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Physicochemical and organoleptic of beef sausages with teak leaf extract (*Tectona grandis*) addition as preservative and natural dye

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Abstract

The purpose of this study is to utilize teak leaves (*Tectona grandis*) as natural dye and preservative compunds in beef sausages. Teak leaf extract was obtained by ethanol extraction. Further identification indicated that the extract contains some active compounds including alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids and glycosides. Given some active compounds are known to display as natural dye and/or preservative properties for food, we aim to utilize it is interesting to know the effect of addition of teak leaf extract on beef sausage. *In vitro* antimicrobial analysis proved that teak leaf extract inhibits the growth of pathogenic bacteria, including *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomons aeroginosa*. In this study, beef sausage was treated by the addition of teak leaf extract in various concentrations: 0% (control), 0.5% and 1% (w/w). Microbiological characteristic of the sausages was analyzed during storage. Addition of teak leaf extract had no affect on pH values, water binding capacities, water contents, ash contents and protein contents of the sausages. However, the treatments significantly increased the red value of the sausages with L, a, b and °HUE values which were significantly different ($P < 0.05$). Nutritional quality of the sausages with the addition of teak leaf extract to 1% met the SNI (Indonesian National Standard) for sausages. Organoleptic testing (hedonic and hedonic quality testing) showed that the panelists remarkably prefered all beef sausages. Microbiological analysis showed that sausages withteak leaf extract had the ability to inhibit *S. aureus*.

Introduction

Beef sausage is one of the popular foods among children and adults. Nitrite is often used as ingredient upon sausage manufacturing as it facilitates the formation of specific color and flavor in processed meat products (Liu *et al*., 2010). However, usage of nitrite is considerably risky for human health as it might react with secondary and tertiary amines in meat producing nitrosamines, which are carcinogenic compounds (Lawrie, 1998). In certain limit, nitrite is considerably safe to be used on food products, from 40 to 100 ppm (ESS/3598, 2005). Accordingly, it remains widely and legaly used in Indonesia as a preservative food (Health Regulation of The Republic of Indonesia No 033/Menkes/Per/XI/12).

Despite it is considerely safe at a certain limit, excessive consumption of nitrite negatively impacts human health. Nitrite represents a less stable oxidation state of nitrogen and therefore can be further reduced to various compounds or oxidized to nitrite. Nitrite may endogenously react with secondary amines to form pounds or oxidized to nitrite. Nitrite may endogenously react with secondary amines to form N-nitrosamines at low pH values, as is the case in the gastric environment of mammals (Reinik *et al*., 2008). Given the risk of nitrite, attempts to find alternative food dry and preservative agents are considerably important.

Teak leaf extract (*Tectona grandis*) is promising to be used as natural additives in order to avoid the negative impacts of nitrite. Tectona grandis contains natural pigment called anthocyanin that produces maroon color (Ati *et al*., 2006). Teak leaf extract also contains antimicrobial compounds such as flavonoids, alkaloids, tannins, anthraquinone and naphthoquinone which inhibit the growth of bacteria (Purushotham *et al*., 2010). Altogether, teak leaf extract is promosing not only for food coloring, but also for food preservation.

Khrisna and Jayakumaran (2010) and Khrisna *et al.* (2011) report that antibacterial substance of Tectona grandis was classified as antraquinone which inhibits pathogenic bacteria of *S. aureus* and *K. pneumonia*, the compound was shown as non toxic agent to normal human cells. In addition, Tectona grandis also plays as natural drug additives. Nayeem and Karvekar (2012) studied about analgesic and anti-inflammatory activity of *Tectona grandis*. The result revealed that the frontal and the mature leaves

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of *Tectona grandis* showed remarkable analgesic and inflammatory activities.

Despite application taek leaf extract as food coloring and preservative agents were widely studied (Siva, 2007; Nirmala *et al*., 2012; Setiawan *et al.*, 2013), however, to our knowledge, there is no study on its application on beef sausages. Indeed, the sausages are risky to be contaminated by pathogenic bacteria (Lindqvist and Lindblad, 2009) and its natural color is relatively not attractive for consumer. It is interesting to be known the effect of teak lef extract as coloring and preservating agents in beef sausage.

In order to pathogenic contamination risk on beef sausage, low temperature storage is widely used. Lindqvist and Lindblad (2009) stated that population of pathogenic bacteria of the sausages was decreased during storage at low temperature (refrigerator) during 20 days of storage. Storage at low temperature might increase the length of adaptation phase of microbial growth resulting increasing the sausages's shelf life. This evidence is consistent to Zhou *et al.* (2010) reported that chilling is a critical for meat hygiene, safety, shell life, appearance and eating quality. All together, this work is aimed to investigate combination of low temperature storage and addition of teak leaf on characteristics of beef sausages with the addition of leaf extract teak (*Tectona grandis*) as preservative and natural dyes.

