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Utilising rice-based ingredients for cheese-analogue cakes

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Abstract

Rice represents a suitable option for bakery products owing to its versatility and gluten-free properties. This study aims to develop cheese-analogue cake using rice-based ingredients to create layered rice cake and non-layered rice cake. The cake formulation comprises 6.9% rice flour and 9.2% fermented rice relative to the total ingredients. This formulation yields a pronounced strong cheese flavour in the cheese-analogue layered rice cake and cheese-analogue rice cake (non-layered) without adding cheese. Sensory evaluations indicate that both developed cakes receive high scores for cheese taste and overall acceptance compared to commercial cheesecake. Analysis reveals that both developed rice cakes contain all nine essential amino acids for human health. The total essential amino acid content in the layered and non-layered rice cake is 29.69 mg/100g and 30.91 mg/100g, respectively. Additionally, the rice cakes exhibit γ-aminobutyric acid (GABA) within the range of 1.27 to 1.93 mg/100g. These findings demonstrate the potential of rice in diversifying bakery products to accommodate various dietary requirements and preferences, offering gluten-free alternatives with desirable cheese flavour and nutritional value.

Keywords: *rice-based cakes, cheese-analogue, essential amino acids, GABA*

Introduction

Asian countries boast a diverse range of indigenous rice varieties that offer significant promise for applications in value-added food processing. A recent study, conducted with the support of the Malaysian Agricultural Research and Development Institute's (MARDI) 2022 Budget Initiative Project, has underscored the substantial potential of rice as a valuable resource in product development (Nicholas et al. 2015). Significantly, the intrinsic versatility and gluten-free characteristics of rice, have prompted investigations into their suitability for incorporation into bakery products. The strategic utilisation of rice-based ingredients within the bakery industry not only enhances product diversity but also serves as a means of optimising the utilization of local resources (Jan et al. 2021).

This study is dedicated to the innovative applications for rice-based ingredients, focusing specifically on rice flour and fermented rice known as 'tapai' in Asian countries such as Malaysia and Indonesia. The primary objective is to develop a cheese-analogue flavoured cakes, encompassing both basic and multi-layered rice-based variations, using established commercial techniques. A cheese-analogue food product also referred to as a cheese substitute or cheese alternative, is a non-dairy product designed to replicate the taste, texture, and functionality of traditional dairy cheese. These analogues are typically made from plant-based ingredients such as nuts, rice, soybeans, or coconut oil (Hans-Peter 2001 & Bedale 2020). Additional components such as nutritional yeast, tapioca starch, and various seasonings may be incorporated to enhance flavour and texture. While rice-based cheese analogues may not perfectly replicate the exact taste and texture of dairy cheese, they offer a versatile alternative for individuals who are lactose intolerant, adhere to a vegan lifestyle, or have dietary restrictions prohibiting the consumption of dairy products.

This study involves comprehensive evaluations of sensory attributes and nutritional composition of the

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developed rice cakes, with a specific focus on amino acids and γ-aminobutyric acid (GABA) content, as well as microbiological analysis and shelf-life assessment. The primary objective is to develop cheese-analogue cake to diversify bakery choices for consumers. By utilising rice as the primary ingredient, scientists and food technologists are exploring innovative methods to replicate the taste, texture and functionality of traditional dairy cheese.

Materials and method

The formulation and processing method of rice-based cheese analogue cakes are based on the information from previous research conducted by MARDI (Nicholas et al. 2014), with some adjustments in the formulation and newly added ingredients based on the requirements. Rice flour and fermented rice are utilised in the cake formulation to evaluate their suitability in producing layered rice cake and conventional rice cake (non-layered).

Raw material

Rice flour

The production of rice flour employed the 'dry milling method', wherein the rice was finely ground using Orimas's medicinal disk milling machine (model DM1500) and sifted through a metal sieve sized at number 80 – 100 mesh (*Figure 1*).

Figure 1. Rice flour used in cake processing

Fermented rice

Fermented rice was produced through fermentation of cooked rice using MARDI's inoculum, which contains a blend of microorganisms such as *Amylomyces oryzae, Hansenula anomala*, and *Saccharomycopsis fibuliger* (Yeoh & Merican, 1986). Glutinous rice should be thoroughly rinsed and soaked for 12 – 16 hours in ample of water at room temperature. Following this, the excess water was drained, and the rice was thoroughly rinsed with clean water. The rice was then cooked using clean water. Once cooked, the rice was spread evenly to a thickness of 2 cm on a plastic-lined surface. After cooling, the inoculum was evenly dispersed over the rice. Approximately 80g of rice was placed into plastic containers and covered. The fermentation process was allowed to proceed for 45 hours at room temperature until the rice became fermented (Yeoh et al. 1992). The fermented rice was stored at a chilled temperature 4 – 5 °C until it was ready for use. It is then blended using a blender to obtain rice paste for use in rice cake production (*Figure 2*).

Figure 2. Fermented rice paste used in cake processing

Additional ingredients

Other ingredients used in rice cake production include butter, eggs, sugar, sweetened condensed milk, fresh milk, coconut cream, flavouring agents (Horlicks malt and vanilla), emulsifier (ovallette), salt, citric acid and white colouring agents (optional). Ovallette was added to improve the homogeneity and stability of the mixture, ensuring a smooth and consistent texture. Salt was included to balance the flavours, while citric acid was used to adjust the acidity levels and enhance the cheese-analogue taste of the developed cake. The formulation of layered rice cake and conventional rice cake (non-layered) is shown in *Table 1*.

