Effect of thyme/cumin essential oils and butylated hydroxyl anisole/butylated hydroxyl toluene on physicochemical properties and oxidative/microbial stability of chicken patties

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ABSTRACT In this study, effects of thyme/cumin essential oils (EO) and butylated hydroxyl anisole (BHA)/butylated hydroxyl toluene (BHT) on physicochemical properties and storage stability of chicken patties were compared in different storage periods (0, 3, 7, 14, 21, and 28 d). It was found that there were significant differences between physicochemical properties of patty samples treated with EO and the synthetic antioxidants. The EO showed similar performance to those of BHA and BHT in limiting TBARS values of chicken patty samples. Effect of thyme EO was significant and remarkable, not allowing any coliform bacteria to grow in the samples. Given that EO were obtained from natural sources, the data suggested that the EO might be more useful than their synthetic counterparts, BHA and BHT, as additives for chicken patties to maintain oxidative/microbial stability and increase shelf life.

Key words: chicken patty, thyme/cumin essential oil, butylated hydroxyl anisole/butylated hydroxyl toluene, oxidative stability, microbial stability

INTRODUCTION

Chicken meat is a very popular food commodity worldwide, and its consumption has increased over the last decades in many countries. Chicken meat has many desirable nutritional characteristics such as low lipid content and relatively high concentration of polyunsaturated fatty acids, which can be further increased by specific dietary strategies (Bourre, 2005). However, a high degree of polyunsaturation accelerates oxidative processes, leading to deterioration in meat flavor, color, texture, and nutritional value (Mielnick et al., 2006). Major strategies for preventing lipid oxidation are the use of antioxidants and restriction of access to oxygen during storage with vacuum-packaging (Tang et al., 2001). Antioxidant additives are added to fresh and further processed meats to prevent oxidative rancidity, retard development of off-flavors, and improve color stability (Nam and Ahn, 2003). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used to control lipid oxidation in meat. These have been reported to be effective at a level of 200 ppm in reduction of lipid oxidation (Shahidi et al., 1987). However, use of these types of antioxidants is controlled because of their carcinogenic potential (Sherwin, 1990; Chen et al., 1992; Whysner and Williams, 1996; Sun and Fukushima, 1997). In addition, some cooked meat products such as chicken patties are susceptible to bacterial growth due to their ideal nutrient composition. Therefore, an effort should be exerted to apply some agents having both antioxidant and antimicrobial activities to maintain quality and extend shelf life of such meat products.

Natural antioxidants of plant origin have been introduced to improve lipid stability as well as enhance sensory properties of food. Antioxidant properties of such plant origin materials are mainly attributed to their phenolic contents; thus, their antioxidant action is similar to synthetic phenolic antioxidants (Durling et al., 2007; Fecka and Turek, 2008; Sellami et al., 2009). For example, essential oil (EO) of thyme (Thymus vulgaris L.), a perennial plant of family Lamiaceae, is abundant in thymol, carvacrol, cuminol, or eugenol, and was reported to show a moderate inhibition of LDL oxidation (20–27%; Teissedre and Waterhouse, 2000). Cuminum

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cyminum L. is an annual plant of family Umbelliferae. Major constituents in cumin that have potential to show antioxidant properties were determined to be gamma-terpinene, 2-methyl-3-phenyl-propanal, myrtenal, and glucopyranosides (Takayanagi et al., 2003; Jalali-Heravi et al., 2007).

The aim of the present study was to determine and compare effectiveness of the synthetic antioxidants (BHA/BHT) and EO of thyme and cumin on the physicochemical properties and oxidative/microbial stability of chicken patties as well as to reveal potential use of such natural plant materials in poultry products.

**MATERIALS AND METHODS**

**Materials**

The day after slaughter, boneless meat from 37-d-old chickens on polyfoam plates overwrapped with polyvinyl chloride stretch film were obtained from a poultry processor (Beyipi Co., Bolu, Turkey). In the production of patties, the chicken meats including breast (68%), thigh (21%), and skin (11%) were used. The fresh chicken meat (moisture, protein, and fat contents, and pH value of 70.24%, 21.01%, and 5.32%, and 6.28, respectively) was transported to the laboratory under refrigerated conditions. All chemicals and reagents used for the study were of analytical grade. Butylated hydroxyanisole and BHT were procured from Sigma Chemical Co. (St. Louis, MO). Dried thyme (Thymbra spicata L.) and cumin (Cuminum cyminum L.) spices were obtained from a local market in Konya, Turkey. The samples were transported in polypropylene bags, and were dried to a constant weight and kept in room temperature until analysis. These spices were botanically identified at Department of Biology Science of Selcuk University, Konya, Turkey.

