แก่นเกษตร 47 (5) : 1099-1112 (2562). KHON KAEN AGR. J. 47 (5) : 1099-1112 (2019). /doi:10.14456/kaj.2019.100.

Effects of addition of Fang (*Caesalpinia sappan* L.) extract powder on qualities and storage stability of reduced-nitrite Vienna sausage

Wiranpat Olanwanit[1] and Thanakorn Rojanakorn[2*]

ABSTRACT: This study investigated the influence of Fang extract powder on the quality attributes and storage stability of reduced-nitrite Vienna sausage. The 75 ppm nitrite or reduced-nitrite Vienna sausage samples with 0, 1, 2, 3 and 4% Fang extract powder were produced. The sample with 125 ppm nitrite (control) was also prepared for comparison. With increasing Fang extract powder, redness (a*) and yellowness (b*) of reduced-nitrite samples increased ($P \leq 0.05$) while pH value and residual nitrite content decreased ($P \leq 0.05$). All reduced-nitrite sausage samples containing Fang extract powder were redder and yellower than the control ($P \leq 0.05$). All sensory liking scores of the reduced-nitrite sample with 3% Fang extract powder were not significantly different from the control ($P \leq 0.05$). Storage stability study (4±1°C for 30 days) revealed that the TBA value of the 3% Fang extract powder reduced-nitrite sample and the control sample with 125 ppm nitrite increased with storage time while their residual nitrite decreased. The microbial counts of both samples throughout 30 days storage period were in the permission limits of the Thai industrial standard. Therefore, they were considered safe.
Keywords: Vienna sausage, storage stability, fang extract powder, reduce nitrite

Received February 22, 2019

Accepted May 23, 2019

[1] Student Master of Science Program in Food Technology, Khon Kaen University 40002

[2] Thesis Advisor Lecturer, Department of Food Technology, Faculty of Technology, Khon Kaen University 40002

[*] Corresponding author; e-mail address: thajoj@kku.ac.th

Introduction

Vienna is a cured emulsion type sausage which is popular in Thailand because of its palatability (Wimontham and Rojanakorn, 2016). It is produced from lean meat, fat and other ingredients. Nitrite is one of the most important curing ingredients commonly used in emulsion type sausages which are kept for an extended period in a cold state. Based on Thai regulation, the maximum permitted level of nitrite in Vienna sausages is 125 mg/kg or ppm (BTIS, 2006). Nitrite gives the meat and meat products a pinkish-reddish color and prevents the growth and deadly botulism toxin formation by Clostridium botulinum. In addition, nitrite slows down the progress of rancid flavor caused by lipid oxidation and provides the unique flavor to the products (Wimonthum and Rojanakorn, 2016). During cooking, N-nitosamines, which are suspected of being carcinogenic substances, can be formed by the reaction of nitrite and secondary amines and amino acids in meat products (Riazi et al., 2016). Additionally, large amount of nitrite in the human diet may induce the disorder known as methemoglobinemia, which can be fatal (Cassens, 1997). Due to the potential danger of nitrite, researches on the quality of reduced-nitrite meat products containing natural colorants have increased (Eyiler and Oztan, 2011). The nitrite reduction may, however, conduce to lowered consumer satisfactoriness of meat products, especially their color after a long period of storage time (Wimonthum and Rojanakorn, 2016). Natural additives with stable red or pink color can be used as a nitrite substitute because they are healthy and safe (Eyiler and Oztan, 2011; Wimonthum and Rojanakorn, 2016). Furthermore, some natural materials have acceptable sensory attributes (i.e., color and flavor), exhibit effective antimicrobial and antioxidant activities and seem to maintain or improve the color of lowered-nitrite meat products (Bazan-Lugo et al., 2012). Riazi et al. (2016) reported that incorporation of dry red grape pomace (1% and 2%) into reduced-nitrite dry-cured sausage (60 ppm sodium nitrite) increased the shelf-life, improved health-promoting properties and stability of the sausage samples. Deda et al. (2007) demonstrated that 100 ppm nitrite frankfurters with acceptable quality could be manufactured by adding 12% tomato paste. Eyiler and Oztan (2011) reported that the ingoing amount of nitrite in frankfurters could be reduced from 150 ppm to 50 ppm by adding 2% tomato powder without detrimental effect on the product quality. Wimontham and Rojanakorn (2016) reported that the high quality reduced-nitrite Vienna sausage could be produced by using a combination of 75 ppm nitrite and 1.0% gac aril powder.

