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# Formulation, Characterization, and *In Vitro* Simulated Gastrointestinal Fluid Analysis of Chewable Yogurt Tablet Incorporated with Corncob Fiber

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#### ABSTRACT

This study formulates and evaluates a novel functional food, corncob fiber-infused chewable yogurt tablets, to enhance nutritional value. The tablets have the potential to alleviate gastrointestinal symptoms in the elderly and combat malnutrition in selective eaters, potentially replacing multiple supplement tablets. Four batches of tablets underwent rigorous evaluation, considering physicochemical properties, shelf life, and probiotic viability in simulated gastrointestinal conditions. All tablets exhibited robust stability

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joshlyn\_chan@outlook.com (Yong Lin Chan) nurulaini.jamal92@gmail.com (Nurul Aini Jamalullail) tancp@upm.edu.my (Chin Ping Tan) yazid.manap@gmail.com (Mohd Yazid Abdul Manap) teck.kim@mpob.gov.my (Teck Kim Tang) lee.yeeying@monash.edu (Yee Ying Lee) engtong.phuah@utb.edu.bn (Eng Tong Phuah) omlai@upm.edu.my (Oi Ming Lai) \*Corresponding author against simulated fluids (85–90% survival rate) and met desired physicochemical benchmarks. Notably, F1 had the lowest hardness (9.50 kp/cm<sup>2</sup>), while tensile strength showed no significant variance (0.93–1.18 N/mm<sup>2</sup>) between tablets. However, F3 and F4 displayed significantly longer disintegration times (41.11–52.82 min). After three months, the average bacterial viability was 7 log no. CFU/g, highlighting the tablets' potential to deliver intact probiotics for immediate beneficial effects upon consumption. Thus, these chewable yogurt tablets offer a promising means to deliver probiotics effectively while addressing specific dietary challenges.

*Keywords*: Chewable yogurt tablet, corncob fiber, physicochemical properties, shelf-life study, simulated gastrointestinal fluid analysis

## INTRODUCTION

Yogurt is normally obtained via milk fermentation by lactic acid bacteria such as Lactobacillus bulgaricus and Streptococcus thermophilus (Cajigas, 1990). It is a betterdigestible dairy product with several known health benefits due to its high probiotics, calcium, and protein content (Kumar & Mishra, 2004; Mckinley, 2005). Past research has demonstrated that the lactic acid bacteria in yogurt act as probiotics, improving the properties of the indigenous microflora in the human gastrointestinal tract and reducing the risk of developing irritable bowel syndrome (EFSA Panel on Dietetic Products, Nutrition and Allergies [NDA], 2010; Guarino et al., 2013; Xie et al., 2023).

Due to people's awareness of its alimental values, yogurt consumption has increased tremendously in the past decade, making it the second-largest segment of the Asia-Pacific dairy market (26.28%) in 2023 (Mordor Intelligence, n.d.). As one of the functional food components potentially offering additional health benefits to customers, yogurt is undoubtedly worth studying (Granato et al., 2020). It has enormous potential to become the next big

thing in the functional food industry, with a projected market value of USD 63.96 billion by 2029 (Mordor Intelligence, n.d.). Yogurt's high digestibility is one of the main factors contributing to the rising yogurt consumption in the Asia-Pacific region (Y. L. Chan et al., 2019). Asians avoid dairy products in their diets and shopping decisions since most (up to 95%) are lactose intolerant (Goh et al., 2018). Asians, often lactose intolerant, can still enjoy the benefits of dairy and probiotics by choosing yogurt owing to its low lactose content (3.5-6%)(Y. L. Chan et al., 2019). Hence, innovation in product development of yogurt's diversity to meet specific nutritional needs is crucial in contributing to and broadening the dairy market share and income.

Many new yogurt products have been developed over the past decades, such as plant-based yogurt, Greek yogurt, drinkable yogurt, yogurt pie, prebiotic enriched yogurt, yogurt ice cream, yogurt gummies, and yogurt puff (Malaysian-German Chamber of Commerce and Industry & Brandt, 2015). Yogurt products, however, are primarily categorized into two groups: (1) set yogurt, where probiotics are directly inoculated without fermentation, and (2) stirred yogurt, which is fermented by lactic acid bacteria (i.e., probiotics) (Hersh, 2021). The drawbacks of products from the group that does not undergo fermentation include lower adherence to existing food standards regarding labeling (e.g., active bacterial count) and the need to include additional ingredients (e.g., fillers, preservatives, flavorings, food coloring, sweeteners, acid regulators, and food texture modifiers) to achieve the desired organoleptic properties of the product (Faccia, 2020; Y. L. Chan et al., 2019). Although direct incorporation of freeze-dried probiotic strains can guarantee the number of bacteria in pharmaceutical products, it fails to offer the advantages of fermentation, such as the production of natural aromatic compounds (like acetaldehyde), beneficial metabolites (like folic acid), and the conversion of lactose to galactose (Baglio, 2014; Li et al., 2023).

Moreover, researchers have successfully enhanced daily fiber intake by integrating fiber into yogurt, developing a novel functional food with a wide range of advantageous effects. Several studies reported that fiber enhances intestinal microflora growth and gastrointestinal immunity (Dabija et al., 2018; Daud et al., 2018; Gilliland, 1990; Shah, 2007). A person's health may also be improved by consuming more fiber as it lowers the risk of developing constipation, obesity, diabetes, cancer, hypercholesterolemia, gastrointestinal issues, ulcerative colitis, hyperlipidemia, hypertension, and heart disease (Hoppert et al., 2013; Ramirez-Santiago et al., 2010; R. K. Robinson, 1992; Tomic et al., 2017).

