Effect of incorporation of Gac *(Momordica cochinchinensis)* aril powder on the qualities of reduced-nitrite Vienna sausage

Wimontham, T. and *Rojanakorn, T.

Department of Food Technology, Faculty of Technology, Khon Kaen University, 40002, Khon Kaen Thailand

Abstract

This study was aimed to determine the effect of Gac aril powder on the quality and storage characteristics of reduced-nitrite Vienna sausage. Initially, Vienna sausage with 125 ppm $NaNO_2$ without Gac aril powder (control) and 75 ppm $NaNO_2$ with 0, 0.5, 1.0 and 1.5% (w/w) Gac aril powder were produced. With increasing Gac aril powder, redness (a^*), yellowness (b^*) and lycopene content of the reduced-nitrite samples increased while the lightness (L^*), pH, cooking loss and residual nitrite decreased ($p \leq 0.05$). All reduced-nitrite samples prepared with Gac aril powder were darker and redder than the control ($p \leq 0.05$). The Vienna sausage produced from a combination of 75 ppm $NaNO_2$ and 1.0% Gac aril powder had sensory likability scores comparable to the control sample with 125 ppm $NaNO_2$ ($p > 0.05$). The quality changes of the 1.0% Gac aril reduced-nitrite sausage and the control sample with 125 ppm $NaNO_2$ during storage at 5 ± 1^oC for 28 days resulted in residual nitrite of both samples being lowered with storage time, while their TBA values rose ($p \leq 0.05$). Based on microbial counts, both samples were considered safe.

Introduction

Vienna is one of the most popular emulsion type sausages in Thailand because of its unique color, flavor and taste. It is a comminuted and cured product prepared from one or more types of raw meat. Nitrite is widely used as a key curing agent in the cured meat product since it results in the characteristic pink color and flavor, inhibits growth and neurotoxin formation by *Clostridium botulinum,* and retards development of oxidation rancidity (Cassens, 1997). Despite all of its desired properties, the safety of nitrite to human health has been questioned (Morita *et al*., 1998). Nitrite can cause the formation of carcinogenic N-nitrosamines in cured meat products by reacting with secondary amines and amino acids in muscle proteins. In addition, residual nitrite in cured meat products may form N- nitrosamines in the gastro-intestinal tract (Cassens, 1997). Due to its potential health risk, studies on production of reduced-nitrite meat products have increased (Deda *et al.*, 2007; Eyiler and Oztan, 2011). Reduction of nitrite may, however, account for lowered consumer acceptability of cured meat products, especially their color after a long shelf life. Natural additives can proxy for nitrite and they are believed to be healthy and safe (Eyiler and Oztan, 2011). In addition, natural additives have consumer acceptance, antioxidant activity, acceptable sensory characteristics (i.e., color and flavor) and seem to improve or maintain the color of reduced-nitrite meat products (Bazan-Lugo *et al.*, 2012). For examples, Liu *et al*. (2010) reported that 25 ppm Chinese dried sausage with acceptable quality could be produced by adding up to 1.5% anka rice, previously inoculated with *Monascus purpureus*. Bazan-Lugo *et al.* (2012) demonstrated that meat batter containing 100 ppm nitrite and 2.0% paprika had greater redness than the control sample with 150 ppm nitrite.

Lycopene is one of the carotenoids found in many agricultural products including tomatoes, watermelon, red grapefruit and red pepper giving them their characteristic red color (Goula and Adamopoulos, 2005). It was reported that consumption of lycopene decreased the risk of cardiovascular disease and certain types of cancer (i.e., prostate and lung) (Goula and Adamopoulos, 2005; Østerlie and Lerfall, 2005). Owing to the health benefit of lycopene and its characteristic red color, tomato products including tomato paste, tomato powder have been used to produced reduced-nitrite emulsion-type sausage of relatively high quality and acceptability (Deda, 2007; Eyiler and Oztan, 2011;Bazan-Lugo *et al.*, 2012).

Gac (*Momordica cochinchinensis* Spreng), especially the aril part is another source of lycopene. Several researchers have reported that the amount of lycopene in Gac aril ranged between 0.380 mg/g and 3.053 mg/g (Aoki *et al*., 2002; Ishida *et al.*, 2004; Vuong *et al*., 2006). In Vietnam, the aril part of Gac

*Corresponding author.
Email: *thajoj@kku.ac.th*

is used as a colorant for cooking red glutinous rice (Aoki *et al*., 2002) and in Thailand this part of the fruit is cooked and eaten with chili paste or cooked in a curry (Kubola and Siriamornpun, 2011). Even if the Gac aril has been used in some food products, details on utilization of the fruit aril in production of reduced-nitrite meat products is very limited, so the objective of this study was to evaluate the effect of Gac aril powder on the factors of quality and storage stability of reduced-nitrite Vienna sausage.

