Journal of Experimental Food Chemistry

Chung et al., J Exp Food Chem 2016, 2:3
DOI: 10.4172/2472-0542.1000114

Research Article Open Access

Kkuaripepper (*Capsicum annum* L.) and Olive Oil Effects on Quality Characteristics of Pork Sausage Studied by Response Surface Methodology

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Received date: May 11, 2016; **Accepted date:** October 5, 2016; **Published date:** October 22, 2016

Abstract

Response surface methodology was used to investigate the effect and interactions of processing variables such as kkuaripepper powder (1.0-5.0%), olive oil (3-13%) on physicochemical, textural and sensory properties of cooked pork sausage. It was found that L* and a* values decreased; however, water-holding capacity, and a* values increased, respectively, as the amount of kkuaripepper increased. Olive oil addition decreased water-holding capacity, moisture contents, and L* values of the sausages. The adhesiveness depended on the kkuaripepper and olive oil added, as its linear effect was positive at $p<0.01$. Kkuaripepper addition increased the springiness and chewiness of sausages. The preference of color, flavor, taste, and overall quality depend on the addition of kkuaripepper and olive oil. The optimum formulation for pork sausage with kkuaripepper powder was 2.99 g of kkuari pepper powder and 9.12 g of olive oil.

The results of this optimization study would be useful for meat industry that tends to increase the product yield for sausages using the optimum levels of ingredients by RSM (Response surface methodology).

Keywords: Kkuaripepper (*Capsicum annum L.*); Pork sausage; Cooking parameters; Response surface methodology; Sensory quality

Introduction

According to the economic development, quality of life improvement and increasing concern about the health of modern life, the palatability and functionality of food are receiving attention as well as the ability to sustain life, and meat and meat products are becoming to the high-quality livestock product with intake convenience and a lot of functional materials in addition to the existing images of common protein foods [1]. In order to produce meat products with the functionality and safety, it is realized that the needs for the environment-friendly natural preservatives and food additives with superior cell function regulating effect [2,3] are important.

Pepper (*Capsicum annuum* L.) is a perennial herbaceous plant belonging to eggplant (Solanacea) and it was originated from South America. It is widely cultivated throughout the temperate region [4], and it is one of the most consumed vegetable in worldwide. Especially in Korea, it has been widely used for a long time as one of the main ingredients of the representative food, kimchi [5], and it contains a variety of nutrients such as vitamin A, C as well as capsaicin, etc. Recently, there are various studies about prevention effect of metabolic syndrome, anti-cancer, inflammatory, antioxidant effects of pepper ingestion [6,5]. Kkuaripepper was developed for a raw food or boiled food because it has soft flash and less spicy than normal pepper [7]. For the studies about kkuaripepper, most researchers handled kkuaripepper restrictively in the study for the antioxidant effect of green pepper and apoptosis effect on breast cancer cell line [5], the anti-obesity effects of green pepper water extracts [8] and so on, which means that the research is not sufficient in food science aspect. In addition, despite the pepper contains many bioactive compounds, many studies are focused on the capsaicin in red pepper and flavonoids in green pepper, and especially the antioxidant effect of kkuaripepper and the foods using this have hardly been studied.

In recent years, the consumer demands for healthier meat and meat products with reduced level of fat, cholesterol, decreased contents of sodium chloride and nitrite, improved composition of fatty acid profile and incorporated health enhancing ingredients are rapidly increasing worldwide. Fat in processed meat products contributes functional and organoleptic characteristics, and plays an important role in the formation of stable meat emulsions [9]. Fat replacements or substitutes are ingredients that contribute a minimum of calories to formulated meats and alter flavor, tenderness, mouth feel, viscosity and other sensory and processing properties [10,11]. The replacement of animal fat with vegetable oils in meat products has been found to be an efficient and successful tactic to increase the nutritional value of meat foods by decreasing saturated fatty acids levels and adding natural antioxidants as pepper, and turmeric.

Sausage is a kind of meat products to improve storage and taste, which is evaluated as a source of protein, iron, minerals and vitamin B complex [12]. Sausage contains more than 30% of fat, which has a lot of effect on taste, flavor, texture and juiciness and processing quality after cooking. However, excessive fat intake through the meat product is correlative with obesity, hypertension, atherosclerosis and disease of the coronary artery system. Therefore, according to the increased interest in the consumer's health, it has been developed that the fat in sausage was reduced or replaced by vegetable fat, and the substances with a variety of physiological function activity was added [13]. Consumer who is interested in food containing natural ingredients has motivated the food industry to evaluate the effectiveness of naturally occurring components of food for functional purposes other than their commonly recognized ones. According to the need for the customer's interest in health, the studies for the application and development of the meat products with functional natural substances have been necessary, so kkuaripepper can be thought as an additive to meet the needs because there are many applications using in Korean life.

The purpose of this study was to investigate the acceptable effects of kkuaripepper powder on ground pork and to focus on the possibility to use kkuaripepper in cooked pork products. To obtain ideal combination levels, the importance of determining the optimum levels of these replacements in sausages comes into prominence. Response surface methodology (RSM) is an effective tool to find these optimum levels of the processing variables for the parameters studied. Therefore, we studied the effect of processing variables such as kkuaripepper powder (1.0-5.0%), olive oil (3-13%) on cooking properties of pork sausages and to find the levels of processing variables to maximize and minimize the cooking parameter.

