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Natural curing of fermented sausage using vegetable powders

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Abstract

Fermented sausages contain a unique flavor derived from lactic acid, which is generated by microorganisms. In order to produce the desired color, the addition of nitrite, which is used as a curing agent, is important. However, there is considerable carcinogenic potential. However, naturally curing agents may be more acceptable, and green vegetables also have potential. Therefore, 3 types of powder from indigenous vegetables (*Alliumcepa var. aggregatum*, AA/ *Coriandrum sativum*, CS/ *Allium sativum* L., AS) were prepared using vacuum drying at 60 ˚C. These powders were added to the ground sausage recipe at 0.1 % prior to fermenting at 4 ˚C for 24 h and at 37 ˚C for 24 h. The results showed that the pH and acidity values of all the sausages had not differed, suggesting typical fermentation. The redness values of the samples with vegetable powders were higher than those of the control (without adding nitrite), which indicates natural curing. Although no external nitrite was added, this result was concomitant with higher nitrite levels. In addition, the total plate count suggested that the addition of AS powder had inhibited microbial growth, while the growth of lactic acid bacteria had been promoted. The sensory evaluation revealed that regardless of the type of vegetable powder used, all sausages had been comparably acceptable. Therefore, AS powder may have the potential of becoming a natural curing agent in the production of fermented sausages.

Keywords: Fermented sausage, Natural curing, Vegetable powder, Nitrite

1. Introduction

Fermented sausages are one of famous meat products spreading throughout the Thailand, especially in the Northeastern part of the county. These are normally made from pork, back fat, and skin, and cooked rice is used as the carbon source. Natural fermentation, carried out by the action of lactic acid bacteria, is crucial for developing the unique flavor and the sour taste of the sausages. The addition of nitrite is necessary to provide the desired color and flavor. Moreover, it has the ability to inhibit microorganisms that spoil the product, as well as to inhibit pathogens, such as *Clostridium botulinum* [1]. In addition, it is believed to retard the oxidative rancidity by scavenging the free radicals, which are generated by oxidation [2]. However, under the conditions of processing, reactions between the nitrite and the amines in meats may lead to the formation of the carcinogen, *n-nitrosamines*. The intake of nitrite in high amounts may place human health at risk. Therefore, the application of nitrite additives in meat products is limited. In fact, according to the regulations of Codex standard, only 80 mg/kg of detectable nitrite residue is allowed in meat products [3]. Avoiding the utilization of nitrite in meat products may offer an alternative for health-conscious consumers. In order to avoid utilizing nitrites in meat products, alternative products for consumers concerned about their health may need to be created [4]. Moreover, the natural curing of meat products by adding natural nitrite, which is an indirect addition of nitrite, could prove to be more acceptable.

Vegetables are good sources of nitrate and both powdered forms and concentrated extracts have been commercially developed [5]. In particular, celery is being used as a natural curing agent in meat products [6]. Leeks have also been reported to have the potential to cure meat in fermented sausage products [7]. However, other indigenous vegetables may also have ability to serve as sources of nitrate. There are some vegetables, which are served as side dishes with fermented sausages, such as Chinese coriander (*Coriandrum sativum*, CS). In addition, the leaves of green shallots or spring onions (*Allium cepa var. aggregatum*, AA) and the leaves of garlic (*Allium sativum* L, AS) are normally discarded when these are harvested in bulk. By harvesting these leaves, drying them, and grinding them into a powder, they could be used as a natural curing agent for fermented sausages. Such a strategy should not affect the quality of the sausages and should make the product more acceptable for consumers. However, information concerning the effect that adding these vegetable powders can have upon the quality of the fermented sausage has not yet been documented. Therefore, the aim of this study was to investigate the curing effects of the indigenous vegetable powders on the physical, microbial, and sensorial qualities of fermented sausages.

