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# Impact of chickpea aquafaba-based emulsion on the physicochemical, nutritional, rheological and structural characteristics of little millet (*Panicum sumatrense* Roth.) flour cake

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Recent patterns in food consumption indicate that consumers have a strong desire for vegan, gluten-free, and healthy diets. This study investigates the utility of chickpea aquafaba and little millet flour (LMF) as alternatives to egg and refined flour, respectively, for developing egg-free and gluten-free cake recipes. The cake and batter were analyzed for their physicochemical, structural, sensorial, rheological and textural properties, with a shelf-life study conducted over a period of 12 days ( $30 \pm 2$  °C). The batter's density ( $1.13 \pm 0.01$  g cm<sup>-3</sup>) and viscosity ( $15796.7 \pm 0.09$  cp) increased with the addition of LMF, and the batter predominantly exhibited an elastic behavior with  $G' > G''$  for all cake formulations. These characteristic changes in cake batter caused the cake's weight to increase (by 10%) while causing its height, baking loss, volume, specific volume and symmetry index to decrease. The textural characteristics showed that adding LMF to cakes enhanced the hardness ( $145.00 \pm 9.45$  N) and firmness ( $19.36 \pm 2.12$  N) and decreased their cohesion, chewiness, gumminess, springiness and resilience. The image analysis showed more uniform bubble distribution in wheat flour (WF) cake than in LMF cakes, and an increase in LMF content resulted in a drop in cell circularity. The microbial degradation of cakes was observed from the 6th day of storage. LMF cake samples exhibited good texture and physical and sensory attributes comparable to those of the WF cake sample. Therefore, the study demonstrated the potential use of alternative ingredients, such as LMF and aquafaba emulsion, in the production of egg-free and gluten-free cakes. These ingredients could facilitate the scaling up of sustainable production practices for baked goods in the future.

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## Sustainability spotlight

This study explores sustainable food technology through ingredients like chickpea aquafaba and little millet flour (LMF) as alternative ingredients for developing egg-free and gluten-free cakes. With a rising consumer demand for vegan and healthy diets, the utilization of such ingredients not only reduces dependence on traditional animal-based products but also promotes the use of underutilized grains like LMF, which require fewer resources and support agricultural biodiversity. By transforming a waste product (aquafaba) into a functional baking component, this research exemplifies the principles of a circular economy, minimizing food waste and enhancing sustainability. Additionally, evaluating other sugar alternatives, such as unprocessed foods like raw honey, brown rice syrup, and jaggery, could significantly improve the health and sustainability of cakes produced in the industrial sector. The findings demonstrate that LMF cakes can achieve sensory and textural qualities comparable to those of conventional wheat flour cakes, making them a viable option for scaling up the production of sustainable baked goods that cater to diverse dietary needs. This work highlights the potential for innovative, environmentally friendly practices in the baking industry, contributing to a more sustainable food system.

## 1. Introduction

Aquafaba, which translates to “bean water” in Latin, is a novel plant-based protein that has been increasingly used as an emulsifier. It is the residual water left from cooked beans (especially chickpeas), which is rich in water soluble proteins and complex carbohydrates.<sup>1</sup> Its unique proportion of soluble carbohydrates and proteins comes through the soaking and cooking process. As an outcome, a more adaptable material, *i.e.*

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aquafaba, is obtained that can withstand a wide range of pH values and temperatures with multifunctional properties, exhibiting gelling, emulsifying, foaming, and water- and oil-holding capacities.

People with phenylketonuria, a condition that affects protein metabolism, can now enjoy foods that are typically egg-free using aquafaba as an alternative. Additionally, those with egg allergy also follow a typical plant-based diet. Plant-based diets using pulses as an alternative to animal proteins have offered the most practical features as an essential protein source for use in culinary applications.<sup>2</sup>

Plant-based meals and nutrients are now widely available worldwide, offering a viable alternative to animal-based foods like dairy products, eggs, meat from cattle and poultry, and seafood.<sup>3</sup> This shift acknowledges their benefits in terms of ethical considerations, environmental sustainability, and human health.<sup>4</sup> Currently, one of the main focuses is on the development of egg alternatives. Aquafaba contains about one fourth of the protein relative to egg white by dry weight.<sup>5</sup> Stantiall *et al.*<sup>6</sup> reported that aquafaba can be utilized as an ingredient in place of egg white owing to its good gelling and foaming properties.

Health challenges such as phenylketonuria, egg allergy, excessive cholesterol, changes in dietary choices and religious convictions, along with monetary incentives, can all contribute to the replacement of eggs.<sup>7</sup> Eggs have unique functional properties that make them highly versatile for various food applications, especially in the preparation of cakes in the bakery industry. In addition to egg less cakes, gluten free cakes are also in demand due to allergic reactions and other health related concerns like celiac disease, diabetes and obesity, which are linked to the active ingredients in cakes, like wheat flour (WF).<sup>8</sup> To overcome this issue, wheat alternatives like millets have been recently researched for obtaining nutritional benefits and comparable textural properties.<sup>9,10</sup>

Little millet provides minerals, essential amino acids, and vitamin B complex—all of which are frequently deficient in our main meal diets.<sup>11</sup> A phytochemical study has demonstrated that this millet has a lower glycemic index and greater antioxidant levels when compared to other dietary crops.<sup>12</sup> Eating cereals made from these whole grains lowers the risk of cardiovascular disease and diabetes.<sup>13</sup> Aquafaba has been recently explored by few authors as an egg-replacer in baked goods especially cakes.<sup>14–17</sup> However, research on gluten free millet cakes using aquafaba has not yet been explored.

Thus, the current research aims to explore the feasibility of producing gluten free egg less cakes using little millet flour (LMF) and chickpea aquafaba with the goal of improving the nutritional and functional attributes similar to those of conventional cakes.

## 2. Materials and methods

### 2.1 Materials

The ingredients including chickpea, refined WF (maida), little millet (*Panicum sumatrense* Roth.), refined sugar, milk powder (Nestle Everyday Tea's Perfect Partner; ~76% Solid Non Fat

(SNF) and 15% fat), baking powder (Weikfield baking powder), vinegar (Dr Vaidya's apple cider vinegar) and vanilla essence (Flavor Mate) were purchased from a local supplier in Varanasi, Uttar Pradesh, India (82.9739° E, 25.3176° N). Little millet and sugar were powdered using a conventional mixer (Butterfly Smart mixer, 750 W), and screened with a 60-mesh size sieve to separate the particles with a smaller size (<200 μm). Analytical reagent (AR) grade chemicals were obtained from standard companies (Hi-Media, India, and Sigma Aldrich, India) for all preparation and analysis, and the reagents were made fresh using standard procedures.

### 2.2 Aquafaba preparation

After cleaning, 100 g of chickpea was soaked at 4 °C for 16 h. The aquafaba was prepared based on the method followed by He *et al.*<sup>18</sup> Soaked chickpeas were mixed with filtered water (1 : 4), placed inside a glass jar, and autoclaved for 30 min at 115 °C. After that, it was left to cool for 24 h at room temperature, which was later filtered and placed in a refrigerator (2 to 4 °C) until further analysis.

### 2.3 Sponge cake preparation

The sponge cakes using little millet flour (LMF) were prepared according to Mustafa *et al.*<sup>14</sup> with some modification. Using a hand blender (Braun MQ9047X, New Castle, USA), 110 mL of aquafaba and 10 mL of apple vinegar were combined, and the mixture was whipped for 7 min at an optimal speed until the majority of the aquafaba became frothy and little liquid persisted. When the frothy blend was whipped into a dense peak, 130 g of granulated sugar was added. Using a paddle, 130 g of WF, 7 g of baking powder, and 13 g of milk powder were carefully added and blended into the froth. Lastly, 5 mL of vanilla essence was added and mixed gently. The ratio of WF to LMF was varied to 100 : 1, 75 : 25, 50 : 50, 25 : 75 and 1 : 100 on weight basis and the samples were labelled as C-100, T-25, T-50, T-75, and T-100, respectively (Table 1). The samples of cake batter were then transferred to 14.7 × 7.7 cm rectangular pans and baked in a conventional micro-oven (Panasonic NN-DS596, Japan) set at 150 °C for 10 min and 180 °C for 20 min. The pans were then taken out of the micro-oven after baking and allowed to cool for 30 min at room temperature.

**Table 1** The formulation combination for sponge cake with respect to different ingredients<sup>a</sup>

	C-100	T-25	T-50	T-75	T-100
Aquafaba (mL)	110.0	110.0	110.0	110.0	110.0
WF (g)	130.0	97.5	65.0	32.5	—
LMF (g)	—	32.5	65.0	97.5	130.0
Sugar powder (g)	130.0	130.0	130.0	130.0	130.0
Milk powder (g)	13.0	13.0	13.0	13.0	13.0
Baking powder (g)	7.0	7.0	7.0	7.0	7.0
Vinegar (mL)	5.0	5.0	5.0	5.0	5.0
Vanilla essence (mL)	10.0	10.0	10.0	10.0	10.0

<sup>a</sup> C-100: 100% WF; T-25: 75% WF + 25% LMF; T-50: 50% WF + 50% LMF; T-75: 25% WF + 75% LMF; T-100: 100% LMF.



## 2.4 Physical measurements of the cake and batter

All the analyses of batter were done in triplicate using the procedures outlined by He *et al.*<sup>18</sup> The quantity of air included into a batter can be determined by the measurement of the batter density ( $\rho$ ). The batter density ( $\rho$ ) was measured using an Elcometer 1800 pycnometer (Manchester, UK). The viscosity of the batter was determined using a Brookfield DV-11 + Pro Viscometer (Middleborough, MA; spindle no. 4 (S64)) set at 5 rpm at room temperature ( $21.8 \pm 2$  °C). The pH of the cake batter was determined using a pH meter (Model 1761, Jenco Electronics Ltd, Taiwan) at a temperature of 25 °C.

