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Optimisation of functional sausage formulation with konjac and inulin: using D-Optimal mixture design

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Abstract: In this study, we applied the D-optimal mixture design method to optimise prebiotic sausage formulation with inulin, konjac (*Amorphophallus konjac* L.), and starch. Also, we investigated the effect of each component individually as well as their mixtures on cooking characteristics, texture, colour and sensory properties of prebiotic sausages. The results of this study revealed that the increase in inulin content in the formulations of sausages led to lower frying loss, and increased water holding capacity (WHC), lightness, and overall acceptability. The incorporation of konjac increased the cooking yield, hardness, cohesiveness, redness, and yellowness. On the other hand, konjac added into the sausage formulation decreased overall acceptability. The mixtures of inulin, konjac, and starch improved the cooking characteristics and overall acceptability of the sausages without significant negative effect on the color or sensory properties. The results of the study clarified that the optimum amounts of inulin, starch, and konjac were 2.09; 2.76; and 0.146 %, respectively. The obtained results make it possible to use the combination of these components to produce prebiotic sausage.

Keywords: Inulin, konjac, sausage, functional, formulation

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INTRODUCTION

In recent years, unhealthy food habits and stressful life style have significantly increased the risk of serious health disorders such as obesity, cancer, high blood cholesterol, and coronary heart diseases. This has created and increased demand for new health products with enhanced nutritional value. As a result, a number of research have been conducted in order to develop foods that are designed to improve digestive system health. One of these approaches is the development of functional foods using probiotics or prebiotics. Prebiotics can improve the host health by stimulating the growth of beneficial bacteria in gastrointestinal tract [12]. Along with the nutritional value of a functional product, its structural properties, such as water holding capacity (WHC) and sensory characteristics, and effective cost should be taken into consideration [23].

Inulin is a dietary fibre that has been approved by WHO as a safe prebiotic. It is a well-known and successful food ingredient in meat industry due to its unique ability to enhance both taste and texture in various processed meat products through binding water, forming gel and mimics the oral tactile sensation of fat. The effectiveness of inulin has been approved in many investigations in a wide range of processed meat products such as scalded sausages, canned meat products, meat balls, liver pâté, and fermented sausages [19].

Konjac glucomannan, a neutral polysaccharide made from the tuber *Amorphophallus konjac*, is another prebiotic that is known for its important technological properties and its ability to improve health. USDA recently accepted the use of konjac as a binder in meat and poultry products. Studies suggested konjac has the ability to lower serum cholesterol, serum triglyceride, glucose, bile acid levels and laxative effect as well (Yang *et al.*, 2017).

Some investigations reported that appropriate amounts of konjac in the diet could help prevent diabetes

Table 1. Sausage samples with konjac (K), inulin (I), and starch (S) in a three component constrained D-optimal mixture design

Samples	Ingredients, %		
	K	I	S
1	0.5	0	4.5
2	0.375	1.188	3.438
3	0	0	5
4	0	2.5	2.5
5	0.5	0	4.5
6	0.5	4.5	0
7	0	0	5
8	0	5	0
9	0.125	3.688	1.188
10	0.375	3.438	1.188
11	0.5	2.25	2.25
12	0.25	4.75	0
13	0.125	1.188	3.688

and aid gradual weight loss. Several studies used konjac as a fat substitute, emulsifier, and gelling and thickening agent in various meat products, such as low-fat frankfurter, bologna sausage, hot dogs, pepperoni, and summer sausage [10]. Usually, the use of konjac in large amounts decreases the firmness of meat products, and its combination with other ingredients such as inulin, starch or carrageenan, could moderate undesirable effect.

Mixture design methodology is a new method to determine an effect of each ingredient in the formulation of processed meat products and demonstrate the result of ingredient interactions by applying reduced numbers of experimental trials [1].

It should be noted that this is the first investigation on effects of inulin and konjac on the physical and sensory properties of functional sausage. Hence, the objective of this research is to determine the influence of adding inulin, konjac, starch, and their mixtures on properties of sausages using the D-optimal mixture design and develop the optimal formulation to produce a high quality sausage.

