

Evaluation of Sensory Profiles, Protein Digestibility, and Mineral Composition in Mocaf-Multi-grain Noodles with Different Binding Agents

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Abstract

The demand for healthier instant noodle alternatives has increased due to dietary restrictions and nutritional concerns. This study explored the sensory profiles, protein profiles and digestibility, as well as the mineral composition of instant noodles made with modified cassava flour (mocaf), multi-grain flours (mung and kidney beans), and different binder (carboxymethyl cellulose (CMC), *Caulerpa lentillifera* (latoh), and carrageenan). Three formulations were prepared using 55 % mocaf, 30 % wheat flour, and 15 % multi-grain with different binders. Sensory evaluations included hedonic tests, Quantitative Descriptive Analysis (QDA), and Principal Component Analysis (PCA). Protein digestibility, free amino acid profiles, and mineral content were also analyzed. Noodles with CMC binder were well-accepted, exhibiting desirable sensory characteristics such as bright color, uniform appearance, chewy texture, and moderate umami aroma and taste. PCA revealed that the characteristics of noodles with CMC binders were related to preference attributes, including texture fragility, aftertaste, and overall acceptability. Protein digestibility analysis showed that noodles with carrageenan had the highest protein digestibility (55.10 %), followed by latoh (48.18 %) and CMC (43.90 %). Noodles with latoh had the highest increase in total amino acid content after digestion (105 %), compared to CMC (58 %) and carrageenan (58 %). Carrageenan as a binder resulted in the highest potassium and iron content, while latoh contributed to increased magnesium, sodium and zinc levels. These findings provide valuable insights into the development of nutritionally enhanced instant noodles using alternative ingredients. Further research on optimization and consumer acceptance is recommended to enhance the market potential of these innovative formulations. Further research on optimizing the binder types and concentrations in relating to consumer acceptance is recommended to enhance the market potential of these innovative formulations.

Keywords: Additives, Amino acids, CMC, *Caulerpa lentillifera*, Carrageenan, Instant noodles, Mineral content, Protein digestibility, Sensory profile

Introduction

Instant noodles are widely consumed worldwide, and their demand is increasing annually. According to a report by the World Instant Noodles Association (WINA), global consumption reached 120.2 billion servings in 2023. This popularity is primarily attributed

to their convenience, affordability, long shelf life, and adaptability to local cuisines. However, despite their widespread consumption, instant noodles are often criticized for their limited nutritional value, particularly their low protein and mineral content and poor

digestibility [1,2]. Increasing awareness of these issues has shifted consumer preferences towards healthier and more sustainable alternatives, particularly in Indonesia, where instant noodle consumption remains among the highest in the world, reaching 12.52 billion servings annually [3]. Additionally, the heavy reliance on wheat flour presents another challenge in terms of nutritional diversity and sustainability. To address these concerns, this study aimed to develop alternative formulations by incorporating functional ingredients to enhance the nutritional quality of instant noodles. This increasing awareness has led to a shift in consumer preferences towards healthier and more sustainable instant noodle alternatives, particularly in Indonesia, where consumption remained among the highest globally, reaching 12.52 billion servings. Furthermore, dependence on wheat flour is also a matter of concern [4]. To address these issues, this study aimed to investigate alternative formulations incorporating functional ingredients.

Alternative noodle ingredients have garnered attention due to their potential to enhance nutritional value and accommodate dietary restrictions. Traditional wheat flour, while a staple, is frequently criticized for its low nutritional profile, particularly in terms of dietary fiber and essential nutrients [5]. Given the significant role of diet in health, the widespread consumption of nutritionally deficient instant noodles is a pressing issue that this study aimed to address by exploring alternative formulations. The incorporation of alternative ingredients not only addresses these deficiencies, but also introduces various health benefits [6]. One promising alternative is modified cassava flour (mocaf) combined with multi-grain flour as a base for instant noodles. Mocaf and multi-grain were selected because of their high fiber content, essential minerals, and gluten-free nature, making them ideal candidates for improving the nutritional profile of instant noodles. Mocaf flour is produced from a cassava fermentation process carried out by lactic acid bacteria, which increases the nutritional value of the flour. One of the main advantages of mocaf flour is its high fiber content, gluten-free nature, and higher content of minerals, such as calcium and phosphorus, compared to wheat flour, making it highly suitable for individuals with gluten intolerance or celiac disease [7].

Utilizing multi-grain flour, which integrates grains like kidney beans and mung beans known for their rich nutritional content and functional attributes, particularly in terms of protein and fiber, can significantly boost the nutritional value of food products [8,9]. This type of flour is typically higher in protein, fiber, and essential vitamins than traditional wheat flour [10]. Mung beans are rich in protein (22.63 - 25.84 g/100 g) and carbohydrates (54.9 - 58.82 g/100 g), with high levels of essential amino acids, particularly lysine, phenylalanine, and leucine, as well as polyphenols (2.36 - 3.05 mg GAE/g) and flavonoids (1.42 - 2.22 mg QE/g); they also exhibit strong antioxidant activity [11]. Kidney beans contains dietary fiber (29.32 - 46.77 %), resistant starch (9.16 - 18.09 %), and protein (22.06 - 32.63 %), with a balanced essential amino acid ratio (0.29 - 0.36) and predominant polyunsaturated fatty acids (47.54-67.26 %), while being low in lipids (1.05 - 2.83 %) and sugars (1.55 - 9.07 %); they also contain γ - and δ -tocopherols (12.83 - 68.35 μ g/g) and phenolic compounds (0.25 - 3.79 mg GAE/g), contributing to significant antioxidant activity [12]. Multi-grain flour, containing both mung beans and kidney beans, offers a low glycemic index and limits starch digestion, which helps in managing blood sugar levels effectively [13,14]. The combination of mocaf with nutrient-dense multigrains holds the potential to enhance the nutritional profile of noodles. Mixing mocaf and multi-grain flour can yield a flexible and durable dough suitable for various uses, such as bread and pasta [15]. Multi-grain flour can also reduce starch digestibility, aiding in blood sugar management [10]. The combination of mocaf with nutrient-dense multigrains holds the potential to enhance the nutritional profile of noodles. Mixing mocaf and multi-grain flour can yield a flexible and durable dough suitable for various uses, such as bread and pasta [11]. The substitution of multi-grain flour can enhance chewiness and adhesiveness when balanced with gluten-containing flours, helping to achieve the desired texture for mocaf noodles as a substitute for wheat noodles [13].

Since partial replacement of wheat flour with mocaf eliminates gluten function, a binding agent is necessary to create a texture that resembles conventional instant noodles [16]. This study used 3 types of binding agents, namely CMC, *Caulerpa lentillifera* (latoh), and carrageenan. CMC, latoh, and carrageenan as binding agents and for enhancing the elastic characteristics of

noodles are significant, particularly in the context of substitute gluten formulations. Each of these ingredients provides unique functional properties that can improve the texture, stability, and overall quality of the noodle products. The ability of CMC to form a gel-like structure when hydrated contributes to moisture retention, which is essential for preventing noodles from becoming dry or brittle during storage. In addition, CMC can enhance the cooking yield and water absorption of noodles, resulting in a product that is more appealing to consumers [4]. Latoh, or sea grape, is a green seaweed that has garnered attention for its potential as a functional ingredient in food products. Latoh is rich in carbohydrates and fiber, proteins and amino acids, lipids, fatty acids, minerals, and vitamins [17]. The polysaccharides present in latoh can provide gelling and thickening properties, which can help mimic the elasticity normally provided by gluten in traditional noodle formulations and potentially enhance the water retention capacity of noodle dough. Furthermore, latoh is rich in dietary fiber and essential nutrients, which can improve the nutritional profile of noodles while contributing to their texture and taste. Carrageenan is a natural polysaccharide sulfate extracted from edible red seaweed. Carrageenan is widely used in food products, particularly for its gelling, thickening, emulsifying, and stabilizing properties. It is extracted from red and purple seaweeds and contains a mixture of polysaccharides [18]. The binder agent replaces gluten as the main binder in noodle making. Hydrated gluten in wheat flour forms a viscoelastic network providing elasticity, extensibility, and cohesiveness to the dough, essential for rolling and forming noodles. A strong binding network reduces cooking losses and improves noodle integrity [19].

