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Research Paper

Development, Characterization, and Comparative Analysis of Halal-Compliant Fortified *Caulerpa lentillifera* Jelly with Enhanced Nutritional and Antioxidant Properties

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Abstract

Oxidative stress is linked to many chronic diseases, making antioxidant-rich functional foods important for health. This study aims to evaluate the nutritional composition and antioxidant capacity of fortified *C. lentillifera* jelly (FJ), control jelly (CJ), and commercial jelly (MJ) using mineral analysis, proximate analysis, and antioxidant assays, followed by Principal Component Analysis (PCA). The fortified jelly, formulated without gelatin or other non-Halal ingredients, ensures compliance with Halal dietary requirements while maintaining desirable product characteristics. The results showed that FJ exhibited enhanced antioxidant activity, with DPPH and ABTS scavenging capacities of 68% and 45%, respectively. These antioxidant activities indicate the ability of the fortified jelly to scavenge free radicals, which are implicated in cellular damage and oxidative stress-related diseases. In addition, FJ had lower sodium content than MJ, suggesting its potential as a healthier alternative. The relatively lower moisture content in FJ (50.34%) compared to MJ (88.28%) contributed to a higher concentration of nutrients and carbohydrates within the product matrix. PCA explained 97.4% of the total variance, clearly differentiating the jelly samples based on their compositional attributes. PC1 was primarily associated with potassium, fat, and ash, while PC2 was influenced by antioxidant activity and selected minerals, including magnesium and zinc, which were more prominent in FJ. Overall, these findings suggest *that C. lentillifera*-fortified jelly may help reduce oxidative stress and support better health as a Halal functional food.

Keywords: *Antioxidant, Caulerpa lentillifera, Functional Food, Jelly, Principal Component Analysis*

INTRODUCTION

The increasing global burden of non-communicable diseases (NCDs), including obesity, diabetes, and cardiovascular disorders, has intensified the need for healthier dietary strategies. Functional foods, which provide physiological benefits beyond basic nutrition, have attracted significant attention across the scientific and industrial sectors (Chen & Martirosyan, 2021). This growth was further supported by rising consumer awareness and health concerns (Granato et al., 2020). This trend is further supported by recommendations from the World Health Organization (WHO) to reduce free sugar intake and encourage healthier dietary patterns through the consumption of nutrient-dense foods, aiming to reduce obesity and other non-communicable diseases (WHO, 2015).

Among emerging functional ingredients, marine resources such as macroalgae have

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attracted increasing interest due to their rich nutritional composition and bioactive compounds. In particular, *Caulerpa lentillifera*, commonly known as sea grape, is known for its high dietary fiber, essential minerals, and secondary metabolites such as polyphenols and flavonoids, which contribute to overall potential health benefits (Syakilla et al., 2022). Furthermore, its growing relevance in aquaculture systems and food applications further highlights its potential as a sustainable functional food ingredient.

To enhance its applicability, incorporating plant-based substances such as *C. lentillifera* into more stable, consumer-friendly food matrices is necessary. Hydrocolloid-based products, such as confectionery jelly, offer a promising delivery system due to their hydrophilic nature. This could help in the effective incorporation and uniform dispersion of water-soluble bioactive compounds while maintaining desirable sensory characteristics, including texture, flavor, and appearance (Cappa et al., 2015; Cedeño-Pinos et al., 2020). Furthermore, confectionery-based products like jelly are accepted across different consumer groups and have increasingly been explored as carriers for functional food ingredients, such as vitamins, minerals, and antioxidants (Yudhistira et al., 2024).

Nevertheless, most conventional jelly formulations rely on animal-derived gelatin for the stability, texture, and sensory quality of the end products. This creates a need for alternative formulations based on non-animal hydrocolloids, particularly in the development of innovative confectionery products with broader consumer acceptability (Sulaiman et al., 2023). The development of gelatin-free jelly products is particularly important in expanding healthier confectionery options for consumers seeking Halal-compliant and plant-based alternatives. Despite the well-documented nutritional properties of *C. lentillifera* (Syakilla et al., 2022), its application in confectionery systems remains limited. In particular, there is insufficient information regarding its functional performance and compositional characteristics within the final product formulation.

