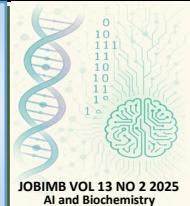




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## Effect of Rice Starch with Different Levels of Amylose Content on the Proximate Composition, Textural, and Thermal Properties of Plant-Based Cheese

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### Abstract

A major challenge in formulating plant-based cheese lies in achieving the springiness and meltability of dairy cheese, and starch shows promise in fulfilling these textural roles. However, the functional properties of starch are mainly determined by its amylose and amylopectin content. This study aimed to determine the effect of rice starch with different levels of amylose content on the proximate, textural, and thermal properties of palm milk-based cheese analogue (PMCA). Palm milk was the main ingredient in the production of PMCA as a milk fat replacement, with chickpea flour as the source of protein. A total of 4 samples were produced with 0% starch as negative control (Sample A), 2% medium-amylose rice starch (B), 2% low-amylose rice starch (C), and 2% corn starch as positive control (D). Sample C exhibited significantly higher hardness and springiness while reduced melt ability as compared to Sample B and D. The PMCA had considerably high protein (9-14%) as compared to the literature (0.11-3.00%) and low fat (12-15%) content, that suggested chickpea flour and palm milk be considered as primary ingredients in high-protein with low-fat cheese analogue. Sample C demonstrated significantly higher ( $p<0.05$ ) hardness and springiness with smaller specimen expansion in meltability as compared to samples B and D, supported by the analysis of scanning electron microscopy. In conclusion, low-amylose rice starch showed a better choice to be applied in plant-based cheese products to obtain mimicked textural characteristics with dairy cheese.

### INTRODUCTION

Originating from fermented cow's milk, cheese production involves curdling and coagulation to form curds and whey, subsequently processed to achieve its distinctive texture and flavor [1]. It stands as a significant source of essential nutrients, including proteins, calcium, and vitamins, vital for general well-being [2]. Capitalizing on current trends, the advancement of cheese substitutes that offer comparable protein content to dairy

presents a market-disrupting opportunity. These alternatives would meet the needs of environmentally and ethically conscious consumers, alongside those with dairy allergies or intolerances [3]. Moreover, they can serve as economical alternatives, particularly beneficial for lower-income populations and areas with limited availability of cow's milk. Cheese analogue, also described as a cheese-like product or imitation cheese, is made by blending different individual constituents, including plant-based or non-dairy fat and protein, through optimum thermal and

mechanical energy to achieve a preferred texture and functional properties. Cheese analogues made from vegetable-derived ingredients have lower costs compared to dairy milk and are preferred in the pizza manufacturing industry [4, 5]. Cheese analogues consumed due to specific dietary preferences (vegetarian, religious compliance, lactose intolerance, and allergies) are usually made from soybeans, rice, nuts, nutritional yeast, and other non-dairy products [6]. Vegetable oils have been used to replace milk fat in plant-based cheese analogues (PBCA) for the advantages of low cholesterol and preventing non-transmissible diseases [7].

Previous studies reported on the utilization of palm kernel oil as a milk fat substitute in Feta cheese [8], and *kashar* cheese with palm oil was highly accepted through sensory evaluation [9]. Palm milk, also known as palm-based *santan*, is a creamer processed from palm oil. Unlike coconut milk, which is rich in medium-chain saturated triglycerides, palm milk contains a balanced ratio of saturated and unsaturated fatty acids, along with tocotrienols [10]. Unlike palm oil, the application of palm milk in plant-based products, specifically plant-based cheese, is underexplored. Legumes have been widely used in developing nutritious food products or as functional ingredients, with chickpea as one of the legumes with the highest amount of protein content [11]. Due to the excellent nutrition profile and functional properties of chickpeas, the development of chickpea-based products as a source of protein is encouraged [12-15].

