

Development of Mushroom Sausage Added with Tamarind Seed Flour of Dong Kui Community Enterprise, Food Production and Processing Phetchabun Province

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Abstract: This research aimed to develop mushroom sausage added with tamarind seed flour to substitute for cooked rice. The use of tamarind seed flour attempted to reduce production costs and add value to tamarind seeds for maximum benefits. The research design was a 2^k Factorial Design with three factors, that consist of mushroom, tamarind seed flour and Textures of minced pork, these are defined as the values A, B, C, respectively. From the variance analysis, the significant factors were AC, AB, ABC, resulting in a statistically significant response to pH values, firmness values and sensory perception at a 95% confidence level. The experiments showed that the pH values after three and five days of storage were standard. The pH values were lower than standard on the seventh day of storage. The color test also showed that the sausage gave the most beautiful color that met the Community Enterprise members' desire. The analysis results of mushroom sausage added with tamarind seed flour with the longest storage time of five days showed that the appropriate factors were mushroom content of 135 grams, tamarind seed flour of 30 grams and the level of fine grinding because it has the highest pH values, firmness and sensory. The economic analysis also showed that the production cost of the appropriate factors with ingredients step 4 to develop a formula for sale, which weighed 315 grams and gave five pieces of sausage per production, was about 32 baht in total. The production cost was four baht cheaper than the production cost of cooked rice-added mushroom sausage, which is 36 baht per production. If the Community Enterprise can produce and sell 1,500 pieces of sausage a day, they will reduce 1,200 baht of production cost per day or 36,000 baht per month.

Keywords: Mushroom sausage; tamarind seed flour; 2^k factorial design



1. Introduction

Phetchabun province is located in the north of Thailand. Fertile soil conditions and abundant water supplies make Phetchabun an excellent cultivation area. Most of the population cultivates primary cash crops, including field crops, horticulture, and vegetables. Furthermore, Phetchabun is a tourist province with several tourist attractions that can be visited all year round. Tamarind is a primary and well-known field crop of Phetchabun. It can be eaten fresh and raw or processed and produced with different tamarind products. Dong Kui Community Enterprise, Food Production and Processing Phetchabun Province and sells OTOP processed mushroom and tamarind products to individuals and tourists. The products are also exported nationally and internationally. In light of the high demand for processed tamarind products, some tamarind residues, such as tamarind shells and seeds [1], are left unused. Fieldwork to study problems and effects revealed that the community enterprise would like to add value to these tamarind residues for maximum benefits. However, the community enterprise, nowadays, burns the tamarind residues, causing air pollution.

Mushroom sausage is a popular healthy diet for all generations [2]. Dong Kui Community Enterprise has produced processed sour pork and mushroom sausage. However, most of the

mushroom sausage available in the market is similar. Dong Kui Community Enterprise has tried to develop mushroom sausage with a distinct flavor to add value and increase sales figures. Moreover, the developed mushroom sausage will help decrease production costs and increase the incomes to the members of the Community Enterprise. Thus, the researchers came up with an idea to reduce production costs during the production process. The tamarind seeds are roasted and ground into flour. The tamarind seed flour is used to substitute for cooked rice as a sour-floured agent.

Cooked rice is used to trigger lactic acid, resulting in sourness in typical sausage. The properties of lactic acid adjust acidity in food, create sourness, and preserve and prevent deterioration of food products [3]. According to a study of tamarind seeds' properties, the flesh of tamarind seeds contains healthy components and various nutrients, such as carbohydrates, fibers, protein [4], minerals like calcium, iron, and phosphate, cholesterol-lowering agents [5]. Lactobacillus-grouped microorganisms, which contribute to sourness in sausage during the fermentation process (AOAC), are also found. Based on the gathered information, the researchers would like to substitute the tamarind seed flour for cooked rice since the tamarind seed flour has similar minerals and nutrients as



cooked rice. Furthermore, cooked rice in the market nowadays is expensive and must be cooked before use, causing complexity to the production process. The use of tamarind seed flour would also create a body of knowledge and uniqueness of the sausage production for the Community Enterprise of Phetchabun. This research applied the Design of Experiment to determine the ingredients at each level in sausage production and used it to analyze the most suitable production formula.

