International Journal of Agricultural Technology 2024 Vol. 20(1):265-274
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The effect of black rice extract powder and black rice bran oil on quality and shelf life of fresh ground beef patties during refrigerated storage

Panatuk, J.[1], Uriyapongson, J.[2] and Uriyapongson, S.[3*]

[1]Faculty of Animal Science and Technology, Maejo University, Chiangmai, 50290, Thailand; [2]Department of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen, 40002, Thailand; [3]Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand.

Panatuk, J., Uriyapongson, J. and Uriyapongson, S. (2024). The effect of black rice extract powder and black rice bran oil on quality and shelf life of fresh ground beef patties during refrigerated storage. International Journal of Agricultural Technology 20(1):265-274.

Abstract According to the result, all samples had rised the microbiological count and the value of the reactive chemical thiobarbituric acid after storage for six days. However, the beef with black rice extract powder 2.0% (w/w) + 0.2% (w/v) black rice bran oil were the most effective applications to improve the color appearance and 6 days of shelf life with acceptable reactive substance value and microbial count level. These findings seem to be the ground beef may benefit from a natural biopreservative solution consisting of black rice bran oil and black rice extract powder.

Keywords: Beef patties, Black rice, Color appearance, Lipid oxidation

Introduction

One of the most popular beef products eaten globally is beef patties; nevertheless, the process of grinding increases the surface area of the meat, which is relatively rich in unsaturated fatty acids and more prone to lipid oxidation during manufacturing and storage (Dave and Ghaly, 2011). Colour appearance, Lipid oxidation, and microbiological deterioration are critical elements that impact the quality and safety of fresh meat. Additionally, the breaking releases oxygenated substances like aldehydes and ketones, which may make meat lose its colour and nutritional value (Addis *et al.,* 2015). Typically, bacterias are limited to muscle fragment surface. Although, grinding might change how germs are distributed throughout the ground beef, making it more prone to degradation (Bae *et al.,* 2011). Furthermore, because mincing exposes fatty acid fractions to pro-oxidants (free iron in muscles), which produces free radicals and leads them to become rancid fast, it undermines the

[*]**Corresponding Author:** Uriyapongson, S; **Email:** suthipng@kku.ac.th

integrity of muscular membranes (Muthukumar *et al.,* 2014). These are all adversely impacted by the state of the economy and consumers. Conventional techniques for preserving fresh meat and its by-products with chemical additions are recognised practises. Meat contains many antibacterial chemical components, such as organic acids, sulphites, nitrites, and sodium chloride (Dave and Ghaly, 2011). However, since synthetic substances are thought to induce carcinogens, their usage has become less acceptable in recent years (Tang *et al.,* 2001). Due to their safety, modern customers need that meat products include natural components. The amount of research done on the natural ingredients found in meat has grown recently. To extend the shelf life of their meat products, several plant extracts with strong potential as antioxidants and antibacterial agents are added. For example, phenolic acids, tannin, carotenoids and flavonoids, (Naveena *et al.,* 2008; Devatkal *et al.,* 2010). Certain plant extracts, such pomegranate, blackberry, and cranberry, have been effectively used to study the antioxidant and antibacterial properties of meat (Ganhão *et al.,* 2013; Firuzi *et al.,* 2019; Tamkutė *et al.,* 2019).

Numerous bioactive substances, such as tannin, flavones, phenolics, anthocyanins, tocols, sterols, essential oils, and γ-oryzanols, are present in the oil extracted from black rice (Deng *et al.,* 2013). Black rice extract powder and rice bran oil have effective antibacterial qualities, particularly against pathogenic bacterial strains. Despite the assumption that black rice bran oil would increase the meat's and meat products' shelf life, we are unaware of any research that has examined the impact of either plant extract on the quality of freshly made beef patties. Thus, the objectives were to investigate the black rice bran oil and extract powder affected the safety and quality of ground beef patties kept in a refrigerated storage for six days.

