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Comparison Between Natural and Synthetic Antioxidants in Beef Products: A Meta-Analysis

Perbandingan Antara Antioksidan Alami dan Sintetik pada Produk Daging Sapi: Analisis Meta

K. A. Fadhil[1], T. Suryati[1*], & A. Jayanegara[2]
[1]Department of Animal Production and Technology, Faculty of Animal Science, IPB University
[2]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University
Jl. Agatis, Kampus IPB Dramaga, Bogor 16680, Indonesia
*Corresponding author: t-suryati@apps.ipb.ac.id
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ABSTRACT

Synthetic antioxidants are created through artificial synthesis, whereas natural antioxidants are created through natural synthesis in plants, animals, and microbes. Although synthetic antioxidants have been used previously, there has been a growing need in recent years for natural antioxidants, largely due to the negative consequences of synthetic antioxidants. Therefore, many current studies have focused on finding natural antioxidants in diverse plants. This study aimed to compare the effectiveness of natural and synthetic antioxidants in beef products using a meta-analysis approach. The method included the stages of identification, selection, and conformity check. The selection process resulted in 12 articles obtained from the electronic database of Harzing's Publish and Perish software. The meta-analysis model used in this study was random-effect model involving a positive control group (synthetic antioxidants) and an experimental group (natural antioxidants). The effect size and confidence interval were computed using the OpenMEE software, while the summary size and Egger's test were obtained using JASP software. Results revealed that the addition of natural antioxidants showed comparable quality to synthetic antioxidants, as indicated by similar values between the two groups for the peroxide value, carbonyl content, metmyoglobin content, cooking loss, pH, and hue angle (P>0.05). Natural antioxidants showed a higher value on *b* (yellowness) and chroma (P<0.05). Parameters indicating lower value (P<0.05) occurred in TBARS, *a* (redness), and *L* (lightness). In conclusion, the addition of natural antioxidants in beef products demonstrates comparable results and could be used as alternatives to synthetic antioxidants.

Key words: color change, oxidation, pH, physicochemical, TBARS

ABSTRAK

Antioksidan sintetik diproduksi melalui sintesis buatan, sedangkan antioksidan alami dihasilkan melalui sintesis alami pada tumbuhan, hewan, dan mikroba. Meskipun antioksidan sintetik telah digunakan sebelumnya, telah terjadi peningkatan kebutuhan dalam beberapa tahun terakhir untuk antioksidan alami, terutama karena konsekuensi negatif dari antioksidan sintetik. Oleh karena itu, banyak penelitian saat ini berfokus pada menemukan antioksidan alami pada tanaman yang beragam. Penelitian ini bertujuan untuk membandingkan efektivitas antioksidan alami dan sintetik pada produk daging sapi dengan menggunakan pendekatan meta-analisis. Metode tersebut meliputi tahapan identifikasi, seleksi, dan pengecekan kesesuaian. Proses seleksi menghasilkan 12 artikel yang diperoleh dari database elektronik perangkat lunak Harzing's Publish and Perish. Model meta-analisis yang digunakan dalam penelitian ini adalah *random-effect* yang melibatkan kelompok kontrol positif (antioksidan sintetik) dan kelompok eksperimen (antioksidan alami). *Effect size* dan interval kepercayaan dihitung menggunakan perangkat lunak OpenMEE, sedangkan *summary size* dan uji Egger diperoleh dengan menggunakan perangkat lunak JASP. Hasil penelitian menunjukkan bahwa penambahan antioksidan alami mempunyai kualitas yang sebanding dengan antioksidan sintetik, yang ditunjukkan dengan kesetaraan nilai antara kedua kelompok untuk nilai peroksida, kandungan karbonil, kandungan metmioglobin, susut masak, pH, dan *hue angle* (*P*>0,05). Antioksidan alami menunjukkan nilai yang lebih tinggi pada *b* (kekuningan) dan *chrome* (*P*<0,05). Parameter yang menunjukkan nilai lebih rendah (P<0,05) terjadi pada TBARS, *a* (kemerahan), dan *L* (ringan). Kesimpulannya, penambahan antioksidan alami pada produk daging sapi menunjukkan hasil yang sebanding dan dapat digunakan sebagai alternatif antioksidan sintetik.

