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SHELF-LIFE AND BIO-FUNCTIONAL ATTRIBUTES OF TRADITIONAL RICE CAKES OF THE KHASI AND JAINTIA TRIBES OF MEGHALAYA, INDIA

Bethsheba **Basaiawmoit**[1], Birendra Kumar **Mishra**[1]*, Subrota **Hati**[2]
[1]Department of Rural Development and Agricultural Production, North-Eastern Hill University, Tura Campus, Chasingre-794002, Meghalaya, India
[2]Department of Dairy Microbiology, Kamdhenu University, Anand-388110, Gujarat, India
*Email: birendramishra14@gmail.com

Abstract
Rice cakes are a traditional delicacy of the ethnic tribes in Meghalaya. In the current study, three popular rice cakes, viz., Putharo, Pusla, and Pusyep, have been evaluated for shelf-life and bio-functional study for 1, 3, and 5 days. Pusyep rice cake had higher pH on day 1 & 5, protein content on day 1, produced more vitamin B_2 on day 3, and exhibited antimicrobial activity against S. aureus, E. coli, and S. typhi. Putharo rice cakes have higher pH on day 3, protein content on days 3 & 5, produce the most vitamin B_2 on days 1 and 5, and have yeast and mould count on all three storage days. The Pusla rice cake had the highest total titratable acidity, carbohydrate content, and antioxidative activity of all storage days. Pusla and Putharo produced the most acetic acid on day 3. The coliform count was absent for all rice cake types. According to our findings, personal hygiene education and safety management during rice cake preparation are required to ensure hygienic conditions and to prevent microbial contamination of the rice cakes caused by the equipment used. The current study provides information on the bio-functional properties, microbial and physico-chemical analysis of three traditional rice cakes, which can aid in the improvement of our dietary strategy for individual health management.

Keywords: antimicrobial, antioxidative, *Pusla*, *Pusyep*, *Putharo*, shelf-life

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1. INTRODUCTION

Oryza sativa, or rice, is the second common cereal crop and a staple food in the world. It is a member of the Gramineae family and the Oryzoides subfamily (Uma Devi 2012). Rice-based foods are a popular custom for major tribal people in India, mainly in the north-eastern region, where they have been preparing them for centuries. The vast majority of tribes prepare rice cakes at home with locally sourced ingredients (Das and Deka 2012). Rice improves the nutrient density and vigor of the everyday diet. Rice and rice-based cuisine have long been a staple of Indian cuisine. In the fields of study, research, investigation, and scientific credentials, the indigenous knowledge of tribal communities has been granted due weight (Ray et al. 2016). It has been discovered that rice flour is among the best flours for preparing celiac patients' foods because of its distinctive taste, dietary values, and hypoallergenic properties. Therefore, its use in the making of puddings and baby foods is growing (Gujral and Rosell 2004). The dietary habits of many of the tribes and communities are mainly rice-based in northeast India, and rice is the most common food for the majority of them (Das and Deka 2012). Rice cakes are naturally fermented, traditional cereal-based items that are commonly consumed as food items in India (Agrawal et al. 2000). In comparison to raw unfermented ingredients, they contribute significantly to nutrition as vitamin resources, particularly Vitamin B-complex, calories, and protein (Srilakshmi 2003). Rice cakes, locally known as "*ki kpu*" in the Khasi & Jaintia hills, come in a variety of flavors and are made using traditional techniques (Umdor et al. 2016). Rice cakes have a high status in Meghalaya, where they are an important part of social rites, rituals, and socio-cultural life. They are commonly consumed during festivals and provide a source of income for many people. Rice-based snacks are inexpensive and nutritious, as they contain very little oil or sugar (Agrahar-Murugkar and Subbulakshmi 2005). The rice and its fermented products have sparked global interest because of their superiority features, specific caloric values, and higher adequacy (Steinkraus 1994). This research looked into the shelf-life analysis of three popular rice cakes from Meghalaya, viz., *Putharo*, *Pusla*, and *Pusyep* rice, and their bio-functional analysis was investigated.

2. MATERIALS AND METHODS

Procurement of raw materials, reagents and chemicals

Raw materials such as *Mynri* rice (local variety), black sticky rice, jaggery, and baking soda were obtained from the local market. The High-Profile Liquid Chromatography (HPLC) standards for Short-Chain Fatty Acid (SCFA) (acetate, butyrate, and propionate), and Vitamin (B_2 and B_{12}) were derived from Sigma-Aldrich, India. The vitamin production media and other selective media were obtained from HiMedia, India.

Preparation of rice cakes

The *Putharo* (PT), *Pusla* (PSL), and *Pusyep* (PSY) rice cakes were made using a local variety of *Mynri* rice (500 grams) and black sticky rice (30 grams) for each rice cake. The rice was soaked separately in water for an hour. The water was discarded and the rice was ground into powder with the help of a mixer grinder.

For the preparation of the PT rice cake, 250 ml of warm water was poured onto the powdered rice and mixed well until a uniform paste was achieved. A spoonful of the paste was spooned into a hot burning "*saraw*" (traditional round flat earthen baking pot), which was then sealed with a *saraw* lid and baked for 2 minutes. For the PSL rice cake, the powdered rice was combined with a 150 ml concoction of melted jaggery (150 grams), tea leaves (5 grams) and water (to achieve the desired color) along with 1 gram of baking soda. The mixture was kneaded until a smooth dough was formed. The dough was split into small balls. It was then stored and packed tightly in *Sla met* leaves (local name)/*Phyrnium pubinerves*. Each packet was transferred into an aluminum pot and warm water was poured over it till it covered all the packets inside. It was sealed with a lid and cooked for 20 minutes. The PSY rice cake was prepared by moistening the powdered rice by sprinkling it with 50 ml of cold water. The rice was sieved through a traditional bamboo sieve to remove any clumps, and left for 6 hours for fermentation before use. To achieve the desired shape, the moistened rice was transferred to a small flat-base aluminum bowl of 7 cm in diameter and covered with a sterile muslin cloth. The covered bowl was transferred and placed upside down on top of a round, narrow-mouthed aluminum pot filled with boiling water and steamed for 2 minutes. (Umdor et al. 2016)

Sensory Evaluation

The sensory evaluations were carried out by a panel of ten people, which consisted of students from the age group between 21-25 years from the Rural Development and Agricultural Production (RDAP) department, North-Eastern Hill University (NEHU), who are primarily from the Khasi and Jaintia Hills districts and are familiar with traditional rice cakes as part of their diet. They were given written instructions and asked to rate the three rice cake samples for taste, color, appearance, odor, flavor, texture, tenderness, and overall liking (Gacula and Singh 1984) using a nine-point hedonic scale (0= Dislike extremely to 9= Like extremely). Three rice cakes were sensory evaluated and kept for up to 5 days at 6°C in the refrigerator and 33°C at room temperature.

