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Application of High Encapsulation of Nutritional and Bioactive Compounds from Black Bean (*Cajanus* sp.) in Functional Ice Cream

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ABSTRACT

Functional ice cream enriched with encapsulated black bean extract (*Cajanus* sp.) represents a type of functional food. This concept aligns with the definition of functional foods as food products containing nutritional components, dietary supplements, or bioactive compounds that specifically enhance the function of certain body parts, improve overall health, boost the immune system, prevent specific diseases, or reduce the risk of illnesses. Functional foods are enriched with specific components or ingredients, such as minerals, vitamins, fatty acids, dietary fiber, bioactive compounds, or probiotics. The key ingredients used in this functional ice cream, based on the best research findings, include 55% low-fat milk powder, 15% encapsulated black bean extract, and 1% carboxymethyl cellulose. Supporting ingredients consist of 6% cornstarch and 26% powdered sugar. The ice cream contains 127,56 ppm of anthocyanins, with total phenolic, flavonoid, and antioxidant activity values of 32,07 mg GAE/g, 40,73 mg QE/g, and 11,08 IC50 (µg/mL), respectively. The unsaturated fatty acids identified in the product include linoleic acid, eicosatrienoic acid, octadecatrienoic acid, and eicosenoic acid. The functional ice cream, with the optimal formulation of 15% encapsulated black bean extract, 55% low-fat milk powder, and 1% CMC, offers a comprehensive nutritional profile. It contains 23.47% protein, 13.60% fat, 14.85% moisture, 3.28% ash, 15.90% total fiber, 28.94% carbohydrates, and 38.21% total dietary fiber. Additionally, the ice cream exhibits antioxidant activity with an IC50 value of 11.08 µg/mL. Phytochemical screening of the functional ice cream revealed the absence of alkaloids, while confirming the presence of terpenoids, flavonoids, polyphenols, and saponins, indicating the presence of bioactive compounds.

1-Introduction

Black peas (*Cajanus sp.*), locally referred to as "kacang lebui" on Lombok Island, are rich in bioactive compounds, including anthocyanins, polyphenols, flavonoids, phenolics, and terpenoids. Research has demonstrated that the phenolic content of *kacang lebui* ranges from 30.501 to 78.363 mg GAE/g, significantly surpassing the levels found in *Phaseolus lunatus* (0.11–9.72 mg GAE/g) and *Vigna angularis* (8.18 ± 0.12 mg GAE/g). Classified as moderate to high in phenolic content, *kacang lebui* falls within a range of 3,000 to >5,000 mg GAE/100 g, with anthocyanin levels between 107.120 and 153.350 ppm [1-3].

Given the potential of local ingredients as sources of bioactive compounds and the increasing demand for natural antioxidants in functional food products, the development of microencapsulation technology is essential. This advanced technique offers enhanced protection for the bioactive compounds in *kacang lebui*, outperforming conventional extraction methods. Moreover, it provides a foundation for product characterization, facilitating the industrial-scale development of functional foods enriched with these valuable compounds [4,5,18,19].

The inherent vulnerability of free bioactive compounds to environmental factors such as oxygen, heat, and light necessitates careful handling to ensure their effective utilization. Microencapsulation produces encapsulated particles that provide optimal protection for these critical compounds. This technology, a subset of encapsulation techniques, transforms materials into particles ranging in size from micrometers (1–1000 µm) to nanometers, enhancing their stability. The fortification of functional ice cream with encapsulated bioactive compounds derived from kacang lebui is an ideal application[16-17]. This approach is supported by existing literature, as the ice cream manufacturing process does not involve high temperatures, a critical factor in preserving the stability and efficacy of bioactive compounds. Therefore, this study aims to determine the optimal composition for producing ice cream that incorporates encapsulated kacang lebui extract, with the best nutritional and bioactive compound content [7-9].