Materials and methods

The local abattoir in Chiangmai Province, Thailand, sold fresh beef chuck muscles from a single corpse. In February 2020, this experiment was carried out. Within two hours after slaughter, the beef was collected, sealed in a low-density polyethylene bag, and transported in less than twenty minutes to the "Meat Quality Laboratory of the Faculty of Animal Science and Technology" at Maejo University in Thailand. Before usage, this beef was refrigerated for 4 hours at 4°C. The black rice extract from Xi'an Rongsheng Biotechnology Co., Limited in China. The black rice bran oil extracted by using a cold press, the black rice bran oil was filtered through a 20µ screen. Analytical grades of all chemicals and reagents were utilised.

After trimming the beef of any loose connective tissue and separable fat, it was chopped into tiny cubes and minced with a meat grinder (Model WD 114, Germany) fitted with 3mm fine and 8 mm coarse plates. After the ground meat, the black rice extract powder and bran oil were combined with the treatments. Following a one-minute blending period, 200 grammes of treated ground beef were divided among the experimental groups and placed within a plastic tray. In this experiment, a completely randomized design (CRD) was used. The eight treatments included the sample untreated (negative control), treating the positive control with 0.02 percent butylated hydroxytoluene (w/w), and treating the beef samples with 1.0 and 2.0 percent (w/w) powdered black rice extract. Black rice bran oil concentrations of 0.2 and 0.4% (v/w) as well as combinations of 1.0 percent black rice extract powder +0.2% and 2.0 percent black rice extract powder+0.2% were applied to the beef samples in five replications each. The samples were put in a fridge at a temperature of 4±1°C after being covered in plastic film. During a 6-day storage period, the samples were examined for pH, colour, thiobarbituric acid reactive chemicals value, and microbiological count every three days.

pH and colour

A digital pH meter (HI61228, Hanna, USA) with a combination glass electrode was used to measure the pH of the meat suspension, which was made by mixing 10g of minced beef with 50ml of distilled water for one minute (Muthukumar *et al.,* 2014). AMSA: "American Meat Science Association" protocol was used while analysing the meat's colour. With D65 illumination, an observer angle of 10 degrees and an aperture size of 3.18cm, the colour parameters of the beef surface, L*(lightness), a*(redness), and b*(yellowness), were estimated with a colorimeter (Konica-Minolta CR-400, Osaka, Japan).

Thiobarbituric Acid Reactive Substances* (TBARS) *value

To analyse the TBARS value, an extraction procedure was followed outlined by (Tarladgis *et al.,* 1960). After adding 10g of minced beef and 40mL of distilled water, the mixture was blended for ten minutes. 2.5mL of a 0.02M trichloroacetic acid solution was mixed with 2.5mL of homogenised beef liquid. After thoroughly mixing the slurry, it was heated for one hour at 100 °C in the water bath. After cooling under running tap water, the mixture was centrifuged for 10 minutes at 4000RPM. At 538 nm, the absorbance was measured in the supernatant. The amounts for TBARS were given in mg MDA/kg of sample (mg of malonaldehyde per kilogramme).

Microbiological quality

The "Official method of analysis of AOAC International" (1995) approach was followed to calculate the TPC (Total Plate Count). In order to determine the microbiological count, 25g of minced beef sample were added to 225mL of sterile peptone water containing 0.1percent, and the mixture was homogenised for two minutes using a Stomacher. For the purpose of calculating the total plate count, 1 ml of the proper serial dilutions were plated using the pour plate technique and incubated for 24 hours at 35°C.

Statistical analysis

A completely randomized design (CRD) was used for the experimental design. IBM® SPSS Statistics V20.0 was used to analyse and compare the mean values of the parameters under study using an analysis of variance (SPSS Inc., USA). At a 95% confidence level ($P \leq 0.05$), for comparing the means, Duncan's multiple range test was used.

Results

Effect of black rice extract powder and black rice bran oil on pH and color appearance

All treatments had an average pH between 5.45 and 5.82. For all treatments, there was no difference in pH ($P > 0.05$). The colour characteristics of treated and control fresh ground beef samples after cold storage is listed in Table 1. The addition of 2.0% black rice extract powder + 0.2% black rice bran oil affected on low lightness of ground beef patties in comparison with other treatments on 0 day ($P < 0.05$). Lightness of beef patties during the storage had no impact on the addition of black rice bran oil at two levels.

