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Effects of natural colorants on physicochemical properties and sensory acceptance of Nham

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Abstract Nham is a traditional fermented meat product of Thailand made from ground pork. In the production process, chemicals such as nitrate or nitrite compound are commonly used as the ingredients to maintain its pink color. However, if these chemicals are used in improper amounts, they may be harmful to the customer's health. In this study, the effects of three natural colorants, rosella flower powder, tomato powder, and safflower powder at three levels (0.2, 0.4 and 0.6% w/w) were reported by comparing with the control formula and the formulas containing sodium nitrite. The results showed that, pH values decreased and total acidity represented by lactic acid content increased continuously as the fermentation time increased. An increase in natural colorant content resulted in decrease in lightness (L*) and increase in redness (a*) and yellowness (b*). All three types of natural colorants at all levels affected the texture characteristics and sensory acceptance ($p<0.05$), but had no effect on proximate composition of Nham ($p\geq0.05$). All types of natural colorant at all levels resulted in higher hardness, adhesiveness, cohesiveness, springiness and chewiness compare to the control formula. Nham samples with 0.4% and 0.6% tomato powder showed no difference in sensory scores for color, flavor, taste, and firmness compared to the control formula ($p\geq0.05$). Nham sample with 0.4% tomato powder showed the highest sensory scores for all attributes. Thus, 0.4% w/w tomato power was shown to be the optimum natural colorant as an alternative to nitrate and nitrite in Nham products.

Keywords: Nham, Natural colorant, Rosella flower powder, Tomato powder, Safflower powder

Introduction

Nham is a traditional fermented meat product of Thailand made from ground pork. It has a sour taste from lactic acid produced from the fermentation process by natural lactic acid bacteria. The ingredients which are pork loin, shredded pork skin, salt, cooked rice, ground garlic and sugar are mixed, wrapped and bundled with a banana leaf or packed in tightly closed container to ensure a minimum airflow. In general, the fermentation process of Nham takes

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about 3-5 days at room temperature. The well-fermented Nham is reddish-pink and has a pH of 4.45-4.55. Color is often used by consumers as an indicator to determine the duration of fermentation and quality deterioration. The pink or reddish-pink color in Nham is the result of the oxidization of myoglobin in pork to methioglobin (Valyasevi *et al*., 1999; Kwanmuang, 2011).

In the production process, chemicals such as nitrate or nitrite are commonly used as the ingredients to maintain its pink color and to achieve unique taste and better flavor compared to the meat products cured by adding only sal (Shahidi and Pegg, 1991). There are 4 types of nitrate and nitrite compounds commonly used in fermentation: potassium nitrate (KNO_3), sodium nitrate ($NaNO_3$), potassium nitrite (KNO_2), and sodium nitrite ($NaNO_2$). In addition to improving color quality of Nham products, nitrate and nitrite compounds inhibit the growth of *Clostridium botulinum*, a pathogen that thrives on food in sealed, airtight containers and inhibit oxidative rancidity, a cause rancidity in meat products (Cassens, 1997; Pourazrang *et al*., 2002).

Thai Industrial Standards, Naem (fermented ground pork) TIS. No.1219. (2004), has set the permissible amount of sodium nitrate or potassium nitrate in Nham products at not exceeding 500 milligrams per kilogram of raw materials and sodium nitrite or potassium nitrite at not exceeding 125 milligrams per kilogram of raw materials. In case of a combination of sodium or potassium nitrate and sodium nitrite or potassium nitrite, the total content shall not exceed 125 milligrams per kilogram of raw materials. These chemicals are only allowed in sausages, Nham and Chinese sausage. If nitrates and nitrites are added to Nham in excessive quantity, the remaining substances in Nham will break down into free radicals and react with an amine to form nitrosamine, a carcinogen that isharmful to human (Kramlich *et al*., 1973; Tirasarot and Thanomwong, 2014).

Nowadays, consumers are increasingly focusing on health care. This is reflected by the selection of natural foods or with more natural, non-synthetic ingredients or minimal use of chemicals in food processing. Natural colorants derived from vegetables, fruits or microorganisms in food products are an alternative to synthetic colors for health-conscious consumers. A number of studies have focused on the use of natural colorants instead of synthetic one.

For example, Sanoppa *et al*. (2021) studied the use of Monascus colorant derived from fermentation of banana with *Monascus purpureus* as a substitute for nitrite in Nham products, a study by Tirasarot and Thanomwong (2014) studied the used of colorant powder derived from fermentation of broken-milled rice with *M. purpureus* to replace nitrite in Nham products. a study by Rojsuntornkitti *et al*. (2010) studied the use of red pigment from pigment from *Monascus* spp. isolated from ankak to replace nitrite in Chinese sausage and

smoked sausage. In addition, there have been the studies on the addition of tomato powder in dry fermented sausage (Calvo *et al*., 2008), the use of tomato poweder and ankak powder in in Chinese sausage (Suksripaisal and Theprugsa, 2017) and the effects of tomato powder in Chinese sausage (Saksomboon *et al*., 2020).

According to various studies, natural colorants are increasingly used in food products, especially in processed meats. Therefore, this study aimed to investigate the effects of three natural colorants (rosella flower powder, tomato powder and safflower powder) at three levels of 0.2, 0.4, 0.6 % w/w on the quality of Nham products. It is to obtain natural colorants that are suitable to be used instead of synthetic chemicals in Nham products to provide safe products and reduce the use of chemicals that can be harmful to consumers, if excessive amounts of these chemicals are used.

Materials and methods

Preparation of tomato powder, rosella flower powder and safflower powder

The tomato powder used in this study was prepared from whole red tomatoes from the local market, Pathumthani, Thailand. The tomatoes were washed, and tomato peel and flesh were ground using a masticating juicer to separate the juice from the pulp. The tomato pulp was then spread on an aluminum tray and drying was done using a tray drier at 75 ℃ for 15 hours. After that, dried tomato pulp was ground into fine powder by using a dry grinding machine at a moderate speed for 15 minutes. The fine tomato powder was seperated using a 60-mesh sieve. For safflower powder and rosella flower powder, they were ground for 15 minutes using a dry grinding machine. Tomato powder was then packed and stored in a sealed container. Tomato powder, rosella flower powder and safflower powder are shown in Figure 1.

