Evaluation of the effect of yellow konjac flour- κ -carrageenan mixed gels and red koji rice extracts on the properties of restructured meat using response surface methodology

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Abstract In the present study, the effect of two independent variables, yellow konjac flour- κ -carrageenan (KFC) mixed gels and red koji rice (RKR) extracts for the development of restructured meat product, was investigated using central composite design of response surface methodology (RSM). The assessed physical characteristics were hardness, water holding capacity (WHC), and color ($^{\circ}$ hue) of the restructured meat products. The second order regression models with high R^2 value were significantly fitted to predict the changes in hardness, WHC and color. The results showed that the predicted optimum formula of restructured meat were the addition of KFC mixed gels at 10.21% and RKR extracts at 6.11%. The experiments results validate these optimum formula and found to be not statistically different at 5% level. Thus, the RSM was successfully employed and can be used to optimize the formulation of restructured meat.

Keywords Formulation · Functional ingredient · Low grade meat · Physical properties · Restructuring method

Introduction

Meat is considered to be a highly nutritious and valued food. It is an important source of high biological value protein and essential micronutrients (including iron, selenium, zinc, folic acid, and vitamin D and B₁₂). However, these positive attributes have often been obscured because of the prominence of several negative attributes. The latter include the perception that meat contains high amounts of fat which is rich in saturated fat (Scollan et al. 2006) and several studies have shown that high intake of meat is associated with increased risk of cancer (Norat et al. 2005; Ferguson 2010; Oostindjer et al. 2014). Therefore, efforts are being made to modify the meat to a “healthier” food by modifying the lipid and fatty acid contents or by adding a series of functional ingredients, such as, fiber, vitamins, mineral, or antioxidants (Fernández-Ginés et al. 2003; Rodríguez-Carpena et al. 2012).

The objective of the meat industry is to provide palatable and healthy products at a reasonable price. Economic pressure to minimize cost and maximize product utilization encourage the development of new products using low grade meat. Restructuring meat refers to the utilization of manufacturing steps to create a consumer-ready product. Restructured meat products include the use of low grade meat that are partially or completely disassembled and then reformed into the same or different form (Gadekar et al. 2015). In this study, we demonstrated that the physical properties of low grade meat can be enhanced by restructuring method. The products are beneficial as they provide improved product characteristics such as texture and shape of the product.

A variety of ingredients and natural foods have been used to enhance the physical and chemical properties of restructured meat, for example, the use of natural

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antioxidants prolong the shelf-life of restructured goat meat product (Gadekar et al. 2014), the use of vegetable oil as a back-fat replacer improve the fatty acid profile (Rodríguez-Carpena et al. 2012), the addition of fiber able to enhance the psychochemical and sensory properties of restructured meat (Gupta et al. 2015), and various non-meat ingredients such as soya, barley, oat, and walnut can be utilized for incorporation of bioactive components in meat products (Gupta and Sharma 2017).

Yellow konjac flour (KF) is produced from the tubers of *Amorphophallus muelleri*, which have yellow natural color. This flour has a special interest because of its particular characteristics and has been used widely in food industry (Widjanarko et al. 2014). It has been used to reduce the fat content of frankfurters (Jiménez-Colmenero et al. 2010), fresh sausages (Osburn and Keeton 1994), and chicken nuggets (Akesowan 2016). κ -Carrageenan has a broad range of applications in the food industry because of its water-binding, thickening, and gelling properties (Verbeken et al. 2005). Many researchers have been investigated the use of carrageenan, alone or combined with other ingredients in a variety of comminuted meat products (Chin et al. 1999; Pietrasik and Duda 2000). Previous report showed that the addition of κ -carrageenan to ham has resulted in good water binding, decreased cooking losses and improved texture (Shand et al. 1994). Konjac flour is known to interact synergistically with κ -carrageenan, which produce a mixed gels with increased elasticity and strength and with a greater resistance towards syneresis (Kaya et al. 2015). Therefore, we expect that the use of yellow konjac flour- κ -carrageenan mixed gels able to improve the physical properties of restructured meat.