Kata kunci: fisikokimia, oksidasi, perubahan warna, pH, TBARS

INTRODUCTION

Meat and meat products are an important part of the diet. Complete content, including protein, vitamins, and fatty acids, can meet human nutritional needs. Meat is easily damaged due to its abundant and complete nutritional content. The main cause of damage in meat is microbes and oxidation reactions. Such damage can result in changes in sensory properties and rejection by consumers (Domínguez *et al.* 2019).

Autooxidation is the main process that causes damage to meat and meat products. This process involves the interaction of unsaturated fatty acids with oxygen. The autooxidation process generally involves a combination of three phases: initiation, propagation, and termination. Free radicals characterize the initiation phase; the propagation phase occurs when reactive compounds are increased, and then the termination phase is indicated by reactive compounds reacting with each other to form non-reactive compounds (Domínguez *et al.* 2019).

Antioxidants inhibit oxidation damage in food, such as changes in taste, color and nutritional value (Shahidi & Zhong 2010). Synthetic antioxidants are widely used in the food industry because they already have mature technology and low prices. Synthetic phenolic antioxidants inhibit oxidation, maintain food stability, and extend the shelf life of food. However, excessive and improper addition of synthetic antioxidants can harm human health (Kim *et al.* 2016). Several synthetic antioxidants that are widely used in food include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) (Rodil *et al.* 2012).

Sources of natural antioxidants can be classified based on their origin (plant, animal, or bacterial) and chemical structure (phenol, tocopherol, or vitamin C). Natural antioxidants from plants come from fruit, tea, seeds, herbs, vegetables, cereals, or trees (Xu *et al.* 2017). Various parts of plant organs such as leaves, fruits, stems, or roots contain high concentrations of antioxidants and vary depending on the plant species. Plant parts were used directly as antioxidants (pure or juice) or after extraction and purification (Karre *et al.* 2013; Shah *et al.* 2014). Many studies have been conducted on applying plant extracts, seasonings, and fruit flour in the bakery, dairy industry, and meat products (Caleja *et al.* 2017). The content of vitamins A, C, E, and polyphenolic compounds derived from fruits and vegetables are used as antioxidants, anticancer, or disease prevention (Xu *et al.* 2021). This antioxidant extract is associated with products for health-conscious consumer groups (Santos-Sánchez *et al.* 2017).

Consumer and food manufacturers have recently increased interest in natural food additives. Consumers prefer foods with natural additives over synthetics (Carocho *et al.* 2015). Studies also showed that consumers who get more information about food additives tend to choose food additives derived from natural ingredients (Bearth *et al.* 2014). The ideal use of natural antioxidants comes from fruits and herbs (Santos-Sánchez *et al.* 2017). Antioxidants are used to extend shelf life and ensure food safety. The main foods that use antioxidants are mainly meat products, oils, fried foods, spices, dairy products, bakeries, and snacks (Baines and Seal 2012).

Meta-analysis is a study process used to integrate the results of individual studies with statistical methods in order to obtain an overall score (summary effect) (Shorten and Shorten 2013). This method provides a mechanism of data synthesis compared to narrative studies. In addition, meta-analysis can assess the distribution of data from summary effects by sub-group analysis (Borenstein *et al.* 2009). This study aimed to compare the effectiveness of natural and synthetic antioxidants to the physicochemical characteristics of beef products.

The use of plant components as natural antioxidants in meat and meat products has already been extensively researched. However, In the food sector, the usage of synthetic antioxidants has not been substituted. Comparison between the two antioxidants was carried out to determine the feasibility of natural antioxidants as substitutes for synthetic antioxidants in beef products. This study aimed to compare the effectiveness of natural and synthetic antioxidants in maintaining the physicochemical characteristics of beef products using a meta-analysis approach.

METHODS

The scientific papers were searched using Harzing's Publish and Perish software on the Google Scholar database option. The search process was conducted using the keywords: "effect of natural antioxidants", "TBARS", and "beef products". The limit for the number of papers sought is 1000 journals. This study used articles published from 2011-2021. The obtained journals list was then entered into the Mendeley Reference Manager 2.70.0 software. The criteria for the journals to be analyzed are research journals that are published internationally and in English.