2-Materials and Methods

Materials

The materials used in the preparation of encapsulated black bean (*Cajanus* sp.) extract include high-quality kacang lebui harvested at 3 months of age from a reliable source in Gunungsari Village, West Lombok. Other materials include n-hexane, 90% ethanol, 70% ethanol, Whatman no. 1 filter paper, filter paper, nitrogen gas (N2), *Rhizopus* sp. culture, PDA medium, maltodextrin coating agent, and additional components for forming encapsulated extract.

The main and supporting materials for this research include microencapsulated particles produced using the best methods and treatments from the previous research, low-fat milk powder, carboxymethyl cellulose (CMC), skim milk, granulated sugar, cornstarch, egg yolks, salt, 90% ethanol, 70% ethanol, Whatman no. 41 filter paper, screening chemicals for physicochemical and phytochemical analysis, profiling, antioxidant activity, and total anthocyanins, phenolics, and flavonoids.

The research equipment used in the third year includes an ice cream maker, freezer, Memmert oven, glassware, pH meter, thermometer, Barnstead SHKE2000 shaker, TLC set, Kiesel Gel GF254 TLC plates, UV rays (366-254nm), Buchi rotary evaporator, Konica Minolta CR10 colorimeter, UV-1700 PharmaSpec spectrophotometer, UV-Vis 1240 spectrophotometer, vortex, micropipette, and GC-MS. Additionally, equipment required for forming the microencapsulated particles includes an electric dryer, microwave, maceration-percolation apparatus, analytical balance, centrifuge, pH meter, autoclave, thermometer, incubator, microbiological analysis equipment, vortex, micropipette, sonicator, spray drying equipment, and glassware. This comprehensive list of materials and equipment is essential for the successful microencapsulation and ice cream production processes in this study.

Methods

The raw material in this study, in the form of encapsulates, incorporates the treatments and optimal stages from previous research [1,2].. The

[DOI: 10.22034/FSCT.22.160.299]

encapsulates are obtained through a series of steps, including kacang lebui extraction, optimization of the pre-microencapsulation process, and the formation of encapsulates. The process optimization in this research employs Response Surface Methodology (RSM) with a Central Composite Design, involving three factors: low-fat milk powder concentration (S) at 35%, 40%, and 45%; encapsulate concentration (E) at 20%, 15%, and 10%; and CMC concentration (C) at 0.5%, 1%, and 1.5%. The chosen value for α, with k = 3, is calculated as 2^(k/4) = 2^(3/4) = 2^0.75 = 1.682, rounded to 1.68. The factor levels are then set based on the average concentration of each material at the central point, with factors a = 0, b = 0, and c = 0. The factor levels are determined by taking the central point of each factor from the average concentration of each material. The physicochemical characterization of whole kacang lebui and its powder includes the analysis of protein content, fat content, carbohydrate content, moisture content, ash content, and total fiber using the AOAC International method[11]. Total anthocyanin and dietary fiber (DF) content are measured according to the method of Asp et al. (1983)[12], while total phenolic and total flavonoid contents are based on the method from ULP (2015)[10].

Qualitative testing to confirm the presence of bioactive compounds in the extract is performed through phytochemical screening, which includes testing for alkaloids, terpenoids, steroids, flavonoids, and polyphenols. Flavonoid screening is conducted using several reagents: a 1N NaOH solution for a basic test, 1N HCl and 1N H_2SO_4 for acid tests, ferric chloride for a specific reaction, and thin-layer chromatography (TLC). Secondary metabolite and fatty acid profiling are carried out using GC-MS, following the method from ULP (2015) [10]. The main parameters in the third phase of the research are anthocyanin content and the determination of the highest anthocyanin concentration, which are obtained through the Response Surface Methodology (RSM) process, consisting of phase 0, phase 1, and phase 2 testing. The phase 1 diagnosis is evaluated through the lack of fit test. If the lack of fit shows a significant value, the linear model or interaction model cannot be used to model the response. A lack of fit is considered significant when the p-value is less than 0.05 (α = 5%). If significant, the curvature is then examined, which may indicate that the response can be modeled using quadratic or cubic equations if the p-value for curvature is less than 0.05 (α = 5%), suggesting the presence of a curve or an optimal point. The models used in Design Expert include linear, two-factor interaction (2FI), quadratic, and cubic models. Research parameters include the physicochemical quality of microencapsulates, antioxidant activity, as well as screening, profiling, and bioactive compound content. The main data on anthocyanin content from the treatments, including expanded treatments, will be processed with the Design-Expert DX 7.1.5 program for statistical analysis.

