Identification of Main Factors Affecting Quality of Thai Fermented Pork Sausage (Sai Krok Prew)

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ABSTRACT

Plackett-Burman design was used to identify the main factors affecting the qualities of Sai Krok Prew (Thai fermented pork sausage). Starter cultures (Lactobacillus plantarum, Pediococcus cerevisiae and Micrococcus varians), sugar and salt were found to be the main factors while ground garlic, white pepper, coriander seed, sodium nitrate and sodium nitrite the minor.

Key words: Fermentation, Pork sausage, Starter cultures, Plackett-Burman design, Quality

INTRODUCTION

Sai Krok Prew is a traditional Thai fermented pork sausage. It is usually made from minced pork, salt, garlic, sugar, white pepper, coriander seed, cooked sticky rice and minced pork lard . After fermentation, it must always be cooked before eating. The interest in using starter cultures for sausage fermentation arises in parallel to the trend towards industrial sausage production, short ripening time and standardisation of product properties (Lücke, 1995). There are several reports on the utilization of starter cultures in the manufacture of fermented sausage (Wiriyacharee et al., 1991; Garriga et al., 1996; González and Díez, 2002; Leroy et al., 2002). In this study, a mixed starter cultures of lactic acid bacteria and nitrate-reducing bacteria were used to improve the quality of Sai Krok Prew. *Lactobacillus plantarum, Pediococcus cerevisiae* and *Micrococcus varians* were used together. Also the ingredients including sugar, salt, sodium nitrate, sodium nitrite, ground garlic, white pepper, coriander seed and cinnamon were studied to identify the main variables affecting the quality of Sai Krok Prew. These variables could be too many to study if using factorial experiments. In this experiment, the 9 factors would require 512 runs for a full factorial or 256 for a half-factorial design. Plackett-Burman design which has prove itself in many industrial situations is reputed to be most efficient in screening large number of variables (Stowe and Mayer, 1966; Anderson, 1981; Earle and Anderson, 1985). Therefore, it was used in this study.

MATERIALS AND METHODS

Materials

Minced pork (lean meat), minced pork lard and cooked sticky rice (50:25:25) were called "meat system" in this study. They were purchased from a local market (Chiang Mai, Thailand). Cooked sticky rice was rinsed through drinking water (1 L drinking water per 150 g cooked sticky rice) to remove the sticky layer. Garlic was peeled and then ground in a universal food processor (Model CombiMax 750 : Braun, Germany) for 1 min. Other ingredients, white pepper, coriander seed, cinnamon, sugar, salt, sodium nitrate and sodium nitrite were weighed base on each formulation. Three starter cultures were used in sausage preparation. *Lactobacillus plantarum* in Lactobacilli MRS broth (Difco Laboratories, USA) was incubated in an incubator (Model D-6450 Hanau : Heraeus, Germany) at 30˚C for 24 hr. This procedure was also used for *Pediococcus cerevisiae*. *Micrococcus varians* was maintained in brain heart infusion broth (BHI, Difco Laboratories, USA) at 30˚C for 48 hr inside an incubator. These three starter cultures were then mixed together before use.

Sample preparation

Minced pork was mixed thoroughly with salt, sodium nitrate and sodium nitrite in a mechanical mixer (Model 5K5SS : KitchenAid, USA) for 1 min. Then ground garlic, white pepper, coriander seed, cinnamon and sugar were added and mixed for 1 min. Cooked sticky rice was mixed additionally for 2 min, then minced pork lard was added and mixed for 2 min. In case where starter cultures had to be added, their suspensions were poured into the mixture finally and mixed for 1 min. The mixture was then stuffed into 2.3-cm diameter collagen casings (Nippi casing, Nippi Incorporated, Japan) and tied with thread. Each piece of sausage was of ~2.5 cm in length. After that, the sausages were kept at 30˚C for 24 hr in an incubator where fermentation took place before being randomly selected for analysis.

Experimental design

In this study, 9 variables (ground garlic, white pepper, coriander seed, cinnamon, sugar, salt, sodium nitrate, sodium nitrite and starter cultures) were used as input variables. The most suitable Plackette-Burman design (N=12) was selected and provided with two dummy variables from which the error was estimated. Table 1 shows the full design matrix with each variable at two levels, "+" denoting high level and "+" low level. The 12 runs were done in random order so that no "order" bias would be introduced. The quantity of each varible at low level and high level was presented in Table 2. To calculate the effect of any variable, the average of the results at low level of that variable was subtracted from the average of the results at the high level of the same variable. Similar calculations were made for each of the effects including those of the dummy variables. The calculated effects of the dummy variables were used to test the significance of the real effects. These calculations were performed by means of Plackett-Burman design (Wiriyacharee, 1996).

