International Food Research Journal 28(5): 976 - 986 (October 2021)

Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of *Dimocarpus longan* var. *obtusus* seed aqueous extract on lipid oxidation and microbiological properties of cooked pork patties during refrigerated storage

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Article history

Received: 26 June 2020
Received in revised form: 11 March 2021
Accepted: 29 March 2021

Keywords

longan seed,
oxidative stability,
antimicrobial activity,
cooked pork patties

Abstract

Meat spoilage is a predominant cause of rancidity, colour, texture, and flavour alterations in meat products. The use of natural compounds as antioxidants in food products has been of considerable interest for consumers in recent years. The purpose of the present work was therefore to determine the peroxide value, thiobarbituric acid reactive substances, physicochemical, and sensory characteristics of Lumyai Thao (LT) (*Dimocarpus longan* var. *obtusus*) seed aqueous extract incorporated in cooked pork patties during storage at 4°C for 21 days. Different concentrations of LT seed aqueous extract (0, 0.05, 0.10, and 0.20% (w/w)) and butylated hydroxyanisole (BHA) (0.01% (w/w)) were added to cooked pork patties. Results revealed that the addition of 0.20% LT seed aqueous extract was the most effective in inhibiting lipid oxidation in cooked pork patties, and the inhibition was observed to be stronger than that with BHA treatment ($p < 0.05$). LT seed aqueous extract at 0.20% could significantly preserve the colour of cooked pork patties ($p < 0.05$). The increasing concentration of LT seed aqueous extract decreased the moisture content, while the textural properties of cooked pork patties were enhanced. LT seed aqueous extract at 0.20% effectively inhibited the growth of microorganisms in cooked pork patties. A sensory analysis using a 9-point hedonic scale showed that the treatments of LT seed aqueous extract (0.05 - 0.20%) exhibited no significant difference in terms of appearance, taste, and overall acceptability of cooked pork patties in comparison to control ($p > 0.05$). The present work demonstrated the benefit of LT seed aqueous extract at 0.20% as a natural antioxidant, and that it may be used as a food preservative to extend the shelf life of cooked pork patties, and possibly other meat products.

Introduction

Dimocarpus longan var. *obtusus*, also known as Lumyai Thao (LT) in Thai, is a subtropical tree that belongs to the Sapindaceae family. It is a 6-m tall creeping tree found only in Thailand (Janick, 2010). The edible fruit is sweet and juicy, with a width of 2.5 cm and containing a single round dark-brown seed (Lithanatudom *et al.,* 2017). The seeds are often discarded after fruit processing, and considered as agricultural waste.

Previous studies have reported that dried longan (*D. longan* Lour.) seed contained high levels of phenolic compounds such as gallic acid, corilagin, and ellagic acid with strong antioxidant activities (Huang *et al.,* 2012; Rangkadilok *et al.,* 2012). The safety concerned with using longan seed has been explored, and it has been reported that there are no adverse effects after the acute and repeated doses (4 and 13 weeks) of oral administration in longan seed extract-treated mice (Worasuttayangkurn *et al.,* 2012). In a recent study, *D. longan* var. *obtusus* seed aqueous extract was reported to contain high total phenolic compounds including gallic acid, and exhibited a potential antioxidant activity and anti-inflammatory effects in RAW 264.7 cells (Nitteranon, 2018).

Lipid oxidation and bacterial spoilage are the main causes of deterioration in the quality of meat and its products during storage. This deterioration alters colour, odour, texture, and nutritive values, resulting in a short shelf life. In particular, cooked meats are notably susceptible to lipid oxidation than raw meat because high cooking temperatures affect the release of free radicals which develop off-flavour in cooked meat (Amaral *et al.,* 2018). Furthermore, ground meats are more vulnerable to pathogenic bacteria during processing and storage. Owing to meat

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DOI: https://doi.org/10.47836/ifrj.28.5.11

grinding, the meat surface area is increased and exposed to air, leading to the loss of intracellular components and contamination by microorganisms (Park and Chin, 2010).

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), and propyl gallate (PG) have been widely used in the meat product industry to delay lipid oxidation, and extend the shelf life of meat and its products (Shah *et al.,* 2014). However, the safety associated with the use of synthetic antioxidants may plausibly cause a major concern for consumers due to their potential toxicological and carcinogenic effects reported in previous studies. For instance, several studies have reported that BHA may lead to DNA repair failure, genotoxicity, carcinogenicity, and reproductive toxicy (Jeong *et al.,* 2005; Vandghanooni *et al.,* 2013). Moreover, it has been affirmed that the administration of BHT promotes tumour formation in mouse lungs (Meier *et al.,* 2007).

Natural resources such as fruits, vegetables, and herbs are excellent sources of antioxidant phenolic compounds. Therefore, the replacement of synthetic antioxidants by natural products has attracted a lot of attention due to the latter's safer nature. A number of studies have demonstrated the usefulness of alternative natural products to be added in meat products to inhibit lipid oxidation and colour loss. Various extracts that contain antioxidant and antimicrobial activities from several plant sources such as tomato (Kim and Chin, 2017), grape seed (Brannan, 2008), and vine tea (Zhang *et al.,* 2019) have exhibited a positive effect on meat and meat products. However, extensive studies on the addition of LT seed aqueous extract to meat products have not been conducted. Therefore, the objectives of the present work were to assess the effect of LT seed aqueous extract on lipid oxidation, physicochemical, microbiological, and sensory characteristics on cooked pork patties during storage at refrigerated temperature (4°C). To the best of our knowledge, this is the first study to demonstrate the possibility of utilising the LT seed aqueous extract as a food preservative in meat products.