Fang or Sappan (Caesalpinia sappan L.) is a tropical plant which is grown and distributed in Southeast Asia including Thailand. It belongs to the Leguminosae family and has long been used as a traditional Thai and Chinese medicine (Nirmal et al., 2015).The heartwood of the plant is a good source of brazilin, a red dye, which is responsible for pharmacological activities including anti-inflammatory, anti-photoaging, hypoglycemia, anti-allergic and antioxidant activity (Nirmal et al., 2015). The Fang extract is traditionally prepared by boiling the wood pieces in water and the obtaining red extract is used as a colorant for Thai dessert and some drinks. Even though the red extract of Fang has been used as a colorant in some food formulations, details on the use of the extract in reduced-nitrite meat products is very limited, so the objective of the current study was to investigate the influence of Fang extract powder as a food colorant on quality characteristics and storage stability of reduced-nitrite Vienna sausage.

Materials and Methods

Materials

The Fang powder was purchased from a traditional drug store in Khon Kaen. The Fang extract was prepared by boiling 10% (w/w) Fang powder in water then filtered through the Whatman paper No1. The filtrate or Fang extract was mixed with 10% (w/w) egg white and 15% (w/w) maltodextrin (DE 16) then whipped to form foam in a mixer (Kitchen Aid Model ULM-400, USA) at a maximum speed of 1400 rpm for 5 min. The resulting foam was evenly spread on the stainless steel plates (15.5x27 cm) at a thickness of 6±1 mm and dried in a tray dryer (Model ED 115, Germany) at 70±1°C with a constant air velocity of 0.5 m/s for 55 min (Suthaporn, 2018). The dried foam obtained was scraped off from the plates then powderized, packed in aluminum foil pouches and stored at 4±1°C until used.

Pork and pork fat were bought from a local meat market in Khon Kaen. The excess fat and connective tissue in the fresh pork was removed in order to get the lean meat. The lean meat and frozen pork back fat were separately ground through a 12 mm and 3 mm plate respectively.

Methods

The current study was planned as 2 steps. In step 1, six Vienna sausage samples with different amounts of nitrite and foam-mat dried Fang extract powder were produced. Five treatments of reduced-nitrite (75 ppm nitrite) sausages with different foam-mat dried Fang extract powder concentrations (0, 1, 2, 3, and 4% by weight of raw batter) were prepared. The control sample with 125 ppm sodium nitrite (the maximum permitted level in emulsion type sausages in Thailand) was also prepared for comparison. In the current study, 75 ppm nitrite was used for the production of reduced-nitrite sausage samples because this level can inhibit the growth and toxin formation of Clostridium botulinum as reported by many researchers (Jafari and Eman-Djomeh, 2007; Tompkin, 2005; Eyiler and Oztan, 2011; Wimontham and Rojanakorn, 2016)

All cooked sausage samples were vacuum packed and stored at 4±1°C for 1 day before evaluating for cooking loss, pH, texture profile analysis, color, amount of residual nitrite and sensory evaluation. All treatments in step 1 (~3.0 kg each) were conducted in duplicate.

In step 2, the control sausage sample (125 ppm nitrite) and 75 ppm nitrite sausage sample with an appropriate concentration of foam-mat dried Fang extract powder chosen from step 1 was produced, vacuum packed (350±5 g/pack) and stored at 4±1°C for 30 days. The storage stability of the samples was evaluated in terms of pH, nitrite residue, Thiobarbituric acid value (TBA), and microbial counts at day 1, 10, 20 and 30. All treatments in step 2 (~ 3.0 kg each) were conducted in duplicate.

The Vienna sausage was prepared as per Wimontham and Rojanakorn (2016) with some modifications. The ground meat (1590.0 g) with 1/3 ice cubes (210.0 g) was chopped in a silent cutter (Cutter M11N, NMH Maschinen, Germany) at high speed for 1 min then sodium chloride (52.8 g), soduim tripolyphosphate (3.0 g), sodium ascorbate (2.1 g), and 1/3 ice cubes (210.0 g) were incorporated and chopped at high speed for 2 min. The test nitrite (125 ppm as a control), test Fang extract powder (0, 1, 2, 3, and 4% by weight of raw batter (3008.0 g) mixed with 75 ppm nitrite), spices and seasonings (15.0 g) were added and chopped for 2 min at high speed. Minced pork fat (585.0 g) and the ice cubes remained (210.0 g) were then added to the meat mixture and further chopped for another 2 min at high speed. The temperature of the meat mixture during chopping was controlled at 12±1°C by intermittent adding of ice cubes and monitoring by the thermometer. The raw batter form each sausage formulation was extruded in a 15 mm diameter plastic casing using a stuffer (TWF-12, DICK, Germany), hand linked at 12.5 cm intervals and cooked in hot water (80±1°C) until the cored temperature reached 72±1°C. The cored temperature of the sausage samples were measured and monitored by using a probe thermometer with a temperature range of -40°C to 200°C (Extech 39240, USA).The cooked sausage samples were cooled in cold water (4±1°C) and the plastic casing removed. The samples were vacuum packed in PE/Polyester bags and chilled (4±1°C).

