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Concentration Optimization of Meat, MOCAF (Modified Cassava Flour), and Purified Porang Flour-κ-Carrageenan (PPFC) Mixed Hydrocolloid Gel for Restructured Sliced Meat Formula using Response Surface Methodology

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Abstract

This study aims to determine the optimum formula in restructured sliced meat production using the Box-Behnken Design of Response Surface Methodology (BBD-RSM) to obtain the best yield, hardness, and water-holding capacity (WHC). The concentration of the meat used was 30 to 40 %, MOCAF (modified cassava flour) was 10 to 15 %, and purified porang flour-κ-carrageenan (PPFC) mixed hydrocolloid gel was 7.5 to 12.5 %. The alterations in yield, hardness, and WHC of restructured sliced meat were significantly predicted by the second-order regression models with high R^2 values. The results revealed that the combining meat composition of 35.41 %, MOCAF of 11.58 %, and purified porang flour-κ-carrageenan (PPFC) mixed hydrocolloid gel of 12.50 % was predicted as the optimum formula for restructured meat with characteristics as follows; 99.62 % yield, 133.63 g hardness, and 68.76 % WHC. The verification results through actual research were not significantly different ($p > 0.05$) from the predictions optimal formula, with characteristics as follows; 99.07 % yield, 131.63 g hardness, and 68.45 % WHC.

Keywords: Restructured sliced meat, Modified cassava flour, Purified porang flour-κ-carrageenan mixed gel, Response surface methodology

Introduction

Based on Indonesian statistics (BPS) data in 2017, beef production by the province during 2016 - 2017 increased by 2.56 %. In 2016 it was 518,484.03 tons, then it increased to 531,756.98 tons by 2017. Along with the increasing demand for beef, the production level of processed beef products is predicted to expand, including restructured meat. Restructured meat is processed meat that combines small pieces with natural protein or other ingredients to form large meat, similar to steaks and roasts [1,2]. Meat restructuring technology utilizes low-quality carcass pieces as value-added products [2]. The restructured meat products well-known to the public include sausages, meatballs, nuggets, burgers, or restructured sliced meat. An important aspect that needs to be considered in the restructured meat products manufacture is that the products have physical characteristics resembling meat, such as the hardness level and water binding capacity. The composition of meat, binders, and fillers mainly influences the physical characteristics of restructured meat products.

In commercially restructured meat products, a binding material is generally added as inorganic materials such as sodium tripolyphosphate (STPP). However, much research has been conducted on using organic binders in restructured meat. One potential alternative binder sourced from organic material is hydrocolloid gel from purified porang flour and κ-carrageenan), the 2 food ingredients easily found in Indonesia.

Porang flour (PF) is a processed product from native porang (*Amorphophallus muelleri* Blume) tubers from Indonesia with a characteristic yellow color on their tubers [3-5]. This flour has unique characteristics and has been applied in various food industries for thickeners, binders, fat substitutes, and other purposes. Generally, PF was further purified using ethanol solvent to obtain the purified porang flour (PPF), which has a composition of glucomannan content, and calcium oxalate content in the purified porang flour was 76.02 - 87.79 % in dry-based and 0.06 - 0.31 %, respectively [3,6-8]. Then, the PFF was transformed into a gel for applications in food products. Previous studies have reported that adding konjac glucomannan increased the plant-based fishball analogue's hardness, chewiness, and gel strength [9]. Furthermore, the application of konjac gel alone or in combination with vegetable powders improved the physicochemical and sensory properties of low-fat frankfurter-type sausage, such as WHC, cooking loss, emulsion stability, texture, and all sensory attributes [10].

Carrageenans are sulfated galactose and anhydrogalactose polymers that are taken from red seaweed. Global production of carrageenan seaweed, cultivated and wild, in 2019 amounted to 11,685,174 wet tons, and Indonesia is the largest carrageenan seaweed-producing country, with the production of 9,795,400 wet tons according to data from FAO [11]. The kappa type of carrageenans is the most frequently used in food technology out of the 3 main types iota, kappa, and lambda [12]. κ-carrageenan has been extensively utilized in the meat industry because of its excellent water-holding, thickening, and gelling capacities [11,12]. Previous studies reported that the addition of 1 % κ-carrageenan concentration was able to produce the highest hardness and rheological parameters (G′, G″, and G*) of restructured chicken breast ham compared to other concentrations [14]. Yasin *et al.* [15], reported that the optimum addition of κ-carrageenan to improve the texture properties, cooking yield, and WHC of the restructured chicken ball was found at 1.28 % through the Central Composite Rotatable Design. Ayadi *et al.* [16], also reported that the carrageenan addition increased the water holding capacity, hardness, and cohesiveness but decreased the emulsion stability in turkey meat sausage. However, PPF and κ-carrageenan can be used individually or in combination as binders. PPF and κ-carrageenan produces a gel with a more elastic and smooth texture that prevents syneresis [17]. The application of carrageenan-konjac flour gel mixed produced the low-fat restructured pork nuggets with characteristics similar to the all-pork nuggets [18]. In another study, Widjanarko *et al.* [19] reported that the best restructured sliced meat products were obtained by adding about 10 % hydrocolloid gel from PPF and κ-carrageenan.