3-Results and Discussion

Selection of the Best Model

In this study, Response Surface Methodology (RSM) was used to optimize the formulation of ice cream with a combination of low-fat milk powder, encapsulation, and carboxymethyl cellulose (CMC) concentrations on anthocyanin content. Choosing the best model in RSM analysis is crucial to ensure that the selected model has both accuracy and good predictive capabilities. In this process, several models were tested, including linear, two-factor interaction (2FI), quadratic, and cubic models, to determine which best fits the research data.

Based on the analysis results, the quadratic model was proposed as the best model for this study. This is supported by a significant Sequential p-value of 0.0394, indicating that the addition of a quadratic component to the model significantly contributes to the variation in the data. Additionally, the Lack of Fit p-value of 0.8704 suggests that the quadratic model does not poorly fit the data, meaning that it is appropriate for the observed data. Furthermore, the Adjusted R^2 value for the quadratic model is 0.7873, and the Predicted R^2 value is 0.7236, indicating that this model has good predictive ability and explains approximately 78.73% of the variation in the measured data. While the cubic model had a slightly higher Adjusted R^2 value of 0.7953, it was not chosen due to its insignificant Sequential p-value of 0.3737. This suggests that adding the cubic component did not lead to a meaningful improvement in the model. Therefore, the quadratic model was selected as the best model to describe the effects of the combination of low-fat milk powder, encapsulation, and CMC on the anthocyanin content in ice cream.

Table 1. Selection of the Best Model

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0,7020	0,5561	0,4976	
2FI	< 0.0001	0,8332	0,7458	0,6903	
Quadratic	**0,0394**	**0,8704**	**0,7873**	**0,7236**	**Suggested**
Cubic	0,3737	0,8773	0,7953	0,6364	Aliased

Assumption Evaluation

Evaluating the assumptions in Response Surface Methodology (RSM) is an essential step to verify the model's validity and reliability. The key assumptions to be met include the normality of residuals, homoscedasticity (the consistency of residual variances), and the appropriateness of data transformation. To assess these assumptions, tests were performed using the P-P Plot for normality, the residual vs. predicted plot, and the Box-Cox plot for determining the optimal power[14-15].

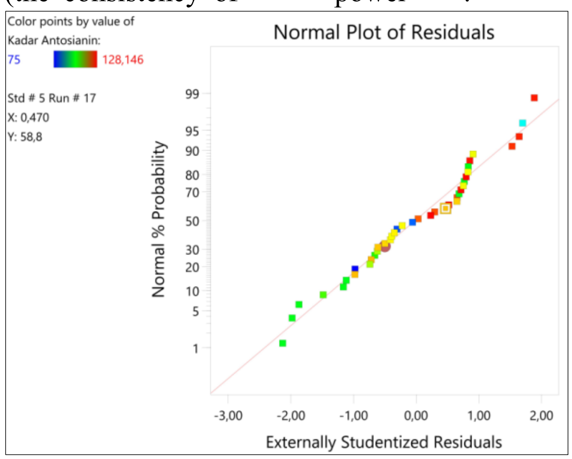

Figure 1. Normality Assumption Evaluation

The assumption check results indicate that the model meets the necessary criteria for the validity of the analysis. The P-P Plot for normality shows that the observation points align with the diagonal line, suggesting that the residuals are normally distributed. Residual normality is a crucial assumption, ensuring that the model's errors are random and unbiased, which means the analysis results can be considered valid.