Cooking loss was determined by measuring weight of ten Vienna sausage samples before and after cooking in hot water (80±1°C) until the core temperature reached 72±1°C. The weight difference was used to calculate the percentage of cooking loss as per Andres et al. (2006).

The pH of cooked sausages was measured after blending 10 g of sample with 90 ml distilled water for 30s using a pH meter (Denver Instrument, USA) as per Wimontham and Rojanakorn (2016).

The surface color of cooked sausages expressed as lightness (L*), redness (a*) and yellowness (b*) was measured at three different points on each sausage link using a Minolta colorimeter (CR-300, Konica Minota, Japan) calibrated with a white standard tile (L*=95.26, a*=0.89, b*=1.18). Five links of sausage from each treatment were used for this analysis (Wimontham and Rojanakorn, 2016).

Texture profile analysis of cooked sausage samples was evaluated using a texture analyzer (TA-XT2i, Stable Micro System, UK). Fifteen transversely cut slices (each 15 mm diameter, 15 mm high) from each treatment were taken and compressed twice to 40% of their original height using 25 kg cylindrical probe (35 mm diameter). The cross-head speed was set at 2.0 mm/s (Wimontham and Rojanakorn, 2016). The textural parameters including hardness, springiness, cohesiveness, adhesiveness and chewiness were interpreted using the software provided by the instrument supplier.

The residual nitrite content in cooked sausages was determined as per Rincon et al. (2003) with some modifications. Briefly, sausage samples were homogenized with warm water (65±1°C) for 20 min and filtered through the Whatman paper No1. N-(1-naphthyl)-ethylenediamine dihydrochloride and sulphanilamide were added to the filtrate to form a red azo compound. The absorbance at 583 nm of the solution was measured by using spectrophotometer (Lamda 25, Perkin Elmer, USA). The nitrite content was calculated based on a standard curved prepared from sodium nitrite and expressed as mg/kg or ppm. In the current study, an equation for nitrite standard curve was y=0.0029x with R2 =0.994, where y is absorbance value at 538 nm and x is nitrite concentration.)

Sensory evaluation of cooked Vienna sausages was performed using 30 untrained taste panels who were asked to evaluate their likability on appearance, color, odor, texture, taste and overall liking by using 9-point hedonic scale test (9=like extremely and 1= dislike extremely) (Eyiler and Oztan, 2011). The taste panels were the member of the faculty of Technology, Khon Kaen University and had experiences in sensory evaluation of meat products. Two slices (2.5 cm long at 35±1°C) of each sausage formulation coded with 3-digit random number were randomly served to the taste panels in individual booths. The taste panels were told to rinse the palate with water between samples (Meilgaard et al., 1991).

The thiobarbituric acid (TBA) value, an index of degree of oxidative rancidity, of all cooked sausage samples was determined as per Hayes et al. (2013) with some modifications. Briefly, three sausage links from each treatment were randomly sampled and blended in a blender for 2 min. Ten gram of ground samples were mixed with 20 ml of thichloroacetic acid (10%) and homogenized for 1 min then centrifuged (Sorvall® RC6 Plus, Themo Electron corporation, USA) for 30 min at 2000g at 5°C. The mixture was filtered through Whatman paper No 1 and 2 ml of the filtrate was mixed with 2 ml thiobarbituric acid (TBA) in a glass tube. The tube was placed in a water bath set at 90±1°C for 20 min. After cooling, the absorbance was read at 532 nm (Lamda 25, Perkin Elmer, USA) and the TBA value was expressed as mg malonaldehyde per kg.

Microbiological quality of Vienna sausage samples was assayed using the standard method of AOAC (2000). About 25 g samples were taken aseptically from each treatment, transferred to a sterile plastic bag and homogenized for 2 min with 225 ml sterile peptone water (0.1%) to make a 10-1 dilution using a stomacher (Lab-Blender 400, Seward Medical, London UK). Further dilution was prepared using 0.1% sterile peptone water. For all microbiological count methods, duplicate plates were prepared and the results were expressed as log CFU per gram sample. The suitable decimal dilutions were used to determine the total plate count using plate count agar and the plates were incubated at 37±1° for 48 h. Yeast and mold count was determined by using potato dextrose agar and the incubation condition was 25±1°C for 5 days. Baired Parker agar was used for Staphylococcus aureus determination by placing the plates in an incubator at 37±1° for 48 h. Tryptose sulfite cycloserine agar and Clostridium welchii egg york agar were used to determine the number of Clostridium perfringens and the plates were incubated at 35±1° for 24 h in an anaerobic condition by using an aerobic jar (BBL, GasPak system, USA)

In the current study, the completely randomized design (CRD) and randomized complete block design (RCBD) were applied for step 1 whereas the split plot, storage time as a main plot and sausage formulation as a subplot, was used for step 2 (Wimontham and Rojanakorn, 2016). The experiments were performed in duplicate and results were expressed as means with standard deviations. Analysis of variance and Duncan's new multiple range test were performed to identify differences among the means at 95% confidence level using SPSS for Windows version 23.