Materials and methods

Reagents and chemicals

Trichloroacetic acid (TCA), thiobarbituric acid (TBA), methanol, butylated hydroxyanisole (BHA), potassium iodide, chloroform, acetic acid, and sodium thiosulfate were purchased from Sigma Chemical Co. (St Louis, MO, USA). Plate count agar (PCA) was purchased from HiMedia (Mumbai, India).

Preparation of LT seed aqueous extract

Fresh LT fruits were collected from the Taladlang community, Chonburi, Thailand. The fruits were washed, peeled, and the seeds were dissected. The LT seeds were washed with distilled water, and dried at 50°C for 72 h using a tray dryer (OFM, Thailand). The dried seeds were pulverised using a hammer mill through a 25-mm sieve (Schmersal, Germany). Next, the LT seed powder was extracted twice with distilled water at a ratio of 1:10 (w/v) using a shaking incubator at 40°C for 2 h. The extract was then separated by centrifugation (Thermo Scientific Sorvall, USA) at 6,000 *g* and 25°C for 20 min, and filtered twice using Whatman No. 1 filter paper. The crude extract was concentrated under reduced pressure at 40°C using a rotary evaporator (Buchi, Switzerland), and lyophilised in a freeze dryer (Christ Alpha, Germany). The colour of crude LT seed aqueous extract was light brown. The extract was kept at -20°C until further analysis.

Preparation of pork patties

Fresh pork meat (*longissimus dorsi*) and pork back fat were purchased from Charoen Pokphand Foods PCL (Bangkok, Thailand), and minced in the ratio of 80:20 using a meat mincer (Rewedo, Italy). Sodium chloride (1% (w/w) of meat) was added to all meat treatments after mincing. The treatments were then divided into five different formulas: control (untreated), pork patties with 0.01% (w/w) BHA, and pork patties combined with 0.05, 0.10, and 0.20% (w/w) LT seed aqueous extract. The concentrations of the LT seed aqueous extract were used in accordance with a previous study of safety evaluation of the addition of longan seed extract to treated animals (Worasuttayangkurn *et al.,* 2012). Following the addition of the extracts and the antioxidant, treatments were thoroughly hand-mixed. Next, 20 g of minced pork was formed in a patty mould with 6 cm diameter and 1.2 cm height. All meat treatments were cooked in an electric convection roaster (VCR-999, Viva) at 150°C until the internal temperature reached 75°C (approximately 10 min). After cooling at 25°C, cooked patties were packaged in pouches, and stored at 4°C for 21 days. All treatments were taken at a duration of 0, 3, 7, 14, and 21 days of refrigerated storage. Three replicates for each treatment and storage duration were performed.

Determination of peroxide values of cooked pork patties

The PVs of all treatments were determined according to AOCS (1997). Cooked pork patties (5 g) were extracted with 30 mL of an organic solvent mixture (chloroform:acetic acid, 2:3). Next, 1 mL of potassium iodide was added, vigorously shaken for 1 min, kept in the dark for 5 min, and added with 30 mL of distilled water. Starch solution (1% (w/v)) was added to the mixture as an indicator. The PVs were evaluated by titrating the iodine liberated from potassium iodide with 0.01 N sodium thiosulfate standard solution until colourless. The PVs were expressed as milliequivalents of free iodine per kg of meat sample, using Eq. 1:

$$\text{PV (meq peroxides/kg of sample)} = (N \times V \times 1000) / W \quad \text{(Eq. 1)}$$

where, N = concentration (N) of sodium thiosulfate solution, V = volume (mL) of sodium thiosulfate solution, and W = sample weight (g).

Determination of thiobarbituric acid reactive substances of cooked pork patties

The TBARS was determined according to Buege and Aust (1978) with slight modifications. Briefly, 3 g of cooked pork patties were homogenised with 10 mL of solution containing 0.375% (w/v) TBA and 15% (w/v) TCA in 0.25 N HCl. The treatments were heated for 15 min in a water bath at 100°C. After heating, the treatments were cooled with tap water and centrifuged at 6,000 *g* and 25°C for 20 min. The supernatant was removed and measured at 532 nm using UV-VIS spectrophotometer (Shimadzu, Japan). The TBARS value was expressed as equivalents of mg of malonaldehyde (MDA) per kg of sample, using Eq. 2:

$$\text{MDA (mg/kg of sample)} = Abs_{532} \times 2.77 \quad \text{(Eq. 2)}$$

Instrumental colour measurement of cooked pork patties

The surface colour of cooked pork patties was determined with lightness (CIE L*-value), redness (CIE a*-value), and yellowness (CIE b*-value) using a colorimeter (CM-3500d, Konica Minolta, Japan) with CIE standard illuminant D65, 10° standard observer angle, and 8 mm aperture size. The instrument was calibrated to standard white tiles (CM-A90) before analysis. The colour of cooked pork patties was measured after exposure of the sample to the room temperature for 15 min. Afterward, a spectrally pure glass was placed between the samples and the equipment. The average of nine different points of each sample was determined on days 0, 3, 7, 14, and 21 of refrigerated storage. The total colour difference (ΔE*) was calculated (AMSA, 2012) using Eq. 3:

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad \text{(Eq. 3)}$$

pH, moisture content, water activity of cooked pork patties

For pH determination of the cooked pork patties, 10 g of cooked pork patties were homogenised for 2 min at 9,500 rpm in 50 mL of distilled water. The pH value was then determined using a digital pH meter (Sartorius, Germany). A moisture analyser (Ohaus, USA) was used to determine the moisture content, while a water-activity meter (Aqualab, USA) was used to determine the a_w. All experiments were performed in triplicate. The average value of readings was reported on days 0, 3, 7, 14, and 21 of refrigerated storage for each treatment.