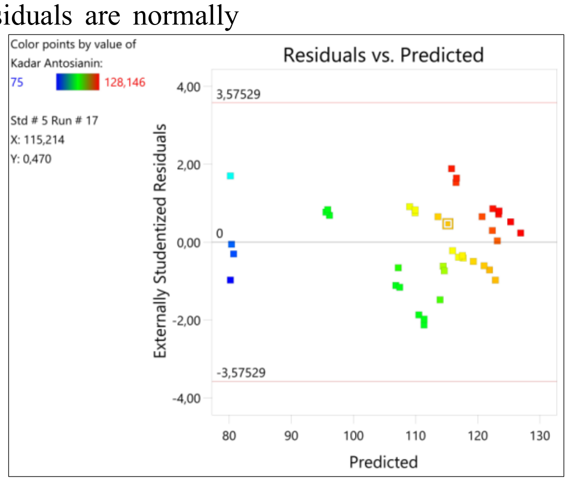

Figure 2. Homogenity Evaluation

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Next, the residual vs. predicted plot shows a random scatter of points without any specific pattern, indicating that the assumption of homoscedasticity is met. This means that the residual variance is constant across the range of predicted values, suggesting that the model do es not exhibit heteroscedasticity, which could undermine the reliability of the predictions.

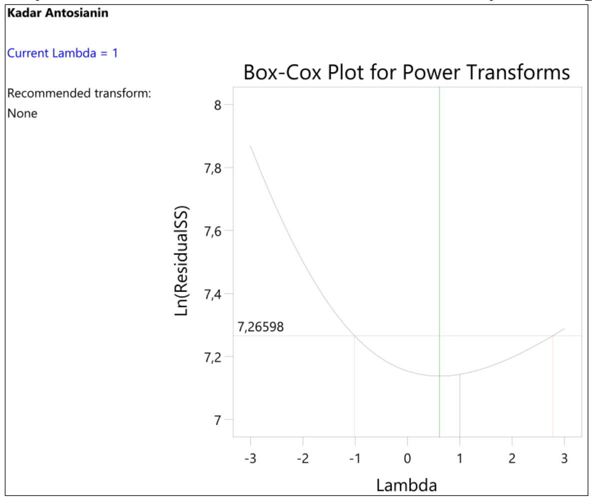

Figure 3. Evaluation of box cox transformation

The Box-Cox plot for power transformation, with a lambda value of 1, indicates that no further data transformation is necessary, as the data already satisfy the assumption of linearity. This lambda value confirms that the model is appropriate for the original data without requiring any changes to the data distribution. Overall, the assumption checks support the validity of the model used in this study, ensuring that the results and conclusions are reliable and trustworthy.

ANOVA Analysis

Analysis of Variance (ANOVA) was used to evaluate the significance of the quadratic model that describes the effect of low-fat milk powder, encapsulation, and Carboxymethyl Cellulose (CMC) on the anthocyanin content in ice cream. ANOVA helps identify factors that significantly affect the measured response as well as interactions between these factors. The ANOVA results also include a "Lack of Fit" test to assess the model's adequacy in fitting the observed data. The ANOVA results show that the quadratic model is overall significant in explaining the variation in anthocyanin content in the ice cream, with an F-value of 17.04 and a p-value of < 0.0001. This indicates that the model is effective in predicting the measured response. Among the individual factors, low-fat milk powder (A) and encapsulation (B) have a significant effect on anthocyanin content, with p-values of 0.0067 and 0.0328, respectively. This suggests that changes in the levels of low-fat milk powder and encapsulation significantly affect the anthocyanin content in the ice cream. In contrast, CMC (C) did not make a significant contribution to the response, as indicated by a p-value of 0.8537.