Modified cassava flour, known as MOCAF, is a product of the fermentation of cassava using the lactic acid bacteria, and it is usually used for wheat flour substitution in food product applications [20,21], but in the study, the MOCAF has function as a filler in restructured sliced meat production. The abundance of MOCAF in Indonesia is not known yet, due to the difficulty in finding data from The Central Bureau of Statistics Indonesia. The demand for MOCAF from several countries abroad is 80 - 100 tonnes per month [22]. It can be noted that MOCAF quickly got it's in Indonesia. MOCAF has unique properties, such as having high viscosity and WHC and being easily dispersed in food systems, making it suitable for application as a food composition in semi-wet products [23]. Several previous studies reported the application of MOCAF as filler in meat restructured, especially nuggets. MOCAF increases mushroom chicken nuggets' hardness, gumminess, chewiness, and colours [24]. Hence, based on the preceding explanation and the author's knowledge, the optimum formulation of meat, MOCAF, and purified porang flour-κ-carrageenan (PPFC) mixed hydrocolloid gel concentration to manufacture restructured sliced meat has yet to be discovered. Thus, finding the optimum formula from the concentration of meat, MOCAF, and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel is necessary to produce the expected characteristics of restructured sliced meat. The Response Surface Methodology is one of the commonly employed methods.

Response Surface Methodology is a powerful and effective tool for process optimization when the independent variables have a combined impact on the desired response. RSM is a group of mathematical and statistical systems that effectively develop, improve, and optimize processes such as formulating new products [25]. Therefore, the aimed to determine the optimum formula for meat, MOCAF, and purified porang flour-κ-carrageenan (PPFC) mixed hydrocolloid gel concentrations in the making of restructured sliced meat using Response Surface Methodology (RSM) with Box-Behnken Design (BBD) approach to obtain products with the best yield, hardness, and water holding capacity (WHC).

Materials and methods

Materials

Brisket meat cuts were purchased from a local butcher at a traditional market (Dinoyo, Malang, Indonesia). In contrast, cattle were slaughtered at Malang City abattoir, East Java Province, Indonesia. The meat was taken from Ongole-grade in Indonesia, called *Peranakan Ongole* (PO), 3-year-old beef carcasses, and dry-ageing for 24 - 48 h before being purchased. The meat was frozen at −20 °C and stored at this temperature until used. Spices such as garlic, pepper, onion, nutmeg, and salt, were purchased at Dinoyo Market, Malang, East Java Province, Indonesia. MOCAF and K-Carrageenan were provided by the La

Tanza store in Depok, West Java, Indonesia, and Kappa Carrageenan Nusantara in Pasuruan, East Java Province, Indonesia, respectively. Skim milk and red food colouring (Cl 14720) was obtained from Prima Rasa Store in Malang City, East Java Province, Indonesia. Soy protein isolates were acquired from Nine stores in Surabaya, East Java Province, Indonesia. Purified porang flour (PPF) was obtained from the Pilot Plant Laboratory, Faculty of Agricultural Technology, University of Brawijaya, Malang, East Java Province, Indonesia. The PPF contained ≥ 80 % in the dry base of glucomannan and 0.21 % in calcium oxalate content.

Gel preparation

The gel was made by mixing purified porang flour (PPF) and κ-carrageenan based on the study of Kaya *et al.* [26], with modification. A 0.5 g purified porang flour (PPF) and 2 g κ-carrageenan were put in a 250 mL glass beaker and then added hot water (80 °C) to 75 ± 5 mL. The solution was transferred into a 100 mL measuring glass then room temperature water was added to reach 100 mL. Afterward, the solution was transferred into 250 mL Erlenmeyer and was shaken using a rotary shaker for 5 min to form a hydrocolloid gel. The hydrocolloid mixture gels stir slowly in water, using stirrer rods to press against the beaker glass wall.

Restructured sliced meat preparation

The preparation of restructured sliced meat was based on the Widjanarko *et al.* [19], method with modification. Briefly, beef was removed from the fat and its binding tissue. The meat was cut into small pieces of ± 1×1×1 cm^3 and then weighed according to the specified concentration, as listed in Table 1. 1.5 % salt and 3 % ice flake were added and then mashed using a chopper. 1.5 % salt and 3 % ice flake were added, then were mashed using a chopper (Phillips HR-2939, Indonesia) for 1 min. MOCAF and purified porang flour-κ-carrageenan (PPFC) mixed hydrocolloid gels were added according to the determined concentration listed in **Table 1**, then were smoothed using a chopper for 1 min. 2.5 % skim milk, 1.2 % garlic, 0.5 % pepper, 0.2 % nutmeg, 0.6 % onion, and 25 % dye which had been dissolved in water, were added and then mashed using a chopper for 1 min. Next, soy protein isolates with a varied concentration (0.5 - 15.5 %) were added to complete the percentage of the dough to reach 100 %. The mixture was smoothed using a chopper for 1 min. The dough was poured into a stainless-steel mould with a diameter of 8 cm coated in plastic wrap, then steamed for 30 min at around 80 ± 5 °C. Lastly, the dough was cooled at room temperature for 1 h and then put in a refrigerator (Sharp FRV-310X, Indonesia) at −20 °C before further analysis. The formulations of restructured sliced meat are listed in **Table 1**.