Processing parameters

The formulation and processing steps for both layered rice cake and conventional rice cake (non-layered) are shown in *Table 1* and *Figure 3*. The layered rice cake was produced by creaming butter with sugar for about 8 – 10 minutes until it was homogenized. Subsequently, eggs, malt flavouring, emulsifier (ovallette), sweetened condensed milk, coconut cream (seri kaya) and vanilla flavouring were added and stirred until smooth. Then, finely blended fermented rice, white colouring, evaporated milk, salt and citric acid were added, then the mixture was stirred until homogenised. Rice flour and wheat flour were added to the mixture and mixed for 5 minutes until a cake batter was formed. It is imperative to ensure that the mixing process is not prolonged after both flours are incorporated.

A thin layer of cake butter was spread on the inner surface of a 7 x 7 inch cake mold. Parchment paper was placed at the bottom before pouring the cake batter, to facilitate the removal of the cake from the mold after baking. Subsequently, 90 grams of cake batter was poured into the mold and leveled. An electric oven was preheated using the top heating mode. The oven should be preheated for 5 minutes until the temperature reaches 130 – 150 °C. The cake batter was baked at 180 °C for 7 – 10 minutes. Upon removal from the oven, the cake was gently pressed on the surface with a cake press to achieve a dense and uniform cake layer. Furthermore, 90 grams of cake batter was added on top of the first layer and baked again. This process was repeated several times as per the desired number of layers. The resulting rice-based layered cake is illustrated in *Figure 4*.

In producing conventional rice cakes (non-layered), the raw materials and preparation method of the cake batter remain the same as the layered rice cake processing method described above. Once all the raw materials were mixed and homogenised, the resulting cake batter was poured entirely into a 7 x 7 inch mold, leveled and then baked in an oven at 180°C for 50 – 60 minutes, utilising both top and bottom heating elements of the oven. No layering method for the cake batter is required. The resulting rice-based cheese-analogue cake is illustrated in *Figure 5*.

Table 1. Formulation of cheese-analogue rice cakes (both for conventional non-layered and layered cakes)

Ingredients	Percentage (%)
Rice flour	6.9
Wheat flour	4.6
Butter	23.0
Egg	23.0
Fermented rice paste	9.2
Sugar	8.3
Condensed milk	9.2
Evaporated milk	3.7
Ovallete	1.4
Salt	0.3
Citric acid	0.2
Coconut cream (seri kaya)	4.6
Malt flavour (Horlick)	4.6
Vanilla flavour	0.1
White colouring	0.9

Figure 4. Rice-based cheese analogue layered cake

Figure 5. Rice-based cheese analogue cake

Figure 3. Processing steps of cheese-analogue layered rice cake and cheese-analogue rice cake (non-layered)

Sensory evaluation

Sensory evaluation was conducted using a 7-point hedonic sensory test to determine the best formulation for rice cakes. The evaluation involved 30 participants who rated various sensory characteristics, including aroma, colour, texture, sweetness, taste and overall acceptance. The study aimed to compare the rice-based cheese analogue cakes with several commercially available cheesecakes (Poste et al. 1991).

Proximate and microbiological analysis

The analysis of the rice cakes was carried out in duplicate. Proximate analysis conducted included the determination of moisture, protein, fat, carbohydrate, energy, sodium, sugar, dietary fibre, and ash content. Moisture, ash and dietary fibre were determined by using standard AOAC methods (AOAC 1990 and 1993). Protein and fat were determined according to in-house method 0506 and 0511, respectively based on Pearson's Chemical Analysis of Food (Harold 1981). Carbohydrate content was calculated according to in-house method 0512 based on the Method of Analysis for Nutritional Labelling (AOAC 1993) by subtracting the values of moisture, protein, dietary fibre, fat and ash from 100. Energy was determined according to AOAC standard method (AOAC 1993) by calculating from the amount of protein, fat and carbohydrate (Nicholas et al. 2023). Microbiological testing covered Total Plate Count, Coliform Count, and Yeast and Molds Count (AOAC 2023).

Determination of GABA content and amino acid profiles

Analysis of amino acid profile and γ-aminobutyric acid (GABA) was carried out for the produced rice cakes by using Ultra-Performance Liquid Chromatography (UPLC) as described by Koh et al. (2012). The amino acids standards (2.5 mM of amino acids and 1.25 mM of cysteine) were bought from Waters (USA) including histidine, serine, arginine, glycine, aspartic acid, glutamic acid, threonine, alanine, proline, cysteine, lysine, tyrosine, methionine, valine, isoleucine, leucine and phenylalanine. The γ-aminobutyric acid (GABA) was purchased from Sigma-Aldrich (USA).

Initially, a stock solution of GABA at a concentration of 2.5 mM was prepared by dissolving GABA in 0.1 N HCl. A working standard solution, including all amino acids and GABA, was prepared by diluting the stock solutions with deionized water to achieve a final concentration of 100 pmol/µl for each amino acid, except cysteine, which was set at 50 pmol/µl. This solution was then derivatized with 70 µl of AccQ-Tag™ Ultra borate buffer and 20 µl of AccQ™ Fluor reagent according to the standard UPLC amino acid analysis protocol. The separation of GABA and amino acids was carried out using an AccQ-Tag™ Ultra column (2.1 mm x 100 mm, 1.7 µm) at a flow rate of 0.7 ml/min, with the column temperature maintained at 55°C. Detection was performed at a wavelength of 260 nm. Quantification was achieved through calibration curves generated by injecting known amounts of amino acids standard and GABA as external standards with known retention times. The total essential amino acids were calculated based on the sum of phenylalanine, threonine, methionine, leucine, isoleucine, lysine and valine. All analyses were performed in triplicate.

