30.6, 31.5 and 31.8 at 0, 1, 3, and 6 months of storage.

The a* value increased more in the control sample than in the treated samples over time. Other reports have shown that antioxidant treatment tends to promote lower lightness values upon storage. The a* value of patties decreased when held under chilled (4°C) display conditions for 0, 3 and 6 days after treatment with different antioxidants (ginseng: 6.07, 4.89, 2.92; mustard: 7.18, 6.82, 4.43; sage: 5.75, 5.00, 2.62; tea catechins: 6.76, 6.28, 5.49). In contrast, a* values increased at day 3 but decreased at day 6 in patties treated with aloe vera (6.75, 7.11, 4.05), rosemary (5.59, 6.52, 4.73), or soy protein (5.84, 6.31, 4.52) (McCarthy *et al.*, 2001). In this study, a similar increase was observed at early time points. However, the data showed a persistent increase in a* value for control (untreated) duck meatballs and meatballs treated with *Polygonum minus* or BHT, whereas the values for duck meatballs treated with *Cosmos caudatus* decreased with increased storage time.

The b* and c* values also decreased at early time points but increased from later time points until the end of 21 days of storage. Hue angle play an important role in determine the colour stability of meat and meat products (Luciano *et al.*, 2009). The hue angle

of raw sirloin steaks was measured in a study by John *et al.* (2005), who showed an increase in hue angle over 21 days of storage. These authors found that hue angles at 7, 14, and 21 days of storage were 34.1, 34.0, and 37.7 for CO packaged samples, 44.6, 46.7, and 61.1 for O₂ packaged samples, and 43.2, 53.0, and 52.7 for vacuum-packaged samples. Similar results were observed for duck meatballs in this study at early storage times, though samples treated with *Polygonum minus* and BHT showed decreasing hue angle values after an initial increase.

Folding Test

The effect of different antioxidant treatments on folding test results is shown in Figure 5. At 0 days, all samples received a perfect score regardless of whether they had been treated with an antioxidant. No significant difference ($P < 0.05$) was observed among the antioxidant treatments at any time point, indicating that the observed decrease in folding test scores over time is more influenced by storage time than by the type of antioxidant. Folding test scores ranged from 3.00-3.08 after 21 days of storage.

Oxidation reduces the nutritional value of meat. The action of free radicals damages proteins, resulting in the loss of their functions. The accumulation of oxidized proteins is promoted by free metals, and active peptides in the meat likely lose their functionality (Descalzo & Sancho, 2008).

The decreasing protein quality of the meat in duck meatballs over storage time caused a decrease in binding between the meat, flour, and other ingredients. Finally, the folding of the duck meatballs will decrease in quality relative to the folding observed on the date of manufacture. Decreased folding values indicate a loss of texture quality.

Aerobic plate counts

The effect of different antioxidant treatments on the aerobic plate count of meatballs is shown in Figure 6. The initial aerobic plate counts were 3.04-3.23 log₁₀ CFU/g (Fig. 6); and during the first 12 days of storage the count in all sample formulations remained below 6 log₁₀ CFU/g. Significant effects for different antioxidant treatments were observed between 15 and 21 days of storage time; at 18 days, the plate count of the control sample (8.06 log₁₀ CFU/g) was significantly higher than those of the BHT-, *Polygonum minus*-, and *Cosmos caudatus*-treated samples (7.25, 7.27, and 7.09 log₁₀ CFU/g, respectively). BHT, *Polygonum minus*, and *Cosmos caudatus* treatment had no significant effects at day 21, however.

Other researchers have studied the antibacterial properties of many natural and synthetic antioxidants. For example, a mixture of rosemary and licorice extracts as reported by Zhang *et al.*, (2009) exhibited strong effects against *Listeria monocytogenes* and other bacteria that can cause meat spoilage.

Camo *et al.* (2008) reported the effects of rosemary active film, oregano active film, and rosemary extract applied to lamb meat before packaging in a high-oxygen atmosphere and storage under illumination (24 h) at $1 \pm 1^\circ\text{C}$ for 0, 5, and 8 days. That study showed an increase in microbial counts in all samples with increased storage time. Control used in study reached the final values of 7-8 log₁₀ CFU/cm². Samples treated with rosemary extract, rosemary active film, or oregano active film showed lower counts throughout the storage period (5-6 log₁₀ CFU/cm²).

