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*Original Article*

# Effect of purple corn cob extract powder and black rice bran oil on quality and shelf life of fresh beef

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## Abstract

This study aimed to assess the ability of purple corn cob extract powder (CEP) and black rice bran oil (RBO) on extending the shelf life of fresh beef during cold storage. The beef samples were treated with different concentrations of CEP (2% and 3% [w/w]) and RBO (0.2% and 0.4% [v/w]) and their combinations (CEP 1% + RBO 0.2% and CEP 2% + RBO 0.4%), and compared to an untreated sample and a positive sample treated with 0.02% butylated hydroxytoluene (w/w). The results showed that the microbial count and thiobarbituric acid reactive substance (TBARS) values increased in all samples during storage up to 9 days, whereas the antioxidant activities were unstable. However, the beef with 2% and 3% of CEP and CEP 2% + RBO 0.2% were the most effective applications to improve shelf life up to 5 days with an acceptable level of microbial count and TBARS value.

**Keywords:** black rice bran oil, purple corn extract, beef, safety, shelf life

## 1. Introduction

Beef (*Longissimus lumborum*) is one of the most dominant foods consumed in Thailand (Osothongs *et al.,* 2016). Beef consists of water, protein, fat, and minerals (Dave & Ghaly, 2011). A shortage of storage seems to be a common problem in many meat markets. Fresh meat is very susceptible to spoilage as a result of chemical (fat oxidation) and enzymatic activities (Dave & Ghaly, 2011). At present, improving the characterization of food safety and quality is recognized as an important aspect for sustainable food productions. The preservation of meat is necessary during storage and transportation for long distances without deterioration of texture, color, and nutritional value (Dave & Ghaly, 2011). Currently, new preservation techniques are being developed to improve the preservation process in order to prolong the shelf life of fresh meat by maintaining both the natural appearance and safety, for example, chemical and non-thermal techniques. Moreover, synthetic phenolic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroquinone, and proplygallates have been extensively used as chemicals to control oxidative deterioration in meat and meat products (Dave & Ghaly, 2011). Generally, there are many natural antimicrobial compounds which are widely used as alternative preservatives in meat products, such as essential oils (Burt, 2004; Rasooli, 2007) and spices (Sema, Nursel, & Suleyman, 2007).

Purple corn and black rice bran are great sources of anthocyanin and phenolic compounds (Kapcum, Uriyapongson, Alli, & Phimphilai, 2016; Pedreschi & Cisneros-Zevallos, 2007). These compounds provide various biological activities (Jing, Noriega, Schwartz, & Giusti, 2007). Furthermore, colored rice bran contains high levels of anthocyanins and phenolics as well as tocols and γ-oryzanol that play important roles in antioxidant potency (Zhang *et al.,* 2006; Jang & Xu, 2009; Mutana & Prasong, 2010). According to Arpan,

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Praveen, and Singh (2013), rice bran oil possesses anti-bacterial properties against selected strains, such as *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Previous research showed antioxidant properties and phenolic constituents from spices that effectively inhibited microbial growth and lipid oxidation in meat and meat products (Zhang *et al.,* 2015; Krishnan *et al.,* 2014). Thus, natural preservation treatment not only increases the quality of meat and shelf life but also improves the functional health properties. However, no research has been done on the application of purple corn cob extract powder (CEP) and black rice bran oil (RBO) to preserve the shelf life in fresh beef. Therefore, the objectives of this study were to determine the effects of CEP and RBO on the qualities and safety in fresh beef during cold storage.

## 2. Materials and Methods

The *longissimus lumborum* muscle (13 kg) of fresh beef from one carcass was purchased and collected from a local slaughterhouse in Khon Kaen Province, Thailand. The black rice bran was obtained from the Plant Breeding Research Center for Sustainable Agriculture, Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. The black RBO was cold press extracted from black rice bran using an oil compressor with 7.5-hp motor (Model: SF-JR, Mitsubishi, Japan). The crude RBO was filtered through a 20 µ sieve and kept in a glass bottle before use. The purple corn cob was obtained from the Siam Miragro Co., Ltd. (Khon Kaen Province, Thailand). The purple corn cob was dried at 60 °C using a hot air oven until the final moisture content of 10% was reached. The epidermis layer of the cob was extracted using hot water at 85 °C. The purple anthocyanin extract solution was encapsulated with a carrier agent and dried to obtain a purple powder using a spray dryer. All chemicals and reagents were analytical grade.