1A:tomato powder 1B: rosella flower powder 1C:safflower powder

Figure 1. Natural colorants derived from tomato, rosella flower and safflower powder

Preparation of Pork and pork skin

Pork loin and pork skin

Pork loin and pork skin used in this study were purchased from Makro Deapartment Store, Pathum Thani province. Pork loin was cleaned. Fat, fascia, and tendons were removed and trimmed. Pork loin was sliced into 3 x 3 x 3 cm pieces, then placed in a plastic bag, and kept at -18 ℃ until further process. Excess fat was removed from pork skin. The pork skin was then boiled for 10 minutes, cut into small pieces, placed in a plastic bag and kept in a cold room (5 ℃).

Rice

Rice (Leung-Pra-Tiew) was rinsed two times and water was added to the rice about 1 inch above the top of the rice. Rice was then cooked in a rice cooker. Other ingredients were salt (Thai Refined Salt Company) and garlic purchased from Si Mum Mueang Market Pathum Thani province which was peeled and ground finely using an electric grinder.

Preparation of Nham

Ingredients for Nham include 700 grams of finely minced pork, 300 grams of pork skin, 40 grams of garlic, 30 grams of cooked white rice, and 20 grams of salt. All ingredients were thoroughly mixed and then put about 50 grams of mixed ingredients in a plastic bag. The plastic bag containing Nham was tied tightly by expelling all the air inside the bag to keep it in an anaerobic state and then kept at 30 ℃ for 3-4 days.

Effects of type and quantity of natural colorants in Nham products

Eleven treatments (formulas) of Nham products were prepared using three different types of natural colorants (flower powder, tomato powder, and safflower powder) at three levels (0.2, 0.4, and 0.6% w/w) The results were compared with the control formula (without any natural colorants and sodium nitrite) and the formulas containing 0.0875 grams of sodium nitrite. According to Thai Industrial Standard for Nham (fermented ground pork), TIS. 1219-2547, the amount of sodium nitrite used in Nham shall not exceed 125 milligrams per kilogram of raw material. Nham samples are shown in Figure 1.

Physicochemical properties of Nham products

pH

The pH was measured by using a pH meter (PB-20 Sartorius, Germany) every 12 hours, until pH value of the sample ranged between 4.4-4.5.

Total acidity

Total acidity was analyzed by determining the lactic acid content (AOAC, 2000) every 12 hours.

Color

Lightness (*L**), redness (*a**) value and yellowness (*b**) values of Nham products were measured using a colorimeter (Hunter Lab model Colorflex45/0, USA).

Texture analysis

Texture analysis was performed by using a texture analyzer (Stable Micro System, TA-XT Plus, UK) and a cylinder probe with a diameter of 50 mm at the pre-test speed of 0.8 mm/s, test speed of 0.8 mm/s, and fixed post-test speed of 8.0 mm/s. The distance was 40%. The parameters measured in the texture analysis were hardness, adhesiveness, springiness, cohesiveness, and chewiness (Suksripaisal and Theprugsa, 2017).

Proximate composition: Proximate composition analysis was performed in triplicate for moisture, ash, crude protein, crude fat, crude fiber and carbohydrate contents using the AOAC standard method (2000).

Sensory acceptance evaluation

Sensory acceptance evaluation of eleven formulas of Nham was performed using the 9-point hedonic scale with 50 untrained panelists. Five formulas were tested with a 3-minute break before other 6 formulas were tested. The attributes in this test included color, flavor, taste, firmness (cohesiveness), and overall liking.

Statistical analysis

Statistical analysis was performed using a computer software. The physico chemical properties were analyzed using the completely randomized design (CRD). Sensory data was analyzed using the random complete block design (RCBD). Analysis of variance (ANOVA) and difference in means between treatments were analyzed using Duncan's new multiple range test at 95% confidence interval.

2A: Control

2B: Sodium nitrite

2C : rosella flower powder 0.2%

2D: rosella flower powder 0.4%

2E: rosella flower powder 0.6%

2F: tomato powder 0.2%

2G: tomato powder 0.4%

2H:tomato powder 0.6%

2I: safflower powder 0.2%

2J: safflower powder 0.4%

2K:safflower powder 0.6%

Figure 1. Nham samples containing different types and levels of natural colorants

Results

Physicochemical properties of Nham products

The pH value and total acidity of Nham products

In this study, the pH value and total acidity were represented by lactic acid content of Nham samples which produced by using three different types of natural colorants at three different levels were compared with the control formula without any natural colorants as shown in Figure 2-3. The pH value of each treatment decreased continuously when the duration of fermentation increased. During day 1 or 24 hours, the pH values of all treatments showed no significant differences. Nham samples were the samples containing rosella flower powder, tomato powder and safflower powere at 0.2%, 0.4%, and 0.6%, control formula and the formulas containing sodium nitrite.

After 48 hours of fermentation, pH values of all samples decreased sharply, especially Nham samples are contained the natural colorants derived from tomato powder and rosella flower powder. Comparison of pH value with total acidity revealed that lactic acid content increased as the pH value decreased. Higher level of natural colorants derived from tomato powder and rosella flower powder resulted in increased acid content. The production of acid in Nham is continuously done until content of acid safe for consumption was obtained, corresponding to a pH value of less than 4.5, within the determined duration. This indicated that all three types of natural colorants did not affect the fermentation.

Color analysis

The lightness of all eleven Nham samples increased with the increased fermentation time (Figure 5). At the beginning of the fermentation, the control formula and the formulas containing the addition of sodium nitrite showed higher lightness (L*) compared to redness (a*) and yellowness (b*), respectively. As the fermentation time increased, redness and lightness (L*) significantly increased, while yellowness (b*) decreased. The addition of tomato powder, rosella flower powder, and safflower powder at 0.2%, 0.4%, and 0.6% by weight increased redness (a*) and yellowness (b*) of Nham sample, while lightness (L*) was not different from the control formula. The levels of all three types of natural colorants in Nham found to increase in the levels of all natural colorants resulted in lower lightness (L*) and higher redness (a*) and yellowness (b*). Meanwhile, an increase in the duration of the fermentation resulted in increased redness (a*) and yellowness (b*).