Although previous studies have examined seaweed-based foods with respect to formulation and physicochemical properties, limited attention has been given to the comprehensive nutritional and functional evaluation of macroalgae-fortified confectionery products. A hydrocolloid-based *C. lentillifera* jelly formulation was previously optimized using a D-optimal design approach (Nasir et al., 2023). Building on that formulation, this study highlighted a novel approach that combines the development of the fortified jelly with a comprehensive assessment of its composition, mineral content, and antioxidant activity, and multivariate compositional differentiation using Principal Component Analysis (PCA). This integration provides new insights into how functional ingredients influence product characteristics and differentiation, an area that has not been widely discussed previously.

Therefore, this study aims to evaluate the nutritional and functional performance of the *C. lentillifera*-fortified jelly compared with control and commercial jelly products. This includes (i) examining proximate composition, mineral content, and antioxidant activity, and (ii) identifying the key variables contributing to compositional differentiation among jellies using PCA. This study contributes to both practical and scientific aspects through advancing the development of plant-based functional confectionery and providing integrated evidence on nutritional enhancement and compositional characterization.

RESEARCH METHODOLOGY

Experimental Design

This study employed a quantitative experimental design to evaluate the nutritional composition and antioxidant properties of a *C. lentillifera*-fortified jelly formulation. Three group samples, including fortified jelly (FJ), control jelly, and commercial jelly (MJ), were prepared and compared based on their nutritional advantages. All analyses were performed in triplicate (n = 3), except for mineral analysis, which was conducted in duplicate due to instrumental constraints to ensure reproducibility and analytical reliability.

Extraction of *C. lentillifera*

C. lentillifera samples were thoroughly washed to remove impurities and subsequently stored at −80 °C for 24 h before freeze-drying at −85 °C (Beta 2-8 LDplus freeze dryer, Martin Christ, Germany) for three days. The dried samples were then milled into a fine powder (approximately 100–200 µm) using an electric blender (BL-70PR1NMY; Toshiba, Japan). The powder was reconstituted in deionized water at a 1:20 (w/v) ratio. Ultrasonic-assisted extraction (UAE) was carried out using a Branson 450 Digital Sonifier (USA) equipped with a 25.4 mm probe at 40% amplitude for 7 min. The resulting extract was centrifuged at 9,000 rpm for 20 min and subsequently filtered using Whatman No. 1 filter paper to obtain a clear extract, following a procedure adapted from previous work (Nasir et al., 2023).

Formulation of the Fortified Jelly

C. lentillifera jellies were prepared using a controlled heating and gelation process. The formulation employed in this study was based on a previously optimised hydrocolloid system developed using a D-optimal design approach (Nasir et al., 2023), which served as the formulation basis for the present investigation.

For comparative evaluation (Table 1), three types of jelly samples were prepared: fortified jelly (FJ), control jelly (CJ), and commercial jelly (MJ). The control jelly was formulated without *C. lentillifera* extract, while the commercial jelly was selected based on similarity in product type and ingredients profile to ensure comparability and minimize formulation bias. All formulations were standardized with respect to processing conditions to maintain consistency across samples.

Table 1. Ingredients used in the formulation of the jellies

Ingredients/Sample (%)	*C. lentillifera* jelly (FJ)	Control jelly (CJ)	Commercial jelly (MJ)
C. lentillifera extract	14.35	-	-
Sucrose	23.00	23.00	ND
Fructose	21.70	21.70	ND
Κ-carrageenan	26.00	26.00	ND
Locust bean gum	13.00	13.00	-
Calcium chloride	1.95	1.95	-
Water	-	-	ND
Flavor	-	-	ND
Citric acid	-	-	ND

Note: The ingredients for commercialized jelly (MJ) are unknown; ND is a component present, but its amount is unknown.