Proximate analysis of the cake was conducted to measure its moisture content, protein content, ash content, fat content, carbohydrate content and calorific value.<sup>19</sup> The physical measurements including baking loss, cake weight and volume of each cake formulation were performed according to Gómez *et al.*<sup>20</sup> For determining the baking loss, a vernier caliper was used to measure the cake's height, breadth and length in centimeters. The cake's weight (g) was measured in triplicate using an electronic weighing scale (Mettler Toledo, JB1603-C, Switzerland). The difference between the total mass of the dough before baking ( $W_u$ ) and the weight of the cake after baking ( $W_c$ ) was used to quantify the baking loss of the cake samples. A laser sensor with a BVM-L370 (TexVol Instruments, Viken, Sweden) was used to measure the cake's volume. The ratio of the size of the cake's volume to its weight provided the cake's specific volume.

According to Grossi Bovi Karatay *et al.*,<sup>21</sup> the symmetry index was calculated after 24 h of storage using eqn (1). This measurement is taken at specified positions to ensure a representative sample of the cake's overall structure. For each cake slice, the height measurements were taken from the center of the slice ( $C$ ), height at one quarter ( $B$ ) and height at three quarters ( $D$ ) of the cake length.

$$\text{Symmetry index} = 2 \times C - B - D \quad (1)$$

## 2.6 Significant rheological characteristics of the prepared doughs

The dynamic rheological measurements of storage modulus ( $G'$ ), loss modulus ( $G''$ ) and  $\tan \delta$  ( $G''/G'$ ) were performed according to Li *et al.*<sup>22</sup> using a rheometer (AR2000ex, TA Instruments, New Castle, USA) with a parallel plate geometry (40 mm in diameter) at 1 mm gap. The dough sample was obtained by manually mixing 2 g of prepared batter with 1.3 mL of distilled water and rested for 20 min to relax any residual stress. The frequency sweep test was carried out at a frequency of 0.1–10 Hz and a strain of 0.1%. The proportion of  $G''$  to  $G'$  was used to determine  $\tan \delta$ .

## 2.7 Instrumental textural profile analysis

The interior crumb texture characteristics of the sponge cake and exterior crust were analyzed in accordance with He *et al.*<sup>18</sup> The texture profile analysis was performed in a Texture analyser (Texture Pro CT V1.6 build, Brookfield) using Texture Lab Pro Software, Version 1, 13-002, and a TMS-Pro Texture Press (Food

Technology Corp., Sterling, VA). Sponge cake pieces of  $25 \times 15$  mm dimension were cut out to make the crumb, making sure to leave out the outermost layer and borders. At a cross-head speed of  $100 \text{ mm min}^{-1}$ , each sample was vertically crushed to 50% of its initial height (7.5 mm). Hardness cycle 1 (N), hardness cycle 2 (N), firmness (N), gumminess (mm), chewiness (N), cohesiveness, resilience (mJ) and springiness (mm) were obtained from the software and an average of triplicate readings was calculated.

## 2.8 Image analysis

Image analysis of the sponge cake was done according to Tsatsaragkou *et al.*<sup>23</sup> Cakes were sliced vertically into 1.5 cm thick pieces. Each slice was photographed at 300 dpi for precision. The cake height was measured with Image J 2.9.0 software (National Institutes of Health, Palo Alto, CA, USA) and the scanned picture was analyzed for obtaining the crumb cell structure. To perform the aforementioned analysis, a  $3.5 \times 3.5$  cm portion with a uniform thickness was clipped from the center of each slice. The image was divided into colour channels first, to which contrast was added, and at last the image was binarized following a grayscale threshold. The image was later analyzed for cell count, average size and number of bubbles and the measurements were taken in triplicate.

## 2.9 Bright field microscopy

Sponge cake crumb structure was imaged using bright field microscopy (Olympus CX23, Olympus, Japan) as per the protocol given by Tsatsaragkou *et al.*<sup>23</sup> with LED illumination and  $30 \mu\text{m}$  magnification. The image was first divided into color channels using Image J 2.9.0 (National Institutes of Health, Palo Alto, CA, USA), then the contrast effect was increased, and finally the image was binarized following the grayscale threshold. Cell circularity and average cell area ( $\text{mm}^2$ ) were computed by eqn (2). The values range from 0.0 to 1.0 for cell circularity. A circularity value of 1.0 denotes a circle that is perfectly circular, whereas values closer to 0.0 suggest an increasingly extended polygon. Triplicate measurements were taken from the center of each cake.

$$\text{Cell circularity} = 4\pi \left( \frac{\text{Area}}{\text{Perimeter}^2} \right) \quad (2)$$

## 2.10 Fourier transform infrared spectroscopy analysis of the sponge cake

The infrared spectra of the materials were obtained using a PerkinElmer Spectrum 100 FTIR spectrometer with the KBr pellet technique, at room temperature. Pellets were formed by gradually combining the samples with KBr powder that had been micronized. Each sample underwent twenty scans, with a spectral resolution of  $4 \text{ cm}^{-1}$ , and spectra were collected between 4000 and  $400 \text{ cm}^{-1}$  wavenumber. Regular background spectrum collection was done to ensure data accuracy and dependability.



### 2.11 Shelf-life studies

The shelf-life study of cakes was done for 12 days and measurements of moisture, protein, ash, total solids, fat, weight gain, total plate count, coliform, yeast and mold were performed. The samples were kept in a PET box at room temperature ( $30 \pm 2$  °C). 10 g of cake sample was ground in 90 mL of a sterile sodium chloride solution (0.9%) diluted for microbiological analysis in accordance with the protocol of Salfinger and Tortorello.<sup>24</sup>

**2.11.1 Total viable bacterial count, mold and yeast and coliform bacteria count.** For the total plate count, total plate count culture medium was used taking the 1st or 2nd dilution. For yeast and mold count, potato dextrose agar was used taking the 1st dilution. For coliform bacteria, eosin methylene blue agar was used taking the 1st dilution. The plates were incubated at  $37 \pm 1$  °C and  $30 \pm 1$  °C for total plate count, yeast and mold count and coliform count, respectively, for 24 or 48 h.

### 2.12 Sensory evaluation

A sensory examination was done on cakes that were baked using LMF and served as 2.5 cm cubes. Thirty semi-trained panelists (15 each male and female), aged between 22 and 25 years, were selected and samples were served at  $25 \pm 2$  °C. The samples were presented to panelists in a random order and they were requested to assess the samples' color, appearance, texture, flavor, aftertaste and overall acceptability based on the 9-point hedonic scale. In the rating system, 1 denotes strong dislike and 9 denotes high liking.

### 2.13 Statistical analysis

Each experiment was conducted in triplicate, and the results were expressed in terms of mean  $\pm$  SD. Using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA), ANOVA (one-way analysis of variance) and Turkey's test ( $p < 0.05$ ) were performed.

## 3. Results and discussion

### 3.1 Physicochemical properties of the cake and batter

**3.1.1 Cake.** The results of physicochemical analysis of the cake and batter are shown in Table 2. The crumb's moisture content varied from  $18.69\% \pm 1.03\%$  (T-100) to  $24.37\% \pm 1.54\%$  (C-100) where the samples with LMF had the least ( $18.69\% \pm 1.03\%$ ) and WF ( $24.37\% \pm 1.54\%$ ) had the highest crumb moisture content. Similar results were observed for crust's moisture content where T-100 had  $13.84\% \pm 1.05\%$  while C-100 had  $16.04\% \pm 0.83\%$ ; however, the values were not significant ( $p \geq 0.05$ ). A proportional fall in moisture content was found to occur when the amount of LMF was significantly ( $p < 0.05$ ) increased, which might be due to the higher absorption rate of WF when compared to LMF. This observation was consistent with Njintang *et al.*<sup>25</sup> who found similar trends in composite bread's moisture with the addition of taro flour. The range of the ash content and calorific value was found to be  $1.24\% \pm 0.04\%$  (C-100) to  $1.44\% \pm 0.03\%$  (T-100) and  $344.00 \pm 1.02$  kcal (C-100) to  $378.00 \pm 1.47$  kcal (T-100), respectively. The results of the investigation demonstrated that the increase in ash content and calorific value can be due to the increase in LMF resulting

**Table 2** The physicochemical analysis and cake and batter characterization of various optimized cakes incorporated with aquafaba and little millet<sup>a</sup>