Material and Methods

Chemical reagents and Culture media

Methycellulose (Methocel® MC with 27.5-32% methoxyl basis, SIGMA, USA) and food grade sodium nitrite were purchased from PKC Chemical Company, Thailand. The reagents for lycopene determination including tetrahydrofuran, acetonitrile and methanol are HPLC grade (Fisher Chemical, UK). Sulfanilamide, N-(1-naphthyl)-ethylenediamine dihydrochloride and sodium nitrite for residual nitrite determination are AR grade (Fluka, Germany). Thiobarbituric acid (AR grade) used for monitoring the degree of lipid oxidation is a product of Fuka company, Germany. Media for microbial enumeration including PCA (plate count agar), PDA (potato dextrose agar), Baired Parker agar, Tryptose sulfite cycloserine agar and *Clostridium* welchii egg yolk agar are products of Himedia company, India.

Preparation of Gac aril powder

Fresh and fully ripe, red colored Gac was obtained from the Faculty of Agriculture, Khon Kaen University. The fruits were washed with tap water, left to dry at ambient temperature ($30\pm1^{o}C$) then cut in half. The whole seed covering with aril was manually removed and the red aril manually separated: this was thoroughly mixed to obtain a uniform sample. Gac aril (14.7^{o}Brix) was mixed with 1.5% w/w methylcellulose. The mixture was then whipped to a foam in a Kitchen Aid mixer (Model ULM-400, USA) at 1400 rpm for 25 min. The stable foam was evenly spread on stainless steel plates (15.5×27 cm.) at a thickness of 1mm and dried at $70^{o}C$ for 60 min at a constant air velocity of 0.5 m/s (Auisakchaiyoung and Rojanakorn, 2015). The respective moisture content and water activity of the foam-mat dried sample was 6.59 % (db) and 0.28. The sample contained 690.16 µg/g lycopene and 83.43 µg/g β-carotene.

Experimental set-up

This study was divided into 2 experiments. In experiment A, Vienna sausage with 125 ppm sodium nitrite ($NaNO_2$), the maximum permitted level in meat products in Thailand was used as the control (Treatment 1). The other 4 reduced-nitrite (75 ppm $NaNO_2$) sausages with different amounts of Gac aril powder (0, 0.5, 1.0 and 1.5 % by weight of batter) were prepared for a comparison. After vacuum packing in plastic bags and storage at $5\pm1^{o}C$ for 2 days, all treatments were evaluated for their color, texture profile analysis (TPA), pH, lycopene content, nitrite residue and sensory scores. All treatments in experiment A, (~2 kg each) were replicated 3x from separate meat sources at 3 different times.

In experiment B, Vienna sausage with 125 ppm $NaNO_2$ and reduced-nitrite (75 ppm $NaNO_2$) sausage containing an appropriate amount of Gac aril powder selected from Exp A was produced, vacuum packed and stored at $5\pm1^{o}C$ for 28 days. During storage, the samples were randomly withdrawn to evaluate their pH, lycopene content, nitrite residue, TBA value, and microbial counts at day 7, 14, 21 and 28. All treatments in experiment B (~2 kg each) were replicated 3x from separate meat sources at 3 different times.

Production of Vienna sausage

Fresh, boneless pork legs and fresh pork back fat were purchased from a local meat market. The fresh pork was trimmed of separable fat to obtain the lean meat. The lean meat and partially frozen pork back fat were separately ground through a 12 mm and 2.5 mm plate respectively. The ground meat (1060.0 g) plus 1/3 of ice (140.0 g) was blended in a bowl chopper (cutter M11N, NMH Maschinen, Germany) at low speed for 1 min. Other ingredients were added in the order: NaCl (35.2 g), sodium tripolyphosphate (2.0 g), sodium ascorbate (1.4 g) and ice (140.0 g). The mixture was chopped at high speed for 2 min, then test $NaNO_2$ (125 and 75 ppm), test Gac aril powder (0, 0.5, 1.0 and 1.5% only for 75 ppm $NaNO_2$), spice and seasoning (10.0 g), starch (88.0 g) and ice (140.0 g) were added and chopped for an additional 2 min. Ground pork fat (390.0 g) and the remaining ice (140.0 g) were then incorporated to the meat mixture and further chopped for another 2 min at high speed. The mixture was chopped for a total of 7 min, and the final temperature of meat blend was ca. $15^{o}C$. Raw meat batter was then stuffed into a 14 mm diameter Nojak cellulose casing (Viscase SA Bagnold Cedex, France) using a stuffer (TWF-12, DICK, Germany), hand linked at 12 cm intervals and cooked in hot water ($80^{o}C$) until the core temperature reached $72^{o}C$. The cooked sausages were cooled in water ($4^{o}C$) then vacuum packed in PE/Polyester bags and chilled ($5+1^{o}C$).