Physicochemical analysis

According to AOAC, 1990, the pH, total titratable acidity, total protein content, and total carbohydrate content of the samples were determined.

Determination of acidity

Using phenolphthalein as an indicator, 10 g of batter was disseminated in distilled water (100 ml) and titrated with 0.1 N NaOH until a light pink colour was observed. The pH of the rice cake batter was measured using a pH metre (Hanna instruments, Model HI96107) at 1, 3, and 5 days.

Microbial analysis

The experiment was carried out based on the technique given by (Thokchom and Joshi 2012) with some modifications. In a sterile mortar and pestle, 10 grams of rice cake sample was mixed thoroughly & treated with sterilized distilled water (90 mL). After that, a 1 mL liquid sample was taken from this solution and thoroughly mixed with 4 mL of sterile 0.1% PBS solution, followed by serial dilutions. The sample was diluted from 10^{-1}-10^{-4} dilutions. A 200 µL of the sample was taken and plated on Violet Red Bile Agar (VRBA) (M049, HiMedia, India), & Peptone Yeast Dextrose Agar (YPDA) (M671, HiMedia, India), for the coliform count and yeast & mold count respectively. VRBA plates were incubated at 37 ± 1 °C for 24 h & YPDA plates were incubated at 32 ± 1 °C for 48 h. This was carried out on the 1st, 3rd, and 5th days of the sample storage study. Each dilution was repeated five times, and all plate counts were expressed as log CFU/ml.

Estimation of protein and carbohydrate content

The protein content was determined using the Biuret method with some modifications. One ml of sample was blended with 3 ml of Biuret reagent. The samples were kept at room temperature for 10 minutes in the dark. An Ultra-Violet (UV) spectrophotometer was used to measure the absorbance at 540 nm and a standard curve of Bovine Serum Albumin (BSA) was used to calculate protein content. The Anthrone method was used to determine the carbohydrate content of rice cakes. Anthrone (200 mg) was dissolved in 100 ml of 95 % v/v H_2SO_4. In a test tube, 1 mL of sample was mixed with 4 mL of Anthrone reagent. Following that, the mixture was incubated in an 80 °C water bath for 10 minutes and further cooled to room temperature. The absorbance was measured using a UV spectrophotometer at 630 nm, and the carbohydrate content was calculated using a glucose standard curve.

Bio-functional analysis

Antimicrobial Activity

Agar well diffusion assay techniques were used to determine the antimicrobial activity (Hati et al. 2014) and it was assessed against six test organisms; *Bacillus cereus* ATCC 14459, *Staphylococcus aureus* MTCC 114, *Listeria monocytogenes* and *Enterococcus faecalis* NCDC 115, *Salmonella typhi* NCTC 5017, and *Escherichia coli* ATCC 25922, collected from Department of Dairy Microbiology, SMC College of Dairy Science, Kamdhenu University, Anand, Gujarat.

Antioxidative Activity

The antioxidative activity of rice cakes was based on their ability to scavenge 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (Hati et al. 2013), and it was calculated by determining the decrease in absorbance at different concentrations and using the following equation:

$$E = [(Ac-At)/A] \times 100$$

where At = absorbance of tested samples and Ac = the absorbance of ABTS radical respectively.

Determination of SCFA production

SCFA was measured using HPLC according to the procedure given by LeBlanc et al. (2017) with a few modifications. Rice cake samples were finely homogenized. 5 ml of the sample was blended with 45 ml of water and vortexed for 5 min. The samples were then passed through Whatman paper no. 42. Before injecting into the HPLC system, the samples were passed through a 0.45 µ syringe filter. For use in HPLC testing, the column was washed twice with phosphoric acid (0.01%) to remove contaminants and salts. Once the cartridge had been washed, organic acid was eluted using phosphoric acid (0.01%) at a 0.5 ml per minute flow rate, and the oven temperature was kept at 40 °C. The absorbance of eluting was observed at 210 nm.

Determination of Vitamin B_2 and B_{12} production

The determination of Vitamin B_2 and B_{12} was studied and quantified by HPLC analysis as illustrated by Taranto et al. (2003) with few modifications. 5 ml of rice cake samples were finely homogenized and blended with water (45 ml). For 1 minute, it was vortexed vigorously and further centrifuged for 10 minutes at 10,000 × g. The supernatant was then passed via a syringe filter of 0.45-µ. Using HPLC, the supernatant was studied further for the production of Vitamin B_2 & B_{12} in rice cakes. Employing a microinjector, a filtrate of 20 µl was injected into the HPLC system (Shimadzu, LC-10, Japan) for assessment. An RP18 end-capped column, a 40 × 4 mm Guard column & a 250 × 4.6 mm LiChroCART column (Chromolith-Merck), were fitted to an isocratic HPLC system. Employing a Hilton injector with a 20 µl loop, the sample was loaded into the system. For 2 hours, the salts & other contaminants from the column were washed twice using distilled water and the cartridge was washed using water. Keeping the oven temperature at 40°C, acetonitrile (50%) with a 0.3 ml per minute flow rate, cobalamin was eluted. Employing a UV detector at 284 nanometers, the absorbance of eluting was monitored.

Statistical analysis

One-way Analysis of Variance (ANOVA) was employed and evaluation was made using the Scheffe test, taking $p \leq 0.05$ as the least significant difference by applying IBM SPSS Ver. 20 Statistical Program and Origin Ver. 9.0 was used for graphical presentation. The findings were stated as M ± SD. The values depicted in the research were a mean of five individual replicates.