2. Materials and Methods

2.1 Main Ingredients Used in the Experiment

Tamarind seed flour is the main ingredient in the experiment. To begin with, wash tamarind seeds, as shown in Fig. 1A. Then roast the clean tamarind seeds at 120 degrees Celsius for 15 minutes [6]. Slightly pound the roasted tamarind seeds to crack the shells from the flesh, as shown in Fig. 1B. Ground the roasted

tamarind seed flesh thoroughly to get tamarind seed powder. Before application, sift the flour through a 60-mesh sieve, as shown in Fig. 1C. The process of grinding and sifting is operated using a high-performance machine from Freshy Thai Co., Ltd.

The mushroom preparation process in this research uses three types of fresh mushrooms with the aim of reducing the consumption of mixed pork in order to reduce the cost of purchasing expensive pork at present which mushrooms have properties that provide nutritional value, are easy to find, inexpensive. Fig. 2A illustrates the Bhutan oyster mushroom, which contains higher protein than meat and helps reduce cholesterol and fat in blood vessels. Fig. 2B shows the Shiitake mushroom, which helps boost bone strength, and Fig. 2C presents the king oyster mushroom that helps prevent cancers and builds an effective immune system in the body [7].



(A) Tamarind seeds



(B) Roasted tamarind seeds



(C) Ground tamarind seed flour

Fig. 1 Preparation process of tamarind seed flour



(A) Bhutan oyster mushroom (B) Shiitake mushroom (C) King oyster mushroom

Fig. 2 Three types of fresh mushrooms

2.2 Experimental Design

This research applied a 2^k Factorial Design [8] with three specified factors, namely ground pork, three types of mushrooms, and tamarind seed flour. Each of the factors contains two levels which were “low” and “high,” as shown in Table 1. The textures of minced pork and three types of mushrooms are currently recommended by the community enterprises as ingredients and tamarind seed flour was the value from the preliminary experiment to determine the minimum and maximum values of mixing. The response values of the three specified factors toward the ten experimental formulations, were pH values,

firmness values, and sensory. Because Center point per block = 1 is added as well, which is caused by 3 factors, 2 of which are numerical values (center value) + 1 text factor (no mean value) is therefore equal to $8+2 = 10$ experiments as shown in Table 2. For the reliability of the experiments, test the power for the general full factorial design of three factors at a power value of 0.90, two times were obtained. To reduce the probability of error in the experiment, the researcher performed it three times. Consequently, there were 30 experiments in total [9].

Table 1 The design level of the experimental factors

Factor	Low	High
Textures of minced pork	Coarse minced pork	Fine minced pork
Three types of mushrooms (grams)	105	135
Tamarind Seed Flour (grams)	30	50

Table 2 The experimental formulations

Run Order	Textures of minced pork	Three types of mushrooms (grams)	Tamarind Seed Flour (grams)
1	Coarse minced pork	105	30
2	Coarse minced pork	135	30
3	Coarse minced pork	105	50
4	Coarse minced pork	135	50
5	Fine minced pork	105	30
6	Fine minced pork	135	30
7	Fine minced pork	105	50
8	Fine minced pork	135	50
9	Coarse minced pork	120	40
10	Fine minced pork	120	40

2.3 Production Methods of Mushroom Sausage Added with Tamarind Seed Flour

The production process of tamarind seed flour-added mushroom sausage was based on the ten formulations. The main factors in the experiments were the textures of minced pork, which were coarse and fine minced pork, the amount of the three types of mushrooms, which was 105 grams, 120 grams, and 135 grams, and the amount of tamarind seed flour, which was 30 grams, 40 grams, and 50 grams. The storage time was three days, five days, and seven days. The production process is shown in Fig. 3 and the details are as follows.

Step 1 Wash all of the mushrooms and remove the hardened lower stems. Then steam the clean mushrooms for 40 minutes. After that, squeeze the juice out of the mushrooms and let them cool down.

Step 2 Mix the tamarind seed flour with little water. Then steam the mixture for 30 minutes.