Chemical and physical analysis

Uncooked fermented sausage, Sai Krok Prew which fermented at 30˚C for 24 hr from twelve treatments were taken for analysis. The pH was measured (AOAC, 2000) by pH meter (Model HI 9321: Hanna, Portugal). Total acidity was analyzed as lactic acid (AOAC, 2000) as well as the Hunter Lab values were measured with chroma meter (Model CR-310 :

Minolta Camera Co.Ltd., Japan). The shear force was analyzed by Instron Universal Testing Machine (Model 5565 : U.S.A.).

Sensory analysis

Uncooked fermented sausages were determined for color by test panel. Cooked fermented sausages, Sai Krok Prew which fermented at 30˚C for 24 hr and then cooked in a cooker at 180-240˚C for 30 min were also evaluated on color, saltiness, sourness, sour flavor, stickiness and juiciness. The sensory panel was comprised of 10 trained graduate students. The ideal ratio profile technique (Wiriyacharee, 1996) was used for the test. The ratio 1.00 indicated that no improvement had to be made for that attribute of the sample. The more the value was near 1.00, the more sausage was of good quality. The ratio less than 1.00 suggested that there must be an increase of that attribute while the ratio more than 1.00 indicated that the attribute must be decreased.

Table 1. Plackett-Burman matrix (N=12) for determination of the effects of a variable at two levels.

		Input variables										
Treatment	Experimental order	Ground garlic	White pepper	Coriander seed	Cinnamon	Sugar	Salt	Sodium nitrate	Sodium nitrite	Starter cultures	Dummy	Dummy
1	(3)	+	+	-	+	+	+	-	-	-	+	-
2	(6)	+	-	+	+	+	-	-	-	+	-	+
3	(8)	-	+	+	+	-	-	-	+	-	+	+
4	(2)	+	+	+	-	-	-	+	-	+	+	-
5	(11)	+	+	-	-	-	+	-	+	+	-	+
6	(5)	+	-	-	-	+	-	+	+	-	+	+
7	(12)	-	-	-	+	-	+	+	-	+	+	+
8	(1)	-	-	+	-	+	+	-	+	+	+	-
9	(7)	-	+	-	+	+	-	+	+	+	-	-
10	(9)	+	-	+	+	-	+	+	+	-	-	-
11	(4)	-	+	+	-	+	+	+	-	-	-	+
12	(10)	-	-	-	-	-	-	-	-	-	-	-

Table 2. Quantity of 9 input variables at low and high level.

Variables	Quantity	
	low level (-)	high level (+)
Ground garlic	5.00% of meat system	10.00% of meat system
White pepper	0.50% of meat system	1.00% of meat system
Coriander seed	0.50% of meat system	1.00% of meat system
Cinnamon	0.00% of meat system	0.20% of meat system
Sugar	0.50% of meat system	1.00% of meat system
Salt	1.00% of meat system	2.00% of meat system
Sodium nitrate	0.02% of meat system	0.05% of meat system
Sodium nitrite	0.01% of meat system	0.0125% of meat system
Starter cultures	0 cfu/g meat system	$3x10^6$ cfu/g meat system*

* This amount derived from *Micrococcus varians*, *Pediococcus cerevisiae* and *Lactobacillus plantarum at* 10^6 cfu/g meat system for each bacteria.