Material and Methods

Plant material

To produce kkuaripepper (*Capsicum annum L.*) powder, we purchased kkuaripepper from NongHyup cultivated at Yeoncheon-gun, Gyeonggi-do in 2014, and then dried in vacuum freeze dryer (MCFD 8505, Ilshin Lab Co., Seoul, Korea).

Experimental design

In order to calculate the optimal combination ratio, Design-Expert version 8 (Stat-Ease Inc., Minneapolis, Minnesota, USA) program was used for experimental design, which was conducted according to the central composite design (CCD) method of response surface methodology (RSM). Through the preliminary experiment, kkuaripepper powder (A) and olive oil (B) were set as the two independent variables which are the factors with the greatest impact on the taste and texture among the quality characteristics of kkuaripepper pork sausage, and the minimum and maximum range of each factor were determined that kkuaripepper powder is 1.00~5.00 g and olive oil is 3.0013.00 g.

To check the response of each factor, we used perturbation plot, contour plot and response surface plot. The experimental point of central composite design method was formed with the center point (0.00), ± α point (Axial point) and ± 1 level point (Factorial point), which has repeated point for model prediction and lack-of-fit. The combination ratio of the completed experimental design is shown in Table 1. We established that the dependent variables includes the physiochemical properties such as pH and salinity, mechanical properties such as chromaticity (lightness, redness, yellowness) and texture (hardness, adhesiveness, springiness, chewiness, gumminess, cohesiveness), antioxidant properties, and sensory properties (color, flavor of pepper, taste of pepper, tenderness, overall quality).

Standard No.	Variable level								
	Kkuaripepper powder (g)	Olive oil (g)	Pork (g)	Salt (g)	Starch (g)	Garlic (g)	Sodium phosphate (g)	Black pepper (g)	Nutmeg (g)
1	1	3	100	1.6	3	0.6	0.3	0.25	0.05
2	5	3							
3	1	13							
4	5	13							
5	1	8							
6	5	8							
7	3	3							
8	3	13							
9	3	8							
10	3	8							

Table 1: Experimental design for pork sausages with Kkuaripepper (*Capsicum annum L.*) and olive oil by response surface design.

Sausage preparation

In order to determine the standard combination ratio and manufacturing conditions of the kkuaripepper pork sausage, we produced the sausage following the general method investigated by literature review and preliminary experiments [14]. Salt (Baeksul Co., Korea), phosphate (Eselnara Co., Korea) and ice which was 5% of the weight of meat were added to minced pork ham, which were mixed in food mixer (SF-100, Samwoo, Daegu, Korea) for 2 minutes. And then the secondary mixing was performed for 2 minutes after adding kkuaripepper powder, olive oil (Baeksul Co., Korea), nutmeg (I.S.F.I Spices, Belgium) and starch (Tureban Co., Korea). The core temperature was maintained under than 10. The batter finished secondary mixing was packed in a collagen casing (Collagen Sausage Casing, Nippi Collagen Ind, Ltd., Shizuoka, Japan), which was boiled at 70 for 35 minutes. The samples were cooled for 10 minutes and then vacuum packed with poly ethylene (PE) film to store at refrigeration temperature (4°C).

Physicochemical characteristics analysis

Determination of pH: pH was measured applying the method of Ranggana [15]. Five gram portions of raw sausage and 45 mL of distilled water were mixed, which was homogenized by Bag Mixer 400 (Interscience, France) for 90 seconds, and measured at 20 with pH meter (F-51, HORIBA, Tokyo, Japan) using buffers of pH 4.0 and 7.0 for calibration.

Determination of salinity: The same sample as pH measurement was used to measure salinity using digital salt meter (ES-421, ATAGO, Tokyo, Japan), the detection principal of ATAGO salt meters is that of electric conductivity method which detects and converts electrolytes to NaCl concentration. The Mohr method, also known as a silver nitrate titration method, utilizes the characteristic of silver nitrate that reacts with chloride ions to measure the salinity % but digital salt meter measure the salinity by creating a conversion table between the two testing methods, correlation between the set of results. Test was repeated three times and the mean value was calculated.

Determination of water holding capacity: Water holding capacity was analyzed applying the method of Verbeken et al., and the sample was prepared by centrifuge at 4, 1200 rpm for 30 minutes with ten gram of boiled sausage. The water holding capacity was calculated by the weight before and after centrifuge.

$$Water\ holding\ capacity\ (\%) = \frac{\text{Weight ofsample after centrifuge (g)}}{\text{Weight ofsample before centrifuge(g)}} \times 100$$

Determination of moisture content: Moisture content was measured by atmospheric drying method by heating at 105 using a dry oven (SW-90D, Sanwoo, Seoul, Korea).

Determination of total phenolic content: Total phenolic content of kkuaripepper pork sausage was measured by the method of Liu et al. which was modified Folin-ciocalteu method of Talcott et al. The absorbance was measured by spectrophotometer (UV-Visible Spectrophotometer, V-530, Jasco Co.,Tokyo, Japan) at 750 nm. Total phenolic contents were analyzed with the calibration curve of garlic acid (Sigma-Aldrich Co., Saint louis, Missouri, USA).

