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Optimization of Analog Meat Formula Made from "Gepak Kuning" Soybean Flour, Carrageenan, and Pectin Using the Mixture Design Method

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ABSTRACT

The development of analog meat products signifies a significant progression in plant-based food innovation, aiming to replicate conventional meats' nutritional and sensory characteristics, such as beef, goat, and chicken. This study specifically examined the process of producing analog meat using soy flour as the main protein source, with carrageenan and pectin serving as the key dietary fiber and texturizing agents. Employing Design Expert 11.0.0 for Mixture Design, we prioritized parameters such as dietary fiber, protein content, and organoleptic qualities. The optimal formulation consisted of 44.42% soy flour, 16.2% carrageenan, and 9.38% pectin. This formulation produced an analog meat product with a protein content of 18.13% and dietary fiber levels of 23.19%, aligning with industry standards for plant-based alternatives. Sensory evaluation results, with an average acceptance score of 5.3 out of 7, indicate strong consumer appeal. These findings demonstrate the practical viability of this composition for commercial plant-based meat production, offering a nutritious and sustainable alternative to traditional meat. The study provided valuable insights for food manufacturers seeking to develop high-fiber. These protein-rich analog meat products cater to the increasing demand for healthier and environmentally friendly food options.

1. Introduction

Meat is a crucial source of essential nutrients, including protein, vitamins, minerals, fats, saturated fatty acids, and cholesterol, which play a key role in shaping dietary habits (Sundari et al., 2015). However, research has consistently linked higher meat consumption to increased blood cholesterol levels, leading many individuals to adopt vegetarian diets or shift toward plant-based foods. This growing preference underscores the need for innovation in developing nutritious and appealing plant-based alternatives to meet consumer demand. These plant-based alternatives not only replicate meat's sensory and textural attributes but also offer superior nutritional benefits, empowering consumers to make informed dietary choices. This innovation includes creating plant-based protein products that aim to replicate meat's sensory and textural attributes, often referred to as analog meats. These analog meats are designed to emulate various characteristics of traditional meat, including texture, fiber content, moisture content, visual appeal, coloration, and flavor (Riyanto et al., 2022). Consumer acceptance of these products largely depends on how closely they replicate the sensory experience of real meat while offering improved nutritional benefits. A key strategy in developing high-quality meat analogs is incorporating functional food ingredients that enhance texture, optimize mouthfeel, and improve nutritional value. Hydrocolloids such as carrageenan and pectin play a crucial role in mimicking the juiciness and structure of meat by improving water retention and gel formation, which enhances the overall texture and mouthfeel of meat analogs.

Additionally, soy protein, a widely used plant-based protein source, provides the necessary structure and chewiness to create a meat-like texture, making it a key ingredient in analog meat formulations. Beyond texture, including protein- and fiber-rich ingredients, enhance the nutritional profile of these products, catering to the growing consumer demand for healthier food options. These advancements in ingredient formulation and processing techniques have significantly improved meat analogs' sensory and functional properties, making them more appealing and acceptable to consumers. As a result, these innovations have expanded the market potential for plant-based meat, reinforcing its role as a viable, sustainable, and nutritious alternative to conventional meat.

The incorporation of soybean flour as the principal ingredient in the formulation of analog meats is primarily due to its high protein content. Among various soybean varieties, the Gepak Kuning, an organic soybean, demonstrates a protein level of approximately 35.38% and a fat content of 15.10% (Widyasakta, 2018). These organic soybeans offer significant nutritional benefits while presenting a reduced environmental footprint, positioning them as a more sustainable choice for health-conscious consumers. Riyanto et al. (2022) highlight that an effective formulation for analog meats consists of 40% soy flour, with additional components such as fillers and binders, namely carrageenan and pectin. Carrageenan functions as a thickening agent, imparting elasticity to the analog meat. Its capacity to interact with macromolecules, including proteins, alongside the presence of hydrophilic galactans, plays a crucial role in establishing a gel matrix that effectively retains moisture. Furthermore, pectin contributes to the product's quality by enhancing water-binding properties, which subsequently influences key attributes such as protein content, color brightness, cooking loss, and the overall texture compactness of the analog meat.