The primary objective of this study was to assess the influence of 3 binders on the sensory characteristics, protein profiles and digestibility, as well as the mineral composition of mocaf-multi-grain instant noodles. The findings of this investigation were anticipated to offer valuable insights into the development of more nutritious instant noodle products, as well as address consumers' demands for nutritionally superior fast-food options. This research on mocaf-multi-grain instant noodles represents a significant contribution towards the transformation of globally consumed fast food into healthier alternatives, with potential implications for public health nutrition.

Materials and methods

Materials

This study utilized multi-grain flours and modified cassava flour (mocaf) to prepare the noodle samples. Mocaf was produced using cassava sourced from Grobogan Regency, Central Java, while flours derived from kidney beans, mung beans, and sorghum were obtained from local farmers in the region. The formulation also included water and high-protein wheat. Binding agents used in the preparation included carrageenan, latoh (*Caulerpa lentillifera*), and CMC (Blanose, France). The chemical reagents used for analysis included Walpole buffer, pepsin, NaOH, phosphate buffer (EC 3.4.23.1), pancreatin (EC 232-468-9), H₂SO₄, boric acid, BCG-MR, and orthophthaldehyde (OPA). All chemicals were procured from Sigma-Aldrich (Missouri, USA).

Methods

Mocaf flour production and noodles preparation

Mocaf flour and noodles production was prepared following the method outlined by Wahjuningsih *et al.* [20]. Fresh cassava tubers were washed, peeled, and thinly sliced (2 - 3 mm thickness). Tapioca waste was used as a stater by dissolving 20 mL per liter of water. The cassava slices were submerged in this solution for 24 h, thoroughly washed, and dried in a cabinet dryer for 12 - 24 h until their moisture content reached 10 - 12 %. The dried cassava slices were then ground using a flour mill and sieved with an 80-mesh sieve to produce fine mocaf flour.

The noodles were produced by combining mocaf flour 55 % of the formulation, 30 % wheat flour, 6.14 % kidney bean flour, and 8.86 % mung bean flour. The binder utilized comprised 2 % of the total flour. The binders employed were CMC, latoh, and carrageenan. The dry ingredients were thoroughly combined in a receptacle, then mixed with water at a ratio of 1:0.5 (w/v). The mixture underwent steaming for 15 min before being processed through an extruder to form noodle dough. The wet noodles were subsequently steamed and dried at 50 °C for 12 h to produce instant dry noodles. The study involved 3 samples, namely CMC Multi-grain Noodles (MCMC), Carrageenan Multi-grain Noodles (MCarrageenan), and Latoh Multi-grain Noodles (MLatoh).

Sensory analysis

Sensory evaluation of cooked noodles was conducted to assess product acceptance using a hedonic scale and to evaluate product characteristics through QDA [21,22]. The Health Research Ethics Commission of Dr. Moewardi General Hospital approved the study under the reference number 2.907/XII/HREC/2024. The panel consisted of 15 trained individuals who underwent a screening process aligned with the Indonesian National Standard [23]. The noodle samples were prepared by boiling for 7 min before sensory evaluation. Screening process included assessments of basic taste perception, basic aroma perception, and normal color perception. Additionally, panelist sensitivity was evaluated using a discrimination test (triangle test), ranking test, and rating/scoring test. For the hedonic test, panelists evaluated sensory attributes such as color and appearance preferences, texture preferences (chewiness), texture preferences (fracturability/brokenness), mouthfeel preferences, taste, aftertaste, and overall acceptance. 9-point hedonic scale (1: Disliked extremely; 9: Liked extremely) [22]. In the QDA [22,24], panelists described sensory attributes including color appearance, elasticity, fracturability, stickiness in the mouth, overall likeness to instant noodles, savory/umami taste, seaweed taste, beany flavor, beany taste, floury taste, bitter aftertaste, savory/umami aftertaste, seaweed flavor, savory/umami flavor, and sweet taste. These 2 results were subsequently combined into 1 matrix by PCA testing [24,25], with biplots illustrating the relationship between products and sensory attributes. Biplot analysis was utilized to identify the attributes that contribute most significantly to variation between products, identify groups of products with similar sensory characteristics, and evaluate correlations between hedonic and descriptive attributes. This approach facilitates the identification of key attributes that influence product acceptance and can assist in more targeted product development.

Total protein content and in vitro protein digestibility

The total protein was analyzed using Kjeldahl method [26]. An in vitro protein digestibility technique described by [27], was employed to assess protein digestibility, involving successive breakdown with

pepsin and pancreatin (Sigma Aldrich, USA). Dry raw noodles sample of 0.5 g was mixed with 5 mL of Walpole buffer. Gastric digestion commenced with the introduction of 2 % pepsin (40,864 units/mg), followed by a 2-h incubation at 37 °C in a Memmert Water bath WNB 29 (Germany). The pepsin reaction was halted by adding 0.5 M NaOH. For intestinal digestion, the pH was adjusted to 7.5 using phosphate buffer and 0.5 M NaOH, before introducing 4 % pancreatin (31,304 units/mg). The mixture underwent further incubation at 37 °C for 2 h. The digestion process was terminated by heating at 80 °C for 15 min. Subsequently, the products were centrifuged at 11,000× g for 15 min to separate the soluble and insoluble components. The soluble phase was used for protein content after digestion was quantified using the Kjeldahl method.

$$\text{Protein digestibility} = \frac{\text{Protein content after digestion with enzymes}}{\text{Total protein content}} \times 100 \% \quad (1)$$

Free amino acid profiles

HPLC (High Performance Liquid Chromatography) (Thermo Dionex UltiMate 3000) was employed to examine the free amino acid profiles, following the methodology outlined by [28]. Free amino acid analysis was performed by hydrolyzing a 60 mg sample with 4 mL of 6N HCl at 110 °C for 24 h. The hydrolysate was cooled to room temperature, neutralized to pH 7 with 6N NaOH, diluted to 10 mL with deionized water, and filtered using a 0.2 mm Whatman filter. A 50 µL aliquot of the sample was mixed with 300 µL of OPA (Orthophthalaldehyde) solution, stirred for 5 min, and injected (10 µL) into an HPLC system. The separation was performed using a LiChrospher 100 RP-18 (5 µm) column with a mobile phase consisting of Solution A (CH₃OH:50 mM sodium acetate: Tetrahydrofuran, 2:96:2, pH 6.8) and Solution B (65 % CH₃OH) at a flow rate of 1.5 mL/min. The gradient elution profile was set as follows: 0.1 min (100 % A, 0 % B), 15 min (0 % A, 35 % B), and 30 min (0 % A, 100 % B), followed by a stop at 35 min. Detection was carried out using a Thermo Dionex UltiMate 3000 RS Fluorescence Detector with excitation and emission wavelengths of 300 and 500 nm, respectively. Free amino acid analysis was conducted on samples both before and after

digestion using the in vitro digestibility method described previously.