Fiber is an indigestible plant carbohydrate that travels through the stomach and intestines unchanged. There are two types of fiber: soluble (i.e., pectins, gums, and mucilage) and insoluble (i.e., cellulose, hemicelluloses, and lignin). Insoluble fibers are usually used as food fortification ingredients due to their beneficial function in relieving fiber-lacking-associated illnesses such as constipation (Bertolino et al., 2015; Dabija et al., 2018; Sah et al., 2016). Poor fiber intake is also one of the major contributors to the development of illnesses associated with malnutrition (Daud et al., 2018; R. K. Robinson, 1992). According to research by Reynolds et al. (2019) dietary fiber intake of at least 25 to 29 g per day was associated with a significant risk reduction for a range of critical outcomes (Reynolds et al., 2019). Despite this, many people fail to achieve the lower end of the suggested range.

Corn is the third main cereal crop in the world, after rice and wheat. The increase in corn production worldwide has led to an increase in corncob waste. These corncobs contain a significant amount of fiber (38.52%) and offer a desirable natural corn flavor (Lee et al., 2019). Moreover, it is a good bulking agent as it can promote satiety and play an essential role in weight management (Lee et al., 2018). Thus, the fiber powder from corncob can be a suitable insoluble fiber source that could be used as a food fortification ingredient.

Consumers increasingly seek convenient and quick nutritious foods to support a healthy diet. The development of these chewable yogurt tablets, enriched with corncob fiber, aims to address swallowing difficulties in the elderly and children while also offering a potential alternative to improve the nutrient intake among selective eaters, potentially reducing the need for multiple supplement tablets (Rana et al., 2011).

Thus, this study aims to formulate and assess chewable yogurt tablets enriched with corncob fiber. It investigated the impact of different formulation factors on the tablets' physical and chemical attributes (titratable acidity, hardness, tensile strength, pH, friability, disintegration, color profile), as well as the survival and stability of lactic acid bacteria (probiotics) within the tablets when subjected to simulated gastrointestinal conditions. Additionally, the study conducted a three-month shelflife analysis of the optimal chewable vogurt tablet formulation under refrigerated conditions (4°C) to compare its lactic acid bacteria (probiotics) viability against a control group.

#### MATERIALS AND METHODS

#### Materials

Skim milk powder, whey protein isolate, fish gelatin powder (Halal), and zip-lock plastic bag were purchased from Edible Food Sdn. Bhd. in Kuala Lumpur, Malaysia. De Man-Rogosa-Sharpe (MRS) agar and peptone water were obtained from Oxoid, United Kingdom. Magnesium stearate, silicon dioxide, lactose, mannitol, talcum powder, starch, and sodium starch glycolate were procured from R&M Chemicals, Malaysia. Phenolphthalein, sodium hydroxide, hydrochloric acid, sodium acetate, acetic acid, ethanol and sterile Petri dish were obtained from Fisher Scientific (Malaysia). Corncob was purchased from NSK Trade City Sdn. Bhd. in Kuala Lumpur, Malaysia.

#### Methods

## Preparation of Corncob Fiber (CF) Powder

Corncobs were cleaned with distilled water and cut into small pieces. After that, corncob pieces were dried in an oven (Memmert, Germany) at 60°C for 36 hr. Dried corncob pieces were ground into finer powder using a heavy-duty blender (Waring Commercial, USA). After that, they were sieved at 100 amplitudes using a vibratory sieve shaker AS 200 basic (Retsch, Germany) with a 40-mesh sieve. The fine powder obtained was sealed in a zip-lock plastic bag and stored at -18°C prior to any analysis (Lee et al., 2019).

#### **Preparation of Fermented Yogurt Powder**

A spray-dried fermented yogurt powder, possessing an active bacterial count of not less than 9 log no. CFU/g, was procured from Universiti Putra Malaysia. The manufacturing process involved spray drying a 10% skim milk solution, subjected to anaerobic fermentation at 38±2°C, with a 2% bacterial strain inoculation. The bacterial strain consisted of a mix-strain culture of Lactobacillus delbrueckii subsp. bulgaricus BSL1 (ATCC® 11842<sup>TM</sup>) and Streptococcus salivarius subsp. thermophilus (ATCC® 19258<sup>™</sup>) in a 1:1 ratio. The spray drying process parameters were maintained at an inlet temperature of 110±5°C, outlet temperature of 60±5°C, and a feed rate of 20 ml/min. The oral administration of this product is constrained in accordance with the guidelines set forth by the American Type Culture Collection (ATCC) (n.d.a, n.d.b).

#### **Preparation of Chewable Yogurt Tablet**

A chewable yogurt tablet was prepared via direct compression based on the formulation Khokra et al. (2012) suggested with some modifications. All the ingredients were weighed individually according to the formulation (F1, F2, F3, and F4) shown in Table 1 and sieved at 100 amplitudes by using a vibratory sieve shaker AS 200 basic (RETSCH, Germany) with a 40-mesh sieve. After that, all the ingredients were blended in a zip-lock plastic bag for 10 min. The above blend was then lubricated with glidants (silicon dioxide, talc, and magnesium stearate) for 2 min. The final blend was compressed into 18 mm diameter right circular cylinder tablets of 1,000

mg weight each using a manual tablet compression machine at 100 MPa.