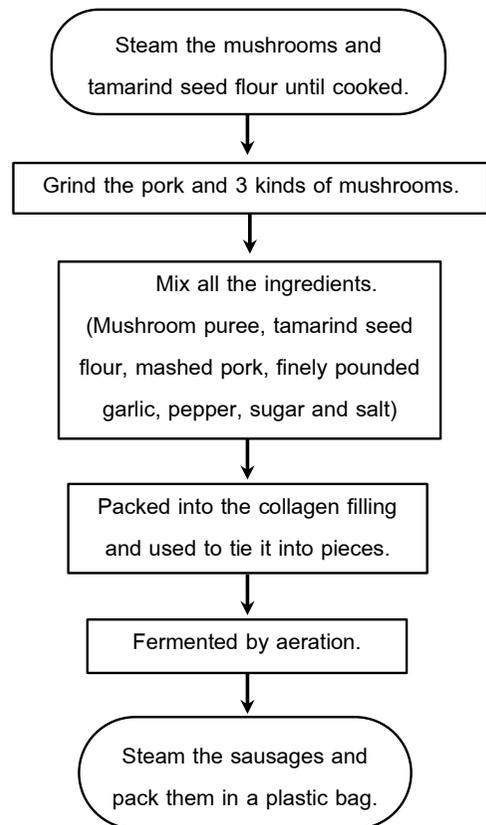


Fig. 3 Flowchart of Mushroom Sausage Making Process



Step 3 Mince pork with fat and the three types of mushrooms in a grinder whose mincing fineness level is adjustable accordingly to specified factors.

Step 4 Mix three types of minced mushrooms, tamarind seed flour, 60 grams of minced pork with fat, 10 grams of pounded garlic, 5 grams of pepper, 5 grams of sugar, and 10 grams of salt.

Step 5 Stuff the mixture into collagen casings using a sausage packing machine. Prepare the collagen casings by soaking them in water until soft. Tie the sausage with threads into pieces of 9-10 centimeters in length.

Step 6 Leave the sausage indoors with airflow for 24 hours for fermentation to take place.

Step 7 Steam the sausage for 20 minutes. Then pack the sausage into plastic bags and seal the bags thoroughly. Keep the bags in a regular section in a refrigerator to test pH values and colors.

3. Results and Discussion

The production of mushroom sausage for all ten formulations showed that general characteristics of the mushroom sausage conformed to community product standards for mushroom sausage production (CPS 1156/2549) [10] with natural colors and smell, soft texture, and no additives or other strange or irrelevant ingredients detected. The physical evaluation of the mushroom sausage was done to test three properties, namely pH value test, texture firmness test, and color test based on

(AOAC: 1990) [2]. Each formulation was repeatedly tested three times, and comparisons between the developed mushroom sausage and the sausages available in the market were also made.

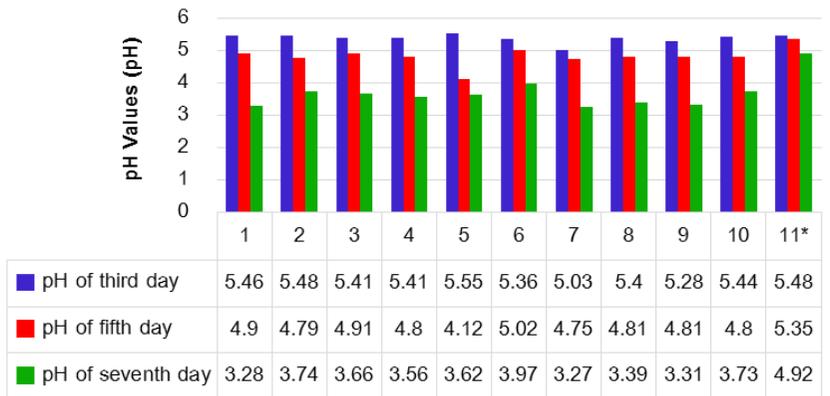
In the analysis for variance, each factor was determined as shown in Table 3. From the variance analysis, it was found that the Significant factors AC, AB, ABC had P-values of 0.039, 0.00, and 0.049 respectively with a response. Statistically significant pH response at 95% confidence level.

