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Comparison of antioxidant properties in different herbal fresh sausages

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Abstract The antioxidant properties of different herbal fresh sausages was evaluated. Sensory evaluation of five herbal adding to fresh sausage recipes No.1 – No.5 were examined. Two highest overall acceptability scores of sausages were selected and subsequently studied on antioxidant properties by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cation decolorization and thiobarbituric acid reactive substances (TBARS) methods. The results showed that the most favorite of cooked herbal fresh sausages recipes were No.2 and No.5. Therefore, two of these recipes were further studied for antioxidant properties. The DPPH and ABTS of sausage No.5 was higher than sausage No.2 and sausage contained 0.01% butylated hydroxytoluene (BHT) ($P<0.05$). However, TBARS of sausage recipes No.2 and No.5 were higher than 0.01% BHT ($P<0.05$) which indicated that sausage recipes No.2 and No.5 was more rancid than the recipe with 0.01% BHT. This finding displayed the beneficial herbal fresh sausages as healthy food.

Keywords: antioxidant properties, fresh sausages, herbs

Introduction

Fresh sausage is one of the most popular meat products worldwide. It's cheap, delicious, easy to prepare and mostly processed from meat trimmings (Ali *et al.*, 2018). Basically, this product contains excellent source of valuable nutrients normally in meat products (proteins, iron, vitamins, etc.) (Pintado *et al*., 2018). Fresh sausage is made from beef, veal, pork, lamb, poultry or from any combination of these meats. The most common sausage seasonings are salt, pepper, mace, red pepper, chili, garlic, ginger, lemon bark, cinnamon, onion, cumin, monosodium glutamate and celery etc. (FAO., 1985) depending on local preparations (Salinas *et al*., 2014). The mixture is stuffed in natural casings and these products are kept in the refrigerator until consumption. The freezer at temperature 0-4℃ are applied for long-term storage (Georgantelis *et*

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al., 2007). Freezing protects the product against bacterial spoilage but not oxidative rancidity, because the salt assisted the catalytic activity (FAO., 1985). Therefore, to maintain quality product, antioxidant preservatives are applied. The preservatives currently used are chemicals, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (Hugo and Hugo, 2015). Nowadays, consumer concern in the relation between diet and health (Nirmala *et al.* 2018). Consumers are increasingly interesting in health and foods which are more natural (Witzela *et al*., 2019). The consumers concern about synthetic antioxidants and their potential adverse effects coupled with the demand for natural antioxidants, especially of plant origin, has increased in recent years (Jagajothi *et al*., 2013). Therefore, natural food ingredients from plant were increasingly in demand (Lorenzo *et al.*, 2014).

A variety of plant materials are categorized as herbs or spices. They have been used since ancient times to improve sensory characteristics of food as preservatives for their nutritional and healthy properties and generally recognized as safe (Gracia, 2015). Natural antioxidants are frequently used in food products to prevent the oxidation of fat and oil in foods (Kirschweng *et al.*, 2017). Antioxidant activities of herbs, spices, vegetables and other extracts is similar to that of the synthetic additives. (Pereira *et al.*, 2017). Herbs are also known to have nutritional value of, antioxidants, antimicrobial and as medicine (Kawiji *et al*., 2017). It has been reported that most of plants and herbs contain natural antioxidants (Poh *et al*., 2018). For example, kaffir lime has been used by Indonesian, especially in the form of the fruit and leaves as food seasonings and ingredients in traditional medicines because it contains alkaloids, polyphenols, essential oils, tannins and flavonoids (Kawiji *et al*., 2017). In addition, lemongrass is a rich source of flavonoids and vitamin C. The natural flavonoids are also attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Sanaa *et al*., 2015). The medicinal plants have become a focal point to improve the present and future health (Rashid *et al*., 2016).

Therefore, the aim of this study was to compare antioxidant properties in different herbal fresh sausages for healthy beneficial food.

Materials and methods

Beef preparation

The fresh lean beef and fat are the main ingredients of fresh sausages. Beef without connective tissue and fat were obtained from Bangkok, Thailand.