Mineral content

The mineral content analysis was conducted using a wet digestion method employing microwave digestion [29]. The raw noodle samples weighing 1 g of the test portion was transferred into a digestion vessel. Prior to digestion, for Sn analysis, a mixture of 2.5 mL HNO₃ and 7.5 mL HCl was added, and the sample was left to stand for 15 min. The digestion process was carried out using a microwave digester until the sample was completely dissolved. The digested sample was then transferred to a 50 mL volumetric flask, to which an yttrium internal standard with a concentration of 100 mg/L was added. The solution was then diluted with ultrapure water to the mark and homogenized. The test solution was filtered using a 0.20 µm RC/GHP syringe filter to ensure solution purity before measurement. The intensities of metal elements such as Ca, Fe, Na, Zn, Mg, K, and Y were measured using an ICP-OES system at their specific wavelengths (Ca: 317.933 nm, Fe: 238.204 nm, Na: 568.821 nm, Zn: 213.857 nm, Mg: 285.213 nm, K: 766.491 nm, Y: 371.029 nm). The measurement results were analyzed based on a standard calibration curve with the equation $Y = bx + a$, where the metal or mineral content was calculated using formula:

$$\text{Mineral content} = \frac{A_{\text{spl}} - a}{b} \times V \times fp \quad (2)$$

Note:

A_{spl} = sample intensity.

a = intercept of standard calibration curve.

b = slope of the standard calibration curve.

fp = dilution factor.

V = final volume of test solution (mL).

W_{spl} = test portion weighting weight (g).

V_{spl} = test portion pipetting volume (mL).

Data analysis

The results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics 26, including analysis of variance (ANOVA) followed by Duncan's multiple range test to identify significant differences, with a significance threshold set at 5 %.

Results and discussion

Sensory profiles of noodles

Sensory profiling is essential for evaluating a product's organoleptic characteristics. This study employed 3 primary methodologies: The hedonic test, QDA, and PCA. The hedonic test assesses consumer preference, QDA enables trained panelists to quantify specific sensory attributes, and PCA analyses and visualises multivariate data from QDA to identify patterns and relationships between attributes. The integration of these methods provides a comprehensive understanding of sensory characteristics, consumer preferences, and attribute relationships, which is crucial for product development and optimization [22].

Hedonic test

The hedonic test results indicate variations in consumer preference for noodles with different binder additives. Although no significant difference for the hedonic test results in all samples. Based on the data obtained in **Table 1**, MCMC consistently scored the highest in almost all sensory attributes (7.00 ± 1.94), followed by MCarrageenan (6.40 ± 1.84) and MLatoh (6.10 ± 2.18). MCMC excelled in the color and appearance category with a score of 6.50, far surpassing MCarrageenan, which reached only 5.00. Although the differences between binders were not statistically significant, the obtained scores suggest that different binders influence consumer preference, particularly in terms of noodle color and appearance. This indicates that MCMC has higher visual appeal.

Table 1 Level of preference (Hedonic Test) for noodles with various binder additives.

Sample code	Color and Appearance	Texture/ Crispness	Texture/ Fragility	Lift in mouth	Taste	Aftertaste	Overall
MCMC	6.50 ± 1.78 ^a	6.10 ± 1.91 ^a	6.00 ± 2.05 ^a	6.50 ± 1.78 ^a	7.20 ± 1.55 ^a	5.67 ± 1.94 ^a	7.00 ± 1.94 ^a
MCarrageenan	5.00 ± 2.31 ^a	5.00 ± 1.83 ^a	4.60 ± 2.32 ^a	5.30 ± 1.95 ^a	6.50 ± 1.51 ^a	6.20 ± 210 ^a	6.40 ± 1.84 ^a
MLatoh	6.40 ± 2.07 ^a	5.50 ± 2.64 ^a	4.80 ± 2.20 ^a	5.70 ± 1.70 ^a	6.10 ± 2.42 ^a	5.30 ± 1.95 ^a	6.10 ± 2.18 ^a

Note: The scale used is 1 - 9 which means extremely dislike - extremely like. Different letter notations in same coloumn (a, b, and c) indicate a significant difference.

**Figure 1** Mocaf noodles with different binder agents: MCMC (left), MLatoh (middle), and MCarrageenan (right).

Taste preferences were highest for MCMC (7.20 ± 1.55), followed by MCarrageenan (6.50 ± 1.51) and MLatoh (6.10 ± 2.42). This result indicating that the binder influences flavor retention. CMC might enhance taste perception due to better water retention and starch interaction, as suggested by [32,33]. In contrast, Latoh contributed to a slightly different flavor profile, possibly due to its marine-derived components [17]. The overall preference scores suggest that CMC-based noodles were the most favored, likely due to their balance in texture, mouthfeel, and taste. This finding is consistent with previous research that highlighted CMC's role in improving dough stability and sensory attributes. However, the acceptance of carrageenan and Latoh-based noodles indicates potential for further optimization, particularly in texture modification and flavor enhancement. The current findings align with prior research on hydrocolloid-based noodle formulations. Study by Shere *et al.* [4], demonstrated that CMC enhances texture, similar to our results. Additionally, Anggraini and Do [18] and Ayadi *et al.*

[31] reported that carrageenan leads to a softer and more elastic texture, comparable to our observations. However, our study uniquely highlights the potential of Latoh as a novel binder, which has not been extensively explored in previous literature. This outcome aligns with earlier research highlighting the significance of texture and taste in consumer food preferences. As noted by [34], the intricate interplay between aroma, flavour, and texture significantly impacts how consumers perceive food products. The traditional process of making noodles generally involves the use of wheat flour, as it contains gluten, which plays a crucial role in forming a gluten network that provides good elasticity to the final product. In this study, wheat flour is still used due to the limitations of alternative binders in achieving a similar level of elasticity. However, its proportion is minimized. Despite the reduced amount of wheat flour, the resulting texture remains elastic, as the binder helps compensate for the reduced gluten content.

The superior sensory attributes of MCMC stem from CMC's properties as a binding agent, which

enhances product texture and appearance. CMC forms stable gels and increases viscosity, improving noodle texture and appearance. High scores in color and appearance suggest that CMC results in brighter, more consistent colors, and attractive presentations. MCMC's excellent texture and crispiness of MCMC indicate that CMC provides a better noodle structure by maintaining moisture and elasticity. The highest taste score for MCMC shows CMC enhances flavor, likely due to its water-binding and moisture retention properties. These findings are valuable for the future development of multi-grain noodles. CMC effectively enhanced overall sensory quality, although MCarrageenan's superiority in aftertaste suggests the potential to combine both binding agents. This is supported by research from [4], which showed that the addition of CMC to noodles enriched with fiber from spinach can improve texture, taste, and overall acceptance.

Quantitative Descriptive Analysis (QDA)

Sensory evaluation of 3 types of mocaf-multi-grain noodles, CMC Noodles (MCMC), Carrageenan Noodles (MCarrageenan), and Latoh Noodles (MLatoh), was conducted using QDA (**Figure 2**). The findings are presented in radar charts, illustrating various sensory attributes, including the flavor, texture, and visual aspects of the 3 samples. The 3 binders give different sensory to the noodles produced.