Results and Discussion

As seen in Table 1, the color parameters of the sausage were affected by sausage formulation ($P \leq 0.05$). The sausage sample with 75 ppm nitrite had lower redness (a*) than the sample with 125 ppm ($P \leq 0.05$) nitrite due to a reduction of nitrite concentration whereas the lightness (L*) and yellowness (b*) were not significantly different ($P \geq 0.05$). Wimontham and Rojanakorn (2016) reported the lower redness (a*) of Vienna sausage containing 75 ppm nitrite as compared to the one with 125 ppm nitrite. As seen in Table 1, level of Fang extract powder did not affect the lightness (L*) of reduced-nitrite sausage samples, however incorporation of 1.0% up to 4.0% Fang extract powder in the sausage containing 75 ppm nitrite insignificantly increased the lightness (L*) as compared to the control with 125 ppm nitrite ($P \geq 0.05$). This may be because of the presence of maltodextrin and egg white in Fang extract powder. The redness (a*) of the control sausage (125 ppm nitrite) was not significantly different from the reduced-nitrite samples (75 ppm nitrite) with 2% Fang extract powder ($P \geq 0.05$) but lower than the reduced-nitrite samples containing 3 and 4% Fang extract powder($P \leq 0.05$). Therefore, addition of 3 and 4% Fang extract powder to reduced-nitrite sausage resulted in a redder sample as compared to the control ($P \leq 0.05$). This is because brazilin in Fang extract powder is responsible for the red color (Nirmal et al., 2015). Brazilin is the major compound naturally occurring in the Fang heartwood. It is one of the water soluble flavonoids, which is well known as the natural red color dye for staining. It has been used as a natural red color in food or beverages (Nirmal et al., 2015). The results of the current study agree with Deda et al. (2007) who reported that an increase in tomato paste concentration in frankfurters resulted in an increase in redness. Eyiler and Oztan (2011) demonstrated that a rise in ingoing amount of tomato powder in frankfurters resulted in the increased value of redness.

Table 1 Some physicochemical parameters of different Vienna sausage formulations

Quality parameters	Nitrite 125 ppm (control)	Nitrite 75 ppm	Nitrite 75 ppm+1% FEP*	Nitrite 75 ppm+2% FEP	Nitrite 75 ppm+3% FEP	Nitrite 75 ppm+4% FEP
Lightness (L*)						
Redness (a*)	66.92±0.77[a]	66.88±0.23[a]	67.15±0.26[a]	67.20±1.15[a]	67.72±0.61[a]	67.76±0.50[a]
Yellowness(b*)	2.69±0.14[b]	1.74±0.07[d]	2.30±0.30[c]	2.64±0.29[b]	3.28±0.27[a]	3.38±0.77[a]
Cooking loss (%)	8.05±0.33[bc]	7.71±0.38[c]	7.62±0.20[c]	8.37±0.37[b]	9.61±0.19[a]	10.09±0.38[a]
pH	1.61±0.03[a]	1.58±0.18[a]	1.56±0.03[a]	1.57±0.06[a]	1.58±0.04[a]	1.62±0.16[a]
Residual nitrite (mg/kg)						
	6.46±0.18[a]	6.56±0.11[a]	5.84±0.02[b]	5.82±0.11[b]	5.80±0.12[b]	5.77±0.12[b]
	46.82±1.34[a]	32.04±1.89[b]	29.26±1.02[c]	27.22±1.12[d]	25.03±1.09[e]	23.97±1.16[e]

*FEP = Fang extract powder

Values within the same row having different superscripts were significantly different ($P \leq 0.05$)

Table 2 Textural parameters of different Vienna sausage formulations

Quality parameters	Nitrite 125 ppm (control)	Nitrite 75 ppm	Nitrite 75 ppm+1% FEP*	Nitrite 75 ppm+2% FEP	Nitrite 75 ppm+3% FEP	Nitrite 75 ppm+4% FEP
Hardness(kg_f)	3.57±0.41[abc]	3.28±0.10[c]	3.36±0.03[bc]	3.75±0.12[a]	3.71±0.12[ab]	2.17±0.18[d]
Adhesiveness(kg.sec)	-0.04±0.59[b]	-0.04±0.57[b]	-0.04±0.70[b]	-0.04±0.92[b]	-0.04±0.32[b]	-0.02±0.27[a]
Springiness	3.53±0.13[ab]	3.23±0.12[b]	3.54±0.30[ab]	3.54±0.22[ab]	3.64±0.21[a]	2.53±0.13[c]
Cohesiveness	0.30±0.01[a]	0.29±0.01[a]	0.29±0.01[a]	0.29±0.01[a]	0.29±0.01[a]	0.26±0.01[b]
Chewiness(kg_f)	3.52±0.46[bc]	2.55±0.55[c]	3.45±0.25[bc]	3.86±0.31[ab]	4.20±0.30[a]	1.48±0.23[d]