Red koji rice, also known as “Hong Qu” or “Ang-kak”, is a red-colored product of rice made by culturing a *Monascus purpureus* on rice grains which become impregnated with intense red pigments that is produced by the mold (Su et al. 2005). Red koji rice, which is commercially available in the form of powder, is commonly used as additive, preservative, and food colorant (Gordon et al. 2010). Meat consumers use discoloration as an indicator of the freshness of meat, therefore their purchasing decisions are influenced by the color of meat more than any other quality factor. Myoglobin is the principle protein responsible for the red color of fresh meat, accordingly oxidation of myoglobin in meat lead to discoloration (Mancini and Hunt 2005).

Response surface methodology (RSM) is a mathematical and statistical technique for testing multiple process variables and estimate their linear, quadratic, and interaction effects (Khare et al. 2015). This tool is specifically used to determine the interaction between factors and responses to predict an optimum processing condition. The advantage of using RSM is the reduction in the number of

experiments needed as compared to a full experimental design. RSM has been used to optimize different quality parameters for meat and food product development (Tiwari et al. 2008; Khare et al. 2015). The objective of this work was to investigate the combined effect of yellow konjac flour- κ -carrageenan (KFC) mixed gels and red koji rice (RKR) extracts concentration on the changes in hardness, water holding capacity (WHC), and color of restructured meat using response surface methodology (RSM). To the best of our knowledge, this is the first report of the development of restructured meat with the use of yellow konjac flour- κ -carrageenan mixed gels and red koji rice extracts in the formulation.

Materials and methods

Materials

Low grade meat and other ingredients were purchased from a local market in Malang, Indonesia. κ -carrageenan flour was obtained from Kappa Carrageenan Nusantara, Pasuruan, Indonesia. Red koji rice powder and modified cassava flour were purchased from a local store in Malang, Indonesia. Yellow konjac flour was produced at our laboratory, which is passing through a 36-mesh sieve.

Experimental design and statistical analysis

RSM was carried out with a central composite design (CCD) with two independent factors, those are konjac flour- κ -carrageenan (KFC) mixed gels (X_1) and red koji rice (RKR) extracts (X_2) (Table 1). For each factor an experimental range was based on the results of preliminary experiments.

Three responses: hardness, water holding capacity (WHC), and color, were chosen as dependent variables. The complete design consisted of 13 experimental points including 5 centre points, 4 factorial points and 4 axial points. Data from the central composite design were analyzed by multiple regressions to fit into the empirical second order polynomial model, as shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1, j=2}^{k-1, k} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where, β_0 ; β_i ; β_{ii} and β_{ij} are regression coefficients in the intercept, linear, quadratic and interaction terms, respectively; X_i ; X_{ii} ; X_{ij} are the independent variables; Y are responses and ε is a random error component.

Design Expert 7.1.0 trial version (State-Ease, Inc.) was used to obtain the coefficients of the quadratics polynomial

Table 1 Independent variables and factor levels in the central composite design

Independent variables	Factor level				
	−1.414	− 1	0	1	1.414
Konjac flour- κ -carrageenan (KFC) mixed gels (% , X_1)	2.93	5	10	15	17.07
Red koji rice (RKR) extracts (% , X_2)	4.59	5	6	7	7.41

model. The quality of the fitted model was expressed by Lack of fit value, R^2 value, and Adjusted R^2 value and its statistical significance was determined by an F-test.

Konjac flour- κ -carrageenan (KFC) mixed gels preparation

Konjac flour- κ -carrageenan (KFC) mixed gels were prepared according to literature procedure (Kaya et al. 2015), which produce a mixed gels with a greater resistance towards syneresis. Konjac flour (1 g) was put in a volumetric flask, then 100 mL of hot water (80 °C) was added. The mixture was stirred and placed into a 250 mL Erlenmeyer flask. The flask was placed into a shaking water bath at 80 °C and the mixture was stirred continuously for 2 h until the konjac flour gel was formed. κ -Carrageenan gel was prepared with the similar procedure to prepare konjac flour gel. κ -Carrageenan powder (3 g) was added with 100 mL of hot water (80 °C). Then the mixture was placed in a shaking water bath at 80 °C and stirred continuously for 2 h until the κ -carrageenan gel was formed. Konjac flour- κ -carrageenan (KFC) mixed gels were prepared by mixing konjac flour gel and κ -carrageenan gel. Then the mixed gels were used in the subsequent experiments for restructured meat formulation.

Red koji rice (RKR) extracts preparation

Red koji rice powder (20 g) was dissolved in 160 mL of water. Then the mixture was stirred and filtered. The filtrate, which is extracts of red koji rice, was collected and used in the subsequent experiments.