**Extraction of EO**

Essential oils of thyme (Thymbra spicata L.) and cumin (Cuminum cyminum L.) were obtained by steam distillation from leaves, stems, and flowers. Air-dried and finely ground aerial parts of thyme and cumin were placed in a flask where distilled water was also added (1 L water in a 2-L flask for a 500-g sample), and subjected to a continuous steam distillation in a Clevenger apparatus for 3 h. The oils obtained were dried over anhydrous sodium sulfate and, after filtration, stored in dark glass bottles at 4°C until added into chicken patty samples.

**Preparation of Chicken Patties**

After meat was immediately transferred to the laboratory under cold conditions, it was ground through a 3-mm plate grinder (Tefal, Le Hachoir 1500 W, Guangdong, China). Each batch of patties was prepared to include 88.5% chicken meat, 10% rusk flour, and 1.5% salt. The EO of the selected spices were added into related meat batter batches at levels of 200 and 500 µL/kg, whereas the BHA and BHT were separately added at levels of 100 and 200 mg/kg. The BHA and BHT were dissolved in 5 mL of sunflower oil before addition, and the same quantity of oil was added to other meat batter batches to maintain uniformity. Then, batter batches were kneaded and homogenously blended in a laboratory type mixer (Kitchen Aid Food Grinder Mixer Attachment Model FGA, St. Joseph, MI) to achieve homogeneity. The batter batches were shaped into patties with 40.0 mm diameter and 11 mm thickness so that each weighed between 25 and 30 g. Experimental raw patty samples were placed on polystyrene trays. Each batch of patties was vacuum packaged by using a vacuum sealer (Original Henkelman Vacuum Systems Boxer 42, Hertogenbosch, Holland) in a polystyrene tray and wrapped with a high barrier film (water vapor permeability of 8.5 g/m²/24 h at 38°C, 90% RH, and oxygen permeability PE/PA film 160 cm³/m²/24 h at 23°C 0% RH, Polinas Ltd., Istanbul, Turkey) and stored at 4°C in a refrigerator (Arcelik, 5192 NF No-Frost, Istanbul, Turkey) for 0, 3, 7, 14, 21, and 28 d.

**Determination of Physicochemical Properties**

For pH determination, sample (10 g) was homogenized in 100 mL of distilled water for 1 min using a blender (Waring Commercial Blender, New Hartford, CT). Then, pH was measured using a pH meter (pH 315i/SET WTW, Weilheim, Germany; Ockerman, 1985). Analysis of metmyoglobin (MetMb) content was performed as described by Krzywicki (1982). Ground raw chicken patty (5 g) was placed into a 50-mL polypylene centrifuge tube, and 25 mL of ice-cold phosphate buffer (pH 6.80, 40 mM) was added into the tube. The mixture in the tube was homogenized for 10 s at 13,500 rpm with an Ultra-Turrax T25 homogenizer (Janke and Kinkel, Staufen, Germany). The homogenized sample was allowed to stand for 1 h at 4°C and centrifuged at 4,500 × g for 30 min at 4°C (Nuve centrifuge equipped with swing-out rotor, NF-800-R model, Ankara, Turkey). The supernatant was filtered through Whatman No. 1 filter paper, and absorbance was read at 572, 565, 545, and 525 nm by scanning the visible spectrum with a spectrophotometer (Hitachi U-1800 model, Tokyo, Japan). Then, percentages of MetMb were calculated based on these absorbance values according to Krzywicki (1982) using the following equation:

\[
\text{MetMb} = \left[ -2.51(A_{572}/A_{525}) + 0.777(A_{565}/A_{525}) + 0.8(A_{545}/A_{525}) + 1.098 \right] \times 100,
\]

where A = the related absorbance.