Table 2. ANOVA Results for Quadratic Model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6472,42	9	719,16	17,04	< 0.0001	**significant**
A- low-fat powdered milk	357,76	1	357,76	8,48	0,0067	
B-Enkapsulat	211,37	1	211,37	5,01	0,0328	
C-CMC	1,46	1	1,46	0,0346	0,8537	
AB	291,94	1	291,94	6,92	0,0133	
AC	14,72	1	14,72	0,3489	0,5592	
BC	16,79	1	16,79	0,3979	0,5330	
A^2	162,31	1	162,31	3,85	0,0592	
B^2	396,38	1	396,38	9,39	0,0046	
C^2	1,60	1	1,60	0,0378	0,8471	
Residual	1265,84	30	42,19			

[DOI: 10.22034/FSCT.22.160.299]

Lack of Fit	1167,84	29	40,27	0,4109	0,8704	**not significant**
Pure Error	98,00	1	98,00			
Cor Total	7738,25	39				

The interaction between low-fat milk powder content and encapsulation (AB) was found to be significant, with a p-value of 0.0133 ($p<0.05$), indicating that these two factors interact to affect the anthocyanin content. However, other interactions, such as between low-fat milk powder and CMC (AC), and between encapsulation and CMC (BC), did not show significance, with p-values of 0.5592 and 0.5330, respectively. The quadratic effect of encapsulation (B^2) was also significant on anthocyanin content, with a p-value of 0.0046, suggesting a significant non-linear effect of encapsulation. Meanwhile, the quadratic effect of low-fat milk powder content (A^2) approached significance (p-value 0.0592), while CMC (C^2) did not show a significant effect (p-value 0.8471). The Lack of Fit test yielded a p-value of 0.8704 ($p>0.05$), indicating that the model fits the data well and there are no patterns left unaccounted for. This reinforces the validity of the quadratic model in describing the influence of the combination of these factors on anthocyanin content in the ice cream.

Response Surface and Contour Analysis

The 3D response surface graph and the contour plot above are used to visualize the interaction between low-fat milk powder content (A) and encapsulation level (B) on anthocyanin content in ice cream, with CMC (C) held constant. The 3D response surface graph provides a three-dimensional representation, while the contour plot offers a more detailed two-dimensional view of how the combination of these two factors affects the anthocyanin content, measured in ppm. Both graphs illustrate the variation in anthocyanin content through color gradations, where blue represents the lowest anthocyanin levels and red indicates the highest. This investigation aims to identify the optimal combination of low-fat milk powder content and encapsulation level that can significantly increase anthocyanin content in the ice cream product.

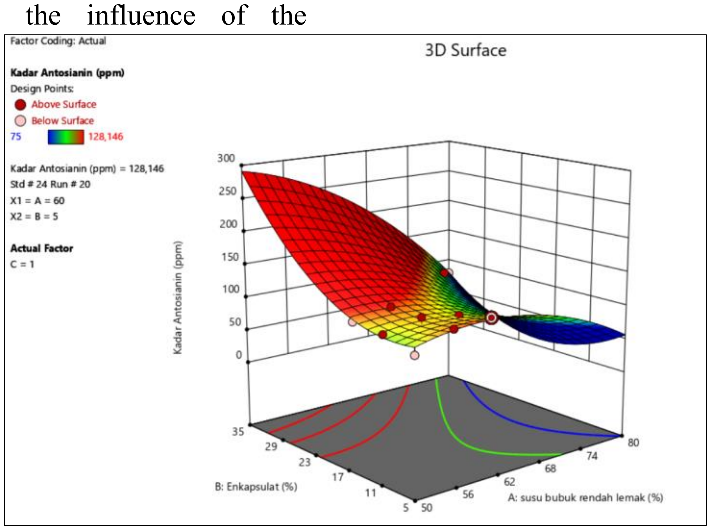

Figure 4. 3D Response Surface

Based on Figure 4, which presents the 3D graph, it is evident that there is a significant interactive relationship between the low-fat milk powder content (A) and encapsulation level (B) concerning the anthocyanin content in ice cream. The response surface shows variations in color, reflecting the anthocyanin levels, ranging from blue (low content) to red (high content). The red areas on the surface indicate the combinations of factors A and B that result in the highest anthocyanin levels, while the blue areas represent combinations that lead to the lowest anthocyanin content. The optimal combination for achieving