Proximate Analysis of the Jellies

The moisture analysis was performed using the oven method (MS 1119:1988). Crude fat content was determined by the Soxhlet extraction method, crude protein was analyzed according to AOAC 13.009 (1975), and ash content was measured according to AOAC 962.06 (2000). All the proximate analyses were conducted in triplicate, and standard AOAC methods were followed to ensure accuracy and reproducibility. The total carbohydrate content was calculated by difference, using Equation 1:

$$Carbohydrate, \% = 100 - (moisture + fat + crude\,protein + ash + crude\,fibre) \quad (1)$$

The total energy value (kcal/100 g) was estimated using standard conversion factors, where protein and carbohydrate were multiplied by 4 kcal/g and fat by 9 kcal/g (Mohd Naeem et al., 2017).

Mineral Analysis

About 0.25 g of the sample was accurately weighed and transferred into the digestion vessel. A volume of 10 mL of 70% nitric acid (HNO_3) was added, and the mixture was allowed to pre-digest for 10 min before microwave digestion. The digestion process was performed using a PerkinElmer Titan MPS System (USA) under a multi-step temperature and pressure program: initial stage at 160 °C (5 min ramp, 5 min hold, 90% power), followed by 190 °C (3 min ramp, 25 min hold, 100% power), and a final cooling stage at 50 °C. After digestion, the samples were dissolved in concentrated hydrochloric acid and then diluted to 100 mL with deionized water. The solution was filtered before being analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (PerkinElmer, USA). The samples were prepared in duplicate, and calibration was performed using a standard reference solution to ensure the analytical accuracy (Mohd Naeem et al., 2017).

Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) Inhibition

The DPPH analysis was conducted on the samples due to its suitability for evaluating free radical scavenging activity related to oxidative stress. Approximately 2.5 g of the fortified jelly was diluted and homogenized in 5.0 mL of distilled water. An aliquot of 50 µL was mixed with 980 µL of a 0.3 mM DPPH solution prepared in methanol. The mixture was vortexed, then incubated in the dark at room temperature for 30 minutes. Next, the absorbance was measured at 517 nm using a BioTek microplate reader (Thermo Fisher Scientific, USA) against a blank of DPPH solution without the sample extract. The DPPH radical scavenging activity (%) was calculated using Equation 2 (Bibi Sadeer et al., 2020).

$$\text{DPPH scavenging activity, \%} \frac{(\text{Abs.control} - \text{Abs.sample})}{(\text{Abs.control})} \text{X100} \quad (2)$$

Abs. control= Absorbance of DPPH scavenging + ethanol
Abs. sample=Absorbance of DPPH scavenging + sample

2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$)

The ABTS assay was employed to evaluate antioxidant capacity via radical scavenging activity, a relevant metric for assessing the potential of functional foods to reduce oxidative stress. The ABTS solution was prepared by mixing 2.45 mM potassium persulfate with 7 mM ABTS in a 1:10 ratio and incubating in the dark for 14-16 hours. The method was slightly modified from that of Masri et al. (2023), in which ethanol was used as a solvent to improve compatibility with food-based samples. For the analysis, 100 µL of diluted jelly extract was mixed with the 3.9 mL $ABTS^+$ solution and incubated for 10 minutes in a dark room at room temperature. Next, absorbance was measured using a BioTek microplate reader (Thermo Fisher Scientific, USA) at 734 nm. The $ABTS^+$ solution and ethanol were used as the negative control, while the ascorbic acid was used as the positive control in triplicate. The $ABTS^+$ radical scavenging activity was then calculated using

Equation 3.

$$\text{ABTS Scavenging activity, \%} \frac{(\text{Abs.control} - \text{Abs.sample})}{(\text{Abs.control})} \text{ X } 100 \quad (3)$$

