Optimizing Formulation and Fermentation Time of Thai Fermented Pork Sausage (Sai Krok Prew) Using Starter Cultures

Panthitra Phromraksa[1], Pairote Wiriyacharee[2*], Lakkana Rujanakraikarn[3] and Pattawara Pathomrungsiyungkul[4]

[1] *Department of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50200, Thailand*
[2] *Department of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50200, Thailand*
[3] *Food Technology Program, School of Agro-Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[4] *Department of Food Engineering, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50200, Thailand*

**Corresponding author. E-mail: deanagro@chiangmai.ac.th*

ABSTRACT

Ingredients (0.7-2.5% sugar and 1.5-3% salt), raw materials (35-65% minced pork, 15-35% minced pork lard and 20-40% sticky rice) and starter cultures (Lactobacillus plantarum, Pediococcus cerevisiae and Micrococcus varians at 4-8 Log cfu/g of each bacteria) in Sai Krok Prew formulation were optimized. Fermentation time (24, 36 and 48 hr) was also investigated. Sausage qualities were determined by measuring pH, total acidity as lactic acid, color Lab, shear force and sensory evaluation. The suitable formulation was the addition of 1.6% sugar, 2.07% salt, 6 Log cfu/g for each bacteria and using minced pork, minced pork lard and sticky rice at ratio of 46.84:23.60:29.56. The optimum fermentation time was 24 hr.

Key words : Fermented sausages, Starter cultures, Product development

INTRODUCTION

Sai Krok Prew or Sai Kork E Sarn, a kind of Thai fermented sausage, is made from pork, pork lard, cooked rice and seasoning, stuffed into pork casing or other edible casings and then fermented until becoming sour, and cooked before eating (Thai Industrial Standards Institute, 1994). Starter cultures have been used in the sausage production to reduce fermentation time, ensure low residual nitrate and nitrite contents in the end product and standardize the organoleptic characteristics (Hugas and Monfort, 1997). Lactic acid bacteria as well as *Micrococcaceae* strains, important microorganisms used as starter cultures in meat fermentation, improve safety and stability of the product, extending the shelf life and provides diversity resulting in new sensory properties as well as health benefits by probiotic characteristic (Lücke, 2000).

In our previous study (Phromraksa et al., 2003), Sai Krok Prew, using starter cultures strains of *Lactobacillus plantarum, Pediococcus cerevisae* and *Micrococcus varians* at amount 10^6 cfu/g meat system for each bacteria had higher total acidity, more redness measured by instrument and lower pH, and was more acceptable on color, sourness and sour flavor as evaluated by panel than sausage without starter cultures. The initial concentrations of starter cultures may vary in accordance with the type of sausage and species of bacteria. For example, *L. plantarum, P. cerevisae* and *M. varians* amounts of 10^3,10^6 and 10^3 cfu/g of meat system respectively were supposed to be in Nham (Boonthanom et al., 1994). *L. plantarum* and *Staphylococcus carnosus* (50%) were added to obtain an initial concentration in the ground meat at 107 cfu/g for preparing the dry fermented pork sausage (Ibañez et al., 1996). *L. sake* CL35 was inoculated to obtain an initial population of 10^3-10^4 cfu/g in chorizo (González and Díez, 2002). However, there is no report about the optimum initial concentration of *L. plantarum, P. cerevisae* and *M. varians* in Sai Krok Prew production.

Sucrose and sodium chloride are typical ingredients for Sai Krok Prew preparation. In a previous study (Phromraksa et al., 2003), the authors identified that both ingredients were the main factors affecting the qualities of Sai Krok Prew. Various sugar including sucrose are added to the formulation to achieve the desired flavor, texture, and yield characteristics (Bacus, 1984). The amount of carbohydrate added are important because they determine the rate and extent of lactic acid formation and the composition of the sausage microflora (Lücke, 1998). Sodium chloride added in the raw sausage mixtures affects microorganism growth, interacts with the myofibrillar and solubilizes proteins which form a sticky film around meat particles and contributes obviously to the taste of meat products (Lücke, 1998). The suitable content of these two ingredients would be determined in the present study.

In our previous study (Phromraksa et al., 2003), the 'meat system' which consisted of pork, pork lard and cooked sticky rice, another carbohydrate source for lactic acid bacteria, was fixed in all treatments because we aimed to screen the effect of other ingredients. Thus, further study would be to investigate the optimum ratio of meat system for activity of starter cultures and development of sensory qualities of Sai Krok Prew. Mixture design, a statistical experiment planning, is used to investigate the suitable amounts of three independent variables in food formulation development (Wiriyacharee, 2004).