Table 1 The formulation for restructured sliced meat.

Formulation materials	**The formulation of restructured sliced meat (%)**																
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Meat	35	35	35	35	35	30	40	30	40	30	40	30	40	35	35	35	35
Ice flake	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
MOCAF Flour	12.5	12.5	12.5	12.5	12.5	10	10	15	15	12.5	12.5	12.5	12.5	10	15	10	15
Hydrocolloid gels	10	10	10	10	10	10	10	10	10	7.5	7.5	12.5	12.5	7.5	7.5	12.5	12.5
Skim milk	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Garlic	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Pepper	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nutmeg	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Onion	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Water	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Soy protein isolate	8	8	8	8	8	15.5	5.5	10.5	0.5	15.5	5.5	10.5	0.5	13	8	8	3
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Yield analysis

The yield was determined based on the method of Khalil [27], by dividing the weight of the restructured meat sample after cooking (B) by the weight of the restructured meat dough before cooking (A) multiplied by 100 % using the following equation;

$$Yield = \frac{B}{A} \times 100\%$$

where A is restructured meat dough before cooking, and B is the weight of the restructured meat sample after cooking

Hardness

The CT3 Texture Analyzer Brookfield (AMETEK, USA) equipped with a TA-39 probe was used to evaluate the hardness of samples. The analysis was conducted at room temperature (25 ± 3 °C) [19]. Briefly, the fried restructured sliced meat samples were chopped into cube shapes ($30\times30\times30$ mm^3) and subjected to the compression test with 2 cycles of measurement using the following parameters: Target puncture depth of 10 mm, hold time of 0 s, trigger load of 6.8 g, test speed of 10 mm/s, and return speed of 10 mm/s.

Water holding capacity (WHC)

The determination of WHC was carried out based on the method of Zamorano and Gambaruto [28]. A sample of about 0.3 g was placed in the center of the filter paper (number 341), which weight is known. Furthermore, the filter paper was folded into 2 parts and placed in the middle of the glass plate ($200\times200\times5$ mm^3). The filter paper containing the sample was closed by a glass plate, and the weigher (3.5 kg) was put on the glass plate for 5 min. Next, filter paper containing water was weighed. The free water content was determined by reducing the weight of the filter paper containing water with the weight of the initial filter paper. The WHC resulted from reducing the total water content with free water content.

Experimental design

Determination of the optimum concentration of meat, MOCAF, and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel on the yield, hardness, and water holding capacity (WHC) values were carried out using Response Surface Methodology (RSM). The Design Expert 7.0 program performed formula optimization calculations and repeated verification 3 times. The optimization process was carried out by Box-Behnken Design (BBD) using 5 center points and twelve factorials, which resulted in 17 experimental treatments, as listed in **Table 2**.

Statistical analysis

The formula optimization calculations were performed by the Design Expert 7.0 trial version program (State-Ease, Inc., USA). The optimum response was determined by choosing the most appropriate response model. The selection of the most appropriate model was based on evaluating the sequential sum of squares model, lack of fit test, and model summary statistics. The chosen model was a model with a p-value of less than 0.05. Multiple regressions analyzed data from BBD to fit into the empirical second-order polynomial model, as shown in the following equation;

$$Y = \beta_0 - \beta_1 * X_1 + \beta_2 * X_2 + \beta_3 * X_3 + \beta_{12} * X_1 * X_2 + \beta_{13} * X_1 * X_3 - \beta_{23} * X_2 * X_3 + \beta_{11} * X_1^2 - \beta_{22} * X_2^2 - \beta_{33} * X_3^2$$

where; β_0; β_1, β_2, β_3; β_{12}, β_{13}, β_{23}; and β_{11}, β_{22}, β_{33} are regression coefficients for the intercept, linear, quadratic, and interaction coefficients, respectively, and X_1, X_2, and X_3 are the coded independent variables indicating yield (%), hardness (g) and WHC (%) of the restructured sliced meat respectively. The predicted optimum formula of restructured sliced meat and verification were calculated for the T-test at *p*-value < 0.05 using MINITAB version 17 (Minitab, Inc., USA).