They were cut into small pieces and then ground minced beef and fat were kept in refrigerator at 0-4˚C before sausage making.

Mixed herbs preparation

Fresh herbs used in this study were shallot, garlic, coriander root, chili pepper, red curry paste, shrimp paste, turmeric, kaffir lime leaf, kaffir lime skin, galangal, lemongrass, black pepper, cilantro, rice flour, parsley, fingerroot and basil. Five recipes of mixed herbs were compared (No.1-No.5) as shown in Table 1.

Herbal Fresh Sausages preparation

The ingredients of herbal fresh sausage were minced beef (48.23%), fat (32.15%), salt (0.56%), sugar (0.56%), monosodium glutamate (0.16%) and mixed herbs (18.33%). All ingredients mentioned above were mixed. The mixture was stuffed in sheep casing, and stored at 0-4˚C before further analysis. Sausages were prepared for 4 treatments including 1) sausage adding BHT (Acros, Belgium) 2) sausage adding BHT and seasoning (salt, sugar and monosodium glutamate) 3) and 4) herbal fresh sausages which selected by consumers.

Analytical samples preparation

The samples were cooked in an oven at 180 ℃ for 15 mins (inner temperature 72 ℃). Then, samples were prepared according to the method of Jung *et al.,* (2010). Each sausage sample recipes (3 g) were homogenized with distilled water 15 ml for 1 min. Adding chloroform 10 ml and mixed by centrifugation (4,000 rpm for 15 mins) (Avanti JA-20, Beckman Coulter, USA). Lipids and supernatant were separated. The supernatant was used for DPPH and ABTS further analysis.

Sensory evaluation

Sensory evaluation was carried out for uncooked and cooked sausages. Sausages were examined for their appearance, odor, buying decision and overall liking. The recipes were coded with three digits random number. There were 30 panelists in all sensory evaluation tests. Sensory attributes were evaluated using a seven points hedonic scale (Pimental *et al*., 2016) from 1 =

"dislike extremely", 2 = "dislike very much", 3 = "dislike", 4 = "neither like nor dislike", 5 = "like", 6 = "like very much" and 7 = "like extremely".

Table 1. The composition of herbs seasoning mix using in different sausage recipes

Ingredients	Mixed herbs				
	No.1	**No.2**	**No.3**	**No.4**	**No.5**
1. Shallot	√	-	√	-	-
2. Garlic	√	-	√	√	√
3. Coriander Root	√	-	-	-	-
4. Chili Pepper	√	-	-	-	-
5. Red Curry Paste	√	√	-	-	√
6. Shrimp Paste	√	-		-	-
7. Turmeric	√	-	√	-	
8. Kaffir Lime Leaf	√	√	√	-	√
9. Kaffir Lime Skin	√	-	√	-	-
10. Galangal	√	-	-	-	-
11. Lemongrass	√	√	√	√	√
12. Black Pepper	-	-	√	√	√
13. Culantro	-	-	-	-	√
14. Rice Flour	-	-	-	√	-
15. Parsley	-	-	-	√	-
16. Fingerroot	-	√	-	-	-
17. Basil	-	√	-	-	-

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity (DPPH, Sigma, Germany) was analyzed using method of Alam *et al*., (2013). The DPPH solution was prepared by mixing 0.2 mM DPPH in ethanol. The control was determined using 2 ml of distilled water with 2 ml of DPPH solution. The mixture of 2 ml of distilled water with 2 ml of ethanol was used as a blank of control. The samples used 2 ml of meat supernatant with 2 ml of DPPH solution and the blank of sample used 2 ml of meat supernatant with 2 ml of ethanol. The decrease in absorbance (Abs) was measured at 517 nm after 30 min. incubation at room temperature in the dark. The percentage of inhibition was calculated as:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A\text{-}B) - (C\text{-}D)}{(A\text{-}B)} \times 100$$

where A is the absorbance of the control, B is the absorbance of the blank of control. C is the absorbance of the sample and D is the absorbance of the blank of sample. All experiments were done in triplicate.