Packaging

Long-term storage of rice cakes for the shipment purpose to distance locations and prolonged storage, permitted food preservatives namely potassium sorbate at the amount of less than 2g/kg could be added into the cake formulation. The prepared cake was packaged following methods outlined in previous studies conducted by MARDI for cake products (Nicholas et al. 2014). The rice cake was first wrapped with PVC plastic or stretch wrap, then placed into airtight plastic packaging of oriented Nylon/LLDPE type, with an oxygen absorber placed between the two packaging layers. The oriented Nylon/LLDPE packaging plastic was completely sealed to prevent air from entering the package. The packaging method is illustrated in *Figure 6*.

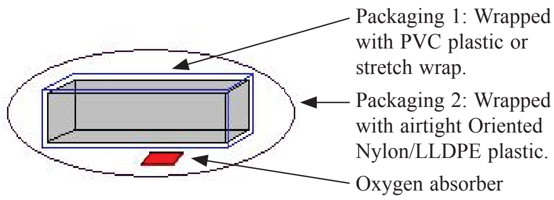

Figure 6. Packaging of rice cakes for prolonged storage

Shelf-life study

The developed cake samples were stored in a refrigerated environment at temperatures ranging from 4 – 10 °C, with a targeted shelf life of 3 months. Shelf-life assessment encompassed various aspects, including microbiological analysis, colour analysis expressed in L*, a*, b* notation, measurement of moisture content, water activity (A_w), pH levels, and sensory evaluation.

Data analysis

Statistical analysis was performed using SPSS. Data were statistically analysed via analysis of variance. The Duncan Multiple Range Test was employed to discern the significance between the samples.

Results and discussion

Development of rice-based cheese-analogue cakes

The development of rice-based cheese-analogue cakes represents a good approach to meet the demand for alternative dairy products. Through a series of formulation development and sensory evaluations, this study identified the best ratio of rice flour to wheat flour for both layered and conventional non-layered rice cakes is 60:40% respectively, a finding consistent with previous research on incorporating rice flour and wheat flour in layered cake (Nicholas et al. 2014). This finding slightly aligns with a study conducted by Saiyavit and Sujin (2000), which highlighted the optimal ratio of 40:60% of triticale flour to wheat flour for cake production. Triticale flour mentioned by Saiyavit and Sujin (2000) is a derivative of the hybrid grain triticale resulting from the crossing of wheat and rye, possessing gluten akin to wheat flour. This property renders it adept at forming cohesive dough suitable for various baking applications, including bread and cakes. However, it is noteworthy that while Saiyavit and Sujin (2000) utilised 60% wheat flour in their study, our investigation successfully reduced the reliance on wheat flour to 40%, while concurrently increasing the usage of rice flour to 60%. This strategic adjustment was aimed at mitigating gluten content in the cakes, considering rice's characteristics as a gluten-free ingredient. By reducing the proportion of wheat flour and incorporating a higher percentage of rice flour, the study aimed to cater to consumers with gluten sensitivities or those seeking gluten-free alternatives. This adjustment not only aligns with evolving dietary preferences but also underscores the versatility and potential of rice flour as a substitute in bakery formulations.

Incorporating rice flour, wheat flour and fermented rice paste with other mentioned ingredients in this study, has successfully created a cheese-analogue cake that does not contain any actual cheese as an ingredient. The optimal formulation of the rice-based cheese-analogue cakes is shown in *Table 1*. The formulation includes 6.9% rice flour and 9.2% fermented rice, based on the total weight of the ingredients. Additionally, the incorporation of a range of flavouring ingredients especially fermented rice paste, butter, eggs, sugar, salt, citric acid, and optional white colouring contributed to the sensory attributes and overall quality of the cakes. The utilisation of fermented rice paste, produced through the fermentation of cooked rice or glutinous rice, played a crucial role in enhancing the flavour profile and texture of the cakes. The aroma and taste of the fermented rice paste contribute significantly to the cheese-like characteristics of the developed rice cakes, as the flavour of cheese is a result of the fermentation process. Our preliminary study indicates that rice cakes produced with the same amount of rice flour but without the fermented rice paste did not develop a cheese-like flavour in the resulting cakes. The presence of microorganisms such as *Amylomyces oryzae, Hansenula anomala*, and *Saccharomycopsis fibuliger* facilitated the conversion of starch to sugar and contributed to flavor development during the fermentation process. Subsequently, the fermented rice paste was incorporated into the cake batter, imparting a distinctive cheese-analogue taste and aroma.

Furthermore, the addition of an emulsifier, ovallette, contributed to the homogeneity and stability of the cake batter, ensuring a smooth and consistent texture in the final product. Generally, the use of emulsifiers in bakery products aims to improve dough handling, product quality and shelf-life extension. Emulsifiers have the capability of promoting emulsions or stabilization of emulsions by their effect on the interfacial tension of the product (Orthoefer & Kim 2019). As the trend continues toward healthier products that contain less fat, sugar and sodium, emulsifiers will play an important role as functional ingredients in baked products (Stauffer 2005). The inclusion of salt in rice cakes in this research helped to balance the flavours, while citric acid served to adjust the acidity levels and enhance the cheese-analogue taste. The processing parameters employed in the production of both layered and conventional rice cakes were carefully optimised to achieve the desired texture and structural integrity. For layered rice cakes, a meticulous layering process involving the pouring and baking of multiple cake batter layers was employed, resulting in a visually appealing and multi-textured final product. In contrast, conventional rice cake (non-layered) was slightly easy to produce by pouring the entire cake batter into a mold and baking it as a single unit.