There were some reports about fermentation time for various sausages using starter cultures. Among them were 2 days at 30˚C for Nham (Wiriyacharee et al., 1993), 3 days at 14±1˚C for fermented pork sausage (Ibañez et al., 1996) and 4 days at 20-22˚C for salami (Böhme et al., 1996). The rate of fermentation and the ultimate pH of the meat product are directly influenced by the specific formulation and processing conditions, as well as the type and activity of the culture employed (Bacus, 1984). Therefore, fermentation time for Sai Krok Prew using starter cultures would be investigated. The main restricting factor was the sensorial qualities of products.

The objectives of this study were to optimize amount of sucrose, salt, pork lard, cooked sticky rice and starter cultures and to determine the suitable fermentation time of Sai Krok Prew.

MATERIALS AND METHODS

Materials

Minced pork (lean meat), minced pork lard, cooked sticky rice, garlic and white pepper were purchased from a local market (Chiang Mai, Thailand). Cooked sticky rice was rinsed through 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. Sodium nitrate and sodium nitrite (food grade) were bought from a local chemical shop. 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.

Fundamental sausage formulation

Minced pork, minced pork lard and cooked sticky rice (50:25:25) were called “meat system”. Other ingredients were : ground garlic (10), white pepper (1.00), coriander seed (1.00), sodium nitrate (0.05), sodium nitrite (0.0125), sucrose* (0.70) and salt* (1.50) (weight % based on meat system weight). Three starter cultures* were added at 6 Log cfu/g meat system for each bacteria.

Note : When the ingredients and starter cultures with sign* were the independent variables which were studied, their new levels would be defined as present in the experiment design context.

Fundamental sausage production

The required quantities of minced pork, salt, sodium nitrate and sodium nitrite were mixed in a mechanical mixer (Model 5K5SS : KitchenAid, USA) for 1 min. Then ground garlic, white pepper, coriander seed 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. Finally, starter cultures were poured into the mixture and mixed for 1 min. The mixture was then stuffed into 2.3-cm diameter collagen casings (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.

Note : In Experiment 4, fermentation time was the independent variables which must be studied. More detail was described in the experimental design part.

Experimental design and statistical analysis

This research was composed of four experiments as described below.

Experiment 1 : Determination of the optimum content of sugar and salt

Sugar and salt, two main factors, consisted of five levels : lowest level, low level, center level, high level and highest level (Table 1). A 2^2 factorial experiment in central composite design was used (Table 2). All analyzed qualities and levels of two main factors were computed by using SPSS 10.0 to form the stepwise multiple regression equations which

were coded equations. Then these equations were decoded by using Mathcad 7 professional program. These decoded equations could demonstrate the effect of both main factors on sausage qualities and be used to determine the suitable amount of each factor. Both suitable amounts would be used in the following experiments.

Table 1. Quantities of sugar and salt at lowest, low, center, high and highest level.

Factor	Lowest level	Low level	Center level	High level	Highest level
($-\alpha$)	(-1)	(0)	(+1)	($+\alpha$)	
Sugar = a	0.70%	0.96%	1.60%	2.24%	2.50%
Salt = b	1.50%	1.72%	2.25%	2.78%	3.00%

Table 2. Treatments planned by a 2^2 factorial experiment in central composite design.

Treatment	Code*	Sugar (%)	Salt (%)
1	(1)	0.96	1.72
2	a	2.24	1.72
3	b	0.96	2.78
4	ab	2.24	2.78
5	(αa	0.70	2.25
6	+αa	2.50	2.25
7	(αb	1.60	1.50
8	+αb	1.60	3.00
9	cp_1	1.60	2.25
10	cp_2	1.60	2.25
11	cp_3	1.60	2.25

* a = sugar; b = salt; + = high level; – = low level; (1) = control; and cp_1, cp_2, and cp_3 = center point

Experiment 2 : Determination of the optimum amount of starter cultures

A 2^3 factorial experiment with 3 center points design was used to investigate the effect of each starter culture on sausage qualities and to conclude the optimum amount of each bacteria for Sai Krok Prew production. Eleven-treatment sausages were prepared, using the fundamental sausage formulation as described in the Experiment 1 but varying the amount of three starter cultures as shown in Table 3. Statistical analysis of data was performed similar to the Experiment 1. The optimum amount concluded from this study would be used in sausage production of following experiments.