The formulation of analog meats using soy flour, carrageenan, and pectin necessitates careful consideration to achieve the desired sensory and functional properties. Conventional formulation techniques often fail to provide predictive values and elucidate the interactions among the various raw materials. As Galvan et al. (2021) noted, the application of Mixture Design has gained traction in enhancing the conditions necessary for processing, developing, or formulating innovative products. This study utilized Design Expert 11.0.0 software, applying the Mixture D-Optimal model to optimize the formulation process. This model accommodates multiple components, ranging from 2 to 24, and employs a comprehensive design methodology that includes response surface analysis, optimization techniques, and subsequent validation of the outcomes. The primary objective of this research was to determine the optimal formulation ratios for producing analog meats with the chosen ingredients.

2. Methodology

2.1. Material

The research utilized local soybeans of the Gepak Kuning variety sourced from the Jember district, Indonesia. The formulation process for the analog meat incorporated a diverse range of ingredients, including kappa-type carrageenan (Maoli brand), pectin (Tamina brand), and essential flavorings such as garlic, onion, shallot, coriander, sugar, and lime leaves, all obtained from Tanjung Market in Jember District, Indonesia. Supplementary components comprised pepper powder (Ladaku brand), salt (Cap Kapal), and Worcestershire sauce (Lotus Flower brand). For the analytical part of the study, various chemical compounds were utilized, including distilled water, n-hexane, and two types of ethanol (95% and 78%). Additional reagents included acetone, pancreatin enzyme, pepsin enzyme, hydrochloric acid (HCl), sodium hydroxide (NaOH), selenium, boric acid, sulfuric acid (H2SO4), along with pH indicators such as methyl red and bromocresol green. This comprehensive approach facilitated a thorough examination of the formulation and its characteristics.

2.2. Methods

2.2.1. Preparation of soybean flour

The preparation process of the yellow gepak soybean flour for producing analog meats is characterized by several critical steps to ensure quality and texture. Initially, the soybeans are sorted to remove any impurities. They are then soaked in water for three hours, a step that facilitates the hydration of the seeds and enhances their subsequent cookability. This is followed by blanching the soaked soybeans at a temperature of 90°C for five minutes, which aids in deactivating anti-nutritional factors and improves the color and texture of the final product. After blanching, the wet soybeans undergo a thorough washing process, where they are drained in a sieve for 30 minutes. This step is essential to remove residual heat and excess moisture, contributing to the overall moisture content management necessary for further processing. The next phase involves dehydration, where the soybeans undergo an oven-drying process lasting 24 hours. This extensive drying period ensures the soybeans achieve a low moisture content, creating a suitable base for the subsequent grinding process. Finally, the dried soybeans are ground using a blender and sifted to achieve a particle size of 60 mesh. This specific size is crucial for the consistency and textural properties of the analog meat, allowing for optimal ingredient integration in further formulations.

2.2.2. Design of experiments

A d-optimal mixture design experiment was conducted to optimize the formulation of analog meat, emphasizing the maximization of protein content, dietary fiber levels, and organoleptic qualities. The formulation incorporated three primary ingredients: soy flour (39.5% to 44.45%), carrageenan (16.2% to 21.2%), and pectin (6.8% to 11.8%). These specified ranges served as input parameters in the Design Expert 11 software, allowing for a systematic approach to generating an optimal analog meat formulation. As a result, a total of 16 experimental runs were established, which are detailed in Table 1 of the study. Determining the optimal formula response based on maximum protein and dietary fiber values. This optimization process aimed to balance the final product's nutritional quality and sensory appeal.

Table 1. Optimization formula by mixture design method (Design Expert 11.0.0)

No	Soybean (g)	Carrageenan (g)	Pectin (g)	Protein (%)	Dietary fiber (%)	Organoleptic qualities
1	39.50	21.20	9.3	14.4	15.11	4.5
2	39.50	21.20	9.3	14.6	15.02	4.5
3	39.88	18.33	11.8	15.3	23.20	4.9
4	39.88	18.33	11.8	15.6	23.24	4.9
5	41.08	20.61	8.3	16.8	20.50	4.7
6	41.42	16.78	11.8	17.0	21.64	4.9
7	41.80	18.94	9.3	18.1	21.27	5.0
8	41.80	18.94	9.3	18.8	21.11	5.2
9	41.80	18.94	9.3	18.8	21.11	5.0
10	41.80	18.94	9.3	18.3	22.04	5.2
11	42.14	21.06	6.8	20.1	16.09	4.9
12	43.12	16.20	10.7	20.5	19.65	4.7
13	43.26	17.65	9.1	20.5	20.54	4.8
14	43.58	19.62	6.8	21.1	18.83	5.0
15	39.87	18.32	11.80	15.63	23.24	4.9
16	4.07	20.60	8.31	16.81	20.5	4.7