Materials and Methods

Teak leaf extraction

The extraction of teak leaf was performed based on according to Rachmadani (2001) using ethanol extraction as common method for plant compounds extraction. Firstly, fresh teak leaf was oven-dried at 60°C for 24 hours, chopped and blended to get teak leaf powder. Two hundreds mililiters of 96% ethanol then added into 20 g of teak leaf powder (10:1 ratio) and was boiled using waterbath at 70°C for 2 hours. The mixture was centrifuged at 6,000 rpm for 15 minutes. Ethanol was then removed by air-dry evaporation until no trace of ethanol and remained one-fifth of the initial volume.

Antimicrobial activity test of teak leaf extract

Antimicrobial activity of teak leaf extract against pathogenic bacteria was assayed by using agar diffusion method (Hechard *et al*., 1992). One mililiter of pathogenic bacteria culture was firstly diluted with 10^6 CFU mL^{-1} and pipetted into a petridish. Twenty mililiters of Muller Hinton agar (MHA, Difco) media was added on each petridish with a temperature of 50°C, homogenized by moving the hockey stick in an "eight-shape" and solidified. Five milliliters diameter wells were then made on solid pathogenic-containing MHA agar with a whole punch or borer. Fifty mililiters of concentrated extract was pipetted into each well. Petridished then incubated at 37°C for 24 hours. The inhibition was indicated by the presence of clear zone around the wells. The diameter of inhibiton zone was measured by calipers at four different places.

Phytochemical test

A teak leaf extract of teak leaf is known to contain various type of active compounds belong, the presence of these compounds in our ethanolic teak leaf extract were confirmed by series of phytochemical tests (Harbone, 1987). The tested compounds were alkaloids, tannins, flavonoids, saponins, steroids and triterpenoids.

For alkaloid test, ten gram of crushed leaves was added by 1.5 mL of chloroform and 3 drops of ammonia. Chloroform fraction was separated and acidified with 5 drops of 2 M H_2SO_4. Acid fraction was divided into 3 tubes then each added reagent Dragendorf, Meyer and Wagner. Presence of alkaloids characterized by the formation of a white precipitate on Meyer reagents, reagent red precipitate Dragendorf and a brown precipitate in the reagent Wagner.

For flavonoid test, 0.5 g of leaves was added to methanol and then heated up submerged. Five drops of 2 M H_2SO_4 was added into the filtrate. Formation of red color upon the addition of H_2SO_4 indicating the presence of flavonoid compounds.

For saponin test, 0.5 g of leaves was added by appropriate amount of water and heated to 60°C for five minutes. The solution was cooled at room room temperature and then reheated for ± 10 minutes. The presence of saponin is indicated by the presence of foam, afterall.

For triterpenoids and steroids test, one g of leaf was added by 2 mL of 100% ethanol, heated and filtered. The filtered fraction was then added to the filtrate evaporated ether. Ether layers were added with Liebermen Burchard reagent (3 drops of acetic anhydride and 1 drop H_2SO_4). Red or purple color indicates formation of triterpenoids, while green color indicates the presence of steroids.

For tannin test, water was added into 5 g of leaves and boiled for a few minutes. Filtered and the filtrate was added with 3 drops of $FeCl_3$. Formation of dark blue or greenish black indicates the presence of tannins.

Sausages processing

Sausage making was started with the preparation of the material and then the meat was ground. Milling was done by using a food processor. Meat 500 g and 0.5% STPP included. The next stage was the addition of 10% chopped ice cubes, 5% starch, 10% skim milk, 2% garlic, 0.5% white pepper, 0.5% ginger powder, 0.5% coriander, 0.5% nutmeg and 1.2% salt. The percentage of ingredient based on 100% of meat. Addition of teak leaf extract (0.5% and 1%) was done in this step and the dough homogenized by two-times milling process. The dough wasput into stuffer then filled into casings and then boiled for 60 minutes at a temperature of 60 to 65^{o}C.

Physicochemical analysis

In order to confirm the effect of addition of teak leaf extract on color formation and other physicochemical paramters of the sausage, following parameters were analyzed: color, pH value, water binding capacity, and water activity (a_w).