STUDY OBJECTS AND METHODS

1. Experimental design. To determine the optimum proportions of the prebiotic sausage formulation, we used Design-Expert (7.1.5) software. D-optimal design was used with three components: konjac (K), inulin (I) and starch (S). The experimental design and the amounts of the relevant ingredients used are shown in Table 1. The component ranges were as follows: $0 < K < 0.5$; $0 < I < 5$; and $0 < S < 5$. Design-Expert software designed 13 samples. Effects of inulin, starch, and konjac on properties of sausage were evaluated, and optimum combination was determined. For optimization, depending on the influence of each factor; the combination of factors that led to the best responses was determined.

2. Sausage preparation. We prepared minced meat for sausage according to a basic formulation. The minced meat consisted of 55% lean beef meat with fat content of about 12.8 ± 1%, 10% soybean oil, 2.2% wheat flour, 1.5% sodium chloride, 0.35% sodium polyphosphate, 0.012% sodium nitrate, 0.02% ascorbic acid, 0.2% red pepper, 0.2% ginger, 0.1% savory, 0.2% garlic powder, and 17.418% water. All the ingredients were mixed in a 3,000 RPM cutter (Talsa Bowel cutter 15, Spain). Then 13 sausage samples were produced (5 kg each). Each sample contained 4,750 g of the minced meat and various proportions of konjac, inulin, and starch treatment (Table 1). The sum of starch, inulin, and konjac in each sample was 5%. Sausages were stuffed into polyamide casings and cooked in a steam oven at 80°C for 60 min until reaching an internal temperature of 72 ± 3°C. Inulin (Inulin Frutafit TEX®) and konjac flour was obtained from Roosendaal (the Netherlands) and Shandong (China), respectively.

3. Physical properties

3.1. Water holding capacity (WHC). The WHC of the sausages was measured using the method described by Asgharzadeh *et al*. and Méndez-Zamora *et al.* [5, 18]. About 0.3 g of sausage was placed between two filtre papers and then placed between two 12×12 cm plates. Four kg force was applied for 20 min. The released liquids in the paper were considered as meat-free water. WHC was calculated using Eqs. (2) and (3). The experiment was performed in triplicate for each sample.

$$\% \text{ of free water} = [(Iw-Fw)/Iw]\times 100, \tag{2}$$

$$WHC = 100 - \% \text{ of free water}, \tag{3}$$

where Iw is the initial weight of the sample (0.3 g) and Fw is the final weight.

3.2. Cooking yield. A slice of raw sausage 3 mm in thickness was cooked on a hot plate at 160°C for 2 min according to the procedure described by Amini *et al.* [4]. Cooking yield was calculated using the initial and final weights and expressed in g/100 g the initial sample weight. Three replicates were carried out for each sample.

3.3. Frying loss. Frying loss was determined based on the procedure described by Bengtsson *et al*. with some modification [6]. Sliced cooked sausages, 1 cm in thickness, deep fat fried in a fryer (moulinex, DR5), maintained at 174°C, for 2 min until the center temperature reached 72–73°C and then left to cool at room temperature. The frying loss was calculated by weighing the samples before and after frying. The test was done in triplicate for each sample.

4. Texture profile analysis (TPA). Texture profile analysis (TPA) was evaluated using an Instron M350-10CT (500 N load cell, England, Rochdale). The textural parameters were determined according the Procedure described by Bourne [7]. Textural measurements included hardness and cohesiveness.

5. Colour. Four samples from each formulation were used to evaluate internal colour (cross-section) of the sausages. For that, we used 2 cm cross-sections of recently cut sausage. The colour values of the samples were determined using a Chromo meter (CR-400, Minolta Co, Konica, Japan) with D65, 2° observer to objectively measure CIE Lab values (L* relative lightness, a* relative redness and b* relative yellowness). Colorimeter calibrated with white standard plate (L* = 94, a* = 0.3158, b* = 0.3322). The calculated results were expressed with mean value of these measurements.

6. Sensory evaluation. Sensory analyse was performed according to the international standards (ISO, 1985) in the sensory laboratory at the National Nutrition and Food Technology Research Institute (NNFTRI). Private stands under white fluorescent lights were prepared for each panelist. Samples of each formulation were presented randomly for panelists. Tap water was available to clear the taste between samples. 15 panelists, 7 men and 8 women, comprising of postgraduate students of food science and technology were asked to evaluate characteristics using a 9-point hedonic scale. The age of the panelists ranged from 20 to 40 years old. The panelists were trained with two training sessions in the product and terminology. Overall acceptability of the samples was scored as follows: 1 (extremely dislike) to 9 (extremely like).