3.1 Test of pH Values of Mushroom Sausage

The pH values tests presented in Fig. 4 were done using a pH meter to compare the pH values based on the community product standards of mushroom sausage production coded CPS 1156/2549, which specifies that the acceptable pH values should be 4.5-5.5. According to the tests, the pH values of the ten formulations of mushroom sausage with the two types of minced pork, the amount of the three types of mushrooms, and the amount of tamarind seed flour

Table 3 The analysis for variance

Factor	Name
A	mushroom
B	tamarind seed flour
C	Textures of minced pork



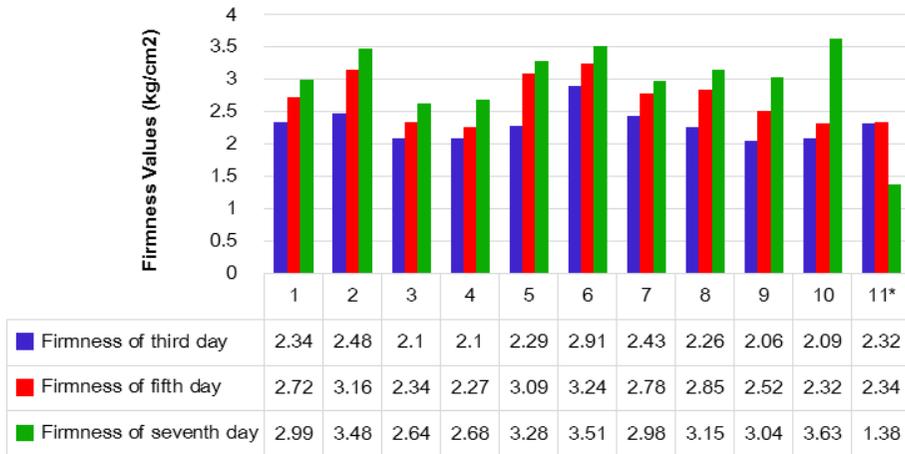
Note: 11* Is sausage in the market

Fig. 4 The comparisons of pH values on the third, fifth, and seventh days

were not significantly different. The factor that significantly affects the pH values was storage time. The mushroom sausage on the third and fifth days of storage showed pH values of 4.12-5.55, which conformed to the standards. However, the pH values of the mushroom sausage on the seventh day of storage were 3.27-3.97, which were lower than the standard values. On the seventh day of storage, the mushroom sausage was rotten and deteriorated. It means that the storage time and the pH values have direct variation. The longer the storage time takes, the more the pH values decrease. Moreover, the longer storage time will increase the number of microorganisms that produce lactic acid. Consequently, similar to the sausage available on the market, the pH values of the developed mushroom sausage reduce accordingly to the length of the storage time [12].

3.2 Textual Firmness Evaluation of Mushroom Sausage

Fig. 5 displays an analysis of mushroom sausage's firmness using the texture analyzer. The analysis showed that all of the ten formulations of the developed mushroom sausage had a higher firmness value when the storage time was three, five and seven days, respectively. This property made the developed mushroom sausage different from the cooked rice-added sausages available in the market. Cooked rice triggers the formation of lactic acid during the fermentation process; therefore, typical sausages lose their firmness when the storage time is long. Moreover, the texture does not stick together. Contrastively, the developed mushroom sausage contains mushrooms and tamarind seed flour. Since mushrooms and tamarind seed flour have particular properties that create viscosity,



Note: 11* Is sausage in the market

Fig. 5 The comparisons of textual firmness on the third, fifth, and seventh days

the sausage's firmness increases [13, 14]. The sixth formula with thoroughly minced pork, 135 grams of mushrooms, and 30 grams of tamarind seed flour resulted in the highest level of firmness. Owing to the fineness of minced pork and the high amount of mushrooms, the adhesion of mushroom fibers took place [4].

3.3 Color Evaluation of Mushroom Sausage

Fig. 6 illustrates the physical characteristics of mushroom sausage added with tamarind seed flour on the third, fifth, and seven days of storage. The color changed from white to dark red when the storage time increased [12]. Regarding the color tests using a color meter of all the ten formulations presented in Table 4, the mushroom sausage's color was white on the third day of storage, the color value is between $L^*=56.71$ $a^*=11.73$ $b^*=28.27$, showing that the tamarind seed flour was creating lactic acid.