Measurement of pH

The pH of beef sausages were measured according to Erkkila *et al.* (2001) by using "Hanna" pH meter, that has been calibrated at pH 4 and 7 prior to measurement. Electrodes were inserted into the inner part of the sausage and pH value was read.

Water holding capacity

Water Holding Capacity (WHC) was measured according to Pouttu and Puolanne (2006). Ten gram of sample was placed into centrifuge tubes. Ten mililiters of water was added and then centrifuged at 6000 rpm for 30 minutes. The volume of supernatant was measured with a 10 mL measuring cup. Bound water was calculated based on the following formula: the difference in initial water (10 mL) with a volume of supernatant expressed in g/g assuming a specific gravity of water is 1 (g/mL).

Measurement of a_w (water activity)

Measurement of water activities were performed based on Ravyts *et al*. (2010) using a "Novasina" a_w meter. The measurement was conducted according to manufacturer's manual.

Color measurement

Color was measured according to Pereira *et al.* (2010) with slight modifications by using chromameter CR 300. Brightness intensity denoted with L-values (luminousity), a-values (red color intensity) and b-values (yellow color intensity). a and b values were used to determine ^{o}HUE. ^{o}HUE was considered to determine the color of the product.

Sensory evaluation

To confirm whether or no the addition of teak leaf extract on sensory properties of the sausage, sensory evaluation was performed based on modified method of Setyaningsih *et al*. (2010). The hedonic quality test and hedonic test (Test A) of the sausages were conducted using a scale of 1 to 5. Fivety untrained and 40 semi-skilled panelists were used for hedonic and hedonic quality tests, respectively.

Nutritional composition

To confirm the effect of addition of teak leaf extract on nutrional composition of the sausage, proximate analysis was performed based on standard method of AOAC (2005). The analysis includes moisture, protein, fat, and ash contents by proximate. Carbohydrat content was calculated by difference from the proximate analysis.

Microbiological analysis

To confirm the function of teak leaf extract as preservative agent for the sausage, microbiological analysis was conducted on the sausage. Combinatorial effect of the leaf extract and low temperature storage on microbial population of the sausage, the analysis was also conducted on the sausage stored at low temperature (refrigerator, about 4^{o}C) for 12 days.The analysis was performed based on Bacteriological Analytic Methods (2002). Twenty-five g of sample was weighed, put in 225 mL of BPW solution and homogenized. The mixture was sterile pipetted into 9 mL of BPW solution to obtain 10^{-1} dilution. The solution was further step-wise diluted up to 10^{-6} dilution.

Quantitative microbial analysis total plate count (TPC)

The analysis was performed based on Bacteriological Analytic Methods (2002). One mililiter of 10^{-4} to 10^{-6} dilutions of the stock culture were put into the petridish (triplicate). PCA media (plate count agar/ Oxoid) which has been cooled to 45°C ± 1°C poured into each petridish as much as 15-20 mL, and then kept until solidified. Total bacteria was counted after incubation at 34-36^{o}C for 24-48 hours.

Quantitative analysis of Staphylococcus aureus

One mililiter sample of 10^{-1} to 10^{-3} dilutions were transferred to a sterile petridish (triplicate) and then poured to BPA media (Baird Parker Agar/Oxoid) with pour plate method (Bacteriological Analytic Methods, 2002). *S. aureus* colonies were black surrounded with yellow color.

Quantitative analysis of Escherichia coli

One mililiter of of 10^{-3} to 10^{-5} dilutions was transferred to a sterile petridish (triplicate). Eosyn Methylen Blue Agar (EMBA, Oxoid media) was added into that petridish. Isolation was conducted using pour plate method (Bacteriological Analytic Methods, 2002). Twenty mililiters of sample was poured and then homogenized. Once the agar solidified, petridishes were incubated upside down at 37°C for 24 hours. Colonies of *E. coli* were seen in a greenish color under the light exposure.

Quantitative analysis of Salmonella spp

The analysis was performed based on (Bacteriological Analytic Methods, 2002). Suspension was obtained from 10^{-1} to 10^{-3} dilutions, 1 mL for each of it and put into a petridish (triplicate). 15-20 mL of cold XLDA media (Xylose Lysine Deoxycholate Agar, Oxoid) was poured into a petridish that already contained a sample suspension, then homogenized. Set the petridish until the media solidified and then incubated upside down at 37°C for 24-48 hours.

Statistical analysis

Data on the physical properties and nutrients statistically analyzed using analysis of variance (ANOVA) for a Completely Randomized Design based on Steel and Torrie (1995). Post hoc analysis was performed by Tukey multiple comparison test. Data of microbiological properties during storage were processed using factorial Completely Randomized Design. Kruskal-Wallis test as a statistical test was used to process organoleptic data.

