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Potential of Biopigments from *Monascus purpureus* Went as Natural Food Colorant for Philippine Native Sausage (*Longganisa*)

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Abstract. Potential of *Monascus* pigments as substitute to sodium nitrite ($NaNO_2$) in Philippine native sausage, *longganisa*, was evaluated. Three pigment levels (1, 2, and 3%) were added and tested for their effects on the microbial, physicochemical and sensory properties of the product. *Monascus* pigments, regardless of concentration, showed good microbial inhibition against bacteria and yeast. Both *Monascus* pigments and $NaNO_2$ had no significant effect on the moisture content, titratable acidity, and water activity of the sausages. All *Monascus*-treated sausages received flavor ratings comparable with that of nitrite-treated samples. However, sausages treated with 2 and 3% *Monascus* pigments had pronounced off-flavor which resulted in their significantly lower over-all acceptability ratings. *Longganisa* added with 1% *Monascus* pigments received the highest over-all acceptability, with ratings comparable with that nitrite-treated sausage. Therefore, addition of *Monascus* pigments at 1% level could be a natural alternative to the use of nitrite in *longganisa*.

Keywords: *angkak, monascus purpureus*, nitrite, *longganisa,* sausage, pigments

1. Introduction

The fungus *Monascus purpureus* has been the subject of intensive research because of its potential as natural colorant and dietary supplement. *Monascus* fungi produce at least six major related pigments which can be categorized into three groups based on color: (1) yellow pigments: monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$), (2) orange pigments: monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$), and (3) red pigments: monascorubramine ($C_{23}H_{27}NO_4$) and rubropuntamine ($C_{21}H_{23}NO_4$). The fungus is traditionally cultivated on starch-containing substrate such as rice, resulting in a fermented product known as red mold rice or *angkak*. This fermented product is also known as *beni-koji* in Japan, *hung-chu, hongqu, zhitai* in China, *rotschimmelreis* in Europe, and red mold rice in the United States [1].

Considerable interest has been devoted to the application of *angkak* as a nitrate (NO_3^-) and nitrite (NO_2^-) substitute for the preservation of meats [2]. NO_3^- and NO_2^- as sodium and potassium are used in processed meat products specially cured products because they impart and stabilize red meat color, inhibit some spoilage and food poisoning anaerobic microorganisms such as *Clostridium botulinum*, delay the development of oxidative rancidity, and contribute to color and flavour development [3]-[7]. However, NO_2^- can react with secondary amines to form carcinogenic compounds called nitrosoamines. This compound constitutes the major adverse effect of NO_2^- because of its possible cancer induction [8]. Thus, research on the use of natural colorants, such as *Monascus* pigments, as substitute for NO_3^- and NO_2^- in meat products has become a universal interest. In 2007, Pattanagul et al. [1] reported that red pigments from *angkak* at 1.6% could actually enhance the color of meat sausages. Meanwhile, De Carvalho et al. [9] stated that red

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Monascus pigment is a potential substitute for NO_2^- in meat products and for synthetic colors such as erythrosine or FD & C Red #3. The said pigment could also be used as substitute to NO_2^- in *nham*, Thai traditional fermented pork sausage [10]. In the work of Shehata et al. [11], it was also revealed that consumers preferred Egyptian fresh beef sausage with *Monascus* pigments. According to Fink-Gremmels et al. [2], meat products containing *Monascus* pigments were generally classified better tasting than products without *Monascus*. Moreover, Kraboun et al. [12] found out that 1.0% *Monascus* pigments could lower the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) in Chinese sausage during storage. They also reported that sausage with this level of pigments was the most accepted in terms of odor and taste. A similar observation was reported by Rhyu [13]. It was found that addition of 2% *Monascus* pigments effectively inhibited the PV and TBARS in bologna-type sausage. In 2012, El-Kholie et al. [14] reported that beef burgers with *Monascus* pigments showed higher nutritional values compared with control samples. The microbial load of the burger with *Monascus* pigments was also reduced after 90 d of storage.

In the Philippines, the native sausage is locally known as *longganisa* [15]. Like most processed meat products, *longganisa* is usually cured with $NaNO_2$ or potassium nitrite (KNO_2) for the same reason stated above. In an effort to offer healthy option to Filipino consumers, this study aimed to evaluate the potential of *Monascus* pigments as substitute to $NANO_2$ in *longganisa*.

2. Methodology

2.1. Source and Preparation of Microbial Inoculum

M. purpureus was obtained from the Institute of Food Science and Technology, College of Agriculture, University of the Philippines Los Baños, College, Laguna, Philippines. The fungus was cultivated on Potato Dextrose Agar (PDA) slants and incubated at 30 ℃ for 7 d. The spores were the aseptically scraped-off with 5 mL of sterilized distilled water. The concentration of the spore suspension was adjusted to a rate of 1.0 x 10^8 spores mL^{-1} using a hemocytometer under high power objective of microscope [16].