Determination of DPPH free radical scavenging activity: The sample was prepared by the same method with the total phenolic content analysis and it was measured by applying the method of Brand et al. The absorbance was measured by spectrophotometer (UV-Visible Spectrophotometer, V-530, Jasco Co., Tokyo, Japan) at 517 nm, and DPPH free radical scavenging activity was calculated using the follow formula.

$$DPPH\ free\ radical\ scavenging\ activity\ (\%) = \left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right) \times 100$$

Mechanical characteristics analysis

Color measurements: The instrumental color of boiled kkuaripepper pork sausage were measured using a Chroma Meter (CR-300, Minolta Co., Ltd, Osaka, Japan), calibrated to white standard plate(L*=97.26, a*=- 0.07, b*=1.86).

Texture measurements: All sausage samples were subjected to texture profile analysis by applying the method of Bourne (1978) with three replicates using a texture analyzer (TA-XT Express v2.1, Stable Micro Systems Ltd., London, England) with the Texture Expert Program. Texture attributes such as hardness, adhesiveness, springiness, chewiness, and cohesiveness were analyzed. The analysis condition was shown in Table 2.

Analysis condition	
Instrument type	TA-XT Express, Stable Micro Systems
Type	TPA (texture profile analysis)
Probe	36 mm cylinder radius probe - SMS P/35
Pre-test speed	1.00 mm/s
Test speed	5.00 mm/s
Post-test speed	5.00 mm/s
Test distance	3.00 mm
Time	5.00 sec
Trigger Force	50.00 g

Table 2: Operating conditions for texture analyser.

Sensory characteristics analysis

Sensory evaluation was performed according to the seven point hedonic scale. The 25 panelists with trained palate, who are majoring in the food and nutrition course in Sookmyung Women's University. Color, flavor of pepper, taste of pepper, tenderness and overall quality were evaluated by 7 points scaling (1 point means the worst, 7 point means the best) which means that the higher acceptability and preference gives the closer points to 7 points.

Statistical analysis

For the optimization analysis for the 10 kind of kkuaripepper sausage which designed by the central composite design (CCD), the quantity of kkuaripepper powder (A) and olive oil (B) were selected through the graphical optimization and the numerical optimization in canonical model using Design-Expert version 8 (Stat-Ease, Inc., Minneapolis, Minnesota, USA) program, and the optimum point was selected by point prediction. In order to determine the impact of two independent variables, the quantity of kkuaripepper powder and olive oil, on the dependent variables, physicochemical, mechanical and sensory properties, the relationship between the variables was analyzed using perturbation plot, contour plot and response surface plot in the statistics program. Among the optimum points proposed by numerical optimization, desirability was calculated according to the following formula, and we obtained the optimum point showing the highest desirability.

$$D = (d_1 \times d_2 \times \ldots \times d_n)^{\frac{1}{n}} = \left[\prod_{i-1}^{n} d_i\right]^{\frac{1}{n}}$$

D=overall desirability, d=desirability, n=response.

Preparation of optimized kkuaripepper pork sausage with kkuaripepper powder and olive oil

According to optimized kkuaripepper powder and olive oil content, minced pork was assigned to one of the following two treatments: Sausage was prepared by adding kkuaripepper powder at 2.99 g (KS);

Control sausage addition without kkuaripepper powder (CS). Olive oil (9.12 g) and the spices were added to two treatments equally as described before by the same way.

Result and Discussion

pH

The results of the model significance, equation and coefficients of determination of the model equations to examine the effects of the independent variables (kkuaripepper and olive oil) on pH of the sausage are shown in Table 3. The pH values didn't differ statistically (p>0.05) among the assays, and the linear model to affect independently each factor was selected, and it was not significant.

Salinity

The salinity of the kkuaripepper pork sausage was in a range of 0.100.15, and the quadratic model which shows interaction between kkuaripepper power and olive oil was selected, but it did not show significant difference (p>0.05) (Table 3).

Response	Model	Mean±S.D.	$R^{2\,a}$	F-value	Prob>F	Polynomial equation[b]
pH	Linear	6.02 ± 00.04	0.5509	4.29	0.0607	6.02-0.047A-003B
Salinity (%)	Quadratic	0.13 ± 0.01	0.7201	2.06	0.2521	+0.11-0.01A-003B-003AB +0.011A2-003B2
Water holding capacity (%)	Linear	95.11 ± 0.91	0.7283	9.38*	0.0105	95.11+0.66A-1.46B
Moisture content (%)	Quadratic	59.50 ± 0.41	0.9843	50.21**	0.0011	58.72-0.12A-2.32B +0.77AB-0.37A2+ 1.66B2
Total phenol (mg/g)	Quadratic	0.66 ± 0.10	0.8885	6.37*	0.0485	0.47+0.17A+0.035B-0.033AB+0.24A2+0.066B2
DPPH scavenging activity (%)	Quadratic	35.43 ± 4.40	0.9635	21.20*	0.0056	28.49+16.91A+0.57B-1.06AB+11.56A2+0.051B2
L	Linear	53.89 ± 0.96	0.9124	36.43***	0.0002	53.89-3.34A-0.32B
a	Quadratic	-3.78 ± 0.23	0.9975	321.80***	0.0001	-4.98-3.49A-0.065B-0.19AB+1.92A2+0.094B2
b	Quadratic	14.70 ± 0.38	0.9925	105.17***	0.0002	15.83+3.34A+0.66B+0.7AB-1.15A2-0.72B2

[a]0<R2<1, close to 1 means more significant; [b]A: Kkuaripepper (*Capsicum annum* L.) powder , B: Olive oil

[*] p<0.05, [**] p<0.01, [***] p<0.001

Table 3: Analysis of predicted model equation for physicochemical characteristics of pork sausages with Kkuaripepper(*Capsicum annum* L.).