3. RESULTS AND DISCUSSION

Sensory evaluation

Since ancient times, the tribal people of Meghalaya have been important specialists in ethnic skills in the preparation of rice-source diets (Umdor et al. 2016). The sensory analysis of the rice cakes stored at 6°C revealed that as the storage days passed, the sensory score provided by the panelist decreased, with appearance values ranging from (7.66-5.50), (8.0-5.26), (7.40-5.26), color (7.83-5.66), (7.80-5.50), (7.60-5.50), odor (7.20-5.70), (8.20-7.0), (7.80-6.50), taste (7.20-5.26), (7.40-5.0), (7.60-5.0), flavor (7.60-5.60), (8.60-5.26), (7.40-5.0), texture (7.60-5.26), (7.40-5.26), (7.80-5.50), tenderness (7.60-5.76), (7.0-5.26), (7.40-5.26) and overall liking (7.40-5.26), (7.60-5.76), (7.0-5.50) for PT, PSL, and PSY rice cakes respectively. The rice cakes, stored at 33°C, were discarded by the panellist on the third day as the product became stale and spoiled with a rancid off-odour and off-taste. As a result, since the panelists favored the rice cake sample stored at 6°C, it was carried forward for further analysis; the panelists' overall sensory score was PSL > PT > PSY. However, because each rice cake has its own distinct preparation and flavor, they cannot be compared and must be judged on an individual basis. The sensory evaluation of PT, PSY, and PSL rice cakes was found to vary significantly from one another at a 5% ($p \leq 0.05$) level. The product was acceptable due to the humble culinary procedure of baking, steaming, & boiling with few components (Umdor et al. 2016). Studies done by Sumnu et al. (2009) revealed that the gluten-free rice cakes showed different sensory scores when cooked in different methods and ovens used, which was similar to our studies for the different rice cakes. Other studies conducted by Chuang and Yeh (2006), found that the sensory characteristics of *mochi* (oriental rice cake of China, Japan, and Taiwan) differed depending on the addition of ingredients and that each panelist's preference test produced different results. It has been observed that the sensory scores are declining as the number of storage days progresses. The shelf-life analysis was completed until the 5^{th} day of storage as the panelists rejected the rice cake samples on subsequent days. Therefore, storing the samples after day 5 comes with a rancid off-odor and off-taste, which also corresponds to the findings of Manaois et al. (2013). Ji et al. (2007) discovered similar results in their *MiGao* study (Chinese steamed cake), where the cake after only three days became completely spoiled in storage. The rice cake's water and moisture content may play a vital role in bacterial progression. (Table 1)

Table 1: Sensory evaluation of rice cake samples

Parameters	Storage days	Storage temp. (^{0}C)	Rice cake samples		
			Putharo (PT)	*Pusla* (PSL)	*Pusyep* (PSY)
Appearance	1	6	7.66 ± 1.040^{a}	$8.00 \pm 0.000^{\alpha}$	7.40 ± 0.529^{A}
		33	6.83±0.577	7.00±0.000	6.00±0.000
	3	6	6.50 ± 1.500^{b}	$7.80 \pm 0.721^{\beta}$	6.00 ± 0.000^{B}
		33	-	-	-
	5	6	5.50 ± 1.500^{c}	$5.26 \pm 0.461^{\gamma}$	5.26 ± 0.461^{C}
		33	-	-	-
Colour	1	6	7.83 ± 0.763^{d}	$7.80 \pm 0.721^{\delta}$	7.60 ± 0.529^{D}
		33	6.53±0.577	6.40±0.360	6.20±0.346
	3	6	6.76 ± 1.123^{e}	$6.73 \pm 0.461^{\varepsilon}$	6.26 ± 0.461^{E}
		33	-	-	-
	5	6	5.66 ± 0.763^{f}	$5.50 \pm 0.50^{\zeta}$	5.50 ± 0.500^{F}
		33	-	-	-
Odour	1	6	7.20 ± 0.346^{g}	$8.20 \pm 0.346^{\eta}$	7.80 ± 0.346^{G}
		33	6.66±0.288	6.00±0.000	6.80±0.346
	3	6	6.26 ± 0.251^{h}	$7.00 \pm 0.000^{\theta}$	6.50 ± 0.000^{H}
		33	-	-	-
	5	6	5.70 ± 0.754^{i}	$5.26 \pm 0.461^{\iota}$	5.00 ± 0.000^{I}
		33	-	-	-
Taste	1	6	7.20 ± 0.346^{j}	$7.40 \pm 0.529^{\kappa}$	7.60 ± 0.529^{J}
		33	6.66±0.288	6.80±0.346	6.20±0.346
	3	6	6.76 ± 0.642^{k}	$7.00 \pm 0.000^{\lambda}$	6.26 ± 0.251^{K}
		33	-	-	-
	5	6	5.26 ± 0.461^{l}	$5.00 \pm 0.000^{\mu}$	5.00 ± 0.000^{L}
		33	-	-	-
Flavour	1	6	7.60 ± 0.000^{m}	$8.60 \pm 0.529^{\nu}$	7.40 ± 0.346^{M}
		33	6.60±0.173	6.00±0.000	6.40±0.529
	3	6	6.76 ± 0.642^{n}	$7.26 \pm 0.461^{\xi}$	6.26 ± 0.230^{N}
		33	-	-	-
	5	6	5.60 ± 0.173^{o}	5.26 ± 0.461^{o}	5.00 ± 0.000^{O}
		33	-	-	-
Texture	1	6	7.60 ± 0.529^{p}	$7.40 \pm 0.529^{\pi}$	7.80 ± 0.200^{P}
		33	6.60±0.173	6.20±0.346	6.80±0.346
	3	6	6.50 ± 0.500^{q}	$6.26 \pm 0.461^{\rho}$	6.50 ± 0.500^{Q}
		33	-	-	-
	5	6	5.26 ± 0.251^{r}	$5.26 \pm 0.461^{\varsigma}$	5.50 ± 0.000^{R}
		33	-	-	-
Tenderness	1	6	7.60 ± 0.529^{s}	$7.00 \pm 0.000^{\sigma}$	7.40 ± 0.000^{S}
		33	6.60±0.173	6.40±0.360	6.20±0.346
	3	6	6.26 ± 0.461^{t}	$6.00 \pm 0.000^{\tau}$	6.00 ± 0.000^{T}
		33	-	-	-
	5	6	5.76 ± 0.251^{u}	$5.26 \pm 0.251^{\upsilon}$	5.26 ± 0.251^{U}
		33	-	-	-
Overall liking	1	6	7.40 ± 0.529^{v}	$7.60 \pm 0.529^{\varphi}$	7.00 ± 0.000^{V}
		33	6.80±0.200	6.90±0.173	6.40±0.360
	3	6	6.76 ± 0.251^{w}	$6.50 \pm 0.500^{\chi}$	6.76 ± 0.208^{W}
		33	-	-	-
	5	6	5.26 ± 0.461^{x}	$5.76 \pm 0.404^{\psi}$	5.50 ± 0.500^{X}
		33	-	-	-

Data are mean ± SD of ten replicates (n = 10) for each observation. '-': discarded samples. Data with separate superscripts were assigned to illustrate statistically significant differences from each other using Scheffe's test with a p-value of 0.05.