Sensory evaluation

The sensory evaluation of rice cakes, both layered and non-layered, alongside commercial cheesecakes was conducted to assess various attributes including aroma, texture, sweetness, cheese flavour, and overall acceptance. The sensory results, as shown in *Table 2* revealed significant findings regarding consumer perception and acceptance of the developed rice cakes compared to their commercial counterparts.

In terms of aroma, texture (specifically softness), sweetness, cheese flavour, and overall acceptance, both variants of the developed rice cakes emerged with the highest scores. Notably, the rice cakes exhibited a remarkable cheese flavour despite not incorporating cheese ingredients in their formulation. Both layered and non-layered rice cakes received commendable sensory scores for cheese flavour, indicating a strong resemblance to traditional cheesecakes. The sensory scores for cheese flavour were substantially higher for both layered (5.90 ± 0.88) and non-layered (5.73 ± 1.04) rice cakes compared to commercial cheesecakes (5.13 ± 0.97) and commercial layered cheesecakes (5.06 ± 0.86). This suggests that the developed rice cakes successfully captured the essence of cheese flavour, surpassing that of their commercial counterparts. The significant distinction in cheese flavour between the developed rice cakes and commercial cheesecakes underscores the efficacy of the formulation strategy employed in enhancing the cheese profile.

Furthermore, the overall acceptance scores for both layered (6.33 ± 0.66) and non-layered (5.83 ± 0.98) rice cakes were notably higher compared to commercial cheesecakes (5.43 ± 1.00) and commercial layered cheesecakes (5.26 ± 0.78). This indicates strong preferences among consumers for the developed rice cakes, emphasising their superior sensory attributes and

Table 2. Sensory evaluation results of rice cakes (non-layered and layered) compared to commercial cheese cakes (non-layered and layered)

Attributes	Cheese-analogue rice cakes		Commercial cheesecakes	
	Cheese-analogue layered rice cake	Cheese-analogue rice cake (non-layered)	Commercial layered cheesecake	Commercial cheesecake
Colour	6.50 ± 0.57 [a]	5.66 ± 0.92 [b]	5.90 ± 0.95 [b]	5.60 ± 0.93 [b]
Aroma	5.96 ± 0.85 [a]	5.63 ± 1.03 [ab]	5.10 ± 1.15 [b]	5.86 ± 1.13 [a]
Texture	6.16 ± 0.83 [a]	5.66 ± 1.12 [ab]	5.46 ± 1.10 [ab]	5.26 ± 1.55 [b]
Sweetness	5.83 ± 1.14 [a]	5.66 ± 1.18 [a]	5.33 ± 0.88 [a]	5.20 ± 1.37 [a]
Cheese flavour	5.90 ± 0.88 [a]	5.73 ± 1.04 [ab]	5.06 ± 0.86 [c]	5.13 ± 0.97 [bc]
Overall acceptance	6.33 ± 0.66 [a]	5.83 ± 0.98 [ab]	5.26 ± 0.78 [b]	5.43 ± 1.00 [b]

* Mean values in the same row with the different letters are significantly different ($p < 0.05$)

overall palatability. The observed strong cheese flavour in the developed rice cakes, despite the absence of cheese ingredients in their formulation, suggests the successful integration of flavouring agents or techniques that mimic the characteristic taste of cheese. This innovative approach not only highlights the adaptability of rice-based formulations but also paves the way for developing cheese-analogue products catering to diverse consumer preferences and dietary requirements.

Proximate, nutritional composition and microbiological analysis

The analysis of proximate values and nutritional content, as presented in *Table 3*, reveals similarities between the layered rice cake, non-layered rice cake and commercial layered cheesecake and commercial non-layered cheesecake. Notably, the ash content in both rice cakes was slightly higher (3.0 – 3.8%) compared to commercial layered cheesecake (0.5%) and commercial cheesecake (1.2%), which suggests a similarity of those in cereal ingredients particularly the germinated barley reported by Sharma et al. (2012). This elevation in ash content suggests a relatively higher mineral content in both rice cakes compared to their commercial cheesecake counterparts. The microbiological analysis of fresh layered rice cake and non-layered rice cake revealed no detectable growth of microorganisms in Total Plate Count, coliform, yeast and molds tests, mirroring the findings observed in commercial cheesecakes.

The presence of essential amino acids in food products is crucial for human health, as these amino acids cannot be synthesized by the human body and must be obtained through diet. As highlighted by Kathy and Jilian (2023), the human body requires a balanced intake of essential amino acids for proper growth and functioning. Analysis results (*Table 4*) indicate that the non-layered rice cake contains 30.91 ± 0.41 mg/100 g of essential amino acids, while the layered rice cake contains a total of 29.69 ± 0.75 mg/100 g. These findings underscore the nutritional value of the developed rice cakes, providing essential amino acids necessary for human health.

Table 5 provides a detailed breakdown of the essential and non-essential amino acids present in the developed rice cakes, further elucidating their nutritional composition. The presence of essential amino acids in the rice cakes highlights their potential as a source of essential nutrients, contributing to a balanced diet. Additionally, the analysis reveals the presence of γ-aminobutyric acid (GABA) in rice cakes, with respective values of 1.93 ± 0.22 mg/100 g in layered rice cake and 1.27 ± 0.02 mg/100 g in non-layered rice cake (*Table 4*). GABA is a bioactive compound known for its potential health benefits, including its role in lowering human's nervous system and lowering the blood pressure (relaxation and stress reduction) (Castanho 2023).