Mold counts

The effect of different antioxidant treatments on mold count is shown in Figure 7. Mold was not detected at 0 days storage. At 3 and 6 days, no more than 10 mold colonies were found; this is the minimum number for counting mold in foods (data not shown). At 9 days, the mold plate counts in the duck meatballs were 1.98, 2.12, and 3.23 log₁₀ CFU/g (Fig. 7). A significant effect of antioxidant addition was shown at day 21, when the mold count in the control sample (2.39 log₁₀ CFU/g) was significantly higher than those in the BHT-, *Polygonum minus*-, and *Cosmos caudatus*-treated samples (2.16, 2.20, and 2.21 log₁₀ CFU/g, respectively). Samples treated with BHT, *Polygonum minus*, and *Cosmos caudatus* did not exhibit any significant increases in mold count between 9 and 21 days of storage.

Fernández-López *et al.* (2005) conducted a study of antimicrobial treatments in beef meatballs and noted that no molds or yeasts were detected in any cooked meatball samples. Although this study just focuses on mold count without any specific mold identification in the duck meatballs treated with *Cosmos caudatus* were less susceptible to mold growth than those treated with *Polygonum minus* or BHT, although no significant effect was observed by the end of storage period.

Conclusions

Both *Cosmos caudatus* and *Polygonum minus* showed potential antioxidant effects on duck meatballs stored at $\pm 4^\circ\text{C}$ for 21 days. In some assays, *Cosmos caudatus* and *Polygonum minus* treatment gave better results than the synthetic antioxidant BHT. However, the antioxidants used in this study

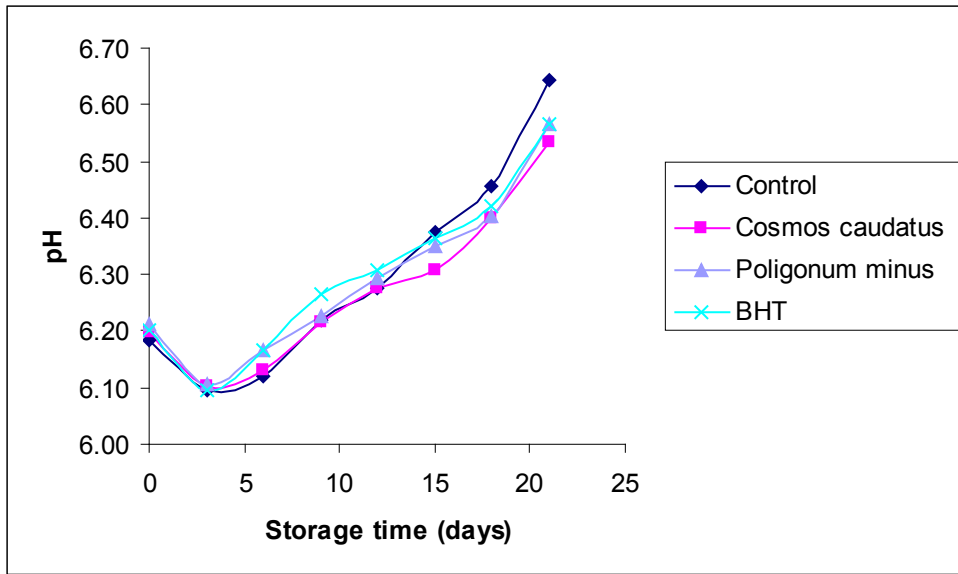


Figure 1. pH of duck meatballs treated with *Cosmos caudatus*, *Polygonum minus*, and BHT over 21 days of storage