ABTS radical scavenging activity

The ABTS radical scavenging activity of herbal fresh sausages was determined according to the method of Slima *et al*. (2018) with slightly modification. The $ABTS^{\cdot+}$ solution was the result of the reaction between 7 mM 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+}$, Sigma, Canada) reagent in distilled water and 2.45 mM potassium persulfate ($K_2S_2O_8$, Unilab, New Zealand). The mixture was incubated in the dark at room temperature for 12–16 h. It was diluted with ethanol to an absorbance of 0.70 (± 0.02) at 734 nm. The test was done by mixing 0.3 ml meat supernatant with 3 ml of diluted $ABTS^{\cdot+}$ solution for 6 mins at room temperature. The blank of sample was determined using 0.3 ml of meat supernatant with 3 ml of ethanol. The mixture of 0.3 ml of distilled water with 3 ml of diluted $ABTS^{\cdot+}$ solution was used as a control and the blank of control used 0.3 ml of distilled water with 3 ml of ethanol. The reduction of the $ABTS^{\cdot+}$ radical was calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \frac{(A\text{-}B) - (C\text{-}D)}{(A\text{-}B)} \times 100$$

where A is the absorbance of the control, B is the absorbance of the blank of control. C is the absorbance of the sample and D is the absorbance of the blank of sample. All experiments were done in triplicate.

Evaluation of lipid oxidation

Lipid oxidation was performed using the distillation method to analyse thiobarbituric acid reactive substances (TBARS) as adapted from Cooper *et al*., (2017). A 10 g sample of meat product with 48 ml of distilled water and 1 ml of 0.2 % of BHT were homogenized. Homogenate was then poured into a digestion tube and tubes were vortexed and rinsed with 30 ml of distilled water. The homogenise was transferred into another tube4 with 1 ml of 5N HCl immediately before distillation. After distillation, Adding 5 ml of sample with 5 ml of thiobarbituric acid reagent (TBA, Sigma-Aldrich, Germany) into a glass tube and vortexed individually. Tubes were boiled in water bath for 35 mins at 95˚C immediately and then removed from the water bath. Tubes were submerged into an ice bath for 35 mins. The absorbance was measured at 538 nm using a spectrophotometer.

Statistical analysis

The experimental design was a completely randomized design. All analyses and data reported were performed as the mean ± standard deviation.

Sensory evaluation was conducted using SPSS version 23 software (IBM). Statistical analysis was performed in SAS Version 9.0 (SAS Institute Inc., 2002) to analyse the effect of ABTS, DPPH and TBARS. The differences among group averages were evaluated by Duncan's Multiple Range Test.

Results

Sensory evaluation of fresh herbs sausages

The sensory evaluation results of uncooked and cooked herbal fresh sausages were shown in Table 2. The uncooked sausage evaluation found that only recipes No.3 was accepted as score 5.57 (mean like or nearly like very much). However, the highest overall liking score of cooked sausage were observed in No.2 and No.5 as the overall acceptance score showed 5.17 and 5.14, respectively. These scores could interpret as consumer like and trend to like very much as shown in Table 2. Therefore, two herbal sausage recipes No.2 and No.5 were selected for further studies.

Table 2. Sensory evaluation scores for uncooked herbal fresh sausages (A) and cooked herbal fresh sausages (B) treated with different ingredients