Texture analysis of cooked pork patties

The effect of LT seed aqueous extract on the texture of cooked pork patties was examined using a texture analyser (TA.XT Plus, Stable Micro Systems, UK). The treatments were re-heated in an electric oven set at 150°C until the internal temperature reached 75°C. The cooked pork patties were then equilibrated to room temperature. Instrumental cutting force (g) measurements were performed on a total of 12 cooked pork patties for each treatment. Each patty (6 cm diameter and 1.2 cm height) was cut once with a Warner-Bratzler blade device attached to a texture analyser with a cross head speed of 2 mm/s and 25 kg load cell. The average firmness and toughness values were calculated and reported on days 0, 3, 7, 14, and 21 of refrigerated storage for each treatment.

Microbiological analysis of cooked pork patties

Ten grams of cooked pork patties were homogenised for 5 min with 90 mL of 0.85% (w/v) sterile NaCl solution. Serial dilutions were prepared, and 0.1 mL of aliquot from each dilution was plated in duplicates on a standard PCA. The inoculated plates were incubated at 37°C for 24 h to determine the total viable counts. All counts were expressed as log CFU (colony forming unit)/mL.

Sensory evaluation of cooked pork patties

Cooked pork patties were determined for sensory attributes using a 9-point hedonic scale by 15 trained panellists recruited from the Department of Food Science and Technology, Rajamangala University of Technology Tawan-ok, Thailand. The panellists were selected on the basis of their experience in sensory evaluation techniques. Patties were warmed in an electric oven adjusted to 150°C until the internal temperature reached approximately 75°C, and served warm to the panellists with a randomly coded number. The sensory parameters: appearance, colour, odour, taste, texture, and overall acceptability were evaluated (1 = dislike extremely and 9 = like extremely).

Statistical analysis

Data were represented as mean value ± standard deviation (SD). Statistical comparison between control and groups with various concentrations was performed using a *t*-test. For multiple comparisons, a one-way ANOVA was followed by Duncan's Multiple Range Test (DMRT). $p < 0.05$ was considered significant. Data analyses were performed using SPSS software v. 17.0.

Results and discussion

PV and TBARS of cooked pork patties

PV is the indicator of the primary phase of lipid oxidation products. The effect of LT seed aqueous extract on PV in cooked pork patties is illustrated in Figure 1a. PV of untreated cooked pork patties continuously increased during refrigerated storage until spoilage occurred after 14 days. In contrast, pork patties treated with LT seed aqueous extract (0.05, 0.10, and 0.20%) and synthetic antioxidant BHA had significantly lower ($p < 0.05$) PV than those of control after storage for 21 days. The levels of PV in cooked pork patties increased over time in the following order: 0.20% LT < 0.01% BHA < 0.10% LT < 0.05% LT < control. Therefore, LT seed aqueous extract appeared to effectively regulate peroxide level of cooked pork patties during 21 days of storage at 4°C.

Peroxide compounds are highly unstable, and degrade rapidly to form a large number of secondary oxidative products such as aldehydes, ketones, and acids (Ross and Smith, 2006). TBARS is a method to evaluate secondary oxidative products that cause rancidity and deteriorate meat quality. In Figure 1b, a continual increase in MDA values can be observed in the control group throughout 14 days of storage ($p < 0.05$). The addition of LT seed aqueous extract at different concentrations (0.05 - 0.20%) and 0.01% BHA in pork patties could significantly lower the MDA value (lipid oxidation) as compared to