Source: Primary data, 2024

2.2.3. Analog meat production

The preparation of the analog meat involves a systematic approach to ingredient incorporation and processing. The primary constituents constitute 70% of the total composition, primarily soybean flour, carrageenan, and pectin. In comparison, the remaining 30% encompasses various spices, including 1.5% garlic, 1.5% shallot, 1.5% onion, 15.0% water, 1.5% sodium tripolyphosphate, 1.0% mushroom stock, 1.5% pepper, 1.0% coriander, 1.5% salt, 1.5% sugar, 1.5% Worcestershire sauce, and 1.0% lime leaves. The initial phase thoroughly homogenizes the dry components to ensure a uniform mixture. Following this, water is gradually incorporated to achieve a semi-wet dough consistency. The spice mixture is then integrated into the dough. The dough is subjected to a two-step kneading process: initial manual kneading is followed by mechanical kneading using a food processor for 15 minutes, a critical step for achieving a consistent texture. Subsequently, the prepared mixture is shaped into patties using molds with precise dimensions—2 cm in thickness and 5 cm in diameter. Cooking is executed under controlled conditions, wherein the patties are steamed at a regulated temperature of 150°C for a duration ranging between 15 to 30 minutes. Following the cooking process, the analog meat is vacuum-packaged to ensure its preservation and subsequently stored in a freezer to uphold its freshness and extend shelf life. This systematic processing not only enhances the quality of the final product but also assures its stability for future consumption.

2.2.4. Analysis

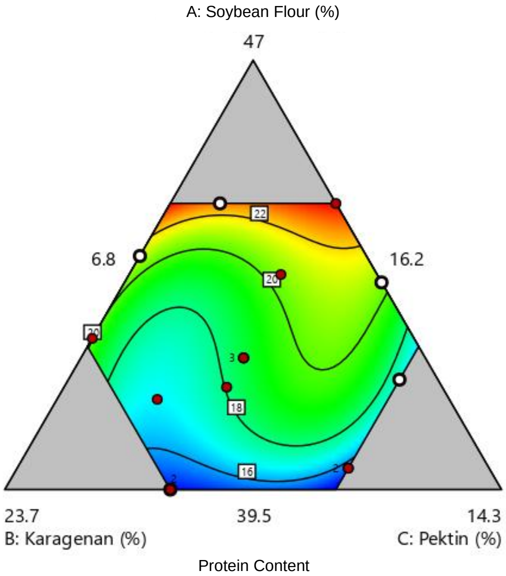
Protein was assessed by the Kjeldahl method (AOAC 945.18-B, 2005), and dietary fiber content was determined through an enzymatic method per AOAC 991.43 (AOAC International, 2005). Sensory analysis: A total of 30 panelists participated in the organoleptic testing process, ensuring a diverse representation of opinions and experiences regarding the analog meats. The evaluation used a hedonic test with a 7-point scale to assess participants' preferences: (1) dislike very much; (2) dislike; (3) dislike slightly; (4) neither like nor dislike);

(5) like slightly; (6) like; and (7) like very much. A hedonic score of 7 or higher on a nine-point scale usually indicates highly acceptable sensory quality; hence, a product achieving this score could be used confidently as a good illustration of 'target' quality (Everitt, 2009).

3. Results and Discussion

3.1. Protein Content

A mixture design was developed to optimize the protein content in analog meats, as outlined in Table 1. When dealing with mixture variables, the ability to combine levels is limited, as changes to one variable must consider the levels of the other variables. In this context, the variables represent the mixture's components, and their total must always remain constant at 100% (Santos Felix et al., 2018). The design was performed to optimize the best proportion of the three materials used (soybean flour, carrageenan, and pectin).