Despite its potential, using chickpea milk presents several challenges in plant-based cheese production. Its naturally mild and nutty flavor might not be suitable for all cheese types, making it difficult to achieve a flavor profile comparable to traditional

dairy cheese. Furthermore, while chickpea milk offers some creaminess, it may fall short in replicating the exact texture and consistency of dairy milk, especially for varieties like soft or aged cheeses [3]. Starch is one of the functional ingredients used in cheese analogue production to modify desired texture and functional properties. Starch was added in the cheese analogue formulation at 2 to 4% as a replacement for rennet with relatively low cost and great availability [16]. Specific types and combinations of starches are essential to achieve a desirable melt and avoid a gummy or overly firm texture upon heating. High amylopectin starches generally contribute more to meltability by forming a weaker gel and improving viscoelasticity [17]. In this study, the effect of rice starches with different levels of amylose content on the proximate, textural, and thermal properties of palm milk-based cheese analogue was determined.

## MATERIALS AND METHODS

### Raw Materials

Palm milk, vinegar, and nutritional yeast were purchased from a local supermarket located in Putrajaya, Selangor, Malaysia. Chickpea flour was purchased from an online vendor through an e-commerce app, while papain and carrageenan were purchased from a local supplier. The low-amylose (12% amylose) and medium-amylose (23% amylose) rice varieties were purchased from a wholesaler in Sekinchan, Selangor, Malaysia.

### Starch Extraction

The rice starch was prepared through an alkaline steeping method [18]. The extraction procedure is summarized in Fig. 1.

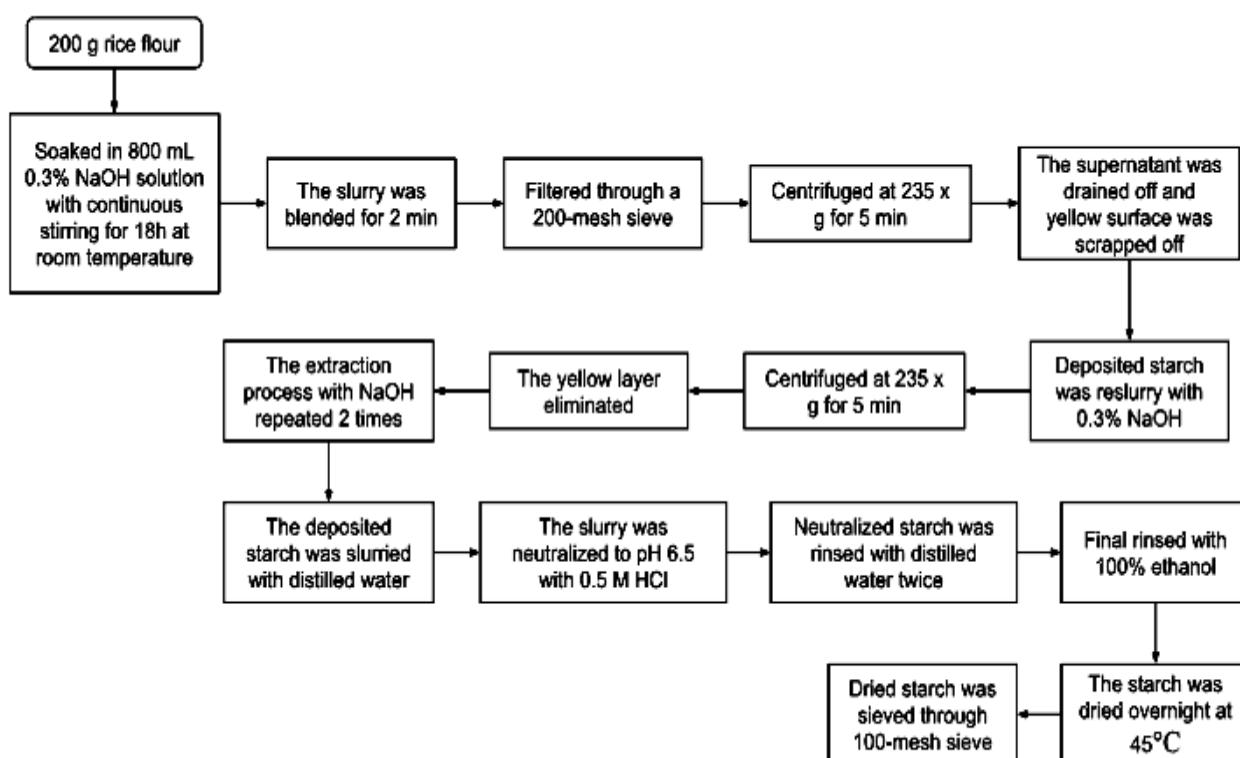


Fig. 1. Starch extraction procedure with alkaline steeping method. Note: NaOH, sodium hydroxide; HCl, hydrochloride acid.

