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

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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 shelf-life of fresh beef during cool 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 and positive treatment (0.02% butylated hydroxytoluene (w/w)). The results showed that microbial count, and thiobarbituric acid reactive substances (TBARS) values increased in all samples during storage up to 9 days, whereas, antioxidant activities were unstable. However, the beef with 2% and 3% of CEP and CEP 2% + RBO 0.2% were the most effective application to improve shelf-life up to 5 days, with the 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 compositions, consisting 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 deteriorating 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 chemical for controlling oxidative
deterioration in meat and meat products (Dave & Ghaly, 2011). Generally, there are many
natural antimicrobial compounds which widely used as alternative preservation in meat
products, such as essential oils (Burt, 2004; Rasooli, 2007) and spice (Sema, Nursel, &
Suleyman, 2007).

Purple corn and black rice bran are a 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). Beside this, colored rice bran contains high levels of
anthocyanins, phenolics as well as tocols and γ-oryzanol that play an important role in
antioxidant potency (Zhang et al., 2006; Jang & Xu, 2009; Mutana & Prasong, 2010).
According to Arpan, Praveen, and Singh (2013), rice bran oil possessed anti-bacterial
effect against selected strains, such as *Escherichia coli*, *Pseudomonas aeruginosa* and
*Staphylococcus aureus*. The previous research showed antioxidant properties and
phenolic constituents from spices that effectively inhibited microbial growth and lipid
oxidation on 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
improve the functional health properties. However, no research has been done on the
application of CEP and RBO on the preservation of shelf-life in fresh beef. Therefore,
the objectives of this study were to determine the effect of CEP and RBO on the qualities and safety in fresh beef during cooling storage.

2. Materials and Methods

Fresh beef was purchased from a local slaughterhouse in Khon Kaen Province, Thailand. The fresh beef approximately 13 kg of Longissimus lumborum muscle from one beef carcass was collected from slaughter house.

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 RBO was cold press extracted from black rice bran using oil compressor with 7.5 horsepower (Model: SF-JR, Mitsubishi, Japan). The crude RBO was filtered by 20 micron sieve and then kept in glass bottle before using. The purple corn cob was obtained from the Siam Miragro Co., Ltd. (Khon Kaen Province, Thailand). The purple corn cob was dried under 60°C using hot air oven until final moisture content reached 10%. The cob was epidermis section and was extracted using hot water (at 85°C). The purple anthocyanin extract solution was encapsulated with carrier agent and dried to obtain purple powder using spray dryer. All chemicals and reagents used 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 laboratory refrigerator under 4±1°C within 1 h after slaughtering. The meat was cut perpendicular to muscle fiber direction. Each sample had an average weight of 100 g. 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. The treatments of this experiments were set as the following: meat sample with 2% CEP (2 g), meat sample with 3% CEP (3 g), meat sample with 0.2% RBO (0.2 mL), meat sample with 0.4% RBO (0.4 mL), meat sample with 1% CEP and 0.2% RBO, meat
sample with 2% CEP and 0.2% RBO, negative control (NC) (untreated beef) and positive control (PC) (meat sample with 0.2% BHT). Each sample was packed in low-density polyethylene bags and stored at 4±1°C. The samples were collected and determined for physical and chemical properties in 3 days interval for 9 days of storage time.

2.2 Antioxidant activities (AOA)

A 10 g of ground beef sample was extracted with 30 ml methanol for 3 h under temperature 60°C using a shaker. The extract was filtered through filter paper before determine for AOA using 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay (DPPH) and 2,2’-azino-bis(3-ethylbenzothiazoline-6-solfonic acid diammonium salt) cation decolorization assay (ABTS). The DPPH assay was estimated using the protocol developed by Leong and Shui (2002) with some modifications. Briefly, 0.1 mM DPPH solution in method was freshly prepared. A 100 μL of 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 spectrophotometer (Shimadzu UV 1800, Japan). 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, radical cation of ABTS (ABTS\(^{++}\)) was generated by reacting ABTS (7 mM) with potassium persulphate (4.95 mM) with the ratio 1:1 (v/v) for 12 h at room temperature in the dark. The ABTS\(^{++}\) stock solution was diluted with phosphate buffer solution to absorbance values 1.0 AU at 734 nm using UV/Vis spectrophotometer. A 40 μL sample extract was mixed with 4 mL of ABTS\(^{++}\) 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 was analyzed according to Tarladgis, Watts, Younathan, & Dugan (1960) with some modifications. A 10 g of ground beef was homogenized with 40 mL of distilled water for 10 min. The homogenized meat liquid by 2.5 mL was transferred to a test tube and then added with 2.5 mL of TBA reagent (0.02 M 2-thiobarbituric acid in distilled water). The mixture was mixed well and incubated in a boiling water bath for 1 h. The temperature of mixture was cooled down to room temperature and then centrifuged at 4,000 rpm for 10 min by centrifuge (Z-200 A, HERMLE, Wehingen, Germany). The supernatant part of solution was determined the absorbance at 538 nm by spectrophotometer. The result was expressed as mg of malonaldehyde per kg sample (mg MDA/kg).