(A)				
uncooked fresh herbs sausages	**Appearance**	**Odor**	**Buying Decision**	**Overall Liking**
No.1	4.47 ± 1.20^{a}	4.23 ± 1.28^{a}	4.17 ± 1.21^{a}	4.30 ± 1.12^{a}
No.2	4.70 ± 1.15^{a}	4.53 ± 1.07^{ab}	4.70 ± 1.26^{ab}	4.70 ± 1.26^{a}
No.3	5.77 ± 1.14^{b}	5.23 ± 1.04^{c}	5.53 ± 1.04^{c}	5.57 ± 0.97^{b}
No.4	4.20 ± 1.19^{a}	4.37 ± 0.93^{ab}	4.23 ± 1.19^{a}	4.27 ± 1.17^{a}
No.5	4.50 ± 1.22^{a}	4.43 ± 0.97^{ab}	4.37 ± 1.16^{a}	4.77 ± 0.94^{a}
(B)				
cooked fresh herbs sausages	**Appearance**	**Odor**	**Texture**	**Overall Liking**
No.1	4.30 ± 1.06^{ab}	4.30 ± 1.42^{a}	4.47 ± 1.36^{ab}	4.37 ± 1.22^{a}
No.2	4.77 ± 1.14^{bcd}	5.17 ± 1.24^{bc}	5.17 ± 0.89^{bc}	5.17 ± 0.95^{bc}
No.3	4.97 ± 1.25^{cd}	4.52 ± 1.12^{ab}	4.48 ± 1.09^{ab}	4.52 ± 0.99^{a}
No.4	4.00 ± 1.60^{a}	4.53 ± 1.50^{ab}	4.50 ± 1.43^{ab}	4.43 ± 1.55^{a}
No.5	5.22 ± 1.03^{d}	5.48 ± 1.07^{c}	5.32 ± 1.02^{c}	5.45 ± 1.00^{c}

1 = dislike extremely, 2 = dislike very much, 3 = dislike, 4 = neither like nor dislike, 5 = like, 6 = like very much and 7 = like extremely

$^{a-c}$ Means within the same column with different letters are significantly different ($P<0.05$).
All values were expressed as mean ± standard deviation.

Determination of Antioxidant Activities

In this study, antioxidant analysis were conducted into 4 treatments 1) 0.01% BHT, 2) 0.01% BHT adding seasoning (salt, sugar and monosodium glutamate) as a positive control, 3) herbal fresh sausages No.2 and 4) herbal fresh sausages No.5.

DPPH radical scavenging activity

The DPPH radical scavenging activity of herbal fresh sausages are displayed in Table 3. Comparison antioxidants properties of 2 herbal fresh sausage and other recipes 0.01% BHT and 0.01% BHT adding seasoning were performed. The results found that the herbal fresh sausages showed different value of % DPPH activity. The % DPPH in herbal fresh sausages exhibited higher than the sausage adding BHT ($p<0.05$). The % DPPH in herbal fresh sausages No.5 and No.2 were 68.85% and 46.47, respectively. The % DPPH in sausage containing BHT and BHT addition seasoning were 25.04 and 21.41, respectively (Table 3). As a result of this, it could be concluded that the herbal fresh sausages contained antioxidants higher than ground beef adding 0.01% BHT and 0.01% BHT adding seasoning.

ABTS radical scavenging activity

The ABTS value of herbal fresh sausages were shown in Table 3. It was found that the herbal fresh sausages showed different value of % ABTS activity. The recipe No.5 (65.44%) revealed the highest of % ABTS activity compared to other recipes. The %ABTS value of herbal fresh sausage No.2 was 48.64% which higher than the sausage adding BHT ($p<0.05$). % ABTS activity of the ground beef adding 0.01% BHT and 0.01% BHT adding seasoning were 31.80 and 39.96, respectively.

Table 3. The percentage of DPPH and ABTS radical cation decolorization in herbal fresh sausages

Antioxidant Methods	% Inhibition of DPPH radical scavenging activity	% Inhibition of ABTS radical cation decolorization
0.01% BHT	25.04 ± 0.52^{c}	31.80 ± 1.28^{d}
0.01% BHT + Seasoning	21.41 ± 0.94^{d}	39.96 ± 0.64^{c}
No.2	46.47 ± 0.67^{b}	48.64 ± 0.83^{b}
No.5	68.85 ± 0.44^{a}	65.44 ± 0.40^{a}

BHT = butylated hydroxytoluene
BHT+ seasoning = butylated hydroxytoluene + salt + sugar + monosodium glutamate
[a-d] Means within the same column with different letters are significantly different ($P<0.05$).
All values were expressed as mean ± standard deviation.