### 2.1 Sample preparation and statistical analysis

The fresh beef was immediately placed inside polystyrene boxes with ice bags. The meat samples were transferred to a laboratory refrigerator at 4±1 ºC within 1 h after slaughtering. The meat was cut perpendicular to the muscle fiber direction. The samples had an average weight of 100 g. The meat samples were treated with CEP and RBO using split-plot design. The storage time was a main-plot factor and different samples were sub-plot factors. Eight separate treatments were employed in the meat samples: (1) 2% CEP (2 g); (2) 3% CEP (3 g); (3) 0.2% RBO (0.2 mL); (4) 0.4% RBO (0.4 mL); (5) 1% CEP and 0.2% RBO; (6) 2% CEP and 0.2% RBO; (7) negative control (NC) of untreated beef; and (8) positive control (PC) of meat treated with 0.2% butylated hydroxytoluene (BHT). Each sample was packed in low-density polyethylene bags and stored at 4±1 °C. The samples were collected and the physical and chemical properties were determined at 3-day intervals during a storage time of 9 days.

### 2.2 Antioxidant activities (AOA)

Ten grams of ground beef sample was extracted with 30 mL methanol for 3 h at 60 ºC using a shaker. The extract was filtered through filter paper before determining the AOA using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS) cation decolorization assay. The DPPH assay was estimated using the protocol developed by Leong and Shui (2002) with some modifications. Briefly, 0.1 mM DPPH solution was freshly prepared. An amount of 100 µL of the extract sample was mixed with 4.0 mL of DPPH solution and then allowed to stand for 30 min at room temperature in the dark before measurement at 512 nm using UV/Vis spectrophotometry (Shimadzu UV 1800, Japan). The AOA was expressed as mg Trolox per 100 g sample (mg Trolox/100 g).

The ABTS assay was estimated using the protocol developed by Stratil, Klejdus, & Kuban (2006) with some modifications. Briefly, the radical cation of ABTS ($ABTS^{\bullet+}$) was generated by reacting ABTS (7 mM) with potassium persulphate (4.95 mM) at a ratio of 1:1 (v/v) for 12 h at room temperature in the dark. The $ABTS^{\bullet+}$ stock solution was diluted with phosphate buffer solution to absorbance values of 1.0 AU at 734 nm using a UV/Vis spectrophotometer. A 40 µL sample extract was mixed with 4 mL of $ABTS^{\bullet+}$ working solution and then left standing at room temperature for 10 min in the dark before measurement. The AOA was expressed as mg Trolox per 100 g sample (mg Trolox/100 g).

### 2.3 Thiobarbituric acid-reactive substances (TBARS)

The TBARS were analyzed according to Tarladgis, Watts, Younathan, & Dugan (1960) with some modifications. A 10-g sample of ground beef was homogenized with 40 mL of distilled water for 10 min. The homogenized meat liquid (2.5 mL) was transferred to a test tube and 2.5 mL of the thiobarbituric acid (TBA) reagent (0.02 M 2-thiobarbituric acid in distilled water) was added. The mixture was mixed well and incubated in a boiling water bath for 1 h. The temperature of the mixture was cooled to room temperature and then centrifuged at 4,000 rpm for 10 min (Z-200 A, HERMLE, Wehingen, Germany). The absorbance of the supernatant of the solution was determined at 538 nm by a spectrophotometer. The result was expressed as mg of malondialdehyde per kg sample (mg MDA/kg).

### 2.4 Color

The color parameters *(L**, *a**, and *b**) were analyzed according to the color evaluation mythology of American Meat Science Association (AMSA, 2012). The surfaces of the meat samples were evaluated using colorimeter (HunterLab UltraScan XE, VA, USA). The values of illuminant, aperture size, and observer angle were set at $D_{65}$, 3.18 cm, and 10 degrees, respectively. The instrument was standardized before measurements with white and black tiles provided by the manufacturer.