Abs. control= Absorbance of $ABTS^+$ scavenging + ethanol
Abs. sample=Absorbance of $ABTS^+$ scavenging + sample

Statistical Analysis

Minitab Statistical Software (version 21, Minitab LLC, Pennsylvania, USA) was used for statistical analysis. All results were expressed as mean ± standard deviation (SD). One-way ANOVA was used to determine whether there were significant differences between groups, followed by Tukey's post hoc test at $p<0.05$. Principal Component Analysis (PCA) was performed using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/) to identify multivariate relationships among nutritional and antioxidant variables and to classify jelly formulations based on compositional profiles. This approach provides deeper insight into the overall variation and clustering patterns among samples.

FINDINGS AND DISCUSSION

Mineral Analysis, Proximate Composition, and Antioxidant of the Jellies

Evaluation of the nutritional and functional attributes of food products is essential to support their potential health benefits and relevance to consumer demands. In Table 2, the sodium (Na) is relatively high in commercial jelly (MJ) with 58.85 mg/100 g compared to *C. lentillifera* jelly (FJ) with 24.28 mg/100 g and the control jelly (CJ) with 20.93 mg/100 g. Although sodium contributes to product palatability and formulation characteristics, excessive intake has been associated with non-communicable diseases such as hypertension. The World Health Organization (WHO) has recommended limiting sodium intake to below 2,000 mg/day (Vargas-Meza et al., 2023), highlighting the importance of developing lower-sodium alternatives such as *C. lentillifera*-fortified jelly. Furthermore, these findings suggest that FJ may offer a healthier option for sodium intake without compromising product functionality.

A prior study reported that *Caulerpa* species contain a range of essential minerals, including potassium, calcium, and magnesium (Ismail et al., 2020). In the present study, variations in mineral content were observed among the jelly samples, which may be attributed to differences in formulation and ingredient composition. The lower potassium content in FJ compared to MJ may be due to dilution effects arising from the incorporation of multiple ingredients into the formulation, rather than reflecting the intrinsic mineral profile of *C. lentillifera*. Conversely, the presence of calcium and magnesium in the FJ is consistent with the known mineral composition of seaweed, indicating the contribution of *C. lentillifera* to the overall nutritional profile. Notably, zinc was detected exclusively in the FJ, suggesting that the incorporation of *C. lentillifera* enhances the specific availability of this micronutrient. These findings indicate that fortification with *C. lentillifera* contributes to a distinct mineral composition with potential nutritional advantages compared to conventional jelly products.

A significant difference ($p<0.05$) was observed in all variables in the proximate analysis of the jellies. All values were expressed as mean ± standard deviation, and statistical assumptions (normality and homogeneity) were verified before analysis to ensure robustness of the comparison. The higher moisture content was observed in MJ (88%), followed by FJ (50.34%) and CJ (49.12%). The moisture content of the fortified jelly is consistent with values reported in similar plant-based jelly systems (Soedirga & Marchellin, 2021), suggesting comparable structural characteristics. This finding is supported by a previous study on plant-based gels, which is extended here by demonstrating similar behavior in marine-based formulations. The relatively lower moisture content in the FJ may be attributed to the presence of seaweed-derived polysaccharides, which contribute to a denser gel network and reduced free water within the matrix. This structural effect results in a higher concentration of solids, including carbohydrates and fiber-related components, thereby increasing the total carbohydrate content. An inverse relationship between moisture and carbohydrate was observed, where higher moisture content levels in MJ resulted in dilution of solids and lower energy contribution (Kasmiati et al., 2022; Nguyen et al., 2011).