Water holding capacity (WHC)

The water holding capacity (WHC) of each kkuaripepper sausage was in a range of 93.34 to 97.31 (Figure 1), and the linear model to affect independently each factor was chosen. The result was significant within 5%, and the suitability of the model had been recognized by 0.7283 of R^2 value (Table 3).

In the perturbation plot and response surface plot of Figure 1, WHC was increased as kkuaripepper powder was increased on the other hand it was decreased as olive oil was increased. Kkuaripepper powder added is likely to affect the myofibrillar proteins to increase the emulsification which influence WHC [16]. In the study of chicken sausage prepared with turmeric by Yun et al. [13], it has reported that low melting point of olive oil reduces the water holding capacity, which is same as the experimental results. Dzudie et al. [17] reported that if a vegetable fat of corn oil was added to beef patties instead of animal fat, the water holding capacity was reduced. Through these results, it is thought that the water holding capacity of kkuaripepper pork sausage was reduced by the effect of olive oil.

Moisture content

Moisture content was in a range of 57.23~63.51 (Figure 1), quadratic model which is interacting with kkuaripepper powder and olive oil was selected. The p-value was significance within 1%, and it was known that the reliability of the regression variation was relatively high (Table 3). In the perturbation plot and response surface plot in Figure 1, it was turned out that olive oil affected the moisture content than kkuaripepper powder, so the moisture content was reduced as the olive oil addition was increased. This is same as the experiment result by Yun et al. [13], but it was thought that the difference with the result of Chung et al. [18] which said the moisture content increased as olive oil increase was caused by the proportion of subsidiary materials or the kind of materials.

Total phenolic content

Recently polyphenols are attracting attention because it is expected that its antioxidant ability acts as an antioxidant in vivo and contributes to maintain health and to prevent disease [19]. The total phenol analysis result of 10 kkuaripepper sausages is written in Table 3, and the quadratic model interacting kkuaripepper powder and olive oil was selected. In Figure 1, it was shown a tendency to increase the total phenolic content as the kkuaripepper powder amount was increased, which means that the total phenol in kkuaripepper powder itself remained its antioxidant ability after manufacturing the sausage. According to the previous studies for the various varieties of peppers, the jalapeno pepper showed 13.39 mg/kg in the study by Yoon [5], Cheongyang pepper had 23.06 mg/kg of total phenol in the study about the quality analysis for fresh noodle added Cheongyang pepper juice [19]. It was reported that the pepper peel had 5.8~10.5 mg/g and the seed had 12.4~18.5 mg/g, and in the total phenolic content analysis for 275 vegetables in Korea, the average was 0.32~49.26 mg/kg, the

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highest item was garlic of 40.29 mg/kg, and it was followed by pepper of 29.16 mg/kg and spinach of 21.35 mg/kg [20].

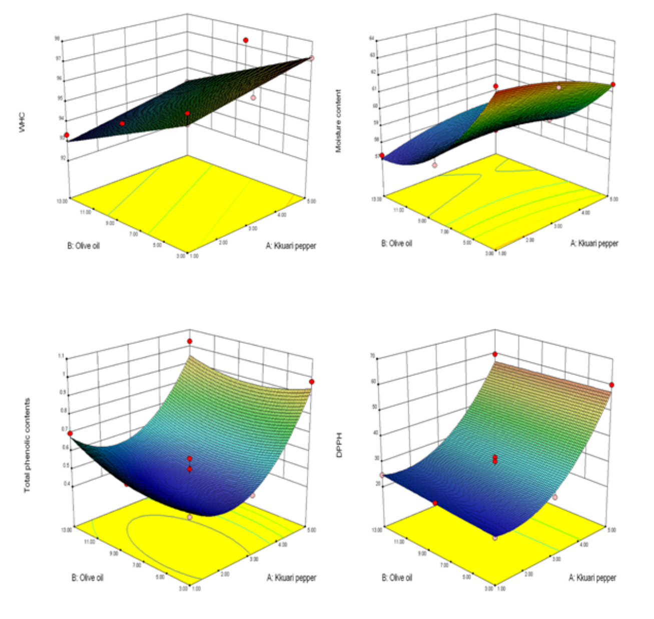

Figure 1: Response surface plot of the physicochemical and antioxidant characteristics of the pork sausages with Kkuaripepper (*Capsicum annum* L.).

DPPH free radical scavenging activity

The result of DPPH radical scavenging activity analysis for kkuaripepper sausage is presented in Table 3. The quadratic model interacting with each factor was selected for DPPH radical scavenging activity, it was significant within 1% and R^2 value was 0.9635, which means that the reliability of the regression variability was very high. Since kkuaripepper powder had more effect on DPPH radical scavenging activity than olive oil, DPPH radical scavenging activity tended to increase as more kkuaripepper added. The report about the antioxidant effect of the green pepper methanol extract by Yoon [5] presented that 200 mg/mL of kkuaripepper showed 8% of scavenging activity, and the study of antioxidactive capacity of 7 common vegetables taken by Korean [21] told that crown daisy had 78.8%, wild parsely had 73.6%, perilla leaf had 46.5% and chive had 27.7% of scavenging activity. Lee et al. [22] compared DPPH radical scavenging activity between thyme and peppermint 70% methanol extract, and they showed 74.5%, and 63.7% of high antioxidant activity respectively. In the result, it is determined that high vitamin C and total phenol in kkuaripepper appear high DPPH radical scavenging activity.