MLatoh demonstrated a significant superiority in terms of taste. This sample exhibited higher intensities across various flavor attributes, particularly sea flavor, umami, and nutty taste, compared to MCMC and MCarrageenan. This indicates that MLatoh provides a richer and more complex sensory experience in the flavor dimension. Meanwhile, MCarrageenan stood out with its sweet taste and slight sea flavor, but showed lower intensity in umami and residual bitterness compared to MLatoh. This sample exhibited better

elasticity, brittleness, and appearance than the other 2 samples.

The superior sensory attributes of MLatoh are likely attributed to its use of a more natural raw material, Latoh seaweed. Previous studies have demonstrated that seaweed-based ingredients tend to provide a stronger umami flavor profile and improved texture due to their natural polysaccharide content, such as agar and alginate, as well as the presence of essential amino acids [35,36]. In contrast, the carrageenan used in MCarrageenan, while capable of producing a chewy texture, often lacks the elasticity of other natural ingredients [37]. In addition to sensory advantages, utilizing seaweed as a raw material also offers significant nutritional benefits. This makes seaweed-based products like MLatoh an attractive option for future food product development. The high nutritional content of seaweed, including fiber, minerals, and vitamins, can add value to multi-grain noodle products.

Principal Component Analysis (PCA)

PCA was conducted on 3 types of mocaf-multi-grain noodle samples (MCMC, MLatoh, and MCarrageenan) to provide comprehensive insights into their sensory characteristics and consumer preferences (**Figure 3**). As a statistical technique for dimension reduction and variable correlation visualization, PCA revealed significant differences among the 3 samples in terms of hedonic attributes and texture [25]. PCA component 1 clearly distinguished between MCarrageenan and MLatoh. MCarrageenan, positioned in the upper left quadrant, was strongly correlated with the hedonic attributes of aftertaste. In contrast, MLatoh, located in the lower left quadrant, negatively correlated with aftertaste but positively correlated with texture elasticity, hedonic color, and overall appearance. This suggests that consumers may prefer MLatoh's visual aspects and texture to MCarrageenan's.

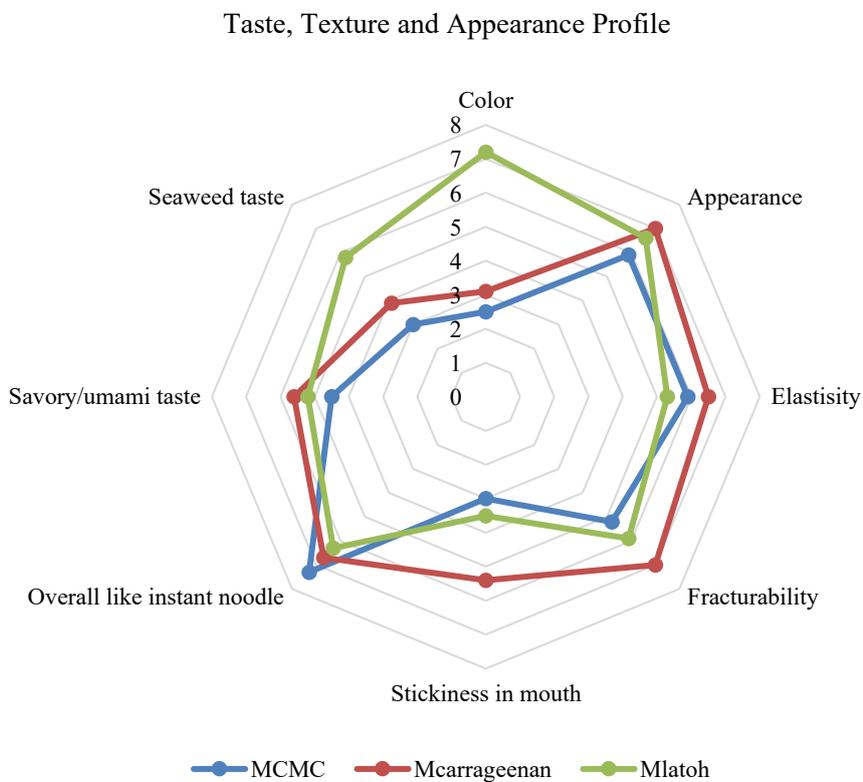
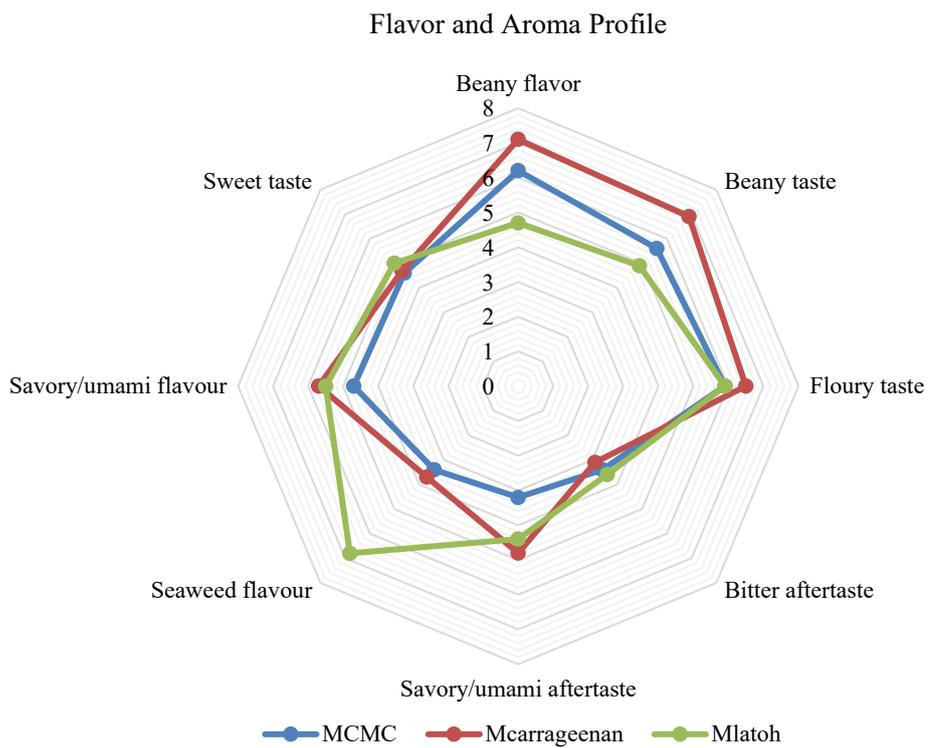


Figure 2 QDA of mocaf-multi-grain noodles with 3 types binding agent.