Despite the higher energy observed in FJ, this increase is primarily associated with reduced moisture content and higher solid concentration rather than excessive addition of simple sugars. The energy contribution is likely derived from complex carbohydrates and polysaccharide components associated with seaweed incorporation. While the energy content of FJ is higher than that of the MJ analyzed in this study, it remains lower than values reported for commercial fruit-based jellies (Teixeira-Lemos et al., 2021). This suggests that the fortified jelly provides a moderate energy profile while maintaining improved nutritional composition. Nevertheless, it should be noted that direct comparison between jelly types may be influenced by differences in formulation and ingredient composition, which can affect physicochemical and nutritional properties (Alghazeer et al., 2022; Barros et al., 2019).

In addition to its nutritional composition, FJ demonstrated enhanced antioxidant potential, supporting its potential as a functional food. For both antioxidant capacities, FJ exhibited the highest DPPH and ABTS scavenging activities at 68% and 45%, respectively. This could be attributed to the bioactive components of *C. lentillifera*, such as caulerpin and other phenolic and flavonoid compounds, which play significant roles in antioxidant and anti-inflammatory properties (Yap et al., 2019). These compounds have the potential to reduce oxidative stress by neutralizing free radicals, thereby improving metabolic health and preventing disease (Yoojam et al., 2021). While control and commercial jellies also exhibited measurable antioxidant activity, this may be associated with ingredients such as carrageenan or other plant-derived components.

In addition, the fortified jelly demonstrated higher DPPH activity compared to previously reported plant-based jelly systems, such as dandelion leaf polysaccharide jelly (Nguyen et al., 2022), highlighting the effectiveness of *C. lentillifera* incorporation in enhancing antioxidant capacity. In contrast to some plant-based systems with lower radical-scavenging activity, the present results indicate improved functional performance in a confectionery matrix. This study not only supports but also extends previous findings by demonstrating the functional performance of marine-based ingredients within a confectionery system.

Table 2. The Comparison of minerals, proximate, and antioxidants of *C. lentillifera*, control jelly, and commercialized jelly

Sample/ parameter	Mineral analysis (mg/100 g)						
	Na	K	Ca	P	Mg	Zn	Fe
Caulerpa jelly (FJ)	24.18±1.0[b]	24.63±1.1[b]	2.03±0.1[a]	2.45±0.1[a]	0.87±0.0[a]	0.97±0.07[a]	0.05±0.01[a]
Control jelly (CJ)	20.93±2.7[b]	42.0±10.4[b]	1.67±0.1[b]	2.14±0.0[b]	0.00±00[c]	0.00±00[b]	0.03±0.00[b]
Commercial jelly (MJ)	58.82±0.7[a]	131.6±6.2[a]	0.00±0[c]	2.02±0.0[b]	0.15±0.0[b]	0.00±00[b]	0.01±0.00[c]
p-value	0.000	0.000	0.000	0.004	0.000	0.000	0.000
	Proximate analysis (% dry basis)						
	moisture	fat	crude protein	ash	crude fibre	total carbohydrat	Energy (kCal/100g)

						e	
Caulerpa jelly (FJ)	50.34±1.1^{b}	0.06±0.01^{b}	0.00±00^{b}	0.16±0.01^{b}	2.04±0.02^{a}	47.39±1.15^{a}	190.10±4.69^{a}
Control jelly (CJ)	49.12±0.5^{b}	0.03±0.01^{b}	0.00±00^{b}	0.12±0.02^{c}	2.00±0.2^{a}	48.73±0.55^{a}	195.19±2.11^{a}
Commercial jelly (MJ)	88.28±2.08^{a}	0.28±0.04^{a}	0.02±00^{a}	0.27±0.01^{a}	1.62±0.04^{b}	9.53±2.17^{b}	40.64±8.32^{b}
p-value	0.000	0.000	0.000	0.000	0.009	0.000	0.000

	Antioxidant, %	
	ABTS	DPPH
Caulerpa jelly (FJ)	45.89±2.9^{a}	68.11±0.0^{a}
Control jelly (CJ)	36.18±1.3^{b}	33.44±2.5^{b}
Commercial jelly (MJ)	38.31±0.8^{b}	24.69±3.2^{c}
p-value	0.002	0.000

Principal Component Analysis

Principal Component Analysis (PCA) was conducted to reduce the dimensionality of the data and identify variables contributing to variation among the jelly samples. The PCA biplot (Figure 1) shows that Principal Component 1 (PC1) accounts for 72.8% of the variance, while Principal Component 2 (PC2) accounts for 24.6%, explaining a total of 97.4% of the variability. The high cumulative variance indicates that the PCA model effectively explains compositional differences among the jelly samples. The distinct clustering of FJ, CJ, and MJ indicates clear compositional differences among the samples, likely driven by ingredient and formulation differences.