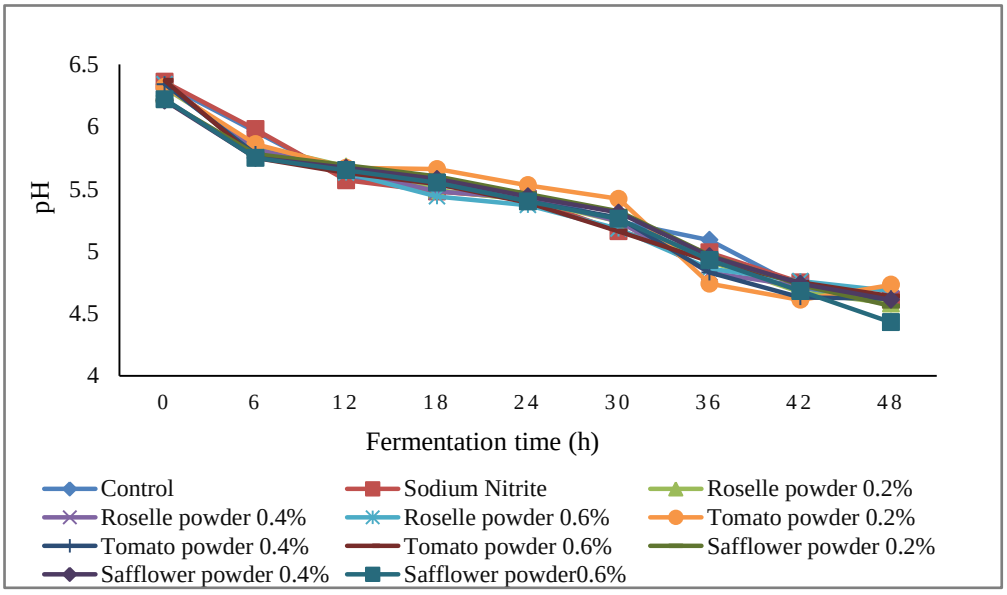


Figure 2. The pH values of Nham samples containing different types and levels of natural colorants