Color

The CIE system, which included L*(lightness), a*(redness/greenness), b*(yellowness/blueness) values, has been shown to be closely associated with sensory perception. The chromaticity analysis result (lightness, redness, yellowness) for kkuaripepper pork sausage is shown in Table 3. The linear model affecting kkuaripepper powder and olive oil independently was selected for the lightness, and the quadratic model interacting with each factor was selected for the redness and yellowness. Lightness, redness and yellowness showed the significance within 1 % and R^2 value was 0.9124, 0.9975 and 0.9925 respectively, so the suitability of each model was recognized (Table 4). In the regression equation, it was shown that kkuaripepper powder had a greater effect on the lightness than olive oil, and the lightness was significantly reduced as the amount of kkuaripepper powder was increased. Thus, an increase in the kkuaripepper powder content resulted in a decrease in the lightness of mainly determined by the presence of denatured-globin hemochromes, which are formed as a result of high temperatures, colored Maillard products upon heating, the physicochemical state of proteins and other meat components [23].

Response	Model	Mean ± S.D.	$R^{2\,a}$	F-value	Prob>F	Polynomial equation [b]
Hardness (g)	Quadratic	2978.06 ± 14.60	0.7277	2.14	0.2407	3225.34+135.46A-180.12B-55.76AB-408.34A2-3.80B2
Adhesiveness (g×s)	Linear	-3.18 ± 0.94	0.7713	11.80**	0.0057	-3.18+1.85A+0.10B
Springiness	2FI	8.69 ± 0.31	0.7456	5.86*	0.0324	8.69+0.39A+0.21B-0.34AB
Chewiness	Quadratic	23401.65 ± 15.68	0.8876	6.32*	0.0492	26140.52+2076.33A-830.72B-1496.57AB-3833.55A2-731.22B2
Cohesiveness	Quadratic	0.91 ± 0.010	0.6889	1.77	0.2997	0.92-004A-003B-003AB-0.013A2-.011B2
Color	Quadratic	3.44 ± 0.20	0.9685	24.59	0.0042**	4.32-0.017A+0.12B-0.062AB-1.36A2-0.11B2
Flavor	Quadratic	3.72 ± 0.33	0.9385	12.2	0.0156*	4.58-0.67A+0.11B+ 0.013AB-1.23A2-0.20B2
Taste	Quadratic	3.77 ± 0.31	0.9259	9.99	0.0223*	4.60+0.46A+0.19B-0.10AB-1.04A2-0.34B2
Tenderness	Quadratic	4.15 ± 0.45	0.6765	1.67	0.3192	4.57-.22A+0.23B+ 0.19AB-0.62A2-0.071B2
Overall quality	Quadratic	3.94 ± 0.44	0.9055	7.66	0.0355*	5.26+0.100A+0.26B-0.063AB-1.26A2-0.94B2

[a]0<R2<1, close to 1 means more significant; [b]A: Kkuaripepper (*Capsicum annum* L.) powder , B: Olive oil

Table 4: Analysis of predicted model equation for the texture characteristics and sensory test of sausages with Kkuaripepper (*Capsicum annum* L.).

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The color changes during heating were most likely associated with the formation of Malillard reaction products (MRP). MRP were reported to be responsible for color changes; however, color differences were not correlated with heating time [24]. The partial replacement of pork back fat with vegetable oils led to a significant modification of the color measured on the surface of the cooked sausages. Sausages treated with vegetable oils showed significantly higher L* values. Also, it is thought to be caused by own color of kkuaripepper powder.

Texture

The result of texture (hardness, adhesiveness, springiness, chewiness, cohesiveness) is shown in Table 4. The hardness of kkuaripepper sausage showed the quadratic model interacting with each factor was selected, but it was not significant. This result is consistent with the report by Hyon [25] which said sausage with mugwort powder did not have significant effect on the hardness of sausage and the experimental result by Kim et al. [26] which was reported that red pepper oil did not cause significant change in hardness. The hardness tended to increase as the amount of kkuaripepper powder added and to decrease after the center point, and it tended to reduce as olive oil added (Figure 2).

Muguerza et al. [27] said that when the fat content of sausage was reduced, the mechanical properties of hardness and firmness were significantly increased, in particular, it was reported that there was a high correlation between hardness and fat loss. With these results, it is believed that the low-fat vegetable oil of olive oil weakened the water binding in this study to form the film inside the sausage than when using the pork back fat. The linear model was selected for the adhesiveness to act dependently, and the p-value was 0.0057 and significant, and the R^2 values were recognized the suitability with 0.7713. The springiness exhibited in a range of 7.34 to 9.07, and 2FI (two factor interaction) model to interact each factor was selected and significant within 5%. As the amount of kkuaripowder and olive oil addition were increased, the springiness showed a tendency to increase, which is thought to be caused by the effect of the dietary fiber in kkuaripepper on the swelling power and water absorption. It was reported that the springiness was reduced when the subsidiary materials with various physiological function were added to meat product [28,29], but it was rather increased in this study, so it is thought that kkuaripepper addition would be able to provide palatability. The chewiness showed quadratic model to interact with each factor was selected as shown in Table 4. The chewiness was decreased from the center point while increased as kkuaripepper powder addition was increased, and it was reduced as olive oil addition was increased. Yun et al. [13] also reported a same tendency that chewiness was reduced as the turmeric powder was added. Because chewiness is one of the important factors to decide the preference of the customers, it is believed that the study to improve the chewiness should be performed.