## Physicochemical Properties Analysis on Chewable Yogurt Tablet

**Color Analysis.** A CR-410 Chroma Meter (Konica Minolta, Inc., Japan) was used to analyze the color of the sample for the values of L\* (lightness), a\* (redness), and b\* (yellowness). A white calibration plate was used to calibrate the system before sample analysis. Samples were filled and sealed into a transparent zip-lock plastic pouch (7.5 cm x 5 cm). The measuring head of the CR-410 Chroma Meter was then placed on top of the samples securely to obtain the color values in triplicate (Lee et al., 2019).

| Ingredients             |       | Form  | ulation |       |
|-------------------------|-------|-------|---------|-------|
| -                       | F1    | F2    | F3      | F4    |
| Intragranular           |       |       |         |       |
| Fermented yogurt powder | 47.50 | 47.50 | 47.50   | 47.50 |
| Skim milk powder        | 2.50  | 2.50  | -       | -     |
| Gelatin powder          | -     | -     | 2.50    | 2.50  |
| Extragranular           |       |       |         |       |
| Whey protein isolate    | 17.50 | 7.50  | 17.50   | 7.50  |
| Corncob fiber powder    | -     | 10    | -       | 10    |
| Mannitol                | 12.50 | 12.50 | 12.50   | 12.50 |
| Maize starch            | 10    | 10    | 10      | 10    |
| Silicon dioxide         | 1.50  | 1.50  | 1.50    | 1.50  |
| Magnesium stearate      | 0.75  | 0.75  | 0.75    | 0.75  |
| Sodium starch glycolate | 3.50  | 3.50  | 3.50    | 3.50  |
| Talc                    | 0.75  | 0.75  | 0.75    | 0.75  |
| Lactose                 | 3.50  | 3.50  | 3.50    | 3.50  |
| Total (%)               | 100   | 100   | 100     | 100   |

# Table 1Composition of chewable yogurt tablet

*Note.* F1 = Chewable yogurt tablet (control); F2 = Chewable yogurt tablet fortified with 10% corncob fiber; F3 = Chewable yogurt tablet added with 2.5% gelatin; F4 = Chewable yogurt tablet added with 2.5% gelatin and fortified with 10% corncob fiber

*Titratable Acidity.* A 6 g sample was crushed into fine powder using a mortar and pestle. After that, the sample was well-mixed with 50 ml of distilled water. The analyte was then added with a few drops of phenolphthalein indicator and titrated with 0.1 M sodium hydroxide (NaOH) solution in triplicate. The titration endpoint was determined by the color change of the analyte from colorless to pink or the pH of the analyte reached pH 8.2 for the dark color sample. The endpoint of titration was recorded in ml of titrant used. The pH of the analyte was measured with a calibrated FIVEGO<sup>™</sup> F2 Portable pH Meter (METTLER TOLEDO, USA). The percentage of titratable acidity can be calculated below using the milliequivalent factor of lactic acid = 0.09 (Sadler & Murphy, 2010).

% Acid = [(ml of NaOH used) x (0.1 N NaOH) x (0.09)] / (Grams of sample) x 100% [1]

*pH Analysis.* Sample preparation for pH analysis was standardized to the method of titratable acidity as mentioned previously. The sample (6 g) was blended with 50 ml of distilled water. The sample was crushed into a fine powder using a mortar and pestle in a prior experiment. FIVEGO<sup>TM</sup> F2 Portable pH Meter (METTLER TOLEDO, USA) was calibrated with pH buffer solutions (pH 4.0, 7.0, and 10.0) prior to analysis. The pH of the sample was measured and recorded using the calibrated pH Meter at room temperature in triplicate (Saint-Eve et al., 2008).

**Tablet Weight Variation Measurement.** Twenty tablets were weighed individually, and the average weight was calculated using Equation 2. Based on the United States Pharmacopeia method, the maximum acceptable percentage difference was set at 5% for tablets weighing more than 324 mg (United States Pharmacopeia [USP], 2012).

$$\mu = (\Sigma x_i) / n \qquad [2]$$

where,  $\mu$  = average weight (mg);  $\Sigma x_i$  = total tablets weight (mg); n = number of tablets.

*Friability.* According to the United States Pharmacopeia method (United States Pharmacopeial Convention [USP], 2015), 10 tablets were de-dusted prior to weighing and the initial weight was recorded. Friability was tested using a friabilator (PI-FTV-01 Pharmag Instruments, India) with a rotation speed of  $25\pm1$  rpm. After 100 rotations, the tablets were collected and de-dusted, and the final weight of the tablet was recorded. Friability was calculated as below:

Friability (%) =  $[(W_i - W_f)/W_i] \times 100\%$  [3]

where,  $W_i = initial$  weight;  $W_f = final$  weight.

**Disintegration.** Based on the United States Pharmacopeia method with some modifications, 6 tablets per sample were tested using tab disintegrator (DT-1000, Pharmag Instruments, India) with basketrack assembly (USP, 2015). A 1 L beaker was filled with 800 ml of distilled water to cover the basket fully and maintained at  $37\pm2^{\circ}$ C. One tablet was added to each glass tube. The basket was moved at a constant frequency rate between  $30\pm1$  cycles per min. The tablet's complete disintegration time was recorded in minutes.

*Hardness and Tensile Strength.* Five tablets' thickness, diameter and hardness were measured using a tablet hardness tester (ezTab400, Pharmag Instruments, India) with a constant loading speed of 20 N/s. The tablet was placed between the platen to allow diametrical compression to be applied up to fracture. The force required to cause fracture on the tablet was recorded as hardness (kilopond, kp). The thickness and diameter of the tablet were reported in centimeters (cm). According to the United States Pharmacopeia method (USP, 2015), tensile strength was calculated as equation below:

$$\sigma = 2F/\pi DH$$
 [4]

where,  $\sigma$  = tensile strength (kp/cm<sub>2</sub>); F = breaking force (kp); D = diameter (cm); H = thickness (cm).

*Hygroscopicity.* The hygroscopicity of the sample was determined using the method reported by European Pharmacopoeia (Barret, 2018). Approximately 0.2 g of sample and blank plastic Petri dish (without lid) were weighed accurately up to 3 decimal places on a calibrated Classic Plus analytical and top-loading digital balances (METTLER TOLEDO, USA). A surplus of saturated ammonium chloride solution (with excess crystal) was placed in the

pit of the desiccator to provide an 80±2% relative humidity (%RH) environment. Samples were placed inside the tightly sealed desiccator and stored for a day in an IF450 convection force air incubator (Memmert, Germany) at a controlled temperature of 25±2°C. After 24 hr of storage, samples were removed from the desiccator and weighed on a calibrated analytical balance. The hygroscopic nature of samples was classified using Table 2. The hygroscopicity of the sample was calculated by the percentage increase in mass as follows:

Percentage increase in mass (%) = 
$$[(M_2 - M_1) \times 100\%] / M_1 - M_0$$
 [5]

where  $M_0(g)$  = weight of blank Petri plate;  $M_1(g)$  = initial weight consisting of Petri plate and sample;  $M_2(g)$  = weight consisting of Petri plate and sample after 24 hr.