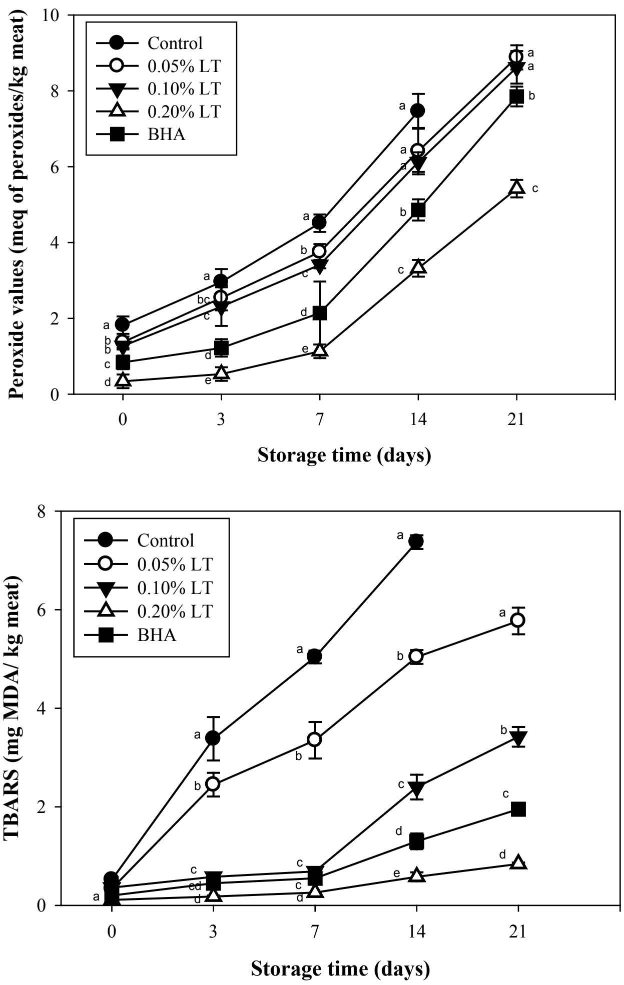

Figure 1. Effect of LT seed aqueous extract at concentrations of 0.05, 0.10, and 0.20%, and 0.01% BHA on (a) PV, and (b) TBARS of cooked pork patties as compared to control on days 0, 3, 7, 14, and 21 under refrigerated storage. Mean values in the same storage time with different lowercase letters indicate significant differences ($p < 0.05$).

control ($p < 0.05$). These results corresponded to PV values. Moreover, cooked pork patties with LT seed aqueous extract at 0.20% concentration had the lowest MDA levels ($p < 0.05$) as compared to BHA and other treatments during a storage period of 21 days. The TBARS levels of 0.20% LT seed aqueous extract treatment ranged from 0.11 mg to 0.84 mg MDA/kg, which were lower than the acceptable limit of TBARS for rancidity (1.0 mg MDA/kg) (Rahman *et al.,* 2015). These results indicate that the bioactive compound from LT seed aqueous extract may efficiently inhibit lipid oxidation, and can plausibly extend the shelf life up to 21 days of storage at 4°C.

Several studies have reported that natural extracts are excellent sources for phenolic compounds, and successfully inhibit lipid oxidation in meat products. For example, the application of hawberry extracts can prevent the formation of secondary lipid oxidation products in ready-to-eat pork patties (Akcan *et al.,* 2017). *Moringa oleifera* leaf extract and buckwheat hull extract can also inhibit lipid oxidation in meat products (Muthukumar *et al.,* 2014; Hes *et al.,* 2017).

A previous study reported that LT seed aqueous extract contains the highest total phenolic compounds (17.04 mg GAE/g of extract), and the strongest antioxidant activity as compared to ethyl acetate and ethanolic extracts (Nitteranon, 2018). The HPLC analysis indicated that gallic acid is the major compound in LT seed aqueous extract which may play a pivotal role in inhibiting lipid oxidation due to its radical scavenging activity. The addition of gallic acid in chitosan coating of pork samples was found to be more effective in lowering lipid oxidation than chitosan coating alone in modified atmosphere packaging (MAP) at 4°C storage (Fang *et al.,* 2018). Furthermore, antioxidant phenolics such as thymol, tannic acid, and gallic acid were assessed with regard to the TBARS values of broiler breast meat, and it was found that gallic acid could conspicuously reduce lipid oxidation in the breast muscle (Starcevic *et al.,* 2015).

Colour of cooked pork patties

Meat colour is one of the most crucial characteristics that influence consumer's acceptability, and storage time can potentially affect the colour of meat and its products. Table 1 shows the effect of LT seed aqueous extract on the colour of cooked pork patties throughout the 21 days of storage at 4°C. Lightness (L*) values of all samples decreased ($p < 0.05$) during storage. On day 0, the L* values were unaffected by adding LT seed aqueous extract and BHA-treated samples ($p > 0.05$). During

Table 1. Effect of LT seed aqueous extract at concentrations of 0.05, 0.10, and 0.20%, and 0.01% BHA on the lightness (L* value), redness (a* value), yellowness (b*), and total colour difference (ΔE*) of cooked pork patties as compared to control on days 0, 3, 7, 14, and 21 under refrigerated storage.