Furthermore, the elevated ash content in the developed rice cakes may be attributed to the higher mineral content inherent in rice and other ingredients utilised in their formulation. Rice is known to contain various minerals such as potassium, magnesium, phosphorus, and calcium, which contribute to overall mineral content. The incorporation of other ingredients such as flour, sugar, and flavouring may also contribute to the ash content, further enriching the nutritional profile of the rice cakes.

Both rice cakes were found to contain all nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) necessary for proper growth and functioning of the body. The amino acid content in these rice cakes may be attributed to raw materials derived from rice, particularly fermented rice 'tapai'.

Packaging and shelf-life study

The packaging of food products plays a critical role in preserving their quality and extending their shelf life. In this study, the packaging methods utilised for the rice cakes incorporated both traditional practices and innovative techniques to ensure product freshness and safety. The storage stability of food products is essential to ensure their quality and safety over time. In this study, the effects of storage on the moisture content, water activity, pH, colour, microbiological, and sensory attributes of developed layered and non-layered rice cakes were investigated and the results are showed in *Table 6*

Table 3. Proximate, nutritional composition and microbiological analysis results of rice cakes (non-layered and layered) compared to commercial cheesecakes (non-layered and layered)

Attributes	Cheese-analogue rice cakes		Commercial cheesecakes	
	Cheese-analogue layered rice cake	Cheese-analogue rice cake (non-layered)	Commercial layered cheesecake	Commercial cheesecake
Energy (kcal/100 g)	460.50 ± 2.12 [ab]	418.00 ± 1.41 [bc]	373.00 ± 38.18 [c]	488.50 ± 3.53 [a]
Protein (g/100 g)	4.75 ± 0.21 [c]	6.20 ± 0.14 [b]	5.95 ± 0.21 [b]	7.30 ± 0.28 [a]
Carbohydrate (g/100 g)	46.95 ± 0.77 [bc]	45.40 ± 0.70 [c]	61.05 ± 1.62 [a]	48.95 ± 0.35 [b]
Fat (g/100 g)	21.35 ± 0.21 [b]	18.35 ± 0.35 [c]	8.78 ± 0.59 [d]	24.70 ± 0.42 [a]
Moisture (g/100 g)	23.95 ± 0.21 [b]	27.85 ± 0.35 [a]	23.65 ± 0.77 [b]	17.55 ± 0.63 [c]
Ash (g/100 g)	3.00 ± 0.14 [a]	3.08 ± 0.02 [a]	0.59 ± 0.02 [c]	1.22 ± 0.02 [b]
Total sugar (g/100 g)	9.40 ± 0.14 [c]	10.10 ± 0.00 [a]	9.65 ± 0.07 [b]	9.80 ± 0.00 [b]
Sodium (mg/100 g)	4.01 ± 0.39 [a]	3.22 ± 0.17 [b]	1.25 ± 0.11 [c]	4.22 ± 0.37 [a]
Dietary fibre (g/100 g)	5.30 ± 0.00	4.00 ± 0.00	8.80 ± 0.00	2.70 ± 0.00
pH value	5.08 ± 0.02 [c]	5.38 ± 0.02 [b]	5.48 ± 0.007 [a]	5.36 ± 0.01 [b]
Total plate count (cfu/g)	NG (<10)	NG (<10)	NG (<10)	NG (<10)
Coliform count (MPN/g)	NG (<3.0)	NG (<3.0)	NG (<3.0)	NG (<3.0)
Yeast and molds (cfu/g)	NG (<10)	NG (<10)	NG (<10)	NG (<10)

*NG - *No growth*
** Mean values in the same row with the different letters are significantly different (p <0.05)*

Table 4. Total essential amino acids, non-essential amino acids, soluble amino acids and GABA content in rice cakes (non-layered and layered)

Samples	(mg/100 g sample)			
	Essential amino acids	Non-essential amino acids	Soluble amino acids	γ-aminobutyric acid (GABA)
Cheese-analogue layered rice cake	29.69 ± 0.75	51.82 ± 1.58	81.50 ± 2.32	1.93 ± 0.22
Cheese-analogue rice cake (non-layered)	30.91 ± 0.41	43.44 ± 2.58	74.34 ± 2.61	1.27 ± 0.02

Table 5. The essential and non-essential amino acid content in cheese-analogue rice cakes

Type of amino acid		Amino acid (mg/100 g sample)	
		Cheese-analogue layered rice cake	Cheese-analogue rice cake (non-layered)
Essential amino acid	Histidine	2.03 ± 0.09	0.92 ± 0.09
	Threonine	3.21 ± 0.07	2.89 ± 0.05
	Lysine	4.84 ± 0.12	4.08 ± 0.07
	Methionine	3.05 ± 0.17	7.17 ± 0.20
	Valine	3.60 ± 0.11	3.57 ± 0.13
	Isoleucine	2.64 ± 0.03	2.47 ± 0.06
	Leucine	5.48 ± 0.10	5.41 ± 0.06
	Phenylalanine	3.23 ± 0.11	3.25 ± 0.08
	Tryptophan	1.63 ± 0.05	1.59 ± 0.01
Non-essential amino acid	Serine	4.96 ± 0.32	4.49 ± 0.12
	Arginine	12.09 ± 0.67	7.51 ± 0.18
	Glycine	3.54 ± 0.10	3.15 ± 0.04
	Aspartic	6.19 ± 0.03	5.61 ± 0.07
	Glutamic	11.22 ± 0.27	9.31 ± 0.32
	Alanine	5.43 ± 0.08	4.25 ± 0.03
	Proline	4.37 ± 0.11	3.93 ± 0.06
	Tyrosine	4.02 ± 0.07	3.69 ± 0.09