7. Statistical and data analysis. Three equation models were fitted to each of the responses (Y) with the independent variables:

$$\text{Linear model: } Y= b1X1 + b2X2 + b3X3;$$

$$\text{Quadratic model: } Y= b1X1 + b2X2 + b3X3 + + b12X1X2 + b13X1X3 + b23X2X3; \text{ and}$$

$$\text{Cubic model: } Y= b1X1 + b2X2 + b3X3 + b12X1X2 + + b13X1X3 + b23X2X3+ b123X1X2X3,$$

where X1is konjac, X2 is inulin, X3 is starch, and b is the regression coefficients calculated from the experimental data by multiple regression.

All parametric tests were performed in triplicate for each experiment and all the data demonstrated the mean and SD (standard deviation). The physicochemical and textural properties were studied using one-way ANOVA independently, and Duncan test was employed to determine differences between the experimental groups ($p < 0.05$). Sensory evaluation was analyzed by the same software using Mann–Whitney U test. Correlation analyses were conducted by using the Pearson correlation model where $p < 0.05$ was taken as significance.

RESULTS AND DISCUSSION

Fitting for the optimal model. The optimal model was fitted according to low standard deviation, low predicted sum of squares and high R-squared. P-values of the acceptable model were lower than 0.05.

For frying loss, cooking yield, hardness and overall acceptability, linear was found the best model. For cohesiveness, a* and b* quadratic was adequately fitted. The model which best matched to water holding capacity and L* were modified special cubic and special cubic, respectively.

Water Holding Capacity (WHC). According to the regression coefficients in Table 3, all three components increased WHC, however konjac had the greatest effect. Interestingly, the mixtures of inulin, starch, and konjac showed a substantial effect on increasing the WHC of sausages. This result is well correlated with results illustrated in Table 2, where samples no. 2 and 11 demonstrated the highest WHC.

The results revealed that, although adding inulin to the formulation of sausage could enhance WHC, the higher levels of inulin (more than 2.5%) decreased the WHC significantly. Sample no. 8 (contained 5% inulin) demonstrated the least WHC. The synergetic effect of konjac and inulin in absorbing water is in agree with the study of Mendez-Zamora *et al*. He involved inulin and pectin in the formulation of frankfurter sausages and showed that the addition of 15% inulin and pectin improved WHC [18]. Studies performed by [9] showed that konjac blend usually had been used as multi-ingredient fat replacer in meat products. In addition, incorporation of konjac blend with carrageenan and starch in low fat bologna increased WHC, produced more stable gel matrix with higher cooking yield and more acceptable texture. López-López *et al.* (described the type of fibers and quantity of their polysaccharides are the factors that influence water holding capacity of product [17]. They mentioned large particles create open structures that enhance the properties of hydration. Álvarez and Barbut also investigated the effect of beta-glucan (BG), inulin, and their mixture on the emulsion stability, and concluded combination of BG and inulin compensa-