### 2.5 Microbial analysis

The total plate count (TPC), and total yeast and mold count are rapid methods described by AOAC (1990). A 25-g sample of fresh beef was aseptically transferred into a sterile Stomacher® bag. The sample was treated with 225 mL

of 0.1% sterile peptone and then homogenized for 2 min using a Stomacher (Stomacher 400 Circulator, Seward Medical Ltd., London, UK). A 1 mL aliquot from each dilution was plated onto a standard plate count agar (PCA) and incubated at 35 °C for 24 h for TPC analysis. For yeast and mold, potato dextrose agar (PDA) was used and incubated at 25 °C for 72 h. The results were expressed as logarithm with the base 10 of colony-forming units per g of fresh beef (log cfu/g).

### 2.6 Statistical analysis

The measurements were done in triplicate (n=3). The analysis of variance was carried out using the Statistical Package for Social Science (SPSS) software (version 19.0). Significant differences between means were assessed using Duncan's New Multiple Range test with the level of significance set at P≤0.05.

## 3. Results and Discussion

### 3.1 Microbial value

The results of the TPC and yeast and mold count are presented in Figure 1a and Figure 1b, respectively. The results found that the microbial counts increased throughout the storage time up to 9 days. The acceptable shelf life according to the Thai FDA standard regulations is a microbial count not more than $5\times10^5$ cfu/g (AOAC, 2000). The acceptable shelf life result of the untreated (NC) sample was 3 days. Most of the beef samples treated by CEP, RBO, and the combination of 1% CEP+0.2% RBO had acceptable shelf lives up to 5 days which was longer than the NC beef. Moreover, 2% CEP+0.2% RBO provided the best effective treatment condition that prolonged the microbial safety in the beef up to 7 days under the standard level of TPC and yeast and mold counts. Based on the above results, it could be implied that the antimicrobial activity of CEP and RBO was possibly related to the presence of the phenolic compounds (Lin, Labbe, & Shetty, 2004; Walsh, 2003). The greater antimicrobial activity of the CEP and RBO treatments on the beef samples might be a complicated mechanism. However, only a few studies have concluded that antimicrobials were proven to be high phenolic compounds (Ramesh & Pattar, 2010; Friedman, 2013). Phenolic compounds are believed to act as antimicrobial substances. They can degrade the microbial cell wall which results in cytoplasmic membrane disruption that leads to a leakage of cellular components and destroys the synthesis of DNA, RNA, and protein translocation. The mechanism of antimicrobial refers to the presence of hydroxyl groups (Cha & Chinnan, 2004; Ramesh & Pattar, 2010; Friedman, 2013).