Equilibrium Moisture Content (EMC). The sample's EMC was determined using the method reported by Callahan et al. (1982). Approximately 0.2 g of sample and bare plastic Petri dish (without lid) were weighed accurately up to 3 decimal places on a calibrated Classic Plus analytical and top-loading digital balances (METTLER TOLEDO, USA). A surplus of saturated salt solution (with undissolved crystal) was placed in the pit of the desiccator to provide a distinct moisture environment. Lithium chloride, potassium carbonate, sodium chloride, potassium bromide, and potassium nitrate created an environment of 11, 43, 75, 83 and 93%RH, respectively. Samples were

| Table 2 |  |  |  |  |
|---------|--|--|--|--|
|         |  |  |  |  |

| Classification         | Criteria                      |
|------------------------|-------------------------------|
| Non-hygroscopic        | Increase in mass 0-0.012% w/w |
| Slightly hygroscopic   | Increase in mass 0.2-<2% w/w  |
| Moderately hygroscopic | Increase in mass 2-<15% w/w   |
| Very hygroscopic       | Increase in mass ≥15% w/w     |

placed inside the desiccator with different relative humidity percentages and tightly sealed. All desiccators were placed into the IF450 convection force air incubator (Memmert, Germany) and stored for a week at a controlled temperature of 25±2°C. After 7 days, samples were detached from the desiccators and the changes in moisture were calculated for each sample by obtaining the final equilibrium weight with a calibrated analytical balance. The initial moisture content of the samples was determined by using an XM120 moisture analyzer (Precisa Gravimetrics AG, Switzerland). The weight change at equilibrium (B) and initial moisture content (A) of the sample was required to determine the percentage moisture dry basis of the sample (P), and EMC values of the sample were calculated from P with the aid of Equations 6 and 7.

$$P = \{[(W \times A/100) - B] \times 100\%\} / [W - (W \times A/100)]$$
[6]

where, P (%) = percentage moisture dry basis; W(g) = initial weight of sample; A(%) = initial moisture content; B(g) = weight difference at equilibrium.

EMC = P / (P + 100) [7]

where, EMC = equilibrium moisture content; P (%) = percentage moisture dry basis. **Proximate Composition Analysis.** Samples were analyzed for their proximate composition based on the official AOAC methods (Latimer, 2023); the parameters that were determined were moisture, ash, fat, crude fiber, protein, and carbohydrate. The calorie content of food was determined from the three main components obtained in proximate composition analysis: carbohydrates, protein, and fat. Four (4) kcal was each provided by 1 g of protein and carbohydrate. However, 1 g of fat in food will provide 9 kcal. The calorie content of the sample was determined with the following formula:

Calorie content (kcal/ 100 g) = (% C x 4 kcal) + (% P x 4 kcal) + (% F x 9 kcal) [8]

where, % C = percentage of carbohydrate; % P = percentage of protein; % F = percentage of fat.

#### **Enumeration of Bacterial Count**

The number of lactic acid bacteria (probiotics) in the sample was analyzed using the pour plate method (Millette et al., 2013). Peptone water and MRS agar were prepared according to the directions recommended by Oxoid (United Kingdom), and autoclaved at 121°C for 15 min at 15 psi

in HV-50 Autoclave (Amerex Instruments, Inc., USA) prior to analysis. One g of the sample (tablet) was crushed into fine powder using a pestle and mortar. After that, the sample was transferred aseptically to a test tube filled with 9 ml of sterile 0.15% (w/v) peptone water and well-mixed using a vortex. Multiple tenfold dilutions in the ratio of 1:10 were required to obtain the targeted concentration, where 1 represents the amount of sample, and 10 represents the total size of the final sample. After the serial dilution, the sample was inoculated in MRS agar and incubated anaerobically at 38±2°C for 48 hr. The anaerobic incubation was carried out using a combustion-modified candle-jar system (Saha et al., 2016). Enumeration of bacteria was carried out in triplicate and reported in log no. CFU/g.

## Stability Analysis of Lactic Acid Bacteria (Probiotics) in Chewable Yogurt Tablet under Simulated Gastrointestinal (GI) Fluid

**Preparation of Simulated GI Fluid.** To study the survival rate of lactic acid bacteria (probiotics) in chewable yogurt tablet samples, simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) was prepared according to the modified method developed by Millette et al. (2013). SGF was prepared by diluting 7 ml of 10 M hydrochloric acid (HCl) and 2 g of sodium chloride (NaCl) with distilled water up to 1,000 ml, and the pH was adjusted to pH 2.0 by using 0.2 M HCl or 0.2 M NaOH. SIF was prepared by diluting 0.9 g NaOH, 6 g bile salt, and 6.8 g monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) with distilled water up to 1,000 ml and the pH

was adjusted to pH 6.8 by using 0.2M HCl or 0.2 M NaOH. Freshly prepared sterile SGF and SIF were stored in an incubator at  $37\pm2^{\circ}$ C for 60 min before the experiment to simulate body temperature (Nagashima et al., 2013).