Results and discussion

Yield, hardness, and WHC

Table 2 shows the result of yield (%), hardness (g), and WHC (%) analysis carried out using the BBD with RSM. In contrast, **Table 3** shows regression coefficients of ANOVA results and significances for meat, MOCAF, and hydrocolloid gel addition and their interactions on yield, hardness, and WHC. The yield of restructured sliced meat ranged from 98.57 to 99.73 %. The yield of restructured sliced meat in this study was higher than in previous studies, such as restructured turkey steak of 84.14 - 88.25 % [29], restructured turkey meat rolls of 90.41 % [30], restructured ostrich ham of 86.00 - 92.5 % [31], and restructured spent hen meat slices of 94.40 - 95.07 % [32]. In this study, the yield of restructured sliced meat was affected by the MOCAF concentration in linear (X_2) and quadratic (X_2^2) terms and hydrocolloid gel concentration in linear terms (X_3). The interaction between independent variables and yield response is revealed in **Figure 1**. Based on the equation in **Table 3** of ANOVA in the yield response, the positive value of coefficients X_2 and X_3 indicates that MOCAF enhancement and hydrocolloid gel concentration increased significantly. The reason is that MOCAF is a filler with high starch content, which can absorb water during cooking so that the cooking shrinkage of the product decreases and the product yield increases. According to, Mbougueng *et al.* [33], as much as 20 % starch content significantly increases the binding capacity of raw patties products and the yield of cooked patties. Li and Yeh [34], added that gelatinised starch would absorb more water, reducing cooking shrinkage and increasing the gel's strength.

Table 2 Yield, hardness, and WHC of restructured sliced meat were obtained for each experimental running of BBD.

Id	Run	Coded variable			Actual variable			Yield (%)	Hardness (g)	WHC (%)
		X_1	X_2	X_3	Meat (%)	MOCAF (%)	Hydrocolloid gel (%)			
0	1	0	0	0	35	12.5	10	99.48	172.20	66.06
0	2	0	0	0	35	12.5	10	99.52	168.70	66.02
0	3	0	0	0	35	12.5	10	99.56	165.55	66.98
0	4	0	0	0	35	12.5	10	99.66	162.15	64.10
0	5	0	0	0	35	12.5	10	99.54	165.85	67.29
1	6	−1	−1	0	30	10	10	99.49	255.20	63.95
2	7	1	−1	0	40	10	10	99.00	120.70	67.41
3	8	−1	1	0	30	15	10	99.73	188.55	59.01
4	9	1	1	0	40	15	10	99.33	88.40	68.91
5	10	−1	0	−1	30	12.5	7.5	99.66	257.60	57.19
6	11	1	0	−1	40	12.5	7.5	99.36	131.80	66.54
7	12	−1	0	1	30	12.5	12.5	99.54	163.60	62.71
8	13	1	0	1	40	12.5	12.5	99.69	64.75	70.85
9	14	0	−1	−1	35	10	7.5	98.57	228.50	63.10
10	15	0	1	−1	35	15	7.5	99.53	165.55	64.54
11	16	0	−1	1	35	10	12.5	99.43	151.05	69.31
12	17	0	1	1	35	15	12.5	99.65	158.30	65.90

Explanation: Variable code X_1 = meat concentration (%), X_2 = MOCAF concentration (%), X_3 = hydrocolloid gel concentration (%), hydrocolloid gel = purified porang flour-κ-carrageenan (PPFC) mixed hydrocolloid gel, WHC = water holding capacity.

Table 3 Regression coefficients of ANOVA result in yield, hardness, and WHC.

	Regression coefficients		
	Y_1[a]	**Y_2[b]**	**Y_3[c]**
Intercept, X_o	95.48	1236.98	−13.26
Linear			
X_1: Meat (%)	−0.28	9.66*	3.13*
X_2: *Modified cassava flour* (%)	1.18*	−95.33*	−3.08
X_3: Hydrocolloid gel (%)	0.25*	−66.55*	5.54*
Interaction			
X_1X_2	1.80	0.69	0.13*
X_1X_3	9.00	0.54	−0.02
X_2X_3	−0.03	2.80*	−0.19
Quadratic			
X_1^2	2.06	−0.50	−0.05*
X_2^2	−0.03*	1.42	9.60
X_3^2	−6.56	0.01	−0.07
Mean			
Standard deviation	0.17	12.14	1.15
R^2	0.84	0.97	0.95
Adjusted R^2	0.64	0.94	0.89
F- (model)	4.12*	29.82*	15.63*
F- (lack of fit)	13.94*	22.88*	0.65

*$p < 0.05$ (significant); [a]Yield (%); [b]Hardness (g); [c]WHC (%)

The increase in hydrocolloid gel concentration significantly increased the products' yield (**Table 3**). It is because the hydrocolloid gel can bind water so that water lost during cooking can be reduced. According to McArdle and Hamill [35], hydrocolloid gel maintains moisture and increases yield, reduces drip loss, and protects products during freezing from muscle tissue damage caused by ice crystals. Purified porang flour and κ-carrageenan in the hydrocolloid gel can bind water to minimize cooking shrinkage and increase product yield. Kaya *et al.* [26], explained that glucomannan might reduce the surface tension of the hydrocolloid gel mixture of purified porang flour and carrageenan so that the elasticity and strength of the gel are increased. Imeson [36], added that carrageenan could bind water and other hydroxyl groups because it has a negatively charged sulfate group along the hydrophilic polymer chain.