Physical and chemical analyses of the sausage samples

The color of fresh cut cross-sections from all cooked sausage samples (9 per treatment) was measured using Minolta colorimeter (CR-300, Konica Minolta, Japan) calibrated with a white standard tile and the results were expressed as L^*(lightness), a^* (redness) and b^* (yellowness).

Texture profile analysis (TPA) of cooked sausages were determined using texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK) according to Tobin *et al*. (2012) with slight modifications. Nine cylindrical slices (each 14 mm diameter, 15 mm long) from each treatment were taken and subjected to a two-cycle compression test using the 25 kg load cell. The samples were compressed to 40% of their original height with a 35 mm diameter probe and a cross-head speed of 2.0 mm/s.

Cooking loss was determined by weight difference before and after cooking ten Vienna sausage samples from each formulation in hot water (80°C) until the core temperature reached 72°C as per Andres *et al*. (2006).

The pH of all cooked sausages was determined as per Deda *et al.* (2007) by blending 20 g of Vienna samples with 80 g distilled water for 30 s. The blended mixtures were read for their pH using pH meter (Mettler-Toledo, China).

Lycopene content of all sausage formulations was determined according to Eyiler and Oztan (2011) with some modifications. A 10 g sample was weighted and extracted with 30 ml of tetrahydrofuran (THF) and filtrated through Whatman filter paper (No:4). The volume of obtained filtrate was adjusted to 50 ml with THF and then kept frozen at -18°C until analyzed. The lycopene content was analyzed using an HPLC technique (Shimadzu LC-20A, Software CLASS-VP pumps, SPD-M20A diode array detector, Cosmosil C-18 (4.6x250 mm. i.d., 5 µm)). An isocratic mobile phase was composed of THF (solvent A)/ acetonitrile (solvent B)/ methanol (solvent C) 35:40:25 at flow rate of 1.0 ml/min. The absorbance was read at 540 nm.

Residual nitrite was analyzed by the ISO 2918-1975 reference method. After a reaction with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride, nitrite was measured spectrophotometrically at 538 nm. The nitrite concentration was calculated based on a standard curve obtained with sodium nitrite and expressed as mg/kg or ppm. Degree of lipid oxidation was monitored through the Thiobarbituric acid (TBA) value as per Deda *et al.* (2007) and expressed as mg malonaldehyde/ kg sample.

Sensory evaluation of the sausage samples

Sensory analyses were performed with 50 untrained panelists, who were the members of the faculty of Technology, Khon Kaen University aged between 20-25 years old. All panelists were asked to evaluate their preference on color, odor, taste, texture, and overall liking by using 9-point Hedonic scale (9 = like extremely, 1 = dislike extremely) test. Before testing, Vienna sausage samples were prepared by steeping in boiling water in individual pans for 2 min. Warm 2.5 cm long pieces from each formulation were randomly distributed for evaluation. Tap water and unsalted crackers were provided between samples to cleanse the palate as recommended by Deda *et al.* (2007).

Microbiological evaluation of the sausage samples

All the microbial parameters of Vienna sausage were determined as per the methods described by AOAC (2000). About 25 g samples were taken aseptically from each treatment, transferred to sterile plastic pouches 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 (Seaward, London). Sterile peptone water (0.1%) was used as a diluent for making further dilution. Duplicate plates were prepared for all microbial enumeration and the counts were expressed as $\log_{10}$ numbers of colony forming units per gram. Plate count agar (PCA; Himedia, India) and potato dextrose agar (PDA; Himedia, India) were used to enumerate total plate count and yeast and mould count, respectively, using the pour plate method. The plates were incubated at $37 \pm 1^{\circ}$C for 48 h and $25 \pm 1^{\circ}$C for 5 days for total microflora and yeast and mould counts, respectively. Baired Parker agar (Himedia, India) was used for enumeration of *Staphylococcus aureus* by incubating the plates at $37 \pm 1^{\circ}$C for 48 h. Tryptose sulfite cycloserine agar (TSC agar) and *Clostridium* welchii egg yolk agar (CWEY) (Himedia, India) were used to enumerate *Clostridium perfringens* counts and the plates were placed in an anaerobic jar (BBL, GasPak system, USA) before incubating at $35 \pm 1^{\circ}$C for 24 h.