	C-100	T-25	T-50	T-75	T-100	
<b>Physicochemical analysis</b>						
Moisture	Crumb (%)	$24.37 \pm 1.54^c$	$23.64 \pm 0.37^c$	$22.17 \pm 0.56^{bc}$	$20.41 \pm 1.65^{ab}$	$18.69 \pm 1.03^a$
	Crust (%)	$16.04 \pm 0.83^a$	$15.83 \pm 0.33^a$	$15.46 \pm 1.65^a$	$14.23 \pm 0.03^a$	$13.84 \pm 1.05^a$
Ash (%)		$1.24 \pm 0.04^a$	$1.29 \pm 0.20^a$	$1.34 \pm 0.05^a$	$1.4 \pm 0.07^a$	$1.44 \pm 0.03^a$
Protein (%)		$13.19 \pm 0.36^c$	$12.94 \pm 0.28^c$	$12.34 \pm 0.52^{bc}$	$11.58 \pm 0.34^{ab}$	$11.13 \pm 0.56^a$
Fat (%)		$16.26 \pm 0.53^a$	$16.61 \pm 0.24^a$	$16.97 \pm 0.42^a$	$17.23 \pm 0.5^a$	$18.35 \pm 0.16^a$
Carbohydrates (%)		$58.48 \pm 0.42^a$	$59.19 \pm 0.36^{ab}$	$59.90 \pm 0.47^b$	$60.37 \pm 0.58^b$	$61.88 \pm 0.42^c$
Calorific value (kcal)		$344.00 \pm 1.02^a$	$347.00 \pm 1.34^a$	$356.00 \pm 1.22^b$	$364.00 \pm 1.11^c$	$378.00 \pm 1.47^d$
<b>Cake characterization</b>						
Length (cm)		$14.60 \pm 0.30^a$	$14.50 \pm 0.20^a$	$14.40 \pm 0.20^a$	$14.40 \pm 0.10^a$	$14.40 \pm 0.30^a$
Breadth (cm)		$7.70 \pm 0.20^a$	$7.70 \pm 0.30^a$	$7.60 \pm 0.40^a$	$7.60 \pm 0.30^a$	$7.50 \pm 0.20^a$
Height (cm)		$4.20 \pm 0.03^b$	$4.20 \pm 0.01^b$	$4.10 \pm 0.05^{ab}$	$4.10 \pm 0.05^{ab}$	$4.00 \pm 0.07^a$
Weight (g)		$170.50 \pm 1.23^a$	$175.25 \pm 0.98^b$	$180.00 \pm 0.15^c$	$184.47 \pm 0.12^d$	$190.03 \pm 1.67^c$
Volume (cm <sup>3</sup> )		$462.84 \pm 0.04^c$	$449.68 \pm 0.3^d$	$448.29 \pm 0.02^c$	$440.09 \pm 0.02^b$	$437.52 \pm 0.04^a$
Baking loss (%)		$11.40 \pm 1.02^a$	$11.09 \pm 0.84^a$	$10.88 \pm 0.65^a$	$10.35 \pm 0.56^a$	$9.30 \pm 0.98^a$
Cake specific volume (cm <sup>3</sup> g <sup>-1</sup> )		$2.71 \pm 0.01^d$	$2.62 \pm 0.05^c$	$2.60 \pm 0.02^{bc}$	$2.53 \pm 0.03^{ab}$	$2.51 \pm 0.01^a$
Symmetry index		$2.60 \pm 0.02^a$	$2.40 \pm 0.03^a$	$2.50 \pm 0.01^a$	$2.20 \pm 0.30^a$	$2.30 \pm 0.24^a$
<b>Batter characterization</b>						
Density (g cm <sup>-3</sup> )		$1.06 \pm 0.01^a$	$1.09 \pm 0.03^{ab}$	$1.11 \pm 0.01^b$	$1.11 \pm 0.02^b$	$1.13 \pm 0.01^b$
Viscosity (cp)		$14450.00 \pm 0.07^a$	$14492.13 \pm 0.07^b$	$15423.44 \pm 0.05^c$	$15678.91 \pm 0.12^d$	$15796.7 \pm 0.09^c$
pH		$6.60 \pm 0.01^a$	$6.70 \pm 0.01^b$	$6.60 \pm 0.01^a$	$6.80 \pm 0.01^c$	$6.70 \pm 0.01^b$

<sup>a</sup> Dissimilar alphabets (a, b, c, d) in the similar row indicate significance variance ( $P < 0.05$ ). C-100: 100% WF; T-25: 75% WF + 25% LMF; T-50: 50% WF + 50% LMF; T-75: 25% WF + 75% LMF; T-100: 100% LMF.



in heavier batter with varied nutrition. This demonstrated that the millet flour with a higher ash content was beneficial for providing various minerals, supporting the claims by Sharoba<sup>26</sup> and resulting in the production of a cake with high ash content.<sup>27</sup> The range of protein content was from 13.19%  $\pm$  0.36% for C-100 to 11.13%  $\pm$  0.56% for T-100. The obtained results can be due to the difference in protein content between WF and LMF. The WF sample had a significantly ( $p < 0.05$ ) higher protein content than the LMF one, and with a decrease in WF ratio the final protein content in cake decreased.<sup>27</sup> These results aligned with the results of Amandikwa *et al.*,<sup>28</sup> where the protein content of wheat-yam flour composite bread decreased with reduced content of wheat flour. The fat and carbohydrate contents were in the range of 16.26%  $\pm$  0.53% (C-100) to 18.35%  $\pm$  0.16% (T-100) and 58.48%  $\pm$  0.42% (C-100) to 61.88%  $\pm$  0.42% (T-100), respectively. The little millet and aquafaba addition resulted in higher fat and carbohydrate content in LMF formulated cakes, however the results were insignificant ( $p \geq 0.05$ ). The results were in line with those of Adasi *et al.*<sup>29</sup> for rock cake produced with the mixture of wheat and millet flour.

The dimensions such as length, breadth, and height (as shown in Fig. 1) and volume of the sponge cake are presented in Table 2. Not much variation in the dimensions of different cake formulations was observed; however, variations in volume, specific volume and weight were found. This could be attributed to the lower content of structure-forming proteins and reduced gluten in the LMF, which led to decreased carbon dioxide gas retention and resulted in a less dense texture. Consequently, the

observed decrease in cake samples was significantly attributed to the dilution of gluten in the wheat mixes. Similar outcomes were obtained by Mudau *et al.*<sup>30</sup> by the addition of millet flour. The cake weights ranged from 170.50  $\pm$  1.23 g (C-100) to 190.03  $\pm$  1.67 g (T-100), with all samples showing significant differences ( $p < 0.05$ ). The heavier dough could be attributed to factors such as particle size, improved moisture absorption, and decreased air entrapment. Kayitesi *et al.*<sup>31</sup> noted that the increased weight and larger particle size of fiber-rich flours result in greater bulk density and water absorption capacity.

Additionally, not much significant ( $p \geq 0.05$ ) variation in cake's baking loss and specific volume was observed, with a decreasing trend in baking loss, being highest for C-100 (11.40%  $\pm$  1.02%) and lowest for T-100 (9.30%  $\pm$  0.98%). de la Hera *et al.*<sup>32</sup> found that lower gluten concentration flours had less baking loss because of their poor water binding capacity. All symmetry index values in the current investigation were positive, indicating no evidence of cake collapse.<sup>33</sup>

**3.1.2 Batter.** The batter density values ranged from 1.06  $\pm$  0.01 g cm<sup>-3</sup> (C-100) to 1.13  $\pm$  0.01 g cm<sup>-3</sup> (T-100), likely due to the heavier weight of the batter with the addition of LMF; however, no significant differences among samples were observed ( $p \geq 0.05$ ). Wu *et al.*<sup>34</sup> demonstrated that the batter density increased with the amount of tamarind seed gum used. Batter viscosity is a crucial physical property that affects the final quality of baked goods, which influences uniform air distribution. Compared to WF added cakes (C-100), there was a significant ( $p < 0.05$ ) increase in the batter's viscosity with the

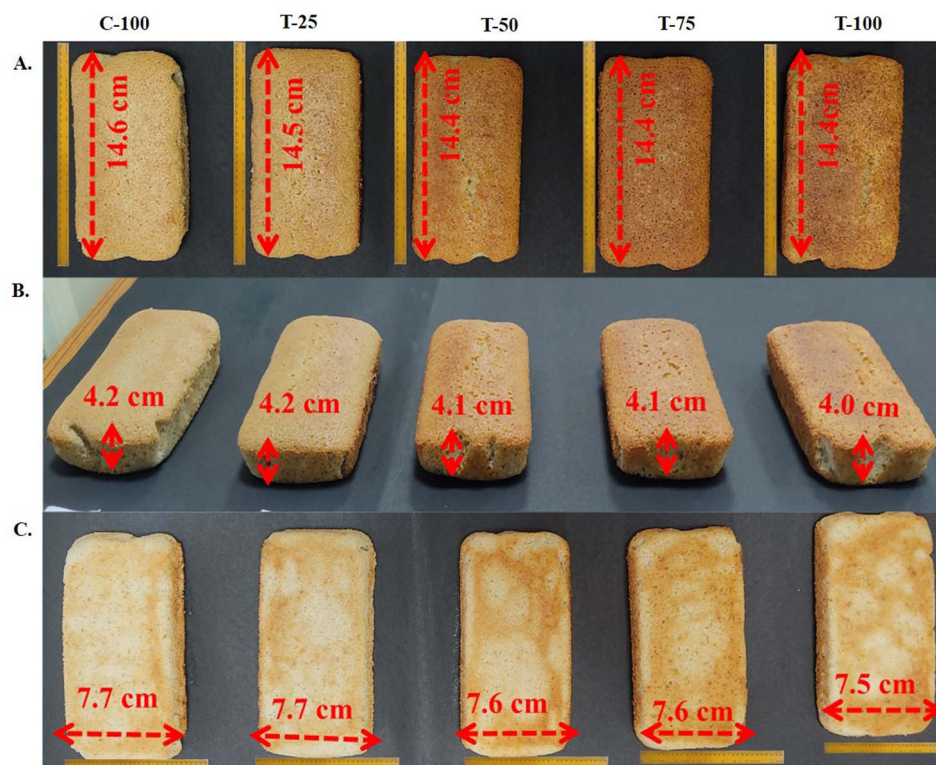


Fig. 1 Physical structural dimensions of aquafaba and little millet flour incorporated cake formulations. (A) length; (B) height; (C) breadth.



addition of LMF. This can be attributed to the higher fiber and fat content of LMF, which reduced the available free water and increased the viscosity.<sup>35</sup> The viscosity values of the batter ranged from  $14450.0 \pm 0.07$  cp (C-100) to  $15796.7 \pm 0.09$  cp (T-100), with no significant differences among samples ( $p \geq 0.05$ ). These results aligned with those of Bhaduri and Mukherjee,<sup>36</sup> who observed that the batter viscosity increased with the amount of quinoa in muffins (3116–48470 cp). The pH levels ranged from 6.6 to 6.8, which was within an ideal pH range for cake batters, *i.e.*, between 6.50 and 7.70.<sup>21</sup>

### 3.2 Rheological characteristics of the prepared dough

Understanding the rheological properties of dough, specifically its viscoelasticity, is crucial for dough preparation. Measuring the batter's rheological properties immediately after preparation offers valuable insights that significantly predict the volume of the cakes together with their cohesiveness.<sup>37</sup> The storage modulus ( $G'$ ) of the batter ranged from 3135.3 Pa (C-100) to 2758.4 Pa (T-100) (at  $0.1 \text{ rad s}^{-1}$ ) (see Fig. 2). The storage modulus of a batter reflects the elasticity or solid nature of the dough, which was highly influenced by the addition of LMF. The addition of LMF enhanced the dough structure and stiffness by forming a more interconnected network. Moreover, the proteins and fibers in LMF contribute to a stronger, more cohesive gel network, which potentially reduced the storage modulus. Unlike WF, LMF lacks gluten, which aids in a stable protein network, resulting in higher  $G'$ . Consequently, the LMF dough exhibited less elasticity compared to the WF batter, resulting in a less elastic structure.