The negative coefficient of X_2^2 indicates that with the higher concentration of the MOCAF squared (**Table 3**), the yield increase to a certain point. Then after reaching the optimum point, the yield decreases significantly with the increase of the products' MOCAF concentration. It might be due to the utilisation of a higher concentration of MOCAF, causing a lower concentration of hydrocolloid gel added to produce 100 % formulas for restructured sliced meat, resulting in a decrease in yield. Hydrocolloid gel has an excellent ability to bind water. So, if the added hydrocolloid gel concentration is low, the product's ability to bind water is low, which causes the product's yield to be low. According to BeMiller [37], konjac glucomannan can absorb water up to 200 times its weight. Pietrasik et al. [38], also mentioned that adding starch prevents excessive water loss and helps to maintain cooking yields either in non-starch control products that are not added by water or added by 20 g of water/100 g of material. It gives the impression that adding starch cannot guarantee an increase in the products' yield that is not added by water or a small amount of water is added.

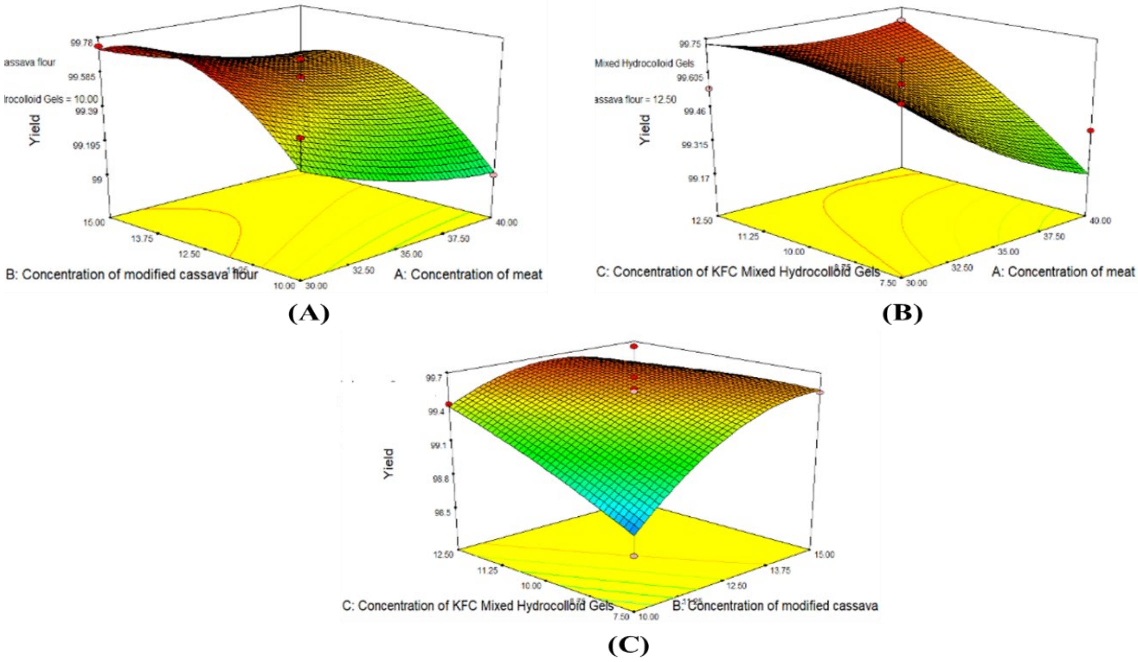

Figure 1 Response surface graph of the effect of meat, MOCAF, and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration on the yield of restructured sliced meat. (A) Interaction of meat and MOCAF concentration, (B) Interaction of MOCAF and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration, and (C) Interaction of meat and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration.

The hardness of restructured sliced meat ranged from 64.75 to 257.60 g. The hardness of restructured sliced meat in this study was higher than those in previous studies, such as restructured meat of 21.40 - 53.10 g [19], and restructured meat with the addition of oyster mushroom of 42.50 - 173.50 g [39]. In this study, the hardness of restructured sliced meat was affected by the meat concentration (X_1), hydrocolloid gel concentration (X_3) in linear terms and interaction between MOCAF concentration and hydrocolloid gel concentration (X_2X_3). The interaction between independent variables and hardness response is revealed in **Figure 2**. Based on the equation of ANOVA of hardness response in **Table 3** showed positive coefficient X_1 indicates that the higher meat concentration, the products' hardness enlarge significantly. It can be caused by the higher meat concentration, the higher the products' protein content, which increases the hardness of the product's texture. A similar trend was found by Haddad [40], who reported that increasing the pale, soft, and exudative (PSE) meats concentration decreased the hardness of restructured cured-smoked pork loin. Cavestany *et al.* [41], and Colmenero *et al.* [42], explained that the high protein content in the product causes the formation of a denser gel matrix which increases the hardness of the product's texture. Cavestany *et al.* [41], agreed that several factors, including differences in formulation and ionic strength, functional properties of meat protein, and fat characteristics, bring the effect of protein concentration on products' texture. In addition, Naruki and Kanoni [43] also found that the texture strength of products is influenced by the interaction of protein molecules with starch molecules that can form a matrix. It is caused during heating, meat protein in actin and myosin changes to form actomyosin, where the addition of starch causes the cavity between the threads of meat protein filled with starch which then undergoes gelatinization to form microcrystalline webs that settle.