Simulated GI Analysis. Two tablets of the same sample were crushed into chunks to simulate the actual swallowing size of the tablet after the chewing process. The tablet chunks were weighed at 1 g and mixed with 9 ml of sterile SGF to form suspension #1. Then, the suspension #1 was incubated at 37±2°C for 120 min. Meanwhile, the remaining part of the tablet (1 g) was crushed into powder and followed with the enumeration of bacterial count to verify the initial count of lactic acid bacteria (probiotics) in the chewable yogurt tablet sample. After incubation, 1 ml of the vortexed aliquot from suspension #1 was added to 9 ml of sterile SIF to form suspension #2 for further incubation (150 min) at 37±2°C. Another 1 ml of the vortexed aliquot was concurrently mixed with 9 ml 0.15% (w/v) sterile peptone water and proceeded with the enumeration of bacterial count to investigate the viability of probiotics in the chewable tablet after two hours of immersion in SGF. After 150 min of incubation, serial dilution was conducted with 1 ml of suspension #2 transferred into a dilution tube with 9 ml of 0.15% (w/v) sterile peptone water to check the final lactic acid bacterial (probiotics) count and evaluate the survival rate of probiotics after a total of 4.5 hr under simulated GI fluid condition (Nagashima et al., 2013).

#### Shelf-life Analysis

Bacterial count, pH, and titratable acidity are the required specifications in most food standards worldwide for yogurt products (Y. L. Chan et al., 2019). Thus, the survival rate of probiotics in the sample during storage in a securely capped amber glass bottle (Schott, Germany) at refrigerated temperature (4±2°C) was evaluated based on the method of Chaikham with some modifications (Chaikham, 2015). The samples with 0, 0.5, 1, 2, and 3 months of storage were extracted aseptically for microbiological enumeration analysis. The titratable acidity and pH of the sample were also analyzed to observe the changes in the sample during storage.

## Data Analysis

All data were obtained in triplicate (n = 3) and subjected to analysis of variance (ANOVA) by using the MINITAB Release 14.12.0 Statistical Software (USA). Ryan Joiner's test was carried out to determine the normality of data distribution. Levene's and Barlett's tests were used to determine the equality of variances. Fisher's least significant difference (LSD) test was carried out to determine the significance of mean differences among samples (Granato et al., 2014). Results were expressed as mean  $\pm$  standard deviation, and the significant level was determined at *p*<0.05.

#### **RESULTS AND DISCUSSION**

## Physicochemical Properties of Chewable Yogurt Tablet

The physicochemical attributes of the chewable yogurt tablets are presented

in Table 3. Ensuring compliance with the United States Pharmacopeia criteria, the tablet size was confined to 22 mm, thus fixing tablet weight and diameter at 1,000 mg and 18 mm, respectively (United States Pharmacopeial Convention, 2015). Minor weight fluctuations emerged due to ingredient loss during sieving and compression, resulting in non-significant differences (p>0.05) among tablets. The weight variation percentage ranged between 0.2 and 3.3%, abiding by the 5% limit prescribed by the United States Pharmacopeia for tablets exceeding 325 mg (United States Pharmacopeial Convention, 2015). The pH and titratable acidity of the samples ranged from 4.30 to 4.47 and 4.94 to 5.25, respectively, aligning with yogurt's pH range suggested by Baglio, from 3.9 to 4.6 (Baglio, 2014). Tensile strength, an indicator of chewability, showed no significant difference (p>0.05) among the samples (0.93-1.18 N/mm<sup>2</sup>). Notably, F1 exhibited the lowest hardness at 9.50 kp/cm<sup>2</sup>, and other samples also maintained hardness levels below 12 kp/cm<sup>2</sup>, in accordance with the United States Food and Drug Administration (US FDA) and Robinson et al. recommendations regarding chewable tablet hardness (Augsburger & Hoag, 2016; R. L. Robinson et al., 2001).

The chewable yogurt tablet was developed to enhance the availability of lactic acid bacteria (probiotics) by bypassing the digestive phase in the digestive system (Renu et al., 2015). For this purpose, a slower disintegration rate in distilled water at 37°C is ideal. As evident from Table 3 data, chewable yogurt tablet variants F3 and F4 exhibited significantly longer