2. Materials and methods

2.1 Materials

The vegetables including Chinese coriander (*Coriandrum sativum*, CS), spring onions (*Allium cepa var. aggregatum*, AA), and garlic (*Allium sativum* L, AS), were obtained from a local market in Nong Khai Province. Then the undesirable parts of the leaves of CS, AA, and AS were removed, and the samples were washed, air dried, and then cut into approximately 10 cm pieces. All samples were dried in a vacuum at 60 ˚C (SK100, Retsch, Germany) until the samples were observed to be completely dried (around 48 h). The dried samples were ground into a powder using a hammer mill with a 0.25 mm diameter sieve (Retsch, Haan, Germany). The powders were kept at -18 ˚C under vacuum conditions until they were ready to be used. Pork and back fat were purchased from the same local market and were then ground separately using a meat grinder (Champ, Kent, U.K.) with a perforation of 3 mm. The N-(1-naphthyl) ethylenediamine ($C_{12}H_{14}N_2$) and Sulfanilamide ($C_6H_8N_2O_2S$) were obtained from Sigma Aldrich (Diegem, Belgium), and the other chemicals used were of analytical grade.

2.2 Fermented sausage preparation

Four treatments of fermented sausage were prepared. First, a control sausage was prepared without adding any nitrite. Each of the vegetable powders was added in the sausage recipe at 0.1%. The ingredients in the sausage recipe were controlled at 50, 25, 10, 5, 5, 2, 1, 1, and 1% for minced pork, pork fat, cooked-skin, garlic, oyster sauce, salt, seasoning salt, and sugar, respectively. Those ingredients were mixed together until a sticky batter was observed (twice mixed for 5 min). Then, all of the batters were stuffed into a collagen casing with the diameter of 2.5 cm. Using cotton rope, the sausages were strung by hand into individual links with lengths of approximately 10 cm each. These samples were incubated in a cold room (4 ˚C) for 24 h, and then they were further incubated at 37 ˚C for 24 h. The obtained samples were kept at 4 ˚C, and within one week, they underwent further analysis.

2.3 Characterization of sausages

2.3.1 pH and acidity measurements

Determination of pH was performed by weighing a sample (10 g) and mixing it with DI-water (90 ml). The pH of the homogenate was reported as the mean value from 3 measurements by using a pH meter (Mettler-Toledo, Thailand). In addition, 25 mL of the homogenate was taken to titrate with NaOH (0.1 M), and phenolphthalein was used as the indicator. The volume of NaOH at the end point was used to calculate the acidity, and this value was expressed as the lactic acid.

2.3.2 The microbial count

2.3.2.1 The total variable count

The microbial count was performed using the homogenate sample (10 g) in sterilized water (90 mL) prior to making the serial dilutions. Then, 1 mL of each sample in each dilution was applied for a pour plate experiment (2 plates per dilution), and the plates were then incubated at 37 ˚C for 48 h. The number of single colonies on each plate was counted in order to calculate number of total microorganisms and was then expressed as $\log_{10}$ of the colony- forming unit per gram of sample ($\log_{10}$ CFU/g).

2.3.2.2 The lactic acid bacteria count

A similar technique was applied to the total microbial count in order to determine the lactic acid bacteria. However, MRS agar was used instead, and sodium carbonate was introduced as the indicator. On each plate, only single colonies with clear zones were counted. These were used to calculate the number of lactic acid bacteria and were expressed as log_{10} of colony forming unit per gram of sample (log_{10} CFU/g).

2.3.3 Color measurement

Color determination was performed on the cross-sectional areas of the samples (0.5 cm in thickness) using a colorimeter (Color meter, JS 555, China). The mean value for *L*, *a*, and *b* were reported from 5 measurements.

2.3.4 Nitrite determination

The nitrite content in the samples was determined according to the Official method (973.31) of the AOAC (2000). Comminuted samples (5 g) were thoroughly mixed with warm DI-water (40 mL) before being transferred to a volumetric flask (500 mL) and heated at 80 ˚C for 2 h. After cooling down, the solution was filtered and the volume was made up by adding DI-water. Thereafter, 45 mL of the clear sample was reacted with Sulfanilamide solution (2.5mL) and was then mixed well for 5 min. Next, NED solution (2.5 mL) was added. Thereafter, the absorbance was read at 540 nm using a spectrophotometer (PG, England). An absorbance of the standard was performed using $NaNO_2$ in the range of concentration from 0-1 μg/mL.