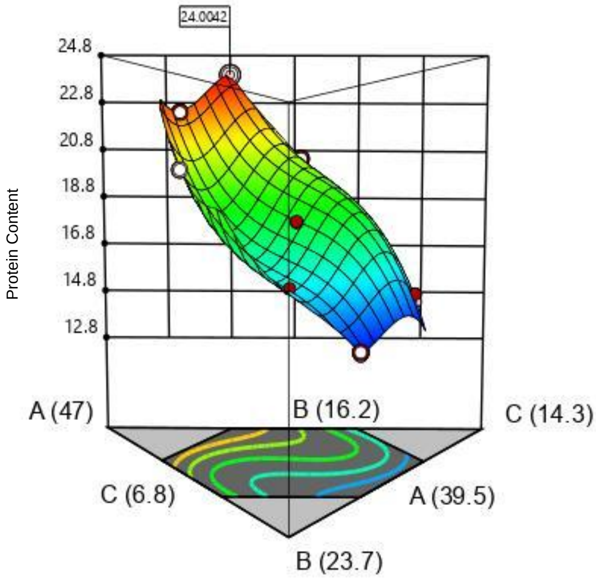


Figure 1. (a) Respon surface and (b) counter plot obtained by Mixture design for protein content optimation

The response surface obtained by mixture design is illustrated in Figure 1A, and the contour plot is illustrated in Figure 1. This surface presented a maximum as the critical point. The coordinates of this point are the proportions of the ingredients that generate the greatest response. The proportions optimized according to the response surface were soybean flour (44.5%), carrageenan (16.2%), and pectin (9.3%), with protein content of 24%, being the maximum inside the experimental domain.

Incorporating soy flour in analog meat products is vital for enhancing protein content, making it a key ingredient in meat alternatives. Research has demonstrated a positive correlation between the quantity of soy flour added and the resulting protein concentration in these products. Notably, the gepak kuning variety of soybeans contains an impressive protein content of approximately 35.38% (Widyasakta, 2018). Supporting this assertion, Valentina Therescova Simbolon et al., 2016) found that increasing soy flour levels significantly elevated nuggets' protein levels. This indicates that strategically integrating more soy flour into imitation meat formulations is a practical and effective method for enriching protein content.

Importantly, it is worth noting that other additives, such as carrageenan and pectin, do not influence the protein content of analog meat, further underscoring the significance of soy flour in these products.

3.2. Dietary Fiber

The study's findings indicate that the formulation comprising soy flour, carrageenan, and pectin in 39.9%, 18.3%, and 11.8% yielded the highest dietary fiber content of 23.24%. According to the guidelines established by the Codex Alimentarius Commission (Codex Alimentarius Commission (CAC), 1997), a food item can be classified as a source of dietary fiber if it contains a minimum of 3% dietary fiber. Furthermore, a food is classified as high in fiber if it contains at least 6% dietary fiber. The dietary fiber content of the analog meat produced in this study ranged from 15.02% to 23.24%, indicating that the formulated product meets the criteria for being classified as high in dietary fiber. This suggests that combining soy flour, carrageenan, and pectin effectively creates analog meat products with significant dietary fiber content, which may contribute to improved nutritional value and health benefits.