RESULTS AND DISCUSSION

Quality of Sai Krok Prew

The results of analysis on chemical, physical and sensory properties for all twelve treatments are shown in Tables 3 and 4. After fermentation at 30˚C for 24 hr, pH and total acidity varied from 4.25±0.03 to 4.66±0.01 and 0.61±0.03% to 0.89±0.04% respectively. In case of Nham, fermented pork sausage, added four starter cultures *(Lactobacillus plantarum, Lactobacillus brevis, Pediococcus cerevisiae and Micrococcus varians),* pH and total acidity varied from 4.35 to 5.99 and 0.45% to 1.52% respectively (Wiriyacharee et al., 1991). L values were between 61.29±0.05 and 64.71±0.27. Range of a values was 9.30±0.14 to 10.70±0.05 while the b values was 16.43±0.12 to 18.79±0.21. The positive values of a and b presented redness and yellowness of Sai Krok Prew respectively. From the measure with Instron Universal Testing Machine, the shear forces were between 20.04±0.33 N to 24.01±0.33 N. In case of sixteen treatments of Nham, shear forces varied from 15.98 N to 33.20 N (Wiriyacharee et al., 1991). This might show that the texture of the whole Sai Krok Prew treatments was less different than that of all Nham treatments. The sensory ratio about color of the uncooked fermented Sai Krok Prew varied from 0.81±0.12 to 0.94±0.12 while from 0.84±0.15 to 1.04±0.08 for the cooked fermented Sai Krok Prew. When comparing those values with ideal ratio 1.00, it might indicate that color of the cooked fermented sausage was more acceptable than that of the uncooked fermented one because the color ratio of the cooked fermented sausage was closer to 1.00 than of the uncooked fermented one. Additionally, the minimum ratio of the cooked fermented sausage was : saltiness 0.81±0.34; sourness 0.91±0.18; sour flavor 0.87±0.24 while the maximum ratio was : saltiness 1.17±0.12; sourness 1.11±0.11; sour flavor 1.08±0.18. Both stickiness and juiciness had ratio less than 1.00.

Effect of ground garlic

Ground garlic reduced pH of Sai Krok Prew significantly ($p \leq 0.20$) (Table 5). This agreed with observation in Nham which was added with raw garlic 3% and 7% of meat system for low level and high level respectively (Wiriyacharee et al., 1991). Fermented meat product composed of garlic had higher lactic acid production rate than without garlic (Niyomvit and Rotejanapaiboon, 1983). Besides, addition of garlic into product could deodorize smell of raw meat and prevent the growth of fungi (Kaewbol, 1982).

Effect of white pepper

It was found that (Table 6) white pepper affected two sensory attributes significantly ($p \leq 0.20$). Color ratio of uncooked fermented sausage and sourness ratio of cooked fermented sausage were increased to ideal ratio (1.00) when using high level of white pepper. However, both low and high levels of white pepper did not affect any chemical and physical properties.

Effect of coriander seed

Using high level of coriander seed increased significantly ($p \leq 0.20$) color of both uncooked and cooked fermented sausage to ideal ratio better than using low level (Table 6).

Table 3. Chemical and physical results of Sai Krok Prew (uncooked) after 24 hours fermentation at 30˚C.

Treatment	pH	Total acidity (%)	Color value			Shear force (N)
			L	a	b	
1	4.35±0.01	0.78±0.05	64.71±0.27	9.95±0.06	16.46±0.34	21.25±0.40
2	4.27±0.01	0.87±0.03	63.35±0.06	10.40±0.03	16.49±0.16	24.01±0.33
3	4.66±0.01	0.61±0.03	61.73±0.33	9.86±0.11	16.60±0.22	22.44±0.22
4	4.40±0.01	0.75±0.07	62.86±0.26	10.18±0.05	16.43±0.12	21.24±0.33
5	4.38±0.01	0.76±0.03	61.36±0.37	10.40±0.03	17.89±0.40	23.37±0.37
6	4.30±0.01	0.85±0.03	62.56±0.17	10.41±0.08	16.50±0.17	23.51±0.07
7	4.47±0.02	0.72±0.05	62.51±0.05	10.64±0.08	17.32±0.12	20.04±0.33
8	4.39±0.01	0.75±0.04	62.80±0.04	10.50±0.13	17.27±0.03	20.11±0.14
9	4.25±0.03	0.89±0.04	63.05±0.13	10.70±0.05	17.03±0.09	20.67±0.24
10	4.58±0.01	0.67±0.03	61.29±0.05	10.17±0.08	16.86±0.21	20.46±0.20
11	4.32±0.01	0.80±0.01	62.33±0.17	10.50±0.12	18.79±0.21	20.63±0.21
12	4.55±0.02	0.68±0.03	62.19±0.05	9.30±0.14	18.07±0.13	21.35±0.40

Table 4. Mean ideal ratio scores of Sai Krok Prew (uncooked and cooked) after 24 hours fermentation at 30˚C.