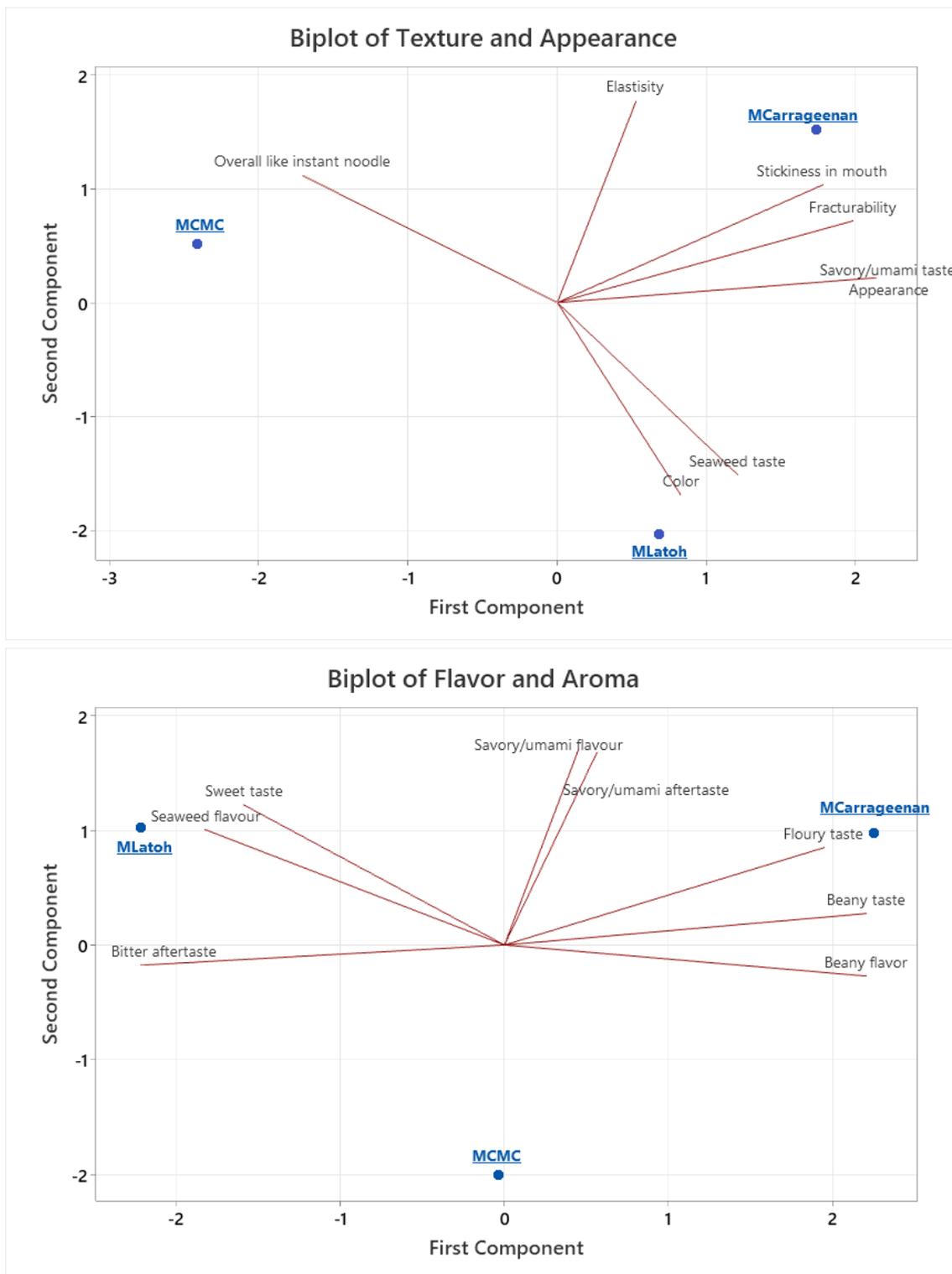


Figure 3 PCA chart of multi-grain noodles; MCMC, MLatoh and Mcarrageenan.

Further analysis revealed the unique characteristics of each sample. MLatoh exhibited notable sea flavor, aftertaste, and elasticity, indicating a complex flavor and texture profile. Caulerpa is rich in nutrients and aromatic derivatives [38]. MCMC was

more associated with mouth chewiness texture and umami or savory taste. This is supported by the findings [39] that the addition of CMC to non-gluten dough can improve the texture character to match the sample with gluten, which is due to the ability of CMC to maintain a

higher water content compared to gluten-free dough. Carrageenan was again linked to a strong aftertaste and distinctive nutty flavor. Consumer preferences for these 3 samples varied. MLatoh exhibited distinct characteristics due to its complex flavor profile, aligning with research suggesting that natural ingredients contribute to more authentic flavors preferred by consumers. Conversely, while Mcarrageenan enhanced certain textural qualities, it tended to create a less favored sensory impression, as indicated by the presence of beany taste, floury taste, and beany flavor (**Figure 2**). The addition of carrageenan in food products, as observed in the Mcarrageenan sample, may provide certain textural qualities but does not significantly enhance flavor. This underscores the importance of balancing texture and taste in the development of food products.

These PCA results offer valuable information for food producers to develop and improve products [24]. Understanding consumer preferences for different sensory characteristics can aid in formulating products that suit market tastes better. Additionally, these findings can serve as a foundation for further research on optimizing ingredient composition and processing techniques to produce multi-grain noodles with superior sensory qualities. Sensory evaluation showed that noodles with CMC binder (MCMC) were well-accepted, exhibiting desirable attributes such as bright color, uniform appearance, and overall resemblance to commercial noodles. PCA indicated that MCMC characteristics were related to preference attributes, including texture fragility, aftertaste, and overall acceptability.

Protein digestibility

Figure 4 displays the results of protein content testing and protein digestibility levels in 3 types of mofaf and multi-grain noodle samples, MCMC, MLatoh, and MCarrageenan. In the protein content test, MLatoh exhibited the highest protein content at 9.16 %, followed by MCMC at 8.86 %, and MCarrageenan with the lowest protein content at 8.13 %. This can be attributed to the ability of CMC to bind with proteins. CMC can form a protective layer around protein molecules during the noodle-making process, thereby preventing protein degradation or release due to high temperatures or intensive processing. This aligns with

[4] findings that spinach noodles containing CMC become more compact due to a thin protective coating surrounding gelatinized starch granules and the robust connection between starch granules and the protein matrix, which contributes to reduced cooking loss during the cooking process. Conversely, the higher protein content in Multi-grain Noodles with Latoh (MLatoh) can be linked to the natural protein content of Latoh. Latoh, the binder ingredient in these noodles, contributes to the overall increase in protein content [40]. Additionally, Latoh acts as a natural hydrocolloid [1] with the ability to bind with proteins and maintain their stability during cooking. This indicates that the protein contained in Latoh not only increases the total protein content but also helps prevent protein degradation during heating. This study presents the protein content of gluten-free noodles as reported by [41], which ranges from 7.52 to 19.3 %. Kidney bean flour and mung bean flour were selected as multi-grain ingredients due to their easy availability and relatively low economic value, making them cost-effective options. Additionally, both are rich in nutrients, particularly high in protein, which is beneficial for formulating low-wheat noodles. Since reducing wheat flour content may lead to a decrease in the protein content of the noodles, incorporating these alternative protein sources helps maintain nutritional quality [11,12]. Moreover, the protein in kidney bean and mung bean flour also contributes to improving the texture of low-wheat noodles [42].