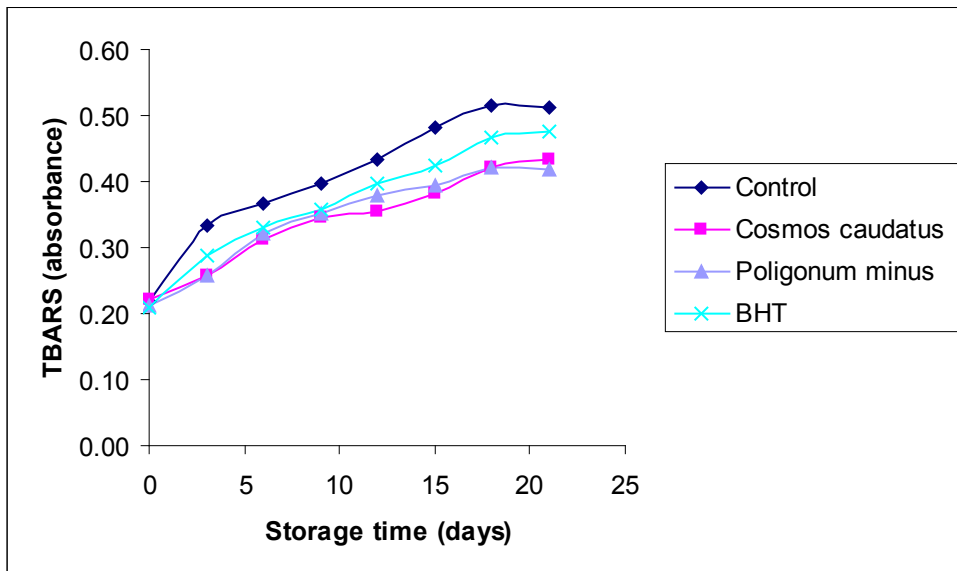


Figure 2. TBARS level of duck meatballs treated with *Cosmos caudatus*, *Polygonum minus*, and BHT over 21 days of storage