Color measurements were performed on chicken patty samples at room temperature (20 ± 2 °C) using a
Chromameter CR-400 (Konica Minolta Inc., Osaka, Japan) with illuminate D65, 2° observer, diffuse/O mode, 8 mm aperture for illumination and 8 mm for measurement. The chromameter was standardized with a white ceramic tile \([L^* \text{ (lightness)} = 98.11, a^* \text{ (redness)} = -0.53, \text{ and } b^* \text{ (yellowness)} = 2.21]\) before measurements. The \(L^*, a^*, \text{ and } b^*\) color coordinates were determined according to the Commission Internationale d’Eclairage \(L^*a^*b^*\) color space system. Visual impression of color is formed from hue-angle \(h = \tan^{-1}(b^*/a^*)\) and chroma \(C^*\) (Shelf Life) before measurement. The chromameter was standardized according to the Commission Internationale d’Eclairage \(L^*a^*b^*\) color space system. Visual impression of color is formed from hue-angle \(h = \tan^{-1}(b^*/a^*)\) and chroma \(C^* = (a^{*2} + b^{*2})^{1/2}\). For color measurements, American Meat Science Association guidelines were followed (Hunt et al., 1991). Measurements were made directly upon samples and carried out 6 times. Average of 6 replicate measurements was used to calculate hue angle \((h)\), which represents relative position of color between redness and yellowness and chroma \((C^*)\), which assesses color intensity. The physicochemical properties of the chicken patty samples were measured on 0, 3, 7, 14, 21, and 28 d of storage.

**Determination of Storage Stability (Shelf Life)**

Thiobarbituric acid reactive substance (TBARS) values were determined colorimetrically using 2-TBA (Turladgis et al., 1960) with a UV-visible spectrophotometer (Hitachi U-1800 model). Ten grams of blended sample, with 2.5 mL of concentrated HCl and 97.5 mL of distilled water, were distilled, and 5 mL of the distillate was collected and treated with 5 mL of TBA reagent. After boiling for 35 min, optical density was measured at 532 nm. A standard curve was prepared using 1.1.3.3-tetraethoxypropane. The TBARS values were calculated by multiplying absorbance readings at 532 nm by a factor of 7.03 determined from the standard curve. The TBARS values were expressed as milligrams of malonaldehyde per kilogram of sample.

For determination of microbial stability of the chicken patty samples, chicken meat used in patty preparation was aseptically obtained from a commercial company (Beypi Co., Bolu, Turkey). The meat was immediately transferred to the laboratory under cold chain conditions and ground through the grinder (Tefal, Le Hachoir 1500 W, Guangdong, China). To obtain patty samples, the ground meat was allocated into 8 groups (4 for thyme/cumin EO and 4 for BHA/BHT), and each of them was separately and aseptically added with the previously described EO and synthetic antioxidants at different concentrations. To be able to determine exact antimicrobial effect of the essential oils and synthetic antioxidants, no ingredient that might have any antimicrobial activity was added into the patty formulation. Each group was mixed, kneaded, and shaped into patties as described above. The raw patty formulations were placed into sterilized stomacher bags (Masticator NR 1147, Spain) and stored in a refrigerator (4 ± 1°C) for 1 h until analysis conducted on 0, 3, 7, 14, 21, and 28 d of storage.

For microbiological analysis, a composite sample (10 g) was formed with portions of at least 3 patties and then homogenized with 90 mL of sterile 1.5% peptone water in a Stomacher 400 (Mayo Homogeniser HG 400V Stomacher, Via Rosmini, Baranzate, Italy) for 1 min. Aliquots were serially diluted up to \(10^{-9}\) in peptone water and plated out following standard methodologies (Gerhardt et al., 1994). Total aerobic mesophilic bacteria (TAMB) were enumerated on plate count agar (Merkck, Darmstadt, Germany) with plates incubated at 37°C for 2 d. Total aerobic psychrotrophic bacteria (TAPB) were enumerated in pour plates of plate count agar (Merck) after incubation at 7°C for 10 d (ICMSF, 1983). Total lactic acid bacteria (LAB) were counted according to AOAC International (1998), in pour plates of DeMan, Rogosa, Sharpe agar (MRS agar, Merck) adjusted to pH 5.6 after incubation at 30°C for 2 d under anaerobic conditions (NuAire CF AutoFlow Incubator, model NU-1500E, Plymouth, MN). Coliform bacteria (CB) were enumerated according to AOAC International (1998), in pour plates of violet red bile agar (Merk) after incubation at 37°C for 24 h. Molds and yeasts were enumerated according to AOAC International (1998), in pour plates of potato dextrose agar (Oxoid, Hampshire, UK) acidified to pH 3.5 (±0.1) with sterile 10% tartaric acid after incubation at 25°C for 5 d. All the enumeration results were expressed as log cfu per gram.