Table 3. Treatments planned by a 2^3 factorial experiment with 3 center points design.

Treatment	Code*	*Micrococcus varians* (Log cfu/g meat system)	*Pediococcus cerevisiae* (Log cfu/g meat system)	*Lactobacillus plantarum* (Log cfu/g meat system)
1	(1)	4	4	4
2	a	8	4	4
3	b	4	8	4
4	ab	8	8	4
5	c	4	4	8
6	ac	8	4	8
7	bc	4	8	8
8	abc	8	8	8
9	cp1	6	6	6
10	cp2	6	6	6
11	cp3	6	6	6

* a = *M. varians;* b = *P. cerevisiae;* c = *L. plantarum;* (1) = control; and cp_1, cp_2, and cp_3 = center point

Experiment 3 : Determination of the optimum ratio of meat system

A mixture design (Wiriyacharee, 2004) was used to determine the optimum ratio of meat system. Sausages of each treatment were produced from different ratio of meat system (Table 4). The optimum ratio determined from this study would be used in next experiment.

Table 4. Treatments planned by a mixture design.

Treatment	Minced pork (%)	Minced pork lard (%)	Sticky rice (%)
1	65	15	20
2	45	35	20
3	45	15	40
4	35	35	30
5	35	25	40

Experiment 4 : Determination of the optimum fermentation time

Three replications of sausages were manufactured according to formulation developed through the preceding experiments. The fundamental sausage production was used for every treatment. The only variation in the process was fermentation time. Sausages were kept at 30˚C for 24, 36 or 48 hr. A completely randomized design was used in this study to determine the optimum fermentation time. Analyses of variance were performed, using the program SPSS 10.0. The differences of means between pairs were resolved by LSD test to obtain the confidence intervals. Significance was defined at $p \leq 0.05$.

Chemical and physical analysis

pH values were measured (AOAC, 2000) by pH meter (Model HI 9321: Hanna, Portugal). Total acidity values were analyzed as lactic acid (AOAC, 2000) and Hunter Lab values were measured by chroma meter (Model CR-310 : Minolta Camera Co.Ltd., Japan). Shear force values were analyzed by Universal Testing Machine (Model 5565 : Instron, U.S.A.).

Sensory analysis

Uncooked fermented sausages were determined for color by test panel while cooked fermented sausages were evaluated on color, saltiness, sourness, sour flavor, stickiness and juiciness. Cooking sausages was done in a cooker at 180-240˚C for 30 min. The sensory panel was comprised of 10 trained graduate students. The ideal ratio profile technique (Wiriyacharee, 2003) 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 the 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.

RESULTS AND DISCUSSION

Determination of the optimum content of sugar and salt

Range of chemical and physical, and sensory qualities of sausages is shown in Tables 5 and 6 respectively.

Table 5. Range of chemical and physical qualities of 11 treatments of sausages*.

Value	pH	Total acidity (%)	Color value			Shear force (N)
			L	a	b	
Min.	4.27±0.03	0.76±0.04	58.86±0.24	9.36±0.28	16.13±0.11	20.97±0.50
Max.	5.01±0.01	0.95±0.07	65.86±0.36	11.61±0.09	17.49±0.88	26.38±0.46

*Values in the table are mean ± standard deviation.

Table 6. Range of sensory qualities of 11 treatments sausages*.

Value	Uncooked fermented sausages	Cooked fermented sausages					
	Color	Color	Saltiness	Sourness	Sour flavor	Stickiness	Juiciness
Min.	0.94±0.11	0.94±0.21	0.88±0.15	0.90±0.12	0.92±0.07	0.85±0.27	0.74±0.27
Max.	1.14±0.18	1.05±0.18	1.17±0.39	1.03±0.07	1.01±0.12	0.97±0.16	0.90±0.17

*Values in the table are mean ± standard deviation.

Stepwise multiple regression analysis indicated that pH, color of uncooked sausages and saltiness were affected by salt and/or sugar (Eqs. 1, 2 and 3). Other qualities were not affected by these two factors.

$$pH = 5.05999 - 0.85199\ S + 0.23644\ S^2 \quad ; R^2 = 0.7110 \quad (1)$$

$$\text{Color of uncooked sausages} = 0.98577 - 0.22257\ Su + 0.08190\ S + 0.06955\ Su^2 \quad ; R^2 = 0.8380 \quad (2)$$

$$\text{Saltiness of cooked sausages} = 0.77294 + 0.10669\ S \quad ; R^2 = 0.8480 \quad (3)$$

Where S = Salt content (%) and Su = Sugar content (%).