Sensory properties

We measured color, flavor, taste of pepper, tenderness and overall quality of 10 sausages manufactured with kkuaripepper powder and olive oil as independent variables, and the regression formula of the result is in Table 4. The preference for color showed the quadratic model interacting between independent variables was selected. It was shown the significant result within 1% and R^2 was 0.9685, so the suitability is recognized. The preference for flavor and taste of pepper was in a range of 2.404.90 and 2.604.85, respectively. The quadratic model was used like as the chromaticity analysis, and the significant result was shown within 5% and R^2 value was 0.9385, which was recognized the suitability of the model. Looking at the coefficient of determination for the regression equation, it was found that the taste of pepper had a large effect on the taste of kkuaripepper sausage. These results were consistent with the purpose of this study which wants to give a positive impact on the palatability of sausage by adding kkuaripepper powder. As more kkuaripepper powder and olive oil were added, the preference for the taste was decreased while increasing. So, we can know that the excessive kkuaripepper powder may reduce the preference for the taste. The preference for tenderness was in a range of 2.905.00, and the quadratic model was also selected but p-value was not significant. As kkuaripepper powder content was increased, the preference for the tenderness was decreased sharply from the center point after increasing, and the preference for the tenderness was increased as the amount of olive oil was increased (Figure 3).

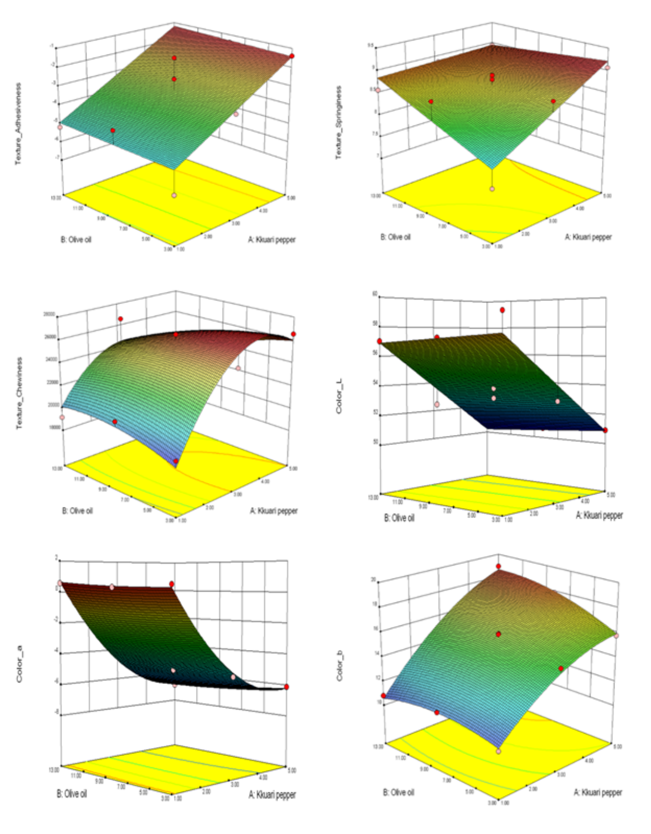

Figure 2: Response surface plot of the texture and color characteristics of the pork sausages with Kkuaripepper (*Capsicum annum* L.).

This result is consistent with the study by Cofrades et al. [29] reported that the amount and kind of dietary fiber affect the texture because the moisture absorption capacity and swelling power of kkuaripepper have an effect on the texture of meat product. The overall suitability exhibited a range of 2.90 to 5.75, and quadratic model was also applied, which showed a significant result within 5% and R^2 value was 0.9055. So it was recognized as the suitability of the model.

Optimization of kkuaripepper sausage

In this study, optimization was applied within the experimental kkuaripepper and olive oil concentrations in order to determine the optimum formulation for sausages. Using the numerical optimization technique in the Design Expert software, optimum levels of kkuaripepper powder and olive oil were determined by superimposing the contour plots of all the responses (physicochemical, texture parameters and sensory properties). The final goal was to obtain a functional sausage with a high sensory acceptance and suitable physicochemical properties.