Physico-chemical analysis
Determination of acidity

PT, PSY, & PSL rice cake pH was observed to decline from (6.622 to 6.296), (6.674 to 6.446), and (6.414 to 6.150) respectively. Furthermore, as the number of days rose, the rice cake pH was found to decline in order. Rice cakes' acidity has a major impact on shelf life, taste, flavor, and overall product acceptance. The titratable acidity in PT and PSY remains constant for the entire storage period, whereas the PSL rice cake increases bit by bit as the number of storage days is enhanced. PSL rice cakes accounted for the highest titratable acidity ranges from (0.027 to 0.054), while PSY rice cakes reported the lowest titratable acidity on all three days. The pH and titratable acidity of PT, PSY, and PSL rice cakes were found to vary notably from one another at a 5% ($p \leq 0.05$) level (Table 2). A decreased pH and an increase in titratable acidity were observed during the storage study of different rice cakes. The rise and fall of pH levels also show an important role in the growth of certain pathogens, this statement is follows Lee et al. (2006) findings, where pH levels have been shown to influence microorganism growth, and the minimum pH value for *B. cereus* growth was discovered to be 4.35 (Raevuari and Genigeorgis 1975). According to Choi et al. (2013), in the analysis of *Jeolpyeon* (Korean rice cake), increasing total titratable acidity ranging between 0.05-0.20 % and decreasing pH ranging between 6.5-5.1 play a beneficial role in inhibiting fungal development and prolonging shelf-life. Studies done by Park et al. (2016) have found the pH of rice cake to be 5.73. Other related findings were achieved by Navarro et al. (1991), for Philippine fermented rice cake (*puto*), in which a decline in pH & total titratable acidity in rice cake have been observed. In research conducted by Rani et al. (2019) on the fermentation of idli batter, there was a marked increase in titratable acidity and a fall in pH, which may be associated with the existence of lactic acid bacteria (LAB) in the course of fermentation. The pH value of rice cake was discovered to decrease as the boiling time increased, which was following PSL rice cake in this study. Gernah et al. (2012) stated a related discovery in the study of *Zogale*, showing confirmation of a steady decline in pH with boiling interval, leading to acidity. This may be as a result of the leaching of alkaline constituents such as minerals and proteins in boiling water. (Table 2).

Microbial analysis

Microbial analysis was performed on Yeast and Mould (Y & M) and the coliform count.

Table 2: pH & total titratable acidity and microbial analysis of rice cake samples.

Parameters	Storage periods (days)	*Putharo* (PT)	*Pusyep* (PSY)	*Pusla* (PSL)
pH	1	6.622 ± 0.011[a]	6.674 ± 0.029[d]	6.414 ± 0.030[g]
	3	6.542 ± 0.045[b]	6.470 ± 0.073[e]	6.302 ± 0.048[h]
	5	6.296 ± 0.056[c]	6.446 ± 0.035[f]	6.150 ± 0.025[i]
TTA (% acid)	1	0.018[a]	0.009[b]	0.027[c]
	3	0.018[a]	0.009[b]	0.036[d]
	5	0.018[a]	0.009[b]	0.054[e]
YPDA (log CFU/ml)	1	2.218±1.247[a]	Absent in 1ml	Absent in 1ml
	3	3.878±0.447[b]	Absent in 1ml	Absent in 1ml
	5	3.295±0.078[a, b]	3.698±0.030[c]	2.218±1.247[d]
VRBA (log CFU/ml)	1	Absent in 1ml	Absent in 1ml	Absent in 1ml
	3	Absent in 1ml	Absent in 1ml	Absent in 1ml
	5	Absent in 1ml	Absent in 1ml	Absent in 1ml

Values were denoted as mean±SD from five replicates (n=5) for individual observation. Values with different superscripts were assigned to illustrate statistically significant differences from each other by Scheffe's test at $p \leq 0.05$; TTA-Total Titratable Acidity

The Y & M count of PT rice cake was observed to vary significantly between day 1 (2.218 log CFU/ml) & day 3 (3.878 log cfu/ml) at 5% ($p \leq 0.05$), while day 5 (3.295 log cfu/ml) reported a midway Y & M count without differing significantly from either of days 1 & 3; whereas, the Y & M for PSY and PSL rice cake was not detected for day 1 & day 3 except for day 5 (3.698 & 2.218 log cfu/ml) respectively.The coliform count was not detected in any of the three types of rice cakes for a shelf-life period of 5 days. The Y & M count of PT, PSY, and PSL rice cakes was found to vary significantly by 5% ($p \leq 0.05$) from each other. Day 5 showed mixed results; PSL & PSY differed appreciably, whereas PT had reported in-between results. In the study, only PT rice cake documented the Y & M count on days 1 & 3, while on day 5, PSY rice cake reported the highest Y & M count, and PSL rice cakes reported the lowest. Overall, the Y & M count reached its peak on day 3 (3.878 log cfu/ml for PT rice cakes). In an analysis of Korea's rice cakes, *Sirutteok, Garaetteok*, and *Gyeongdan*, Jeong et al. (2012) discovered that the microbial contamination was, Not detected (ND)-1.9, 2.4-4.5, 1.0-2.1, and1.2-2.1 log CFU/g for coliforms, total aerobic bacteria, *B. cereus*, and fungi respectively. Microbial contamination of rice cakes was found to multiply at the time of soaking & grinding processes. However, during the steaming process, they declined. Another study done by Wang et al. (2016) discovered that the particular microbial degrees of yeast and mould, *B. cereus*, & coliforms of Korean rice cakes were in the range of ND (Not detected) to 2.7, ND-3.7, & ND-3.6 log CFU/g respectively, for the full duration of rice cake processing, which follows our findings. Sang et al. (2015) found comparable results in their study of (*NianGao*), a traditional Chinese rice cake, where the Y & M count was observed to increase slowly (range of 1.0-2.87 log cfu/g for a storage period of 90 days) and remained low, just as for PSY & PSL rice cakes in this study, indicating that there is a lag period for bacteria to adapt to their new surroundings, which was also in agreement with Ji et al. (2007) in their study of the microbiological characteristics of rice cakes. (Table 2)