Database created in Google Sheets included author's name, type of product, type of meat, unit, type of natural additive, the form of natural additive, type of synthetic additive, number of replications, average, standard deviation, effect size (d), the variance of d, and standard error from d. The heterogeneity test in this study used I^2. The number of replicates, mean and standard deviation was divided into experimental (natural antioxidants) and positive controls (synthetic antioxidants).

Data Analysis

Data analysis was carried out using a meta-analysis approach. The calculation of summary size using Hedge's d (Borenstein *et al.* 2009). Data processing was done by importing a comma-separated value (CSV) file from Google Sheets into OpenMEE to calculate effect size and standard error. Effect size and standard error were then moved to Google Sheets in a separate column. The complete CSV file was then imported into the JASP 0.16.2 software to calculate the summary size, heterogeneity and Egger's tests (publication bias).

RESULTS AND DISCUSSION

Searching processes for articles in the Google Scholar database regarding the physiochemical characteristics of beef products with various types of antioxidants yielded 441 articles. The collected articles then went through a selection process. Articles with the same title and author and those with different substances were removed from the database. The number of articles collected for the meta-analysis process after assessing suitability was 12 articles (Figure 1).

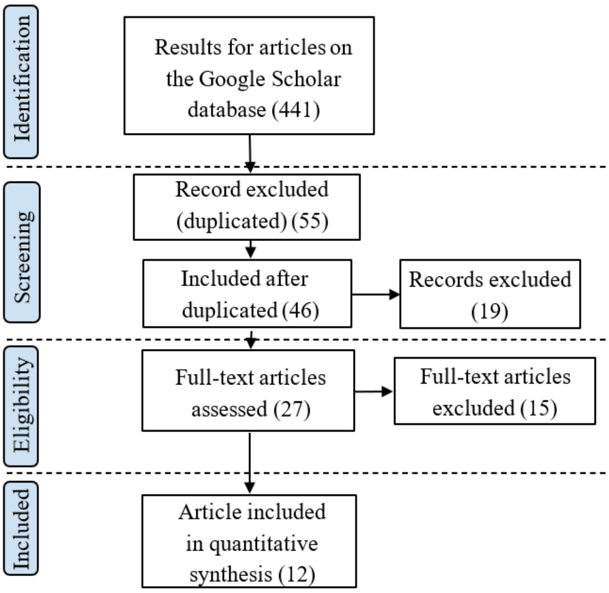

Figure 1. Meta-analysis article inclusion diagram

Table 1 presents the types of beef products, sources of antioxidants, and the forms of antioxidants from selected articles. The types of beef products collected in this meta-analysis were derived from tea, grape seed, cloves, black cumin, golden thread, *Moringa oleifera*, dried plums, rosemary, butterbur, broccoli, *Bidens pilosa*, lemon, *chamnamul*, fatsia, and *Caesalpinia decapetala* while synthetic antioxidants as comparison are BHA, BHT and nitrite. The types of beef products collected in this study are patty, raw beef, minced beef, and ground beef. The forms of natural antioxidants obtained from the articles found included extracts, purees, and essential oils. Several indexes of variation should follow adequate and comprehensive data (e.g., SD, SE) and the significance level ($P<0.05$) to interpret the experimental result.

Physicochemical Characteristics

The computational results of the meta-analysis in this study contain information on summary size, upper and lower bounds of the 95% confidence interval, total studies, and total replications. Summary size showed a positive value indicates a higher parameter value in natural antioxidants, while a negative summary size indicates a lower parameter value. The non-significant difference can be indicated by the confidence interval of the summary size, which contains 0 (zero).

Meta-analysis was carried out on thirteen parameters of physicochemical characteristics, including TBARS, pH, metmyoglobin content, carbonyl content, cooking loss, and peroxide value. The number of studies on the calculated parameters ranged from 11 to 151 (Table 2).

Thiobarbituric Acid Reactive Substances (TBARS)

The studies used for the meta-analysis of the TBARS value were 151. All replicates for the calculation of the meta-analysis of the TBARS value were 952. Products with natural antioxidants had a higher TBARS value than products with synthetic antioxidants ($P<0.05$). The Egger's test showed that there was no publication bias in the TBARS ($P>0.05$) (Table 2).