Evaluation of lipid oxidation

The evaluation of lipid oxidation by TBARS were demonstrated in Table 4. There was nonsignificant difference of herbal fresh sausage No.2 (0.318 mg MDA/kg meat) and No.5 (0.333 mg MDA/kg meat). The TBARS value of the ground beef adding 0.01% BHT and 0.01% BHT adding seasoning (0.106 and 0.137 mg MDA/kg meat, respectively) were significant lower than the herbal fresh sausage recipes No.2 and No.5 as shown in Table 4.

Table 4. The TBARS values (mg malonaldehyde/kg meat) of different Ingredients in herbal fresh sausages

Sample	TBARS (mg MDA/kg meat)
0.01% BHT	0.106 ± 0.03^{b}
0.01% BHT + Seasoning	0.137 ± 0.04^{b}
No.2	0.318 ± 0.01^{a}
No.5	0.333 ± 0.05^{a}

BHT = butylated hydroxytoluene
BHT+ seasoning = butylated hydroxytoluene + salt + sugar + monosodium glutamate
[a-c] Means within the same column with different letters are significantly different ($P<0.05$).
All values were expressed as mean ± standard deviation.

Discussion

Five herbal fresh sausages were evaluated by 30 consumers. Two recipes, No.2 and No.5 obtained higher score than other recipes and then selected for further antioxidant study. Due to the composition of herb in sausage No.2 was basil, while No.5 contained black pepper and cilantro which theses herbs were not addition to other recipes. The comparison of antioxidant properties in different herbal fresh sausages by DPPH, ABTS and TBARS methods were determined. The percentage of DPPH in herbal fresh sausages were higher antioxidants activities than the sausages adding BHT due to herb, which contained several compounds such as polyphenolics, flavonoids, lignans, and terpenoids (Rather *et al.,* 2016). Antioxidant properties of polyphenolics arise from their high reactivity as hydrogen or electron donors from the ability of polyphenol derived radical to stabilise and delocalize the unpaired electron (chain braking function) and from their potential to chelate metal ions (termination of Fen-ton reaction). This assay of DPPH radicals are the most popular spectrophotometric methods for determination of the antioxidant capacity of foods and vegetable extracts. Both chromogens and radical compounds can directly react with antioxidants (Tohma *et al.,* 2016). In ABTS

method, similarly %ABTS in herbal fresh sausages higher than sausage adding BHT. The decolorization of $ABTS^{\cdot+}$ radical reflected the capacity of an antioxidant herbs to donate electron or hydrogen atoms to inactivate this radical cation. The $ABTS^{\cdot+}$ radical reactions involved electron transfer and took place at a much faster rate compared to DPPH radicals. Furthermore, various factors like stereoselectivity of the radicals or the solubility of the tested sample in different testing systems and functional groups present in the bioactive compounds had been reported to affect the capacity of the sample to react and quench different radicals (Abed *et al*., 2014). Free radical scavenging is one of the mechanisms involved in inhibiting lipid oxidation and is commonly used to estimate antioxidant activity (Jin *et al.*, 2018).

In this study, TBARS value of sausage adding BHT demonstrated lower than both herbal fresh sausages, leading to delay the development of rancidity in sausages (Shahidi and Zhong. 2005). However, consumers are concerned about the safety of synthetic food additives (Ismail and Yusuf. 2017). Even though, two herbal fresh sausage recipes were more rancid than 0.01% BHT. This concern has led to a great interest in natural additives with antioxidant properties. Thus, natural additives were readily accepted by consumers, as they were considered natural, which commonly used to delay the development of rancidity in food products (Sallama *et al.*, 2004). Lipid oxidation is one of the main factors affecting the quality characteristics of meat products during storage period since it influenced the development of rancidity and product nutritive values, colours, and flavours.

In conclusion, five herbal fresh sausage recipes were studied on consumers acceptance. Two out of five recipes, No.2 and No.5 were selected for antioxidant properties comparison. Both herbal fresh sausage recipes showed higher antioxidant than sausage containing BHT. However, sausage adding BHT delayed the rancidity performed longer shelf life. This finding indicated that herbal fresh sausage showed beneficial for consumers healthy, even though the shelf life shorter.

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