The loss modulus ( $G''$ ) of the batter ranged from 1228.6 Pa (C-100) to 1995.44 Pa (T-100) (as shown in Fig. 2). The loss modulus quantifies a material's viscous behavior by indicating the amount of energy dissipated as heat during deformation. An increase in LMF content typically raises the batter's viscosity due to the higher fiber content, which in turn increased the loss modulus.<sup>38</sup> Enhanced hydration from LMF contributes to improved viscosity characteristics, as greater water absorption leads to more energy dissipation during deformation. LMF, with its higher starch content compared to WF, further elevates the batter's viscosity, resulting in a higher loss modulus. Consequently, the LMF batter exhibited a more viscous behavior than the WF batter. Supporting this, Yu *et al.*<sup>39</sup> found that an increase in barley flour proportion led to a higher loss modulus and a lower storage modulus of the dough. The addition of millet flour may have created new interactions between starch and protein, resulting in higher viscosity in the formulated dough.

The  $\tan \delta$  values ranged from 0.39 (C-100) to 0.72 (T-100) (as shown in Fig. 2) with a significant difference among the samples ( $p < 0.05$ ). The  $\tan \delta$  ratio represents the dough's viscosity-to-elasticity ratio, where a lower  $\tan \delta$  indicates a more elastic structure. The data show that the  $\tan \delta$  value initially decreased and then increased with increasing frequency, suggesting that elasticity is not dominant at higher frequencies. Throughout the tested frequencies,  $G'$  consistently exceeds  $G''$ , indicating that elasticity is the predominant characteristic of the formulated sponge cakes. The addition of the low-moisture flour, *i.e.*, the LMF, notably altered the dough's viscoelastic properties. Varying LMF levels resulted in distinct changes in  $G'$

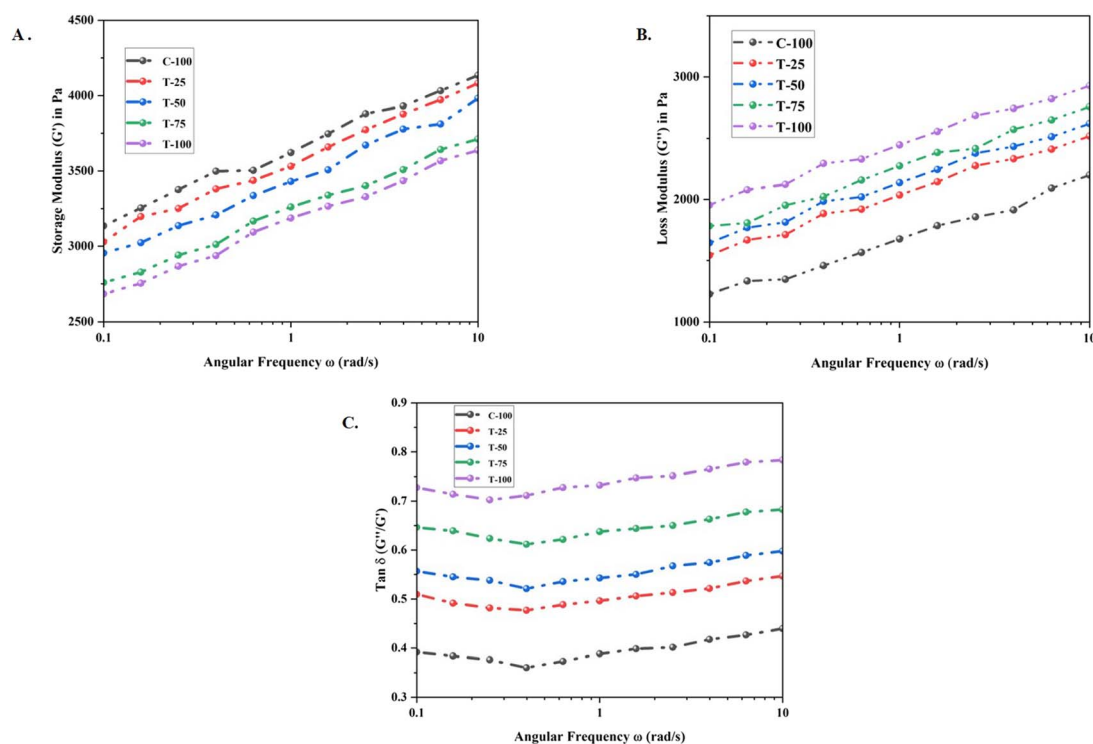


Fig. 2 Dynamic rheological properties of formulated cake batters. (A) Storage modulus ( $G'$ ); (B) loss modulus ( $G''$ ); (C)  $\tan \delta$  ( $G''/G'$ ).



and  $G''$ , likely due to differences in cross-linking and mechanical changes in the gluten network.<sup>40</sup> Consequently, the dough with a more elastic structure (C-100) exhibited the lowest  $\tan \delta$  value.

### 3.3 Instrumental textural profile analysis

Table 3 presents the texture profile analysis for the crust and crumb of cakes. The data indicated that cakes with LMF exhibited higher hardness compared to WF cakes, but the data was insignificant ( $p \geq 0.05$ ). The hardness of the cake crust ranged from  $140.00 \pm 10.24$  N (C-100) to  $145.00 \pm 9.45$  N (T-100); however, not much significant difference between the values was observed ( $p \geq 0.05$ ). For the crumb the hardness values ranged from  $15.00 \pm 1.4$  N (C-100) to  $20.00 \pm 1.69$  N (T-100). The increased hardness observed in cakes with higher LMF content is likely due to a denser batter. Lee *et al.*<sup>41</sup> also reported an increase in cake hardness with the addition of oat bran. With respect to firmness, the crust values ranged from  $16.91 \pm 2.34$  N (C-100) to  $19.36 \pm 2.12$  N (T-100), while crumb values ranged from  $6.56 \pm 1.86$  N for C-100 to  $6.96 \pm 0.96$  N for T-100 with no significant difference among cake formulations ( $p \geq 0.05$ ). Similar trends of crust and crumb firmness were reported by Martinez *et al.*<sup>42</sup> Moreover, significant ( $p < 0.05$ ) variation in chewiness was also observed, which represents the internal strength of the sponge cake, which could be related to the dilution of gluten content by the millet flour. Similar findings were reported by Vinay and Singh.<sup>38</sup> in muffins prepared with pearl millet flour.

Not much variation in cohesiveness, springiness, gumminess and resilience of crust and crumb of cake formulations was observed. However, a decreasing trend with the addition of LMF was observed, which could also be attributed to the reduced

gluten content of LMF. This can be correlated to the results of  $G'$  of cake batter, where the elasticity decreased with decreased gluten content due to LMF addition, leading to reduced cohesiveness. Rajiv *et al.*<sup>43</sup> observed a similar trend in muffins prepared with finger millet flour, where cohesiveness and springiness decreased with increased flour substitution.

### 3.4 Image analysis

Due to water evaporation and gas diffusion, the low viscosity of the batter enhanced the mobility of bubbles, which contributed to surface irregularities. A significant central hole (Fig. 3) was observed in the cake crumb, resulting from substantial gas formation. Turabi *et al.*<sup>44</sup> reported similar findings in rice flour cakes, where low-viscosity batters allowed air bubbles to easily rise to the surface and escape. The samples with WF (C-100) exhibited a significant ( $p < 0.05$ ) increase in expansion height compared to the other samples (Fig. 3), with average bubble sizes categorized as  $<0.5$  mm,  $0.5$ – $1.0$  mm,  $1.0$ – $1.5$  mm,  $1.5$ – $2.0$  mm,  $2.0$ – $3.0$  mm,  $3.0$ – $4.0$  mm,  $4.0$ – $5.0$  mm, and  $>5.0$  mm in a  $3 \times 3$  cm area (Fig. 4). These results suggest improved gas retention and the effective action of leavening agents during baking. The results were consistent with those of Sanz *et al.*,<sup>45</sup> who suggested that dough viscosity influenced bubble incorporation and mobility, both of which are crucial in determining final cake volume. These findings emphasize the importance of batter viscosity in obtaining the overall cake structure. Conversely, T-100, with a greater density and broader range of bubble sizes, resulted in a noticeably smaller cake height. This aligns with the results of Zahn *et al.*,<sup>46</sup> who found that muffins made with added fiber *i.e.* 50% inulin instead of fat had reduced volume without showing significant changes in mass loss during baking.