## Palm Milk Cheese Analogue (PMCA) Formulation and Preparation

The palm milk cheese analogue (PMCA) was produced by adding ingredients amount (% in mass) of palm milk (60%), chickpea flour (6.25%), starch (2%), carrageenan (2%), papain (1.5%), nutritional yeast (1.4%), vinegar (1%), and water by modifying the method from Ferawati et al. [19]. The chickpea flour was weighed and mixed with water for 1 min to achieve a homogenized mixture. The mixture was added with starch, papain, carrageenan, nutritional yeast, and vinegar, and mixed for 1 min. Next, palm milk was added, and the mixture was continued for 30 sec until the mixture thickened. The mixture was double-boiled until it reached a temperature of 60–75 °C to ensure gelatinization. The PMCA was shaped in a square container and stored in a cold room between 5 to 10 °C. The method was repeated with different starches.

## Proximate Analysis

Proximate analysis on moisture content, ash content, crude fat, crude fibre, and carbohydrate was determined using the standard method of the Association of Official Analytical Chemists [20]. The moisture content of the palm milk cheese analogue was determined using a dry oven test. The ash content was obtained through the incineration of the sample in a muffle furnace at a high temperature. The fat content of the sample was based on the weight of fat extracted through Soxhlet extraction. The fibre content of the sample was determined by the gravimetric method. For crude protein, Kjedahl method was used to determine the protein content. The carbohydrate content of the sample was calculated using Equation 1 by subtracting all other components (moisture content, crude fat, crude fibre, protein) from 100.

$$\% \text{ nitrogen} = \frac{V_s - V_b \times N_{acid}}{W} \times 1.4 \times 100 \quad (\text{Equation 1})$$

Where,

$V_s$  = volume (mL) of acid required to titrate the sample

$V_b$  = volume (mL) of acid required to titrate blank

$N_{acid}$  = normality of acid

$W$  = weight of sample (g)

## Texture Profile Analysis (TPA)

Texture profile analysis was conducted using a texture analyser (TA.HDPlus Connect, Stable Micro Systems Ltd., Surrey, UK) equipped with a cylindrical probe (P/36R) of diameter 36mm [21]. The samples were cut into 2 cm × 2 cm × 2 cm cubes and stored in a chiller before analysis. The samples were undergoing a double compression with pre-test speed (2 mm/s), test speed (1 mm/s), post-test speed (5 mm/s), distance (5 mm), and trigger force (5 g). The hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience of the sample were determined.

## Melting Ability

The melting ability of the cheese analogues was evaluated using the Schreiber test which determined the specimen expansion of the sample. The method adopted was referred to Grasso et al. [22] with modifications on the heating temperature and time. The samples were cut into a cylinder with a diameter of 27 mm and a height of 5 mm. The samples were stored in a chiller until testing. The sample was located at the centre of a covered glass Petri dish and heated at 160 °C for 10 min. Next, the samples were left to cool for 30 min at room temperature. The pictures of the samples after heating were taken, and the specimen expansion was measured using a Vernier calliper and reported as specimen expansion.

## Differential Scanning Calorimetry (DSC)

Thermograms of the samples were analysed through a differential scanning calorimeter (DSC823/700, Mettler Toledo, Columbia, Ohio, United States) as described by Grasso et al. [22] with modifications. Samples (9–16 mg) were weighed into standard aluminium pans (Mettler-26763, 40 µL) followed by hermetically sealed. The thermal behaviour of the samples was recorded from 27 °C to 100 °C at a heating rate of 5 °C/min. Then, the Mettler-Toledo STARe system was used to interpret the DSC curve.

## Scanning Electron Microscopy (SEM)

The microstructure properties of the sample were synthesized using the same method that was detailed by [23]. A scanning electron microscope was used to examine the sample at an accelerating voltage of 10 kV. A double-sided stick tape was used to fix the sample on an aluminium stub. The sample was covered with a thin gold film of 10 nm.