Table 2. Cooking and sensory characteristics of experimental sausage samples

RUN	WHC	Cooking yield	Frying loss	Overall acceptability	Hardness	Cohesiveness	L*	a*	b*
1	63.78 ± 0.19	95.09 ± 0.16	21.05 ± 0.21	5.18 ± 0.11	22.63 ± 0.24	0.69 ± 0.01	39.84 ± 0.08	10.98 ± 0.09	15.56 ± 0.16
2	73.08 ± 0.21	93.58 ± 0.14	19.44 ± 0.17	5.57 ± 0.10	23.47 ± 0.21	0.59 ± 0.00	37.56 ± 0.08	8.39 ± 0.11	13.24 ± 0.19
3	59.37 ± 0.14	90.38 ± 0.14	17.59 ± 0.14	5.80 ± 0.10	21.10 ± 0.23	0.66 ± 0.01	38.01 ± 0.07	11.64 ± 0.08	16.19 ± 0.17
4	51.5 ± 0.22	89.76 ± 0.23	16.04 ± 0.12	6.27 ± 0.07	23.71 ± 0.24	0.62 ± 0.00	38.31 ± 0.08	12.27 ± 0.13	16.62 ± 0.16
5	63.77 ± 0.16	95.09 ± 0.16	21.03 ± 0.18	5.16 ± 0.09	22.63 ± 0.24	0.68 ± 0.00	39.83 ± 0.07	10.96 ± 0.07	15.59 ± 0.19
6	68.36 ± 0.28	93.86 ± 0.15	18.29 ± 0.21	6.06 ± 0.10	27.31 ± 0.22	0.62 ± 0.01	40.56 ± 0.07	10.58 ± 0.12	16.56 ± 0.19
7	59.37 ± 0.14	90.4 ± 0.16	17.62 ± 0.18	5.78 ± 0.07	21.09 ± 0.21	0.65 ± 0.00	38.01 ± 0.07	11.64 ± 0.09	16.19 ± 0.16
8	36.01 ± 0.22	89.07 ± 0.22	14.52 ± 0.15	6.83 ± 0.15	26.29 ± 0.21	0.71 ± 0.01	40.58 ± 0.07	18.25 ± 0.07	21.28 ± 0.18
9	54.16 ± 0.23	90.57 ± 0.17	16.20 ± 0.19	6.38 ± 0.12	25.33 ± 0.24	0.60 ± 0.00	38.88 ± 0.07	11.88 ± 0.14	15.97 ± 0.19
10	72.10 ± 0.17	92.97 ± 0.15	18.07 ± 0.19	6.05 ± 0.15	25.84 ± 0.25	0.58 ± 0.02	38.11 ± 0.07	8.98 ± 0.12	14.18 ± 0.18
11	82.96 ± 0.20	94.48 ± 0.15	19.62 ± 0.14	5.64 ± 0.14	25.00 ± 0.25	0.60 ± 0.02	36.78 ± 0.08	8.61 ± 0.14	14.34 ± 0.15
12	52.84 ± 0.14	91.49 ± 0.22	16.42 ± 0.21	6.46 ± 0.15	26.82 ± 0.24	0.61 ± 0.00	40.66 ± 0.06	12.51 ± 0.13	16.64 ± 0.14
13	62.25 ± 0.12	91.23 ± 0.14	17.71 ± 0.14	5.91 ± 0.15	22.74 ± 0.26	0.6 ± 0.010	37.82 ± 0.07	9.44 ± 0.12	13.93 ± 0.19

Table 3. Regression coefficients and correlation for the adjusted model to experimental data in D-optimal mixtures design for physical properties, textural parameters, color parameters, and sensory analysis

Parameter	K	I	S	KI	KS	IS	KIS	Pred-R^2
WHC	103.49	36.00	59.37	284.30	–	15.27	682.63	0.9997
Cooking yield	137.18	89.06	90.40	–	–	–	–	0.9951
Frying loss	10.40	2.90	3.51	–	–	–	–	0.9939
Overall acceptability	–0.01	6.99	5.99	–	–	–	–	0.9579
Hardness	36.44	26.31	21.10	–	–	–	–	0.9908
Cohesiveness	16.15	0.70	0.65	–18.13	–16.89	–0.23	–	0.8950
L*	6.44	40.58	38.01	37.70	55.36	–3.95	–129.30	0.9961
a*	631.78	18.26	11.64	–767.03	–696.53	–10.72	–	0.9981
b*	783.11	21.27	16.20	–899.03	–859.00	–8.41	–	0.9923

ted undesirable effect of fat reduction by increasing WHC [3]. Liu *et al*. also prepared konjac-egg white protein gels and determined that konjac could significantly improve the water retention capacity [15].

Cooking yield and frying loss. The results in Table 3 revealed that konjac with its positive coefficient had significantly ($p < 0.05$) increased cooking yield, while inulin and starch with their negative coefficient decreased this parameter in the product. The samples no. 1 and 5, which contained highest amount of konjac (0.5% konjac, 4.5% starch, and 0% inulin), showed the highest cooking yield and frying loss. As one can see in Table 1, two pair sam-