Effect of black rice extract powder and black rice bran oil on microbiological of beef patties

Results of microbial quality was demonstrated in Figure 1. The results showed that the TPC increased during the storage time in the samples ($P < 0.05$), but the samples treated with black rice bran oil and black rice extract powder increased the shelf-life for 6 days microbial quality by reducing the population of microbial when it was compared to the negative control group ($P < 0.05$). The additional black rice extract powder and black rice bran oil showed that microbial population was similar to the positive control ($P > 0.05$).

Table 1. The colour of fresh ground beef patties that were refrigerated for 6 days and were treated with varying amounts of black rice extract powder and black rice bran oil

Parameter	Treatment	Storage time (day)		
		0	3	6
L*	NC	48.85^{Aa}	49.02^{Aa}	50.29^{Ab}
SE = 0.69	PC	48.72^{A}	48.74^{A}	49.02^{A}
	BREP 1.0%	46.15^{Ba}	45.54^{Cb}	45.78^{Cb}
	BREP 2.0%	44.23^{Ca}	43.12^{Db}	43.18^{Db}
	BRBO 0.2%	48.12^{A}	47.84^{B}	48.57^{B}
	BRBO 0.4%	47.85^{A}	47.87^{B}	47.49^{B}
	BREP 1.0% + BRBO 0.2%	46.38^{Bb}	47.62^{Ba}	47.32^{Ba}
	BREP 2.0% + BRBO 0.2%	45.47^{BCb}	46.78^{Ca}	45.44^{Cb}
a*	NC	32.14^{Ba}	26.25^{Bb}	18.64^{Dc}
SE = 0.73	PC	34.52^{Aa}	28.78^{Ab}	26.94^{Ac}
	BREP 1.0%	33.74^{Aa}	24.29^{Cb}	20.74^{Cc}
	BREP 2.0%	34.89^{Aa}	28.57^{Ab}	26.47^{Ac}
	BRBO 0.2%	33.41^{Aa}	28.23^{Ab}	24.25^{Bc}
	BRBO 0.4%	32.18^{Ba}	29.12^{Ab}	23.47^{Bc}
	BREP 1.0% + BRBO 0.2%	29.31^{Ca}	26.42^{Bb}	24.49^{Bc}
	BREP 2.0% + BRBO 0.2%	32.14^{Ba}	25.87^{Bb}	24.22^{Bb}
b*	NC	22.18^{Aa}	20.13^{Ab}	18.32^{Ac}
SE = 0.61	PC	21.16^{ABa}	21.23^{Aa}	17.97^{Ab}
	BREP 1.0%	18.23^{Ca}	16.12^{Cb}	17.54^{ABa}
	BREP 2.0%	17.61^{Da}	14.18^{Db}	15.48^{Cb}
	BRBO 0.2%	20.28^{Ba}	17.87^{BCb}	16.48^{Bb}
	BRBO 0.4%	21.32^{ABa}	16.25^{Cb}	16.78^{Bb}
	BREP 1.0% + BRBO 0.2%	19.23^{BCa}	17.41^{Bb}	18.32^{Aab}
	BREP 2.0% + BRBO 0.2%	16.98^{Db}	18.62^{Ba}	18.38^{Aa}

$^{A\text{-}C}$ Least square means with different letters within the same day of storage time are significantly different (P<0.05). $^{a\text{–}c}$ Least square means with different letters within the same treatments are significantly different (P < .001). L* represents lightness; a* represents redness; b* indicates yellowness.