	Treatment	Storage time (day)				
		0	3	7	14	21
L*	Control	39.53 ± 0.09^{aA}	37.93 ± 0.13^{cB}	36.99 ± 0.41^{dC}	29.02 ± 0.48^{cD}	-
	0.05% LT	39.79 ± 0.37^{aA}	38.88 ± 0.82^{bB}	37.72 ± 0.51^{cC}	31.53 ± 0.55^{cD}	27.29 ± 0.31^{cE}
	0.10% LT	39.81 ± 0.36^{aA}	38.88 ± 0.40^{bB}	37.90 ± 0.50^{cC}	31.94 ± 0.49^{cD}	27.78 ± 0.76^{cE}
	0.20% LT	40.22 ± 0.63^{aA}	40.05 ± 0.23^{aA}	39.76 ± 0.44^{aB}	37.14 ± 0.66^{aC}	34.60 ± 0.57^{aD}
	BHA	40.17 ± 0.62^{aA}	40.07 ± 0.44^{aA}	39.06 ± 0.22^{bB}	34.12 ± 0.34^{bC}	30.74 ± 0.36^{bD}
a*	Control	4.02 ± 0.14^{bA}	3.14 ± 0.18^{dB}	2.35 ± 0.09^{dC}	1.70 ± 0.20^{dD}	-
	0.05% LT	4.13 ± 0.22^{bA}	3.28 ± 0.09^{cdB}	2.83 ± 0.10^{cdC}	1.03 ± 0.09^{cD}	0.34 ± 0.02^{dE}
	0.10% LT	4.29 ± 0.16^{bA}	3.37 ± 0.05^{cB}	2.92 ± 0.02^{cC}	1.09 ± 0.16^{cD}	0.62 ± 0.04^{cE}
	0.20% LT	4.94 ± 0.04^{aA}	4.85 ± 0.06^{aB}	3.78 ± 0.06^{aC}	3.07 ± 0.09^{aD}	2.70 ± 0.06^{aE}
	BHA	4.39 ± 0.22^{bA}	3.95 ± 0.18^{bB}	3.05 ± 0.18^{bC}	1.80 ± 0.10^{bD}	0.81 ± 0.10^{bE}
b*	Control	10.36 ± 0.07^{aD}	11.16 ± 0.25^{aC}	12.26 ± 0.24^{aB}	16.23 ± 0.38^{aA}	-
	0.05% LT	10.15 ± 0.29^{aE}	10.85 ± 0.25^{aD}	11.70 ± 0.32^{bC}	14.61 ± 0.22^{aB}	17.57 ± 0.31^{aA}
	0.10% LT	10.04 ± 0.14^{aE}	10.77 ± 0.27^{abD}	11.50 ± 0.22^{bC}	13.32 ± 0.22^{bB}	16.20 ± 0.16^{bA}
	0.20% LT	9.52 ± 0.09^{bD}	9.66 ± 0.53^{cD}	9.98 ± 0.03^{dC}	10.86 ± 0.12^{dB}	11.49 ± 0.08^{dA}
	BHA	9.68 ± 0.41^{bE}	10.25 ± 0.49^{bD}	10.56 ± 0.21^{cCD}	11.85 ± 0.41^{cB}	14.23 ± 0.35^{cA}
ΔE*	Control	-	2.13 ± 0.09^{aC}	3.70 ± 0.43^{aB}	12.37 ± 0.38^{aA}	-
	0.05% LT	0.52 ± 0.17^{bE}	1.20 ± 0.48^{bcD}	2.68 ± 0.42^{bC}	9.66 ± 0.50^{bB}	14.78 ± 0.38^{aA}
	0.10% LT	0.55 ± 0.18^{bD}	1.32 ± 0.30^{bC}	1.91 ± 0.33^{cC}	8.79 ± 0.48^{cB}	13.67 ± 0.70^{bA}
	0.20% LT	1.43 ± 0.26^{aC}	1.19 ± 0.34^{bcCD}	0.68 ± 0.10^{eD}	2.78 ± 0.60^{eB}	5.40 ± 0.56^{dA}
	BHA	0.95 ± 0.45^{aCD}	0.74 ± 0.23^{cD}	1.26 ± 0.20^{dC}	6.19 ± 0.19^{dB}	10.25 ± 0.40^{cA}

Mean values in the same column (same storage time) with different lowercase superscripts indicate significant differences ($p < 0.05$). Mean values in the same row (same treatment) with different uppercase superscripts indicate significant differences ($p < 0.05$).

the storage period, 0.20% LT seed aqueous extract significantly decelerated the decrease in lightness of cooked pork patties ($p < 0.05$).

Moreover, it was observed that the addition of 0.20% LT seed aqueous extract significantly increased the a* value as compared to the untreated sample ($p < 0.05$). This may have been due to the dark brown colour of the LT seed aqueous extract (L*: 23.24, a*: 2.01, and b*: 0.44) having a discernible influence on cooked pork patties. However, a significant decrease was observed throughout the 21 days of storage ($p < 0.05$). Several studies found that increasing storage time can lead to the reduction in a* values of cooked meat products (Moroney *et al.,* 2013; Muthukumar *et al.,* 2014; Hes *et al.,* 2017). Loss of redness may plausibly result from the change in colour from red to brown. This can be due to the formation of denatured metmyoglobin in cooked pork patties over the storage period. Interestingly, 0.20% LT seed aqueous extract delayed the decrease in a* value in cooked pork patties stored at 4°C. In addition, the changes in b* value after treating cooked pork patties with LT seed aqueous extract were lower than those of the control throughout the refrigerated storage of 21 days.

The initial values of total colour difference (ΔE*) of LT seed aqueous extract-treated pork patties were between 0.52 ± 0.17 and 1.43 ± 0.26 (Table 1). The addition of 0.20% LT seed aqueous extract exhibited the highest value of ΔE* in cooked pork patties ($p < 0.05$) on day 0. Typically, the colour differences are considered visually detectable when ΔE* values are higher than 2 (Larraín *et al.,* 2008). The ΔE* values of all LT seed aqueous extract and BHA-treated samples were lower than that of the control after day 3 of storage ($p < 0.05$). However, the obtained results showed that ΔE* values of all samples gradually increased during refrigerated storage ($p < 0.05$). Additionally, cooked pork patties that were treated with 0.20% LT seed aqueous extract exhibited the lowest ΔE* during refrigerated storage for 21 days.

Therefore, it may be deduced that 0.20% LT seed aqueous extract can preserve the colour of cooked pork patties over the storage period, exhibiting better results than those of BHA. This finding is concordant with previous studies of natural antioxidants, for example, the use of vine tree extract

Table 2. Effect of LT seed aqueous extract at concentrations of 0.05, 0.10, and 0.20%, and 0.01% BHA on pH, moisture content, and a_w of cooked pork patties as compared to control on days 0, 3, 7, 14, and 21 under refrigerated storage.