| Physicochemical properties of chewable yogurt tablet   | yogurt tablet  |  |   |  |
|--|--|--|---|--|
|  | F1   | F2   | F3  | F4   |
| Weight (mg)  | $998.30 \pm 22.30$   | $973.30 \pm 23.40$   | $985.00 \pm 15.20$  | $966.70 \pm 15.10$   |
| Diameter (mm)  | $18.03\pm0.02$   | $18.01\pm0.01$   | $18.02\pm0.01$  | $17.97\pm0.02$   |
| Tensile strength (N/mm <sup>2</sup> )  | $0.93\pm0.23^{\mathrm{a}}$   | $1.09\pm0.23^{\mathrm{a}}$   | $1.16\pm0.07^{\mathrm{a}}$  | $1.18\pm0.08^{\rm a}$                                      |
| Hardness (kp/cm <sup>2</sup> )   | $9.50\pm2.34^{\rm b}$  | $11.09\pm2.35^{\rm a}$   | $11.86\pm0.65^{\rm a}$  | $12.03\pm0.86^{\rm a}$                                     |
| hd   | $4.47\pm0.01^{a}$  | $4.45\pm0.01^{\rm a}$  | $4.33\pm0.01^{\rm b}$   | $4.30\pm0.01^{\rm b}$                                      |
| Titratable acidity (%)   | $4.94\pm0.03^{\rm b}$  | $5.01\pm0.11^{ m b}$   | $5.19\pm0.08^{a}$   | $5.25\pm0.07^{\mathrm{a}}$                                 |
| Friability (%)   | $0.98\pm0.03^{\rm b}$  | $0.15\pm0.01^{\rm a}$  | $0.95\pm0.03^{\rm b}$   | $0.27\pm0.01^{ m a}$                                       |
| Disintegration (minutes)   | $21.81\pm0.87^{\rm a}$   | $19.80\pm0.84^{\rm a}$   | $52.82\pm4.03^{\rm b}$  | $41.11 \pm 2.73^{b}$                                       |
| Hygroscopicity (%)   | $1.62\pm0.45^{\rm b}$  | $2.43\pm0.50^{\rm ab}$   | $1.94\pm0.17^{ m b}$  | $4.20\pm0.94^{\rm a}$                                      |
| Color profile  | $L^{*}=88.05\pm0.14^{\mathrm{b}}$  | $\mathrm{L}^{*}=88.99\pm0.24^{\circ}$                                | $\mathrm{L}^{*}=88.42\pm0.14^{\mathrm{a}}$                          | $\mathrm{L}^{*}=88.69\pm0.37^{\mathrm{c}}$                 |
| 4  | $a^* = 0.13 \pm 0.03^{a}$  | $a^{*}=0.27\pm0.04^{\mathrm{b}}$                                     | $a^{*}=0.14\pm0.04^{a}$   | $a^* = 0.27 \pm 0.02^b$                                    |
|  | $b^* = 7.94 \pm 0.21^a$  | $\mathrm{b}^{\mathbf{*}}=19.75\pm0.25^{\mathrm{b}}$                  | $b^{\boldsymbol{*}}=7.69\pm0.14^{a}$                                | $b^{\boldsymbol{*}} = 19.61 \pm 0.25^{\mathrm{b}}$         |
| Proximate composition  |  |  |   |  |
| (g/100 g of dry matter)  |  |  |   |  |
| Moisture   | $9.70\pm0.03^{\rm ab}$   | $11.42\pm1.19^{a}$   | $9.03\pm0.09^{ m b}$  | $9.36\pm0.16^{\rm ab}$                                     |
| $\operatorname{Ash}$   | $6.41\pm0.06^{\rm ab}$   | $6.54\pm0.20^{a}$  | $5.92\pm0.08^{ m b}$  | $6.06\pm0.24^{\rm ab}$                                     |
| Crude fat  | $0.67\pm0.01^{\mathrm{a}}$   | $0.58\pm0.06^{a}$  | $0.68\pm0.02^{\rm a}$   | $0.55\pm0.06^{\rm a}$                                      |
| Crude fiber  | $0.04\pm0.01^{ m b}$   | $2.41\pm0.16^{\rm a}$  | $0.03\pm0.01^{ m b}$  | $2.61\pm0.11^{\rm a}$                                      |
| Crude protein  | $29.36\pm0.23^{\rm b}$   | $22.95\pm0.81^{ m d}$  | $31.19\pm0.45^{\mathrm{a}}$   | $25.02\pm0.18^\circ$                                       |
| Carbohydrate   | $53.86\pm0.33^{\rm b}$   | $58.51\pm2.25^a$   | $53.20\pm0.42^{\mathrm{b}}$   | $59.01\pm0.74^{\mathrm{a}}$                                |
| Calorie  | $339.00 \pm 1.41^{\mathrm{a}}$   | $331.50 \pm 6.36^{a}$  | $342.00 \pm 1.41^{a}$   | $341.00 \pm 1.41^{a}$                                      |
| (kcal/100 g of dry matter)   |  |  |   |  |
| <i>Note</i> . F1 = Chewable yogurt tablet (control); F2 = Chewable yogurt tablet fortified with 10% corncob fiber; F3 = Chewable yogurt tablet added with 2.5% gelatin; F4 = Chewable yogurt tablet added with 2.5% gelatin and fortified with 10% corncob fiber. The values represented by different letters in the same row differ significantly ( $p$ <0.05); L* = Lightness; a* = Redness; b* = Yellowness | ol); F2 = Chewable yogurt tabl<br>1.2.5% gelatin and fortified w<br>* = Redness; b* = Yellowness | et fortified with 10% corncob fib<br>ith 10% corncob fiber. The valu | oer; F3 = Chewable yogurt table<br>es represented by different lett | st added with 2.5% gelatin;<br>ters in the same row differ |

Analysis of Chewable Yogurt Tablet with Corncob Fiber

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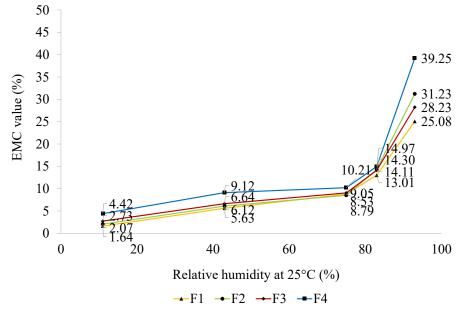
<sup>943</sup> 

disintegration times (41.11–52.82 min) compared to F1 and F2. This outcome indicated that the addition of gelatin powder influenced the disintegration time. Including gelatin decreased tablet porosity due to cross-linking during gelatin, extending the disintegration process (Jones et al., 2011). Consequently, longer disintegration times result in reduced exposure of lactic acid bacteria (probiotics) to stomach acid and bile, aligning with the observations of Augsburger and Hoag (2016). Therefore, chewable yogurt tablets F3 and F4 hold the potential as effective carriers for delivering a higher number of probiotics (lactic acid bacteria) to the host's gut over an extended disintegration period.

Distinguishing trends emerged in color and friability within the tablet samples. Notably, the incorporation of corncob fiber powder significantly influenced the color profile and friability of F2 and F4 (p < 0.05). These variants exhibited a more pronounced yellow hue (b\* value: 19.61-19.75) attributed to carotenes and xanthophylls from corn (Floyd et al., 1995). Additionally, F2 and F4 demonstrated favorable friability (0.15-0.27%), which is essential for chewable yogurt tablets acting as probiotic carriers. Friability gauges the mechanical durability of tablets, shielding internal cells from gastric interactions. As per United States Pharmacopeia (United States Pharmacopeial Convention, 2015), minimal weight loss ( $\leq 1.0\%$ ) post-friability test implies sound structural integrity. It reflects the robust binding capability of corncob fiber powder.