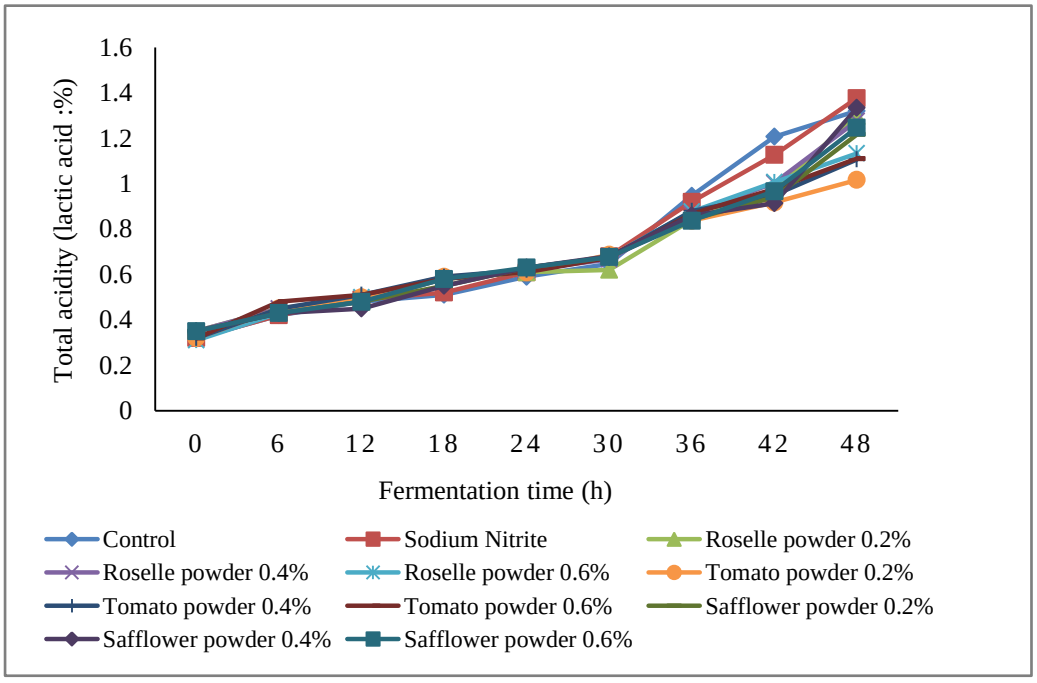
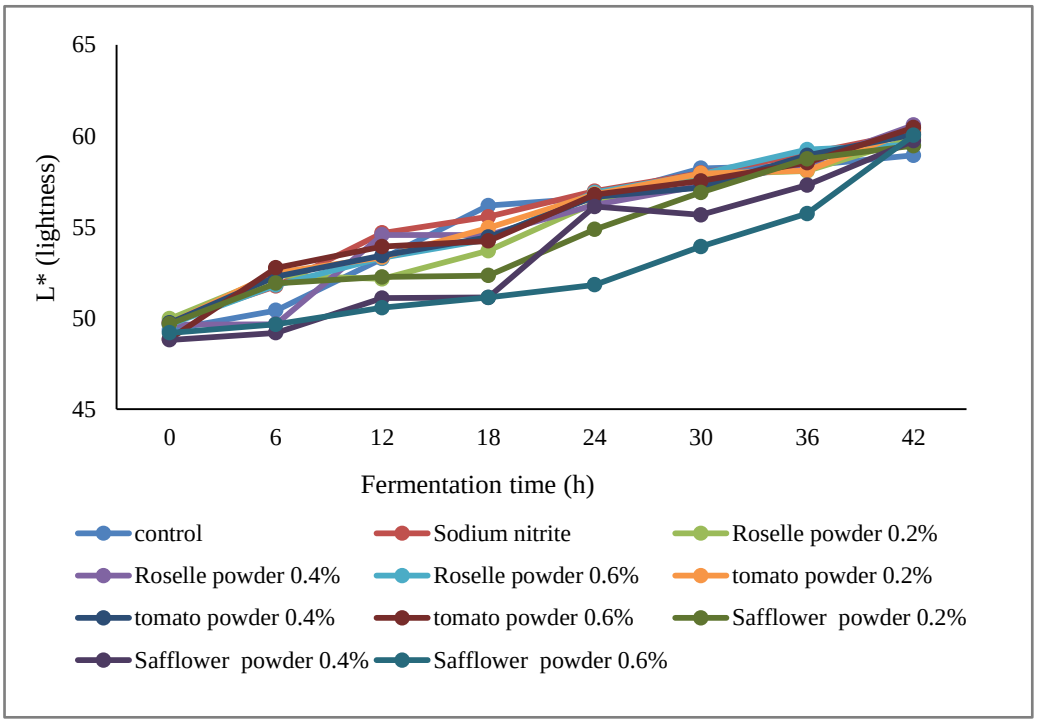
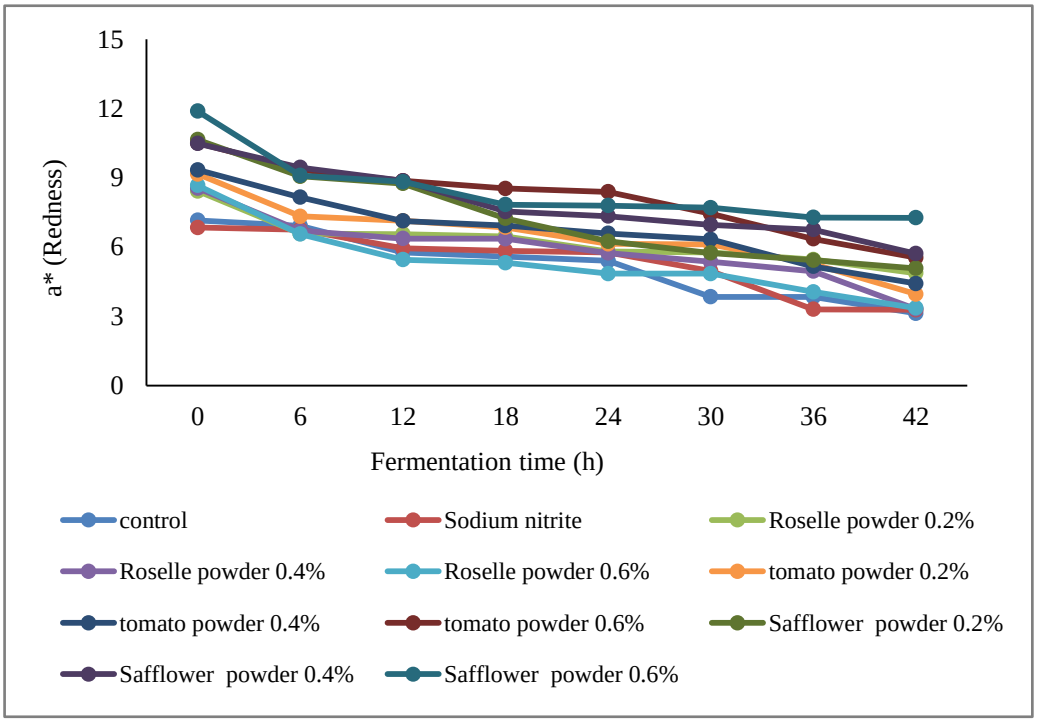


Figure 3. Total acidity of Nham samples containing different types and levels of natural colorants


65
60
55
50
45
L* (lightness)
0
6
12
18
24
30
36
42
Fermentation time (h)
control
Sodium nitrite
Roselle powder 0.2%
Roselle powder 0.4%
Roselle powder 0.6%
tomato powder 0.2%
tomato powder 0.4%
tomato powder 0.6%
Safflower powder 0.2%
Safflower powder 0.4%
Safflower powder 0.6%


15
12
9
6
3
0
a* (Redness)
0
6
12
18
24
30
36
42
Fermentation time (h)
control
Sodium nitrite
Roselle powder 0.2%
Roselle powder 0.4%
Roselle powder 0.6%
tomato powder 0.2%
tomato powder 0.4%
tomato powder 0.6%
Safflower powder 0.2%
Safflower powder 0.4%
Safflower powder 0.6%