2.3.5 Sensory evaluation

To determine the acceptability of the fermented products, a hedonic test (9-point) was applied for sensory evaluation. The sausages were grilled at 220 ˚C for 15 min before being sliced into thicknesses of about 1.0 cm. Then, 3 pieces of the samples were served to 15 panelists (18-45 years of age). The panelists were asked to evaluate the products' acceptability in terms of 'color', 'odor', 'texture', and 'flavor', as well as for overall acceptability.

2.4 Statistical analysis

The mean values among treatments were compared using ANOVA test at the significant level of $P < 0.05$. The means were compared via the Duncan test using SPSS version 16.0 (SPSS Inc., Ils, U.S.A.).

3. Results and discussion

3.1 Physical appearance of sausage

The physical appearance of the sausages is shown in Figure 1. It can be seen that the colors of the sausages with the vegetable powders had not differed with respect to the types of the vegetables used. In addition, the appearance of color was also similar to that of the control (Figure 1A). This suggests that at this level, the addition of vegetable powder could be possible and that the appearance of the color would not be affected. However, by analyzing the physicochemical properties of the sausages, it was shown that the effects of these powders on the quality of the sausages still needs further clarification.

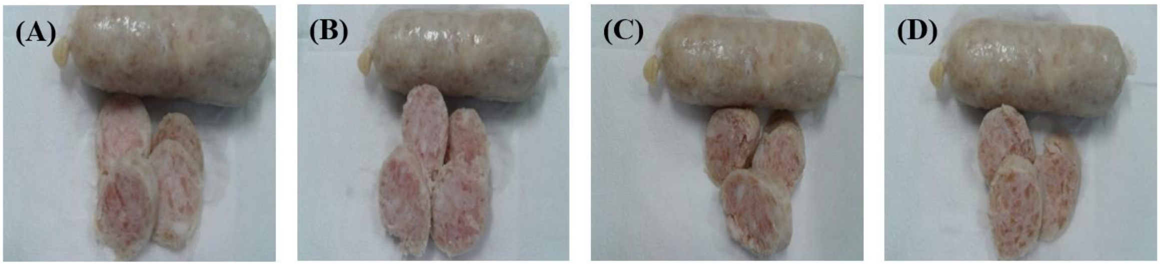

Figure 1 Appearance of the fermented sausages without nitrite (A), AS (B), AA (C), and CS (D).

3.2 Quality attributes

3.2.1 pH and acidity

The pH values of all sausage were found to be about 4.37 as shown in Table 1. This value is much lower than the value of meat (approximately 6.0), which suggests that acid fermentation had occurred. The pH values in our study were lower than those of the fermented sausages with leek powder at similar incubation times [7]. This was because our sausages had been fermented at a higher temperature (37 ˚C for 24 h), while the sausages in the previous report had been fermented at only 20 ˚C. As observed among the treatments, differences in pH values were not seen. As for the samples with the vegetable powders, similar degrees of acidity, which were expressed as lactic acid, were also observed. Most likely, this would represent the action of lactic acid bacteria. A reduction in pH would likely be a result of lactic acid fermentation caused by the action of lactic acid bacteria. A reduction in pH and an increase in the lactic acid bacteria count was reported after leek powder had been added to the fermented sausages [7]. The results indicated that the addition of the vegetable powders had not affected the fermentation process. Therefore, the application of vegetable powders in fermented sausage would not affect the processing technique.

Table 1 The pH and acidity of fermented sausages with different vegetable powders.

Sausage recipe	pH	Acidity (g of lactic acid/g sample)
Control	4.37 ± 0.007[a]	10.55 ± 0.51[a]
AS	4.37 ± 0.007[a]	10.45 ± 0.51[a]
AA	4.34 ± 0.014[a]	10.81 ± 0.01[a]
CS	4.37 ± 0.014[a]	10.45 ± 0.51[a]

The means were determined from 3 measurements. *Different letters in the same column indicate a statistical difference of $P < 0.05$.