Results and Discussion

Leaf morphological identification and phytochemicals test

To confirm the identity of leaves used in our experiments, morphological identification of leaf was performed by using identified teak leaf available the Indonesian Institute of Sciences (LIPI). The identification showed that the leaves used in this research were indeed teak leaves withthe scientific name of *Tectona grandis* L.f. from *Lamiaceae* family. Morphological characteristif of the leaves in our experiment is in agreement to some references of teak leaf morphology (White, 1991; Tewari, 1992; Soerianegara *et al*., 1993).

Phytochemical test was conducted to identify the presence of active compounds in ethanolic teak leaf extract. The results showed that the following active compounds were present in in teak leaf extract: alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides, but no steroid. This result is similar to Purushotham *et al.* (2010), which reported that teak leaves contained flavonoids, alkaloids, tannins, anthraquinone, and naphthoquinone. The compounds has been reported to be able to inhibit the growth of *Psedomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhimurium*, *Escherichia coli*, *Serratia marcescens*, *Citrobacter freondii*, *Pichia pastoris*, and *Streptococcus* spp (Purushotham *et al.*, 2010). According to Mangunwardoyo *et al*. (2009), alkaloids are alkaline compounds containing one or more nitrogen atoms, generally in the form of amino acids. Ramsewak *et al.* (1999) stated that the alkaloid of the plant display antibacterial effect against *Bacillus subtilis*, *Salmonella lutea*, *Pseudomonas vulgaris*, *Echerichia coli* and *Staphylococcus aureus.* Saponins are potential as antimicrobial compounds for their ability to reduce the permeability of bacteria's cell wall, thus they can enter the cytosol and inhibit bacterial growth (Safitri, 2010). In addition, tannins in plants can inhibit microbial activity.

Table 1. Inhibition zone of pathogenic bacteria from *Tectona grandis* leaf extract compared with antibacterial substances

Pathogenic Bacteria	Antibacterial Substance				
	Control	*Tectona grandis* leaf extract	$NaNO_2$	Alcohol	Amoxilin
	----------	----------	mm[b]	----------	----------
B. cereus	0^a	$23.40^b \pm 1.23$	0^a	$11.28^c \pm 1.97$	$18.44^c \pm 1.08$
S. typhii	0^a	$23.63^b \pm 1.76$	0^a	0^a	$26.64^b \pm 2.56$
P. aeroginosa	0^a	$29.02^b \pm 3.66$	0^a	0^a	$23.41^b \pm 2.93$
S. aureus	0^a	$29.20^b \pm 1.52$	0^a	0^a	$26.43^b \pm 3.50$
E. coli	0^a	$12.17^c \pm 0.78$	0^a	0^a	$27.86^b \pm 2.09$

[a] Diameter of inhibition zone including wellbore diameter (5 mm)
[b] Different letters next to the middle value in the same column differ significantly ($P < 0.05$)

According to Naim (2004), action mechanism of tannins as antimicrobial substances related to the ability of tannins to inactivate microbial cell adhesion (molecule attachment to host cell surfaces). Tannins are phenolic compounds that cause damage to the cell wall's polypeptides. Flavonoids are a class of phenols contained in all vascular plants. Flavonoids in plants serves to regulate the growth, organize photosynthesis, regulate antimicrobial, and antiviral, as well as regulate insect works. This is due to a broad spectrum of flavonoids' antimicrobial activity by reducing the target organism's immunity (Naidu, 2000). According to Robinson (1995), triterpenoids is a class of terpenoids which have antifungal, insecticidal, antibacterial, and antiviral properties. Altogether, the presence of these compounds in teak lef extract indicates promising ability of teak leaf extract as antimicrobial substances.

Antimicrobial of teak leaf extract (Tectona grandis)

To address, antimicrobial activity test of teak leaf extract was performed using agar dilution method with Muller Hinton agar media. Formation of inhibition zone in the presence of teak leaf extract is shown in Table 1.

The results on Table 1 showed that teak leaf extract displayed wide spectrum of antimicrobial activity towards *Bacillus cereus*, *Salmonella typhii*, *Pseudomonas aeroginosa*, *Staphylococcus aureus*, and *Escherichia coli.* Antimicrobial activity of teak leaf extract is considerebely comparable to a commercially available antimicrobial substance, amoxicillin. Noteworthy, for unkown reason, the extract inhibits *B. cereus* stronger than that of amoxicillin or alcohol.