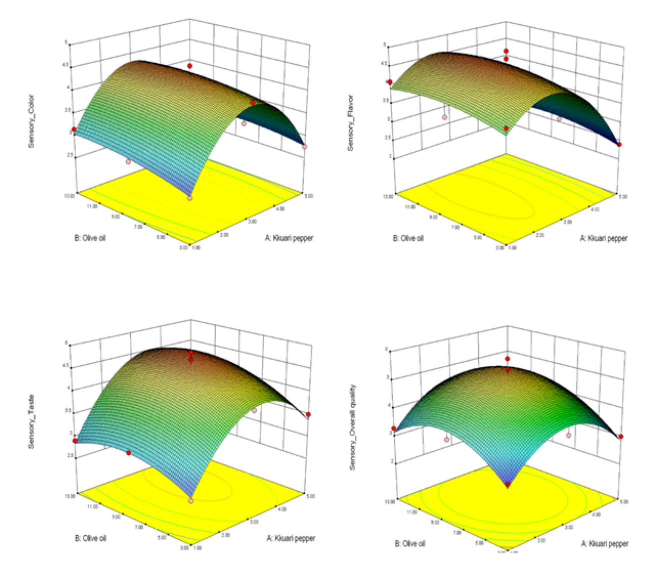

Figure 3: Response surface plot of the sensory characteristics of the pork sausages with Kkuaripepper (*Capsicum annum* L.).

For the kkuaripepper pork sausage optimization, we estimated the numerical optimization of the canonical model by setting the sensory evaluation items (color, flavor, taste, tenderness and overall quality) which were shown significant results within the combination range of independent variables (kkuaripepper powder and olive oil) in a maximum level, and the optimum point showing the maximum desirability was derived by point prediction. Based on these superimposed plots, it was suggested that the optimum formulation of the kkuaripepper sausage was 2.99 g for kkuaripepper powder and 9.12 g for olive oil. The graphical optimization by response model and perturbation plot used in this study was presented in Figure 4, and the characteristics of the dependent variable of the derived optimum point were drawn in the overlay plot of Figure 4. The predicted values for each of response variables were as follow; pH- 6.03, salinity-0.12, WHC-94.78%, L*-52.85, a*- -4.96, b*-15.91, hardness-3184.05 g, adhesiveness- -3.16 g.s, springiness-8.73, chewiness-2924.09, cohesiveness-0.91. In addition, the sensory scores for color, flavor, taste, tenderness, and overall quality were 4.33, 4.59, 4.61, 4.88 and 5.26, respectively (Figure 4). The desirability function approach is one of the most widely used methods for the optimization of the multiple response process. Desirability concept for multi criteria optimization in industrial quality management was introduced by Harrington [30]. It is based on the idea that the quality of a product or process that has multiple quality characteristics is unacceptable when one of them stays outside of some desired range.

Physicochemical, texture, and sensory characteristics of optimized kkuaripepper pork sausage with kkuaripepper powder and olive oil

Salinity and water holding capacity: The salinity of the optimized kkuaripepper sausage was 0.16% and the control group showed 0.14%, which means the salinity of kkuaripepper sausage, was higher than the control group. And the water holding capacity was 95.76% in the optimized sausage and 96.51% in the control group, which indicated that the water holding capacity of the optimized kkuaripepper sausage was lower than the control group, and there was no significant difference in both salinity and water holding capacity (Table 5). On the other hand, this is contradictory result with the study by Bishop et al. [31] reported that the mixing with vegetable oils and non-meat proteins that are replaced during the manufacturing low-fat meat products increased the water holding capacity by increasing the gel-forming action due to the increased binding capacity of fat.

Moisture contents: The moisture content was 59.5% in the optimized sausage and 62.34% in the control group, which means the moisture content of optimized kkuraipepper sausage was lower than the control, and the significance level was observed within 5% (Table 5). Studies on the back fat of press ham replaced with olive oil [28] also showed that the experimental group with olive oil was lower. On the other hand, in the study about the influence of meat product on the moisture content according to the type of sub material as the independent variable, as the amount of the freeze-dried green tea powder was increased, the moisture content was reduced, which is in same with the results of this study [32].

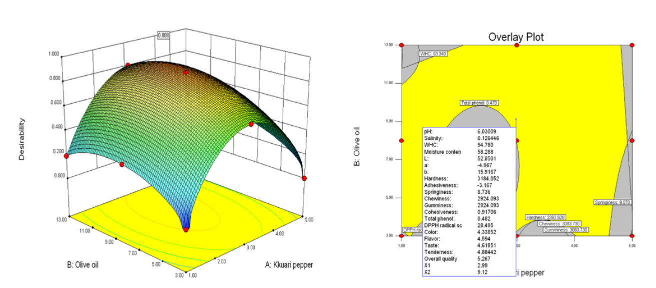

Figure 4: Response surface plot of the desirability and overlay plot of optimized the pork sausages with Kkuaripepper (*Capsicum annum* L.).

Chromaticity: The chromaticity result of the kkuaripepper pork sausage and the control group is shown in Table 5. Lightness, redness and yellowness were all significantly different. But the lightness and redness of the optimized kkuaripepper pork sausage was lower than the control group, while the yellowness was higher in the kkuaripepper powder added sausage. In the result, it was found that kkuaripepper powder affects lowering the lightness and redness, while it has an effect to increase the yellowness.

Total phenolic contents: Result of the total phenolic content of the optimized kkuaripepper pork sausages and the control group was exhibited in Table 5, and showed a significant difference ($p<0.001$). The total phenolic content of the control sausage was 0.18 mg/g, the kkuaripepper sausage was 0.64 mg/g, which indicated that kkuaripepper powder increases antioxidant activity three times. It is determined that the purpose of the present study to develop a functional low-fat meat products to meet the needs of customers for the natural food with various physoplogical activity.