Table 3 Texture profile analysis of the crust and crumb of formulated cakes<sup>a</sup>

	C-100	T-25	T-50	T-75	T-100
<b>Crust</b>					
Hardness cycle 1 (N)	$140.00 \pm 10.24^a$	$135.00 \pm 8.64^a$	$125.00 \pm 14.86^a$	$130.00 \pm 11.34^a$	$145.00 \pm 9.45^a$
Hardness cycle 2 (N)	$95.00 \pm 6.87^b$	$75.00 \pm 6.41^{ab}$	$65.00 \pm 10.53^a$	$70.00 \pm 9.34^a$	$80.00 \pm 7.92^{ab}$
Firmness (N)	$16.91 \pm 2.34^a$	$19.76 \pm 1.68^a$	$18.45 \pm 2.36^a$	$18.62 \pm 3.52^a$	$19.36 \pm 2.12^a$
Cohesiveness	$0.39 \pm 0.01^b$	$0.38 \pm 0.02^b$	$0.34 \pm 0.02^{ab}$	$0.34 \pm 0.02^{ab}$	$0.32 \pm 0.03^a$
Gumminess (mm)	$18.00 \pm 2.21^a$	$17.00 \pm 1.24^a$	$15.00 \pm 3.21^a$	$14.00 \pm 2.36^a$	$13.00 \pm 1.69^a$
Chewiness (N)	$240.50 \pm 3.98^c$	$210.40 \pm 5.34^b$	$205.00 \pm 5.47^b$	$199.50 \pm 4.26^{ab}$	$186.00 \pm 7.23^a$
Resilience (mJ)	$0.25 \pm 0.02^b$	$0.24 \pm 0.02^{ab}$	$0.22 \pm 0.01^{ab}$	$0.21 \pm 0.01^a$	$0.21 \pm 0.01^a$
Springiness (mm)	$8.65 \pm 0.23^c$	$7.98 \pm 0.78^{bc}$	$6.34 \pm 0.46^{ab}$	$5.45 \pm 1.34^a$	$4.84 \pm 0.54^a$
<b>Crumb</b>					
Hardness cycle 1 (N)	$15.00 \pm 1.4^a$	$25.00 \pm 1.02^c$	$25.00 \pm 1.68^c$	$20.00 \pm 1.62^b$	$20.00 \pm 1.69^b$
Hardness cycle 2 (N)	$9.50 \pm 1.34^a$	$15.00 \pm 1.9^b$	$15.00 \pm 1.58^b$	$10.00 \pm 0.72^a$	$10.00 \pm 1.76^a$
Firmness (N)	$6.56 \pm 1.86^a$	$6.84 \pm 1.68^a$	$5.42 \pm 0.86^a$	$6.64 \pm 2.05^a$	$6.96 \pm 0.96^a$
Cohesiveness	$0.50 \pm 0.03^b$	$0.45 \pm 0.01^{ab}$	$0.44 \pm 0.04^{ab}$	$0.43 \pm 0.01^a$	$0.42 \pm 0.02^a$
Gumminess (mm)	$15.00 \pm 3.67^a$	$15.00 \pm 2.02^a$	$13.00 \pm 1.55^a$	$11.00 \pm 1.86^a$	$10.00 \pm 2.68^a$
Chewiness (N)	$95.00 \pm 4.78^c$	$80.00 \pm 8.42^b$	$69.00 \pm 3.24^{ab}$	$63.00 \pm 5.98^a$	$58.00 \pm 3.86^a$
Resilience (mJ)	$0.24 \pm 0.01^a$	$0.24 \pm 0.02^a$	$0.23 \pm 0.03^a$	$0.22 \pm 0.01^a$	$0.22 \pm 0.01^a$
Springiness (mm)	$6.48 \pm 1.12^a$	$6.39 \pm 0.92^a$	$6.23 \pm 0.56^a$	$6.18 \pm 0.86^a$	$5.56 \pm 1.34^a$

<sup>a</sup> Dissimilar alphabets (a, b, c) in the similar row indicate significance variance ( $P < 0.05$ ). C-100: 100% WF; T-25: 75% WF + 25% LMF; T-50: 50% WF + 50% LMF; T-75: 25% WF + 75% LMF; T-100: 100% LMF.



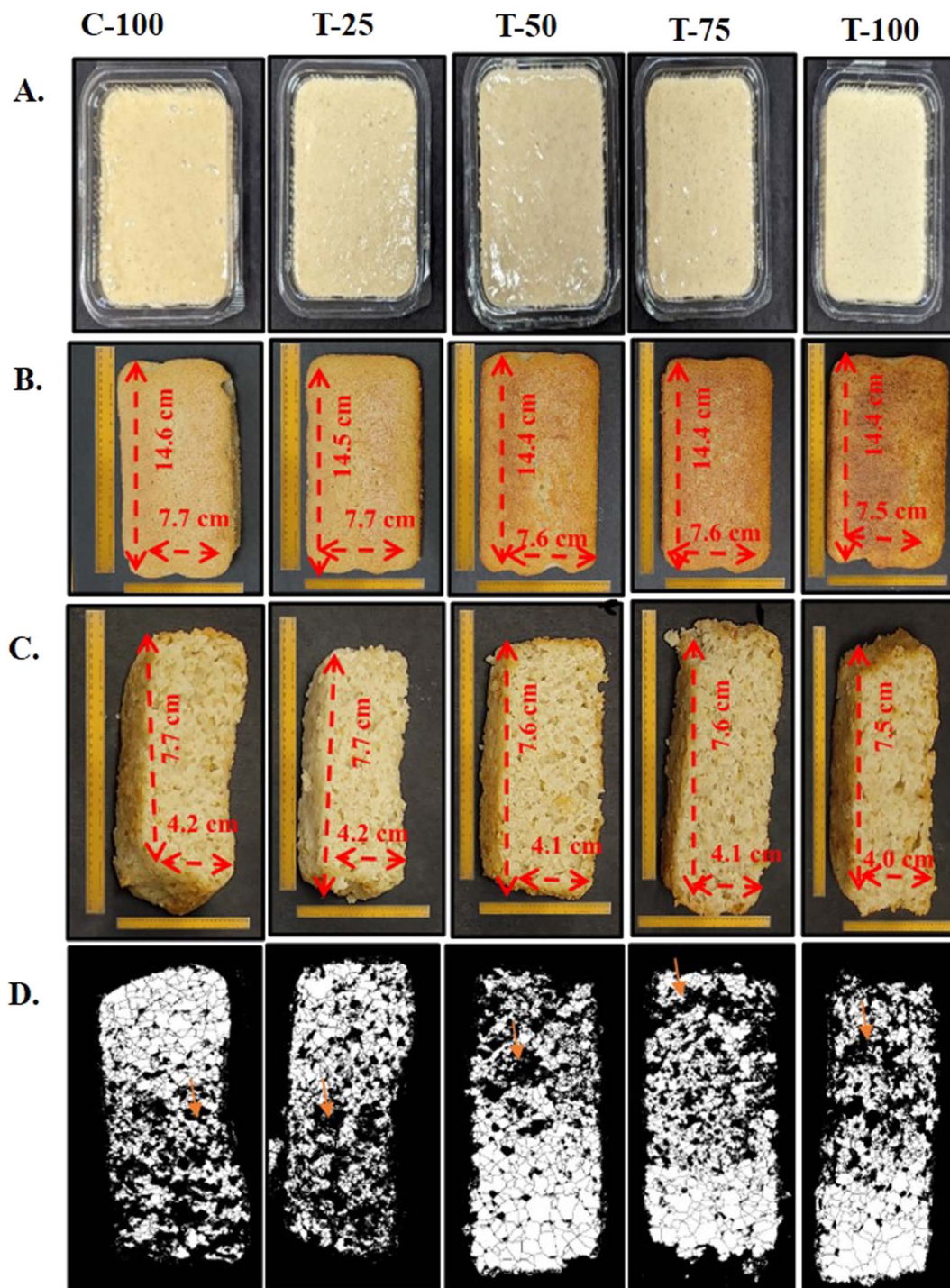


Fig. 3 Image analysis of aquafaba and little millet flour incorporated cakes. (A) Batter; (B) cake; (C) crumb slice; (D) crumb structure and (↓) indicates the central hole.

### 3.5 Bright field microscopy images

The variation in physicochemical characteristics of each cake formulation can be due to the difference in cake morphology. From Fig. 3 and 4 it can be inferred that the crumb structures of all cake formulations differed notably from one another. Specifically, T-100, which did not include WF, restricted the expansion of cakes, resulting in decreased cake height. The cell

circularity and average cell area varied among the crumbs, with T-100 showing larger, more mobile bubbles that were capable of greater growth. Consequently, T-100's crumb structure was characterized by large, interconnected cells and crack-like diffusion channels (Fig. 5). As noted by Kocer *et al.*,<sup>47</sup> increased gas phase mobility facilitated the formation of such diffusion paths. The T-100 cake made solely with LMF had the