In addition to the protein content, protein digestibility is a crucial parameter that reflects how effectively the protein in a product can be broken down and absorbed by the body. Higher digestibility indicates that these proteins are good nutrient sources to provide a high proportion of absorbed peptides and amino acids [43]. The research findings indicated that the protein digestibility of Multi-grain Noodles CMC (MCMC) (43.90 %) was the lowest compared to Mie Multi-grain Noodles Latoh (MLatoh) at 48.18 % and Mie Multi-grain Noodles Carrageenan (MCarrageenan) at 55.10 %. The protein digestibility level is comparable to the findings of [44] on gluten-free noodles, which range from 44 to 75 %. The lower protein digestibility observed in MCMC can be attributed to the ability of CMC to form stronger bonds with proteins. CMC, as a binding agent, interacts with proteins through various

bonds, including hydrogen bonds and electrostatic interactions [45]. These interactions create a more stable matrix structure, resulting in proteins that are more tightly bound and less readily released during digestion in the gastrointestinal tract. Consequently, the protein in MCMC is more challenging to digest and absorb in the body, as reflected by its lower protein digestibility value [30]. In contrast, the bonds between the protein and carrageenan in MCarrageenan tended to be weaker than those formed with CMC. Carrageenan, a sulfated polysaccharide, forms loose bonds with proteins, such as weaker ionic bonds [46,47]. This facilitated the release of proteins during digestion, thereby enhancing their bioavailability in the digestive tract. This is

evidenced by the high protein digestibility value of 55.10 % in MCarrageenan, indicating that the protein in Multi-grain Noodles Carrageenan is more easily broken down and absorbed by the body. For MLatoh, the protein digestibility capability fell between the 2 other samples, with a value of 48.18 %. The latoh hydrocolloid possesses the ability to maintain protein stability during cooking, yet it does not form bonds as strong as CMC. This allows better protein release during digestion, although it is not as efficient as MCarrageenan [48]. Protein digestibility analysis demonstrated that noodles with carrageenan had the highest protein digestibility (55.10 %), followed by latoh (48.18 %) and CMC (43.90 %).

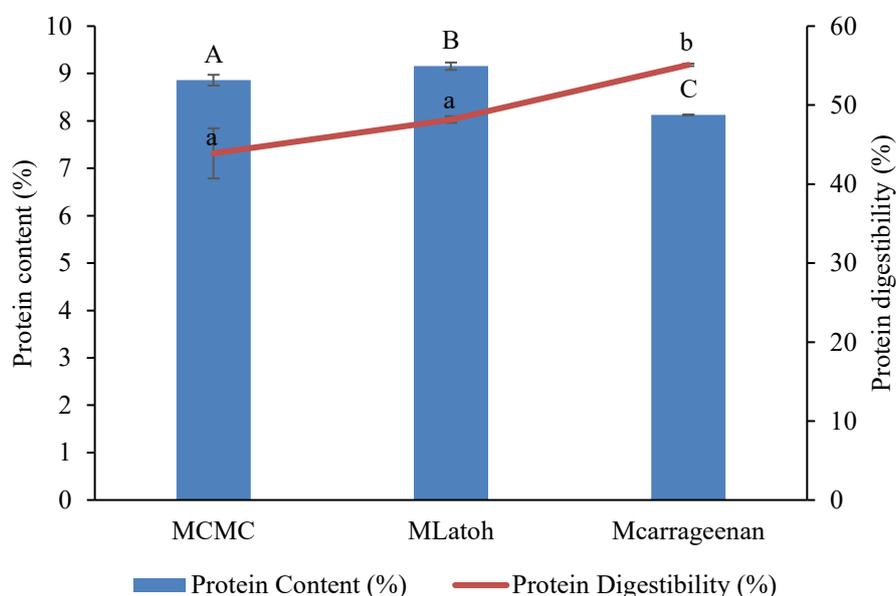


Figure 4 Protein content and protein digestibility in noodle samples. Values are expressed as the mean \pm standard deviation. The different uppercase letters (A, B, and C) indicate significant different values ($p < 0.05$) of protein content. meanwhile different lowercase letters (a, b, and c) indicate significant different values ($p < 0.05$) of protein content.

Free amino acid profiles

This study compares the amino acid profiles of various mocaf-based and multi-grain noodles, differing in binding additives such as CMC, Latoh, and Carrageenan, both in undigested (undigested) and post-digested (digested) samples (Table 2). The results indicate that Multi-grain Noodles with Latoh enhanced amino acid availability after digestion compared to the other 2 noodle types. Specifically, Multi-grain Noodles with Latoh showed an increase in total amino acid content from 38.35 ppm (undigested) to 78.55 ppm

(digested) after digestion, representing an approximate increase of 105 %. The MCMC increased from 38.77 to 61.38 ppm, an increase of 58 %. MCarrageenan from 37.89 to 59.89 ppm, an approximately increase of 58 %. These findings reflect a substantial improvement in free amino acid availability following digestion, consistent with studies indicating that formulations and additives can influence nutrient bioavailability in food products. The bioavailability of amino acids in food products is affected by the composition and additives used [49,50]. Furthermore, according to Loveday [51] food

processing treatments such as heating, gel formation, and enzymatic hydrolysis can significantly enhance or reduce the digestion and absorption rates of amino acids in protein-rich foods. Choosing the right formulation can also improve amino acid bioavailability, leading to

more easily digestible innovative products. These findings support previous research on Multi-grain Noodles with Latoh, demonstrating a significant increase in amino acid availability after digestion.

Table 2 Free amino acid content of multi-grain noodles with different additives in the Undigested (UD) and Post-Digested (PD) phases.

Free amino acid		MCMC	MLatoh	MCarrageenan
Aspartic acid	Undigested	2.85 ± 0.11 ^a	2.66 ± 0.07 ^a	2.60 ± 0.08 ^a
	Digested	4.62 ± 0.06 ^a	4.35 ± 0.80 ^a	4.73 ± 0.33 ^a
Glutamic acid	Undigested	4.63 ± 0.41 ^a	4.69 ± 0.73 ^a	3.82 ± 0.15 ^a
	Digested	8.79 ± 0.13 ^a	7.84 ± 2.32 ^a	8.68 ± 0.47 ^a
Serin	Undigested	2.53 ± 0.05 ^a	2.56 ± 0.15 ^a	2.44 ± 0.01 ^a
	Digested	4.07 ± 0.34 ^a	3.54 ± 0.58 ^a	3.83 ± 0.26 ^a
Glysin	Undigested	3.26 ± 0.1 ^a	3.26 ± 0.02 ^a	3.24 ± 0.08 ^a
	Digested	4.52 ± 0.28 ^a	4.24 ± 0.44 ^a	4.41 ± 0.36 ^a
Threonin	Undigested	3.72 ± 0.05 ^a	3.65 ± 0.04 ^a	3.65 ± 0.04 ^a
	Digested	4.39 ± 0.06 ^a	4.15 ± 0.45 ^a	4.16 ± 0.35 ^a
Arginin	Undigested	2.40 ± 0.09 ^a	2.55 ± 0.10 ^a	2.47 ± 0.04 ^a
	Digested	3.90 ± 0.19 ^a	3.83 ± 0.10 ^a	3.88 ± 0.45 ^a
Alanin	Undigested	2.36 ± 0.08 ^a	2.31 ± 0.04 ^a	2.22 ± 0.08 ^a
	Digested	3.56 ± 0.08 ^a	3.33 ± 0.27 ^a	3.32 ± 0.43 ^a
Tyrosin	Undigested	2.88 ± 0.10 ^a	2.60 ± 0.03 ^a	2.79 ± 0.43 ^a
	Digested	5.46 ± 0.36 ^a	5.52 ± 0.50 ^a	5.54 ± 0.69 ^a
Valin	Undigested	2.76 ± 0.33 ^a	3.11 ± 0.24 ^a	3.21 ± 0.22 ^a
	Digested	4.67 ± 0.39 ^a	5.55 ± 0.91 ^a	4.42 ± 0.04 ^a
Phenilalanine	Undigested	2.53 ± 0.11 ^a	2.47 ± 0.03 ^a	2.43 ± 0.09 ^a
	Digested	3.65 ± 0.14 ^a	3.52 ± 0.57 ^a	3.63 ± 0.06 ^a
Isoleusin	Undigested	2.14 ± 0.10 ^a	2.08 ± 0.03 ^a	2.02 ± 0.06 ^a
	Digested	2.93 ± 0.00 ^a	5.95 ± 0.64 ^b	2.94 ± 0.11 ^a
Leusin	Undigested	2.55 ± 0.21 ^a	2.39 ± 0.06 ^a	2.38 ± 0.13 ^a
	Digested	4.28 ± 0.20 ^a	3.98 ± 0.79 ^a	4.23 ± 0.12 ^a
Lysin	Undigested	4.17 ± 1.07 ^a	4.01 ± 1.01 ^a	4.64 ± 1.71 ^a
	Digested	6.53 ± 1.19 ^a	22.74 ± 5.21 ^b	6.13 ± 0.60 ^a
Total Undigested		38.77 ± 0.08 ^a	38.35 ± 2.30 ^a	37.89 ± 2.75 ^a
Total Digested		61.38 ± 1.86 ^a	78.55 ± 1.91 ^b	59.89 ± 3.46 ^a
Percentage increase (%)		58.33 ± 5.14 ^a	104.84 ± 7.24 ^b	58.06 ± 2.63 ^a