The presence of afore-mentione active compounds in the teak leaf extract might account for its antimicrobial activity. Moreover, Nti *et al.* (2011) highlighted the ability of teak leaf extract to inhibit *B. cereus.* It is taught to be related to the presence of many types of antimicrobial compounds in teak leaf extract which had hydrophilic property due to polar metabolite like tannins. Hydrophilic property allows the active compounds to penetrate liposaccharide of Gram-negative bacterial cell walls (Nti *et al*., 2011).

Inhibition of teak leaf extract against *Salmonella typhii* was significantly higher to that of nitrite and alcohol. *Salmonella typhii*'s inhibition zone was likely due to its sensitive property to the acidic of teak leaf extract. Doores (1993) mentioned that Salmonella typhii is inhibited by propionic acid, acetic acid, and lactic acid similar to the high level ofinhibition in *Pseudomonas aeroginosa. Pseudomonas aeroginosa* could not thrive due to low pH of teak leaf extract, because it was grown optimally at pH 6.6 to 7.0. *Staphylococcus aureus* had an area of inhibition that was not significantly different from amoxicillin. Inhibition of *Escherichia coli* was the lowest since this bacteria poorest grew at acidic pH of teak leaf extract. This bacteria is grow optimally at high pH of 7 to 7.5 (Sugiastuti, 2002). Inhibitory activity of teak leaf extract was significantly different to the control, alcohol, and nitrite was not significantly different from amoxicillin. For unknown reason, the weakest inhibition of teak leaf extract was shown by Escherichia coli. Indeed, this result strongly indicates teak leaf extract has ability to exhibit antimicrobial effect toward pathogenic bacteria.

Microbiological properties of sausages

Despite teak leaf extract indeed displayed antimicrobial activity (Table 1), however it remains questionable if teak leaf extract retains its activity when the extract is unsed in food processing. To address, beef sausages made with the addition of teak leaf extracts were microbiologically analyzed for TPC numbers, the population of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. was conducted during cold storage (refrigerator, about 4°C) on 0, 4, 8, and 12 days (Table 2). Cold storage was conducted because according to Sharma *et al.* (2004) storing at low temperatures produces a slower rate of microbial inactivation when compared to store at room temperature.

Table 2. Microbiological quality of beef sausages

Variables	SNI*	Day of Storage			
		Day-0	Day-4	Day-8	Day-12
TPC					
0%	10^5	$4.54^a \pm 0.00$	$4.35^a \pm 2.42$	$4.72^a \pm 2.26$	$4.55^a \pm 0.00$
0.5%		$4.40^a \pm 2.83$	$4.52^a \pm 2.26$	$4.33^a \pm 1.89$	$3.13^b \pm 1.51$
1%		$4.14^a \pm 0.21$	$4.53^a \pm 0.00$	$3.19^b \pm 1.26$	$3.12^b \pm 1.28$
E. coli					
0%	10^1	0	0	0	0
0.5%		0	0	0	0
1%		0	0	0	0
S. aureus					
0%	10^2	$1.49^a \pm 0.54$	$1.32^a \pm 0.33$	$1.73^a \pm 0.07$	$1.34^a \pm 0.18$
0.5%		$1.18^b \pm 0.02$	$1.16^b \pm 0.34$	$1.21^b \pm 0.21$	$1.02^b \pm 0.64$
1%		$1.39^b \pm 0.34$	$1.09^b \pm 0.34$	$1.12^b \pm 0.00$	$1.01^b \pm 0.23$
Salmonella spp.					
0%	negative	0	0	0	0
0.5%		0	0	0	0
1%		0	0	0	0

* Indonesian National Standart 01-3820-1995, 1995

* Superscript letters in the same row and column indicate significant differences ($P < 0.05$)

Table 2 showed that the addition of teak leaf extract was able to reduce total microbial population of the sausage compared to the control, during 12 days of storage. The result indicated that, to some extent, teak leaf extract kept its antimicrobial activity in the sausage product. The ability to reduce total microbial population in the sausage during storage also strongly indicated that teak leaf extract is promising to be used as preservatives in beef sausages.

We believe that reducing of total microbial population is mainly due to the addition of teak leaf extract instead of low temperature storage. Despite low temperature storage enables to inhibit some pathogenic bacterial strain, low temperature storage at refrigerator could not inactivate some psychrophilic bacteria, such as *Pseudomonas*, *Achromobacter*, *Micrococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediocoocus*, *Falvobacterium*, and *Proteus* (Ulfa, 2007).