Furthermore, corncob fiber-incorporated samples (F2 and F4) displayed moderate hygroscopicity (2.43 and 4.20%) compared to slightly hygroscopic F1 and F3 (1.62 and 1.94%) as per European Pharmacopoeia (Barret, 2018). Corncob fiber's addition likely contributed to hygroscopicity. Despite similar equilibrium moisture content (EMC) among all samples (1.64-14.97%) in relative humidity between 11 to 83% at 25°C, the EMC spiked to 31.23 and 39.25% (F2 and F4, respectively) at 93%RH, reflecting the moderately hygroscopic nature of corncob fiber (Figure 1) (Igathinathane et al., 2005). It aligns with the known moisture sensitivity of these materials.

By analyzing the proximate composition (Table 3), negligible distinctions (p>0.05)were observed in crude fat (0.55-0.68 g/100 g dry matter) and calorie content (331.5–342.0 kcal/100 g dry matter) among all chewable yogurt tablet formulations. However, F2 and F4 exhibited a significant decrease (p < 0.05) in crude protein content (22.95 and 25.02 g, respectively) due to partial substitution of whey protein isolate with corncob fiber powder. On the other hand, the addition of corncob fiber powder remarkably elevated (p < 0.05) crude fiber content in chewable yogurt tablets (F2 and F4) to 2.41 and 2.61 g, respectively, aligning with its high content (38 g/100 g dry matter) as reported by Lee et al. (2019). Rich in dietary fiber, these tablets have the potential to be a functional food through improved fiber and probiotic intake.



*Figure 1*. Equilibrium moisture content (EMC) of chewable yogurt tablets with different formulations (F1, F2, F3, and F4) and different relative humidity (11, 43, 75, 83, and 93%) at 25°C

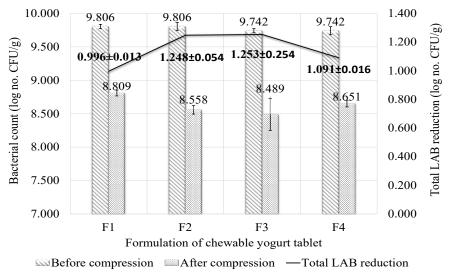
*Note.* F1 = Chewable yogurt tablet (control); F2 = Chewable yogurt tablet fortified with 10% corncob fiber; F3 = Chewable yogurt tablet added with 2.5% gelatin; F4 = Chewable yogurt tablet added with 2.5% gelatin and fortified with 10% corncob fiber

#### Bacterial Count in Chewable Yogurt Tablet

As depicted in Figure 2, the initial bacterial count across the tablet formulations ranged from 9.742 to 9.806 log no. CFU/g. Following the tablet-making process, a reduction of approximately 1 log cycle was observed in the bacterial count, resulting in ranges of 8.489-8.809 log no. CFU/g. Notably, no significant differences (p>0.05)were discerned among the four chewable yogurt tablet variants, indicating that tablet formulation did not significantly influence bacterial count reduction during compression. This reduction is likely attributed to the mechanical stresses encountered during compression, which damage bacterial cell walls and membranes (e Silva et al., 2013; Klayraung et al., 2009). These findings align with E. S. Chan and Zhang's (2002) study, revealing that probiotics endure compression up to 30 MPa without noteworthy viability loss. However, viability gradually diminished to around 85% at 90 MPa, and above 90 MPa, bacterial survival declined linearly, with only 33% retention at 180 MPa.

## Viability of Probiotics after Simulated Gastrointestinal Analysis

The exposure of bile in SIF poses a significant threat to the lactic acid bacteria (*L. bulgaricus* and *S. thermophilus*) within chewable tablets, as bile disrupts the integrity and permeability of the cell membrane in



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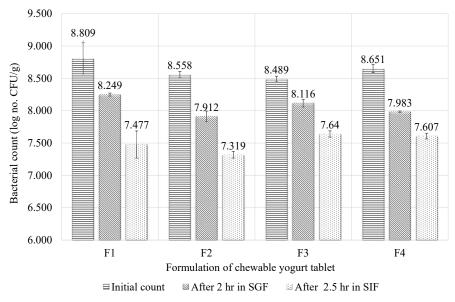
Figure 2. Bacterial count in chewable yogurt tablet before and after compression

*Note.* F1 = Chewable yogurt tablet (control); F2 = Chewable yogurt tablet fortified with 10% corncob fiber; F3 = Chewable yogurt tablet added with 2.5% gelatin; F4 = Chewable yogurt tablet added with 2.5% gelatin and fortified with 10% corncob fiber; LAB = Lactic acid bacteria

Gram-positive bacteria, ultimately causing membrane lysis and degradation (Begley et al., 2005). Survival outcomes of probiotics (lactic acid bacteria) within chewable yogurt tablet samples under simulated GI conditions are depicted in Figure 3. Notably, F3 and F4, benefiting from a resilient proteinaceous matrix formed by gelatin, exhibited relatively stronger resistance to simulated GI fluids compared to F1 and F2. This intricate protein matrix, created by entrapped gelatin, acted as a protective barrier against SIF (Maldonado-Valderrama et al., 2011). Consequently, F3 and F4 displayed a reduction of  $<1 \log$  no. CFU/g, while F1 and F2 encountered a decrease of approximately 1.3 log no. CFU/g over 4.5 hours in simulated GI fluids. This outcome correlates with the gradual disintegration properties of F3 and F4, facilitated by

gelatin. All chewable yogurt tablets performed admirably under GI conditions, yielding remarkable survival rates (85-90%). This success can be attributed, in part, to tablet friability (<1%), as outlined in the 'Physicochemical Properties of Chewable Yogurt Tablet' section. Adequate tablet friability effectively protects encapsulated cells against the challenging GI environment (Govender et al., 2014). A parallel study by Klayraung et al. similarly demonstrated that optimal tablet friability (<1%) sustained probiotic survival rates of up to 89.3% in simulated GI media (Klayraung et al., 2009). Notably, the results identify F4 (incorporating gelatin) as an optimal vehicle for probiotic delivery, enhanced by adding corncob fiber, significantly boosting viable cell delivery to the human intestine.