Note: The results are expressed as mean ± standard deviation (SD). Different letter notations in the same row (a, b, and c) indicate a significant difference.

All types of noodle binders showed a significant increase after digestion and were highest in Multi-grain Noodles with Latoh. Notably, there was a significant increase in Glutamic Acid, increasing from 4.69 ppm (undigested) to 7.84 ppm (digested). Additionally, Phenylalanine increases from 2.47 ppm (undigested) to 3.52 ppm (digested), which is important for protein synthesis and brain function. Isoleucine also showed an increase from 2.08 ppm (undigested) to 5.95 ppm (digested), which is crucial for muscle repair. Research by [52] indicated that the enhancement of essential amino acids after digestion can contribute to physiological functions and muscle recovery. Isoleucine promotes muscle mass through myogenesis and intramyocellular lipid deposition, thereby contributing to physiological function and muscle recovery. Other essential amino acids, such as Lysine, Leucine, and Valine, also showed increased post-digestion values compared to their undigested forms in Multi-grain Noodles with Latoh, reinforcing their role in providing a balanced amino acid profile. Cao *et al.* [53] reported that wheat noodles contain glutamic acid (0.059 mg/g) and methionine (0.063 mg/g) as the most abundant amino acids, followed by aspartic acid (0.022 mg/g), asparagine (0.022 mg/g), serine (0.035 mg/g), glutamine (0.035 mg/g), histidine (0.013 mg/g), glycine (0.027 mg/g), threonine (0.024 mg/g), arginine (0.027 mg/g), alanine (0.028 mg/g), tyrosine (0.009 mg/g), cysteine-S (0.021 mg/g), valine (0.016 mg/g), tryptophan (0.021 mg/g), phenylalanine (0.018 mg/g), isoleucine (0.016 mg/g), leucine (0.0052 mg/g), lysine (0.0048 mg/g), and proline (0.0048 mg/g). The findings indicate a varied amino acid profile, with leucine, lysine, and proline present in the lowest concentrations. Moreover, Liu *et al.* [54] demonstrated that *in vitro* digestion leads to a significant increase in free amino acids, attributed to the enzymatic degradation of the food matrix by proteases in the gastrointestinal tract, which enhances amino acid release and solubility.

Following digestion, Multi-grain Noodles containing CMC and those with Carrageenan demonstrated elevations in various amino acid levels, although not to the same degree as that observed in MLatoh. Research by Wee and Henry [55] notes that certain binding agents may inhibit the digestion process, as the bound protein matrix becomes denser. The

increased digestibility of amino acids in Multi-grain Noodles with Latoh indicated its ability to release amino acids during digestion, thereby enhancing their bioavailability. Appropriate formulation and selection of ingredients can enhance the bioavailability of amino acids in food products. This aligns with the findings of [56], who showed that the right formulation and ingredient selection, such as protein sources, enzymes used in hydrolysis, and peptide enrichment methods, can improve the bioavailability of amino acids in food products.

Total amino acid data provided insight into the overall effectiveness of various multi-grain noodle types in supplying amino acids before and after digestion. By focusing on the total amino acid content in undigested (undigested) and post-digested (digested) samples, we can understand how digestion affects amino acid availability and which noodle variants most effectively enhance it. The increase in total amino acids post-digestion indicates that Multi-grain Noodles with Latoh are highly digestible, with amino acids released efficiently during the digestion process. The use of Latoh (a type of seaweed) likely contributes to this due to its high content of bioactive compounds, known to aid digestion and improve nutrient absorption. Research by Mabeau and Fleurence [57] supports these findings, demonstrating that seaweed-based food products contain compounds with potential nutritional benefits that can enhance nutrient bioavailability in food products. This enhanced post-digestion amino acid profile positions Multi-grain Noodles with Latoh as a standout product in providing essential and non-essential amino acids, making it an ideal food to boost protein intake in daily diets. Gaudichon and Calvez [58] emphasize that the right formulation and ingredient selection can play a key role in enhancing amino acid bioavailability.

Mocaf-multi-grain cellulose-based composition (CMC) may not support the same level of amino acid release as latoh-based noodles. The diminished amino acid release in CMC-based Multi-grain Noodles compared to latoh-based noodles arises from differences in their composition and chemical properties. CMC, a cellulose derivative, possesses a stable polymer structure that is difficult to degrade during digestion, thus limiting amino acid release. Research indicates that

CMC has gel-forming capabilities that can entrap proteins and inhibit degradation by digestive enzymes [59]. Furthermore, the hydrophobic properties of CMC reduce its interaction with water, which is crucial for facilitating enzyme access to proteins [60]. Hydrophobic interactions promote enzyme binding with protein digestion inhibitors [61].

In contrast, Latoh-based noodles, rich in natural polysaccharides such as agar or alginate, possess a more soluble and hydrophilic structure that supports more efficient amino acid release during digestion. Seaweeds like Latoh are also known for their high content of essential plant proteins, which contributes to better amino acid release. According to Matanjun [62], seaweeds, such as *Caulerpa lentillifera*, contain significant amounts of vitamin C and β -tocopherol, and their amino acid profile indicates an amino acid release between 20 and 67 %. Therefore, Latoh naturally has a higher potential for amino acid release than CMC does.

The 58 % increase in total amino acids post-digestion suggests that Multi-grain Noodles with Carrageenan are reasonably effective in releasing amino acids but are not as effective as Multi-grain Noodles with Latoh. David *et al.* [63] noted that carrageenan can impair the proteolytic digestion of whey and inhibit the bioaccessibility of essential amino acids. Although carrageenan can enhance the bioavailability of certain nutrients, its effectiveness can vary depending on the composition and formulation of the product. The carrageenan (derived from seaweed) present in these noodles may contribute to digestibility but appears less effective than the components of Latoh in Multi-grain Noodles with Latoh. Despite the observed increase, Multi-grain Noodles with Carrageenan start with a lower total amino acid content under undigested conditions compared to both Multi-grain Noodles with Latoh and Multi-grain Noodles with CMC, which may affect their overall nutritional value. The initial composition of food

products can influence the digestion process in the human body. Factors, such as protein content, fat, particle size, and bolus viscosity, can affect the breakdown and absorption of food in the digestive tract [64,65].