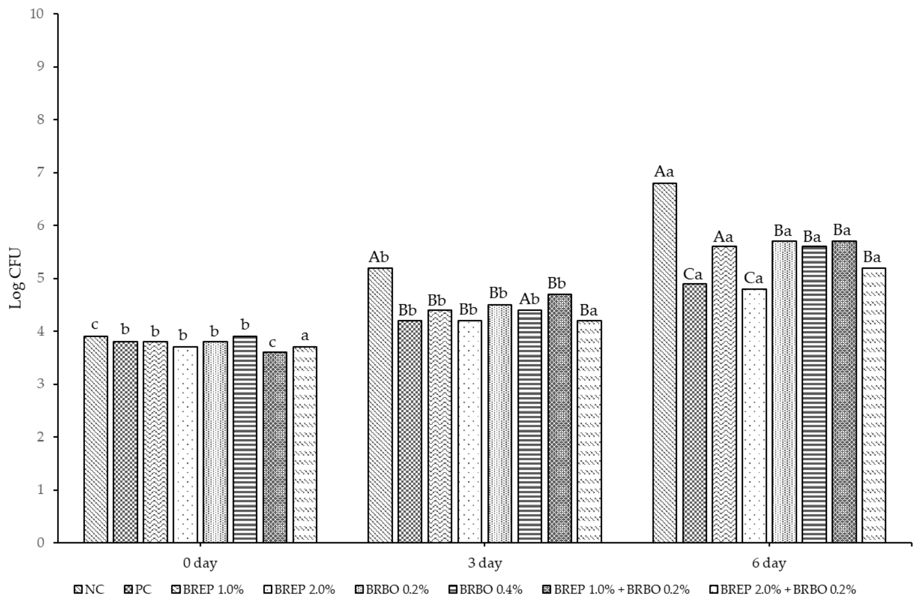

Figure 1. Beef patties TPC (log CFU/g) when treated with varying concentration of treated black rice bran oil and extract powder when refrigerated for six days. A-C Least square means with different letters within the same day of storage time are notably different ($P < 0.05$). a-c Least square means with different letters within the same treatments are notably different ($P < 0.05$). The standard error for treatments and storage time comparison is 0.35.

Effect of black rice extract powder and black rice bran oil on lipid oxidation

For fresh ground beef, lipid oxidation was examined using the thiobarbituric acid reactive compounds value method (Figure 2). Thiobarbituric acid reactive substances value increased in all samples which indicated the formation of aldehydes in fresh ground beef during 6 days of storage ($P<0.05$). The samples with 0.4% black rice bran oil had the high thiobarbituric acid reactive substances value in day 6 but not different with the negative control group, whereas other samples addition with black rice extract powder had the potential to reduce the Thiobarbituric acid reactive substances value.

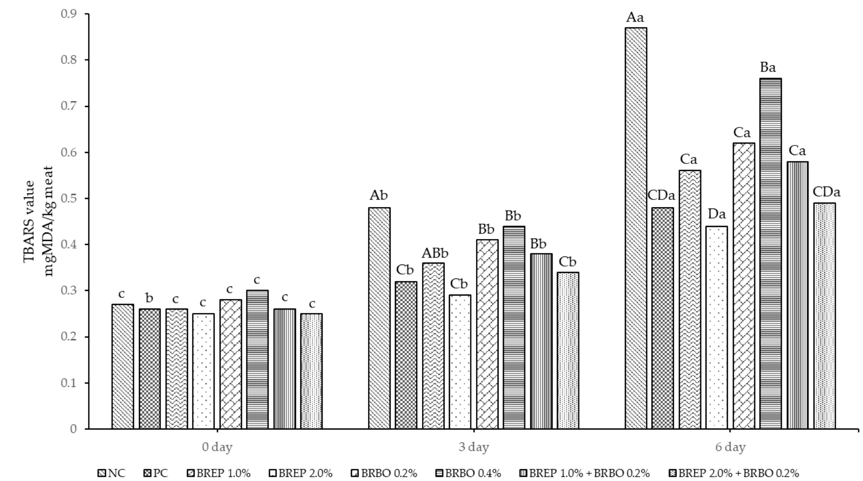

Figure 2. The value of reactive chemicals such as thiobarbituric acid in beef patties that were stored in a refrigerator for six days and were treated with varying amounts of black rice bran oil and extract powder. A-C Least square means with different letters within the same day of storage time are notably different ($P < 0.05$). a-c Least square means with different letters within the same treatments are notably different ($P < 0.05$). The standard error for treatments and storage time comparison is 0.32.