## Statistical Analysis

All samples were analysed in triplicate, except for viscoelastic properties, and differential scanning calorimetry was analysed in duplicate. Minitab statistical software version 21.4 (Minitab, Pty Ltd., Sydney, Australia) was used to conduct a one-way Analysis of Variance (ANOVA) and Tukey's test on the data obtained. The confidence level at 95% ( $p < 0.05$ ) was used to determine significant differences between samples.

## RESULT AND DISCUSSION

### Proximate Analysis

The proximate composition (moisture, ash, fat, fibre, protein, and carbohydrate) was expressed as a percentage and presented in Table 1. The moisture content of all 4 samples had comparable results, ranging from 64.36 % to 64.86 %. There were no significant differences in the moisture content among cheese analogues made with different types of starch or the absence of starch. The moisture content of cheese analogue produced from palm milk in this study was significantly higher than in other studies [22,24,25]. The pulsed-based cheese analogues made from flour of boiled and roasted yellow peas and faba beans showed a comparable result with PMCA ranging from 68.50 to 70.30 %.

The high moisture content of the PMCA was due to the formulation of 76.85% (palm milk, water, and vinegar) liquid phase. The fat and protein concentration of the processed cheese was influenced by its moisture content, in turn modifying the firmness and meltability of the cheese [26]. The firmness of dairy cheese is determined by its moisture levels, with classifications including extra hard (under 51%), hard (49–56%), semi-hard (54–69%), and soft (over 67%) [27]. This moisture-based classification system could be adopted as a benchmark for plant-based cheeses. Based on this, the palm milk cheese analogues (PMCA) developed in this study are classified as semi-hard. The ash content ranges from 0.82 % to 0.89 %, which is lower than the ash content reported by [19,24]. Ash content represents the mineral content in food and is important for nutritional evaluation [28]. The fat content of the 4 samples of PMCA ranged from 12.45% to 15.04%, with no significant difference. PMCA showed a lower fat content as compared to plant-based cheese using canola oil [22] and coconut oil [29] as sources of fat, respectively. Palm milk is derived from palm oil, which is rich in saturated fat, vitamin E, and antioxidants.

The protein content among the 4 samples of PMCA did not have a significant difference, except Sample D showed the highest protein content of 13.48%. Sample A had a slightly higher protein content, which is 9.41 % as compared to Sample B (8.67 %) and C (8.57 %). The PMCA exhibited a better nutritional profile with lower fat content and greater protein content as compared to commercially available plant-based cheese analogues (PBCAs) [30] and pulsed-based cheese analogues with only 4.20 to 6.60 % protein content [19]. However, it is lower when compared to previous research on novel formulations of PBCA with 16 % protein [30]. Fava protein isolates with 91% protein purity in the formulation contribute to the high protein content of PBCA.

A similar result was also shown by Grasso et al. [12], where the cheese analogue produced from chickpea protein concentrate had higher protein content than chickpea flour. Therefore, protein isolate could be a better choice to maximize the protein content in plant-based cheese as compared to its corresponding flour, with consideration of higher raw material cost. The PMCA have a very low fibre content, ranging from 0.00 % to 0.23 %.

The ingredients that contribute to the fibre content include chickpea flour and nutritional yeast, in which both present a small proportion in the formulation, with 6.25% and 1.40% of total mass, respectively. An assumption of no fibre content in Sample C was made as the fibre content in the cheese analogue was too low and unable to be extracted and identified. Besides, the PMCA produced also have a lower carbohydrate content (7.95 to 13.28 %) as compared to other studies [12,22]. The contribution of carbohydrate content in PMCA came from the chickpea flour and starch, with only a small proportion added contributing to the low carbohydrate content. The higher carbohydrate content of plant-based cheese analogs reported by [22] might be due to the starch serving as the major ingredient in the formulation to alter the texture of the cheese analogue.

### Texture Profile Analysis

The moisture content and pH had a significant effect on the textural properties of cheese [31]. However, the PMCA in these studies had comparable moisture content and pH also exhibited different textural properties, especially hardness. **Table 2** shows the textural properties and rheological properties of PMCA.