**Statistical Analysis**

Except for color measurements \((n = 12)\), each parameter was tested in triplicate samples with 2 replications \((n = 6)\). Conventional statistical methods were used to calculate means and standard deviations. Collected data were subjected to statistical analysis using MINITAB for Windows Release 14 (2003, Minitab Inc., State College, PA). Analysis of variance was used to evaluate effect of treatments and storage period \((0, 3, 7, 14, 21, \text{ and } 28 \text{ d})\) on the physicochemical properties and storage stability (shelf life) parameters. After microbiological data were transferred into logarithms of the number of cfu per gram, they were subjected to statistical analysis. When a significant \((P < 0.05)\) main effect was found, mean values were further analyzed using Duncan’s multiple range test (Mstat-C, version 4.00, Michigan State University, East Lansing; https://www.msu.edu/~freed/mstatc.htm; Snedecor and Cochran, 1989). In addition, to indicate the effect of each treatment on shelf life (storage stability) of chicken patties during storage period \((0, 3, 7, 14, 21, \text{ and } 28 \text{ d})\), slope of the line of curve plotted for each stability parameter versus storage time was calculated. The slope values \((m)\) were obtained by fitting each stability parameter values versus storage time to linear equation \([1]\) and calculated by employing linear regression analysis.
RESULTS AND DISCUSSION

Physicochemical Properties

Mean moisture, protein, and fat contents, and pH value of chicken meat used in this research were determined to be 70.24%, 21.01%, 5.32%, and 6.28, respectively. Similar results were reported (Karakaya et al., 2005). Table 1 indicates effect of antioxidative type and storage time on pH values of chicken patties. The pH values of thyme and cumin EO treatments were generally higher than those of BHA and BHT treatments. But, increased levels of these EO were observed to decrease (P < 0.05) pH values. Regarding the effect of storage time, pH values decreased (P < 0.05) prolonged storage, reaching the lowest level after 21 d storage. Similar results were observed in fresh and frozen pork patty samples formulated with plant extracts (McCarthy et al., 2001).

Given that consumer rejection occurred at 40% MetMb in meat products (Greene et al., 1971), MetMb formation should be of a great importance to consumer preference. In this study, formation of MetMb in raw chicken patties is also shown in Table 1. There were significant (P < 0.05) differences among the treatment groups and storage times. In the patty samples treated with thyme and cumin EO, MetMb formation was generally lower than in those treated with BHA and BHT, revealing that EO had more ability to retard formation of MetMb. It should be noted here that cumin EO treatments resulted in the lowest MetMb formation compared with thyme EO and BHA-BHT. These results indicated that EO that can be regarded as natural antioxidants could retard lipid peroxidation longer than the synthetic ones, namely, BHA and BHT. This was thought to result from the well-known fact that EO have strong antioxidative properties due to their phenolic components; especially, thymol, carvacrol, and eugenol (Ruberto and Baratta, 2000) and strong radical scavenging activity of these compounds (Schwarz et al., 1996; Kulisic et al., 2004; Bouzidi et al., 2013). Accordingly, several authors concluded that superoxide anion radical (O−2) can initiate lipid peroxidation, leading to formation of prooxidant substances capable of inducing peroxidation.

\[
y = mx + b, \quad [1]
\]

where \(y\) was each of the stability parameters; \(m\) was the slope of the line of curves plotted for each stability parameter versus storage time for each treatment, \(x\) was the storage time (day), and \(b\) was the intercept (where the line crosses the Y-axis). To compare and report the effect of each treatment on shelf life during the storage period, bivariate correlation analysis was performed to calculate correlation coefficients (\(r\)) between \(m\) values and the corresponding stability values for the same treatment. Bivariate correlations between the variables were analyzed by Pearson’s test using Minitab 14.0 software.
of reacting with oxymyoglobin (OMb) and resulting in MMb formation (Anton et al., 1993). They postulated that OMb could be oxidized not only by lipid-oxid radicals, but also by other prooxidant radicals generated by O−2. This could explain the reason why patty samples treated with EO exhibited lower MetMb formation (Table 1). On the other hand, prolonged storage times gave rise to a general increase (P < 0.05) in MetMb values of patty samples, indicating that MetMb formation was time dependent. The possible reason could be attributed to the limited oxidation level in the vacuum-packed patty samples.