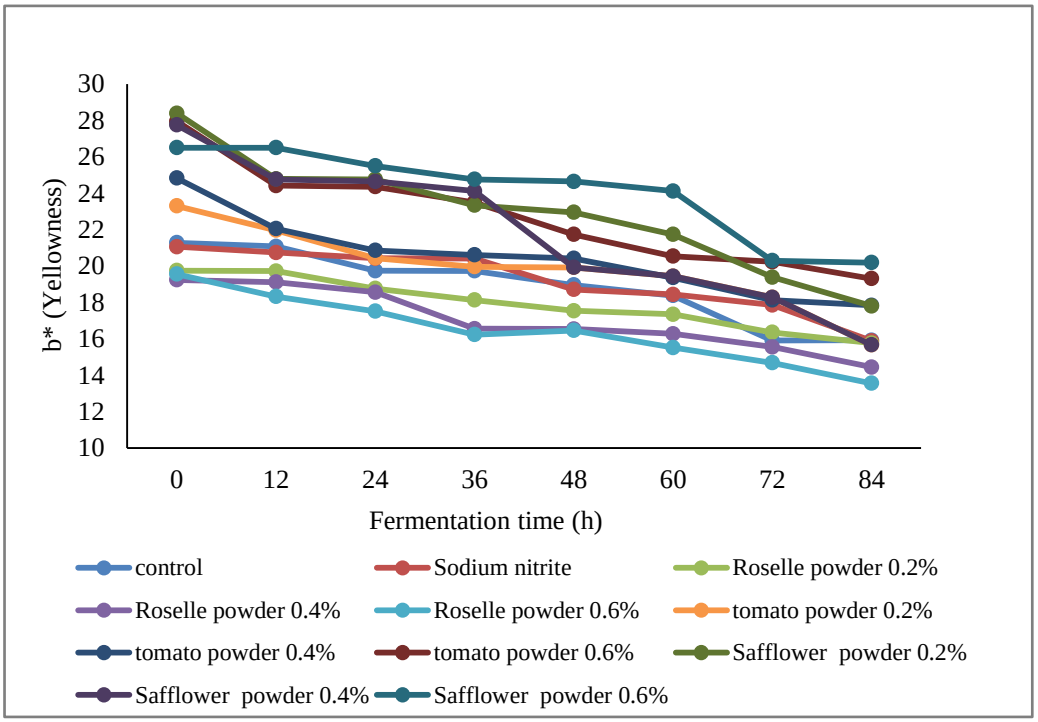


Figure 5. Lightness (L*), redness (a*) and yellowness (b*) of Nham samples containing different types and levels of natural colorants

Texture analysis of Nham

The hardness, adhesiveness, cohesiveness, springiness, and chewiness of all formulas were significantly different ($p<0.05$). Thus, natural colorants derived from rosella flower powder, tomato powder, and safflower powder at 0.2%, 0.4%, and 0.6% by weight affected the texturual charcteristics of Nham. The use of rosella powder at 0.2%, 0.4%, 0.6%, and tomato powder at 0.6% resulted in higher hardness compared to the control formula and the formula containing sodium nitrite. However, the use of safflower powder at all levels gave no difference of hardness compared to the control formula, and hardness was higher than the formula containing sodium nitrite (Table 1).

All three natural colorants at all levels resulted in higher adhesiveness compared to the control formula and the formula contaning sodium nitrite. Rosella flower powder and safflower powder resulted in highest adhesiveness. Safflower powder resuled in higher springiness and the formula containing 0.6% safflower powder had higher chewiness compared to all other formulas.

Table 1. Texture profile analysis of Nham samples containing different types and levels of natural colorants

formula	Percent (%)	Texture Profile Analysis				
		Hardness	**adhesiveness**	**cohesiveness**	**springiness**	**chewiness**
Control	0	298.87 ±1.08^{c}	-2.21 ±1.38^{c}	0.73 ±0.01^{c}	0.74 ±0.01^{b}	353.33 ±0.23^{e}
Sodium nitrite		243.80 ±2.89^{d}	-2.20 ±1.81^{c}	0.67 ±0.05^{d}	0.75 ±0.01^{b}	353.31 ±0.24^{e}
Roselle powder	0.2	358.27 ±0.62^{ab}	-1.33 ±1.35^{a}	0.74 ±0.05^{bc}	0.73 ±0.05^{b}	325.45 ±0.50^{f}
	0.4	383.38 ±1.74^{a}	-1.33 ±1.82^{a}	0.74 ±0.05^{cd}	0.74 ±0.05^{b}	325.56 ±0.46^{f}
	0.6	386.58 ±1.02^{a}	-1.34 ±1.68^{a}	0.73 ±0.15^{bc}	0.74 ±0.07^{b}	438.46 ±0.54^{b}
Tomato powder	0.2	244.21 ±1.24^{d}	-1.67 ±1.51^{b}	0.73 ±0.05^{bc}	0.62 ±0.04^{c}	420.13 ±0.92^{c}
	0.4	288.24 ±0.62^{cd}	-1.67 ±1.39^{b}	0.72 ±0.30^{c}	0.63 ±0.04^{c}	313.48 ±0.46^{g}
	0.6	329.08 ±0.94^{b}	-1.33 ±1.22^{b}	0.75 ±0.15^{abc}	0.62 ±0.01^{c}	418.91 ±0.57^{d}
Safflower powder	0.2	296.12 ±0.80^{c}	-1.33 ±1.49^{a}	0.76 ±0.11^{ab}	0.84 ±0.01^{a}	418.84 ±0.54^{d}
	0.4	298.57 ±2.07^{c}	-1.33 ±1.71^{a}	0.77 ±0.12^{a}	0.84 ±0.05^{a}	419.45 ±0.64^{cd}
	0.6	295.30 ±1.85^{c}	-1.33 ±1.28^{a}	0.77 ±0.29^{a}	0.82 ±0.05^{a}	448.05 ±0.56^{a}

$^{a-f}$ Values with different superscript letters in the same column are significantly different ($p \leq 0.05$).

Proximate composition

Moisture, crude protein, crude fat, crude fiber, and carbohydrate contents of all formulas were significantly different ($p<0.05$), but there was not significantly differed in total ash content ($p>0.05$) (Table 3). The higher contents of rosella flower powder, tomato powder, and safflower powder resulted in higher total ash content (Table 2).

Sensory acceptance evaluation

All three natural colorants at all levels resulted in significant differences ($p<0.05$) in the color, flavor, taste, firmness, and overall liking scores. Nham samples containing 0.4% and 0.6% of tomato powder had significant differences in the color, flavor, taste, and firmness scores compared to that of control sample ($p \geq 0.05$), and Nham samples containing rosella flower powder and safflower powder at all levels showed significant differnces in all sensory attributes ($p<0.05$). The higher levels of rosella flower powder and safflower powder resulted to decrease sensory scores in all attributes. In contrast, tomato powder at higher levels did not affected the sensory scores in all attributes. Nham sample containing 0.4% tomato powder had highest color, flavor, taste, and overall liking scores compared to other formulas (Table 3).