*FEP = Fang extract powder

Values within the same row having different superscripts were significantly different ($P \leq 0.05$)

Cooking loss and pH of the control sausage and reduced-nitrite sausage without Fang extract powder were not significantly different (P≥0.05) indicating that a reduction of nitrite concentration did not influence the pH and cooking loss values of Vienna sausage (Table 1). Wimontham and Rojanakorn (2016) reported that a reduction of nitrite from 125 ppm to 75 ppm did not affect the cooking loss and pH values of emulsion type sausage. The incorporation of up to 4% Fang extract powder in reduced-nitrite sausage did not change the cooking loss value of the sausage samples while increasing Fang extract powder level from 0% to 4% resulted in a reduction of the pH value from 6.46 to 5.77. Furthermore, the higher the concentration of Fang extract powder, the lower the pH value (Table 1). This may be due to the acidic pH of Fang extract powder (pH; 4.98-5.09). Generally, the pH of Vienna sausages is in the range of 6.3-6.6, however the pH value of this sausage depends on the ingredients used in sausage formulation. Furthermore, the standard or specification of the pH of Vienna sausages was not specified (Finer, 2006). Many researchers reported that the sour taste of emulsion sausages could not be detected if the pH values were higher than 5.6 (Finer, 2006; Jafari and Emam-Djomeh, 2007; Eyiler and Oztan, 2011; Wimontham and Rojanakorn, 2016). In case of fermented sausages, their pH values were recorded as 4.5-4.6, which was the threshold of sour taste (Finer, 2006; Cavaheiro et al., 2013). In the current study the pH value of reduced-nitrite sausage samples containing different concentrations of Fang extract powder ranged between 5.77 and 5.84, which was higher than the fermented sausage and did not provide the sour taste. The result of the current study agree with Wimontham and Rojanakorn (2016) and Eyiler and Oztan (2011) who reported that the incorporation of gac aril powder (pH; 4.95-5.18) and tomato powder (pH; 4.48-5.02) lowered the pH of emulsion type sausages.

As expected, the control sausage sample with 125 ppm nitrite had higher (P≤0.05) residual nitrite content than the reduced-nitrite sausage samples. An increase in Fang extract powder concentration in reduced-nitrite samples from 0% to 4% accounted for a reduction of the residual nitrite and from 32.04 to 23.97 (Table 1). The variation in residual nitrite in reduced-nitrite sausages may be due to the lower pH of the samples with a higher amount of Fang extract powder. Similarly, Dedat et al. (2007) reported that the lower the pH of frankfurters, the lower the residual nitrite detected in the product. Liu et al. (2010) demonstrated that the reduced-nitrite Chinese sausage with lower pH exhibited a smaller amount of residual nitrite as compared to the samples with a higher pH. Wimontham and Rojanakorn (2016) observed that the residual nitrite content of reduced-nitrite Vienna sausages decreased with a decrease in their pH. Theiler et al. (1981) likewise reported that the lower the pH value, the faster the rate of nitrite decomposition.

The textural parameters of different Vienna sausage formulations are presented in Table 2. The textural

Table 3 Sensory likability scores of different Vienna sausage formulations

Quality parameters	Nitrite 125 ppm (control)	Nitrite 75 ppm	Nitrite 75 ppm+1% FEP*	Nitrite 75 ppm+2% FEP	Nitrite 75 ppm+3% FEP	Nitrite 75 ppm+4% FEP
Appearance	6.67 ± 1.69^{bc}	6.63 ± 1.39^{bc}	6.37 ± 1.77^{c}	7.27 ± 1.11^{a}	7.07 ± 1.01^{ab}	6.7 ± 1.11^{bc}
Color	6.40 ± 1.44^{ab}	6.3 ± 1.52^{b}	6.93 ± 1.51^{a}	7.13 ± 1.22^{a}	7.13 ± 1.36^{a}	7.16 ± 1.43^{a}
Odor	6.50 ± 1.28^{a}	6.20 ± 1.83^{a}	6.3 ± 1.40^{a}	6.80 ± 1.54^{a}	6.77 ± 1.28^{a}	6.33 ± 1.09^{a}
Texture	7.40 ± 1.16^{a}	6.97 ± 1.07^{a}	6.97 ± 1.30^{a}	7.60 ± 1.13^{a}	6.90 ± 1.83^{a}	4.90 ± 1.73^{b}
Taste	7.13 ± 1.25^{a}	6.27 ± 1.72^{b}	6.33 ± 1.67^{b}	6.9 ± 1.41^{ab}	6.63 ± 1.77^{ab}	6.43 ± 1.57^{ab}
Overall liking	7.03 ± 1.07^{a}	6.0 ± 1.26^{b}	6.47 ± 1.50^{ab}	7.07 ± 1.11^{a}	6.77 ± 1.57^{ab}	5.4 ± 1.50^{c}