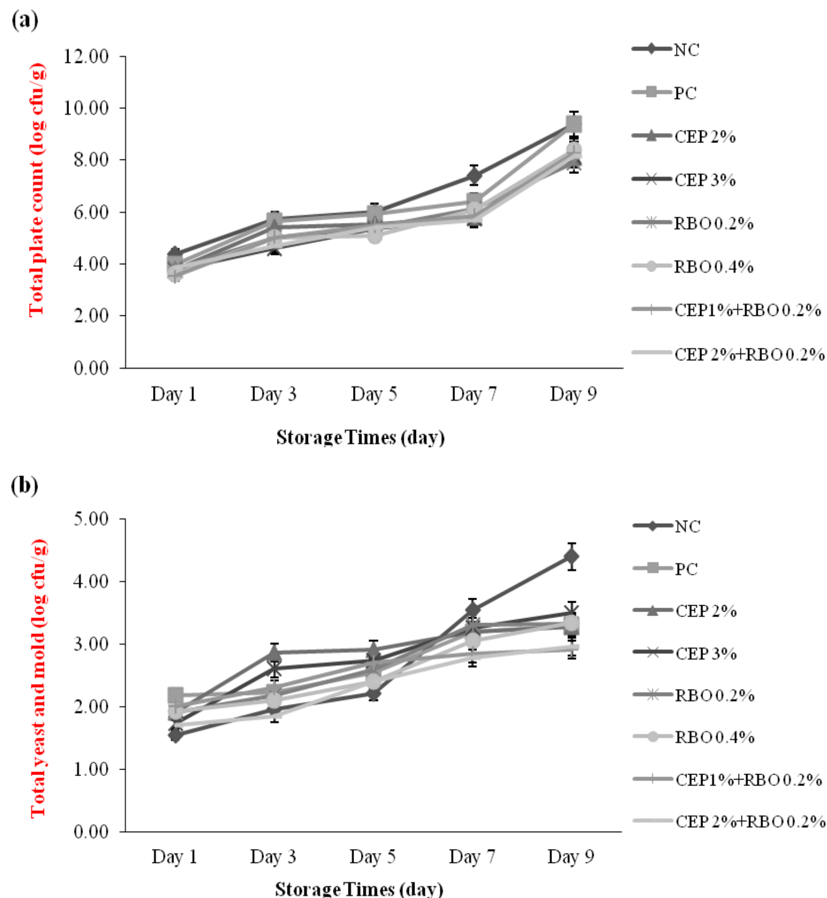


Figure 1. Total plate count (a) and total yeast and mold (b) in fresh beef treated with different concentrations of purple corn cob extract powder (CEP), black rice bran oil (RBO) and their combinations during storage at 4 °C for 9 days.
NC=negative control (untreated); PC=positive control (0.02% butylated hydroxytoluene)

## 3.2 AOA

The ABTS and DPPH assays determined the AOA in the fresh beef samples during storage. On the first day, the sample treated with 3% CEP provided the highest AOA followed by the 0.4% RBO sample (Figure 2). From our previous study, the CEP sample had a higher AOA measured by the ABTS assay (676 mg Trolox/100 g) and DPPH assay (518 mg Trolox/100 g) than the RBO (18 and 52 mg Trolox/100 mL, respectively) (data not shown). The greater AOA of CEP could be attributed to the higher concentration of phenolic compounds (663 mg gallic acid/100 g) than the RBO (12 mg gallic acid/100 mL) (data not shown). In addition, the main antioxidant compounds in the oil extracted from rice bran are tocopherols, tocotrienols, and γ-oryzanol (Thanonkaew, Wongyai, Decker, & Mc Clements, 2015). The declining trends of AOA measured by the ABTS assay were found throughout the storage period. However, an increase in the AOA by DPPH assay was observed after storage for 3 days and then decreased over the storage time. This occurred because the storage time could possibly induce the breakdown of some sensitive phenolic antioxidants that were in the CEP and RBO.

## 3.3 TBARS

Lipids in meat are susceptible to oxidation which causes the breakdown of nutritive fatty acids and yielding off-flavors. Actually, the TBARS test is used to estimate the degree of lipid oxidation by detecting the secondary product of oxidation. So, it can be an alternative way to evaluate the effectiveness of antioxidants (Luo *et al.,* 2007). The results showed that the treatment condition and storage time significantly affected the TBARS value ($P \leq 0.05$) (Figure 3). Fresh beef treated with both concentrations of pure CEP effectively retarded lipid oxidation during storage for 7 days and the TBARS values were between 0.46 and 1.44 mg MDA/kg. These values were lower than the acceptable quality limit value of 2.28 mg MDA/kg according to Campo *et al.* (2006). However, according to our previous results on microbial values, the beef treated with pure CEP treatments provided 5 days of microbial quality. Likewise the sample treated by 2% CEP+0.2% RBO could extend shelf life up to 5 days based on the TBARS value. The protection against fat oxidation of CEP in fresh beef might be due to the presence of high phenolic content that contributed to the marked AOA in preventing lipid oxidation (Zhang, Zhang, Zhang, & Liu, 2010; Velioglu, Nazza, Gao, & Oomah, 1998). The mechanism might be related to the ability of donating hydrogen atoms of the phenolics and neutralizing free radicals by scavenging the free radicals. In the case of beef treated with RBO, lipid oxidation rapidly increased during storage. This result possibly resulted from lipids in the RBO that could cause lipid oxidation and promote the rate of oxidation in fresh beef during storage.