Restructured meat preparation

The low grade meat (chuck beef cuts) samples were cut into small chunks and blended with salt (1%) and ice flakes (5%). Then the samples were coarsely ground with food chopper for 1 min. Modified cassava flour (6%) in water (23%), garlic (0.5%), pepper (0.5%), and konjac flour- κ -carrageenan (KFC) mixed gels were added and mixed. Next, the meat dough samples were added and mixed with red koji rice (RKR) extract, and subsequently steamed at 80 ± 5 °C for 30 min. The steamed restructured meat product then cooled into a room temperature and stored in a refrigerator prior to be analyzed. The proportion of low

grade meat, KFC mixed gels, and RKR extracts in the experimental runs is according to percentages specified in Table 2.

Hardness

The hardness of restructured meat was measured by CT3 Texture Analyzer Brookfield (AMETEK, USA) equipped with TA39 probe. The steamed restructured meat samples were cut into a cubic ($30 \times 30 \times 30$ mm³) and subjected to compression test (target: 10 mm, trigger load: 6.8 g, test speed: 10 mm/s, return speed: 10 mm/s) with two cycles of measurement. The analysis was performed at room temperature.

Water holding capacity (WHC)

Water holding capacity of restructured meat samples were determined using filter paper test method (Zamorano and Gambaruto 1997). 0.3 g of restructured meat sample was placed in a middle of glass plate ($150 \times 150 \times 5$ mm³). A filter paper (number 341) was weighed and put on the top of the sample. Then the weigher (3.5 kg) was put on the sample covered by a glass plate for 5 min. WHC was expressed as the percentage of water retained in the filter paper after weighing.

Color

The color of restructured meat products was observed using handheld Konica Minolta Color Reader CR-10 with 8° illumination angle, measuring area of approx. 8 mm, and CIE Standard Illuminant D₆₅ as an illuminant to measure L^* , a^* , and b^* values of the surface of the meat at room temperature. Two times measurements were conducted for each samples. The $^{\circ}hue$ was obtained from the calculation, $^{\circ}hue = \arctan b^*/a^*$ (Madeira et al. 2003).

Results and discussion

Effects of KFC mixed gels and RKR extracts

The values obtained from the response variables studied in each experimental running are shown in Table 3. The dependent variables, which is influenced by independent

Table 2 Formula of restructured meat according to RSM experimental design

Formula (%)	Formula of restructured meat												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Low value meat (%)	48	48	44	49.41	48	46.59	54	52	48	40.93	42	48	55.07
KFC mixed gels (%)	10	10	15	10	10	10	5	5	10	17.07	15	10	2.93
RKR extracts (%)	6	6	5	4.59	6	7.41	5	7	6	6	7	6	6
Other ingredients ^a	36	36	36	36	36	36	36	36	36	36	36	36	36
TOTAL (%)	100	100	100	100	100	100	100	100	100	100	100	100	100

^aOther ingredients: modified cassava flour 6%, salt 1%, ice flakes 5%, water 23%, garlic 0.5%, and pepper 0.5%

Table 3 Physical properties obtained for each experimental running of central composite design (CCD)

Std	Run	Variable Code		Variable Actual		Responses		
		X ₁	X ₂	KFC mixed gels (%)	RKR extracts (%)	Hardness (g)	WHC (%)	Color (°hue)
12	1	0.00	0.00	10.00	6.00	41.4	72.91	69.60
11	2	0.00	0.00	10.00	6.00	51.2	72.39	69.33
2	3	1.00	− 1.00	15.00	5.00	21.4	71.19	70.31
7	4	0.00	− 1.41	10.00	4.59	25.4	71.00	70.96
13	5	0.00	0.00	10.00	6.00	53.1	73.72	69.88
8	6	0.00	1.41	10.00	7.41	29.8	71.88	70.40
1	7	− 1.00	− 1.00	5.00	5.00	26.7	70.63	71.65
3	8	− 1.00	1.00	5.00	7.00	31.0	70.89	70.16
9	9	0.00	0.00	10.00	6.00	41.9	72.14	69.22
6	10	1.41	0.00	17.07	6.00	24.6	71.42	70.76
4	11	1.00	1.00	15.00	7.00	29.4	71.10	71.29
10	12	0.00	0.00	10.00	6.00	40.8	72.44	69.37
5	13	− 1.41	0.00	2.93	6.00	42.0	70.67	70.47