Fig.1 demonstrates the relationship of pH and salt content (1.5 - 3.0%), based on quadratic equation (Eq.1). Sugar content (0.7 - 2.5%) had no influence on pH. Since lactic-acid-producing bacteria, used as starter cultures in this study, was capable of salt toleration, they could grow and produce lactic acid which had relationship with pH. The curve (Fig.1) indicated that addition of 1.72% salt resulted in the lowest pH.

The effect of sugar and salt content (0.7 - 2.5% and 1.5 - 3.0%, respectively) on uncooked sausage color was demonstrated by response surface plot (Fig. 2) which was plotted according to a quadratic regression mode (Eq. 2) fitted to their relationship.

Color of uncooked sausages (0.99) was closest to 1.00 when adding 1.6% sugar and 2.25% salt. This was due to a salt toleration of starter cultures and their ability to grow by using sugar substrate. Lactic acid produced by them accelerated the formation of nitric oxide which was the important factor for sausage color formation. Moreover, *M. varians* bacteria could reduce nitrate into nitrite, thus enhancing the satisfied red color of product.

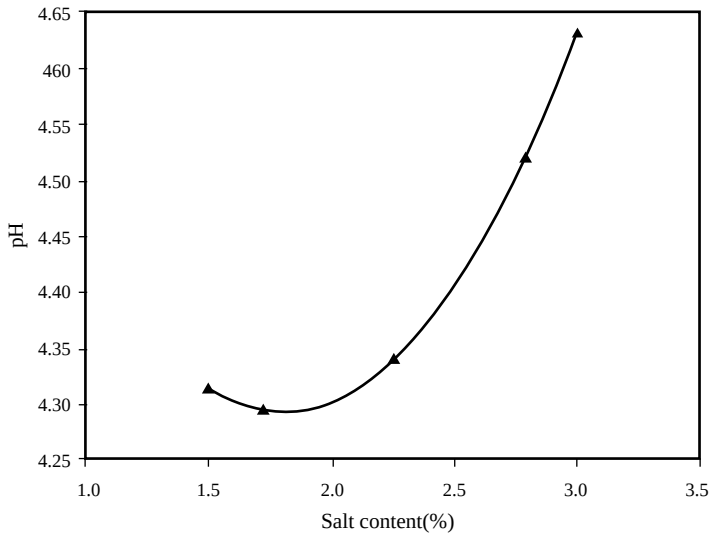

Figure 1. Relationship of salt content (1.5-3.0%) and pH.

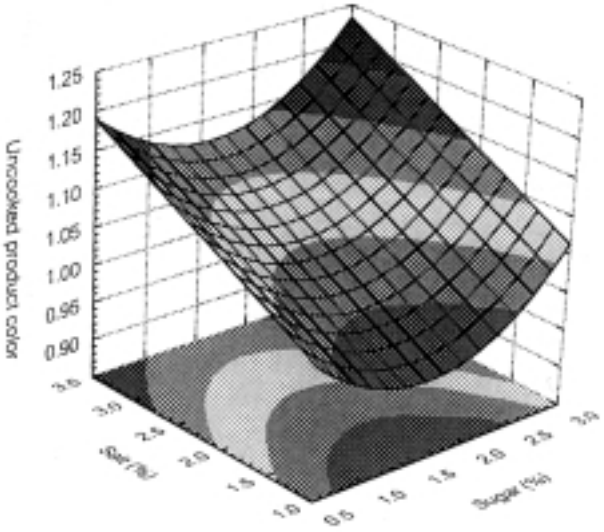

Figure 2. Response surface plot of uncooked sausage color as a function of salt and sugar content.

Saltiness of cooked sausages was a function of salt content. When linear equation of salt content ranged from 1.5% to 3.0%, saltiness developed (Eq. 3). The more salt content added, the more saltiness was accepted. The most saltiness accept, saltiness = 1.01, was for sausages processed with 2.25% salt.

Considering Eqs. 1, 2 and 3, sugar had effect only on color of uncooked sausages as shown in Eq.2. Thus the optimum content of sugar was 1.6%.

Since different salt contents resulted in sausages having the appreciative qualities on pH, color of uncooked sausages and saltiness of cooked sausages, those salt contents had to be averaged to determine the optimum level (Table 7). The suitable content was 2.07% salt. If substitute the suitable content of sugar (1.60%) and salt (2.07%) into Eqs.1, 2 and 3 to predict pH, color of uncooked sausage and saltiness, the results would be 4.31, 0.98 and 0.99 respectively.