*FEP = Fang extract powder

Values within the same row having different superscripts were significantly different ($P\leq0.05$)

Table 4 Changes of chemical and microbiological quality of Vienna sausage samples during cold storage

Sausage sample	Storage time (day)	Residual nitrite (ppm)	TBA (mg malonaldehyde/kg)	Total plate count (log CFU/G)	Yeast and Mold count (log CFU/G
125 ppm nitrite	1	46.08 ± 1.04^{a}	0.031 ± 0.004^{e}	N.d.	N.d.
	10	33.24 ± 1.07^{b}	0.036 ± 0.006^{cde}	N.d.	N.d.
	20	26.67 ± 2.83^{c}	0.043 ± 0.005^{c}	3.55 ±	1.70 ±
	30	16.85 ± 1.48^{e}	0.056 ±	4.82 ±	3.18 ±
75 ppm nitrite +3.0% Fang extract powder	1	25.03 ± 1.09^{c}	0.033 ±	N.d.	N.d.
	10	20.71 ± 1.32^{d}	0.040 ±	N.d.	N.d.
	20	15.29 ± 1.07^{e}	0.057 ±	4.57 ±	3.32 ±
	30	10.31 ± 1.46^{f}	0.070 ±	6.27 ±	4.47 ±

*N.d =Not detected

Values within the same column having different superscripts were significantly different ($P\leq0.05$)

parameters of the reduced-nitrite sausage samples containing up to 3% Fang extract powder were not significantly different from the control sample with 125 ppm nitrite (P>0.05). Additions of 4% Fang extract powder in the reduce-nitrite sausage resulted in the lowest textural parameters (P≤0.05). This might be because maltodextrin in Fang extract powder interfere the protein gel matrix formation of the emulsion meat system, resulting in a weaker gel network and less cohesive texture (Barretto et al., 2015; Rather et al., 2016). Felisberto et al. (2015) studied the effect of prebiotics including polydextrose, inulin, and resistant starch on microstructure of low-fat bologna sausage which related to its textural property by using scanning electron microscopy (SEM). They found that these prebiotics were embedded in the protein gel matrix of low fat emulsion system, resulting in a weaker gel network and less cohesive texture. To get a clear evident of the interference of protein gel matrix formation induced by maltodextrin in Fang extract powder, the microstructure of Vienna sausage samples containing Fang extract powder under scanning electron microscope should be further studied.

Results of sensory evaluation of all sausage samples are presented in Table 3. Almost all sensory attributes except odor were significantly affected (P≤0.05) by sausage formulation. Color likability scores of the control sample with 125 ppm nitrite and the reduced-nitrite sample without Fang extract powder were not significantly different (P>0.05), indicating that an acceptable color in Vienna sausage can be achieved by using 75 ppm nitrite. Wimontham and Rojanakorn (2016) reported that the color liking scores of Vienna sausages containing 125 and 75 ppm nitrite were not significantly different (P>0.05). Sindelar and Milkowski (2011) reported that 25 ppm sodium nitrite was sufficient for producing acceptable color in cured meat products; however, higher concentrations would be necessary to achieve and maintain acceptable cured meat color during a long shelf-life. The reduced-nitrite (75 ppm nitrite) sample with no Fang extract powder addition had lower overall likability score (P≤0.05) than the control sample with 125 ppm nitrite. Addition of 1-3% Fang extract powder resulted in the reduced-nitrite sausage samples with comparable sensory likability scores to the control sample (P>0.05). The lowest texture and overall likability scores were found for the reduced-nitrite sample with 4% Fang extract powder. It has been reported that Brazinlin in Fang heartwood possess various biological activities including antibacterial, anti-inflammatory, anti-allergic and antioxidant (Nirmal et al., 2015). Therefore, the reduced-nitrite sausage sample with the highest amount of Fang extract powder that was still accepted by the panelists should be chosen. Incorporation of 3% Fang extract powder, thus, has beneficial effects on the human health and on the sensory attributes of reduced-nitrite sausage samples.

As seen in **Table 2** and **Table 3** the textural and sensory properties of the reduced-nitrite sausage sample (75 ppm nitrite) containing 3% Fang extract powder and the control sample with 125 ppm nitrite were not significantly different (P>0.05). The reduced-nitrite sausage sample (75 ppm nitrite) containing 3% Fang extract powder was, thus, chosen for storage stability study (step 2).