Statistical analysis

The experiments were conducted in triplicate and results were given as means with standard deviations. Analysis of variance and Duncan's new multiple range test were performed to identify differences among the means using SPSS software version 19. Statistical significance was accepted at 95% probability.

Table 1. Effect of formulation on physical and chemical properties of Vienna sausage

Property	Treatments				
	NaNO$_2$ 125 ppm (control)	NaNO$_2$ 75 ppm +0.0% gac	NaNO$_2$ 75 ppm + 0.5% gac	NaNO$_2$ 75 ppm + 1.0% gac	NaNO$_2$ 75 ppm + 1.5% gac
L*	70.72±1.20[a]	71.94±0.41[a]	67.33±1.72[b]	66.54±0.15[b]	63.96±0.35[c]
a*	1.60±0.18[d]	1.37±0.06[d]	7.28±0.60[c]	10.22±0.45[b]	12.96±0.42[a]
b*	9.75±0.15[d]	9.46±0.13[d]	16.94±0.08[c]	21.57±0.83[b]	25.09±0.70[a]
Cooking loss (%)	2.35±0.19[a]	2.26±0.12[a]	2.15±0.17[ab]	2.00±0.14[b]	1.74±0.15[c]
pH	6.61±0.12[a]	6.57±0.09[a]	6.47±0.16[b]	6.40±0.08[bc]	6.33±0.10[c]
Residual nitrite (ppm)	37.83±0.14[a]	21.05±0.15[b]	20.66±0.05[b]	20.09±0.09[b]	19.80±0.05[b]
Lycopene (mg/g)	n.d	n.d	5.35±0.53[c]	10.34±0.84[b]	14.17±0.65[a]

*n.d.= Not detected
Means within the same row having different letters were significantly different (p ≤ 0.05).

Results and Discussion

Quality characteristics of Vienna sausage with different formulations (Experiment A)

Formulation significantly affected (p ≤ 0.05) the color parameters of the sausage (Table1). Addition of 0.5 up to 1.5% Gac aril powder in reduced-nitrite (75 ppm) sausage resulted in darker (p ≤ 0.05) samples than the control with 125 ppm NaNO$_2$ (p ≤ 0.05). In the same way, reduced-nitrite samples containing Gac aril powder exhibited higher redness (a*) and yellowness (b*) than the control (p ≤ 0.05). The highest value of a* and b* along with the lowest L* (lightness) (p ≤ 0.05) was found for the sample including 75 ppm NaNO$_2$ and 1.5 % Gac aril powder. Therefore, incorporation of Gac aril powder into reduced-nitrite sausage resulted in darker, redder and more yellow (p ≤ 0.05) samples as compared to the control. This is because carotenoids in Gac aril, like lycopene and β-carotene, are responsible of the yellowish red colour (Aoki *et al.*, 2002; Vuong *et al.*, 2006; Kubola and Siriamornpun, 2011). The results of the current study are in agreement with the work of Bazan-Lugo *et al*. (2012) who reported that as the concentration of paprika and tomato paste increased the lightness of nitrite-reduced meat batter decreased while the redness and yellowness increased. Deda *et al*. (2007) also reported that redness and yellowness of frankfurters increased with increasing tomato paste level. In addition the lightness of the product decreased as the level of tomato paste increased. Eyiler and Oztan (2011) demonstrated that an increase in the tomato power adding level resulted in the increased value of redness but decreased value of lightness.

Cooking loss and pH of reduced-nitrite sample without Gac aril powder were slightly lower (p> 0.05) than the control with 125 ppm nitrite, showing that lowering of nitrite level did not affect the cooking loss and pH values of the Vienna sausage (Table 1). As the amount of Gac aril powder in reduced-nitrite samples was increased from 0.5 % to 1.5%, the pH of the samples was significantly decreased (p ≤ 0.05) from 6.47 to 6.33. It may be related to the acidic characteristics of the Gac aril powder (pH: 4.95 -5.18).Eyiler and Oztan (2011) reported that addition of tomato powder (pH: 4.48-5.02) decreased the pH of the frankfurters. The cooking loss of the reduced-nitrite samples decreased (p ≤ 0.05) with increasing amount of Gac aril powder. The sample with 1.5% Gac aril powder showed the lowest (p ≤ 0.05) cooking loss value of 1.74%. It is possibly related to the water sorption property of the Gac aril powder.