Table 2. Proximate composition of Nham samples containing different types and levels of natural colorants

Formula	Percent (%)	Proximate composition (%)					
		Moisture ^{ns}	Ash	Protein^{ns}	Lipid^{ns}	Fiber^{ns}	Carbohydrate^{ns}
Control	0	62.73±0.28	3.36±0.14^{c}	20.41±0.11	9.96±0.21	0.23±0.13	3.31±0.62
Sodium nitrite		62.56±0.16	3.37±0.07^{c}	20.25±0.70	9.88±0.12	0.23±0.14	3.71±0.52
Roselle powder	0.2	62.50±0.53	3.66±0.06^{ab}	20.39±0.27	9.73±0.28	0.25±0.12	3.47±1.15
	0.4	62.66±0.12	3.62±0.04^{b}	20.23±0.15	9.91±0.43	0.24±0.16	3.34±0.82
	0.6	63.64±0.41	3.72±0.05^{ab}	20.24±0.42	9.96±0.21	0.28±0.21	3.16±0.62
Tomato powder	0.2	62.60±0.06	3.70±0.07^{ab}	20.26±0.48	9.85±0.24	0.26±0.17	3.33±0.69
	0.4	62.46±0.11	3.76±0.07^{ab}	20.37±0.84	9.90±0.10	0.33±0.14	3.18±0.90
	0.6	62.22±0.70	3.88±0.14^{a}	20.39±0.45	9.85±0.90	0.37±0.07	3.29±1.51
Safflower powder	0.2	62.50±0.21	3.74±0.05^{ab}	20.32±0.46	9.85±0.28	0.28±0.10	3.31±0.34
	0.4	62.55±0.14	3.84±0.18^{a}	20.33±0.28	9.72±0.22	0.35±0.15	3.21±0.39
	0.6	62.39±0.16	3.84±0.04^{a}	20.58±0.14	9.73±0.16	0.32±0.16	3.14±0.39

$^{a-e}$ Values with different superscript letters in the same column are significantly different ($p \leq 0.05$);
^{ns} = not significantly different ($p > 0.05$).

Table 3. Sensory evaluation of the Nham samples containing different types and levels of natural colorants

Formula	Percent (%)	Hedonic score				
		Colour	flavor	Taste	Texture	Overall Liking
Control	0	5.92±1.29^{ab}	5.52±1.38^{abc}	5.50±1.40^{bcd}	5.86±1.49^{bc}	6.02±1.23^{bcd}
Sodium nitrite		5.58±1.56^{bc}	5.82±1.81^{abc}	6.02±1.59^{ab}	5.94±1.47^{abc}	6.18±1.54^{bc}
Roselle powder	0.2	5.78±1.37^{b}	5.96±1.35^{ab}	6.26±1.35^{a}	6.18±1.28^{abc}	6.36±1.24^{abc}
	0.4	5.06±1.71^{cd}	5.50±1.82^{abc}	5.22±1.93^{cd}	5.08±1.86^{d}	5.54±1.60^{def}
	0.6	3.24±1.70^{e}	4.06±1.68^{d}	5.30±1.37^{cd}	5.80±1.17^{bc}	4.56±1.41^{g}
Tomato powder	0.2	5.86±1.44^{ab}	5.72±1.51^{abc}	5.78±1.29^{abc}	6.44±1.16^{ab}	6.00±1.21^{bcd}
	0.4	6.46±1.38^{a}	6.12±1.39^{a}	6.00±1.14^{ab}	6.34±1.27^{ab}	6.84±1.46^{a}
	0.6	5.84±1.29^{ab}	5.86±1.22^{bc}	6.16±1.37^{a}	6.56±1.32^{a}	6.54±1.60^{ab}
Safflower powder	0.2	5.04±1.79^{cd}	5.28±1.49^{abc}	5.46±1.47^{bcd}	5.44±2.01^{cd}	5.30±1.80^{ef}
	0.4	5.30±1.14^{bcd}	5.52±1.71^{bc}	5.46±1.40^{bcd}	5.62±1.45^{cd}	5.76±1.18^{cde}
	0.6	4.82±1.40^{d}	5.16±1.28^{c}	5.06±1.70^{d}	5.60±1.32^{cd}	5.10±1.50^{fg}

$^{a-f}$ Values with different superscript letters in the same column are significantly different ($p \leq 0.05$)

Discussion

During the fermentation process of Nham, pH decreased and total acidity represented by lactic acid content increased. Nham samples were fermented at room temperature at about 30 ℃ for 3 days. After 3 days, the pH value ranged between 4.4–48, and total acidity was about 0.77–1.60. During 84 hours of

fermentation at 30 ℃, the pH value decreased sharply in the first 24 hours and it droped to 4.8 within 72 hours due to an increase in lactic acid content produced by lactic acid bacteria (Visessanguan *et al*., 2004; Visessanguan *et al*., 2005).

The decreased pH value and increased total acidity provides Nham with sour taste resulted from the fermentation with microorganisms in Nham. The microorganisms are responsible for the production of lactic acid in Nham reported as *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus leichannii*, *Leuconostoc mesenteroides*, and *Pediococcus cerevisiae* (Valyasevi *et al*., 1999).

During the fermentation of Nham, the microorganisms found in the first stage of fermentation that found in pork, including both acid-producing and spoilage microorganisms. After about 24 hours of fermanatation under optimal condition, acid-producing microorganisms multiplied rapidly, while the number of other microorganisms decreased. In the first 3 days of fermentation, heterofermentative lactobacilli and homofermentative cocci (*Pediococcus* spp.) rapidly grew, resulting in higher acid content and lower pH. After three days, the heterofermentative lactobacilli (*Lactobacillus plantarum*) count increased, while other microorganisms decreased because they could not tolerate highly acidic conditions.

Thai Industrial Standards, Ministry of Industry, determined that edible Nham must be fermented for not less than 4 days with pH of lower than 4.5 and lactic acid content should be higher than 0.50%. Coliforms and salmonella cannot grow at this level of lactic acid (Visessanguan *et al*., 2006; Valyasevi *et al*., 1999).