Meta-analysis showed that products with synthetic antioxidants were more effective at inhibiting lipid oxidation than those with natural antioxidants. TBARS values which were also greater in the addition of natural antioxidants than synthetic antioxidants were found in studies of ground pork (Qin *et al.* 2013), sheep nuggets (Das *et al.* 2016), and goat meat (Rababah *et al.* 2011). Comparable TBARS values between natural antioxidants and synthetic antioxidants were found in studies on pork patties (Jia *et al.* 2012) and chicken meat patties (Naveena *et al.* 2008). Research that showed lower TBARS numbers in the addition of synthetic

Table 1. The selected articles for the meta-analysis

Papers	Products	Natural antioxidants	Antioxidants forms	Positive control
Liu *et al.* (2015)	Patty	Catechins tea; grape seed	Extract	BHA
Zahid *et al.* (2020)	Patty	Clove	Extract	BHT
Rahman *et al*. (2021)	Patty	Cumin	Extract	BHA
Zahid *et al.* (2018)	Patty	Golden thread; Clove	Extract	Nitrite
Shah *et al*. (2015)	Raw beef	*Moringa oleifera*	Extract	BHT
Movileanu *et al.* (2013)	Patty	Dried plum; rosemary	Puree; Extract	BHA; BHT
Amin & Edris (2017)	Minced beef	Grape seed	Extract	BHT
Kim *et al.* (2013)	Ground beef	Butterbur; Broccoli	Extract	BHT
Falowo *et al.* (2017)	Ground beef	*Bidens pilosa; Moringa oleifera*	Extract	BHT
Hsouna *et al.* (2017)	Minced beef	*Citrus limon*	Essential oil	BHT
Kim *et al.* (2013)	Patty	Chamnamul; Fatsia	Extract	BHT
Gallego *et al.* (2015)	Patty	*Caesalpinia decapetala*	Extract	BHT

Table 2. Summary size of the physicochemical characteristics of beef products

Parameter	Summary size	Confidence interval (95%)		Total studies	Total replications
		Lower bound	Upper bound		
TBARS	0.339*	0.153	0.524	151	952
Peroxide value	-0.145b	-1.089	0.799	12	72
Carbonyl content	-0.173b	-0.610	0.263	11	88
Metmyoglobin	1.486	-2.347	5.319	18	124
Cooking loss	0.120	-0.197	0.437	23	154
pH	-0.008b	-0.227	0.212	69	544

Asterisk (*) shows a significantly different meta-analysis (P<0.05). Superscripted b shows publication bias based on Egger's test (P<0.05).

antioxidants was found in studies of pork patties (Lorenzo *et al.* 2014) and pork sausages (Lorenzo *et al.* 2013).

The low phenolic content, the polarity of phenolic compounds that cannot enter the emulsion system, or the sugar compounds in the additional ingredients reduce the efficiency of natural antioxidants in inhibiting lipid oxidation. Research on natural antioxidants showed a strong positive correlation between phenolic content and antioxidant activity (Yildirim *et al.* 2005; Shah *et al.* 2015). Fat oxidation occurs more quickly when antioxidants can dissolve with fat in the emulsion system (Berton-Carabin *et al.* 2014). In addition, the high sugar content decomposed into aldehydes during processing and storage (Rodríguez-Carpena *et al.* 2011). Movileanu *et al.* (2013) also explained that ingredients such as dextrose, sorbitol, and other compounds could dilute and reduce the antioxidant effectiveness of dried plums.

Peroxide Value

The studies on peroxide values used for the meta-analysis were 12. The number of replicates involved in the peroxide value meta-analysis was 72. The peroxide value of products with natural and synthetic antioxidants was not significantly different (P>0.05). This is indicated by summary size with a confidence interval containing the 0 (zero) value. The Egger's test showed publication bias in the peroxide value (P<0.05) (Table 2).

The meta-analysis showed that the products with natural antioxidants and those with synthetic antioxidants had similar peroxide value. Research on ground pork with the addition of BHT showed a lower peroxide value than ground beef with pomegranate juice extract or pomegranate seed powder (Qin *et al.* 2013). Peroxide value is an indicator of primary lipid oxidation in meat products (Rahman *et al.* 2021). The process begins with the oxidation of unsaturated fatty acids that produce hydroperoxides. The next step is the alteration of the hydroperoxides into secondary compounds such as hydrocarbons, aldehydes, ketones, alcohols, esters and acids (Kumar *et al.* 2015). In addition to fat oxidation, reactive oxygen also produces peroxides from protein oxidation processes (Lobo *et al.* 2010).