3.2.2 Color

Regardless the types of vegetables used, the lightness (L value) of all sausage samples showed similar values (Table 2). It was reported that the addition of leek powder in fermented sausages had resulted in darker colored sausages [7]. Our results have been found to contradict this previous result and may possibly be due to the different species of vegetables used and their concentrations. Similar lightness values were observed among the samples, and this factor could possibly be attributed to similar acidity. Thus, the redness of the products was increased when compared to that of control (Table 2). This suggests that the addition of vegetable powders had provided color development as a part of the natural curing process. Based on this study, fermented sausages could be cured naturally by using vegetable powders as alternative sources of nitrite. The curing effect could be promoted by adding the juices from the vegetables as well [8]. Based on this study, fermented sausages could be cured naturally by using vegetable powders as alternative sources of nitrite. In addition, a limited amount of nitrite residue was observed to be at an acceptable level according to the Codex standard (not over 80 mg/kg) [3]. The addition of vegetable powders could be an alternative procedure, which could reduce the nitrite content of the fermented sausages without affecting the redness. It was seen that addition of AS powder had exhibited the lowest value of yellowness. Normally, samples, which showed a lower degree of yellowness with a similar redness, had proven to be more desirable than those with a high degree of yellowness. The curing effect of the meat model system was also demonstrated because there had been a reduction of yellowness, which did not affect the redness after the plant-based curing agents had been added [9]. This suggests that the addition of AS had exhibited the highest potential to cure meat pigments during fermentation. Developing pigment during the fermentation of meat product without adding nitrite has been documented [10].

Table 2 The color values of fermented sausages incorporated with different vegetable powders.

Sausage recipes	Color value		
	L	*a*	*b*
Control	58.85 ± 0.05[a]	5.37 ± 0.13[b]	12.97 ± 0.22[a]
AS	59.55 ± 0.93[a]	6.64 ± 0.29[a]	10.66 ± 0.24[b]
AA	58.14 ± 1.06[a]	6.26 ± 0.54[a]	13.33 ± 0.18[a]
CS	56.84 ± 0.77[a]	6.24 ± 0.52[a]	13.38 ± 0.51[a]

The means were determined from 5 measurements. *Different letters in the same column indicate a statistical difference of $P < 0.05$.

3.2.3 The total variable count

The total plate count in the fermented sausages represented all of the microorganisms growing in the sample. The results in Table 3 indicated that the addition of the AS powder had resulted in a reduction of the total number of microorganisms. This suggests that garlic leaf powder could inhibit microbial growth, which would likely extend the shelf life of the product. Garlic extract is well-known to inhibit the growth of *Staphylococcus aureus* (*S. Aureus*) and *Escherichia coli* (*E. Coli*), both of which are present in the environment [11]. In addition, fresh garlic extract has effectively inhibited both pathogenic bacteria [12]. It was found that the antibacterial activity against *E. coli*, *S. aureus*, and *Bacillus subtilis* had been dependent upon the concentration, rather than dependent on the temperature and the time variables [13]. Moreover, the inhibiting effects towards the *Enterobacteriaceae* by curing agents have been reported [14].

Table 3 The total microbial and lactic acid bacterial counts in the fermented sausages with different vegetable powders.

Sausage recipes	Total plate counts (Log CFU/g)	Lactic acid bacterial counts (Log CFU/g)
Control	7.18 ± 0.07^{a}	2.64 ± 0.06^{c}
AS	6.50 ± 0.02^{b}	4.37 ± 0.01^{a}
AA	7.13 ± 0.07^{a}	2.81 ± 0.02^{c}
CS	7.32 ± 0.04^{a}	3.13 ± 0.04^{b}

The means were determined from 2 measurements in 2 replications. *Different letters in the same column indicate a statistical difference of $P < 0.05$.

3.2.4 Lactic acid bacteria

As seen in Table 3, in the sausages with the garlic leaf powder, the highest number of lactic acid bacteria was found. However, when compared to the control, the addition of other vegetable powders showed similar numbers of lactic acid bacteria. This suggests that the addition of garlic leaf powder could inhibit microbial spoilage, while also promoting the growth of lactic acid bacteria. In the sausages fermented with the leek powder, a high lactic acid bacteria count was reported, which resulted in a rapid reduction of pH [7]. However, in this study, a higher lactic acid bacteria count was not concomitant with a lower pH value, for which there might have been two possibilities. Firstly, after the pH of all samples had been reduced to the final value, no further reductions were observed. Secondly, although there could have been some growth of the lactic acid bacteria, lactic acid production had been limited by the action of some compounds, which were present in the garlic leaf powder.