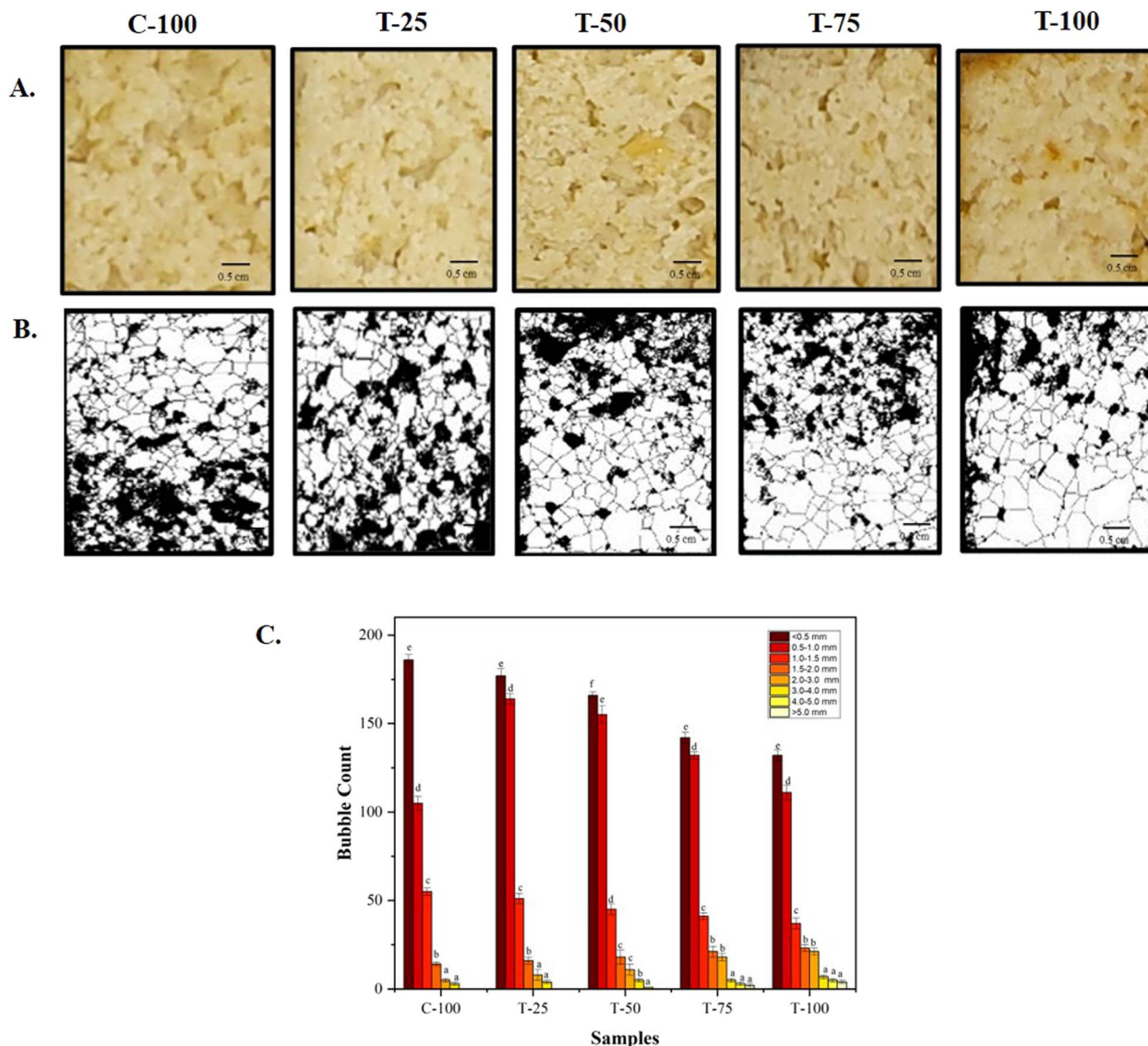


Fig. 4 Bubble count of aquafaba and little millet flour incorporated cake formulations. (A) Crumb ( $3 \times 3$  cm); (B) crumb bubbles ( $3 \times 3$  cm); (C) bubble count ( $3 \times 3$  cm).

least height of all the samples, indicating reduced cake expansion. In contrast, the control cake featured a uniform distribution of smaller cells as compared with other formulated cakes (see Fig. 3 and 4). T-100's crumb showed a more heterogeneous cell distribution, with larger cells and denser regions, reflected by a significant ( $p < 0.05$ ) increase in mean cell size and decrease in circularity values. Specifically, the circularity values for C-100 and T-100 were 0.77 and 0.71, while cell area values were  $0.61 \mu\text{m}^2$  and  $0.78 \mu\text{m}^2$ , respectively (see Fig. 5). This can be attributed to higher loss modulus ( $G''$ ) values, which typically indicates greater energy dissipation, which can result in a less stable structure. If the batter is too fluid due to a high  $G''$ , it may not support the formation of well-defined air cells, leading to a more irregular shape, leading to reduced circularity. These findings aligned with those of Tsatsaragkou *et al.*,<sup>23</sup> who observed lower cell circularity values in cakes and biscuits made with inulin as a sugar replacer.

### 3.6 Fourier transform infrared spectroscopy analysis of sponge cakes

The examination of FTIR spectra (Fig. 6) revealed notable differences among the samples, particularly between C-100 and T-100, while other cake formulations showed minimal variations. Peaks in the  $3800\text{--}2500 \text{ cm}^{-1}$  range were associated with O-H bond stretching, which potentially indicated the water molecules, visibly present in all samples. Specifically, a peak at  $3786.5 \text{ cm}^{-1}$  was observed in both C-100 and T-100. Peaks at  $2931.32 \text{ cm}^{-1}$  and  $2845.14 \text{ cm}^{-1}$  appeared in all samples, which represent the C-H stretching of methoxyl groups related to the lignin component.<sup>48</sup> The peak near  $2845.14 \text{ cm}^{-1}$  was clearly visible in all the samples batter (LMF) except for C-100, inferring the absence of fibre. The  $3500\text{--}3100 \text{ cm}^{-1}$  range, linked to the N-H stretching of amide A, was consistent across all samples, with a peak at  $3447.6 \text{ cm}^{-1}$ .<sup>49,50</sup> Additionally, peaks associated



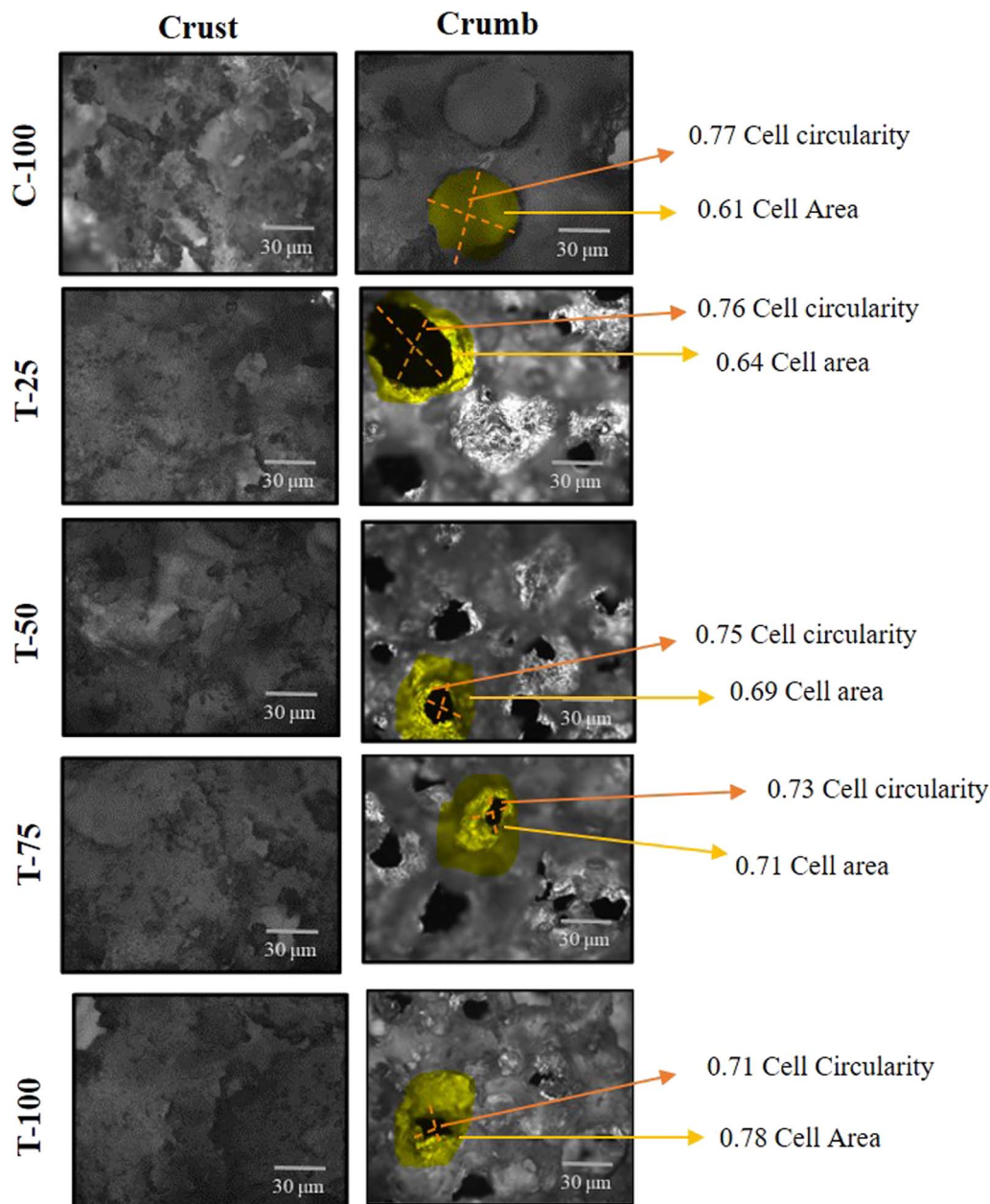


Fig. 5 Bright field microscopic images of aquafaba and little millet flour incorporated cakes representing cell circularity. Sample were: C-100; T-25, T-50, T-75, and T-100 respectively.

with amide groups were identified at  $1660.73\text{ cm}^{-1}$  and  $1454.71\text{ cm}^{-1}$ , representing amide-I and amide-II, respectively. These peaks were significantly diminished with baking, reflecting typical denaturation of proteins while baking.

Carbohydrates, identified by peaks in the  $1200\text{--}900\text{ cm}^{-1}$  range, were present in all spectra, with notable peaks at  $936\text{ cm}^{-1}$ ,  $1024.62\text{ cm}^{-1}$ , and  $1158.41\text{ cm}^{-1}$ . The presence of a peak at  $1158.41\text{ cm}^{-1}$  in T-100 suggests distinct starch derivatives from LMF, influenced by heat treatment and biological origin. This peak corresponds to the crystalline area of starch,<sup>50</sup> while the peak at  $1022\text{ cm}^{-1}$  is the amorphous phase of starch granules and the range of  $995\text{--}900\text{ cm}^{-1}$  marks the molecular order and crystalline areas.<sup>51</sup>

### 3.7 Shelf-life

The shelf-life analysis of the cake is summarized in Table 3, which examines the effects of ambient storage at  $30 \pm 1\text{ }^{\circ}\text{C}$  for over 12 days on the cake's bacteriological quality, particularly when WF is replaced by LMF. During storage, the cake's moisture content, ash content, fat content, and weight decreased, while microbial populations increased. Total plate count showed no significant change during the first 3 days of storage at  $30\text{ }^{\circ}\text{C}$ , suggesting a lag phase for bacterial adaptation. Yeast and mold counts, as well as coliform bacteria, increased more slowly compared to total plate counts and remained low throughout the storage period. By the end of 12 days, the total plate count had risen to  $17.2 \times 10^3 \pm 0.3\text{ CFU g}^{-1}$ , which



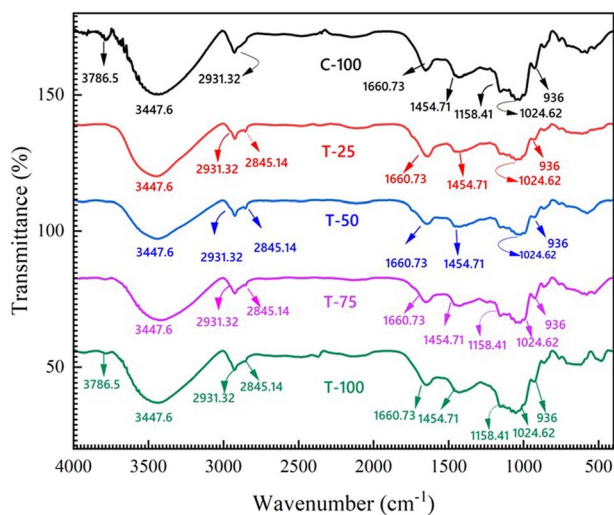


Fig. 6 FTIR fingerprints of aquafaba and little millet flour incorporated cakes.

correlated with a decline in the cake's sensory properties. This increase in total plate count, particularly on the 9th and 12th days, can be attributed to the ideal growth conditions of  $30 \pm 1 \text{ }^\circ\text{C}$ . The temperature range of  $4.4\text{--}60 \text{ }^\circ\text{C}$  is generally recognized as the danger zone where microorganisms proliferate most rapidly.<sup>52</sup> Extended exposure to ambient temperatures can lead to higher microbial loads, potentially reaching unsafe levels, posing significant health risks to humans (Table 4).