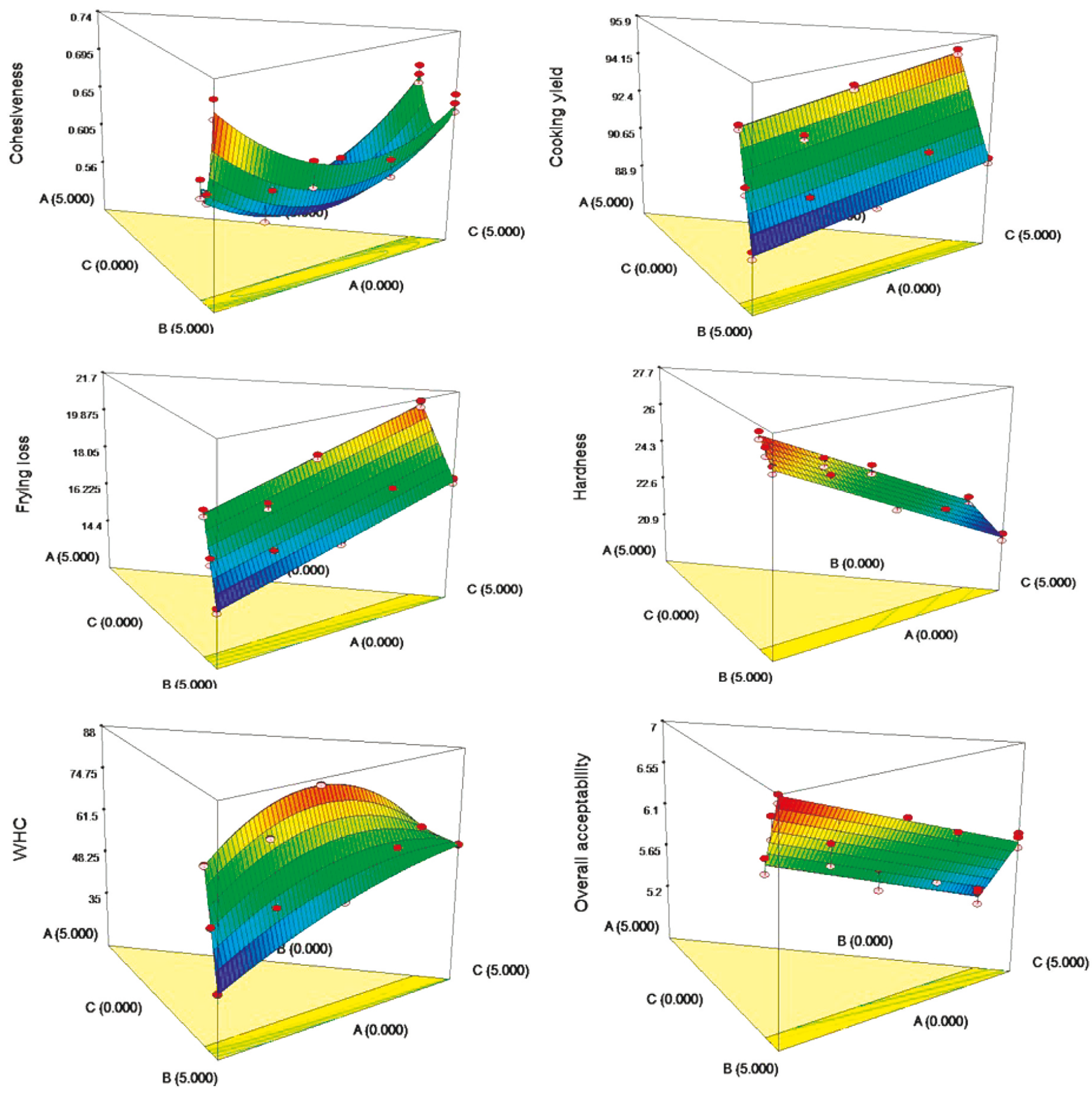

Fig. 1. Contour plots for effectsы of konjac (A), inulin (B)б and starch (C) on water holding capacity (WHC), cooking yield, frying loss, hardness, cohesivenessб and overall acceptability of prebiotic sausage.