Table 6. Comparison of moisture content, water activity, pH and colour of the cakes during shelf-life assessment

Storage duration (month)	Moisture content (%)	Water activity (Aw)	pH	Colour		
				L	a	b
Cheese-analogue layered rice cake						
0	28.41 ± 0.99 [a]	0.88 ± 0.01 [a]	6.28 ± 0.14 [a]	79.04 ± 1.71 [a]	-0.52 ± 0.38 [c]	29.85 ± 1.74 [a]
1	25.80 ± 0.19 [bc]	0.87 ± 0.01 [ab]	5.98 ± 0.08 [b]	79.22 ± 1.89 [a]	-0.86 ± 0.78 [c]	29.42 ± 1.40 [a]
2	26.54 ± 0.60 [b]	0.87 ± 0.02 [b]	6.07 ± 0.06 [b]	79.98 ± 1.43 [a]	5.73 ± 0.73 [b]	27.43 ± 2.03 [ab]
3	25.25 ± 0.61 [c]	0.87 ± 0.00 [ab]	6.22 ± 0.13 [a]	79.53 ± 1.41 [a]	5.01 ± 0.58 [b]	28.26 ± 1.36 [ab]
4	25.21 ± 0.48 [c]	0.87 ± 0.01 [b]	6.09 ± 0.03 [b]	78.68 ± 0.41 [a]	5.46 ± 0.52 [ab]	29.98 ± 1.98 [a]
Cheese-analogue rice cake (non-layered)						
0	35.38 ± 0.28 [a]	0.92 ± 0.01 [a]	6.08 ± 0.05 [b]	82.99 ± 0.88 [a]	-2.28 ± 0.38 [d]	31.95 ± 0.46 [a]
1	33.52 ± 0.72 [c]	0.90 ± 0.00 [b]	6.02 ± 0.03 [bc]	81.84 ± 0.99 [bc]	-1.82 ± 0.50 [d]	31.57 ± 1.06 [a]
2	32.71 ± 0.43 [c]	0.90 ± 0.00 [b]	6.00 ± 0.04 [c]	81.17 ± 1.24 [c]	5.95 ± 0.61 [a]	26.78 ± 0.68 [c]
3	33.54 ± 0.31 [c]	0.92 ± 0.00 [a]	6.03 ± 0.04 [bc]	84.07 ± 1.36 [a]	2.69 ± 0.61 [c]	28.05 ± 0.84 [b]
4	34.49 ± 1.05 [b]	0.92 ± 0.01 [a]	6.15 ± 0.06 [a]	82.51 ± 2.38 [bc]	3.50 ± 0.95 [b]	27.42 ± 0.76 [bc]

** Mean values in the same column with the different letters are significantly different ($p < 0.05$)*

Table 7. Microbiological test of the cakes during shelf-life assessment

Storage duration (month)	Total plate count (cfu/g)	Yeast and molds (cfu/g)	Coliform and *E. coli** (cfu/g)
Cheese-analogue layered rice cake			
0	4.4×10^3	<1.0 x 10	<1.0 x 10
1	4.7×10^3	<1.0 x 10	<1.0 x 10
2	2.1×10^3	<1.0 x 10	<1.0 x 10
3	6.8×10^3	<1.0 x 10	<1.0 x 10
4	2.2×10^4	<1.0 x 10	<1.0 x 10
Cheese-analogue rice cake (non-layered)			
0	2.5×10^2	<1.0 x 10	<1.0 x 10
1	2.0×10^2	<1.0 x 10	<1.0 x 10
2	1.0×10^2	<1.0 x 10	<1.0 x 10
3	<1.0 x 10	<1.0 x 10	<1.0 x 10
4	2.6×10^2	<1.0 x 10	<1.0 x 10

**E. coli not detected*

Table 8. Sensory evaluation of the cakes during shelf-life assessment

Storage duration (month)	Colour	Aroma	Texture	Sweetness	Taste	Overall acceptance
Cheese-analogue layered rice cake						
0	6.15 ± 0.74 [ab]	6.05 ± 0.68 [a]	6.00 ± 0.72 [ab]	5.75 ± 0.91 [a]	5.95 ± 0.60 [ab]	6.10 ± 0.55 [ab]
1	6.54 ± 0.68 [a]	5.90 ± 0.70 [a]	6.36 ± 0.80 [a]	5.72 ± 0.90 [a]	6.27 ± 1.00 [a]	6.27 ± 0.78 [a]
2	6.50 ± 0.52 [a]	5.90 ± 0.31 [a]	6.10 ± 0.73 [ab]	5.70 ± 0.67 [a]	5.90 ± 0.73 [ab]	6.30 ± 0.48 [a]
3	5.73 ± 0.70 [b]	5.06 ± 0.79 [b]	5.66 ± 0.89 [b]	5.53 ± 0.74 [a]	5.40 ± 0.82 [b]	5.53 ± 0.99 [b]
4	5.66 ± 0.89 [b]	4.66 ± 0.89 [b]	5.46 ± 0.63 [b]	5.40 ± 1.05 [a]	4.60 ± 0.98 [c]	4.86 ± 1.06 [c]
Cheese-analogue rice cake (non-layered)						
0	6.10 ± 0.71 [a]	6.00 ± 0.79 [a]	6.40 ± 0.59 [a]	5.70 ± 0.80 [a]	6.25 ± 0.63 [a]	6.40 ± 0.59 [a]
1	6.27 ± 0.78 [a]	5.81 ± 0.98 [a]	6.36 ± 0.92 [a]	5.90 ± 1.22 [a]	6.18 ± 1.16 [a]	6.36 ± 0.80 [a]
2	5.70 ± 0.82 [a]	5.60 ± 0.51 [a]	5.60 ± 0.84 [b]	5.70 ± 0.67 [a]	5.60 ± 0.51 [b]	5.70 ± 0.48 [b]
3	5.93 ± 0.70 [a]	5.40 ± 0.82 [ab]	5.66 ± 0.72 [b]	5.80 ± 0.94 [a]	5.53 ± 0.63 [b]	5.66 ± 0.72 [b]
4	5.86 ± 0.91 [a]	4.80 ± 0.86 [b]	5.66 ± 0.72 [b]	5.66 ± 0.61 [a]	4.93 ± 0.70 [c]	5.06 ± 0.79 [c]