Comparison between Nham samples containing natural colorants derived from rosella flower powder, tomato powder, safflower powder, the control formula and the formula containing sodium nitrite revealed that there were not significant differed in pH values and total acidity. It indicated that all three types of natural colorants at 0.2%, 0.4%, and 0.6% did not affect the fermentation. The fermentation process which continued until the total acidity was achieved according to general standard for Nham production. If these natural colorants are used in higher amount, they affected the total acidity of Nham as tomato powder has a pH of 4.48 (Saksomboon *et al*., 2020) and rosella flower powder had total acidity of 9 mg/100 g and vitamin C of 11 mg/100 g (Mohamed *et al*., 2012). The use of these natural colorants in large amount results in more sour products. During the fermentation process, besides the change in pH and total acidity in food, the color appearance of Nham is an important chemical property that attracts consumers.

In the early stages of fermentation, in the control formula and the formulas containing sodium nitrite, the lightness value (L*) was higher than the formulas using a natural colorants. It could be due to more production of acid by lactic acid bacteria during the fermentation, resulting in denaturation of muscle proteins and increase in light-scattering properties. The shrinkage of the Microfilament lattice also increased reflectivity, lightness (L*) as the fermentation time increased.

As the fermentation time increased, the redness (a*) and yellowness (b*) values of Nham samples increased. The redness and total acidity of Nham samples significantly increased in the fermentation time. Nitrite is converted to nitric oxide and reacts with myoglobin in meat to produce nitrosomyoglobin (NOMb), which is pink. *Micrococcus varians* and *Lactobacillus Plantarum* facilitates faster color changing in Nham (Visessanguan *et al*., 2005). The enzymatic activity of microorganisms also helps to produce nitric oxide. When myoglobin in meat is exposed to oxygen, it transforms into oxymyoglobin, producing a visible red color in the product (Kramlich *et al*., 1973).

In Nham samples containg three types of natural colorants (rosella flower powder, tomato powder, and safflower powder) at three levels (0.2, 0.4, 0.6%), the redness and yellowness in Nham samples increased. It is due to roselle flower powder contains anthocyanin, a red soluble pigment. It is a flavonoid compound a natural colorant (Kirca *et al*., 2006). Tomato powder, tomato peel, and tomato fruit are rich in lycopene, a a reddish-orange pigment, and other carotenoids such as β-carotene, phytoene, phytofluene, and lutein (Choski and Joshi, 2007) and zeaxanthin, a yellow pigment (Domínech-Asensi *et al*., 2013). Safflower contains a yellow substance, Safflower yellow, and red substance, Carthamin, one of flavonoid compounds (Rojanak, 1995). Therefore, adding these 3 three types of natural colorants at each level resulted to increase in the redness and yellowness of Nham samples.

The texture profile analysis after the 4 days of fermentation showed that Nham samples containing sodium nitrite and all three types of natural colorants had higher hardness, adhesiveness, cohesiveness, springiness, and chewiness compared to that of the control formula. It is probable that at the early stage of the fermentation, lactic acid bacteria converted a carbon source and cooked rice to lactic acid, resulting to decrease in pH values when total acidity decreased continuously. Protein was denatured resulting in gel effect, causing the texture of the Nham to become sticky. As the fermentation time increased, microorganism count and total acidity increased, leading to the non-covalent intermolecular interactions. These interactions affected the collagen fibril structure of meat (Visessanguan *et al*., 2006).

Rattanapanone (2014) stated that cellulose is a main component of tomato powder, rosella flower powder, and safflower powder. The molecular structure of cellulose consists of polycrystalline part with sticky characteristics, and noncrystalline part. However, noncrystalline part of cellulose can be transformed into crystals after drying. Cellulose is an insoluble dietary fiber (IDF) that can instantly absorb water on its surface, thus causing swelling. This is because cellulose fibers are dense, coarse fibers with both molecules arranged in the same direction and opposite direction, making the fibers strong and not easily brittle. Its swelling capacity of water and solution is different.

Adding rosella flower powder at 0.2%, 0.4%, and 0.6% and tomato powder at 0.6% in Nham samples resulted in rapid water absorption and higher hardness compared to those of the control formula the the formulas containing safflower powder. This is consistent with a study by Calvo *et al*. (2008) which found that the adding tomato powder at 0.6 – 1.2% by total weight increased the hardness values of dry-cured sausages compared to the control formula.

All eleven formulas for Nham containing three different types of natural colorants (rosella flower powder, tomato powder, and safflower powder at different levels (0.2%, 0.4% and 0.6% w/w) showed no significant difference ($p \geq 0.05$) in the moisture, crude protein, crude fat, crude fiber, and carbohydrate contents. It showed that natural colorants derived from rosella flower powder, tomato powder, and safflower powder at 0.2%, 0.4%, and 0.6% had no effect on the nutritional value of Nham. However, these narural colorants increased the total ash content of Nham samples. The moisture, crude fat, crude protein, crude fiber and carbohydrate contents of Nham samples from all 11 formulas ranged between 63.18%-63.63%, 20.23%-20.58%, 9.72%-9.96%, 0.23%-0.27%, and 3.14%-3.71%, respectively.

According to the Bureau of Nutrition, Department of Health (2018), 100 grams of Nham contains 62.8% moisture, 20.2% protein, 9.9% fat, 0% fiber, 3.8% carbohydrate and 3.3% total ash. The addition of natural colorants had the effects on total ash content in the product. Rosella flower, tomato, and safflower are rich in inorganic substances, minerals or organic substances such as calcium, phosphorus, potassium, iron, magnesium, etc., which results in higher total ash content in the product.

The use of these three natural colorants (rosella flower, tomato powder, and safflower powder) at three different levels (0.2%, 0.4%, and 0.6% w/w) in Nham as an alternative to sodium nitrite, a harmful substance to consumers, which had different effects on sensory scores. The samples containing 0.4% tomato powder obtained the highest sensory scores in all attributes and showed no difference compared to the control formula. In addition, rosella flower powder and safflower powder decreased sensory scores. It is due to the

consumers who are familiar with Nham with a bright red appearance. Therefore, when tomato powder was added to the product, it gave the product an attractive red-orange color to the consumers. Moreover, tomato powder taste was not much sour compared to rosella flower powder, and low pH in longer fermentation time. These characteristics caused the lactic acid bacteria to produce more lactic acid, resulting in discoloration in Nham and the consumer may reject the product. On the other hand, safflower powder gave a different color from the usual color of Nham. Nham samples containing safflower powder gave more yellow in color than red resulting in less sensory scored in all attributes.