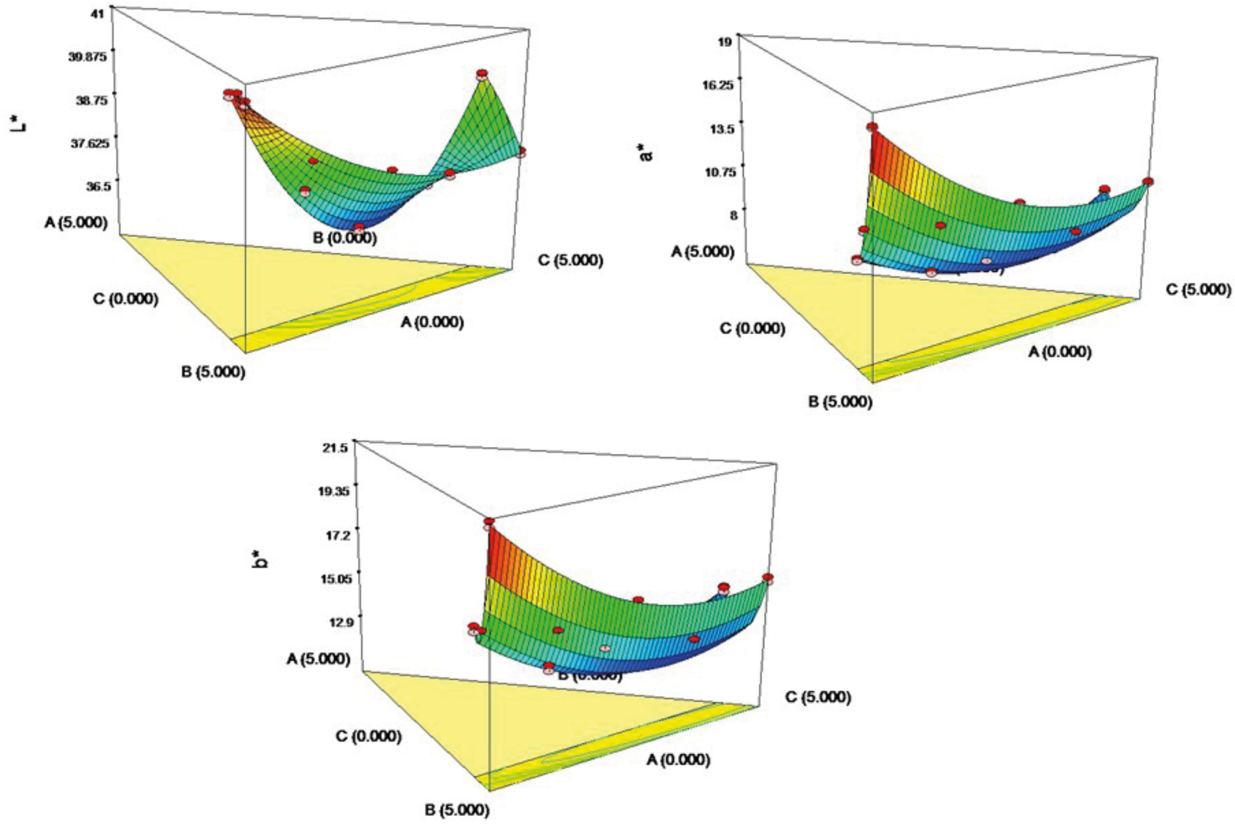

Fig. 2. Contour plots for effectы of konjac (A), inulin (B), and starch (C) on lightness (L*), redness (a*), and yellowness (b*) of prebiotic sausage.

ples are similar: one pair is samples no. 1 and 5, another one is the samples no. 3 and 7. These compositions were defined by Design Expert software to check the repeatability of findings. As it was expected similar compositions have displayed comparable results for cooking and sensory characteristics in Table 2, indicating the repeatability of findings.

Several studies suggested to use konjac in combination with other hydrocolloids. Emir *et al*. discussed that weak junction zones in konjac made it susceptible to heat, and its interaction with other hydrocolloids caused tights junction which made it resistant to cooking or frying [11].

According to regression coefficients (Table 3), inulin caused decrease in cooking and frying loss. The sample no. 8 (with the maximum level of inulin) displayed the least cooking yield and frying loss. This data is similar to that of Afshari *et al*. who indicated although inulin is able to increase WHC and decrease frying loss, but, at higher amounts, it reduced the moisture retention and cooking yield probably due to its porous structures and inability to form a tight gel [1].

Texture profile analysis (TPA). As the hardness analysis showed, konjac had a strong effect on the hardness of sausage, inulin also increased it, while starch, on the contrary, reduced the hardness of the product.