As far as the color properties were concerned, the patty samples treated with EO (except for thyme EO at 0.05%) had lower L* values (brightness) than did those treated with BHA and BHT (Table 1). This could be explained by lower pH values of patties treated with thyme EO (at 0.05%) as well as BHA and BHT, indicating the full reflection of the pH effect. Accordingly, it was reported that low pH is often associated with paleness or increased brightness in meat, which was the case in this study in which the patty samples treated with thyme EO (at 0.05%) or BHA-BHT (at 0.01 and 0.02%) had higher brightness values (Hamm, 1972). The reason could be partly explained based on free water between myofibers and on cut surfaces that might reflect more light because there is an efflux of cell fluid as pH declines (Hamm, 1972). This relationship between free water and brightness in meat and meat products was also reported by several authors (Fernández-López et al., 2000; Pipek et al., 2008).

On the other hand, it should be noted that the cumin EO treatment at 0.02% resulted in the highest a* values. The reason could be related to the fact that this treatment also resulted in the highest pH value (Table 1). In other words, the highest pH value of this treatment probably kept the iron in oxymyoglobin in a reduced state (Fe²⁺). But, at lower pH values, the iron in MetMb was thought to be in an oxidized state (Fe³⁺). Accordingly, it was reported that once the iron is oxidized, it cannot be easily converted back to its reduced state, thus the shift from oxymyoglobin (bright red color) to MetMb (brown color) will be permanent (Jenschke, 2004). Given that MetMb is the compound giving meat to brown color, all these knowledge should explain us the reason why lower redness was observed in the patty samples treated with thyme EO, BHA, and BHT (Table 1). Regarding the other color parameters; namely, b*, h, and C values, although effects of the treatments were significant on these values (P < 0.05), there was no consistent trend between these effects (Table 1). These results indicated that there would be no consistent difference between the application of natural (thyme and cumin EO) and synthetic antioxidants (BHA-BHT) with respect to the color properties of patty samples except for L* and a* values.

As for the effect of storage time on color properties, L* values were observed to increase (P < 0.05), whereas a* values decreased (P < 0.05) as storage time increased (Table 1). This result can be expected and explained based on the aforementioned negative relationship between pH and L* values. As was reported above, pH values decreased (P < 0.05) as storage time increased (Table 1). Another explanation was proposed by Boulianne and King (1995) who showed a relationship between brightness of refrigerated breast fillets of chickens and the loss of heme pigments during prolonged storage times, leading such a decrease in meat brightness. Furthermore, Mallia et al. (2000) observed changes in turkey and broiler breast meat color, at various postmortem times. They reported that L* value of breast muscles increased (P < 0.05) dramatically during storage, which was attributed to increased acidity during storage time.

On the other hand, an inverse trend was observed in the a* values during storage period. Probably, this decrease in redness was associated with effect of pH on the percent myoglobin (Table 1). In other words, a decrease at pH values during the prolonged storage times was thought to cause denaturation in myoglobin (giving redness to meat), thus leading to the observed decrease in redness (a* values) of the patty samples during storage. Accordingly, Trout (1989) reported denaturation effect of pH on myoglobin molecule, leading to obvious color differences in cooked beef muscles. In addition, this result should not be surprising as meat stored in prolonged times would be expected to have predominantly either OMb or MMb, as opposed to deoxymyoglobin, which in turn would predispose meat to faster browning rate (Hunt at al., 1999). On the other hand, storage time had no clear effect on the remaining color parameters (b*, h, and C values).