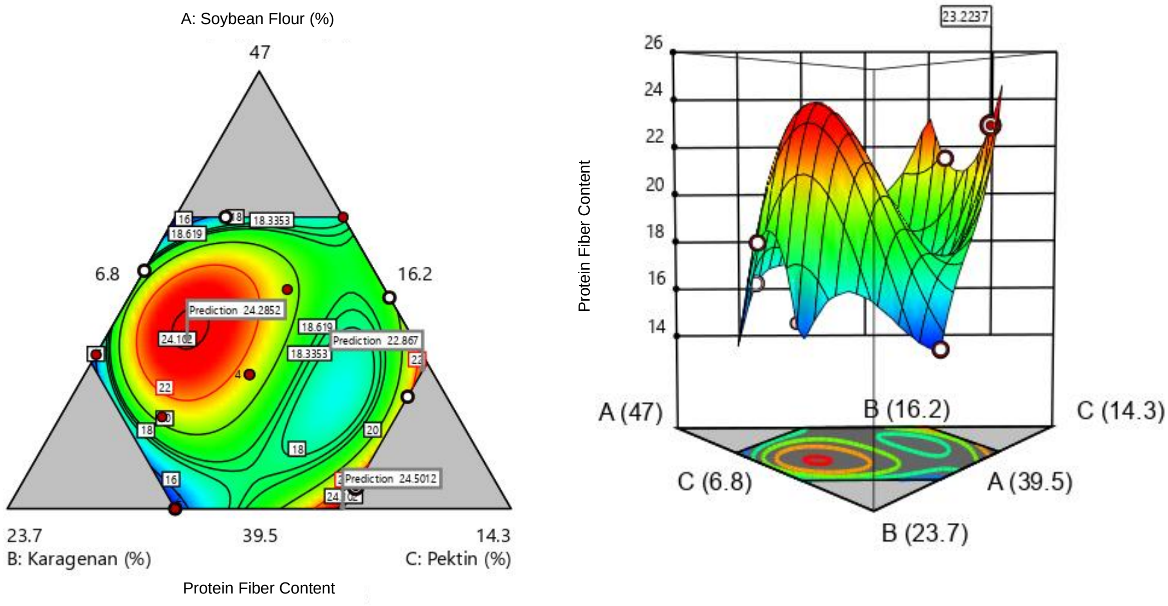


Figure 2. (a) Respon surface and (b) counter plot obtained by Mixture design for dietary fiber optimation

Figure 2 illustrates the influence of various ingredient components on the dietary fiber content of analog meat products. Based on the findings, it is evident that the primary factor affecting fiber content is the incorporation of pectin. In contrast, adding carrageenan and soy flour minimally impacts the fiber levels. Notably, the synergistic effect of combining carrageenan with pectin markedly enhances the dietary fiber content of the analog meat. This observation aligns with the research conducted by (Hutagalung et al., 2016), which indicated that in the formulation of sheet jams using carrageenan and pectin as stabilizers, pectin significantly outperformed carrageenan in contributing to fiber content. Pectin, derived from a mixture of polysaccharides and glycoproteins, possesses favorable solubility characteristics in food matrices and can bind with other components, thereby effectively preserving levels of crude fiber within the product.

3.3. Organoleptic qualities

The study revealed that the optimal preference value for analog meat was observed in a formulation comprising soy flour, carrageenan, and pectin in the following ratios: 44.5%, 17.95%, and 7.55%, respectively. This formulation enhances the overall organoleptic qualities of the analog meat, suggesting that the balance of these components is critical for achieving desirable consumer acceptance.

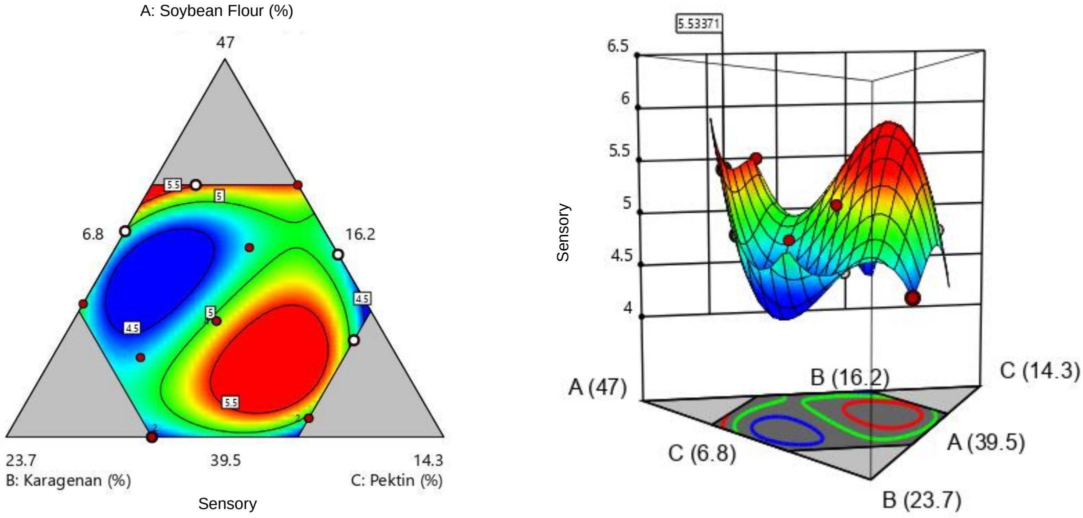


Figure 3. (a) Respon surface and (b) counter plot obtained by Mixture design for organoleptic qualities optimation

The findings illustrated in Figure 3 indicate a positive correlation between the incorporation of soy flour and the preference ratings of panelists. An increase in the quantity of soy flour within the food formulation not only elevates the product's protein content but also enhances the textural properties. Specifically, research (Zurriyati, 2011) suggests that a higher percentage of protein is associated with increased chewiness in meat products. To optimize the sensory quality of food products that utilize an increased soy flour content, it is crucial to supplement this with pectin and carrageenan. As noted by (Safira et al., 2016), pectin serves as a gelation agent, whereas carrageenan works as a stabilizer. The interaction between these two hydrocolloids facilitates the formation of a double helix structure, significantly contributing to the gel strength and overall texture stability. However, it is important to note that excessive amounts of carrageenan may be undesirable due to its bland flavor profile. This can overshadow the desired aroma of other flavorings or additional ingredients integrated into imitation meat products, highlighting the need for careful formulation to balance taste and texture.