Carbonyl Content

The number of studies on carbonyl content used for the meta-analysis was 11. The number of replications involved in the meta-analysis of carbonyl content was 88. Products with natural and synthetic antioxidants had carbonyl levels that were not significantly different (P>0.05). This was indicated by summary size with a confidence interval containing the 0 (zero) value. The Egger's test showed publication bias on the carbonyl content (P<0.05) (Table 2).

The results of the meta-analysis showed that the products with natural antioxidants and those with synthetic antioxidants had similar carbonyl content. Research on pork patties with blackcurrant extract also showed similar results compared to those with BHT (Jia *et al.* 2012). Carbonyl content is the main index of protein oxidation in meat. Carbonyl is formed as a result of the reaction between proteins with aldehydes or oxygen. Amino acid side chains involved in oxidation reactions by oxygen free radicals occur in lysine, proline, arginine, cysteine, threonine, leucine, and histidine (Stadtman and Levine 2003). Phenolic compounds inhibit protein oxidation by acting as metal chelating agents (Estévez and Heinonen 2010). Covalent bonds between phenolic compounds and meat protein can act as a deterrent to carbonyl formation. This mechanism stabilizes phenoxyl radicals bound to proteins, thereby slowing down carbonyl production (Jongberg *et al.* 2013).

Metmyoglobin

The studies used for the meta-analysis of metmyoglobin levels were 18. All replicates used for the meta-analysis of metmyoglobin levels were 124. Products with natural and synthetic antioxidants had metmyoglobin levels that were not significantly different (P>0.05). This was indicated by summary size with a confidence interval containing the 0 (zero) value. Egger's test showed no publication bias on metmyoglobin levels (P>0.05) (Table 2).

The results of the meta-analysis showed that the products with natural antioxidants and those with synthetic antioxidants had similar metmyoglobin levels. Research on rabbit meat patties treated with purslane extract also showed a level of metmyoglobin equivalent to those given added BHT (Wang *et al.* 2021). Metmyoglobin levels in meat indicate the process of changing color from red to brown due to oxymyoglobin oxidation (Lindahl 2011). Suman *et al.* (2014) explained that lipid peroxide products more dominantly cause myoglobin oxidation. Liu *et al.* (2015) also proved that metmyoglobin levels were positively correlated with lipid oxidation and negatively correlated with *a* (redness).

Cooking Loss

The studies used for the meta-analysis of cooking loss were 23. All replicates used for the meta-analysis calculation of cooking loss were 154. Products with natural and synthetic antioxidants had metmyoglobin levels that were not significantly different (P>0.05). This was indicated by summary size with a confidence interval containing the 0 (zero) value. The Egger's test showed no publication bias on the cooking loss percentage (P>0.05) (Table 2).

The results showed that the products with natural antioxidants and those with synthetic antioxidants had similar cooking loss characteristics. Research on fried chicken with *Origanum syracum L.* essential oil and those with BHA also showed comparable cooking losses (Al-Hijazeen 2019). Cooking loss is a combination of liquid and solutes lost due to increasing temperature during cooking. This process causes the percentage of fat and protein to increase due to water loss (Brugiapaglia and Destefanis 2015). During cooking, meat proteins undergo denaturation and cause structural changes such as the damage of cell membranes, shrinkage of meat fibers, and the formation of myofibrillar and sarcoplasmic protein gels (Jung *et al.* 2012).

pH

The number of studies on pH value used for the meta-analysis was 85. The number of replicates involved in the meta-analysis of pH value was 640. Products with natural and synthetic antioxidants had pH values that were not significantly different (P>0.05). This was indicated by summary size with a confidence interval containing the 0 (zero) value. The Egger's test showed publication bias in the pH value (P>0.05) (Table 2).