Estimation of protein and carbohydrate content

The protein content in PT rice cake was observed to be significantly higher at 5% level ($p \leq 0.05$) on day 1 (5.667 mg/ml) and day 3 (5.017 mg/ml) in comparison to day 5 (3.861 mg/ml). Alternatively, protein content in PSY and PSL rice cake was observed to be significantly higher at 5% ($p \leq 0.05$) level for day 1 (8.413 and 6.597) than on day 3 (3.166 and 2.083) and day 5 (2.775 and 1.934) respectively. In all experimental setups, PSY day 1 reported the highest protein concentration value (8.413 mg/ml) and it declined as the number of days advanced. The carbohydrate content in PT and PSY rice cakes was observed to vary at a 5% ($p \leq 0.05$) significant level on each day; day 1 recorded the highest carbohydrate content; whereas, the carbohydrate concentration for PSL rice cakes did not differ significantly at a 5% ($p \leq 0.05$) level for different days. On Day 3, PSL (16.671 mg/ml) and PSY (13.904 mg/ml) recorded the highest and lowest carbohydrate content, while on day 5, PSL (16.492 mg/ml) reported significantly higher carbohydrate content than PT & PSY. The protein and carbohydrate content of PT, PSY, and PSL rice cakes were found to vary at a 5% ($p \leq 0.05$) significant level from one another, and were observed to decrease with shelf-life advancement. The protein content is identified to have a major effect on batter & finishing results (Wilderjans et al. 2010). Research conducted by Mohamed and Hamid (1998), found that the effects of various ingredients were observed in the increase in protein concentrations of steamed leavened rice cakes by using skimmed milk powder and egg white. Park et al. (2016) discovered similar results when preparing a South Korean rice cake with 3.75 g (g/100g) of protein content. Navarro et al. (1991) also reported comparable findings. Lee and Ryu (1992) investigated the protein content of processed *jeung-pyun* (Korean fermented rice cake) and discovered that it varied depending on the ingredients, fermentation time, temperature, and cooking time, which followed our findings. PSL rice cakes illustrated higher carbohydrate content in all settings. This may be due to the addition of jaggery to the rice cake. Moreover, jaggery contains 95 g per 100 g of carbohydrate (Srilakshmi 2003). Similar studies have been found by Kim and Chung (2007) in the study of *Karedduk*, the rice cake of Korea, for the addition of carbohydrate sources. PSL rice cake prepared by packing in *Phyrnium pubinerves* leaves and boiling for 20 minutes may affect carbohydrate concentration. Related findings have been described by Gernah et al. (2012), in which *Zogale* (prepared in Nigeria's northern regions by mixing peanut cake (*kulikuli*) and boiled *Moringa oleifera* leaf) boiled for 20 minutes had a higher carbohydrate value of 5.54 % in comparison to other products boiled for 5, 10, and 15 minutes. (Figure 1 and 2).

Bio-functional properties of rice cake

Antimicrobial Activity

In PT and PSL rice cakes, antimicrobial activity was absent against all the test organisms as evident from Table 3; whereas, PSY rice cake showed antimicrobial activity against *E. coli* (18.00 mm), *S. aureus* (18.00 mm), and *S. typhi* (18.00 mm) for day 1. The day 3 and day 5 antimicrobial activity of PSY rice cake was significantly lower compared to day 1. Several previous studies have revealed that steam cooking rice cakes can reduce the number of foodborne pathogenic bacteria by 0.6 log CFU/g, as well as disintegrate spores (Lee et al. 2006; Okahisa et al. 2008).