Several studies indicated that the addition of konjac into the food matrix increased the hardness of products but it depends to many factors that should be taken into consideration. These factors are the molecular weight and particularly the type of konjac (flour or as hydrolyzed), pH of a food system, presence of salts, and an amount of incorporated konjac and other food ingredients, specially gelling agents. All these studies are in agree that increase in amount of konjac increase the hardness that may not be accepted by consumers. Hu *et al*. reported that konjac glucomannan (KGM) affected functional properties of egg white protein and increased hardness, chewiness, and springiness of the gel samples at a certain concentration [13]. The investigation conducted by Emir *et al*. indicated that the bigger molecular weight of KGM caused the highest hardness and closely the lowest springiness, which had negative effect on the choice of panelists [11]. Akesowan reported that increasing of NaCl resulted increase in links between konjac/κ-carrageenan and konjac/gellan, leading to the increment in the hardness of the produced gel [2]. Purwandari *et al.* used konjac a noodle formulation. They found that the hardness and adhesiveness of noodle significantly increased ($p < 0.05$) and became three times harder than standard Chinese or Japanese wheat noodle [21]. The researchers also indicated that an increase in proportion of water in pregelatinised flour led to increased harness in konjac noodle.

Several studies also determined that the use of powdered inulin resulted in higher moisture loss during cooking. This can affect the texture of a product and increased hardness of burgers, frankfurter sausages and dry-fermented chicken sausages. [1, 18 and 19]. These results are in a good agreement with the results of this study.

Another texture parameter related to meat products is cohesiveness. Adhesiveness and cohesiveness are parameters that play an important role in handling of sau-

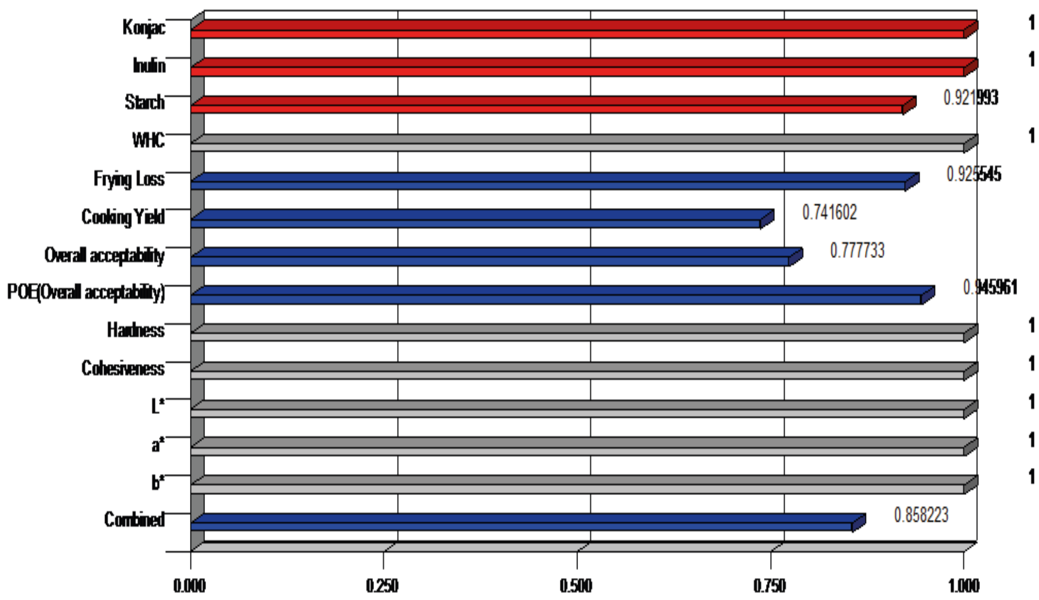

Fig. 3. Desirable plot for optimum formulation.

sages, particularly for the slicing of these products. If products are too adhesive or cohesive, they become undesirably sticky, and it cannot be easily to cut [20]. In the current investigation konjac showed a significant positive effect on cohesiveness of probiotic sausage. On the contrary, combination of konjac with inulin or starch reduced the undesirable effects of using konjac alone and maintained the appropriate adhesiveness and cohesiveness of sausages with improved textural properties. The study [9] documented that when konjac was used as a multi ingredient in the formulation of meat products, unwanted hardness and cohesiveness decreased significantly. The researchers suggested to incorporate konjac in combination with other hydrocolloids Purwandari *et al.* also confirmed that konjac had a substantial effect on the increase of adhesiveness and cohesiveness of noodle: konjac noodle was about ten times more cohesive and sticky than wheat flour noodle [21].