The negative value obtained for each X_2 and X_3 coefficient shows that the higher the MOCAF concentration and hydrocolloid gel concentration, the products' hardness decreases significantly. Increasing concentration of MOCAF causes the gel matrix formed to be not strong enough to bind between the components of the material so that the product texture becomes less compact. Modified potato starch causes a decrease in hardness which is thought to be caused by weakly formed matrix gel bonds. Claus and Hunt [44], also implied that high water content in meat is thought to cause muscle proteins to interact more easily with water than forming cross bridges which raises the hardness of meat.

The hydrocolloid gel in this study was produced from a mixture of purified porang flour and κ-carrageenan, which was added to water. Hence, the hydrocolloid gel water content was relatively high.

These results follow the research of Widjanarko *et al.* [19], which stated that increasing the concentration of purified porang flour-carrageenan mixed gel and red koji rice extract causes a decrease in the hardness and WHC of restructured meat products. Tumieva *et al.* [45], also reported that the incorporation of 0.625 % of konjac gum into carrageenan solution showed an increased hardness of konjac gum-κ-carrageenan mixed gels, but more than 0, 625 % incorporation of konjac flour decreased the gel hardness of konjac gum- κ-carrageenan mixed gels. Mariana *et al.* [39], also stated that the application of concentration of porang flour-mixed κ-carrageenan gel from 2.5 to 10 % significantly increased the hardness of restructured meat, and significantly the harness decreased when the concentration of porang flour-κ-carrageenan gel increased to 15 to 17.07 % applied. The positive coefficient of X_2X_3 indicates that the higher the MOCAF concentration with hydrocolloid gel, the more complex the hardness of restructured sliced meat raises.

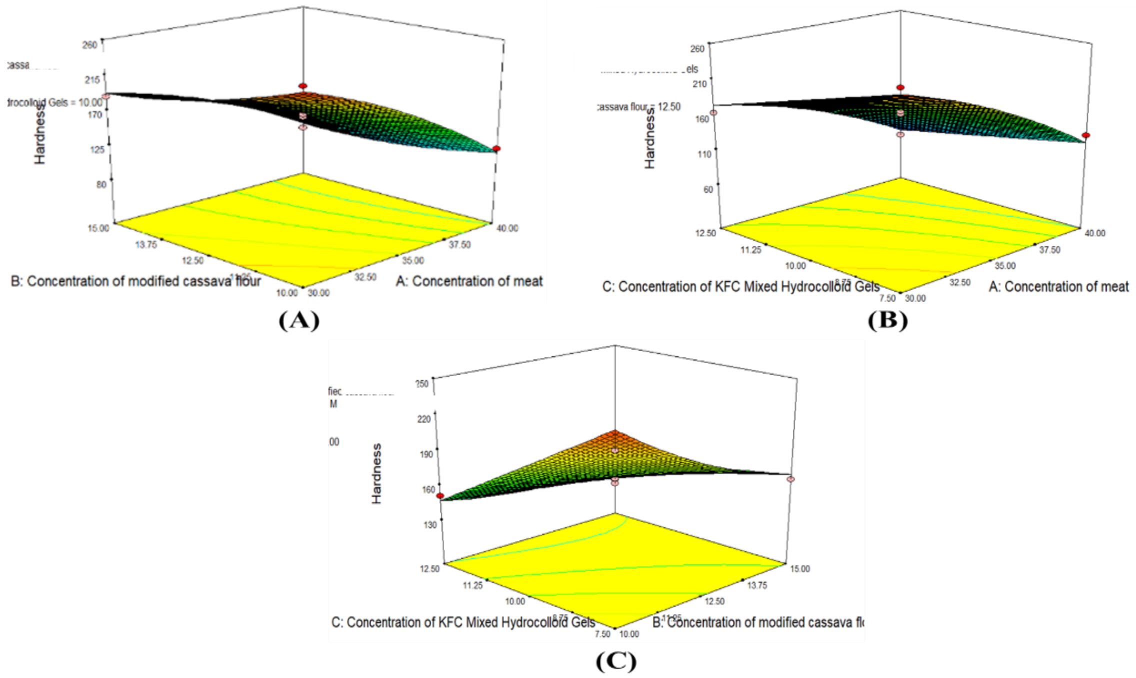

Figure 2 Response surface graph of the effect of meat, MOCAF, and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration on the hardness of restructured sliced meat. (A) Interaction of meat and MOCAF concentration, (B) Interaction of MOCAF and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration, and (C) Interaction of meat and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration.