As expected, an increment of Gac aril powder led to a greater amount of lycopene content in the reduced-nitrite sausage samples (p ≤ 0.05) (Table 1); indicating that the Gac aril powder used in this study was a good source of lycopene (690.16 µg/g of Gac aril power). Aoki *et al.* (2002), Vuong *et al.* (2006) and Kubola and Siriamornpun (2011) reported that Gac aril contains a large amount of lycopene. Our results agreed with those of Eyiler and Oztan (2011) who reported that as the level of tomato powder, a good source of lycopene was increased the level of lycopene was increased. The reduced-nitrite Vienna sausage with and without Gac aril powder had lower (p ≤ 0.05) residual nitrite content than the control produced with 125 ppm NaNO$_2$. The increase in amount of Gac aril powder in the reduced-nitrite samples from 0.0% to 1.5% resulted in a slight decrease (p > 0.05) of the residual nitrite from 6.57 ppm to 6.33 ppm (Table 1). The difference in residual nitrite is probably due to the lower pH of the samples with a higher amount of Gac aril powder. Deda *et al.* (2007) reported that the residual nitrite of frankfurters decreased with a decrease in their pH.

Table 2. Effect of formulation on textural parameters of Vienna sausage

Textural parameter	Treatments				
	NaNO$_2$ 125 ppm (control)	NaNO$_2$ 75 ppm +0.0% gac	NaNO$_2$ 75 ppm + 0.5% gac	NaNO$_2$ 75 ppm + 1.0% gac	NaNO$_2$ 75 ppm + 1.5% gac
Hardness (g$_f$)	2705.12±214.14[a]	2926.76±175.54[a]	2837.17±415.69[a]	2847.50±176.45[a]	2811.01±277.87[a]
Cohesiveness	0.61±0.03[a]	0.60±0.01[a]	0.61±0.003[a]	0.63±0.020[a]	0.62±0.010[a]
Springiness	1.00±0.07[a]	0.95±0.03[a]	0.93±0.04[a]	0.95±0.05[a]	0.95±0.01[a]
Chewiness(g$_f$)	4420.98±242.24[a]	4624.59±282.61[a]	4401.48±641.09[a]	4316.32±413.87[a]	4327.18±339.24[a]
Adhesiveness	-29.48±9.99[a]	-37.06±0.98[a]	-36.04±1.23[a]	-37.18±0.53[a]	-36.14±1.42[a]

Means within the same row having different letters were significantly different ($p \leq 0.05$).

Table 3. Effect of formulation on sensory likability scores of Vienna sausage

Treatment	Sensory likability score				
	Color	Odor	Taste	Texture	Overall liking
125 ppm NaNO$_2$	5.05±1.48[b]	6.11±1.36[a]	6.08±1.31[a]	6.51±1.29[a]	6.20±1.18[b]
75 ppm NaNO2+0.0% gac	5.05±1.47[b]	6.05±1.23[a]	6.11±1.34[a]	6.40±1.39[a]	6.11±1.13[b]
75 ppm NaNO2+0.5% gac	6.60±1.37[a]	6.20±1.38[a]	6.60±1.11[a]	6.57±1.21[a]	6.64±1.03[a]
75 ppm NaNO2+1.0% gac	6.80±1.37[a]	6.11±1.49[a]	6.51±1.35[a]	6.57±1.16[a]	6.77±1.64[a]
75 ppm NaNO2+1.5% gac	7.14±1.82[a]	5.54±1.55[b]	6.25±1.50[a]	6.60±1.39[a]	6.25±1.52[b]

Means within the same column having different letters were significantly different ($p \leq 0.05$).

Liu *et al*. (2010) observed that low-nitrite Chinese sausages with a lower pH also had a lower residual nitrite than the samples with a higher pH. Theiler *et al*. (1981) likewise reported that the faster rate of nitrite decomposition occurs at lower pH values.

Even if the textural parameters of Vienna sausage samples (hardness, cohesiveness, springiness, chewiness and adhesiveness) were not significantly affected ($p > 0.05$) by sausage formulations (Table 2), a decrease in nitrite content from 125 ppm to 75 ppm resulted in an increase ($p > 0.05$) in hardness and adhesiveness of the sausages. Dong *et al*. (2007) reported that nitrite content in cooked sausage had negative correlation with hardness and adhesiveness. They explained that the complex reactions with respect to nitrite might contribute to the textural variation of meat products.