[DOI: 10.22034/FSCT.22.160.299]

the highest anthocyanin content in the ice cream occurs at a low-fat milk powder content (A) in the range of 50-60%, an encapsulation level (B) around 25-30%, and a constant CMC (C) value of 1%. In this combination, the response surface shows a deep red color, indicating high anthocyanin content. This suggests that increasing the low-fat milk powder content along with the appropriate encapsulation level synergistically maximizes the anthocyanin content, while the effect of CMC remains constant at a non-significant value[13]. Thus, adjusting the composition to a higher low-fat milk powder content (A) and an optimal encapsulation level (B) is key to enhancing the anthocyanin content in the ice cream product.

The interaction between the two factors is non-linear, confirming the synergistic effect of low-fat milk powder content and encapsulation in influencing the anthocyanin levels. The optimal combination of these two variables is essential to achieving the highest anthocyanin content in the ice cream, as indicated by the surface pattern produced. This graph reinforces the statistical analysis results, which demonstrate the significance of the interaction between low-fat milk powder content and encapsulation level in the measured response[13].

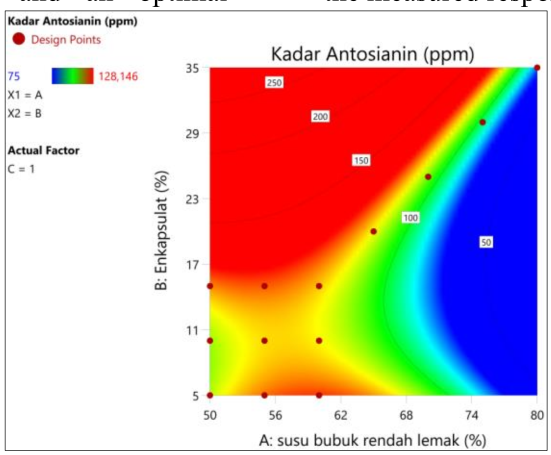

Figure 5. The interaction between low-fat milk powder content and encapsulation level with respect to anthocyanin content

The interaction between low-fat milk powder content and encapsulation level with respect to anthocyanin content is an important factor in determining the final anthocyanin concentration in the ice cream product. As the analysis indicates, these two factors exhibit a significant synergistic effect. When both the low-fat milk powder and encapsulation are optimized, there is a marked increase in the anthocyanin content, demonstrating that their combination plays a crucial role in enhancing the functional properties of the ice cream. From the 3D response surface and contour graphs, it is clear that higher low-fat milk powder content (within the range of 50-60%) combined with an optimal encapsulation level (around 25-30%) leads to the highest anthocyanin levels[13]. This interaction suggests that both factors work together in a non-linear fashion, where small changes in either factor can lead to significant changes in anthocyanin concentration. However, the effect of CMC remains relatively constant and does not significantly influence this interaction. This synergistic effect further supports the idea that the combination of these two variables (low-fat milk powder and encapsulation level) should be carefully adjusted to maximize the health benefits related to anthocyanin content, which is desirable for both its nutritional and antioxidant properties in the ice cream.

Based on Figure 5, the contour plot clearly shows a color gradient ranging from blue to green, yellow, and red, representing the anthocyanin content from low to high. The red areas indicate the highest levels of anthocyanin, while the blue areas represent the lowest levels. The plot indicates that higher low-fat milk powder content (A), particularly in the range of 50-65%, combined with encapsulation levels (B) above 25%, results in higher anthocyanin content, as shown by the red region in the plot. Conversely,

[DOI: 10.22034/FSCT.22.160.299]

lower concentrations of low-fat milk powder and encapsulation levels lead to lower anthocyanin content, represented by the blue areas.

The interaction between these two variables demonstrates that increasing the low-fat milk powder content (A) along with higher encapsulation levels (B) significantly enhances the anthocyanin content in the ice cream. This plot further confirms the previous analysis, which showed that the optimal combination of these two variables effectively increases the anthocyanin levels. The transition areas between green and red indicate specific combinations that require further optimization to achieve the maximum anthocyanin content[13].