**Table 1.** Proximate composition of cheese analogues.

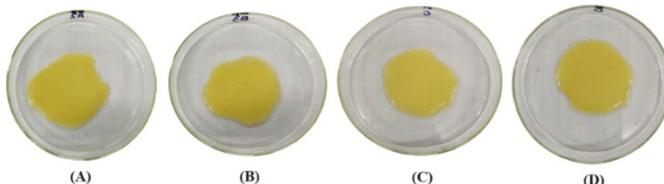
Sample	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)	Crude Protein (%)	Carbohydrate (%)
A	64.76 ± 0.13 <sup>a</sup>	0.89 ± 0.07 <sup>a</sup>	15.04 ± 0.89 <sup>a</sup>	0.23 ± 0.06 <sup>a</sup>	9.41 ± 0.24 <sup>b</sup>	9.66 ± 1.28 <sup>a</sup>
B	64.54 ± 0.46 <sup>a</sup>	0.83 ± 0.02 <sup>a</sup>	13.56 ± 0.73 <sup>a</sup>	0.20 ± 0.03 <sup>a</sup>	8.67 ± 1.28 <sup>b</sup>	12.21 ± 0.91 <sup>a</sup>
C	64.87 ± 0.31 <sup>a</sup>	0.84 ± 0.02 <sup>a</sup>	12.45 ± 1.90 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	8.57 ± 1.53 <sup>b</sup>	13.28 ± 3.70 <sup>a</sup>
D	64.36 ± 0.15 <sup>a</sup>	0.84 ± 0.03 <sup>a</sup>	13.34 ± 1.84 <sup>a</sup>	0.02 ± 0.05 <sup>b</sup>	13.48 ± 1.74 <sup>a</sup>	7.95 ± 2.22 <sup>a</sup>

Note: A = sample without starch, B = sample with medium-amylose rice starch, C = sample with low-amylose rice starch, D = sample with corn starch.  
 Different superscript letters indicate a significant difference ( $p < 0.05$ ) among the same columns.

**Table 2.** Textural properties and rheological properties of palm-milk cheese analogue.

Sample	A	B	C	D
Hardness (g)	824.32 ± 3.32 <sup>c</sup>	697.01 ± 7.70 <sup>d</sup>	937.27 ± 11.14 <sup>a</sup>	854.80 ± 17.90 <sup>b</sup>
Adhesiveness	-44.22 ± 12.67 <sup>a</sup>	-52.32 ± 7.98 <sup>a</sup>	-74.10 ± 32.80 <sup>a</sup>	-78.30 ± 41.60 <sup>a</sup>
Springiness	0.83 ± 0.02 <sup>b</sup>	0.84 ± 0.01 <sup>ab</sup>	0.85 ± 0.02 <sup>ab</sup>	0.87 ± 0.01 <sup>a</sup>
Cohesiveness	0.43 ± 0.11 <sup>a</sup>	0.50 ± 0.03 <sup>a</sup>	0.49 ± 0.08 <sup>a</sup>	0.45 ± 0.04 <sup>a</sup>
Gumminess	356.30 ± 46.90 <sup>a</sup>	461.44 ± 7.12 <sup>a</sup>	454.60 ± 73.40 <sup>a</sup>	387.90 ± 38.50 <sup>a</sup>
Chewiness	296.10 ± 42.90 <sup>a</sup>	387.61 ± 6.36 <sup>a</sup>	386.20 ± 71.00 <sup>a</sup>	337.30 ± 32.20 <sup>a</sup>
Resilience	0.20 ± 0.03 <sup>a</sup>	0.23 ± 0.02 <sup>a</sup>	0.23 ± 0.05 <sup>a</sup>	0.21 ± 0.03 <sup>a</sup>

Note: A = sample without starch, B = sample with rice starch of Putra 2 variety, C = sample with rice starch of NMR variety, D = sample with corn starch. Different superscript letters indicate a significant difference across the same row.