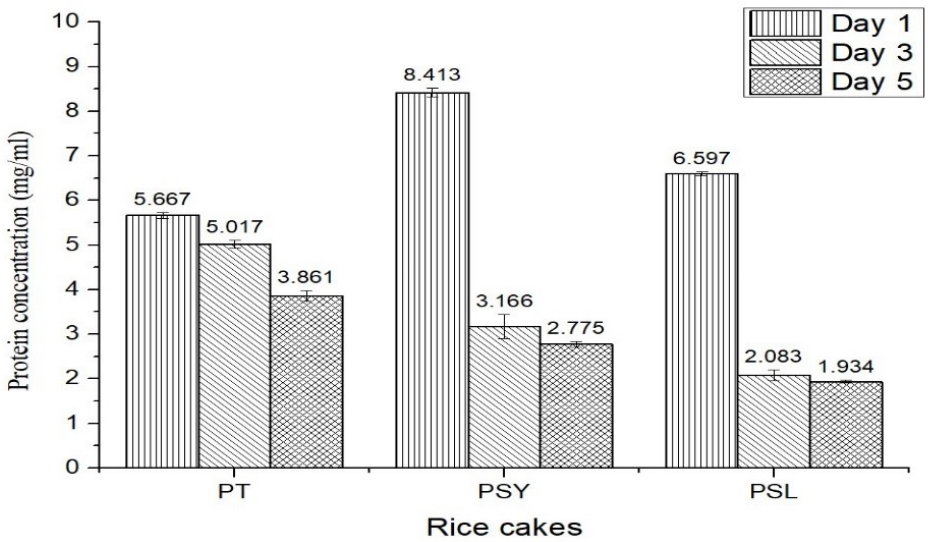

Fig. 1. Protein concentration of rice cakes

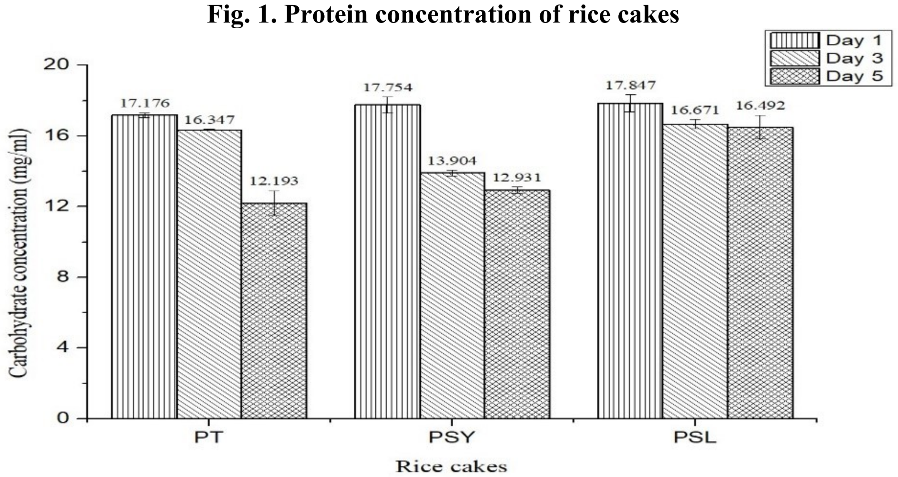

Fig. 2. Carbohydrate concentration of rice cakes

Related findings were found in the analysis of PSY, which is a steam rice cake, which showed antimicrobial activity against *S. aureus* in a range of (15 mm to 18 mm), *E. coli* (17.40 mm to 18 mm), and *S. typhi* (15.20 mm to 18 mm) during the shelf-life study, in which the test organisms were found to be susceptible to steam cooking and can effectively minimize the growth of foodborne pathogens. Okahisa et al. (2008) also reported that heat-resistant bacterial endospores could withstand the steaming of rice cakes. This may be because post-processing measures such as packaging or food handlers may reintroduce microorganisms into the items. The optimal pH for *B. cereus* growth was discovered to be 4.35 (Raevuori and Genigeorgis 1975). Gram-positive microorganisms, for instance, *S. aureus*, are dominant species during storage, according to research. Since *Staphylococci* are among the common genus located on normal human & domestic animal skin, the presence of *S. aureus* in PSY rice cake indicates that it may have entered during rice cake processing (Ji et al. (2007).

Antioxidative Activity

The Antioxidative activity (AOA) of PT rice cake was highest on day 3 (42.74-69.93%), followed by day 1 (34.93-57.82%), and the least AOA was observed on day 5 (24.10-39.44%). For PSY rice cake, the highest AOA was witnessed on day 3 (44.73-59.90%), followed by day 5 (41.34-58.65%) and day 1 (27.74-35.11%). Similarly, in PSL rice cake, the highest antioxidant activity was observed on day 3 (53.50-73.38%), followed by day 5 (45.81-60.63%) and day 1 (42.44-53.91%). The AOA seemed to achieve saturation at the 7th minute for each sample type. Overall, the PSL (42.44 to 73.38%) rice cake illustrated a markedly higher AOA followed by PT (24.10 to 69.93%) and PSY (27.74 to 59.90%). In this study, the findings showed that the AOA of rice cakes increased significantly up to the third day of storage, which is uniform with the results of Hubert et al. (2008), who found that the AOA of rice cakes increased significantly after 6 hours of fermentation, which was later observed to decrease gradually as the fermentation period increased to 48 hours. Park et al. (2016) in the study of AOA in rice cake, discovered that microbial enzymes have the ability to break down the structure of cereal cell walls during fermentation, which can contribute to the formation of various bioactive compounds. This result was also in line with Hur et al. (2014), who discovered that fermentation and synthesis of antioxidant compounds in plant-based foods have a beneficial relationship. Kim et al. (2011) discovered that the antioxidative action of *maejakgwa* (Korean rice cake) declined as the storage interval was enhanced, varying between 19.18-8.19% after 14 days, which was consistent with our findings. (Figure 3).

Table 3: Antimicrobial activity of rice cakes

RICE CAKES	DAYS	*S. aureus* (mm)	*S. typhi* (mm)	*L. monocytogenes* (mm)	*B. cereus* (mm)	*E. coli* (mm)	*E. faecalis* (mm)
PT	1	-	-	-	-	-	-
	3	-	-	-	-	-	-
	5	-	-	-	-	-	-
PSY	1	18.00±1.000^{a}	18.00±0.707^{e}	-	-	18.00±0.707^{c}	-
	3	17.00±0.707^{a}	15.20±0.837^{f}	-	-	17.40±0.548^{d}	-
	5	15.00±0.707^{b}	-	-	-	-	-
PSL	1	-	-	-	-	-	-
	3	-	-	-	-	-	-
	5	-	-	-	-	-	-

Values were depicted as mean±SD from five replicates (n=5) for individual observation. Values with different superscripts were assigned to illustrate statistically significant differences from each other by Scheffe's test at $p \leq 0.05$, ('- 'zone of inhibition is missing, mm-diameter of a zone of inhibition), Borewell diameter- 15mm)