X₁ = variable code for KFC mixed gels, X₂ = variable code for RKR extracts, KFC mixed gels = yellow konjac flour-κ-carrageenan mixed gels, RKR extracts = red koji rice extracts, WHC = water holding capacity

variables, was analyzed statistically, including fix summary model, analysis of variance, normal plots of residual and model graph of responses. The highest hardness and WHC of restructured meat was on run number 5, where the addition of KFC mixed gels on restructured meat formulation was 10% w/w. This may be due to the contribution of the meat level in the restructured meat formulation and the addition of KFC mixed gels improve the physical properties of restructured meat. Konjac flour itself has a strong water-binding ability and when the flour mixed with other polysaccharide, e.g., carrageenan and starch, it exhibits a synergistic effect on protein gelation and water-binding in the production of comminuted meat (Chin et al. 2009). Konjac flour interacts strongly with κ-carrageenan and the interactions produce strong elastic gels which have rupture strength four times higher than that of κ-carrageenan gel alone (Saha and Bhattacharya 2010).

Response surface analysis

The response surface analysis (RSA) on the basis of regression coefficients of responses (hardness, WHC and color) of restructured meat is shown in Table 4. The results showed that the KFC mixed gels and RKR extracts concentrations were fitted to the second-order model and significantly affect the responses at $p < 0.01$. Whereas, lack of fit testing shown not significant. The purpose of lack of fit analysis is to measure the failure of a model to represent the data in the experimental domain at which the points were not out of the regression (Varnalis et al. 2004).

The responses of hardness (Y₁), WHC (Y₂), color (Y₃) and independent variables are mentioned in the following equations:

Table 4 Regression coefficients and significance of the predicted second-order polynomial models for hardness, WHC and color of restructured meat

	Regression coefficients		
	Y ₁ ^a	Y ₂ ^b	Y ₃ ^c
Intercept, X ₀	− 332.69	40.86	103.74
Linear			
X ₁ : KFC mixed gels (%)	3.72	0.88	− 1.23
X ₂ : RKR extracts (%)	118.92	8.90	− 9.21
Interaction			
X ₁ X ₂	0.19	− 0.02	0.12**
Quadratic			
X ₁ ²	− 0.28*	− 0.04**	0.03**
X ₂ ²	− 9.87**	− 0.71**	0.65**
Mean	35.28	71.72	70.26
Standard deviation	5.45	0.52	0.24
R ²	0.84	0.83	0.94
Adjusted R ²	0.72	0.70	0.90
F-value (model)	7.28**	6.66**	22.44**
F-value (lack of fit)	0.62	0.28	0.68

* $p < 0.05$; ** $p < 0.01$; ^aHardness (g), ^bWHC (%), ^cColor (^ohue)

$$Y_1 = -332.69 + 3.72X_1 + 118.92X_2 + 0.19X_1X_2 - 0.28X_1^2 - 9.87X_2^2 \quad (2)$$

$$Y_2 = 40.86 + 0.88X_1 + 8.90X_2 - 0.02X_1X_2 - 0.04X_1^2 - 0.71X_2^2 \quad (3)$$

$$Y_3 = -103.74 - 1.23X_1 - 9.21X_2 + 0.12X_1X_2 + 0.03X_1^2 + 0.65X_2^2 \quad (4)$$

Equations (2) and (3) show a negative quadratic effect on hardness and WHC of the studied factors which were observed. These mean that by increasing concentration of KFC mixed gels and RKR extracts will decrease the hardness and WHC values (Eqs. 2, 3). Increasing KFC mixed gels added into a formula of a restructured meat will increase moisture content of the end products. RKR extracts were prepared by dissolving RKR powder in water. Thus, increasing KFC mixed gels and RKR extracts in the formula of restructured meat product decreased the hardness and WHC. The technological implication of these phenomenon was: That the optimum concentration of KFC mixed gels and RKR extracts for restructured meat formula required to be determined. Decreasing the hardness and WHC of restructured meat product will corroborate the decrease sensory attributes of the product. Glucomannan powder are tasteless, odorless and pure white (Arvill and Bodin 1995). Thus, increasing concentration KFC mixed

gels containing konjac flour added into restructured meat formula, will decrease sensory characteristics of restructured meat product.