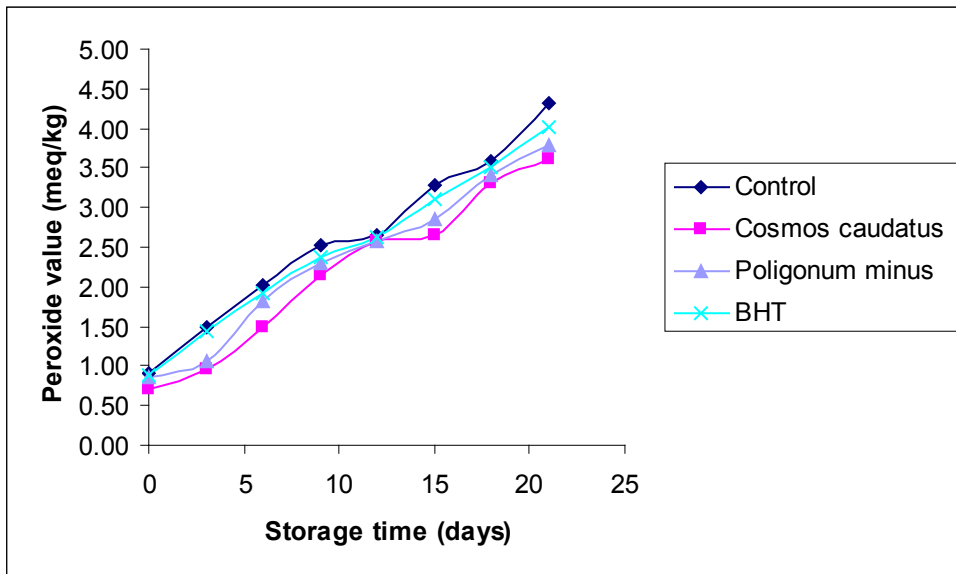
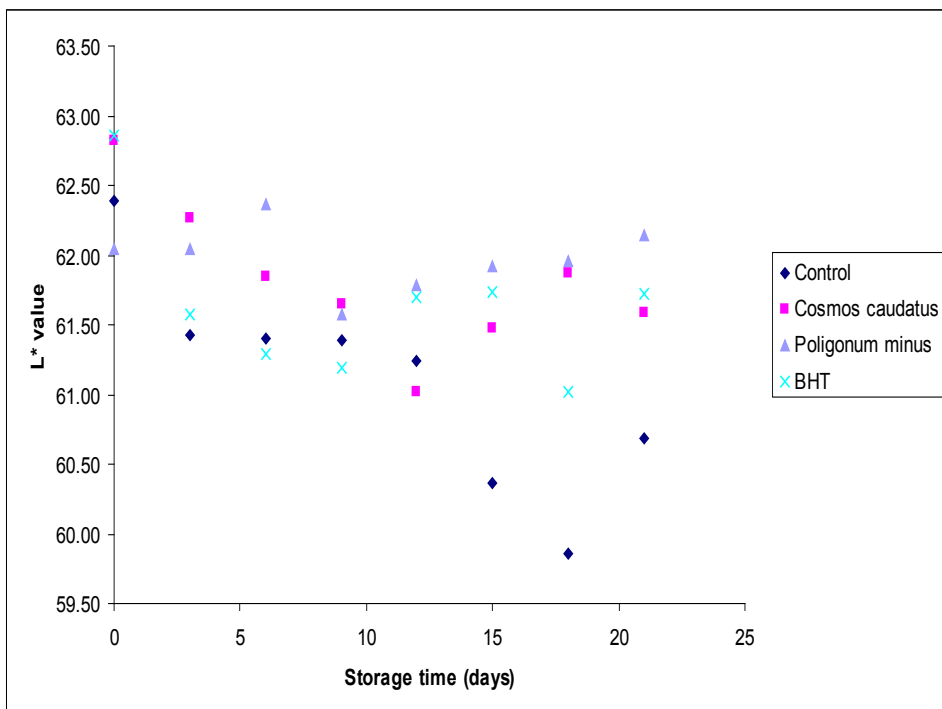
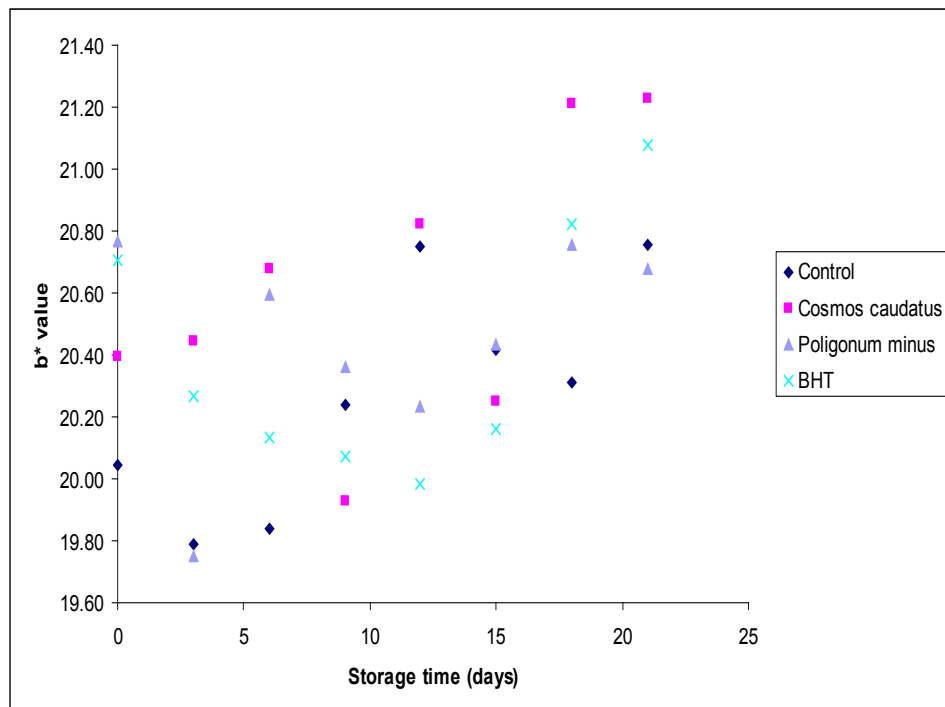
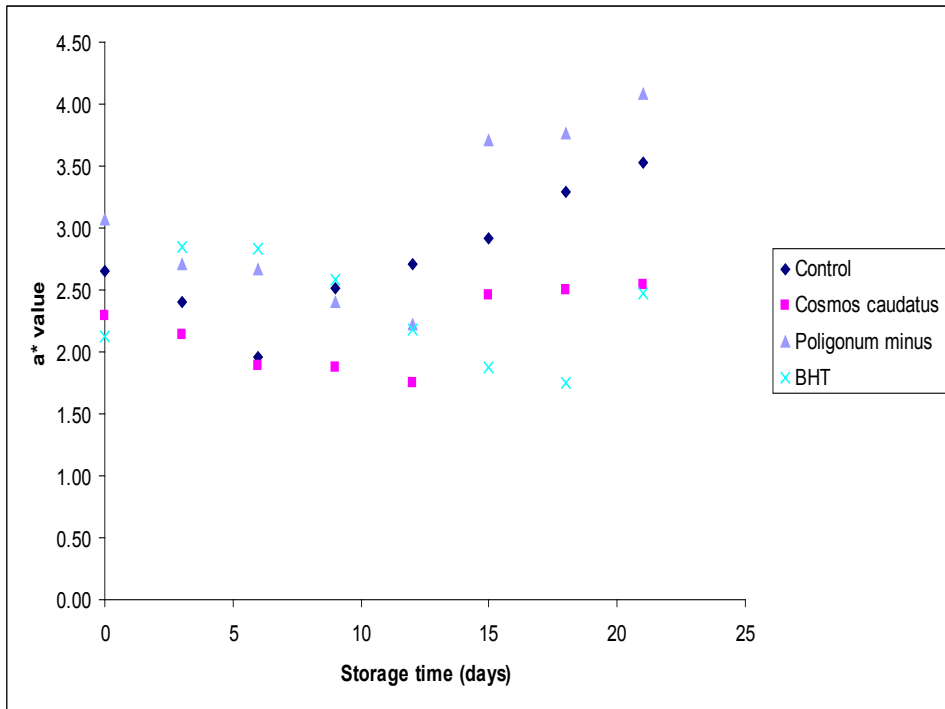