	Treatment	Storage time (day)				
		0	**3**	**7**	**14**	**21**
pH	Control	6.47 ± 0.00^{dA}	6.42 ± 0.00^{dB}	6.38 ± 0.01^{dC}	6.36 ± 0.00^{dD}	-
	0.05% LT	6.51 ± 0.01^{cB}	6.46 ± 0.00^{cC}	6.40 ± 0.01^{cD}	6.52 ± 0.04^{aB}	6.60 ± 0.00^{bA}
	0.10% LT	6.53 ± 0.00^{bB}	6.47 ± 0.01^{bD}	6.50 ± 0.00^{aC}	6.50 ± 0.00^{bC}	6.72 ± 0.01^{aA}
	0.20% LT	6.55 ± 0.00^{aA}	6.48 ± 0.00^{aB}	6.41 ± 0.00^{cC}	6.41 ± 0.00^{bC}	6.56 ± 0.01^{cA}
	BHA	6.50 ± 0.00^{cB}	6.48 ± 0.00^{aC}	6.45 ± 0.01^{bD}	6.50 ± 0.01^{cB}	6.57 ± 0.00^{cA}
Moisture content (%)	Control	45.08 ± 0.12^{aA}	44.85 ± 0.12^{aB}	43.75 ± 0.08^{aC}	42.70 ± 0.04^{aD}	-
	0.05% LT	45.06 ± 0.65^{aA}	44.39 ± 0.04^{bB}	43.44 ± 0.09^{bC}	41.69 ± 0.06^{bD}	41.08 ± 0.06^{aE}
	0.10% LT	45.00 ± 0.11^{aA}	43.96 ± 0.04^{cB}	43.09 ± 0.07^{cC}	41.46 ± 0.06^{bD}	40.92 ± 0.06^{bD}
	0.20% LT	43.99 ± 0.11^{bA}	42.60 ± 0.16^{eB}	40.71 ± 0.05^{eC}	39.26 ± 0.06^{dD}	38.00 ± 0.06^{dE}
	BHA	44.94 ± 0.12^{aA}	43.55 ± 0.05^{dB}	42.71 ± 0.06^{dC}	40.42 ± 0.04^{cD}	39.68 ± 0.04^{cE}
a_w	Control	0.999 ± 0.00^{aA}	0.997 ± 0.00^{aB}	0.997 ± 0.00^{aB}	0.996 ± 0.00^{aC}	-
	0.05% LT	0.998 ± 0.00^{aA}	0.996 ± 0.00^{aAB}	0.994 ± 0.00^{aAB}	0.989 ± 0.00^{aC}	0.979 ± 0.00^{aD}
	0.10% LT	0.999 ± 0.00^{aA}	0.994 ± 0.00^{aAB}	0.994 ± 0.00^{aAB}	0.984 ± 0.00^{bC}	0.943 ± 0.00^{bD}
	0.20% LT	0.998 ± 0.00^{aA}	0.984 ± 0.00^{cB}	0.983 ± 0.00^{cB}	0.905 ± 0.00^{dC}	0.875 ± 0.00^{dD}
	BHA	0.997 ± 0.00^{aA}	0.988 ± 0.008^{bB}	0.987 ± 0.00^{bB}	0.945 ± 0.00^{cC}	0.918 ± 0.00^{cD}

Mean values in the same column (same storage time) with different lowercase superscripts indicate significant differences ($p < 0.05$). Mean values in the same row (same treatment) with different uppercase superscripts indicate significant differences ($p < 0.05$).

can preserve the colour of cooked pork patties (Zhang *et al.,* 2019).

pH, moisture content, and water activity of cooked pork patties

The changes in pH values of cooked pork patties during refrigerated storage for 21 days are shown in Table 2. pH values were affected by treatments and storage days, and varied between 6.27 - 7.02. On day 0, the pH values of LT aqueous extract-treated and BHA-treated cooked pork patties increased as compared to control ($p < 0.05$). These results are congruous with previous studies where the addition of BHA/BHT, rosemary extract, and annatto seed powder were found to increase the pH values of pork patties (Mc Carthy *et al.,* 2001; Cuong and Chin, 2016). Furthermore, during the storage period, pH values of all samples gradually decreased. However, a dramatic increase in pH value of treated groups was observed after a 14-day storage, which may have been due to proteolytic activities of microorganisms into volatile basic nitrogen molecules including ammonia and amines (Verma and Sahoo, 2000).

Moisture content is a vital indicator of the juiciness and shelf life of meat products. The percentage of moisture content of cooked pork patties on day 0 was in the range from 43.99 ± 0.11 to 45.08 ± 0.12 (Table 2). The addition of BHA and LT seed aqueous extract at 0.05 - 0.10% resulted in a non-significant change in the moisture content in cooked pork patties as compared to control ($p > 0.05$). The moisture content of all the samples gradually decreased during refrigerated storage. However, 0.20% LT seed aqueous extract showed a significant decrease in cooked pork patties moisture content ($p < 0.05$) as compared to other treatments. This may plausibly affect the texture quality of meat products.