** Mean values in the same column with the different letters are significantly different ($p < 0.05$)*

The slight decrease in moisture content observed in both layered and non-layered rice cakes after three months of storage suggests minimal moisture loss during the storage period. This indicates the effectiveness of packaging and storage conditions in preserving the moisture content of the rice cakes. Additionally, the relatively unchanged values of water activity (A_w) and pH for both cakes indicate the maintenance of their internal environment, which contributes to the stability of the products.

Regarding colour attributes, minor changes in the L, a and b values were observed for non-layered rice cake throughout the storage period, while no significant changes were noted in the L and b colour attributes for layered rice cake. These observations suggest that the colour stability of the rice cakes was maintained during storage, which is crucial for preserving their visual appeal and consumer acceptance.

Microbiological testing revealed no growth of *E. coli* and no increase in yeast and mold in the layered rice cake and non-layered rice cake during the four-month storage period at a chilled temperature. Furthermore, the Total Plate Count remained at 10^2 cfu/g throughout the four months of storage of the non-layered rice cake, while 10^3 cfu/g in the layered rice cake for three months of storage. For comparison, a study by Ying et al (2007) has mentioned that the shelf-life of cake prepared from rice flour and sticky rice flour, stored at 25 °C, without any preservative, was estimated to be only two or three days, as the Total Plate Count in the product reached 10^3 cfu/g on the third day of the storage. The incorporation of a permitted food preservative, potassium sorbate, in the cake formulation in this study (not exceeding 2000 mg/kg) serves as an additional measure to inhibit microbial growth and enhance shelf life. Potassium sorbate is widely recognised for its effectiveness in controlling the growth of molds, yeasts and bacteria in food products, thereby contributing to the preservation of product quality (Kumar et al. 2015). These findings indicate the microbiological safety and stability of both developed rice cakes for up to three months under chilled storage conditions with the addition of food preservatives employed in the study.

However, sensory evaluation results indicated significant differences in scores for colour, aroma, texture, taste and overall acceptance attributes in both layered and non-layered rice cakes after three months of storage. By the fourth month of storage, low minimum scores of 4.0 (neither liked nor disliked) were obtained for aroma and taste attributes. The decline in sensory attributes suggests a deterioration in product quality over time, despite remaining within acceptable sensory limits during the earlier months of storage.

The observed decline in sensory attributes over the storage period highlights the importance of monitoring product quality throughout its shelf life. While the rice cakes remained acceptable for consumption within the first three months of storage, the decline in sensory scores beyond this period suggests a limited shelf life not exceeding three months.

The rice cake was wrapped with PVC plastic or stretch wrap, serving as a primary barrier against physical damage and moisture loss. Subsequently, the wrapped cake was placed into airtight plastic packaging composed of oriented Nylon/LLDPE material. Oriented Nylon/LLDPE packaging offers excellent barrier properties, including resistance to moisture and oxygen transmission, thereby preserving the freshness and quality of the enclosed products (Chen et al. 2020). The complete sealing of the oriented Nylon/LLDPE packaging plastic ensures a hermetic seal, preventing air (oxygen) ingress and maintaining an anaerobic environment within the package (Oluwadara et al. 2022). This is crucial for inhibiting the growth of aerobic microorganisms and preserving the sensory attributes of the rice cakes over an extended storage period. Additionally, the use of oxygen absorbers in the rice cake packaging reduces the oxidative deterioration of the product and inhibits the growth of aerobic spoilage organisms (Cichello 2015).

Conclusion

The study's findings demonstrate the feasibility of creating cheese-analogue cakes using optimised formulations and processing parameters, presenting a promising alternative to traditional dairy-based cakes. By utilising rice-based ingredients, particularly rice flour and fermented rice, these cakes offer a distinct cheese-like flavour and aroma. Despite the absence of actual cheese, these cakes possess a unique cheese-like flavour and aroma profile. Sensory evaluations highlight superior attributes of the formulated cheese analogue cakes, indicating higher scores for cheese aroma, texture, sweetness and overall acceptability compared to commercial cheesecakes. This underscores the potential of rice-based formulations to meet consumer preferences and the growing demand for sustainable and environmentally friendly food choices while offering nutritional benefits. The developed rice cheese-analogue cakes have a shelf life of up to three months when stored under chilled conditions with added food preservatives. The successful development of these cakes not only diversifies bakery products but also promotes the use of local resources and stimulates innovation in the food processing industry.

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References

AOAC (2023). *Official Methods of Analysis of AOAC International*. (22nd Edition). Association of Official Analytical Chemists Inc.

AOAC (1990). *Official Methods of Analysis*. (15th Edition). Washington, D.C.: Association of Official Analytical Chemists.