Figure 3. Peroxide value of duck meatballs treated with *Cosmos caudatus*, *Polygonum minus*, and BHT over 21 days of storage





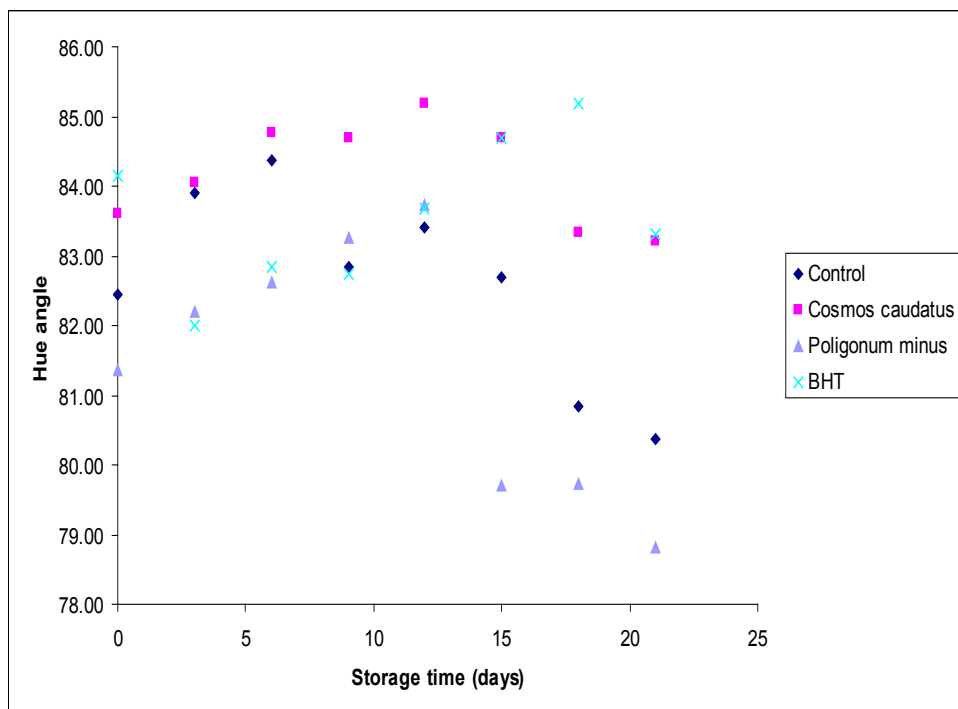
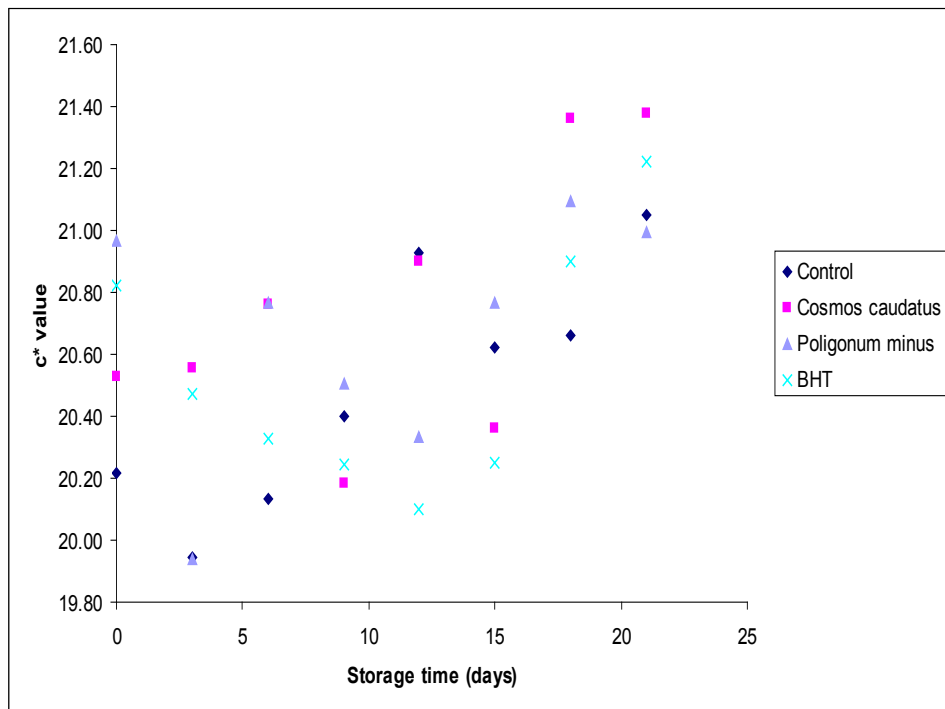