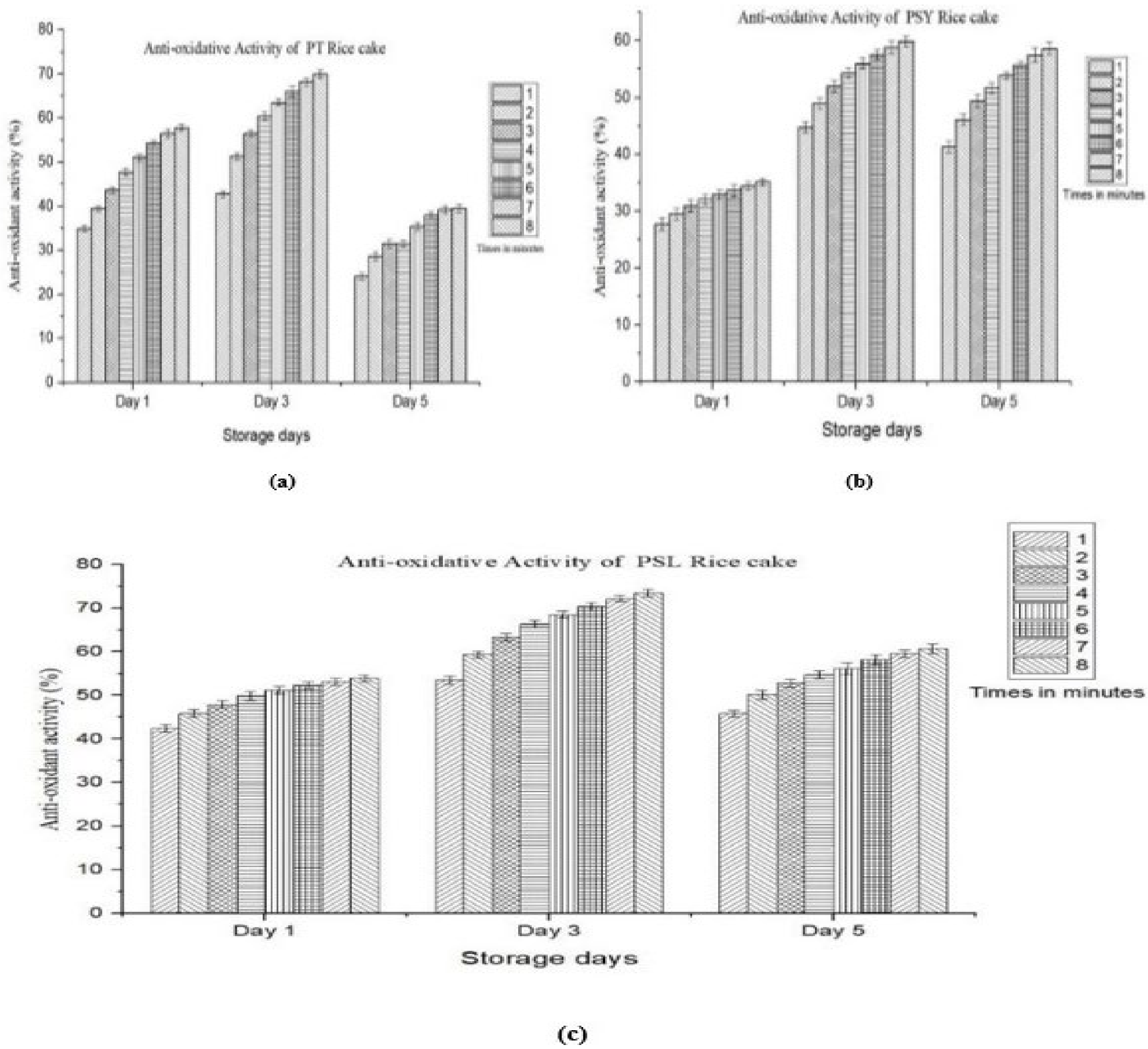

Fig. 3. (a) Antioxidant activity of PT rice cake; (b) Antioxidant activity of PSY rice cake; (c) Antioxidant activity of PSL rice cake

Determination of SCFA production

SCFA was determined for acetate, butyrate, and propionic acid, but only acetic acid (AA) production was noticeable. In the three varieties of rice cakes, day 3 (0.012, 0.01, 0.012 µg/ml) was found to be on the larger side at a 5% ($p \leq 0.05$) significance level compared to day 1 (0.006, 0.007, 0.012 µg/ml) and day 5 (0.005, 0.005, 0.005 µg/ml) for PT, PSY, and PSL respectively. On day 1, PSL and PT AA production were recorded as the highest and lowest, while no significant difference was observed for days 3 & 5. Studies have shown that cooked rice contains resistant starch that can produce SCFA in the colon (Rossi et al. 2005). Research carried out by Nandan et al. (2001), found that rice vinegar has a total acidity of 4-5 % (mainly AA). It was also discovered that AA has antibacterial and antifungal properties, which could be a factor in the antimicrobial action of the rice cakes in this research (Cabo et al. 2002). The glutinous rice was found to contain three of the six organic acids found in common rice cake, i.e., AA, palmitic acid, and 9,12-octadecadienoic acid were the three, while 6-octadecanoic, hexadecanoic, and tetradecanoic acids were also present in common rice cake. Acetic acid was also found to be responsible for the rice cake's sour flavor (Palaniveloo and Vairappan 2013) (Figure 4 and 5).

Determination of Vitamin B_2 and B_{12} production

Vitamin B_2 and B_{12} analysis was carried out in this study, but only vit B_2/riboflavin (RBF) production was detected. The RBF production of day 3 (0.158, 0.228 μg/ml) in PT and PSY rice cakes was found to be on the larger side at a 5% ($p \leq 0.05$) significance level compared to day 1 (0.035, 0.025μg/ml) & day 5 (0.023, 0.016 μg/ml) respectively. The RBF production was not detected in the PSL rice cake.

The RBF production in rice cakes was found to increase until day 3, followed by a decline as shelf-life progressed.

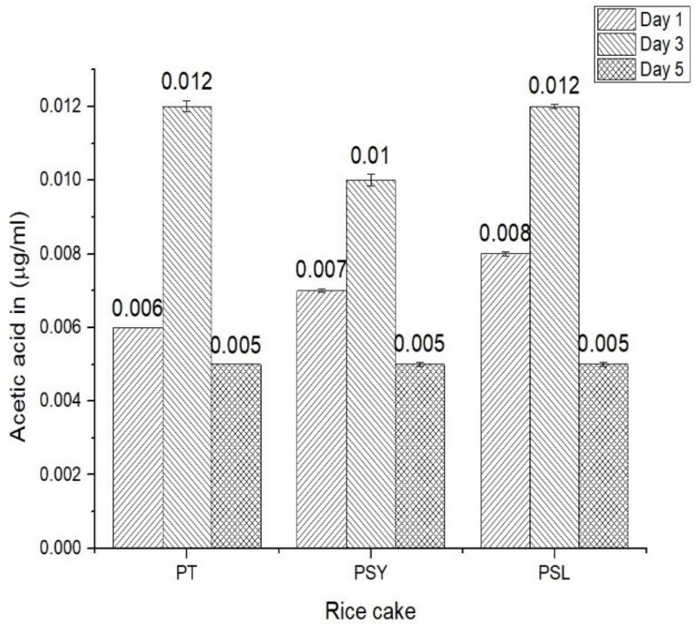

Fig. 4. Acetic acid production of rice cakes over the shelf life

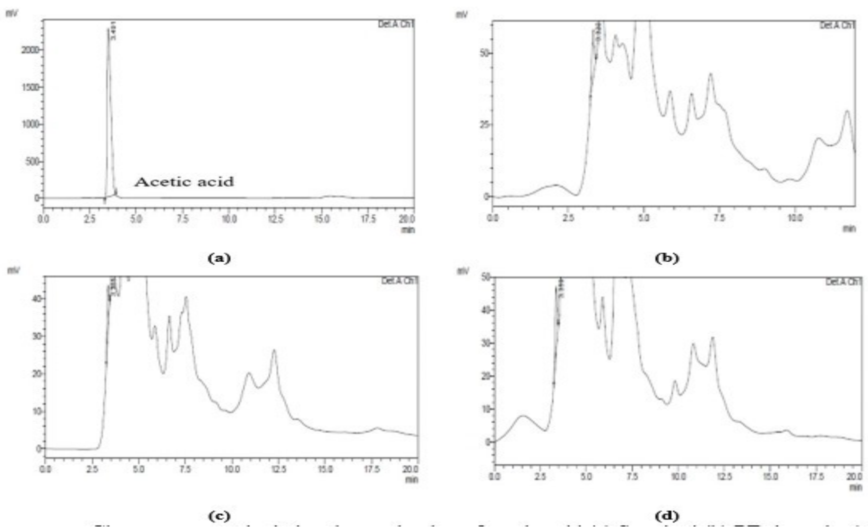

Fig. 5. Chromatograms depicting the production of acetic acid (a) Standard (b) PT rice cake (c) PSY rice cake (d) PSL rice cake