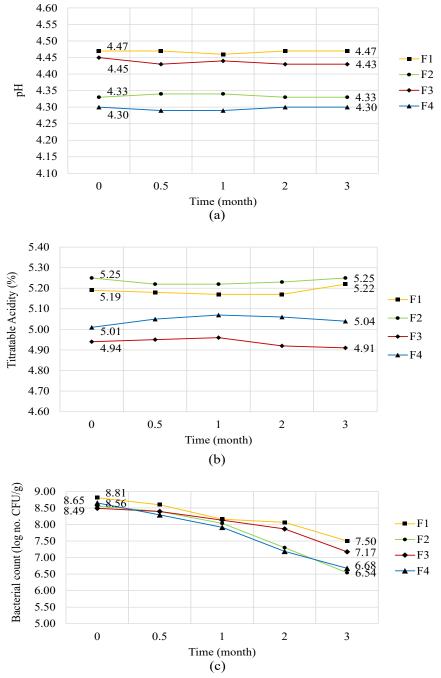
#### Analysis of Chewable Yogurt Tablet with Corncob Fiber



*Figure 3*. Viability of probiotics in chewable yogurt tablet after simulated gastrointestinal analysis *Note.* F1 = Chewable yogurt tablet (control); F2 = Chewable yogurt tablet fortified with 10% corncob fiber; F3 = Chewable yogurt tablet added with 2.5% gelatin; F4 = Chewable yogurt tablet added with 2.5% gelatin and fortified with 10% corncob fiber; SGF = Simulated gastric fluid; SIF = Simulated intestinal fluid

#### Shelf-life Study

In the three-month shelf-life assessment (Figure 4), the tablets exhibited consistent pH and titratable acidity levels, indicating stability. However, the viability of probiotics gradually diminished across all tablets during storage at 4±2°C. While all four chewable tablet samples commenced with a similar initial bacterial count (around 8.6 log no. CFU/g), F2 and F4, incorporating corncob fiber powder, experienced a more substantial 2 log no. CFU/g reduction compared to F1 and F3 (1.3 log no. CFU/g) after three months. This decrease could be linked to corncob fiber powder's slightly higher equilibrium moisture content, impacting bacterial count during storage. Equilibrium moisture content holds significance for storage, handling, and processing due to its role in chemical and enzymatic reactions (Peng et al., 2007). Water presence affects shelf life and quality by triggering chemical and enzymatic reactions (Zhang & Mittal, 2013). Igathinathane et al. (2005) estimated corncob fiber powder's average equilibrium moisture content at  $13.9 \pm 11.1\%$  d.b. across temperatures (10-40°C). Atmospheric moisture continues to permeate the corncob fiber powder until equilibrium is reached. Elevated moisture content could lead to higher dissolved oxygen levels, potentially causing lactic acid bacteria to perish due to oxygen toxicity (Zayed & Roos, 2004). Notably, all tablets' average final bacterial viability remained at 7 log no. CFU/g aligns with worldwide food standards, requiring a minimum probiotic count of 10<sup>7</sup> (Y. L. Chan et al., 2019). Solutions to address



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*Figure 4*. Changes in (a) pH value, (b) titratable acidity, and (c) viability of probiotics during refrigerated storage  $(4\pm 2^{\circ}C)$  for 0, 0.5, 1, 2, and 3 months

*Note.* F1 = Chewable yogurt tablet (control); F2 = Chewable yogurt tablet fortified with 10% corncob fiber; F3 = Chewable yogurt tablet added with 2.5% gelatin; F4 = Chewable yogurt tablet added with 2.5% gelatin and fortified with 10% corncob fiber

this challenge include incorporating food additives, silica gel desiccants, and vacuum packaging (Amarakoon & Navaratne, 2017).

## CONCLUSION AND RECOMMENDATIONS

In this study, all tablets possessed good stability against the simulated GI fluids with an 85–90% survival rate and desired physicochemical properties (weight variation, hardness, tensile strength, pH, and friability). Furthermore, the F4 tablets added with gelatin and incorporated with corncob fiber had a good sustain-released property (longest disintegration time) that enhanced the delivery of probiotics and fiber to human guts. Adding corncob fiber powder in the formulation also imparted a pleasant maize flavor and natural yellowish color (higher b\* value  $\approx$  19) to the chewable yogurt tablet with excellent friability (0.27%). The threemonth stability study showed that all tablets encountered a marginally slow decline in terms of the viability of probiotics and had a final bacterial count ranging between 6.5 and 7.5 log no. CFU/g at the end of storage from the initial bacterial count of approximately 8.6 log no. CFU/g. However, there are no significant changes in physical parameters (titratable acidity and pH) during the three months of storage. Chewable yogurt tablet is considered to have good potential as a new dairy product or alternative supplement, as it is a functional, palatable, attractive, and innovative product that meets modern needs. F4 had decent physicochemical properties and sturdy resistance against the simulated GI fluid. Hence, it could be an

ideal functional product to deliver probiotics and fiber to the guts in the most intact and viable form, which allows them to exert their beneficial effect immediately upon consumption.

For future research, it is suggested that the preservation of lactic acid bacteria in chewable yogurt tablets by using different packaging such as vacuum-packed, aluminum strip-packed, plastic blisterpacked, amber bottle-packed, and packed with desiccants could be investigated to maintain the beneficial properties of probiotics in it. Moreover, an in-depth examination of the sensory attributes of this novel yogurt product may be conducted through methodologies such as volatile profiling, traditional acceptance testing, or text highlighting techniques. This exploration aims to enhance our comprehension of consumer preferences, a pivotal factor in achieving effective market positioning. Finally, an in vivo or clinical study on the treatment of gastrointestinal diseases by using chewable yogurt tablets incorporated with corncob fiber powder could be further researched.

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