Figure 4. L*, a*, b*, c*, and Hue angle of duck meatballs treated with *Cosmos caudatus*, *Polygonum minus*, and BHT over 21 days of storage

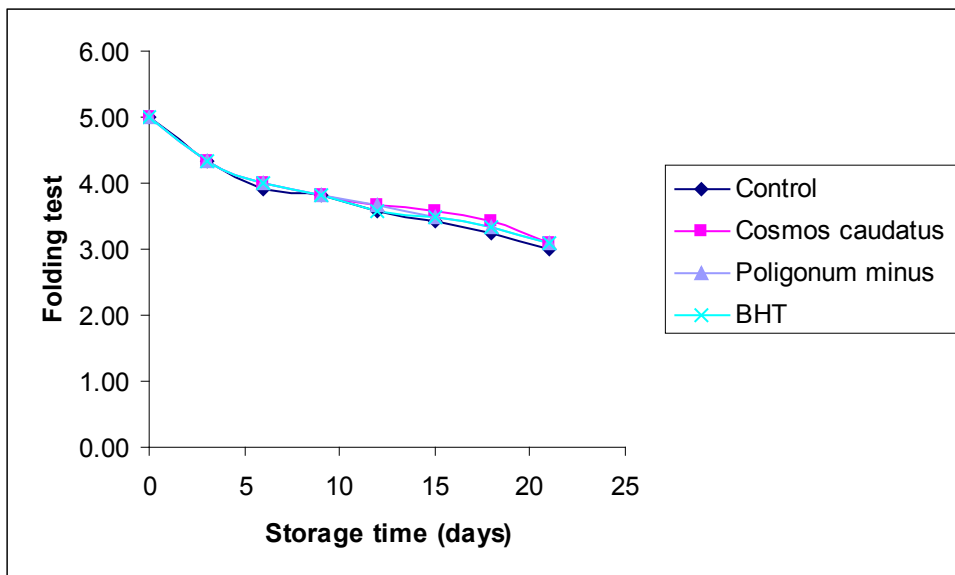


Figure 5. Folding test of duck meatballs treated with *Cosmos caudatus*, *Polygonum minus*, and BHT over 21 days of storage