The study on the effects of natural colorants as subtitutes for sodium nitrate in Nham revealed that the use of 0.4% tomato powder by weight optimum formula that can be improved the color quality of Nham. The use of natural colorants did not affect the fermentation process and nutritional value of Nham. In addition, all three types of natural colorants improved the textural characteristics of Nham, resulting in firm and not too soft texture. Nham sample containing 0.4% tomato powder had the highest sensory scores in all attributes compared to the samples containing natural colorants derived from rosella flower powder and safflower powder, the formulas containing sodium nitrite, and the control formula. Therefore, a natural colorant derived from tomato powder can be used as a substitute for sodium nitrite and as a guideline for developing Nham products that are safe forconsumption and as an approach for adding the value of tomato.

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References

AOAC (2000). Official methods of analysis. Association of Official Analytical Chemists, Washington, DC.

Bureau of Nutrition, Department of Health (2018). Food composition table of Thai food. Thailand, Ministry of Public Health.

Calvo, M. M., Garc ́ıa, M. L. and Selgas, M. D. (2008). Dry fermented sausages enriched with lycopene from tomato peel. Journal of Meat Science, 80:167-172.

Cassens, R. G. (1997). Composition and safety of cured meats in the USA. Journal of Food Chemistry, 59:561-566.

Choski, P. M. and Joshi, V. Y. (2007). A review on lycopene: Extraction, purification, stability and applications. International Journal of Food Properties, 10:289-298.

Domínech-Asensi, G., García-Alonso, F. J., Martínez, E., Santaella, M., MartínPozuelo, G., Bravo, S. and Periago, M. J. (2013). Effect of the addition of tomato paste on the nutritional and sensory properties of mortadella. Journal of Meat Science, 93:213-219.

Kirca, A., Özkan, M. and Cemeroglu, B. (2006). Stability of black carrot anthocyanins in various fruit juices and nectars. Journal of Food Chemistry, 97:598-605.

Kramlich, W. E., Pearson, A. M. and Tauber, F. W. (1973). Processed meats. AVI Publishing Co. Inc., Westport, CT.

Kwanmuang, P. (2011). Development of pork spareribs and middle wing chicken Nham fermentation by using starter cultures and herb gardens. King Mongkut's Institute of Technology Ladkrabang, Bangkok: pp.51.

Mohamed, B. B., Sulaiman, A .A. and Dahab, A. A. (2012). Roselle (*Hibiscus sabdariffa* L.) in Sudan, Cultivation and Their Uses. Bulletin Of Environment, Pharmacology and Life Sciences, 1:48-54.

Rattanapanone, N. (2014) . Food chemistry (5^{th}ed). Bangkok, Thailand, Odeon store Publishing.

Pourazrang, H., Moazzami A. A. and Fazly B. S. (2002). Inhibition of mutagenic N-nitroso compound formation in sausage sample by using L-ascorbic acid and α-tocopherol. Journal of Meat Science, 62:479-483.

Rojanak, N. (1995) . Natural color. Department of Science Service Ministry of Highe Education, Science, Research and Innovation, 43:3-6.

Saksomboon, K., Meemookich, S., Kaewsaad, T., Limroongreungrat, K. and Theprugsa, P. (2020). Effect of tomato powder on quality of Chinese sausage. Int. Journal of Agricultural Technology, 16:711-720.

Sanoppa, K., Meesangket, S., Aemchalee, W. and Wongwan, P. (2021). Effects of Supplementation with Pigment Powders from *Monascus purpureus* Fermented with Pisang Awak Banana (Musa sapientum Linn.) Replace Nitrite in Fermented Pork Sausage (Nham). Journal of KMUTNB, 31:99-108.

Shahidi, F. and Pegg, R. (1991). Novel Synthesis of Cooked Cured-Meat Pigment. Journal of Food Science, 96: 1205-1208.

Suksripaisal, C. and Theprugsa, P. (2017). Use of Tomato and Ang-kak Powder in Chinese Sausage. Journal of Science and technology Thammasat university, 27:252-263.

Rojsuntornkitti, K., Jittrepotch, N., Kongbangkerd, T. and Kraboun, K. (2010). Substitution of nitrite by Chinese red broken rice powder in Thai traditional fermented pork sausage (Nham). International Journal of Food Research, 17:153-161.

Thai Industrial Standards Institute (2004). Thai industrial standard, Naem (fermented ground pork) TIS. No.1219. Thailand, Ministry of Industry.

Tirasarot, J. and Thanomwong, C. (2014). The utilization of Ang-Kak for color in the emulsion fish sausage. Journal of KKU Science, 42:169-175.

Valyasevi, R., Smitinond, T., Praphailong, W., Chowvalitnitithum, C., Kunawasen, S. and Chavasith, V. (1999). The microbiology and development of starter cultures for nham, the Thai pork sausage. In: Tuijtelaars, A. C. J., Samson, R. A., Rombouts, F. M., Notermans, S. (Eds.), Food Microbiology and Food Safety into the Next Millenium. TNO Nutrition and Food Research Institute, A. J. Zeist, pp.709-711.

Visessanguan, W., Benjakul, S., Panya, A., Kittikun, C. and Assavanig, A. (2005). Influence of minced pork and rind ratios on physicochemical and sensory quality of Nham a Thai fermented pork sausage. Journal of Meat Science, 69:355-362.

Visessanguan, W., Benjakul, S. Riebroy, S. and Thepkasikul, P. (2004). Changes in composition and functional properties of proteins and their contributions to Nham characteristics. Journal of Meat Science, 66:579-588.

Visessanguan, W., Benjakul, S., Smitinont T., Kittikun, C., Thepkasikul, P. and Panya, A. (2006). Changes in microbiological, biochemical and physico-chemical properties of Nham inoculated with different inoculum levels of Lactobacillus curvatus. LWT - Food Science and Technology, 39:814-826.

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