The a_w value indicates the availability of water for microbial growth. On day 0, a_w values of cooked pork patties were approximately 0.997 - 0.999, and no significant differences ($p > 0.05$) in a_w values were observed among treatments and control (Table 2). Similar to moisture content, there was a decrease in a_w values of the cooked pork patties over time. However, the a_w value of cooked pork patties added with 0.20% LT seed aqueous extract was significantly lower ($p < 0.05$) than control and other treatments. Therefore, the treatment with 0.20% LT seed aqueous extract appeared to be more stable in controlling microbial growth.

Texture of cooked pork patties

One of the indicators for the edibility of meat products is their texture. The texture profile analysis results of control and treated cooked pork patties are presented in Table 3. In general, the firmness and toughness values of all samples significantly increased throughout the refrigerated storage ($p < 0.05$). On day 0, the addition of LT seed aqueous

Table 3. Effect of LT seed aqueous extract at concentrations of 0.05, 0.10, and 0.20%, and 0.01% BHA on texture analysis (firmness and toughness) of cooked pork patties as compared to control on days 0, 3, 7, 14, and 21 under refrigerated storage.

	Treatment	**Storage time (day)**				
		0	**3**	**7**	**14**	**21**
Firmness (N)	Control	2.17 ± 0.09^{cC}	2.24 ± 0.09^{dBC}	2.30 ± 0.01^{dAB}	2.34 ± 0.09^{eA}	-
	0.05% LT	3.08 ± 0.06^{bD}	3.10 ± 0.04^{cD}	3.26 ± 0.04^{cC}	3.60 ± 0.03^{dB}	3.90 ± 0.05^{cA}
	0.10% LT	3.09 ± 0.06^{bD}	3.10 ± 0.06^{cD}	3.33 ± 0.05^{cC}	3.74 ± 0.03^{cB}	3.95 ± 0.03^{cA}
	0.20% LT	3.36 ± 0.07^{aE}	3.95 ± 0.02^{aD}	4.24 ± 0.07^{aC}	4.89 ± 0.07^{aB}	5.11 ± 0.08^{aA}
	BHA	3.40 ± 0.25^{aE}	3.75 ± 0.08^{bD}	3.85 ± 0.09^{bC}	4.29 ± 0.10^{bB}	4.58 ± 0.22^{bA}
Toughness (N.s)	Control	1.60 ± 0.09^{dBC}	1.60 ± 0.21^{dBC}	1.61 ± 0.08^{dB}	1.63 ± 0.04^{dA}	-
	0.05% LT	1.60 ± 0.47^{dE}	1.68 ± 0.42^{cD}	1.91 ± 0.38^{cC}	2.12 ± 0.53^{cB}	2.39 ± 0.45^{dA}
	0.10% LT	1.65 ± 0.28^{cdE}	1.69 ± 0.30^{cD}	1.94 ± 0.56^{cC}	2.16 ± 0.32^{cB}	2.53 ± 0.28^{cA}
	0.20% LT	1.92 ± 0.44^{aE}	2.40 ± 0.21^{aD}	2.66 ± 0.54^{aC}	3.03 ± 0.44^{aB}	3.27 ± 0.33^{aA}
	BHA	1.68 ± 0.20^{bE}	2.17 ± 0.21^{bD}	2.25 ± 0.19^{bC}	2.61 ± 0.47^{bB}	2.91 ± 0.72^{bA}

Mean values in the same column (same storage time) with different lowercase superscripts indicate significant differences ($p < 0.05$). Mean values in the same row (same treatment) with different uppercase superscripts indicate significant differences ($p < 0.05$).

extracts (0.05 - 0.20%) and BHA caused an increase in firmness and toughness in cooked pork patties as compared to control ($p < 0.05$). Moisture content is one of the most pivotal factors that influence the texture of meat products. Pork patties incorporated with 0.20% LT seed aqueous extract attained the highest firmness and toughness values ($p < 0.05$) that can be related to the lowest moisture content of treated cooked pork patties as compared to control. This result is in agreement with Kumar *et al.* (2015) who asserted that the addition of grape seed extract combined with sea buckthorn extract can considerably increase the hardness and springiness values in pork patties.

Microbiological quality of cooked pork patties

Figure 2 illustrates the results of total viable count (TVC) values of pork patties treated with LT seed aqueous extracts and BHA during refrigerated storage for 21 days. No significant differences ($p > 0.05$) in TVC were observed in all cooked pork patties sample on day 0 of storage. After 3 days of storage, the TVC value of control significantly increased until day 14 of storage, and reached 4.47 log CFU/mL, and then it spoiled. For LT seed aqueous extract-treated samples, it was observed that their TVC values were lower than that of control ($p < 0.05$) in a dose-dependent manner. The TVC of LT seed aqueous extract-treated samples (0.10 - 0.20%) was statistically lower than that of BHA-treated cooked pork patties ($p < 0.05$). LT seed aqueous extract exhibited the potential for antimicrobial activity in cooked pork patties. This may have been due to the phenolic compounds such as gallic acid in LT seed aqueous extract. Gallic acid is a water-soluble phenolic acid with three hydroxyl groups, which could cause irreversible changes in bacterial cell membranes by altering its hydrophobicity and forming pores (Zhao *et al.,* 2015). These results are in agreement with Tseng *et al.* (2014) who opined that the antimicrobial activities of *D. longan* Lour. seed extracts were associated with the presence of phenolic compounds including gallic acid, corilagin, ethyl gallate, and ellagic acid. Several researchers have shown that gallic acid has strong antioxidant and antimicrobial activities against a number of pathogens such as *Escherichia coli, Staphylococcus aureus*, and *Listeria monocytogenes* (Borges *et al.,* 2013; Sorrentino *et al.,* 2018).