2.2. Preparation of *Angkak*

Angkak was prepared following the optimized method described by Dizon [17] using PSB Rc72H (Mestiso), a Philippine rice cultivar with low amylose content. The rice was thoroughly washed with water and then soaked for 10 h. Then, the rice was drained, spread onto cheesecloth, and steamed for 30 min. Approximately 50 g of steamed rice was transferred into 250-mL Erlenmeyer flasks. The moisture content (MC) was adjusted to approximately 35% by adding 4 mL distilled water. The flask was then sterilized at 121 ℃ for 30 min. After cooling, the sterilized rice was inoculated with 5 mL spore suspension. Incubation was carried out for 10 d at 30 ℃. The fermented rice was then taken out from the flask, spread onto clean aluminum tray, and oven-dried at 60 ℃ for 12 h. *Angkak* was then transferred in plastics bags and stored at refrigerated temperature until use.

2.3. Extraction of *Monascus* Pigments

Angkak was ground using sanitized blender and sieved through 0.45 mm mesh. Approximately 0.5 g of the sample was weighed in centrifuge tube and 50 mL of 70% ethanol was added. The resulting solution was shaken for 1 hr. The tubes were centrifuged at 4000 rpm (Allegra 25R, Beckman Coulter, CA, USA) for 15 min. The supernatant was collected and filtered through ordinary filter paper. It was then concentrated in a rotary vacuum evaporator (Eyela, Tokyo Rikakikai Co., Tokyo, Japan) set at 50 ℃ and 70 cm Hg. The concentrated extract was stored at refrigerated temperature until use.

2.4. Preparation of *Longganisa*

The preparation of *longganisa* followed the procedure described by Ibarra [15] with some modifications. The sausage was prepared with the following ingredients: 700 g ground lean meat, 300 g ground fat, 18 g salt, 120 g sugar, 2 g ground black pepper, 2 g monosodium glutamate, 5 g phosphate, 30 mL pineapple juice, 50 g minced garlic, 30 g flour, 30 g skimmed milk, 50 mL water, and imported hog intestine for casting. First, the ground lean and fat were combined, and onto it, all the dry ingredients were added. This was followed by the addition of the remaining ingredients. It was then mixed manually and divided into 5 parts. Three parts were added separately with 1, 2, and 3% *Monascus* pigments. One part was added with 7 g Prague powder#1

($NaNO_2$) and served as positive control. The last remaining part served as the negative control. All the meat patties were then stuffed in hog intestine and tied every six inches long. The sausages were stored at chilling temperature (4 ℃) for 3-day curing.

2.5. Quality Evaluation of *Longganisa*

Total plate, bacterial, yeast, and mold counts of *longganisa* were determined using the standard pour plating technique for microbiological analysis [18]. MC, pH, and titratable acidity were measured based on standard AOAC methods [19]. Water activity (A_w) was determined using a calibrated Lufft Durotherm A_w-Wert-Messer water activity meter.

For sensory evaluation, cooked *longganisa* were cut into a convenient size (about 1 cm thick) with sides and casing trimmed. The samples were coded with random numbers and served in plates. The samples were evaluated by 15 experienced panelists for color, aroma, flavor, off-flavor, tenderness, and over-all acceptability using a 7-point hedonic scale for each trait [20].

2.6. Statistical Analysis

All analyses were done in triplicate including the sensory evaluation. ANOVA and mean comparison analysis using Tukey's HSD test were analyzed using SPSS for Windows version 20 statistical software package (IBM SPSS Statistics, Armonk, NY). The level of significance used was p=0.05.

3. Results and Discussion

Samples of cooked and sliced *longganisa* with and without *Monascus* pigments are shown in Figure 1.

Fig. 1: Sliced *longganisa* with and without *Monascus* pigments.

3.1. Microbiological Properties

Monascus pigments, regardless of concentration, showed microbial inhibition comparable with nitrite-treatment particularly against bacterial and yeast. In terms of total plate count (TPC), the highest inhibition was produced by $NaNO_2$. However, *Monascus*-treated sausages showed considerably lower TPC than that of untreated sample. Meanwhile, no significant differences were observed among the mold counts of the samples.

3.2. Physicochemical Properties

Table I also shows the physicochemical properties of *longganisa*. Among the parameters evaluated, only pH showed significant difference among the samples. Noticeably, all the pH values were considerably higher (6.5 – 6.7) than those of reported (4.5 – 5.2) [21]. It can be noted that *longganisa* samples used in the experiment were only cured and not fermented, hence there was no production of lactic acid, which is responsible for the low pH in other similar products. The nitrite-treated sample had the highest pH value (6.70) among the samples. Meanwhile, treatments with *Monascus* pigments and $NaNO_2$ showed no significant effect on the moisture content, titrate acidity, and A_w of the sausages. The A_w of samples ranged from 0.92 to 0.93 which conformed to the values reported by Rogers and Brimelow [22], which were 0.91-0.97. A_w, not MC, determines the lower limit of available water for microbial growth.