Table 7. Optimum level of sugar and salt.

Quality	Sugar (%)	Salt (%)
pH	-	1.72
Color of uncooked sausages	1.60	2.25
Saltiness of uncooked sausages	-	2.25
Optimum content	1.60	2.07

Determination of the optimum amount of starter cultures

Range of chemical and physical, and sensory qualities of sausages is shown in Tables 8 and 9 respectively. Eqs. 4-10 demonstrate the relationship of some qualities and number of starter cultures.

Table 8. Range of chemical and physical qualities of sausages, varying number of starter cultures*.

Value	pH	Total acidity	Color value			Shear force
		(%)	L	a	b	(N)
Min.	4.28±0.01	0.54±0.03	58.75±0.29	9.31±0.27	15.78±0.18	19.37±0.41
Max.	5.03±0.01	0.81±0.02	66.24±0.44	11.83±0.30	19.07±0.26	29.14±0.44

*Values in the table are mean ± standard deviation.

Table 9. Range of sensory qualities of sausages, varying number of starter cultures*.

Value	Uncooked fermented sausages	Cooked fermented sausages					
	Color	Color	Saltiness	Sourness	Sour flavor	Stickiness	Juiciness
Min.	0.70±0.23	0.80±0.17	0.81±0.20	0.93±0.13	0.83±0.11	0.90±0.31	0.88±0.27
Max.	0.98±0.32	0.94±0.18	1.13±0.36	1.22±0.18	1.02±0.09	1.13±0.31	1.03±0.27

*Values in the table are mean ± standard deviation.

$$L \text{ value } = 72.813 - 5.115\ M + 0.511\ M^2 \quad ; R^2 = 0.7790 \quad (4)$$

$$a \text{ value } = 2.617 + 3.144\ M - 0.201\ P - 0.247\ M^2 \quad ; R^2 = 0.8940 \quad (5)$$

$$b \text{ value } = 18.117 + 0.2615\ M - 0.3765\ P \quad ; R^2 = 0.7350 \quad (6)$$

$$\text{Color of uncooked sausages } = -0.904 + 0.6495\ M - 0.056\ M^2 \quad ; R^2 = 0.9040 \quad (7)$$

$$\text{Sourness of cooked sausages} = 1.33725 - 0.252\ P + 0.07\ L - 0.00656\ PL + 0.02725\ P^2 \quad ; R^2 = 0.9870 \quad (8)$$

$$\text{Sour flavor of cooked sausages} = 0.14197 + 0.22251M + 0.015\ P + 0.01625L - 0.01854\ M^2 \quad ; R^2 = 0.8480 \quad (9)$$

$$\text{Juiciness of cooked sausages} = 1.1085 - 0.02375\ M \quad ; R^2 = 0.8110 \quad (10)$$

Where M = number of *M. varians* (Log cfu/g), P = number of *P. cerevisiae* (Log cfu/g) and L = number of *L. plantarum* (Log cfu/g).

Only one of starter cultures, *M. varians,* affected L value of sausages (Eq.4). Both *M. varians* and *P. cerevisiae* resulted in the change of both a value and b value (Eqs.5 and 6). There was no any relationship between *L. plantarum* number and instrumental color values.

Again, *M. varians* had relationship solely to color of uncooked sausages (Eq. 7). Fig. 3 indicates that the value of this quality (0.98) is closest to 1.00 when sausages are processed with 6 Log cfu/g *M. varians* content. This is in consistence with data reported by Campbell - Platt and Cook (1995) that the suitable amount of *M. varians* to reduce nitrate into nitrite which resulted in the most favorable red formation of sausages was 6 Log cfu/g.

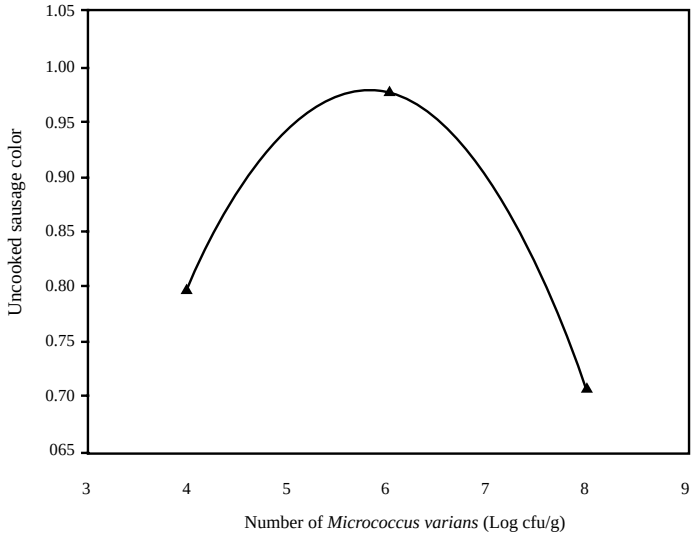

Figure 3. Relationship of number of *Micrococcus varians* and uncooked sausages color.