Despite weak ability of teak leaf extract to inhibit growth of *E. coli*, none of *E. coli* was observed in sausages during storage. It might be mainly due to good sanitation and cold storage. This is in accordance with the opinion of Jones *et al.* (2002) that minimum growth temperature of *E. coli* is 7°C. *S. aureus* is a bacteria that produces enterotoxin causing poisoning to human. These bacteria are found in foods that contain high protein such as sausage, eggs, and so on (Fardiaz, 1989). The result also showed that addition of teak leaf extract effectively inhibited S. aureus in the sausages. Sausages with 0.5% and 1% concentrations of teak leaf extracts were significantly different to that. It implied that teak leaf extracts could effectively inhibited *S. aureus.* Bioactive components in teak leaf extract effectively played as antimicrobial components.

Growth of *Salmonella* spp. was retarded at 50^{o}F (10^{o}C) although it could survive in freezing temperature. *Salmonella* spp. remains grew with or without oxygen and temperatures ranging from 40 to 117^{o}F. Ideally, *Salmonella* will grow at pH 6.5 to 7.5 (Jay, 2000). *Salmonella* spp. was not found in sausage products in three different treatments during 12 days of storage. During storage, *Salmonella* spp. was not found, which indicated a good sanitation of the product during storage. A study of *Salmonella* spp. found that the bacteria could grow at 8^{o}C (Mattick *et al*., 2003), but other study mentioned that its optimal temperature is 4°C (Phillips *et al.*, 1998).

Sausages were made using a variety of additional ingredients including ice cubes, tapioca flour, skim milk, garlic, STPP, salt, coriander, sugar, pepper, ginger, and nutmeg. These materials might have antimicrobial properties and added the three types of sausages: control, sausage with the addition of teak leaf extract 0.5% and 1%. Made sausages tested against total bacteria (TPC), *Salmonella*, *S. aureus*, and *E. coli*. Table 2 shows that the sausage with the addition of teak leaf extract 0.5% and 1% significantly inhibited *S. aureus* compared with controls. These results proved that the teak leaf extract had inhibitory activity against *S. aureus* in sausages.

Altogether, in respect to nutritional quality, the addition of teak leaf extract met the microbial standard for Indonesian market as regulated by Indonesian National Standard (SNI). Indeed, the addition of teak leaf extract up to 1% could produce sausages with appropriate nutritional quality standards.

Physicochemical properties

As teak leaf has been reported to have ability as natural dye, it is interesting to investigate the effect of addition of teak leaf extract on color of the sausage. Physicochemical analysis were performed for color of the sausage including intensity of luminousity (L value), intensity of red color (a value), intensity of yellow color (b value) and degree of HUE. In addition, other physicochemical analysis was also performed for pH, aw and water absorption. Results of analysis of the physicochemical characteristics of beef sausages were shown in Table 3.

The L value represented the luminousity, it indicated lightness (Fernandes *et al*., 2014). Control had the highest brightness intensity. Sausage brightness intensity was also affected by the stability of the pigment. Anthocyanin pigments are stable at low pH which is red at pH 2-4, pH 4-6 (purple), pH 7-8 (blue) and yellow at pH >8 (Reyes and Zevalles, 2007).

Table 3. Physical properties of beef sausages

Variables		Treatments		
		0% Extract	0.5% Extract	1% Extract
pH		5.77 ± 0.11	5.94 ± 0.10	5.81 ± 0.03
WHC		8.97 ± 0.25	8.93 ± 0.15	9.13 ± 0.25
a_w		0.888 ± 0.010	0.892 ± 0.006	0.898 ± 0.008
L	18.17	50.34^{a} ± 1.60	47.5^{ab} ± 2.03	45.3^{b} ± 1.57
a	0.78	4.65^{a} ± 0.61	6.04^{ab} ± 0.31	7.22^{b} ± 0.68
b	-0.72	14.83^{b} ± 0.91	13.80^{ab} ± 0.56	12.67^{a} ± 0.33
°HUE		72.63^{a} ± 1.40 (Yellow Red)	66.34^{b} ± 0.22 (Yellow Red)	60.35^{c} ± 2.08 (Pink)

[a] Superscript different on the same line indicate significant differences (P < 0,05).

The a value corresponds to chromatic colors which were mixture red to green colors. Positive (+a) and negative (-a) values ranged from 0 to 100 for red color, and from -80 to 0 for green color, respectively (Hutchings, 1999). The values of the sausages in our experiment were classified as positive values, indicated that the sausage was considerably reddish. The addition of teak leaf extract could improve the intensity of red color of the sausages, indicating that the teak leaf extract was promising to be used as a natural dye.