The sensory panels were asked to evaluate the effect of sausage formulations on color, odor, taste, texture and overall likability (Table 3). Almost all sensory attributes except texture were significant ($p \leq 0.05$) by sausage formulation. Color likability scores of the control (125 ppm NaNO$_2$) sample and the reduced-nitrite (75 ppm NaNO$_2$) sample without Gac aril powder were not significantly different (p

> 0.05); indicating that 75 ppm NaNO$_2$ is sufficient for an acceptable color in Vienna sausage. DuBose *et al*. (1981) reported that no significant ($p > 0.05$) differences existed for color likability scores among cured ham containing 25, 75 and 125 ppm NaNO$_2$. According to the review by Sindelar and Milkowski (2011), a minimum level between 25 and 50 ppm NaNO$_2$ was enough for acceptable cured meat color in most meat and poultry products; however, higher levels would be necessary to achieve and maintain acceptable cured meat color, during a long shelf-life. Color likability scores of reduced-nitrite samples with 0.5, 1.0 and 1.5% Gac aril powder were not significantly different ($p > 0.05$); however, they were higher than that of the control, suggesting that color likability scores were improved by the addition of Gac aril powder. The reduced-nitrite sausage with 1.5% Gac aril powder showed the lowest ($p < 0.05$) odor and taste likability scores (5.54 and 5.25, respectively); probably due to the unacceptable flavor caused by an excessive amount of Gac aril powder. The overall likability scores of reduced-nitrite samples containing 0.5 and 1.0% Gac aril powder were significantly higher ($p \leq 0.05$) than that of the control. Although no statistically significant difference was detected,

Table 4. Nitrite residue, lycopene content and TBA value of Vienna sausage during storage at
5+1°C for 28 days

Treatment	Storage time (day)	Nitrite residue (ppm)	Lycopene (mg/g)	TBA (mg/kg)
125 ppmNaNO₂	0	37.88±1.21[a]	n.d	n.d
	7	34.01±1.05[b]	n.d	n.d
	14	23.98±0.87[c]	n.d	0.02±0.01[e]
	21	17.50±0.46[d]	n.d	0.29±0.02[c]
	28	17.11±0.74[d]	n.d	0.33±0.02[b]
75 ppmNaNO₂ +1.0% gac	0	20.17±1.05[c]	10.50±0.42[a]	n.d
	7	17.08±0.62[d]	10.12±0.32[b]	0.06±0.01[d]
	14	13.01±0.30[e]	9.88±0.66[c]	0.09±0.01[d]
	21	6.98±0.34[f]	8.53±0.22[d]	0.33±0.01[b]
	28	6.84±0.24[f]	8.01±0.13[e]	0.38±0.0[a]

*n.d.= Not detected
Means within the same column having different letters were significantly different ($p \leq 0.05$).

the sample containing 1.0% Gac aril powder showed higher overall likability than the sample with 0.5% Gac aril powder. Incorporation of up to 1.0% Gac aril powder, thus, has beneficial effects on the sensory attributes of reduced-nitrite sausages. Based on the results of the sensory evaluation, a reduced-nitrite sausage sample (75 ppm NaNO₂) containing 1.0% Gac aril powder was selected for experiment B.

Quality changes of Gac aril reduced-nitrite sausage during storage (Experiment B)

In the current experiment, the control sample with 125 ppm NaNO₂ and the sample containing 75 ppm NaNO₂ and 1.0% Gac aril powder were produced and compared for their quality changes during storage at 5±1°C for 28 days under vacuum packing.

In the control, with a higher nitrite level, nitrite content rapidly decreased ($p \leq 0.05$) from 37.88 ppm at day 0 to 23.98 ppm at day 14, and 15.11 ppm at day 28 (Table 4). In the 1.0% Gac aril reduced- nitrite sample, the nitrite content dropped ($p \leq 0.05$) from 20.17 ppm at day 0 to 13.01 ppm at day 14 then remained constant after 21 days storage (Table 4). The reduction of nitrite content during low- temperature storage may be a consequence of complex nitrite reactions (Dong *et al*., 2007). The results of the current study agreed with Liu *et al*. (2010) who reported that the residual nitrite in Chinese sausage with 100 ppm NaNO₂ drastically declined from 36.2 ppm (at day 0) to 21.0 ppm and 11.7 ppm after storage under vacuum packing at 4°C for 14 and 56 days, whereas the nitrite contents of low-nitrite sausage (with 25 ppm NaNO₂) were relatively constant throughout storage. Dong *et al*. (2007) reported a decrease in nitrite content of cooked sausage containing 150, 100 and 50 ppm NaNO₂ after 0-21 days at 0-4°C. They also observed a gradual decrease in residual nitrite in the 50 ppm NaNO₂ sausage. Deda *et al*. (2007) similarly reported

that nitrite content of the control frankfurter (150 ppm NaNO₂ without tomato paste) and the reduced-nitrite (100 and 50 ppm NaNO₂) frankfurters with 12% tomato paste decreased with increasing storage time. Perez-Rodriquez *et al*. (1996) found that the nitrite content at different concentrations (75, 125 or 250 ppm) in frankfurters rapidly declined until it reached a fairly low level after 18 days of cold storage. Sen and Baddo (1997) reported that the residual level of nitrite in 35 samples of meat products in Canada ranged from 4 to 68 ppm.