Therefore, the sausage's color did not change much, and the sourness was not detected [3]. On the fifth day of storage, the mushroom sausage had a yellowish-white color, the color value is between $L^*=43.63$ $a^*=17.41$ $b^*=32.87$, and the taste was sour, resulting from the lactic acid created by *Pendiococcus cerevisiae*. On the seventh day of storage, the mushroom sausage had a dark reddish color [14]. On the fifth day of storage, the mushroom sausage had a yellowish-white color, the color value is between $L^*=33.80$ $a^*=22.54$ $b^*=38.45$, because the long storage time contributed a darker reddish color. The darker reddish color resulted from a great deal of lactic acid in the sausage [15], which could also cause perishability. Lastly, the color comparison of the developed mushroom sausage and the sausage available in the market revealed that both types of sausage had the same color-changing pattern.



Fig. 6 The characteristics of mushroom sausage added with tamarind seed flour

Table 4 The results of the average color tests of mushroom sausage added with tamarind seed flour

Test details	Color of the third day			Color of the fifth day			Color of the seventh day		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Sausage with tamarind flour (\bar{X})	56.71	11.73	28.27	43.63	17.44	32.87	33.80	22.54	38.45
Sausage in the market (\bar{X})	55.56	9.76	26.63	45.43	15.24	30.94	35.97	20.28	35.34

3.4 Sensory Evaluation of Mushroom Sausage

The sensory analysis of the ten formulations of mushroom sausage added with tamarind seed flour was conducted. The six aspects, namely appearance, color, smell, taste, texture, and overall liking [10], were analyzed using a 9-point Hedonic scale. A panel of 30 people was purposively selected [14], and there was the same number of male and female participants from Dong Kui Community Enterprise, Food Production and Processing Phetchabun Province. The panel was divided into three groups with the same number of 10 people aged between 20-30 years old, 31-40 years old, and 41-50 years old, respectively. Regarding the report of pH value tests, on the seventh day of storage, the mushroom sausage

added with tamarind seed flour had pH values lower than standards; therefore, it was not included in the sensory testing. The sensory testing included only the mushroom sausage after being kept for three and five days.

Table 5 presents the sensory analysis results of the third day which are ten formulations of tamarind seed flour-added mushroom sausage. The sensory analysis revealed that the third mushroom sausage formulation received the highest average of sensory liking at 7.61, showing a high preference level. An evaluation of individual attributes also showed that appearance gained the highest liking average at 8.5 ± 0.94 , followed by texture at 8.05 ± 0.94 [15, 16].

Table 5 The results of sensory analysis of mushroom sausage on the third day of storage

Attribute	Sensory preference score (\bar{X}) \pm S.D.									
	1	2	3	4	5	6	7	8	9	10
Namely appearance	8.2 \pm 0.76	8.3 \pm 0.92	8.5 \pm 0.94	8.3 \pm 0.92	8.3 \pm 1.031	8.45 \pm 1.05	8.2 \pm 0.89	8.25 \pm 0.85	8.2 \pm 1.10	8.35 \pm 1.13
Color	6.75 \pm 1.20	6.9 \pm .788	7.5 \pm 0.94	6.9 \pm 0.78	7.3 \pm 1.08	7.25 \pm 1.01	7.05 \pm 1.276	7.2 \pm 0.95	6.9 \pm 1.02	7.05 \pm 0.68
Smell	7 \pm 1.02	6.9 \pm 0.71	7.4 \pm 1.04	6.9 \pm 0.71	7.25 \pm 1.16	7.05 \pm 1.09	7.15 \pm 0.93	6.8 \pm 1.00	6.7 \pm 0.97	6.8 \pm 0.95
Taste	6.55 \pm 1.26	6.55 \pm 0.94	6.9 \pm 0.96	6.55 \pm 1.19	6.8 \pm 1.05	6.85 \pm 1.30	6.85 \pm 0.87	6.95 \pm 1.05	6.75 \pm 0.91	6.75 \pm 0.91
Texture	7.7 \pm 1.08	7.75 \pm 0.78	8.05 \pm 0.94	8.45 \pm 1.18	8.2 \pm 1.23	7.55 \pm 1.43	8.1 \pm 1.11	7.75 \pm 0.96	7.6 \pm 1.27	7.8 \pm 1.23
Overall liking	7.05 \pm 0.68	7.05 \pm 0.88	7.3 \pm 1.08	6.45 \pm 1.19	7.2 \pm 1.10	7.03 \pm 1.20	7.05 \pm 0.88	7.15 \pm 1.03	6.70 \pm 0.80	7.05 \pm 0.88
Average (\bar{X})	7.21	7.24	7.61	7.26	7.51	7.36	7.40	7.35	7.14	7.30