Mineral profile

The mineral content analysis of mocaf-multi-grain noodles revealed significant differences based on the binding agent (**Figure 5**). Carrageenan as a binder resulted in the highest potassium content, exhibiting statistically significant differences from CMC and latoh. The highest magnesium levels were observed in samples utilizing latoh as a binder, demonstrating significant differences compared to the other 2 samples. Latoh also contributed to increased sodium and zinc levels. Carrageenan use led to the highest iron content among the 3 binders. Calcium content, although relatively lower than other minerals, varied with the highest levels in carrageenan-binders noodles. Generally, CMC as a binder tended to yield lower mineral levels compared to the other 2 binders. This is because the other 2 binders are derived from seaweed, where according to [66] that seaweed is rich in mineral essentials. This phenomenon indicates that the choice of binding agent significantly influences the mineral profile of the resulting mocaf-multi-grain noodles. The variation in mineral content within the final product is primarily influenced by the disparities in mineral composition among the diverse binders. The selection of binding agents can be strategically tailored to meet specific nutritional objectives. For instance, carrageenan can be utilized to enhance potassium and iron content, while latoh can be employed to optimize magnesium, sodium, and zinc levels. The mineral content of the wheat noodles made with 100 % wheat flour showed 51.31 mg of calcium, 3.07 mg of iron, and 0.60 mg of zinc per 100 g [67].

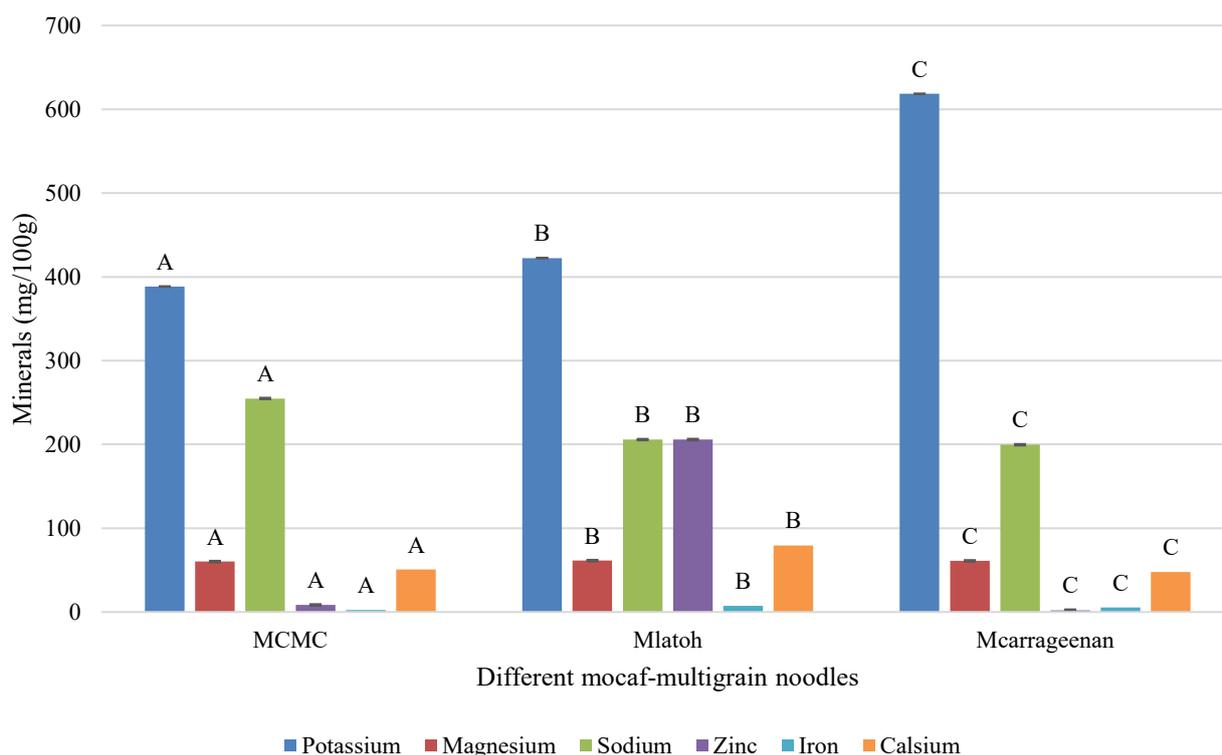


Figure 5 Mineral profiles of Different Moca-Multi-grain Noodles Binders. Values are expressed as the mean \pm standard deviation. The different uppercase letters (A, B, and C) indicate significant different values ($p < 0.05$) in each binder.

Conclusions

This study aimed to evaluate the impact of 3 binders (CMC, lath, and carrageenan) on the sensory attributes, protein digestibility and mineral content of moca-multi-grain-based instant noodles. The results showed significant differences among the noodle formulations in terms of protein digestibility, amino acid profiles, and mineral content ($p < 0.05$). Sensory evaluation showed that noodles with CMC binder (MCMC) were well-accepted, exhibiting desirable attributes such as bright color, uniform appearance, and overall resemblance to commercial noodles. PCA indicated that MCMC characteristics were related to preference attributes, including texture fragility, aftertaste, and overall acceptability. Protein digestibility analysis demonstrated that noodles with carrageenan had the highest protein digestibility (55.10%), followed by lath (48.18%) and CMC (43.90%). The enhanced protein digestibility in carrageenan-based noodles may be attributed to its gelling properties, which facilitate the modification of the protein matrix, thereby improving nutrient availability. The free amino acid profiles showed that noodles with lath had the highest increase

in total amino acid content after digestion (105%), compared to CMC (58%) and carrageenan (58%), likely due to lath's structural properties, which aid in the disruption of the protein matrix during digestion. Carrageenan as a binder resulted in the highest potassium and iron content, while lath contributed to increased magnesium, sodium and zinc content. These differences may be influenced by the distinct properties of the binder materials, which can contribute to varying mineral content and enhance the concentration of specific minerals. Overall, each binder demonstrated unique advantages. Although lath-based noodles were slightly less preferred in sensory evaluation compared to CMC-based noodles, they exhibited superior functional properties, including enhanced bioaccessibility of amino acids and higher mineral content. These findings offer valuable insights for the development of nutritionally enhanced instant noodles using alternative ingredients. Additionally, the study provides recommendations for the commercialization of high-nutrient products, targeting both the general population and specific groups such as those experiencing malnutrition or stunting. Further research is needed to optimize binder

types and concentrations in relation to consumer acceptance to maximize the market potential of these innovative formulations.

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Declaration of Generative AI in Scientific Writing

Generative AI was used solely to assist with language editing. All scientific content was created, reviewed, and approved by the authors.

CRedit author statement

Sri Budi Wahjuningsih: supervision and review; Rohadi: methodology and drafting; Zulhaq Dahri Sihny: data analysis and visualization; Ridha Indri Oktaviani: resources; Mita Nurul Azkia: conceptualization, administration and writing; Dian Haryati: drafting, software and data support.

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