3.2.5 Nitrite content

By the applied method, nitrite residues in the control sausage samples were not detected because external nitrite had not been added. With the addition of vegetable powders, nitrite residues in the fermented sausages were observed even though nitrite residues had not been previously detected in the vegetable powder (data not shown). However, vegetables are considered to be good sources of nitrate, and the nitrate content in garlic has been determined to be 30 ± 5.89 mg/ 100 g [5]. During the fermentation process of the meat, this nitrate can be reduced to nitrite [15]. The development of nitrite in sausage could possibly have been due to the action of the nitrate reducing microorganisms. It is well-known that *Staphylococcus carnosus* (*S. Carnosus*) is one of the most important commercial cultures, which is used for natural curing in meat products [7]. This suggests that the addition of vegetable powders provides a curing effect on the meat pigments, and corresponds to increased redness (Table 2). Although the detected value was much lower than that found in the sausages, which had been fermented with leek powder, the addition of CS showed the highest value. This was due to the absence of a starter culture (*S. carnosus*), as well as a lower concentration of vegetable powder. The nitrite residue, found in this fermented sausage, was low enough to meet the standard of Codex. This suggests that the addition of vegetable powder in this amount is sufficient enough to develop the cured color. In addition, the nitrite residue, which would be left over, would be able to maintain the stability of the color, as well as to carry out other functions, such as inhibiting lipid oxidation, developing the unique flavor, and the preventing the growth of pathogens.

Table 4 The nitrite contents in the fermented sausages with different vegetable powders.

Sausage recipes	Nitrite content (mg/kg)
Control	0.00 ± 0.01^{d}
AS	0.33 ± 0.02^{b}
AA	0.17 ± 0.04^{c}
CS	0.50 ± 0.02^{a}

The means were determined from 2 measurements in 2 replications. *Different letters in the same column indicate a statistical difference of $P < 0.05$.

3.2.6 Sensory evaluation

The control sausage exhibited a similar perception of color and flavor as seen in the similar acceptability of those attributes. A reduction in the acceptability of ‘odor’ was observed in the sausages with the CS powder, which was possibly due to the strong CS odor. It was noted that the addition of AS and AA powders had resulted in a higher acceptability attribute for odor and had been firmer than those of the control. It is possible to hypothesize that the fiber in the vegetable powder could possibly enhance the firmness of the sausages. The result from hedonic test also indicated that the sausages with the CS powder had shown a lower score for overall acceptability than the other samples had. This acceptability was in agreement with the lower acceptability of the odor attribute. This could suggest that the CS powder may have provided an off-odor (or undesirable odor) in this sample. It was seen that the overall acceptability of the samples with the AS and AA had been at the highest level (at about 7 of 9), which suggested that these were highly acceptable.

Table 5 The sensory scores of fermented sausages with different vegetable powders.

Sausage recipes	Color	Odor	Flavor	Texture	Overall acceptance
Control	6.53 ± 0.28^a	6.50 ± 0.34^b	7.07 ± 0.31^a	6.37 ± 0.31^a	7.00 ± 0.27^b
AS	6.93 ± 0.22^a	6.97 ± 0.26^a	7.37 ± 0.22^a	7.63 ± 0.19^a	7.47 ± 0.18^a
AA	6.83 ± 0.22^a	7.07 ± 0.28^a	7.47 ± 0.23^a	7.10 ± 0.29^a	7.47 ± 0.22^a
CS	6.10 ± 0.27^a	6.17 ± 0.30^c	7.28 ± 0.27^a	7.13 ± 0.27^a	6.93 ± 0.32^b

The means were determined from at least 15 panelists. *Different letters in the same column indicate a statistical difference of $P < 0.05$.

4. Conclusion

The vegetable leaf powders have the potential to be used as natural curing agents in fermented sausages as evidenced by the increase in redness, as well as the detection of nitrite residues in the products. The addition of garlic leaf powder could inhibit the total bacterial count, but could also promote the growth of lactic acid bacteria. Based on this study, garlic leaf powder showed the highest potential for becoming a natural curing agent for fermented sausages.

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