Discussion

One of the key factors that determines the quality of raw meat is its colour. The red colour of the meat, which indicates its freshness, is caused by the myoglobin pigment found inside it. However, because of lipid oxidation and microbial spoiling, this pigment is far more vulnerable to oxidative processes when stored (Muthukumar *et al.,* 2014). For that reason, one of the main aims of the addition of natural extract in fresh meat is to increase the red colour (a*). The colour characteristics of the treated and control fresh ground beef samples after cold storage are demonstrated. On day zero, the ground beef patties' lightness was reduced when 2.0% black rice extract powder and +0.2% black rice bran oil were added, as compared to the other treatments. While L* fell dramatically in the control sample after storage, the treated samples showed no change in these values. While all samples showed a drop in a*, the b* values remained to be unchanged during storage. All samples gradually displayed a fall in chroma and a rise in hue°, indicating a decline in redness brought on by metmyoglobin build-up during storage, giving the meat an unappealing colour (Haak *et al.,* 2006).

When the colour of the control beef changed from red to brown, these effects were apparent. In a similar manner, Muthukumar *et al.* (2014) found that L* and b* values of uncooked patties infused with Moringa oleifera leaf extract were constant after 9 days of storage at 4°C. The research suggested that fresh ground beef might retain more of its red colour even after prolonged storage, if the PC dosage is increased. In other study, Formanek *et al.* (2003) found that reducing the proportion of metmyoglobin and raising oxymyoglobin, the addition of rosemary extracts to irradiation that enhanced the colour retention of minced beef during storage.

Thai Agricultural Commodity and Food Standard (TACFS 6000-2004) stated that fresh beef may be reached 5×10^5cfu/g of permissible microbiological counts. The total plate count grew in all samples throughout the course of storage, according to the microbiological quality results; however, the samples that received all extra treatments had a microbial quality shelf life of up to six days. Under general, raw meat may be stored in refrigeration for five to seven days (Mitsumoto *et al*., 2005). It is thought that phenolic chemicals, which have hydroxyl groups in their molecules and are positioned relative to one another in the benzene ring, are responsible for the natural extract's antibacterial properties. Additionally, the black rice bran oil and extract powder used in this experiment have a lipophilic quality and a functional group that may penetrate cell membrane structures and have an antibacterial effect, increasing the cell membrane permeability (Jiang *et al*., 2011).

It is well knowledge that lipid oxidation in meat during storage causes off-flavour, rancidity, and organoleptic changes in the product; nevertheless, these major issues resulted in a loss of functionality, shelf life, nutritional value, customer acceptability, most importantly, and safety (Arab-Tehrany *et al*., 2012). Using the TBARS technique, lipid oxidation in freshly ground beef was examined. All samples showed a rise in TBARS levels, indicating the development of aldehydes in the fresh ground beef after six days of storage. Compared to control, it could be seen that black rice bran oil does not enhance lipid oxidation prevention in fresh beef. The investigated natural extracts, phenols are a major constituent that work as antioxidants to stop meat's fat from oxidising.

Major phenolic diterpene compounds found in black rice extract powder, such as carnosic acid and carnosol, are excellent hydrogen donors that inhibit lipid oxidation (Georgantelis *et al*., 2007), and according to (Tomović *et al.,* 2017), this extract is widely employed as the natural antioxidant in most of the processed meat products. According to these findings, fresh ground beef patties treated with 2.0 percent BREP were shown to be significant levels of active chemicals that prevented fat oxidation and microbial development, as well as extending the patties' shelf life at 4°C for 6-days. At the same time, not a single one of the extracts improved the colour quality. Hence, BREP may be used as a preservative and antioxidant component to protect the meat products from oxidative rancidity without negatively impacting their sensory attributes.

Acknowledgments

The authors gratefully acknowledge the supported by This work was supported by the Post-Doctoral Training Scholarship (PD2562-06), Khon Kaen University, and the Faculty of Animal Science and Technology, Faculty of Science, Maejo University for providing necessary technical facilities in this study.

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(Received: 25 December 2022, Revised: 23 September 2023, Accepted: 14 November 2023)