The biplot simultaneously illustrates both sample distribution and variable contributions, with variables such as ash, potassium, and fat more closely associated with MJ, while antioxidant-related variables (DPPH, ABTS) and selected minerals (magnesium and zinc) are more strongly associated with FJ. This pattern highlights the contribution of *C. lentillifera* to enhancing functional and nutritional properties in the fortified jelly. The separation of clusters further confirms that the incorporation of *C. lentillifera* significantly alters the jelly's compositional profile.

These nutrients, particularly antioxidants and essential minerals, are widely associated with beneficial health effects, including antioxidant defense and physiological regulation (Syakilla et al., 2022). The PCA results, therefore, support the potential of FJ as a nutritionally enhanced alternative to conventional jelly products. Furthermore, the observed compositional differentiation demonstrates the applicability of *C. lentillifera* as a functional ingredient in confectionery-based systems, contributing to the development of value-added functional foods, supporting the physicochemical and antioxidant results, and strengthening the reliability of the study outcomes.

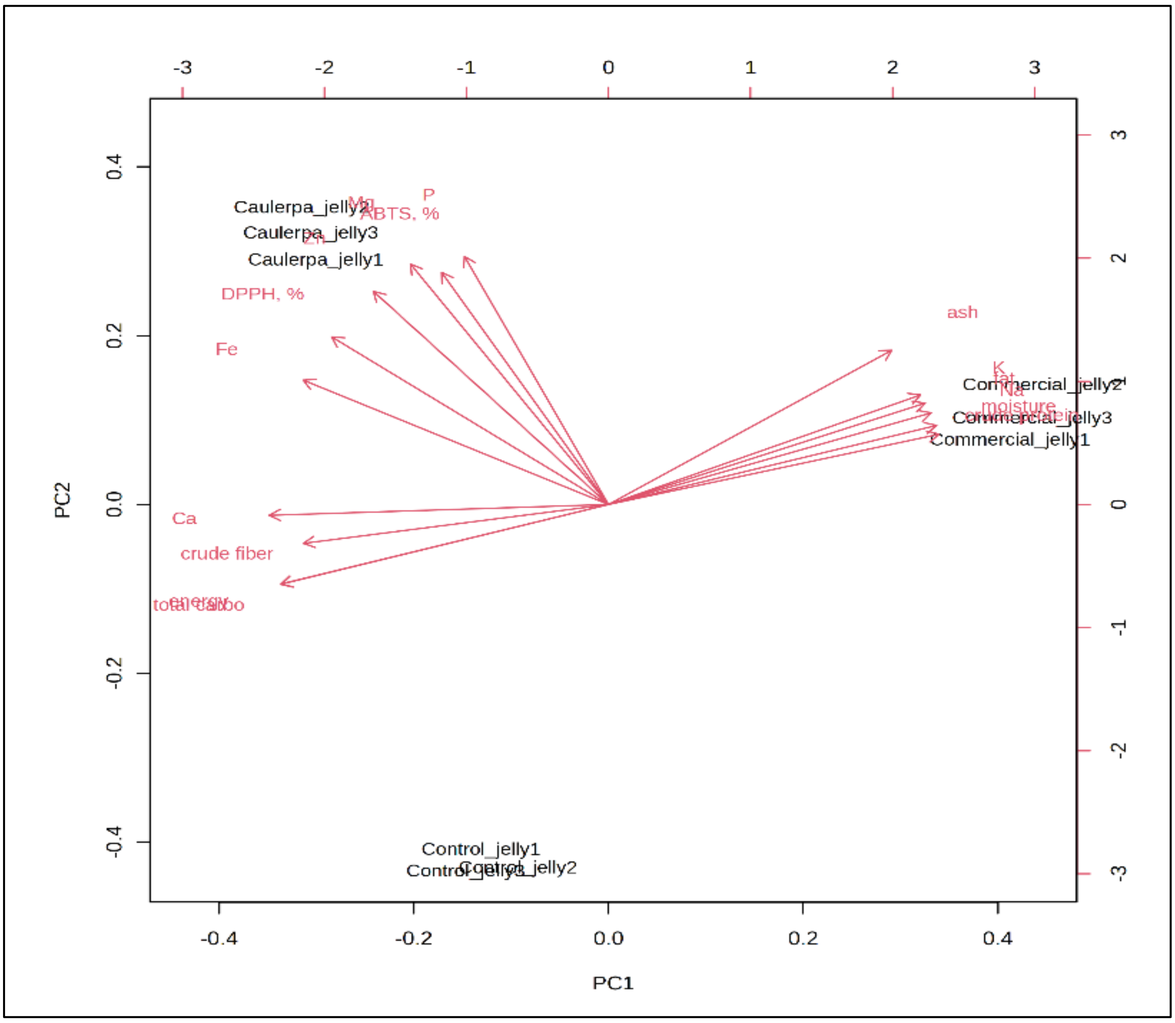

Figure 1. Principal Component Analysis (PCA) biplot of fortified jelly (FJ), control jelly (CJ), and commercial jelly (MJ), illustrating the relationship between samples and compositional variables, including mineral, proximate, and antioxidant parameters.

CONCLUSION

This study demonstrated that fortified *C. lentillifera* jelly differed significantly from both the control and commercial jellies in nutritional composition, antioxidant capacity, mineral profile, and multivariate compositional characteristics identified through PCA. FJ demonstrated higher antioxidant activity and a distinct nutritional profile, including beneficial minerals such as magnesium and zinc, while maintaining a lower sodium content. This indicates that FJ has potential as a healthier confectionery alternative. Principal Component Analysis (PCA) further confirms the contribution of the *C. lentillifera* to