Sourness of cooked sausages was influenced by *P. cereviseae, L. plantarum* and by the interaction between these two starter cultures (Eq.8). When the objective was to develop sourness to 1.00, the highest sourness was located in the vicinity of 6 Log cfu/g for these two starter cultures (Fig. 4).

Effect of three starter cultures on sour flavor of cooked sausages is shown in Eq. 9. Campbell - Platt and Cook (1995) and González and Díez (2002) stated that *M. varians* played an important role on flavor quality of fermented sausages but it had to be used with lactic-acid-producing bacteria to produce fermented sausage flavor which was accepted by consumer.

Response surface plot which demonstrated the effects of any three independent variables could not be plotted, therefore one of these variables had to be fixed as constant. In this case, it was a variable that had a minimum influence on sour flavor. Considering the coefficients of each independent factor in Eq.9, regardless of the positive and negative sign, it was found that the coefficient of *P. cerevisiae* content was the lowest value, therefore number of this bacteria would be fixed as constant. Determination of this constant was performed by substituting contents of three bacteria into Eq.9. as shown partly in Table 10. It was found that the highest sour flavor value (0.997) was obtained by addition of 6 Log cfu/g *P. cerevisiae* content. Thus, new equation of sour flavor, calculated by fixing content of this bacteria would be :

$$\text{Sour flavor} = 0.23197 + 0.22251M + 0.01625L - 0.01854M^2 \qquad (11)$$

Reponse surface plot (Fig. 5), resulting from Eq. 11 indicated that the highest sour flavor value was observed for sausages manufactured with 6 Log cfu/g *M. varians* and *L. plantarum* additions.

M. varians was the only one variable that affected juiciness of products. Their linear relationship is demonstrated in Eq. 10. By substituting juiciness value of 1.00 into Eq. 10, it was found that content of *M. varians* should be ~ 4.6 Log cfu/g. However, 6 Log cfu/g *M. varians* was the optimum number to improve color of uncooked sausages close to 1.00. When substitute M = 6 Log cfu/g into Eq. 10, juiciness from calculation was 0.97 which was a reasonable result.

In conclusion, the optimum amounts of three starter cultures for Sai Krok Prew production were 6 Log cfu/g for each bacteria.

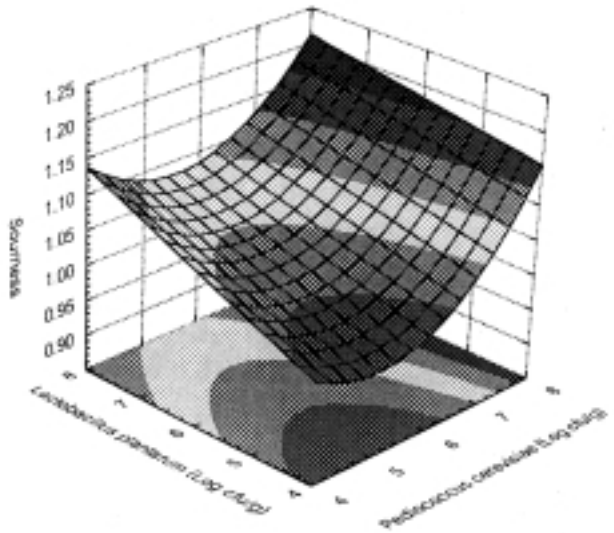

Figure 4. Response surface plot on sourness of cooked sausages as a function of *Pediococcus cerevisiae* and *Lactobacillus plantarum* number.