The WHC of restructured sliced meat ranged from 57.19 to 70.85 %. The WHC of restructured sliced meat in this study was higher than those in previous studies, such as restructured turkey meat rolls at 40.97 % [30], restructured turkey steak at 59.16 - 65.03 % [29], and restructured meat at 70.63 - 73.72 % [19]. In this study, the WHC of restructured sliced meat was affected by the meat concentration (X_1), hydrocolloid gel concentration (X_3) in linear terms, and meat concentration in quadratic terms (X_1^2). The interaction between independent variables and WHC response is revealed in **Figure 3**. Based on the equation of ANOVA of the WHC in **Table 3**, the positive value on each coefficient X_1 and X_3 indicates that the higher the meat concentration and hydrocolloid gel concentration, the higher the products' WHC. A high WHC indicated low water loss during the cooking process. The results showed that the meat and hydrocolloid gels showed an excellent ability to bind water. Beef meat contains protein that can retain water to minimize water loss during cooking or frying. According to Sun *et al.* [46], and Sun and Holley [47], most myofibril proteins contain actin and myosin, which form texture and WHC in comminuted meat products. Carballo *et al.* [48], suggested that the high protein content in the products causes the number of polypeptide chains to increase, which allows them to interact with each other during the heating process so that the formed gel matrix is more stable and brings the loss of the amount of water and fat during the lower process.

Hydrocolloid gel can form a matrix that can bind water during heating. Hydrocolloids can interact with water through the hydroxyl group and reduce free water in the system. The addition of hydrocolloid gel causes the amount of water lost to decrease while WHC increases [49]. In addition, hydrocolloids can form a strong network with protein and starch, which can trap water so that it increases WHC. Chin *et al.* [50], implied that konjac flour could bind vital water that, when mixed with other polysaccharides such as carrageenan and starch, create a synergistic effect in the formation of protein gel and binding of water to make comminuted meat products.

The positive coefficient of X_1X_2 indicates that the higher concentration of meat with MOCAF flour, the higher products' WHC. Proteins can cause it in meat, and starch in MOCAF can interact with binding water to the product mixture. According to Gaonkar and McPherson [51], starch and protein can interact with binding water lost during denaturation due to the heating process in processed meat products. The addition of starch and salt to the production of surimi can increase the solubility of actomyosin and cause the interaction of proteins with proteins to be weak so that the protein matrix becomes open and facilitates the formation of 3-dimensional gel matrices between protein, starch, and water which can increase the products' WHC [52].

The negative value of the X_1^2 coefficient shows that the higher the concentration of squared meat, the higher the products' WHC to the optimum point, which decreases with increasing meat concentration after reaching the optimum point. Decreasing the products' WHC and the increased concentration of squared meat after passing through the optimum point is thought to be due to the high-fat content of the dough due to the addition of meat concentration. Products with high-fat content quickly lose water after cooking. In addition, when the meat concentration in the mixture is increased, the concentration of the hydrocolloid gel used tends to be lower to complete the percentage of the dough in the manufacture of the product so that it can affect the decrease in the products' WHC. Hydrocolloid gel is expected to bind water in higher amounts than meat.