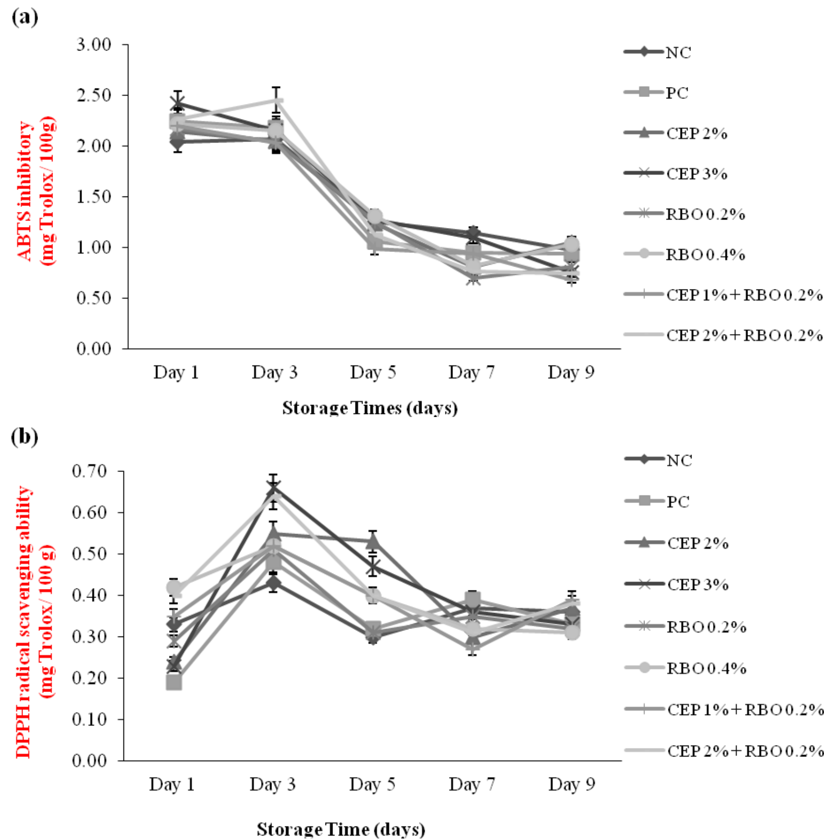


Figure 2. ABTS inhibitory (A), and DPPH radical scavenging ability (B) in fresh beef treated with different concentrations of purple corn cob extract powder (CEP), black rice bran oil (RBO) and their combinations during storage at 4 °C for 9 days.
NC=negative control (untreated); PC=positive control (0.02% butylated hydroxytoluene)

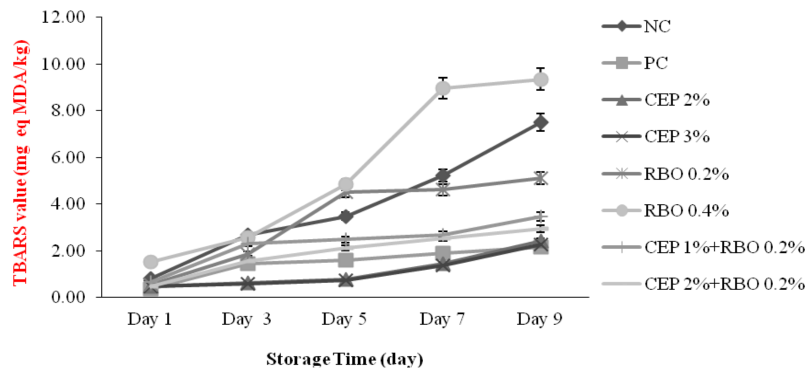


Figure 3. Thiobarbituric acid-reactive substances (TBARS) value in fresh beef treated with different concentrations of purple corn cob extract powder (CEP), black rice bran oil (RBO) and their combinations during storage at 4 °C for 9 days.
NC=negative control (untreated); PC=positive control (0.02% butylated hydroxytoluene)

### 3.4 Color

The color appearance in fresh meat is an important factor for consumers because most consumers have learned through experience that the color of fresh beef is bright red (Neethling, Suman, Sigge, Hoffman, & Hunt, 2017). The color parameters $L^*$, $a^*$, and $b^*$ of fresh beef are shown in Figures 4a, 4b, and 4c, respectively. As expected, the sample treated with the higher concentration of CEP gave a higher $a^*$, while a reduction of $L^*$ was detected due to the effect of the purple pigment called anthocyanin in purple corn. During storage, an increase of $b^*$ was observed in all samples, whereas $a^*$ had a decreasing trend. However, $a^*$ in the samples treated by CEP 3% and CEP 2%+RBO 0.2% remained at a high level during storage. Based on these results, CEP had a natural red color that could be used in fresh beef to improve the color and appearance of the retail cut.