The b value corresponded to chromatic colors which were mixed color between blue with yellow with the range of 0 to 70 for yellow color and the -70 to 0 for blue color (Hutchings,1999). The addition of 1% teak leaf extract lowers the intensity of yellow color of the sausage. Results analysis of variance of ^{o}HUE values showed significant differences in the concentration of 1%, 0.5%, and 1%. The obtained ^{o}HUE value was ranged between 60-72°.

The addition of teak leaf extract had no significantly affect the final pH value of sausages and a_w, but cooking process might cause for the differences on pH and a_w values. pH value of the ground meat that used as material for making the sausage was 5.45, while pH of sausages was 5.77-5.94. pH different between raw meat and cooked sausages ranged between 0.0 and 0.4 depending on the initial pH and the use of phosphate (Puolanne *et al*., 2001). Water holding capacity (WHC) could affect the quality of the sausages. However, the addition of teak leaf extracthad no affect the WHC values of sausages. WHC values might be affected by the pH value. Low pH value would decrease the water binding in a meat (Lonergan and Lonergan, 2005) that result in changing the value of water binding.

Nutritional quality

The afore-mentioned result clearly showed that teak leaf extract might function as preservative and natural dye agents for beef sausage. However, it remains unknown the effect of addition of teak leaf extract on nutritional quality for sausage product. Indonesian National Standard (SNI), No 01-3820-1995 for sausage product was used as a reference in

Table 4. Nutritional properties of beef sausages

Variables	Treatments			SNI (Indonesian National Standard)
	0% Extract	0.5% Extract	1% Extract	Sausage %
		(% bb)		
Water Content	59.42 ± 2.31	60.64 ± 0.77	57.44 ± 1.03	Max. 67.0
Ash Content	2.81 ± 0.44	2.93 ± 0.29	3.07 ± 0.21	Max. 3.0
Fat Content	8.56[b] ± 0.36	11.49[a] ± 0.57	11.81[a] ± 0.33	Max. 25.0
Protein Content	12.44 ± 0.80	12.63 ± 0.71	12.62 ± 1.12	Min. 13.0
Carbohydrate Content	16.77[a] ± 0.94	12.31[b] ± 0.81	15.07[ab] ± 1.72	Max. 8

[a] Different superscript on the same line indicates significant differences (P < 0,05).

assessment of nutritional quality of the sausage in our experiment. The standard highlighted amount of nutritional components (water, ash, fat, and protein) to be in Indonesian market.

The result of nutritional component analysis is shown in Table 4. Water content obtained in this analysis was below the water level according to SNI 01-3020-1995 sausage which was 67.0%. Ash content in foodstuffs corresponds to the large amount of minerals contained in the food. Ash content in the sausage was 2.81-3.07%, and relatively still in the range recommended by SNI. Fat content of sausage were still lower than the maximum fat (25%) content recommended by SNI. Altogether, this product considerebely fits sausage quality standard. Despite the protein content of the sausage in this experiment is slightly below the standard, however the protein content (12.44-12.63) is quite close to minimum content of protein required for sausage (13%). Accordingly, we concluded that protein content of the sausage in our experiment remains suits to the standard. In addition, according to Gonzales-Vinaz *et al*. (2004), protein content in commercial sausage in Spain is about 11.13 to 16.06% and 15.2% in Brazil (Pereira *et al*., 2000).

Despite there is no significant effect of nutritional component by the addition of teak leaf extract, indeed fat and carbohydrat content were affected. The addition of 1% teak leaf extract was significantly higher than that of control. Teak leaf has about 22% crude fat (Gupta and Patle, 1991). Therefore addition of teak leaf extract might increase the fat content of final product higher to that of control. Additionally teak leaves contain triterpenoid compound that was non polar and soluble in hexane solution so it would measurably as crude fat sausage. Water levels were below the sausages protein content according to SNI 01-3020-1995 which was 13%. According to Gonzales-Vinaz *et al.* (2004), protein content in commercial sausage in Spain is about 11.13 to 16.06% and 15.2% in Brazil (Pereira *et al*., 2000).

Carbohydrate levels are above the levels of sausage's carbohydrates level according to SNI 01-3020-1995, which was 8%. Differences in carbohydrate levels were influenced by the ash, fat, and protein contents. Carbohydrate levels are also influenced by the addition of tapioca flour which is a source of high carbohydrate (Directorate of Nutrition, 1995).