Table 6 displays the sensory acceptability evaluation of the fifth day which are ten formulations of mushroom sausage added with tamarind seed flour. The evaluation revealed that the sixth formulation gained the highest average sensory liking level at 7.81, showing a high preference level. The evaluation of individual attributes showed that the texture received the highest average at 8.35 \pm 0.98, followed by the taste at 8.2 \pm 1.05. The two attributes received a higher liking level than the other attributes [11, 16].

According to the comparison of the sensory acceptability toward the ten formulations of the

mushroom sausage added with tamarind seed flour in Tables 4 and 5, all mushroom sausage formulations on the fifth day of storage received a higher acceptability level than on the third day of storage. Specifically, the sixth formulation, which contained thoroughly fine minced pork, 135 grams of mushrooms, and 30 grams of tamarind seed flour, gained the highest overall satisfaction level. Due to the fineness of minced pork and more mushrooms, the taste and texture were good and responded well to the sensory acceptability of all consumers.

Table 6 The results of the sensory analysis of mushroom sausage on the fifth day of storage

Attribute	Sensory preference score (\bar{X}) \pm S.D.									
	1	2	3	4	5	6	7	8	9	10
Namely appearance	6.45 \pm	6.25 \pm	6.95 \pm	6.55 \pm	6.8 \pm	7.05 \pm	7 \pm	6.9 \pm	5.8 \pm	6.6 \pm
	0.99	1.16	0.88	0.88	0.89	1.23	1.02	1.25	1.50	1.39
Color	6.85 \pm	6.9 \pm	7.05 \pm	7.1 \pm	7.2 \pm	7.4 \pm	7.35 \pm	7.5 \pm	7.05 \pm	7.1 \pm
	0.98	1.07	1.23	1.02	0.95	1.09	1.03	0.99	1.31	1.33
Smell	7.7 \pm	7.4 \pm	7.95 \pm	7.9 \pm	7.95 \pm	7.8 \pm	7.85 \pm	7.1 \pm	7.3 \pm	7.85 \pm
	1.17	1.04	1.46	0.78	1.23	1.00	0.98	1.07	1.08	1.28
Taste	8.35 \pm	8.1 \pm	8.5 \pm	8.5 \pm	8.25 \pm	8.2 \pm	8.45 \pm	8.5 \pm	8.25 \pm	8.35 \pm
	0.98	0.96	1.14	1.09	1.11	1.05	1.05	1.09	1.06	1.18
Texture	7.55 \pm	7.55 \pm	8.15 \pm	7.95 \pm	8.1 \pm	8.35 \pm	8.35 \pm	8.3 \pm	7.55 \pm	7.7 \pm
	0.94	0.82	1.03	0.82	0.85	0.98	0.93	0.92	1.31	1.38
Overall liking	7.4 \pm	7.55 \pm	7.95 \pm	7.9 \pm	7.95 \pm	8.05 \pm	7.25 \pm	8.25 \pm	7.7 \pm	7.95 \pm
	1.18	0.75	1.14	0.85	1.05	0.99	1.16	1.01	1.33	1.53
Average (\bar{X})	7.38	7.29	7.76	7.65	7.71	7.81	7.71	7.76	7.28	7.59

3.5 Analysis with Design of Experiment

Fig. 7 illustrates the test results of mushroom sausage fortified with tamarind seed flour. Based on DOE analysis, predictions can be made to determine the optimal mix ratio and raw material grinding level to get the pH values, firmness values, and highest sensory acceptance was the ratio with mushroom content 135 grams, tamarind seed flour 30 grams and the level of fine grinding which the prediction result is 91.45% accurate. Significant factors (P -value ≤ 0.05) were pH values, firmness values, and sensory, with statistically significant differences at the 95% confidence level [17].