There was no discernible difference in PT and PSY rice cakes' RBF production at a 5% ($p \leq 0.05$) level on all storage days. RBF was found to be essential for cellular metabolic movements, specifically in cell build-up & oxidation-reduction reactions (Hati et al. 2019). The RBF content in milled rice was found to be 0.02-0.06 (mg/100 g) (Julio and Hicks 1996). According to Lebiedzinska and Szefer (2006), rice cakes have an RBF concentration of 0.039–0.057mg/100g. The absence of RBF content in the PSL rice cake and the difference in RBF content between the PT and PSY rice cakes could be attributed to the preparation methods and cooking process, which was in line with Rachmani and Muller's (1996) findings that RBF is extremely susceptible to handling provisions, particularly light and heat. Depending on the conditions in which technical processes are performed, vitamins can lose up to 66% of their RBF (Prodanov et al. 2004). Rice and rice products (also wild rice, which contains 0.192 mg/100 g) contain trace amounts of RBF. The reduction in RBF substances may be accredited to leaching & heat destruction. A similar report was investigated by Gernah et al. (2012) for *Zogale* (Nigerian cake) for different samples in which RBF was found to be in a range of 0.19 mg/100 g-0.07 mg/100 g. (Figure 6 and 7).

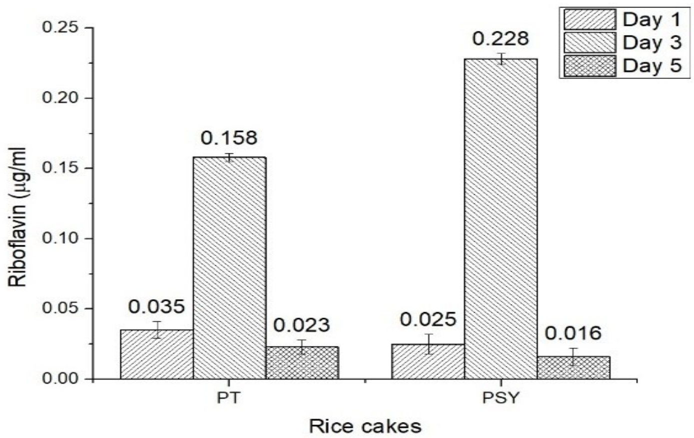

Fig. 6. Riboflavin production of rice cake over the shelf life

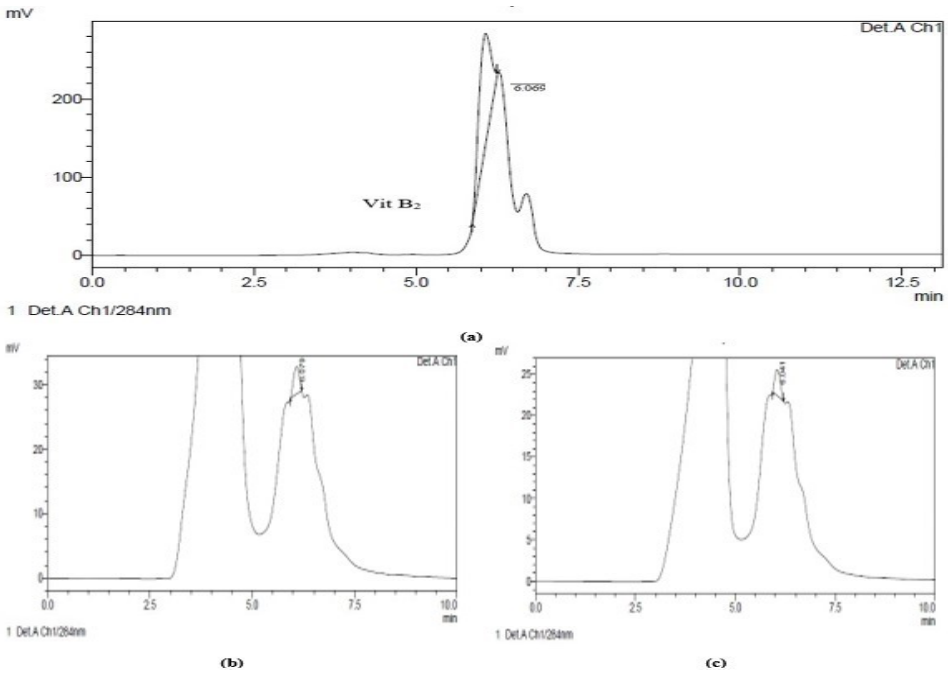

Fig. 7. Chromatograms depicting production of vitamin B_2 (a) Standard (b) PT rice cake, (c) PSY rice cake

4. CONCLUSION

Our findings show that there are significant differences in the nutritional composition of each rice cake. The rice cakes were found to serve as an excellent source of several dietary supplements. The sensory assessment, titratable acidity, carbohydrate content, antioxidant activity, acetic acid, and microbial count are all higher in PSL rice cakes. The PSY rice cake contains more vitamin B_2, has stronger antimicrobial activity, and has a higher pH value. The protein content of PT rice cake was discovered to be the highest. The current study offers details on the bio-functional properties of microbial & physico-chemical analysis of the three traditional rice cakes, which can help to improve our dietary strategy for individual health management. Recently, there has been a surge in public interest in rice cakes, and based on our results, personal hygiene education and safety management during the preparation of the rice cakes are needed to ensure hygienic conditions and to prevent microbial contamination of the rice cakes caused by the equipment used.

5. ACKNOWLEDGEMENT

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