### 2.4 Color

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

### 2.5 Microbial analysis

Total plate count (TPC), and total yeast and mold count are rapid methods as described by AOAC (1990). A 25 g of fresh beef was aseptically transferred into a sterile stomacher bag. The sample was added 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 of aliquot from each dilution was plated onto standard plate count agar (PCA) and incubated at 35°C for 24 h for TPC analysis. For yeast and mold, the 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 triplicates (n=3). The analysis of variance was carried out using the statistic package for the social science (SPSS) software (version 19.0). The significant differences between means were assessed by the Duncan’s New Multiple Range test with significant effect at p<0.05.

3. Results and Discussion

3.1 Microbial value

The results of TPC and yeast and mold count were presented in Figure 1 (a) and (b), respectively. These results found that the microbial counts increased throughout the storage time up to 9 days. The acceptable shelf-life, reported by Thai FDA standard regulation was with the microbial counts not more than $5 \times 10^5$ cfu/g (AOAC, 2000), and then acceptable shelf-life of NC sample (no treated) was 3 days. Most of beef samples treated by CEP, RBO, and the combination at 1% CEP+0.2% RBO had acceptable shelf-life up to 5 days which showed longer than that of NC beef. Moreover, 2% CEP+0.2% RBO provided the best effective treatment condition that prolonged the microbial safety in beef up to 7 days under the standard level of TPC and yeast and mold counts. Based on the above results, it could be noticed that antimicrobial activity of CEP and RBO might be related to the presence of phenolic compounds (Lin, Labbe, & Shetty, 2004; Walsh,
In relation of the results, the greater antimicrobial activity of CEP and RBO treated on beef samples might have complicated structure. However, only a few studies have concluded that antimicrobials were proven by high phenolic compounds (Ramesh & Pattar, 2010; Friedman, 2013). Phenolic compounds are believed to act as antimicrobials substances. They can degrade microbial cell wall which results in cytoplasmic membrane disruption, that lead to a leakage of cellular components and destroying 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).

3.2 AOA

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

Lipid in meat is susceptible for oxidation causing the breakdown of nutritive fatty acid and yielding off-flavors. Actually, TBARS 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 result from Figure 3 showed that the treatment condition and storage time significantly affected the TBARS value ($p \leq 0.05$). Fresh beef treated with both concentrations of pure CEP effectively retarded lipid oxidation during storage for 7 days, with TBARS value in the range between 0.46-1.44 mg MDA/kg, these values were lower than the acceptable quality limit value (2.28 mg MDA/kg) according to Campo et al. (2006), however, according to our previous result of microbial value, the beef treated with pure CEP treatments provided 5 days for microbial quality. Likewise the sample treated by 2% CEP+0.2% RBO, could extend shelf-life up to 5 days regarding 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 mark 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 phenolics and neutralizing free radical by scavenges the free radical. In case of the beef treated with RBO, it could be found that the lipid oxidation rapidly increased during storage. This result could be noted that lipid compositions in RBO can be reactant for lipid oxidation and promote the rate of oxidation reaction in fresh beef during storage.

3.4 Color

The color appearance in fresh meat is important factor for consumer selection 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 were shown in Figure 4 (a), (b) and (c), respectively. As expected, the sample treated with higher concentration of CEP gave the higher in $a^*$, while the reduction of $L^*$ was detected due to the effect of the purple pigment called anthocyanin in purple corn. During storage, the increasing of $b^*$ was observed in all samples, whereas, the trend of $a^*$ was found to decrease. However, $a^*$ in the samples treated by CEP 3% and CEP 2%+RBO 0.2% remained high level during storage. Based on these results, CEP has a natural red color that could be used in fresh beef in order to improve color and appearance of retail cut.

4. Conclusions

The present study showed that the application of CEP at the concentration 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 the acceptable level of microbial counts and lipid oxidation value as well as the color appearance.

Acknowledgments

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References


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)
**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)
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25 Day 1

Storage Times (days)

20.00 25.00 30.00 35.00 40.00 45.00 50.00

NC PC CEP 2% CEP 3% RBO 0.2% RBO 0.4% CEP 1%+ RBO 0.2% CEP 2%+RBO 0.2%

For Review Only

26 Day 1

Storage Times (days)

8.00 10.00 12.00 14.00 16.00 18.00 20.00

NC PC CEP 2% CEP 3% RBO 0.2% RBO 0.4% CEP 1%+ RBO 0.2% CEP 2%+RBO 0.2%

For Review Only

27 Day 1

Storage Times (days)

0.00 2.00 4.00 6.00 8.00 10.00

NC PC CEP 2% CEP 3% RBO 0.2% RBO 0.4% CEP 1%+ RBO 0.2% CEP 2%+RBO 0.2%

For Proof Read only
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)