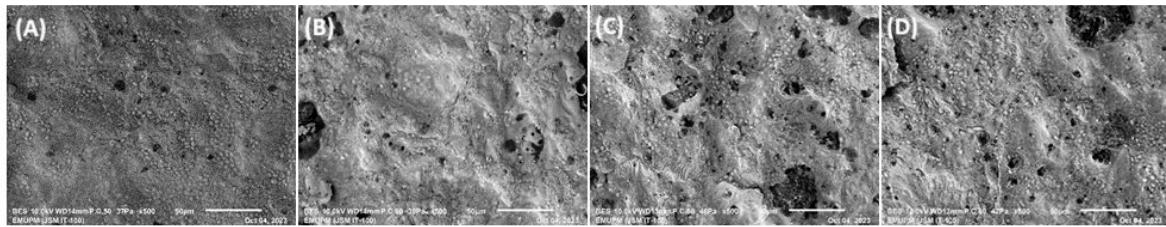


**Fig. 3.** Physical appearance of melted cheese analogues after heating at 160 °C for 10 min. Note: From left to right, A, B, C, and D. A = sample without starch, B = sample with medium-amylose rice starch, C = sample with low-amylose rice starch, D = sample with corn starch.

**Table 3.** Distance travel of the cheese analogues after heating in an oven at 160°C for 10 minutes and the on-set temperature, end-set temperature, peak temperature, and enthalpy change from the differential scanning calorimeter.

Sample	A	B	C	D
Specimen expansion (mm)	27.73 ± 2.53 <sup>a</sup>	23.67 ± 2.66 <sup>ab</sup>	20.69 ± 2.73 <sup>b</sup>	22.46 ± 2.49 <sup>b</sup>
On-set temperature, (°C)	26.98 ± 0.0071 <sup>a</sup>	26.98 ± 0.00 <sup>a</sup>	26.97 ± 0.0141 <sup>a</sup>	26.98 ± 0.00 <sup>a</sup>
End-set temperature (°C)	72.13 ± 2.55 <sup>a</sup>	75.25 ± 1.124 <sup>a</sup>	84.6 ± 16.4 <sup>a</sup>	74.84 ± 0.757
Peak temperature (°C)	30.06 ± 0.0141 <sup>a</sup>	30.22 ± 0.099 <sup>a</sup>	30.18 ± 0.191 <sup>a</sup>	30.15 ± 0.0141 <sup>a</sup>
Enthalpy change, $\Delta H$ (J/g)	103.4 ± 17.5 <sup>a</sup>	114.7 ± 18 <sup>a</sup>	105.72 ± 3.03 <sup>a</sup>	105.38 ± 12.14 <sup>a</sup>

Note: A = sample without starch, B = sample with rice starch of Putra 2 variety, C = sample with rice starch of NMR variety, D = sample with corn starch. A different alphabet of superscript indicates a significant difference among the same row.



**Fig. 4.** Microstructure of PMCA samples at magnification of  $\times 500$ . Note: A = sample without starch, B = sample with rice starch of Putra 2 variety, C = sample with rice starch of NMR variety, D = sample with corn starch.

The types of starches in formulating PMCA had a significant effect on the hardness of the sample, where sample C (937.27 g) was the hardest followed by sample D (854.80 g), sample A (824.32 g), and sample B (697.01 g) as the softest as tabulated in Table 2. Hardness of cheese might be contributed by the amylose/amyllopectin content in the cheese, which mainly contributed by the starch used. High amylose starches, with their more linear structure, tend to form stronger gel networks and exhibit higher retrogradation, leading to firmer textures. This contributed to the higher hardness value in sample C (PMCA using low-amylose rice starch) as compared to sample B (PMCA using medium-amylose rice starch).

The adhesiveness of PMCA falls in the range of -44.19 to -78.3. The large differences in the values between the samples while showing no statistically significant difference might be due to its high standard deviation. Sample A without adding starch had the lowest adhesiveness (-44.19) among the 3 samples with starches. The adhesiveness was the force required to overcome the attractive force between different surfaces, while in sensorial properties, it was the stickiness of the sample to the teeth during the chewing process. According to Zheng et al. [21], the greater the fat content, the greater the adhesiveness. However, this trend is not being observed in this study since there is no significant difference in fat content among the samples.

The starches used had a significant effect on the springiness of PMCA. Sample D made from corn starch had the greatest springiness followed by Sample B and C, both made from rice starch had comparable springiness, and Sample A without starch had the lowest springiness. According to Lee et al. [26], the springiness of cheese is dependent on the protein matrix contributing to the structure and elasticity. When comparing with the protein content, Sample D with the highest protein content had the greatest springiness. However, Sample B and C with lower protein content had greater springiness than Sample A. This indicated that the springiness is not only influenced by the protein content but also the properties of starch used. Meanwhile, the different types of starch used did not affect the cohesiveness, gumminess, chewiness, and resilience as tabulated in Table 2.