Treatment	Uncooked fermented sausage	Cooked fermented sausage					
	Color	Color	Saltiness	Sourness	Sour	Stickiness flavor	Juiciness
1	0.89±0.09	0.97±0.13	1.02±0.03	1.01±0.10	0.98±0.09	0.94±0.12	0.89±0.07
2	0.87±0.14	0.93±0.11	0.85±0.19	1.03±0.06	0.97±0.13	0.93±0.16	0.93±0.07
3	0.91±0.10	0.97±0.13	0.88±0.32	0.93±0.24	0.93±0.24	0.93±0.27	0.92±0.05
4	0.92±0.07	0.95±0.13	0.86±0.28	1.04±0.16	0.98±0.12	0.92±0.19	0.93±0.11
5	0.92±0.08	0.96±0.11	1.06±0.13	1.01±0.07	1.00±0.02	0.94±0.19	0.94±0.07
6	0.84±0.12	0.84±0.15	0.98±0.16	1.06±0.12	1.04±0.09	0.94±0.22	0.88±0.09
7	0.89±0.12	0.97±0.10	0.97±0.24	1.06±0.10	1.01±0.08	0.90±0.19	0.96±0.04
8	0.94±0.12	1.04±0.08	1.02±0.16	1.04±0.13	1.03±0.11	0.91±0.19	0.89±0.02
9	0.90±0.09	0.96±0.09	0.99±0.18	1.11±0.11	1.08±0.18	0.95±0.20	0.93±0.04
10	0.91±0.10	0.97±0.13	1.17±0.12	1.02±0.11	0.99±0.14	0.96±0.21	0.92±0.06
11	0.88±0.10	0.94±0.10	1.04±0.14	1.10±0.10	1.02±0.17	0.98±0.23	0.95±0.04
12	0.81±0.12	0.85±0.16	0.81±0.34	0.91±0.18	0.87±0.24	0.90±0.27	0.92±0.05

Effect of cinnamon

The study showed that there was no significant ($p>0.20$) difference in quality of Sai Krok Prew using both low and high level of cinnamon. At low level, there was no cinnamon while at high level, 0.20% cinnamon was added. In this study, we considered that cinnamon could be omitted from Sai Krok Prew ingredients in order to reduce production cost.

Effect of sodium nitrate

Sodium nitrate significantly ($p \leq 0.05$) affected saltiness, sourness and sour flavor of cooked Sai Krok Prew as well as saltiness ($p \leq 0.15$) (Tabel 6). These attributes approached ideal ratio when using high level of sodium nitrate. Nitrate substance enhanced flavor of fermented meat product causing it to be accepted by consumer more than that added by salt only (Surapanpisit, 1993). High level of sodium nitrate also increased the a value of Sai Krok

Prew significantly ($p \leq 0.20$) which indicated that the product had deeper red color. Nitrates added to meat are reduced via nitrite to nitric acid which can combine with heme pigments to form the red color typical of cured meats (Hutchings, 1994).

Effect of sodium nitrite

Sodium nitrite in curing mixtures for meats is able to develop and fix the color, inhibit microorganisms, and develop characteristic flavors (Lindsay, 1985). Table 6 shows that sodium nitrite affected some sensory qualities of Sai Krok Prew. High level of sodium nitrite significantly ($p \leq 0.20$) increased color acceptance of uncooked Sai Krok Prew to ideal ratio much more than low level. Besides, saltiness and sour flavor were better accepted when using high level of sodium nitrite. Nitrites in meat form nitric oxide which reacts with heme compounds to form nitrosomyoglobin, the pigment responsible for the pink color of cured meats (Lindsay, 1985). In this study, high level of sodium nitrite was 0.0125% which was slightly different from 0.015% sodium nitrite added by González and Díez (2002) in the "chorizo" which is the most popular Spanish dry fermented sausage.

Effect of sugar

High level of sugar improved two sensory qualities of cooked Sai Krok Prew. Sourness and sour flavor of sausage, using high level of sugar were closer to ideal ratio than sausage using low level strongly significantly ($p \leq 0.05$) (Table 6). Usage of sugar at high level decreased pH and increased total acidity significantly ($p \leq 0.05$) (Table 5). The products of carbohydrate fermentation (lactic acid with small amount of acetic acid) give the sausages their typical 'tangy' taste (Lücke, 1995). High level of sugar increased L value and a value significantly ($p \leq 0.20$) (Table 5) which resulted in the increase of whiteness and redness of Sai Krok Prew.

Effect of salt

Table 6 indicates that with more salt, the more saltiness was accepted. Beside the effect of high level of sugar as mentioned above, sourness and sour flavor of cooked Sai Krok Prew also approached ideal ratio significantly ($p \leq 0.10$) with addition of high level of salt. High level of salt, additionally, resulted in color of uncooked and cooked Sai Krok Prew to be more accepted significantly at $p \leq 0.20$ and $p \leq 0.15$ respectively. This might be that *Micrococcus varians* could grow in system at this level of salt and then contribute to the stability of red color in product using nitrate and nitrite.