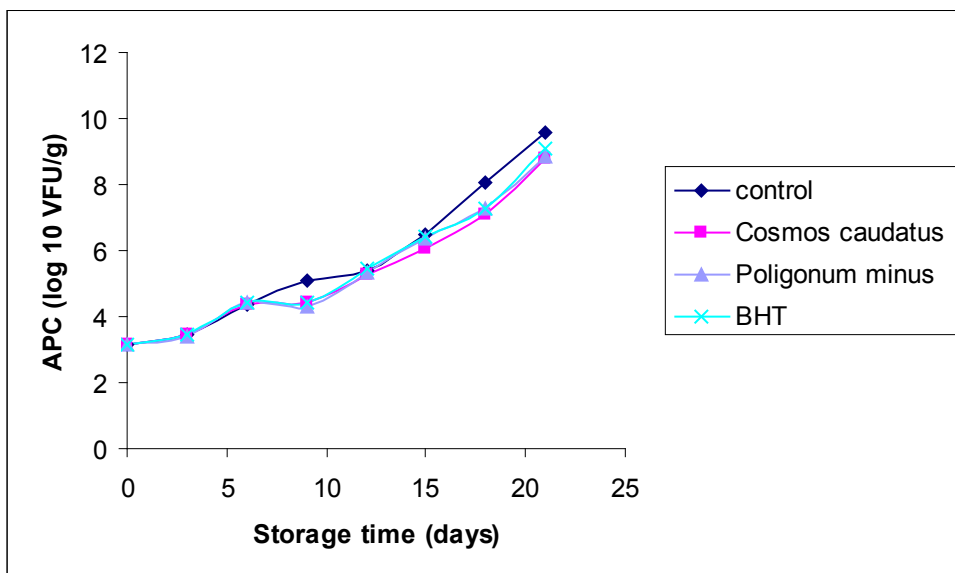


Figure 6. Total bacteria count of duck meatballs treated with *Cosmos caudatus*, *Polygonum minus*, and BHT over 21 days of storage

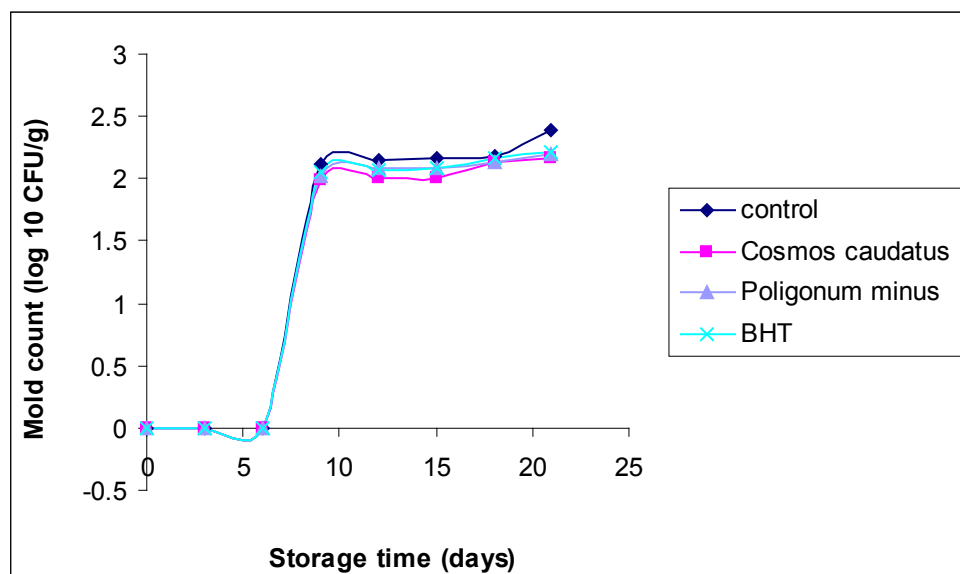


Figure 7. Total mold count of duck meatballs treated with *Cosmos caudatus*, *Polygonum minus*, and BHT over 21 days of storage

had no significant effects on folding test results or microbial growth over the analyzed storage period.

Acknowledgements

The authors acknowledge with gratitude the support given by Universiti Sains Malaysia (USM) and the Malaysian Ministry of Science, Technology and Innovation (MOSTI) through Science Fund research grant 05-01-05-SF0089.

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