Colour. As shown in Table 3 and Fig. 2, all three component separately increased the lightness of the product, while their combination in special cubic model caused decrease in L* value in the sausages. The results indicated that inulin was meaningfully ($p < 0.05$) more effective in enhancing the lightness compared to starch and konjac. The samples no. 6 and 8, which containing the highest amount of inulin, illustrated the most lightness.

According to Table 3, konjac demonstrated a significant ($p < 0.05$) positive effect on a* and b* values causing more reddishbrown product, while its combination with starch or inulin decreased a* and b*. Trespalacios and Pla reported that if when myoglobin and fat content was maintained constant, the color of formulated products was mostly influenced by many factors, including additive ingredients [24]. In the present study, as the protein and fat content was invariable, the color was influenced mainly by mixing ingredients. Amini *et al.* mentioned konjac led konjac to a more reddishebrown of a product by its susceptibility to Maillard browning [4]. Jiménez-Colmenero *et al.* also indicated that the addition of konjac in frankfurter sausage caused decrease ($p < 0.05$) in lightness (L*) and an increase ($p < 0.05$) of yellowness (b*), compared to other samples [14]. The results of another investigation, conducted by Ruiz-Capillas *et al.*, are in agreement with the present study that konjac gel affected color parameter of sausages through decrease in L* and increase in yellowness (a*) [22]. Delgado-Pando *et al*. observed less red, paler ($p < 0.05$), and yellower pâtés as a result of adding konjac [10].

Sensory analysis. The experimental results obtained from the regression coefficient values of overall acceptability (Table 3 and Fig. 1) displayed that increase in the proportion of konjac had a significant ($p < 0.05$) negative effect on the overall acceptability of the product. Inulin showed a positive effect on the acceptability of the product, while starch was not significantly effective. Formulation 12 (contained 0.25; 4.75; and 0% konjac, inulin and starch, respectively) and formulation 9 (0.125; 3.68; and 1.18%) demonstrated the highest overall acceptability score.

Results obtained by sensory analysis highlighted that adding konjac in the amount of up to 0.2% could improve the appearance of sausage. On the contrary, increase in the amount of konjac (more than 0.2%) decreased the overall acceptability significantly ($p < 0.05$). The results emphasized that hardness and cohesiveness are the factors that significantly influence overall acceptability. Increase in proportion of konjac (more than 0.2%) can make the sausage harder and more cohesive than standard sausage which may not be acceptable by consumers. In the other words, consumers would not accept a product with extreme hardness or cohesiveness. Another explanation is that high amount of konjac probably enhances its typical fishy taste/odours. These findings are in a good agreement with the results of Purwandari *et al*. who reported that the addition of konjac glucomannan could improve sensory perception of wheat noodle, while a high level of this ingredient reduced preference, since noodle became too sticky [21]. Lin *et al*. also observed that 1% konjac in reduced-fat frankfurter sausages led to higher scores of sensory overall acceptability [15].

Liu *et al*. also assumed that functional properties of food products could be controlled by adding small amounts of KGM without causing undesirable sensory changes [16].

On the contrary, inulin showed positive coefficient on overall acceptability of sausages and an increase in portion of inulin improved the product flavour. Menegas *et al.* represented incorporation of inulin (maximum level of 7.5%) in reduced-fat sausages made the product more favorable and acceptable by consumers [19].

Mixture proportion optimization and desirability function

The optimisation was done in order to access the optimal amount of each component that had an excessive effect on quality properties of the sausages. The predicted values of the responses are shown in Fig. 3. Our aims were to maximize overall acceptability and cooking yield of the sausages, minimise frying loss, and, at the same time, to maintain WHC, hardness, and cohesiveness within normal range. Having all these criteria taken into consideration, we found that optimal amounts of inulin, starch, and konjak were 2.09; 2.76; and 0.146%, respectively (Fig. 3). The selected mixture achieved 0.858 desirability score. As the desirability value between 0.8 and 1.0 is recognized as acceptable and excellent product, the formulation with 0.858 desirability value was selected as optimal formulation that could provide valuable nutritional and technological properties.

CONCLUSION

In conclusion, the development of functional foods opens up new possibilities for the food industry and consumers. The development of healthier sausage with prebiotics inulin and konjac is a promising direction of research. The physicochemical and sensory characteristics of the prebiotic sausages are conditioned by the formulation. The study demonstrated that the sausage contained 0.146; 2.09; and 2.76% konjac, inulin, and starch, respectively, has high quality and sensorial properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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