improving both nutritional and functional properties. These findings suggest that *C. lentillifera* can be effectively used as a functional ingredient in confectionery products. From a practical perspective, this study provides useful guidance for food developers and Halal functional-food manufacturers in developing plant-based confectionery products with improved nutritional and antioxidant characteristics. From a theoretical perspective, the findings advance current understanding of marine-based fortification and support the application of PCA-assisted compositional characterization in functional confectionery systems. Overall, this study advances the development of Halal functional confectionery by demonstrating the feasibility of *C. lentillifera*-fortified jelly and integrating proximate, mineral, antioxidant, and multivariate analyses to validate its functional-food potential.

LIMITATION & FURTHER RESEARCH

Despite the promising findings, this study was limited to compositional and antioxidant evaluation without assessing consumer-related attributes. In addition, the exact formulation details of the commercial jelly were not fully available, which may influence direct compositional comparisons among products. Future research should include sensory evaluation to determine product acceptability, as well as an investigation into processing consistency and product standardisation. In addition, further studies on storage behaviour and stability may be conducted to complement the present findings, ensuring the practical applicability and quality retention of *C. lentillifera*-fortified jelly.

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AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Nor Atikah Husna Ahmad Nasir conducted data collection, analysis, and manuscript drafting. Yus Aniza Yusof supervised and validated the work. Kaiser Mahmood assisted in manuscript preparation, and Kazunori Kadota contributed to the formulation. Aswir Abd Rashed, Nor Nadiah Abd Karim Shah, and Mohd Hafis Yuswan reviewed and validated the manuscript. All authors approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests

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