Formulation, Nutritional Composition, and Bioactive Compounds of Functional Ice Cream

In this section, the formulation of functional ice cream is discussed, including the selection of ingredients that contribute to its nutritional value and bioactive properties. The focus is on creating a product that not only provides essential nutrients but also offers health benefits through the incorporation of bioactive compounds, such as antioxidants, vitamins, and other functional ingredients derived from sources like local black bean extracts. The composition of the ice cream is carefully designed to optimize both the sensory qualities and the functional health benefits. Key components include low-fat milk powder, microencapsulated bioactive ingredients, and other nutritional additives, such as CMC, which enhances texture and stability. The aim is to balance the flavor, texture, and nutritional profile while ensuring the bioactive compounds remain effective in providing the desired health benefits. By considering the scientific principles of formulation and the properties of various ingredients, the functional ice cream is designed not only as a treat but also as a functional food that contributes to better health outcomes, particularly by boosting antioxidant levels and providing dietary fiber[13].

The ice cream enriched with encapsulates from black bean extract is made using an ice cream maker that is assembled and automated for the process. The resulting ice cream is considered a form of functional food. This is supported by the definition that functional foods are food products that contain nutritional components, food supplements, or bioactive compounds that can specifically enhance the function of certain body parts, improve overall health, strengthen the immune system, prevent specific diseases, and/or reduce the risk of illness. Such functional foods are products enriched or fortified with specific components or ingredients, such as minerals, vitamins, fatty acids, dietary fiber, bioactive compounds, or probiotics. They may also contain organic acids and other functional components.

The main ingredients used, based on the optimal results of the research, include 55% low-fat milk powder, 15% encapsulated black bean extract, and 1% carboxymethyl cellulose (CMC). Supporting ingredients include 6% cornstarch and 26% powdered sugar. All ingredients are mixed and processed in the ice cream maker for 45 minutes. The finished ice cream is then packaged in ice cream containers, labeled, and stored in a freezer to maintain the quality and consistency of the product. The ice cream produced from this process contains 127.56 ppm of anthocyanins. The total phenolic content, flavonoid content, and antioxidant activity are 32.07 mg GAE/g, 40.73 mg QE/g, and 11.08 (IC_{50}, µg/ml), respectively. The unsaturated fatty acids found include linoleic acid, eicosatrienoic acid, octadecatrienoic acid, and eicosenoic acid[20,21].

The functional ice cream with the best treatment, containing 15% encapsulated black bean extract, 55% low-fat milk powder, and 1% CMC, has a complete nutritional composition with protein (23.47%), fat (13.60%), moisture (14.85%), ash (3.28%), total fiber (15.90%), carbohydrates (28.94%), and total dietary fiber (DF) of 38.21%. Furthermore, the ice cream also exhibits antioxidant activity with an IC_{50} of 11.08 µg/ml. Based on the results of the phytochemical screening of bioactive compounds in the functional ice cream, it was found that the ice cream sample does not contain alkaloids, but does contain terpenoids, flavonoids, polyphenols, and saponins[13,20,21].

4-Conclusion

The functional ice cream, with the optimal formulation of 15% encapsulated black bean

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extract, 55% low-fat milk powder, and 1% CMC, offers a comprehensive nutritional profile. It contains 23.47% protein, 13.60% fat, 14.85% moisture, 3.28% ash, 15.90% total fiber, 28.94% carbohydrates, and 38.21% total dietary fiber. Additionally, the ice cream exhibits antioxidant activity with an IC50 value of 11.08 µg/mL. Phytochemical screening of the functional ice cream revealed the absence of alkaloids, while confirming the presence of terpenoids, flavonoids, polyphenols, and saponins, indicating the presence of bioactive compounds.

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6-Conflicts of interest

The authors declare no conflict of interest.

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