## 4. Conclusions

The present study showed that the application of CEP at the concentration of 2% and 3% as well as CEP 2%+RBO 0.2% on fresh beef could effectively extend the shelf life up to 5 days with acceptable levels of microbial counts and lipid oxidation values as well as the color.

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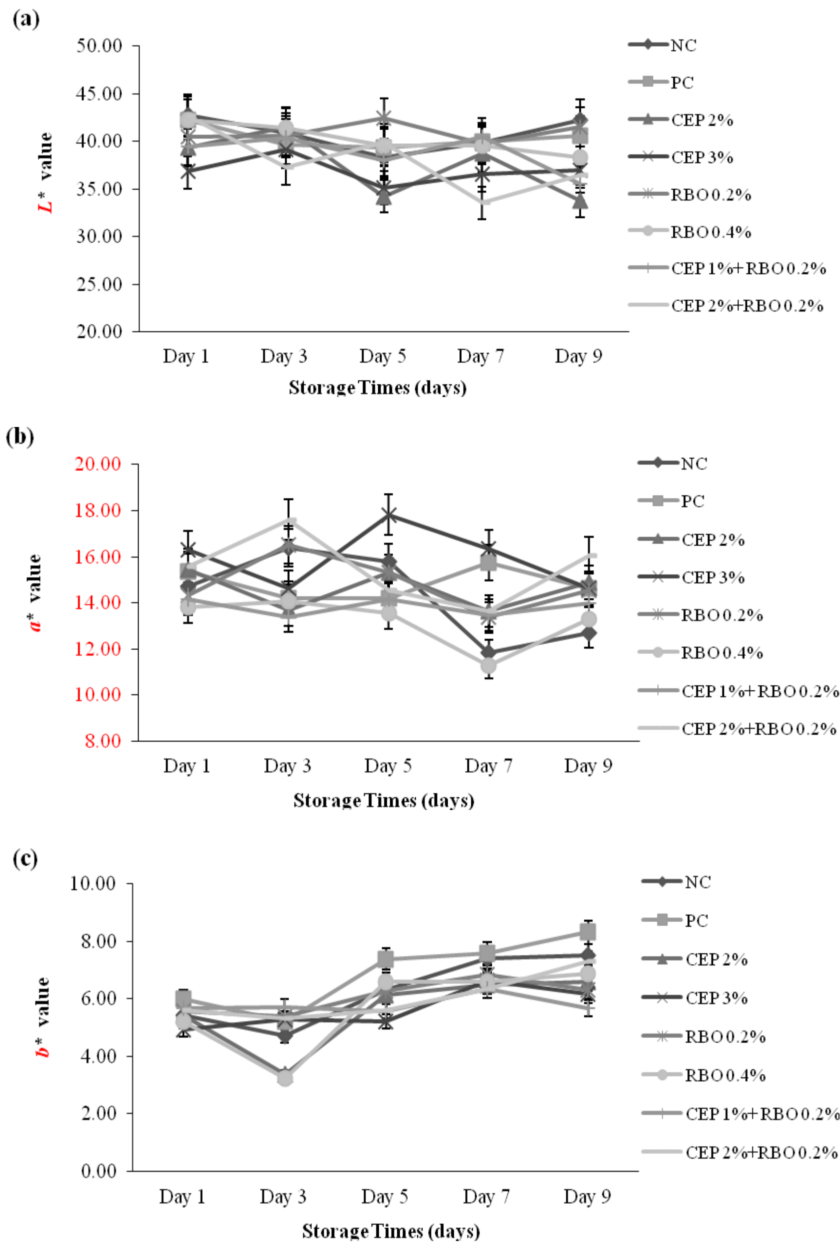


Figure 4. Color parameters of *L** (a), *a** (b) and *b** (c) in fresh beef treated with different concentrations of purple corn cob extract powder (CEP), black rice bran oil (RBO) and their combinations during storage at 4 °C for 9 days.
NC=negative control (untreated); PC=positive control (0.02% butylated hydroxytoluene)


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