3.4. Optimization and Validation Result

The optimization solution using Design Expert 11.0.0 by maximizing the parameters of protein and dietary fiber content is shown in Table 2.

Table 2. Optimum conditions suggestion of analog meat formulation

Soy flour (g)	Carragenan (g)	Pectin (g)	Protein content (%)	Dietary fiber (%)	Organoleptic qualities	Desirability	
44.42	16.20	9.38	23.76	18.02	5.38	0.79	selected

Table 2. presents the optimal formulation consisting of soy flour, carrageenan, and pectin in the proportions of 44.42%, 16.20%, and 9.38%, respectively. This formulation is predicted to yield a dietary fiber content of 18.02% and protein content of 23.76%, with an overall organoleptic rating of 5.39. The calculated desirability value for this formulation is 0.79, indicating that the formulation's accuracy is 79%, leaving a discrepancy of 21%. The desirability function illustrates the proximity of the three factors utilized in this formulation. Desirability is quantified on a scale from 0 to 1, where a value closer to 1 signifies a more ideal optimal solution. Conversely, a value nearing 0 indicates that one or more responses fall outside the acceptable limits.

Validation of an optimum formula based on mixture design is an integral process that involves assessing the alignment between the response values of the optimum solution and the predicted response values, as illustrated in Table 2. This step ensures that the optimization methodology accurately reflects the underlying system behavior and confirms the reliability of the derived solutions. The result of this verification step can be seen in Table 3.

Table 3. Validation of optimum formulation

Respons	Predicted Mean	Std Dev	n	SE Pred	95% PI low	Data mean	95% PI high
Protein (%)	18.01	18.02	3	0.08	17.82	18.13	18.21
Dietary fiber (%)	23.76	23.76	3	0.26	23.14	23.19	24.38
Organoleptic qualities	5.38	5.38	3	0.08	5.19	5.3	5.57

To validate the predicted optimum formulation, an experiment was conducted with three replications based on the suggested composition, which included 44.2% soy flour, 16.2% carrageenan, and 9.38% pectin. As detailed in Table 3, the resultant protein content, dietary fiber, and organoleptic qualities were measured at 18.13%, 23.19%, and 5.3, respectively. When compared to the predicted values presented in Table 3, the verification results fall within the range of the 95% predicted interval (PI) low and 95% PI high. This alignment indicates that the optimum formula is consistent and reliable.

The evaluation of analog meat formulations has revealed significant nutritional properties. In its optimal formulation, the protein content of analog meat is 18.13%, which is comparable to traditional meats such as beef and goat, which contain approximately 24.9 g of protein per 100 g, along with chicken, which provides around 18.20 g of protein per 100 g (Kementerian Kesehatan Republik Indonesia, 2018). This indicates that the blend of soy flour, carrageenan, and pectin in the imitation meat achieves a protein level nearly equivalent to that of chicken meat. Furthermore, the selected analog meat formulation boasts % a dietary fiber content of 23.19%. This positions the product within the high-fiber food category as defined by the Codex Alimentarius Commission (Codex Alimentarius Commission (CAC), 1997). A sensory evaluation yielded an attribute value of 5.3 on a scale of 7, illustrating that the analog meat is well-received by consumers. These findings underscore the potential of analog meats as a viable alternative to conventional meats, offering both comparable protein levels and beneficial dietary fiber

4. Conclusion

The formulation for producing analog meat has been optimized to consist of 44.2% soy flour, 16.2% carrageenan, and 9.38% pectin. This specific combination yields a protein content of 18.13% and a dietary fiber content of 23.19%. Additionally, sensory evaluation indicates a score of 5.3 on a scale 7, suggesting favorable consumer acceptance. This formulation serves as a viable source of protein and offers high dietary fiber, positioning it as a healthy food option that aligns with contemporary consumer preferences.

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