### 3.8 Sensory evaluation

The sensory evaluations revealed significant differences in appearance, color, flavor, body, texture, aftertaste, and overall acceptability among the cakes (Fig. 7). Sensory scores generally improved with increasing amounts of LMF in the cake composition. Notably, the crust color became darker with higher LMF content, reflecting a trend toward more intense coloration. The addition of LMF, known for its nutritional benefits, enriched the overall flavor profile of the cakes, imparting a better taste. The texture of the cake is influenced by its moist and tender

Table 4 Shelf-life study of formulated cakes along with the control at  $30 \pm 1 \text{ }^\circ\text{C}$  for 12 days<sup>a</sup>

		0 <sup>th</sup> Day	3rd Day	6th Day	9th Day	12th Day
Moisture (%)	C-100	24.37 $\pm$ 1.54 <sup>c</sup>	24.01 $\pm$ 0.83 <sup>c</sup>	22.83 $\pm$ 0.11 <sup>d</sup>	20.74 $\pm$ 1.01 <sup>cd</sup>	20.36 $\pm$ 0.34 <sup>c</sup>
	T-25	23.64 $\pm$ 0.37 <sup>c</sup>	23.43 $\pm$ 0.73 <sup>c</sup>	22.28 $\pm$ 0.23 <sup>cd</sup>	21.57 $\pm$ 0.85 <sup>d</sup>	21.38 $\pm$ 0.74 <sup>c</sup>
	T-50	22.17 $\pm$ 0.56 <sup>bc</sup>	21.25 $\pm$ 1.02 <sup>b</sup>	20.91 $\pm$ 0.54 <sup>bc</sup>	19.38 $\pm$ 0.22 <sup>bc</sup>	18.28 $\pm$ 0.69 <sup>b</sup>
	T-75	20.41 $\pm$ 1.65 <sup>ab</sup>	20.14 $\pm$ 0.56 <sup>ab</sup>	19.79 $\pm$ 0.85 <sup>b</sup>	18.48 $\pm$ 0.45 <sup>ab</sup>	17.63 $\pm$ 0.63 <sup>ab</sup>
	T-100	18.69 $\pm$ 1.03 <sup>a</sup>	18.40 $\pm$ 0.34 <sup>a</sup>	18.11 $\pm$ 0.79 <sup>a</sup>	16.82 $\pm$ 0.37 <sup>a</sup>	16.40 $\pm$ 0.91 <sup>a</sup>
Ash (%)	C-100	1.24 $\pm$ 0.04 <sup>a</sup>	1.24 $\pm$ 0.02 <sup>a</sup>	1.25 $\pm$ 0.11 <sup>a</sup>	1.25 $\pm$ 0.12 <sup>a</sup>	1.26 $\pm$ 0.75 <sup>a</sup>
	T-25	1.29 $\pm$ 0.2 <sup>a</sup>	1.29 $\pm$ 0.01 <sup>ab</sup>	1.30 $\pm$ 0.03 <sup>a</sup>	1.31 $\pm$ 0.23 <sup>a</sup>	1.32 $\pm$ 0.56 <sup>a</sup>
	T-50	1.34 $\pm$ 0.05 <sup>a</sup>	1.34 $\pm$ 0.1 <sup>ab</sup>	1.35 $\pm$ 0.04 <sup>a</sup>	1.36 $\pm$ 1.01 <sup>a</sup>	1.37 $\pm$ 0.68 <sup>a</sup>
	T-75	1.40 $\pm$ 0.07 <sup>a</sup>	1.40 $\pm$ 0.07 <sup>b</sup>	1.50 $\pm$ 0.06 <sup>a</sup>	1.50 $\pm$ 0.68 <sup>a</sup>	1.60 $\pm$ 0.22 <sup>a</sup>
	T-100	1.44 $\pm$ 0.03 <sup>a</sup>	1.44 $\pm$ 0.02 <sup>b</sup>	1.50 $\pm$ 0.3 <sup>a</sup>	1.56 $\pm$ 0.45 <sup>a</sup>	1.60 $\pm$ 0.23 <sup>a</sup>
Fat (%)	C-100	13.19 $\pm$ 0.53 <sup>c</sup>	12.99 $\pm$ 0.54 <sup>b</sup>	12.47 $\pm$ 0.43 <sup>c</sup>	11.98 $\pm$ 0.22 <sup>c</sup>	11.02 $\pm$ 0.11 <sup>c</sup>
	T-25	12.94 $\pm$ 0.24 <sup>c</sup>	12.81 $\pm$ 0.75 <sup>ab</sup>	11.84 $\pm$ 0.57 <sup>c</sup>	11.39 $\pm$ 0.17 <sup>c</sup>	10.31 $\pm$ 0.24 <sup>c</sup>
	T-50	12.34 $\pm$ 0.42 <sup>bc</sup>	11.91 $\pm$ 0.55 <sup>ab</sup>	11.60 $\pm$ 0.15 <sup>bc</sup>	11.29 $\pm$ 0.76 <sup>bc</sup>	10.25 $\pm$ 0.87 <sup>bc</sup>
	T-75	11.58 $\pm$ 0.5 <sup>ab</sup>	11.19 $\pm$ 0.98 <sup>a</sup>	10.80 $\pm$ 0.22 <sup>ab</sup>	9.85 $\pm$ 0.71 <sup>ab</sup>	8.79 $\pm$ 0.67 <sup>ab</sup>
	T-100	11.13 $\pm$ 0.16 <sup>a</sup>	11.09 $\pm$ 0.32 <sup>a</sup>	10.50 $\pm$ 0.24 <sup>a</sup>	9.53 $\pm$ 0.62 <sup>a</sup>	8.22 $\pm$ 0.45 <sup>a</sup>
Weight (g)	C-100	170.00 $\pm$ 1.34 <sup>a</sup>	168.00 $\pm$ 0.67 <sup>a</sup>	167.00 $\pm$ 0.81 <sup>a</sup>	164.00 $\pm$ 0.79 <sup>ab</sup>	161.00 $\pm$ 0.56 <sup>bc</sup>
	T-25	171.00 $\pm$ 1.56 <sup>a</sup>	170.00 $\pm$ 1.11 <sup>ab</sup>	168.00 $\pm$ 0.64 <sup>ab</sup>	166.00 $\pm$ 0.69 <sup>b</sup>	163.00 $\pm$ 0.66 <sup>d</sup>
	T-50	172.00 $\pm$ 0.57 <sup>a</sup>	171.00 $\pm$ 1.33 <sup>ab</sup>	168.00 $\pm$ 0.55 <sup>ab</sup>	164.00 $\pm$ 0.67 <sup>ab</sup>	160.00 $\pm$ 0.34 <sup>ab</sup>
	T-75	173.00 $\pm$ 2.1 <sup>a</sup>	171.00 $\pm$ 1.45 <sup>ab</sup>	167.00 $\pm$ 0.43 <sup>a</sup>	163.00 $\pm$ 1.1 <sup>a</sup>	159.00 $\pm$ 0.11 <sup>a</sup>
	T-100	174.00 $\pm$ 1.86 <sup>a</sup>	172.00 $\pm$ 0.95 <sup>b</sup>	169.00 $\pm$ 0.21 <sup>b</sup>	166.00 $\pm$ 1.3 <sup>b</sup>	162.00 $\pm$ 0.38 <sup>cd</sup>
Total plate count (CFU g <sup>-1</sup> )	C-100	10.0 $\pm$ 2 <sup>a</sup>	2.0 $\pm$ 0.1 $\times$ 10 <sup>2b</sup>	7.1 $\pm$ 0.2 $\times$ 10 <sup>2c</sup>	10.3 $\pm$ 0.3 $\times$ 10 <sup>3b</sup>	1.72 $\pm$ 0.03 $\times$ 10 <sup>4b</sup>
	T-25	8.0 $\pm$ 2 <sup>a</sup>	1.5 $\pm$ 0.1 $\times$ 10 <sup>2a</sup>	6.9 $\pm$ 0.3 $\times$ 10 <sup>2bc</sup>	9.4 $\pm$ 0.3 $\times$ 10 <sup>3a</sup>	1.69 $\pm$ 0.04 $\times$ 10 <sup>4 ab</sup>
	T-50	8.0 $\pm$ 1 <sup>a</sup>	1.3 $\pm$ 0.1 $\times$ 10 <sup>2a</sup>	6.6 $\pm$ 0.3 $\times$ 10 <sup>2abc</sup>	8.9 $\pm$ 0.4 $\times$ 10 <sup>3a</sup>	1.64 $\pm$ 0.04 $\times$ 10 <sup>4 ab</sup>
	T-75	7.0 $\pm$ 2 <sup>a</sup>	1.2 $\pm$ 0.2 $\times$ 10 <sup>2a</sup>	6.4 $\pm$ 0.2 $\times$ 10 <sup>2 ab</sup>	8.8 $\pm$ 0.3 $\times$ 10 <sup>3a</sup>	1.63 $\pm$ 0.05 $\times$ 10 <sup>4 ab</sup>
	T-100	6.0 $\pm$ 1 <sup>a</sup>	1.2 $\pm$ 0.2 $\times$ 10 <sup>2a</sup>	6.2 $\pm$ 0.2 $\times$ 10 <sup>2a</sup>	8.6 $\pm$ 0.3 $\times$ 10 <sup>3a</sup>	1.59 $\pm$ 0.02 $\times$ 10 <sup>4a</sup>
Yeast and Moulds (CFU g <sup>-1</sup> )	C-100	4.0 $\pm$ 1 <sup>a</sup>	1.5 $\pm$ 0.3 $\times$ 10 <sup>1b</sup>	3.0 $\pm$ 0.4 $\times$ 10 <sup>2b</sup>	5.0 $\pm$ 0.3 $\times$ 10 <sup>2c</sup>	7.5 $\pm$ 0.3 $\times$ 10 <sup>3c</sup>
	T-25	4.0 $\pm$ 1 <sup>a</sup>	1.5 $\pm$ 0.3 $\times$ 10 <sup>1b</sup>	3.0 $\pm$ 0.3 $\times$ 10 <sup>2b</sup>	4.3 $\pm$ 0.3 $\times$ 10 <sup>2bc</sup>	5.0 $\pm$ 0.4 $\times$ 10 <sup>3a</sup>
	T-50	3.0 $\pm$ 1 <sup>a</sup>	1.0 $\pm$ 0.3 $\times$ 10 <sup>1a</sup>	2.5 $\pm$ 0.2 $\times$ 10 <sup>2 ab</sup>	4.0 $\pm$ 0.4 $\times$ 10 <sup>2 ab</sup>	6.5 $\pm$ 0.3 $\times$ 10 <sup>3b</sup>
	T-75	3.0 $\pm$ 1 <sup>a</sup>	0.5 $\pm$ 0.2 $\times$ 10 <sup>1a</sup>	2.5 $\pm$ 0.2 $\times$ 10 <sup>2 ab</sup>	3.2 $\pm$ 0.4 $\times$ 10 <sup>2a</sup>	5.5 $\pm$ 0.2 $\times$ 10 <sup>3a</sup>
	T-100	3.0 $\pm$ 1 <sup>a</sup>	0.5 $\pm$ 0.1 $\times$ 10 <sup>1a</sup>	2.0 $\pm$ 0.1 $\times$ 10 <sup>2a</sup>	3.7 $\pm$ 0.3 $\times$ 10 <sup>2 ab</sup>	6.5 $\pm$ 0.3 $\times$ 10 <sup>3b</sup>
Coliform bacteria (CFU g <sup>-1</sup> )	C-100	3.0 $\pm$ 2 <sup>a</sup>	1.0 $\pm$ 0.2 $\times$ 10 <sup>1a</sup>	4.0 $\pm$ 0.2 $\times$ 10 <sup>1b</sup>	9.0 $\pm$ 0.3 $\times$ 10 <sup>1d</sup>	14.0 $\pm$ 0.3 $\times$ 10 <sup>1d</sup>
	T-25	3.0 $\pm$ 1 <sup>a</sup>	1.0 $\pm$ 0.4 $\times$ 10 <sup>1a</sup>	3.0 $\pm$ 0.2 $\times$ 10 <sup>1a</sup>	7.0 $\pm$ 0.4 $\times$ 10 <sup>1b</sup>	12.0 $\pm$ 0.2 $\times$ 10 <sup>1b</sup>
	T-50	2.0 $\pm$ 1 <sup>a</sup>	0.7 $\pm$ 0.3 $\times$ 10 <sup>1a</sup>	4.0 $\pm$ 0.2 $\times$ 10 <sup>1b</sup>	8.0 $\pm$ 0.3 $\times$ 10 <sup>1c</sup>	13.0 $\pm$ 0.2 $\times$ 10 <sup>1c</sup>
	T-75	2.0 $\pm$ 2 <sup>a</sup>	1.0 $\pm$ 0.3 $\times$ 10 <sup>1a</sup>	4.0 $\pm$ 0.3 $\times$ 10 <sup>1b</sup>	7.0 $\pm$ 0.4 $\times$ 10 <sup>1b</sup>	11.0 $\pm$ 0.2 $\times$ 10 <sup>1a</sup>
	T-100	2.0 $\pm$ 2 <sup>a</sup>	0.9 $\pm$ 0.2 $\times$ 10 <sup>1a</sup>	3.0 $\pm$ 0.3 $\times$ 10 <sup>1a</sup>	6.0 $\pm$ 0.4 $\times$ 10 <sup>1a</sup>	12.0 $\pm$ 0.2 $\times$ 10 <sup>1b</sup>