#### Thermal properties

Fig. 3 shows the physical appearance of melted cheese analogues after heating at  $160^{\circ}\text{C}$  for 10 min. The types of starches had a significant effect on the melt ability of the PMCA. A distinct trend was observed on the specimen expansion for the cheese analogue where sample A has the highest value (27.73 mm) followed by sample B (23.67 mm), sample D (22.46 mm), and sample C has the lowest value (20.69 mm). Starch reduced the melting ability of the cheese analogue demonstrated by sample A, without adding starch, which showed the greatest specimen extension. The reduced melt ability observed might be due to

gelatinized starch granules immobilizing water and dehydrating the protein matrix, leading to stronger protein-protein interaction [20, 32]. In addition, gelatinized starch increases the mixture's viscosity [33]. Hence, leading to reduced meltability after incorporation of starch. When gelatinized starch forms a continuous network in the food system, the properties of the final products are dependent on the properties of starch, usually causing reduced melting characteristics. The meltability decreases from sample A, followed by sample B, and sample D to sample C. From the aspect of rice starch, sample C (PMCA using low-amylose rice starch) demonstrated a greater extension than sample B (PMCA using medium-amylose rice starch). Plant-based cheese analogues utilizing high amyllopectin starches exhibited significantly greater melt and stretch compared to commercial alternatives, often more closely resembling dairy cheese [22]. Starches rich in amyllopectin (low amylose) have a greater swelling capacity when heated in the presence of water. This leads to the formation of a more viscous and elastic gel network. The viscous nature of the gelatinized amyllopectin network allows the cheese to soften and flow when heated.

The melting ability of PMCA was illustrated in Table 3, expressed in specimen expansion (mm) after heating. The on-set, end-set, peak, and enthalpy changes determined by DSC were tabulated in Table 3. There were no significant differences or trends observed from the data obtained. A comparable result was observed with on-set temperature, end-set temperature, peak temperature, and enthalpy change with a range from  $26.97$  to  $26.98^{\circ}\text{C}$ ,  $72.13$  to  $84.6^{\circ}\text{C}$ ,  $30.06$  to  $30.22^{\circ}\text{C}$ , and  $103.4$  to  $114.7\text{ J/g}$ , respectively. According to Grasso et al. [22], the melting enthalpy increases while the on-set melting temperature decreases when fat content increases, which supports the findings in this study that there was no significant difference in the fat content of PMCA, contributing to the absence of observable differences and trends in DSC analysis.

#### Scanning Electron Microscopy

Fig. 4 shows the microstructure of PMCA samples at a magnification of  $\times 500$ . The sample with no starch shows a denser gel network as compared to samples B, C, and D with 2% starch. [22] reported that amylose content had a greater impact than the type or source of starch on the structure of starch gels and their rheological behaviour. Therefore, there were no differences demonstrated by samples B, C, and D. [20] Based on the comparison between the microstructural properties of dairy cheese with plant-based cheese, plant-based cheese demonstrated a distribution of generally spherical fat globules within the matrix of starch and other hydrocolloids [22]. In addition, Grasso et al. [20] reported that a low occurrence of non-homogenously distributed protein aggregates within the network was observed in a plant-based cheese of slightly higher protein content (3.00%), whereas not observable in other plant-based products of protein content ranging from 0.11 to 0.64%. The findings from the previous

study could be applied to Sample D; however, the analyses should be conducted using Confocal laser SEM or Cryo-SEM to achieve that conclusion.

## CONCLUSION

The palm-milk cheese analogues developed in this study demonstrated a comparable nutritional profile than commercial cheese analog with 12.45 - 15.04 % fat, 8.57 - 13.48 % protein, and 7.95 - 13.28 % carbohydrate. The results of this study declared the potential of palm milk as a milk fat replacement while chickpea as an alternative protein in the production of low-fat, high-protein cheese analog. The incorporation of starches with different amylose content had a significant effect on the hardness and meltability properties of the PMCA. A lower amylose content in the starch used in plant-based cheese formulations generally leads to improved meltability, characterized by a softer texture and the ability to flow and stretch when heated, more closely mimicking the behavior of traditional dairy cheese.

## CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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