Effect of starter cultures

Usage of starter cultures enhanced several qualities of Sai Krok Prew. They decreased pH ($p \leq 0.10$), increased total acidity ($p \leq 0.20$) and a value ($p \leq 0.15$) (Table 5). They also affected color of both uncooked and cooked Sai Krok Prew significantly ($p \leq 0.15$ and $p \leq 0.20$ respectively) (Table 6). From result in the Table 4, color ratio average from six treatments using starter cultures for both uncooked and cooked Sai Krok Prew was closer to 1.00 than from other six treatments which did not use starter cultures. Correspondingly, sourness and sour flavor were more accepted in Sai Krok Prew using starter cultures. This might be because *Lactobacillus plantarum* and *Pediococcus cerevisiae* produced lactic acid which reduced pH of Sai Krok Prew and at low pH system, nitrite which was reduced from

nitrate by *Micrococcus varians* might decompose rapidly to nitrous acid and nitric acid which played on important role in the formation of stable red color in cured meat.

Table 5. Effects and significance level of input variables on chemical and physical qualities of Sai Krok Prew (uncooked).

Input variables	Response variables					
	pH	Total acidity	Color value			Shear force
		(%)	L	a	b	(N)
Ground garlic	-0.060[a]	0.038	0.253	0.002	-0.742	1.433
White pepper	-0.033	0.008	0.223	0.028	0.115	0.020
Coriander seed	0.053	-0.038	-0.337	0.035	-0.138	-0.217
Cinnamon	0.040	-0.008	0.423	0.072	-0.698	-0.223
Sugar	-0.193[d]	0.125[d]	1.143[a]	0.318[a]	-0.105	0.213
Salt	0.010	-0.028	-0.123	0.218	0.578	-1.227
Sodium nitrate	-0.047	0.038	-0.257	0.365[a]	0.025	-0.997
Sodium nitrite	0.033	-0.012	-0.860	0.178	-0.235	0.340
Starter cultures	-0.100[c]	0.058[a]	0.187	0.438[a]	-0.142	-0.033

[a] t-test significance level at 80%, [b] t-test significance level at 85%, [c] t-test significance level at 90%, [d] t-test significance level at 95%

Table 6. Effects and significance level of input variables on sensory qualities of Sai Krok Prew (uncooked and cooked).

Input variables	Response variables						
	Uncooked fermented sausage	Cooked fermented sausage					
	Color	Color	Saltiness	Sourness	Sour flavor	Stickiness	Juiciness
Ground garlic	0.003	-0.018	0.038	0.003	0.003	0.010	-0.013
White pepper	0.027[a]	0.025	0.008	0.013	0.013[a]	0.020	0.010
Coriander seed	0.030[a]	0.042[a]	-0.002	0.000	-0.010	0.010	0.003
Cinnamon	0.010	0.032	0.018	0.000	0.003	0.003	0.007
Sugar	-0.007	0.002	0.025	0.063[d]	0.057[d]	0.017	-0.020
Salt	0.030[a]	0.058[b]	0.152[d]	0.027[c]	0.027[c]	0.010	0.007
Sodium nitrate	0.000	-0.015	0.062[b]	0.077[d]	0.057[d]	0.017	0.013
Sodium nitrite	0.027[a]	0.022	0.092[c]	0.003	0.040[d]	0.010	-0.017
Starter cultures	0.033[b]	0.045[a]	-0.025	0.043[d]	0.040[d]	-0.017	0.017

[a] t-test significance level at 80%, [b] t-test significance level at 85%, [c] t-test significance level at 90%, [d] t-test significance level at 95%

CONCLUSIONS

Starter cultures, sugar and salt significantly affected 7, 6 and 5 out of 13 properties which were analyzed while other input variables affected less than 5. This can be concluded that starter cultures, sugar and salt are the main factors determining the final qualities of Sai Krok Prew while ground garlic, white pepper, coriander seed, sodium nitrate and sodium nitrite are the minor factors.

ACKNOWLEDGEMENTS

The authors would like to thank National Science and Technology Development Agency (NSTDA), Thailand for support the research fund and Faculty of Agro-Industry, Chiang Mai University for kind assistance in conducting the research work.

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