<sup>a</sup> Dissimilar alphabets (a, b, c, d) in the similar row indicate significance variance ( $P < 0.05$ ). C-100: 100% WF; T-25: 75% WF + 25% LMF; T-50: 50% WF + 50% LMF; T-75: 25% WF + 75% LMF; T-100: 100% LMF.



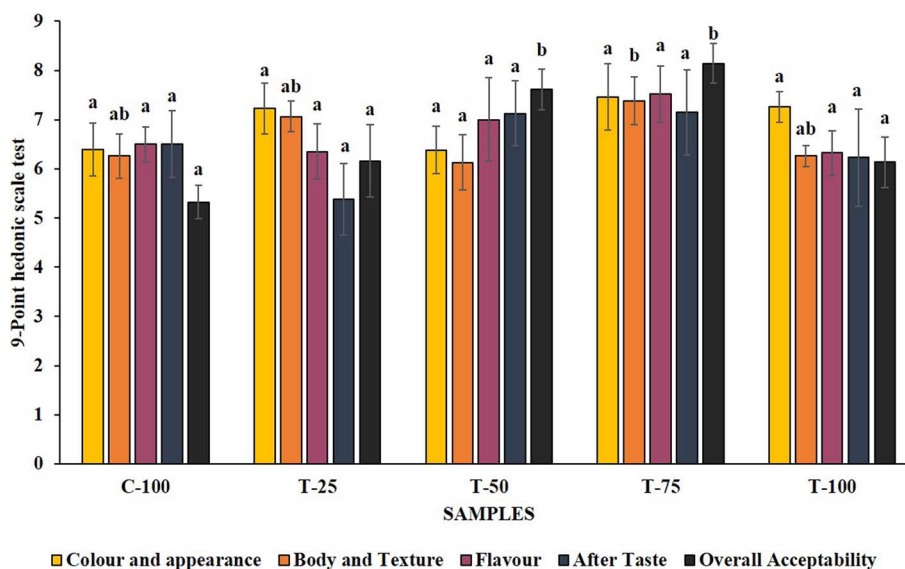


Fig. 7 Sensory evaluation of aquafaba and little millet flour incorporated cake formulations.

mouthfeel. A higher storage modulus ( $G'$ ) indicates a well-developed gluten network that provides a light and airy texture, enhancing the perception of softness. Conversely, a higher loss modulus ( $G''$ ) negatively affects the texture. These results align with rheological characteristics, showing a significantly ( $p < 0.05$ ) lower textural acceptability for cakes incorporated with LMF. However, the flavor score improved with the addition of LMF, with the highest being observed for the sample incorporated with 75% LMF (T-75), influencing the overall acceptance of the cake incorporated with LMF for substituting WF. These findings aligned with the results of Vinay and Singh,<sup>38</sup> who found the highest flavor score for eggless muffins incorporated with pearl millet flour compared to refined WF.

## 4. Conclusion

This study demonstrated the potential utilization of aquafaba based emulsion, which is often discarded as waste. Substituting wheat flour (WF) with little millet flour (LMF) showed a significant increase in calorific value. It was found that for all the cake formulations, there was an increase in both hardness and firmness values with LMF substitution. The differences in cake dimensions with the incorporation of LMF were clearly observed from image analysis, which showed that the C-100 sample had an even distribution of cells while the T-100 sample had a more uneven distribution of cells. The microbial shelf-life analysis revealed higher shelf life for the T-100 cake sample compared to C-100 with an average shelf life of 6 days. Sensory evaluation revealed higher acceptability for LMF cakes. Thus, it was concluded that the substitution of LMF for WF in aquafaba incorporated egg-less cake formulations was successful in improving the sensory properties, shelf life and calorific values without much significant changes in cake quality characteristics. Future research could explore the utilization of LMF and aquafaba in largescale production of eggless

and gluten free formulations for other baked goods for broader application. Furthermore, a comparative analysis with other sugar alternatives, such as unprocessed sugars like raw honey, brown rice syrup, and jaggery powder, could further enhance the health and sustainability aspects of formulated cakes in the industrial sector.

## Abbreviations

WF:	Wheat flour
LMF	Little millet flour
SNF	Solid non-fat
FTIR	Fourier transform infrared spectroscopy
mL	Milliliters
L	Liters
mg	Milligrams
$G'$	Storage modulus
$G''$	Loss modulus
cP	Centipoise
Pa	Pascal
Hz	Hertz
N	Newton

## Data availability

The data supporting this article have been included in the main document.

## Author contributions

Bijjam Madhavi: investigation; methodology; data curation; formal analysis; visualization; validation; writing – original draft. Niveditha Asaithambi: investigation; methodology; data curation; formal analysis; visualization; validation; writing –



original draft; Alok Kumar: investigation; methodology; data curation; formal analysis; Pralay Maiti: visualization; writing – reviewing and editing; Dinesh Chandra Rai: formal analysis; visualization; writing – reviewing and editing; Raj Kumar Duary: conceptualization; visualization; formal analysis; resources; supervision; writing – reviewing and editing.

## Conflicts of interest

The authors declare no conflict of interest.

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