Table 4 showed the chemical and microbiological changes of the control Vienna sausage containing 125 ppm nitrite and the reduced-nitrite sample (75 ppm nitrite) with 3% Fang extract powder during cold storage at 4±1°C for 30 days. As expected the residual nitrite content in both sausage samples sharply decreased with storage time (P≤0.05). The decomposition of residual nitrite during refrigerated storage may be due to the complicated reactions of nitrite (Dong et al., 2007). Many researchers reported the reduction of residual nitrite in meat products with storage time such as Chinese sausage (Lui et al., 2010), frankfurters with 12% tomato paste (Deda et al., 2007) and cooked sausage (Dong et al., 2007).

The TBA value of the sample with 125 ppm nitrite was significantly lower than that of the reduced-nitrite sample containing 3 % Fang extract powder at day 20 and 30 (P≤0.05) indicating that the lowered antioxidant ability caused by a decrease of nitrite cannot be compensated by the Fang extracts powder. Even after 30 days of storage, the TBA value of both samples remained lower than 1.0 mg malonaldehyde /kg, an acceptable range for oxidative rancidity (Verma and Sahoo, 2000).

The TBA value is a measure of oxidative rancidity of fat in foods. The secondary oxidation products react with 2-thiobarbituric acid to form condensation products with maximum absorption at 528-530 nm (Pokorny and Dieffenbacher, 1898). Normally, the secondary oxidation products increase with time resulting in the higher TBA value. It can be concluded that the longer the storage time, the higher the TBA value (Pokorny and Dieffenbacher, 1989). Many researchers reported that the TBA value of emulsion type sausages increased with storage time (Deda et al., 2007; Eyiler and Oztan, 2011; Wimontham and Rojanakorn, 2016).

The total plate counts and yeast and mold counts of both sausage samples were detected after 20 days storage at 4±1°C, after which counts significantly increased (P≤0.05). The sample containing 125 ppm nitrite had lower total plate counts and yeast and mold counts than the reduced-nitrite sample with 3% Fang extract powder. However, the total plate counts of both sausage samples throughout 30 days storage period were less than the permissible limit (5 log CFU/g), as specified by the Thai Industrial Standard (TIS) No. 2300-2549 (BTIS, 2006). It is interesting to note that Staphylococcus aureus, Salmonella spp. and Clostridium perfringens were not detected in both sausage samples throughout the storage period (data not shown).

The results of TBA values and number of microorganisms in both samples shown in Table 4 indicated that the lowered antimicrobial and antioxidant ability caused by a decrease of nitrite cannot be compensated by the Fang extract powder.

Conclusion

An increment of Fang extract powder in reduced-nitrite sausage samples resulted in a redder product with lower residual nitrite content. The reduced-nitrite sausage sample containing 3% Fang extract powder had sensory liking scores comparable to the control sample with 125 ppm nitrite. Based on the storage stability, Fang extract powder could not compensate a reduction of antimicrobial and antioxidant ability caused by a reduction of nitrite. However, reduced-nitrite sample (75 ppm nitrite) containing 3% Fang extract powder was considered safe throughout 30 days storage period. The residual nitrite content in reduced-nitrite sausage with 3% Fang extract powder and the control sample with125 ppm decreased with storage time. The addition of 3% Fang extract powder can, therefore, be used to lower the amount of nitrite added to Vienna sausage from 125 ppm to 75 ppm.

Acknowledgements

The authors would like to thank Khon Kaen University for financial support under "ทุนวิจัยสำหรับคณาจารย์บัณฑิตศึกษา เพื่อให้สามารถรับนักศึกษาที่มีความสามารถและศักยภาพสูงเข้าศึกษาในหลักสูตรและทำวิจัยในสาขาที่อาจารย์มีความเชี่ยวชาญ ประจำปีการศึกษา 2560 จากบัณฑิตวิทยาลัย มหาวิทยาลัยขอนแก่น" project.

References

Andres, S., Garcia, M., Zaritzky, N., and Califano, A. 2006. Storage stability of low-fat chicken sausage. J Food Eng. 72: 311-319.

AOAC. 2000. Official methods of analysis of AOAC international. Volume 2, 17th ed. AOAC international.

Barretto, A. C. D. S., Pacheco, M. T. B., and Pollonio, M. A. R. 2015. Effect of the addition of wheat fiber and partial pork back fat on the chemical composition, texture and sensory property of low-fat bologna sausage containing inulin and oat fiber. Food Science and Technology 35: 100-107.

Bazan-Lugo, E., Garcia-Martinez I., Alfaro-Rodriguez, R. H., and Totosaus, A. 2012. Color compensation in nitrite-reduced meat batters in corporating paprika or tomato paste. J Sci Food and Agri. 92: 1627-1632.