Table 5. Hedonic tests and hedonic quality of beef sausages

Variables	Treatments		
	0% Extract	0,5% Extract	1% Extract
Hedonic Test[a]			
Color	3.88[a] ± 0.72	3.66[ab] ± 0.87	3.34[b] ± 1.00
Aroma	3.68[a] ± 0.96	3.50[b] ± 0.95	3.36[b] ± 0.85
Texture	3.62[ab] ± 0.78	3.96[a] ± 0.83	3.36[b] ± 0.98
General Appearance	3.82[a] ± 0.80	3.96[a] ± 0.81	3.48[b] ± 0.91
Hedonic Quality Test[b]			
Color	2.65 ± 0.80	2.25 ± 0.84	2.75 ± 1.17
Aroma	3.98 ± 0.80	3.93 ± 0.76	3.93 ± 0.83
Texture	3.60 ± 0.59	3.65 ± 0.74	3.85 ± 0.62
General Appearance	3.23 ± 0.93	3.48 ± 0.81	3.60 ± 1.00

[a] Hedonic test: 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, 5 = like a lot
[b] Hedonic quality test:
Color: 1 = very not red, 2 = not red, 3 = slightly red, 4 = red, 5 = very red
Aroma: 1 = smells strong teak leaf, 2 = smells teak leaf, 3 = smells slightly teak leaf, 4 = not smells teak leaf, 5 = very not smells teak leaf
Texture: 1 = very coarse, 2 = coarse, 3 = slightly coarse, 4 = soft, 5 = very soft
General appearance: 1 = very unattractive, 2 = unattractive, 3 = slightly attractive, 4 = attractive, 5 = very attractive
[c] Different superscript on the same line indicates significant differences (P < 0,05).

Sensory acceptability of sausages

The remaining question dealing with the effect of addition of teak leaf extract on panelist acceptability. Despite leaf extract well functions as antimicrobial and natural dye agents for the sausage, it would be unacceptable for market if the sensory badly affected. To address, we performed organoleptic tests to confirm quality sensory of the susage. The tests were devided into hedonic test and hedonic quality test. A hedonic test (hedonic) state like or dislike of a particular product. Hedonic quality test states of good and bad impressions or evaluations of a product, in this case isbeef sausages product. Results of organoleptic analysis on beef sausagescould be seen in Table 5.

Hedonic test

Treatments affected the color parameters by the panelists. Sausages with concentrations of 0% and 0.5% of teak leaf extract were preferred over sausages with the 1% concentration. A panelist preference of color might be seen from the intensity of the brightness. Sausages that had high brightness were more preferred by the panelists. Treatments had no affect the flavor parameters of sausages. The flavor of sausages was affected by many things: like the teak leaf extracts and spices used. Undetectable aroma of teak leaf extract as well as a common spice that used in sausages accounted for indiscrepancy the valuation by panelists. Panelists preferred texture of the sausage with the addition of 0.5% teak leaf extract. Panelists preferred texture that had the highest water content among the others because according Fardiaz *et al.* (1992) texture is influenced by moisture content. The general appearance of the sausage was not affected by the addition of teak leaf extract at any concentrations. However, panelists remarkably preferred the sausages with 0%, 0.5%, and 1% of teak leaf extract additions.

Hedonic Quality Test

Color on the sausages comes from meat, fillers, binders, and ingredients. Treatments had no affect the color of aroma, texture, and general appearance of sausages. Treatments had no affect the bad or good valuations of sausages. Insignificant differences also affected by the reddish resulting color because the red value being obtained was quite low. However, this sausage had an advantage of not smelling like teak leaf. In addition, sausage also had a soft texture that was influenced by moisture content (Fardiaz *et al.*, 1992). The general appearance was quite attractive to the panelists and all hedonic test panelists liked sausages with the addition of 0%, 0.5%, and 1% of teak leaf extracts. Altogether, additional teak leaf extract did not seriously affect sensory quality of the sausages. In overall, panelists have neutral preferences on the product. Further attempts to increase preferences of the panelist are considerably required.

Conclusion

Teak leaf extract functions as antimicriobal and natural dye agents for beef sausages. Addittion of 1% teak leaf extract yielded better sausages in term of microbiological quality compared to the controls. The addition of 1% teak leaf extract significantly increased red color intensity of beef sausages. However, the addition of teak leaf extract at any concentrations had no effect on physical properties such as pH, water binding capacity, a_w (water activity), as well as nutritional quality. Sensory test stated that the panelists remarkably prefered beef sausages with the addition of 0.5% and 1% of teak leaf extracts. Thus, 1% teak leaf extract could be used as preservatives and natural dyes in beef sausages products.

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