AOAC (1993). *Official Methods of Analysis for Nutritional Labelling*. Washington, D.C.: AOAC International.

Bedale, W. (2020). *General Overview of Plant-based Cheeses*. Food Research Institute, University of Wisconsin-Madison.

Castanho, A. (2023). Improving γ-Oryzanol and γ-Aminobutyric acid contents in rice beverage produced with brown, milled and germinated rices. *Foods*, *12*(7), 1476. https://doi.org/10.3390%2Ffoods12071476.

Chen, Y., Chen, H., Lin, S., Huang, S. & Yu, J. (2020). Oriented nylon films with high mechanical and barrier properties for food packaging applications. *Food Chemistry*, *305*, article number 125451.

Cichello, S.A. (2015). Oxygen Absorbers in Food Preservation: A Review. *Food Science and Technology*, *52*(4), 1889 – 1895. https://doi.org/10.1007/s13197-014-1265-2.

Harold, E. (1981). *Pearson's Chemical Analysis of Food 8th Ed. (1981)*. Churchill Livingstone, New York.

Hans-Peter, B. (2001). Cheese analogues: a review. *International Dairy Journal, 11*, 505-515.

Jan, N., Naik, H. R., Gani, G., Bashir, O., Hussain, S. Z., Rather, A. H., Zargar, I. A., Mohd Wani, S. & Amin, T. (2021). Optimization of process for the development of rice flour incorporated low-gluten wheat based pretzels: Evaluation of its physicochemical, thermal and textural characteristics. *Journal of the Saudi Society of Agricultural Sciences, 20*(2), 116-127. https://doi.org/10.1016/j.jssas.2020.12.007.

Kath, W. W. & Jilian, K. (2023). Essential Amino Acids: Definition, benefits and food sources. *Healthline*. Retrieved on 20.02.2024 from https://www.healthline.com/nutrition/essential-amino-acids.

Koh, S. P., Jamaluddin, A., Alitheen, N. B., Mohd-Ali, N., Mohd Yusoff, H. & Long, K. (2012). Nutritional value of tempe inoculated with different strains of Rhizopus: its γ-aminobutyric acid content and antioxidant property. *Journal of Tropical Agriculture and Food Science*, *40*(2), 181-192.

Kumar, D. P., Jayanthi, M., Saranraj, P. & Karunya, K.S. (2015). Effect of potassium sorbate on the inhibition of growth of fungi isolated from spoiled bakery products. *Life Science Archives*, *1*(4), 217-222.

Nicholas, D., Chua, H. P., Mejelan, S., Teresa, A. M., Hazijah, M. H., Lorne, K. & Zakaria, A. R. (2015). Penggunaan bertih beras tradisional Sarawak dalam penghasilan bar granola (The use of Sarawak's traditional puffed rice in the production of granola bar) *Buletin Teknologi MARDI*, *7*, 33-42.

Nicholas, D., Chua, H.P., Siah, W.M. & Koh, S.P. (2023). Application of gelling agent in particle suspension beverage. *Journal of Tropical Agriculture and Food Science*, *51*(1), 15-20.

Nicholas, D., Chua, H.P., Mejelan, S., Teresa, A.M., Hazijah, M.H., Zakaria, A.R. & Lorne, K. (2014). Penggunaan tepung beras dalam penghasilan kek lapis Sarawak (The use of rice flour in the making of Sarawak layer cakes). *Buletin Teknologi MARDI*, *6*(2014), 1-7.

Oluwadara, A., Olumide, A.O., Mariyana, S. & Deyan, S. (2022). Microbial spoilage of vegetables, fruits and cereals. *Applied Food Research*, *2*(1), 200122. https://doi.org/ 10.1016/j.afres.2022.100122.

Orthoefer, F.T. & Kim, D. (2019). Applications of emulsifiers in baked foods. *Food Emulsifiers and Their Applications*. (Third Edition). https://doi.org/10.1007/978-3-030-29187-7_10.

Poste, L.M., Mackie, D.A., Butler, G. & Larmond, E. (1991). *Laboratory Methods for Sensory Evaluation of Food. Publication 1864/E*. Canada Department of Agriculture. Retrieved on 20.11.2023 from https://dn790000.ca.archive.org/0/items/laboratorymethod00otta/laboratorymethod00otta.pdf.

Saiyavit, V. & Sujin, S. (2000). Comparative properties of cakes prepared from rice flour and wheat flour. *European Food Research and Technology*, *211*(2), 117-120. https://doi.org/10.1007/s002179900124.

Sharma, P., Gujral, H. S., & Singh, B. (2012). Antioxidant and polyphenol oxidase activity of germinated barley and its milling fractions. *Food Chemistry*, *134*(2), 742-748. https://doi.org/10.1016/j.foodchem.2009.10.059.

Stauffer, C.E. (2005). Emulsifiers for the food industry. *In book: Bailey's Industrial Oil and Fat Products*. https://doi.org/10.1002/047167849X.bio080.

Yeoh, Q.L., Nor Azmi, M.A., Normah, O. & Normah, A. (1992). *Penghasilan makanan peraman terpilih.* Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI).

Yeoh, Q.L. & Merican, Z. (1986). Innovations in traditional fermented foods. *Teknologi Makanan, MARDI*, *5*(2), 133-138.

Ying, Ji., Zhu, K., Qian, H.F. & Zhou, H.M. (2007). Microbiological characteristics of cake prepared from rice four and sticky rice flour. *Food Control*, *18*(12), 1507 – 1511. http://dx.doi.org/10.1016/j.foodcont.2006.11.005.