Bureau of Thai Industrial Standard (BTIS). 2006. Thai Industrial Standard: Vienna sausage. TIS No. 2300-2549.

Cassens, R. 1997. Composition and safety of cured meats in the USA. Food Chem. 59: 561-566.

Cavalheiro, C. P., Piovesan, N., Terra, L. M., Lovato, M., Terra, N. N., and Fries, L. L. M. 2013. Colorimetric and sensory characteristics of fermented cured sausage with Brazilian ostrich meat addition. Food Sci. Technol.

33: 660-665.

Deda, M. S., Bloukas, J. G., and Fista, G. A. 2007. Effect of tomato paste and nitrite level on processing and quality characteristics of frankfurters. Meat Sci. 76: 501-508.

Dong, Q. L., Tu, K., Guo, L.Y., Yang, J. L. Wang, H., and Chen, Y. Y. 2007. The effect of sodium nitrite on the textural properties of cooked sausage during cold storage. Journal of Texture Studies. 38: 537-554.

Eyiler, E., and Oztan, A. 2011. Production of frankfurters with tomato powder as a natural additive. LWT-Food Sci and Tech. 44: 307-311.

Feiner, G. 2006. Meat products hand book: practical science and technology. Woodhead Pub. Cambridge.

Felisberto, M. H. F., Galvao, M. T. E. L., Picone, C. S. F., Cunha, R. L., and Pollonio, M. A. R. 2015. Effect of prebiotic ingredients on the rheological properties and microstructure of reduced-sodium and low-fat meat emulsions. LWT-Food Sci and Tech. 60: 148-155.

Hayes, J. E., Canonico, I., and Allen, P. 2013. Effects of organic tomato pulp powder and nitrite level on the physicochemical, textural and sensory properties of pork luncheon roll. Meat Sci. 95: 756-762.

heiler, R. F., Sato, K., Aspelund, T. G., and Miller, A. F. 1981. Model system studies on N-nitrosamine formation in cured meat: The effect of curing solution ingredients. J Food Sci. 46: 996-999.

Jafari, M., and Eman-Djomeh, Z. 2007. Reducing nitrite content in hot dogs by hurdle technology. Food Control. 18: 1488-1493.

Liu, D. C., Wu, S. W., and Tan, F. J. 2010. Effect of addition of anka rice on the qualities of low-nitrite Chinese sausage. Food Chem. 118: 245-250.

Meilgaard, M., Civille, G.V., and Carr, B.T. 1991. Sensory Evaluation Techniques, 2nd Edition. CRC Press, Florida.

Nirmal, N. P., Rajput, M. S., Prasad, R. G.S.V., and Ahmad, M. 2015. Brazilin from Caesalpinia sappan heartwood and its pharmacological activities: A review. Asian Pacific Journal of Tropical Medicine. 8: 421-430.

Pokorny, J., and Dieffenbacher, A. 1989. Determination of 2-Thiobarbituric acid value: Direct method. Pure&Appl. Chem. 61: 1165-1170.

Rather, S. A., Masoodi, F. A., Akhter, R., Rather, J. A., and Amin, F. 2017. Effects of guar gum as a fat substitute in low fat meat emulsions. Journal of Food Processing and Preservation. 41: doi.org/10.1111/jfpp.13249

Riazi, F, Zeynali F, Hoseini E., and Behmadi, H. 2016. Effect of Dry Red Grape Pomace as a Nitrite Substitute on the Microbiological and Physicochemical Properties and Residual Nitrite of Dry-cured Sausage. Nutr Food Sci Res. 3: 37-44.

Rincón, F., Martínez, B., and Delgado, J. M. 2003. Detection of factors influencing nitrite determination in meat. Meat Sci. 65(4), 1421–1427http://doi.org/10.1016/S0309-1740(03)00065-2.

Sindelar, J. J., and Milkowski, A. L. 2011. Sodium nitrite in processed meat and poultry meats: A review of curing and examining the risk/benefit of its use. American Meat Science Association. 3: 1-14.

Sutaporn, D. and Rojanakorn, T. 2018. Foam-mat drying behavior of Sappan (Caesalpiniasappan Linn.) extract. p.96-107. in: Proceeding of the 6th national conference innovation and technology for quality of life and sustainable society 24 November 2018. Kasetsart University Chalermphrakiat, Sakon Nakhon.

Verma, S. P., and Sahoo, J. 2000. Improvement in the quality of ground chevon during refrigerated storage by tocopherol acetate preblending. Meat Sci. 62: 403-413.

Wimontham, T., and Rojanakorn, T. 2016. Effect of incorporation of Gac (Momordica cochinchinensis) aril powder on the qualities of reduced- nitrite Vienna sausage. IFRJ. 3:1048-1055.