DPPH free radical scavenging activity: DPPH free radical scavenging activity of the optimized kkuaripepper sausage and the control sausage was shown in Table 5, and they were significantly different (p<0.001) like as the total phenolic content. The control sausage was 4.38%, while, DPPH free radical scavenging activity was significantly increased up to 65.78% as the addition of kkuaripepper powder. Through this, it was found that the antioxidant activity of kkuaripepper powder left after producing the sausage. Therefore, it can be expected more effective antioxidant activity when we take the kkuaripepper powder added pork sausage than normal pork sausage.

Texture: The texture analysis result (hardness, adhesiveness, springiness, chewiness, cohesiveness) of the optimized kkuaripepper sausage was shown in Table 6. The result of hardness, adhesiveness, springiness, chewiness, and cohesiveness were 2603.97 g, -7.29 g.s, 5.28, 6495.46 and 0.45, respectively, and the springiness, chewiness and cohesiveness were significantly different within 1%. The hardness of the kkuaripepper added sausage was low compared to the control group, which is a result similar to the study for the sausage with red pepper seed powder [26] and red yeast rice.

Characteristics	KS[a]	Control	t-value
Salinity (%)	0.16 ± 0.00	0.14 ± 0.01	2.44
Water holding capacity (%)	95.76 ± 0.04	96.51 ± 0.05	-23.42
Moisture content (%)	59.50 ± 0.11	62.34 ± 0.08	7.94*
L	59.60 ± 0.57	62.23 ± 0.43	-23.41***
a	-4.21 ± 0.29	4.15 ± 0.03	-36.49***
b	20.46 ± 0.73	12.49 ± 0.05	23.65***
Total phenol (mg/g)	0.64 ± 0.02	0.18 ± 0.01	24.13***
DPPH radical Scavenging activity (%)	65.78 ± 0.14	4.38 ± 0.12	52.18***

[a]KS, sausage added with Kkuaripepper (*Capsicum annum* L.) powder; control, sausage without antioxidant

*p<0.05, **p<0.01, ***p<0.001

Table 5: Physicochemical characteristics of optimized pork sausage with Kkuaripepper (*Capsicum annum* L.).

In general, it has been reported that increased fat in the sausage causes to increase the hardness, gumminess, cohesiveness and chewiness but the springiness is reduced. In this experiment, we aimed at improving the palatability by replacing the backfat with olive oil, and it was observed the effect on the texture by adding kkuaripepper. In the result, the springiness and chewiness were significantly increased, while the cohesiveness was reduced, so it is considered that the preference for the texture of springiness and chewiness can be increased than the general pork sausage.

Sensory properties: We evaluated the sensory properties (color, flavor, taste, tenderness, overall quality) of the optimized kkuaripepper sausage color (Table 6), and the optimized kkuaripepper sausage showed significantly high score in color (p<0.01), flavor (p<0.001), taste (p<0.001) and overall acceptability (p<0.05) except for the tenderness. In this result, it was confirmed the possibility as a product to enhance the nutritional and sensory properties by the addition of kkuaripepper powder to sausage.

Characteristics	Ks[a]	Control	t-value
Hardness (g)	2603.97 ± 246.37	2781.46 ± 178.46	-1.712
Adhesiveness (gxs)	-7.29 ± 4.13	-10.42 ± 4.79	0.9231
Springiness	5.28 ± 0.79	1.23 ± 0.04	17.124***
Chewiness	6495.46 ± 234.53	3346.10 ± 54.12	3.498**
Cohesiveness	0.45 ± 0.01	0.54 ± 0.02	1.462*
Color	5.60 ± 1.53	3.90 ± 1.58	-3.44***
Flavor	5.55 ± 1.43	3.40 ± 1.72	-4.28***
Taste	5.40 ± 1.39	2.85 ± 1.34	-5.29***
Tenderness	4.75 ± 1.37	3.80 ± 176	-1.9
Overall quality	5.10 ± 1.97	3.55 ± 1.70	-2.66*f

[a]KS, sausage added with Kkuaripepper (*Capsicum annum* L.) powder; control, sausage without antioxidant

*p<0.05, **p<0.01, ***p<0.001

Table 6: Texture and sensory characteristics of optimized pork sausage with Kkuaripepper (*Capsicum annum* L.).

Conclusions

In this study, we tried to develop the pork sausage with unique kkuaripepper flavor and physiological activity by manufacturing the kkuaripepper pork sausage. The optimum point was predicted as 2.99 g for kkuaripepper powder and 9.12 g for olive oil. RSM was successfully used to identify the best combination of kkuaripepper powder and olive oil to create a low fat-functional sausage and to obtain an innovative functional pork sausage with a high sensory acceptance and suitable physicochemical properties. The results obtained in this study will be useful to the meat industry, which tends to decrease the saturated fatty acid content with a concomitant enrichment in the unsaturated fatty acids content. The advantage of the kkuaripepper powder was the pleasant color of the meat product. In addition, the flavor of the cooked pork sausage was maintained, which was not the case when spice extracts, such as rosemary and garlic, were used. The use of plant extracts in meat may alter the product sensory characteristics, particularly flavor and color. Undesirable changes in flavor and color can have a negative effect on meat products prepared with other plant extracts. And further research is necessary to examine the lipid oxidation stability of pork patty and antimicrobial activity of during storage period.

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