Storage Stability (Shelf Life)

The effects of treatment and storage time on oxidative and microbial stability are shown in Table 2. Treatment and storage time had a significant effect (P < 0.05) on TBARS values of patty samples. The lowest TBARS values (0.64–0.66 mg of malonaldehyde/kg of sample) could be achieved by treatment of BHA/BHT at 0.02%. These results indicated that BHA/BHT exhibited higher antioxidant performance compared with EO treatments. However, antioxidant effects of thyme and cumin EO at 0.05% cannot be ignored because at these concentrations, their performances were close to those of BHA/BHT. Their antioxidant performance could be attributed to their phenolic contents found in every part of plant, including fruit, seeds, and leaves (Shahidi, 2000; Sachetti et al., 2005). Gerhardt and Schrotter (1983) reported that antioxidant activity may occur via various mechanisms such as inhibitory effect on lipid peroxidation and via scavenging radicals. On the other hand, during storage period, TBARS values showed a linear increase (P < 0.05; Table 2). The general increase in TBARS values was probably because of formation of secondary lipid oxidation products such as...
aldehydes and other volatile compounds (Kolakowska, 2002).

Table 2 also indicates comparison of effectiveness of EO and BHA/BHT on microbial stability of patty samples. There were differences ($P < 0.05$) between the natural and synthetic treatments with respect to their antimicrobial effects on TAMB, TAPB, LAB, CB, and molds-yeasts. In the patty samples treated with synthetic antioxidants, TAMB number was lower ($P < 0.05$) than in those treated with thyme and cumin EO. On the other hand, cumin EO treatment resulted in the lowest ($P < 0.05$) TAPB number, whereas thyme EO treatment yielded in the highest ($P < 0.05$) number. Regarding LAB number, the highest numbers were observed in the patty samples treated with cumin EO and BHA. On the other hand, EO treatments resulted in generally lower ($P < 0.05$) number of molds-yeasts than did antioxidant treatments. These results showed that spice EO and synthetic antioxidants did not exhibit an obvious superiority to each other in terms of number of TAMB, TAPB, LAB, and molds-yeasts, suggesting that they would provide almost similar microbial stability in chicken patty samples. However, thyme EO exhibited very distinctive ($P < 0.05$) antimicrobial effect on the CB at both concentrations, allowing no-coliorganism showed generally a stable trend until 14 d of storage, but decreasing after this time.

The performance of each treatment to stabilize shelf life parameters might be different during the storage period (0, 3, 7, 14, 21, and 28 d). Therefore, the way to evaluate effect of each treatment during the storage period was to calculate slope of the line of curve plotted for each stability parameter versus storage time. Table 3 indicates the slope (m) values showing the effect of each treatment on shelf life of chicken patties during the storage period (0, 3, 7, 14, 21, and 28 d). From the lowest magnitudes of the slope values (m) for TBARS values, it can be said that BHA/BHT exhibited the highest antioxidant performance during the storage period. Similarly, the lowest TAMB number was observed in the patty samples treated with synthetic antioxidants during the storage period. Table 3 also shows that the highest LAB number was observed in the patty samples treated with cumin EO and BHA, which was similar phenomena presented in Table 2. In addition, from the zero m values calculated for CB number (Table 3), it is seen that thyme EO did not allow coliform bacteria to grow during the storage period; cumin EO treatment resulted in the lowest number. Regarding the number of molds-yeasts, the trend seen in the corresponding
stability values presented in Table 2 was also seen in the m values (Table 3), indicating that EO treatments resulted in lower number of molds-yeasts than did antioxidant treatments. However, different trends were observed in the number of TAPB when the effect of each treatment was evaluated during the storage period. In other words, as different from their corresponding stability values presented in Table 2, the m values calculated for TAPB were the lowest for the patty samples treated with both cumin and thyme EO, indicating that both EO exhibited the lowest antimicrobial effect during the storage time. Accordingly, the relationship between the slope values presented in Table 3 and the stability values presented in Table 2 was also seen in the m values (Table 3), indicating that EO treatments resulted in lower number of molds-yeasts than did antioxidant treatments.

Conclusions

Our results indicated that, like synthetic antioxidants (namely, BHA and BHT), EO of the thyme and cumin spices studied could limit oxidation, extending storage time of chicken patties. Regarding microbial stability, the EO exhibited very good performance, keeping microbial count stable, even not allowing any coliform bacteria to grow in the chicken patties. Given that the EO were obtained from natural sources, it can be suggested that they are more useful than their synthetic counterparts, BHA and BHT, as additives for chicken patties to maintain stability and increase shelf life.

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