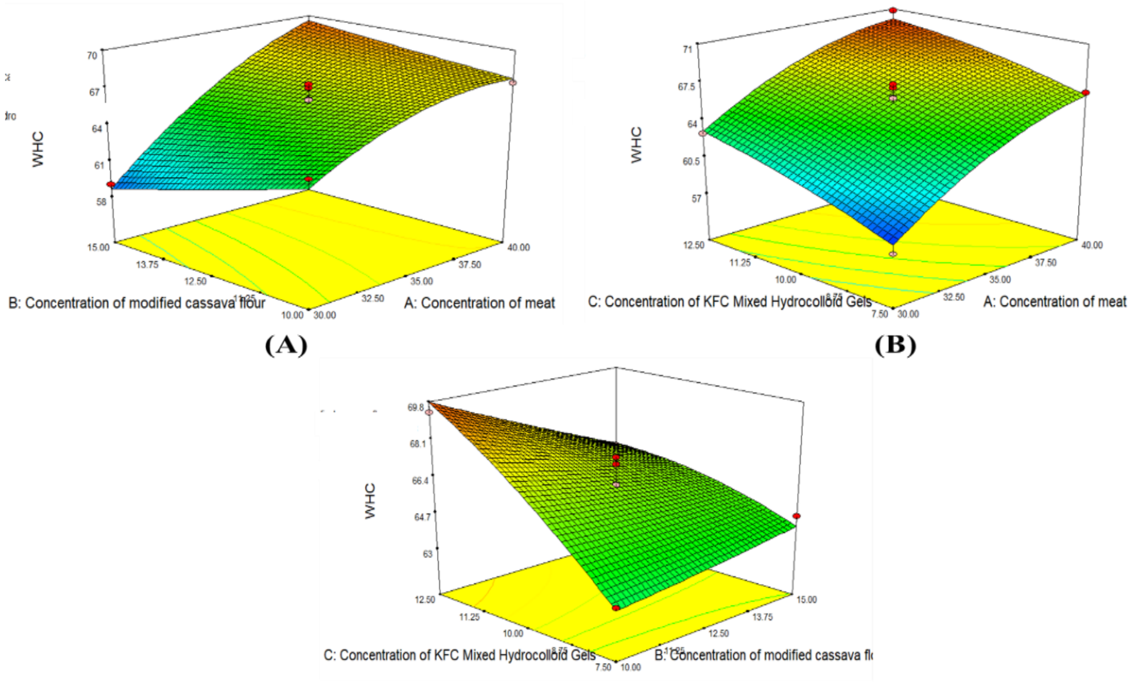

Figure 3 Response surface graph of the effect of meat, MOCAF, and purified flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration on water holding capacity of restructured sliced meat. (A) Interaction of meat and MOCAF concentration, (B) Interaction of MOCAF and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration, and (C) Interaction of meat and purified porang flour-**κ**-carrageenan (PPFC) mixed hydrocolloid gel concentration.

Determination of the predicted optimum formula of restructured sliced meat and verification

Determination of the predicted optimum point using the Design Expert DX 7.0 program requires setting each independent variable's criteria and the observed responses. The criteria for finding the solution of the software DX for the percentages of meat, MOCAF, and hydrocolloid gels were set in the range, while observed responses were set to maximize. The solution of the predicted optimum calculated by the Design

Expert program exhibited that the meat concentration of 35.41 %, MOCAF concentration of 11.58% , and hydrocolloid gel of 12.50 % and resulted in the yield of 99.62 %, hardness of 133.63 g, and WHC of 68.76 %, with desirability equal to 0.915 or 91.5 % and it means that 8.5 % is noises (**Table 4** and **Figure 4**). Three replications of verification were employed to validate the predicted optimum formula. The results revealed a yield of 99.07 %, hardness of 131.61 g, and WHC of 68.45 %, as shown in **Table 4**. The verification results under optimum formulation coincided closely with the prediction values, and a paired t-test indicated no statistically significant differences between them at $p > 0.05$ (**Table 4**). It demonstrates that the verification experiment supports and validates the optimal point prediction.

Table 4 The prediction and verification of the optimum formulation of restructured sliced meat.

	Meat (%)	**MOCAF (%)**	**Hydrocolloid gel (%)**	**Yield (%)**	**Hardness (g)**	**WHC (%)**	**Desirability**
Prediction	35.41	11.58	12.50	99.62	133.63	68.76	0.915
Verification	35.41	11.58	12.50	99.07	131.61	68.46	-
***p*-value**				**0.18**	**0.91**	**0.05**	

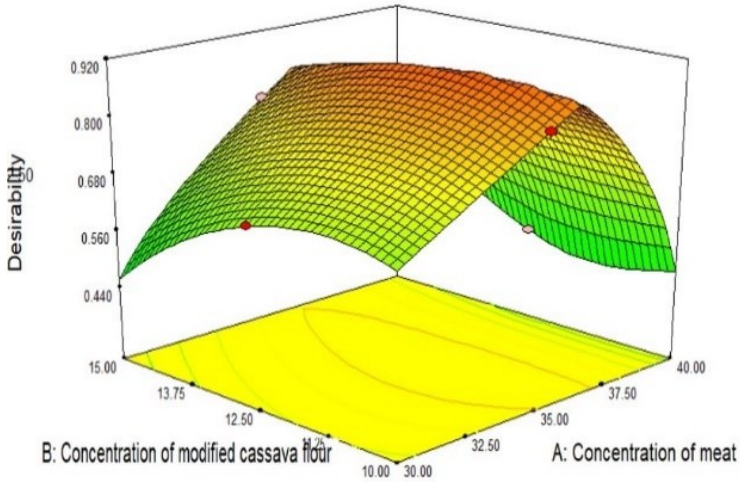

Figure 4 Response surface graph for an optimum point as the Design Expert DX 7.0 predicted of restructured sliced meat.

Conclusions

The yield, hardness, and WHC of restructured meat products are influenced by the composition of the meat, MOCAF, and purified porang flour-κ-carrageenan (PPFC) mixed gels. It was noticed that PPFC-mixed gels are successful as binding agents in making restructured sliced meat. The predicted optimum formula calculated by Design Expert D was the restructured sliced meat containing a meat composition of 35.41 %, MOCAF of 11.58 %, and purified porang flour-κ-carrageenan (PPFC) mixed hydrocolloid gel of 12.50 % in which the optimal formula of restructured sliced meat products had a yield of 99.62 %, hardness of 133.63 g, and WHC of 68.76 %. The verification experiments supported the predicted optimum formula and were not statistically different p-value = 5 % level. Henceforth, it is necessary to scale up restructured sliced meat production on a larger scale, such as a pilot plant scale.

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