Table I: Microbial load (CFU g^{-1}) and physicochemical properties of *longganisa* samples[1].

Sample	Microbial load				Physicochemical properties			
	Total plate count (x 10^5)	Bacterial count (x 10^5)	Yeast count (x 10^4)	Mold count (x 10^3)	pH	Moisture content (%)	Water activity (A_w)	Titratable acidity (%)
(-)	5.70^a	5.90^a	2.73^a	5.33^a	6.50^b	51.76^a	0.92^a	3.63^a
(+)	1.23^c	1.47^b	0.17^b	2.33^a	6.70^a	56.33^a	0.93^a	3.55^a
1%	3.50^b	3.57^{ab}	1.37^b	3.33^a	6.50^b	52.18^a	0.92^a	3.78^a
2%	3.33^b	3.57^{ab}	1.43^{ab}	3.33^a	6.50^b	56.99^a	0.93^a	3.86^a
3%	3.00^b	3.23^{ab}	0.90^b	3.03^a	6.50^b	57.40^a	0.93^a	3.93^a

[1]Mean values with different letters in the same column are significantly different ($P < 0.05$) using Tukey's HSD test (N=3)
*(-) negative control, (+) positive control (with sodium nitrite), values in % refer to the amount (w/w) of *Monascus* pigments added

3.3. Sensory Properties

Color of the samples was highly distinguished by the panelits. Among the samples, sausage treated with 3% *Monascus* pigments was rated as darkest or reddest while untreated sausage as palest. Aroma of sausages treated with 1 and 2% *Monascus* pigments were comparable with that of nitrite-treated sausage. The flavor ratings of all *Monascus*-treated sausages were comparable to nitrite-treated samples. It has been reported that meat products containing *Monascus* pigments were classified by numerous researchers as better tasting than products without the pigments. The relishing effect of *Monascus* could be caused by flavor enhancing oligopeptides produced during the partial hydrolysis of rice proteins by *Monascus* enzymes [3]. Unfortunately, sausages treated with 2 and 3% *Monascus* pigments had pronounced off-flavor mainly attributed to bitterness of the samples. This perception resulted in their significantly lower over-all acceptability ratings. At 1% *Monascus* pigment addition, the off-flavor was no longer perceived. At this level, the sausage obtained off-flavor and over-all acceptability ratings comparable with those of sample treated with $NaNO_2$. This finding agrees with the reports of Pattanagul et al. [1], Kraboun et al. [12], and El-Kholie et al. [14]. Lastly, no significant difference was observed on the tenderness of the sausages (Table II).

Table II: Mean sensory scores of *longganisa* samples[1].

Sample*	Sensory attributes**					
	Color	Aroma	Flavor	Off-flavor	Tenderness	Over-all Acceptability
(-)	1.50^e	3.75^c	3.80^b	1.91^{bc}	4.03^a	3.77^b
(+)	2.40^d	4.05^{bc}	4.90^a	1.63^c	4.86^a	5.03^a
1%	3.59^c	4.38^{abc}	4.74^a	1.75^c	4.68^a	4.89^a
2%	5.15^b	4.89^{ab}	4.47^{ab}	2.58^b	4.20^a	3.90^b
3%	6.15^a	5.29^a	4.45^{ab}	3.36^a	4.31^a	3.82^b

[1]Mean values with different letters in the same column are significantly different ($P < 0.05$) using Tukey's HSD test (N=3)
*(-) negative control, (+) positive control (with sodium nitrite), values in % refer to the amount (w/w) of *Monascus* pigments added
**color: 1-pale pink, 7-dark red; aroma: 1-weak, 7-strong; flavor: 1-weak *longganisa* flavor, 7-full *longganisa* flavor; off-flavor: 1-imperceptible, 7-very perceptible; tenderness: 1-very tough, 7-very tender; overall acceptability: 1-very unacceptable, 7-very acceptable

4. Conclusion

Monascus pigments, regardless of concentration, showed significant inhibition in *longganisa* against bacteria and yeast. Addition of *Monascus* pigments and $NaNO_2$ had no remarkable effect on the physicochemical properties of sausages, except for pH. All *Monascus*-treated sausages obtained flavor ratings comparable with that of nitrite-treated samples. However, sausages treated with 2 and 3% *Monascus* pigments had pronounced off-flavor which resulted in their significantly lower over-all acceptability ratings. Ultimately, the *longganisa* with 1% *Monascus* pigments and nitrite-treated *longganisa* were highly preferred. To the best of our knowledge, this is the first work that proves that addition of *Monascus* pigments at 1% level could be a natural alternative to the use of sodium nitrite in the *longganisa*.

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