As expected, the lycopene content of reduced-nitrite sausages with 1.0% Gac aril powder significantly decreased ($p \leq 0.05$) with storage time (Table 4) This might be due to the oxidation of lycopene, which is the main cause of lycopene degradation during storage. Famurewa *et al*. (2013) reported that the lycopene content of tomato paste packed in a plastic bottle and stored at ambient temperature decreased with increasing storage time. Likewise, Markovic *et al*. (2007) reported a significant reduction of lycopene in tomato purée with storage time, at 5, 15 and 25°C.

The 1.0% Gac aril reduced-nitrite sample had a higher TBA value ($p \leq 0.05$) than the control (with 125 ppm NaNO₂) throughout the storage period (Table 4). The TBA value of the Gac aril reduced-nitrite sausage increased from a respective 0.06 to.09, 0.33 and 0.38 mg malonaldehyde/kg at day 7, 14, 21 and 28. In the control, this value increased from a respective 0.02 to 0.29 and 0.33 mg malonaldehyde / kg at day 14, 21 and 28. Even after 28 days of storage, the TBA value of both samples remained ≤ 1.0 mg malonaldehyde /kg, an acceptable range for oxidative rancidity (Verma and Sahoo, 2000). Melton (1983) reported the TBA values for the detectable oxidized flavors of beef and pork (0.3-1.0 mg malonaldehyde /kg), chicken (1.0-2.0 mg malonaldehyde /kg) and

Table 5. Microbial counts (log CFU/g) of Vienna sausage during storage at 5+1°C for 28 days

Treatment	Storage time (day)	Total plate count	Yeast and Mold	*S. aureus*	*C.perfringens*
125 ppmNaNO₂	0	N.d.	N.d.	N.d.	N.d.
	7	N.d.	N.d.	N.d.	N.d.
	14	N.d.	2.79±0.14ᵈ	N.d.	N.d.
	21	3.77±0.04ᵈ	4.57±0.20ᵇ	4.55±0.06ᶜ	N.d.
	28	4.15±0.15ᶜ	5.11±0.11ᵃ	4.94±0.16ᵇ	N.d.
75 ppmNaNO₂ +1.0% gac	0	N.d.	N.d.	N.d.	N.d.
	7	N.d.	N.d.	N.d.	N.d.
	14	N.d.	3.31±0.31ᶜ	N.d.	N.d.
	21	4.61±0.03ᵇ	4.59±0.11ᵇ	4.55±0.15ᶜ	N.d.
	28	4.94±0.04ᵃ	5.32±0.39ᵃ	5.15±0.16ᵃ	N.d.

*n.d.= Not detected
Means within the same column having different letters were significantly different ($p \leq 0.05$).

turkey (>3.0 mg malonaldehyde/kg). Al-Shuibi and Al-Abdullah (2000) recommended that these values should not be considered reference values for the thresholds of rancid odor in meat, since the TBA values are influenced by many factors. These results indicate that even if the lycopene present in Gac aril powder exerts an antioxidant activity, the incorporation of 1.0% Gac aril powder cannot compensate for the reduced antioxidant activity caused by the reduction of sodium nitrite from 125 ppm to 75 ppm. The results of the current study agree with Deda *et al.* (2007) who reported that the antioxidant activity of 12% tomato paste added to frankfurters did not compensate for the reduction of sodium nitrite from 150 ppm to 100 ppm and 50 ppm.

The total plate counts of both samples were detected at day 21, after which counts significantly increased ($p \leq 0.05$) with storage time (Table 5). The total plate counts of the Gac aril reduced-nitrite sausage during storage were between 4.61 and 4.94 log CFU/g, which was significantly higher ($p \leq 0.05$) than the control sample with 125 ppm NaNO2 (viz., 3.77- 4.15 log CFU/g); notwithstanding, the total plate counts were within permissible limits (5 log CFU/g), as specified by the Thai Industrial Standard (TIS) No. 2300-2549 (BTIS, 2006). The reduced-nitrite sample containing 1.0% Gac aril powder had a higher ($p \leq 0.05$) number of *S. aureus* than the control, but only at the end of storage (28 days). The number of *S. aureus* of Gac aril reduced-nitrite sausage during storage was between 4.55 and 5.15 log CFU/g, whereas the control was between 4.55 and 4.94 log CFU/g (Table 5). As expected, *C. perfingens* in both samples was not detected throughout storage. Yeast and mold counts were detected at day 14 in both samples: the respective number of yeast and mold in the reduced-nitrite sausage containing Gac aril powder was slightly higher ($p > 0.05$) than in the control throughout the storage period (Table 5). The

results of the current study indicate that the addition of Gac aril powder did not reduce the microorganisms in reduced-nitrite Vienna sausages while a higher level of nitrite (125 ppm) inhibited more microorganisms seen as a lower count. Notwithstanding, 75 ppm NaNO₂ was sufficient for controlling the number of microorganisms in Vienna sausages, making them safe for consumption. Sodium nitrite added to Vienna sausage can, thus, be reduced from 125 ppm to 75 ppm with the incorporation of 1.0 % Gac aril powder without any detrimental effect on the quality of the product.