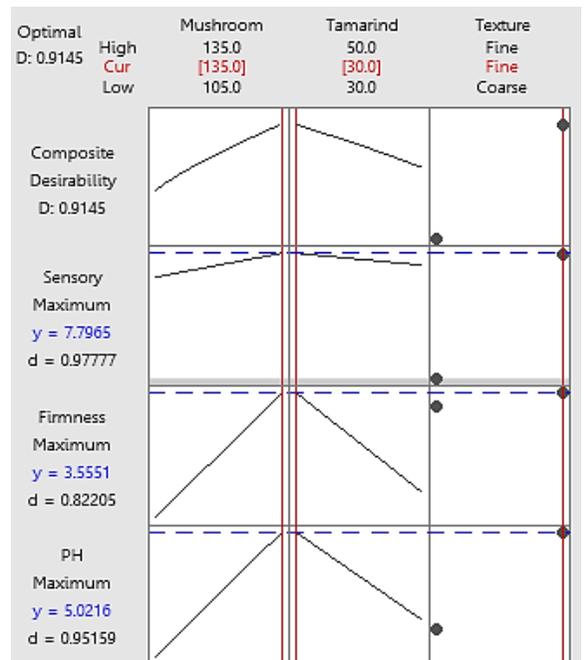


Fig. 7 Test results of mushroom sausage fortified with tamarind seed flour with DOE



4. Conclusion

The 2^k Factorial Design with three experimental factors resulted (mushroom, tamarind seed flour and Textures of minced pork) in the ten formulations of mushroom sausage added with tamarind seed flour. Regarding the experiments, the variable that significantly affected the pH values, firmness, and colors was the length of storage time. Moreover, natural lactic acid formation occurred when the length of storage increased. In turn, the lactic acid triggered the creation of *Lactobacillus* and *Pediococcus* during the first stage of fermentation. Then during the later stage of fermentation, the formation of *Lactobacillus Plantarum* and *Lactobacillus Brevis* took place, causing sourness and a pH value decrease. On the third and fifth days of storage, the pH values conformed to standards. In contrast, on the seventh day of storage, the pH values were lower than the standards.

Unlike the sausages available in the market, the texture firmness evaluation of the mushroom sausage added with tamarind seed flour found that the firmness values changed accordingly to the length of storage, particularly the sixth formulation, which had the highest texture firmness value. Furthermore, the color evaluation revealed that, on the fifth day of storage, the mushroom sausage had a yellowish-white color that met the Community Enterprise members' needs the most. The sensory

analysis also showed that all formulations of the mushroom sausage on the fifth day of storage gained a higher level of satisfaction than on the third day of storage. Besides, the sixth formulation had the highest level of overall satisfaction.

Consequently, it could be concluded that tamarind seed flour helps to trigger lactic acid formation, and it can completely (100%) substitute cooked rice in mushroom sausage production. Typical sausages available in the market contain cooked rice equal to 25 percent of their weight. The transfer of knowledge and production process of mushroom sausage added with tamarind seed flour to the Community Enterprise members revealed a positive economic analysis. If the Community Enterprise members produce the mushroom sausage, based on the sixth formulation with ingredients step 4 to develop a formula for sale, which weighs 255 grams of the total ingredients, they will get the 315 gram processed mushroom sausage per production. This 315 gram mushroom sausage can be tied into five pieces. The production cost of the developed mushroom sausage is 32 baht, and it is cheaper than the production cost of regular sausages with cooked rice, which is 36 baht per production. Therefore, the use of tamarind seed flour reduces the production cost of four baht per production. If the Community Enterprise members produce 1,500 pieces of mushroom sausage per day, they can reduce 1,200 baht of production cost.



In total, they will save around 36,000 baht of the production cost per month.

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