As shown in Table 4 and Eqs. (2–4), both of the independent variables, the KFC mixed gels and RKR extracts, have quadratic effect on the hardness, WHC and color of restructured meat with R² value or coefficient of determination higher than 80%. The R² value of all of responses exceed 80%, indicating a high proportion of variability as explained by the data. High R² value, which is close to 1, is desirable (Noordin et al. 2004). A high R² coefficient ensures a satisfactory adjustment of the quadratic model to the experimental data. Therefore, the response surface model developed in this study was adequate.

KFC mixed gels and RKR concentrations showed negative quadratic impact ($p < 0.01$) on hardness and WHC, and no significant interaction between KFC mixed gels and concentration of RKR extracts (Table 4). Hardness is one of the physical properties affected by the addition of hydrocolloids, such as xanthan gum, in the restructured meat product (Rather et al. 2015). The hardness of a restructured meat product made from minced biceps femoris meat was significantly influenced ($p < 0.05$) by k-carrageenan addition with 40 and 50% of added brine (Patraşcu et al. 2014). These studies supported that hardness of restructured meat product was significantly affected by the addition of hydrocolloids. Response surface models and contour plots depicted in Fig. 1 showed that the KFC mixed gels had positive linier and negative quadratic effect due to the elastic properties of the gels, which is caused by the interaction of konjac flour with κ-carrageenan and induced by the present of meat in the formulation. Similar finding was reported previously, where meat level had positive linier and quadratic effect on hardness of chicken meat noodles (Khare et al. 2015). The ability of meat to retain moisture is an important quality characteristic. Losses of moisture in meat product is important from economic point of view, sold and distribution and mobility of water in muscle and meat had a influence quality attributes like juiciness, tenderness and appearance (Pearce et al. 2011). The use of KFC mixed gels enhanced the water holding capacity of the restructured meat product.

The interactions of KFC mixed gels and RKR concentrations showed positive linier and quadratic effect on the color of restructured meat (Table 4). The optimum response of the color affected by mixed gels and RKR concentrations was produced in the center of contour plots of color (C1), which is indicated by a node (Fig. 1). This predicted contour response of the color indicate minimum response. The increases of KFC mixed gels and RKR concentrations minimize the brown color development of cooked restructured meat. The addition of red koji rice in Chinese sausages produce darker red color, whereas the

Fig. 1 Contour and response surface plots of hardness (A1, A2), WHC (B1, B2), color (C1, C2) of restructured meat, and contour and response surface plots of desirability (D1, D2) for predicted optimum

color of sausages without red koji rice were light red (Liu et al. 2010). The red koji rice extracts as a natural food colorant produced restructured meat with more desirable color.

Predicted optimization and verification

Criteria setting to obtain optimization process with Design Expert was set as follow: hardness is in the range, maximum WHC, and minimum color. The results showed that the optimum formulation of making restructured meat was as follows: the predicted optimum KFC mixed gels was 10.21% w/w and RKR extracts concentration was 6.11% w/w. These results produce restructured meat with the hardness, WHC and color of about 45.65 g, 72.74% and

results of a quadratic effect of interactions between KFC mixed gels and RKR extracts concentration

64.48 °hue, respectively, and the desirability of about 76.2%. The obtained desirability means the independent variables affect 76.2% on responses and 33.8% comes from noises. Verification step was conducted to make sure the predicted results were not biased towards the practical value with the objective of each response produce maximum yield using deduced optimal condition. The recheck experiments were conducted in three replications. The results showed that the hardness, WHC and color of restructured meat were 43.83 ± 1.82 g, 72.74 ± 0.002 and 69.34 ± 0.14 °hue, respectively. Those values were very close (less than 5% difference) to the predicted values using model. Less than 5% differences in responses between the predicted results and repeated experiments confirmed that the model was quite accurate.

Conclusion

RSM is a useful tool for the optimization of restructured meat formulation. The hardness, WHC, and color of the restructured meat products were affected by the addition of KFC mixed gels and RKR extracts. The predicted values were in agreement with the experimental values and found to be not statistically different at 5% level. The models developed in this study can be used to estimate the desired physical quality of restructured meat products with the addition of KFC mixed gels and RKR extracts. The optimized formula predicted by the models was KFC mixed gels 10.21% w/w and RKR extracts concentration 6.11% w/w.

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