Conclusion

Increments of Gac aril power were added to reduced-nitrite Vienna sausage; resulting in redder and darker sausage with higher lycopene content and lower cooking loss. The Vienna sausage produced from a combination of 75 ppm NaNO₂ and 1.0% Gac aril powder exhibited sensory scores comparable to the control sample with 125 ppm NaNO₂. The residual nitrite in 1.0% Gac aril reduced-nitrite sausage and the control sample with 125 ppm NaNO₂ decreased with storage time, while their TBA values increased with time. Based on microbial counts, the reduced-nitrite sausage containing 1.0% Gac aril powder was considered safe. The incorporation of 1.0% Gac aril powder can, therefore, be used to reduce the amount of nitrite added to Vienna sausage from 125 ppm to 75 ppm.

References

Al-Shuibi, A. and Al-Abdullah, B. 2002. Substitution of nitrite by sorbate and the effect on properties of mortadella. Meast Science 62: 473-478.

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

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

Aoki, H., Kieu, M. T. N., Kuze, N., Tomisaka, K. and Chuyen, V. N. 2002. Carotenoid pigments in gac fruit (*Momordica cochinchinenensis* Spreng). Bioscience, Biotechnology, and Biochemistry 66(11): 2479–2482.

Auisakchaiyoung, T. and Rojanakorn, T. 2015. Effect of foam-mat drying conditions on quality of dried Gac fruit *(Momordica cochinchinenensis)* aril. International Food Research Journal 22(5): 2025-2031.

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. Journal of Science Food and Agriculture 92: 1627-1632.

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

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

Deda, M. S., Bloukas, J. G., Fista, G. A. 2007. Effect of tomato paste and nitrite level on processing and quality characteristics of frankfurters. Meat Science 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.

DuBose, C. N., Cardello, A. V. and Maller, O. 1981. Factor affecting the acceptability of low-nitrite smoked, cured ham. Journal of Food Science 46: 461-463.

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

Famurewa, J. A. V.,Ibidapo, P. O. and Olaifa, Y. 2013. Storage stability of tomato paste packaged in plastic bottle and polythene stored in ambient temperature. International Journal of Applied Science and Technology 3(6): 34-42.

Goula, M. A. and Adamopoulos, G. K. 2005. Stability of lycopene during spray drying of tomato pulp. LWT-Food Science and Technology 38: 479-487.

Ishida, B. K., Turner, C., Chapman, M. H. and McKeon, T. A. 2004. Fatty acid and carotenoid composition of Gac (*Momordica cochinchinensis* Spreng) fruit. Journal of Agricultural and Food Chemistry 52: 274-279.

ISO 2918. 1975. Internal standards meat and meat products-determination of nitrite content. Geneva: International Organization for Standardization.

Kubola, J. and Siriamornpun, S. 2011. Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (Momordica cochinchinensis Spreng). Food Chemistry 127: 1138–1145.

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 Chemistry 118: 245-250.

Markovic, K., Hruskar, M. and Vahcic, N. 2007. Stability of lycopene in tomato purée during storage. Acta Alimentaria 36(1): 89-98.

Melton, S. L. 1983. Methodology for following lipid oxidation in muscle foods. Food Technology 38(7): 105-111.

Morita, H., Sakata, R. and Nakata Y. 1998. Nitric oxide complex of Iron (ii) myoglobin converted from metmyoglobin by Staphylococcus xylotus. Journal of Food Science 63: 352-355.

Østerlie, M. and Lerfall, J. 2005. Lycopene from tomato products added minced meat: effect on storage quality and colour. Food Research International 38: 925-929.

Perez-Rodriguez, M. L., Bosch-Bosch, N. and Garcia-Mata, M. 1996. Monitoring nitrite and nitrite residues in frankfurters during processing and storage. Meat Science 44: 65-73.

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.

Theiler, 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. Journal of Food Science 46: 996-999.

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

Vuong, L.T., Adrian, A. F., Laurie, J. C. and Suzanne, P. M. 2006. *Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated. Journal of Food Composition and Analysis 19: 664-668.