Products with natural antioxidants and those with synthetic antioxidants have similar pH characteristics. Research that also resulted in a comparison of equal pH values between natural and synthetic antioxidants was found in ground pork products (Qin *et al.* 2013), chicken meat patties (Naveena *et al.* 2008), and lamb nuggets (Das *et al.* 2016). However, research on pork patties with chestnut extract had a higher pH than the addition of BHT (Lorenzo *et al.* 2013). A low pH value indicates a decrease in the quality of meat products. The decrease in pH is caused by the acid produced by lactic acid bacteria and the release of FFA due to fat decomposition (Sun *et al.* 2011). The presence of bacteria also causes an increase in pH, as described by Muthukumar *et al.* (2014) that bacteria produce ammonia from amino acid metabolism processes when stored glucose is unavailable.

Color Values

The number of studies of *a* (redness), *L* (lightness), *b* (yellowness), chroma, and hue angle used for the meta-analysis was 110, 79, 64, 27, and 19, respectively (Table 3). Meta-analysis showed significantly different *a*, *L, b,* and chroma values (P<0.05). On the other hand, the hue angle showed a non-significant difference between the two antioxidants (P>0.05). Egger's test showed publication bias on *L*, chroma, and hue angle (P<0.05) (Table 3).

Color is an indicator of quality and freshness consumers use before buying meat (Jeong *et al.* 2015). Buyers directly reject meat products only from the appearance of color (Soriano *et al.* 2018). The results showed the products with natural antioxidants had lower *a* and *L* than those with synthetic antioxidants. The red color of meat products is influenced by the heme protein, haemoglobin and myoglobin. These proteins are red in the reduced form and brown in the oxidized form (Sabeena *et al.* 2012). Oxidation of myoglobin produces free radicals that increase lipid oxidation and cause color changes (Bekhit and Faustman 2005). Research on pork patties showed that products with the addition of pomegranate rind powder, pomegranate juice, pomegranate seed powder, or black currant extract have higher *a* than those with the addition of BHT (Jia *et al.* 2012; Qin *et al.* 2013). The comparable value between the addition of BHA and BHT compared to those with the addition of dried plum puree occurred in the study of raw pork sausage (Gonzalez *et al.* 2008). Research on chicken meat patties showed that the addition of BHT had a higher *a* than those with the addition of pomegranate juice (Naveena *et al.* 2008). Research with higher *L* in products with the addition of synthetic antioxidants compared to the addition of natural antioxidants also occurred in pork patties (Jia *et al.* 2012; Qin *et al.* 2013), pork sausages (Gonzalez *et al.* 2008), and chicken patties (Naveena *et al.* 2008).

The meta-analysis showed that products with the addition of natural antioxidants had higher *b* and chroma than those with synthetic antioxidants. Research that showed higher *b* in products with natural antioxidants than those with synthetic antioxidants was also shown by pork sausage (Naveena *et al.* 2008). However, a lower *b* value in the addition of natural antioxidants occurred in pork patties (Qin *et al.* 2013) and chicken patties (Naveena *et al.* 2008). Research on chicken meat patties with BHT has a higher

Table 3. Summary size of the color characteristics of beef products

Parameter	Summary Size	Confidence interval (95%)		Total studies	Total replications
		Lower bound	Upper bound		
a (redness)	-0.721*	-1.047	-0.396	110	698
L (lightness)	-0.290*b	-0.478	-0.102	79	506
b (yellowness)	0.512*	0.179	0.846	64	416
Chroma	0.907*b	0.069	1.745	27	200
Hue Angle	-0.006b	-0.779	0.766	19	152

Asterisk (*) shows significantly different meta-analysis results (P<0.05). Superscripted b shows publication bias based on the Egger's test (P<0.05).

chroma than those with pomegranate juice (Naveena *et al.* 2008).

The meta-analysis showed that the products with the addition of natural and synthetic antioxidants had the same hue angle characteristics. Comparable hue angle values occurred in studies comparing the addition of BHT and those with pomegranate juice to chicken meat patties (Naveena *et al.* 2008). The color change parameter shows different comparisons between products with the addition of natural and synthetic antioxidants. This is thought to be due to the influence of pigments from natural antioxidants carried during application. These pigments include the purple pigment in clove extract (Gonzalez *et al.* 2009) or the green pigment in plants containing chlorophyll (Milne *et al.* 2015).

CONCLUSION

Physicochemical characteristics show comparable quality between beef products with natural and synthetic antioxidants. However, the color characteristics of the beef products showed mixed results. This study showed that synthetic antioxidants can be replaced by natural antioxidants to maintain the quality of beef products.

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