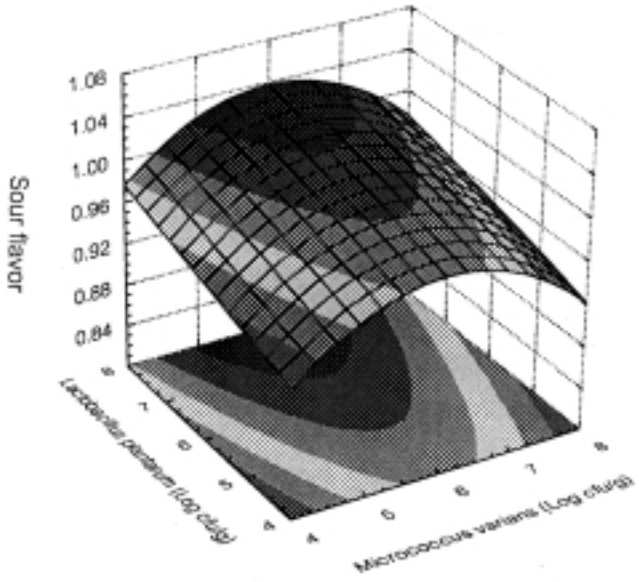

Figure 5. Response surface plot on sour flavor of cooked sausages as a function of *Micrococcus varians* and *Lactobacillus plantarum* number.

Table 10. Substituted Sourness Equations Varying Number of Starter Cultures.

Content (Log cfu/g)			Substituted Equations of Sourness	Ideal ratio of Sourness
M	P	L		
8	4	8	$0.14197 + 0.22251(8) + 0.01500(4) + 0.01625(8) - 0.01854(8)^2$	0.925
4	8	8	$0.14197 + 0.22251(4) + 0.01500(8) + 0.01625(8) - 0.01854(4)^2$	0.985
8	8	8	$0.14197 + 0.22251(8) + 0.01500(8) + 0.01625(8) - 0.01854(8)^2$	0.985
6	6	6	$0.14197 + 0.22251(6) + 0.01500(6) + 0.01625(6) - 0.01854(6)^2$	0.997

M = *M. varians*, P = *P. cerevisiae* and L = *L. plantarum*.

Determination of the optimum ratio of meat system

Sensory qualities of five treatments (data not shown) were analyzed serially by linear regression, partial derivatives with Lag range technique and POM linear program to investigate the suitable ratio of meat system to each sensory quality. The results studied by POM linear program are shown in Table 11. Overall, the suitable ratio of meat system (46.84 ± 1.43% mince pork, 23.60 ± 1.17% mince pork lard and 29.56 ± 1.49% sticky rice) was obtained by averaging values in the same column of Table 11.

Table 11. The optimum ratio of meat system from a linear program POM.

Quality	Minced pork (%)	Minced pork lard (%)	Sticky rice (%)
Color of uncooked sausage	47.89	23.28	28.83
Color of cooked sausage	45.27	23.72	30.99
Saltiness of cooked sausage	45.87	22.69	31.45
Sourness of cooked sausage	45.33	24.87	29.80
Sour flavor of cooked sausage	47.23	25.13	27.64
Stickiness of cooked sausage	47.15	22.49	30.36
Juiciness of cooked sausage	49.14	23.01	27.87
Average	46.84	23.60	29.56
Standard deviation	1.43	1.17	1.49

Determination of the optimum fermentation time

Table 12 shows the chemical, physical and sensory qualities of Sai Krok Prew fermented for 24, 36 and 48 hr. Increase in fermentation time significantly ($p \leq 0.05$) decreased pH and increased total acidity. This means that lactic-acid-producing bacteria, used as starter cultures, can produce more lactic acid when fermentation time is longer. No significant difference in L and b values (lightness and yellowness, respectively) ($p \geq 0.05$) was found. However, the differences between a values (redness) were significant ($p \leq 0.05$). The highest redness was found in products fermented for 24 hr. Again, fermentation time had no significant effect ($p \geq 0.05$) on shear force.

Varying the hour of fermentation had significant effect ($p \leq 0.05$) on five sensory qualities (Table 12). The most acceptance on color of uncooked sausages was found in samples fermented for 36 hr. However, their values were not significantly ($p \geq 0.05$) different from those of samples fermented for 24 hr. Cooked products from incubation for 24 hr was most acceptable on color and sourness. There were no significant differences ($p \geq 0.05$) in sour flavor between treatment fermented for 24 hr and 48 hr. Their values were nearest to 1.00. Sausages fermented for 48 hr were evaluated. It was found that their juiciness was 1.00, similar to that of products fermented for 24 hr ($p \geq 0.05$). However, panelists found no significant difference in saltiness and stickiness for all treatments.

In conclusion, fermentation of Sai Krok Prew for 24 hr resulted in products with good qualities in terms of chemical, physical and sensory properties. Also, it contributed to the reduction of production cost and to the increase of product yields.