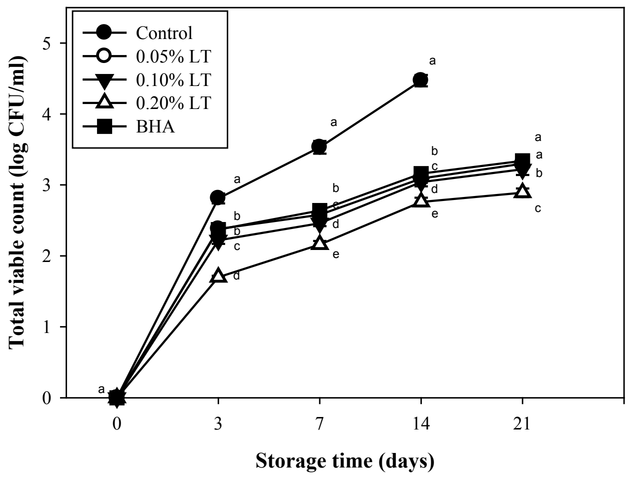

Figure 2. Effect of LT seed aqueous extract at concentrations of 0.05, 0.10, and 0.20%, and 0.01% BHA on TVC values of cooked pork patties as compared to control on days 0, 3, 7, 14, and 21 under refrigerated storage. Mean values in the same storage time with different lowercase letters indicate significant differences ($p < 0.05$).

Table 4. Effect of LT seed aqueous extract at concentrations of 0.05, 0.10, and 0.20%, and 0.01% BHA on sensory analysis of cooked pork patties as compared to control on day 0 under refrigerated storage.

Treatment	Sensory attribute					
	Appearance	Colour	Odour	Taste	Texture	Overall acceptability
Control	7.60 ± 0.73^{a}	7.07 ± 0.88^{ab}	6.27 ± 1.03^{b}	7.27 ± 1.28^{a}	7.00 ± 0.65^{a}	7.13 ± 0.91^{a}
0.05% LT	7.67 ± 0.81^{a}	7.40 ± 0.63^{a}	7.07 ± 0.96^{a}	7.27 ± 1.06^{a}	6.87 ± 1.24^{a}	7.07 ± 0.96^{a}
0.10% LT	7.27 ± 0.59^{a}	6.80 ± 0.67^{bc}	7.20 ± 0.41^{a}	6.67 ± 0.90^{a}	7.13 ± 0.51^{a}	7.07 ± 0.45^{a}
0.20% LT	6.53 ± 0.51^{a}	6.47 ± 0.51^{c}	6.87 ± 0.74^{a}	6.67 ± 0.81^{a}	6.67 ± 0.48^{b}	6.93 ± 0.18^{a}
BHA	7.27 ± 0.88^{a}	7.20 ± 1.01^{ab}	7.07 ± 1.10^{a}	7.13 ± 1.12^{a}	7.00 ± 1.24^{a}	6.87 ± 1.06^{a}

Mean values in the same column (same sensory attributes) with different lowercase superscripts indicate significant differences ($p < 0.05$).

Sensory quality of cooked pork patties

Sensory analysis of cooked pork patties at the beginning of storage was determined, and the results are shown in Table 4. The addition of BHA or LT seed aqueous extracts (0.05 - 0.20%) had no considerable effect on sensory parameters (appearance, aroma, taste, and overall acceptability) of cooked pork patties as compared to control ($p > 0.05$). However, texture scores of cooked pork patties were the lowest with the addition of 0.20% LT seed aqueous extract ($p < 0.05$). Furthermore, the addition of 0.20% LT seed aqueous extract also affected the colour of cooked pork patties. This could be due to the dark brown colour of LT seed aqueous extract (a* value: 4.94, and ΔE* value: 1.43). Muthukumar *et al.* (2014) reported that the addition of *Moringa oleifera* leaf extract did not impact the overall acceptability in ground pork patties. Moreover, the incorporation of dried pumpkin pulp and seed mixture in beef patties was as acceptable as the control (Serdaroglu *et al.,* 2018). Conversely, Carpenter *et al.* (2007) reported that the incorporation of grape seed extract at different concentrations caused negative perception in cooked pork due to an undesired increase in redness.

Conclusion

The addition of 0.20% LT seed aqueous extract manifested a preservative effect on lipid oxidation that can prospectively extend the shelf life of cooked pork patties with a lower microbiological growth as compared to the control sample throughout the 21 days of refrigerated storage. The addition of 0.20% LT seed aqueous extract also increased the firmness and toughness values, decreased moisture content, and preserved the surface colour of cooked pork patties throughout the refrigerated storage period. No significant differences in the appearance, taste, and overall acceptability were observed on the addition of LT seed aqueous extract in cooked pork patties. Therefore, these results suggest that 0.20% LT seed aqueous extract can be used as an alternative source of natural antioxidant and antimicrobial agents in cooked pork patties, and possibly in other meat products as well.

Acknowledgement

The present work was financially supported by Rajamangala University of Technology Tawan-ok. The authors acknowledge Ms. Duangruthai Muenhan and Mr. Yensira Veeratananant for technical assistance and statistical analysis.

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