Table 12. Chemical, physical and sensory qualities of sausages fermented in different time.

Quality	Fermentation time (hr)		
	24	36	48
pH	4.48 ± 0.02c	4.10 ± 0.01[b]	3.96 ± 0.01[a]
Total acidity (% as lactic acid)	0.73 ± 0.01[a]	1.18 ± 0.04[b]	1.27 ± 0.04[c]
L value	61.90 ± 0.29	62.80 ± 0.15	62.34 ± 0.38
a value	10.63 ± 0.20[b]	10.40 ± 0.05[b]	10.03 ± 0.02[a]
b value	17.72 ± 0.18	17.81 ± 0.09	17.94 ± 0.06
Shear force (N)	20.45 ± 0.02	20.24 ± 0.04	20.11 ± 0.23
Color of uncooked sausages	0.98 ± 0.02[a]	0.99 ± 0.03[a]	1.12 ± 0.02[b]
Color of cooked sausages	0.99 ± 0.03[a]	1.06 ± 0.02[b]	1.04 ± 0.03[b]
Saltiness of cooked sausages	1.08 ± 0.03	1.06 ± 0.02	1.11 ± 0.02
Sourness of cooked sausages	0.99 ± 0.01[a]	1.10 ± 0.01[b]	1.15 ± 0.02[c]
Sour flavor of cooked sausages	1.05 ± 0.02[b]	0.85 ± 0.01[a]	1.08 ± 0.01[b]
Stickiness of cooked sausages	0.97 ± 0.03	0.96 ± 0.02	0.97 ± 0.02
Juiciness of cooked sausages	1.03 ± 0.02[b]	0.98 ± 0.01[a]	1.00 ± 0.02[ab]

[a-b] Means (± S.D.) within the same row for similar test items followed by different superscripts are different ($p \leq 0.05$).

ACKNOWLEDGEMENTS

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

REFERENCES

AOAC. 2000. Official methods of analysis. AOAC International, Maryland, USA.

Bacus, J. 1984. Utilization of microorganisms in meat processing. Research Studies Press Ltd., England.

Böhme, H. M., F. D. Mellett, L. M. T. Dicks, and D. S. Basson. 1996. Production of salami from ostrich meat with strains of *Lactobacillus sake, Lactobacillus curvatus* and *Micrococcus* sp. Meat Science 44(3):173-180.

Boonthanom, S., P. Wiriyacharee, and N. Chinprahast. 1994. Effect of sodium nitrate and sodium nitrite on Nham quality produced with glucono-delta-lactone and mixed starter cultures. Journal of Agriculture 10(3):296-312.

Campbell-Platt, G., and P. E. Cook. 1995. Fermented Meat. Blackie Academic and Professional, Glasgow, UK.

González, B., and V. Díez. 2002. The effect of nitrite and starter culture on microbiological quality of “chorizo”–a Spanish dry cured sausage. Meat Science 60 :295-298.

Hugas, M., and J. M. Montfort. 1997. Bacterial starter cultures for meat fermentation. Food Chemistry 59:547-554.

Ibañez, C., L. Quintanilla, C. Cid, I. Astiasaran, and J. Bello. 1996. Dry fermented sausages elaborated with *Lactobacillus plantarum - Staphylococcus carnosus* part I : effect of partial replacement of NaCl with KCl on the stability and the nitrosation process. Meat Science 44(4):227-234.

Lücke, F. K. 1998. Fermentation sausages. p.441-483. In B. J. B. Wood (ed) Microbiology of fermented foods. Blackie Academic and Professional, London.

Lücke, F. K. 2000. Utilization of microbes to process and preserve meat. Meat Science 56: 105-115.

Phromraksa, P., P. Wiriyacharee, L. Rujanakraikarn, and P. Pathomrungsiyungkul. 2003. Identification of main factors affecting quality of Thai fermented pork sausage (Sai Krok Prew). CMU. Journal 2(2):89-96.

Thai Industrial Standards Institute, 1994. Standard of Thai industrial standards institute : fermented pork sausage TSI 1266-1994. Ministry of Industry, Thailand.

Wiriyacharee, P. 2003. Sensory evaluation. Department of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand.

Wiriyacharee, P. 2004. Advanced experimental design. Department of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand.

Wiriyacharee, P., L. Rujanakraikarn, and A. Kuntiya. 1993. Nham product development using mixed bacterial starter cultures 1. Suitable source of carbohydrate for Nham production. Journal of Agriculture 9(1):51-60.