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Research Article

Jackfruit Seed as a Natural Source for Protein and Mineral Enrichment of Yogurt

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A significant proportion of the global population is currently suffering from protein and mineral malnutrition. Food enrichment or fortification is an effective strategy being utilized worldwide to fight malnutrition. The objective of the study was to extract protein and minerals from an underutilized natural source of jackfruit seed and to incorporate these nutrients into a widely consumed food yogurt. Protein isolation was achieved through the removal of the major component starch from jackfruit seed flour (JSF) followed by spray drying to get jackfruit seed protein isolate (JSPI). Mineral extraction was performed from the residuals after protein extraction. Four different yogurt samples were formulated enriched with varying concentrations of extracted protein (8%, 6%, 4%, and 2%) and a constant mineral concentration of 747 mg/100 g of yogurt. A plain yogurt served as the control sample (S5), which was not enriched with protein and mineral. The yogurts were successfully enriched with protein and minerals in this study. The sensory evaluation experiment suggested that the yogurt sample (S2) prepared with 6% protein and 747 mg/100 g mineral secured better sensory acceptance than any other sample prepared in this study. Shelf-life study showed that the yogurts were safe for consumption up to 12 days when stored under refrigeration temperature and 4 days when stored at room temperature.

1. Introduction

Approximately 10% of the global population is suffering from malnutrition and inadequate energy consumption [1]. Africa has the highest prevalence of malnutrition or undernourishment, with 20.2% of its population affected, and in Asia, 9.1% of the population is similarly impacted. The occurrence of undernourishment in Bangladesh is among the highest affected countries in the world. About 11.4% of the total population and 30.2% of children under 5 years are suffering from stunted growth due to malnutrition in this country [2]. Diets in those affected regions are often deficient in essential nutrients, particularly protein and minerals [3].

Proteins are macromolecules, made of amino acids, serve as building blocks, provide structural support, and perform various functions including acting as hormones, enzymes, and biochemical catalysts in the body [4]. Deficiency of protein in the diet can lead to pervasiveness of underweight and stunted growth in children as well as inadequate protein malfunctions [5]. Minerals also have a crucial role in regulating normal status of bone, muscles, immune system, and

nervous system of the body [6, 7]. Mineral deficiencies are often termed as "hidden hunger" and have an adverse effect on human health. About 2 billion individuals worldwide are affected from mineral deficiency [8]. Fortification or enrichment of food with these essential nutrient components can be an immediate and sustainable solution to overcome these deficiencies. This strategy is already in use and is considered a cost-effective public health intervention [9, 10].

The researchers and food manufacturers are on continuous lookout for new plant proteins with distinct and known characteristics due to growing customer demand for plant-based proteins [11–20]. Jackfruit (*Artocarpus heterophyllus* Lam) is widely grown in Southeast Asia, particularly in Bangladesh, India, and Vietnam. The fruit contains juicy edible pulps, each of which surrounds a seed that makes up 8 to 10% of the total weight of a jackfruit. These seeds are high in protein and carbohydrates, and a rich source of food minerals [21–26]. The seed of jackfruit contains up to 7.04% protein, while seed flour contains up to 16.01% protein, on dry basis [27]. So, jackfruit seed could be a promising new source of plant protein and minerals [28]. Several studies have been conducted on the isolation and characterization of proteins from jackfruit seed [27, 29–31]. However, to the best of our knowledge, no comprehensive study was done on the extraction of minerals from JSF. Therefore, comprehensive researches are needed that includes isolation of those nutrients, and method optimization is required to use the isolated nutrients in food formulations.

Yogurt is a fermented dairy food product prepared by fermentation of milk and vastly consumed worldwide. It is also suitable for people with lactose intolerance [32]. It can be a good carrier for protein and mineral fortification [33]. Fortification of yogurt with whey protein concentrates, fat, fiber, antioxidants, minerals, and other bioactive components has been practiced by other studies [27, 29–32, 34–45].

Therefore, the study is aimed at (1) extracting protein and minerals from JSF, (2) preparing yogurt enriched in protein and minerals, and (3) analyzing the physiochemical attributes, sensory assessment, and storability of the prepared yogurt.

2. Materials and Methods

2.1. Materials. Dry seeds of ripe jackfruits (*Artocarpus heterophyllus* Lam.) were collected from the local vegetable market of Gazipur, Bangladesh. Whole milk was procured from Bangabandhu Sheikh Mujibur Rahman Agricultural University's farm in Gazipur. Yogurt was obtained locally and utilized as a starting culture. Chemical and others were used from the agroprocessing laboratory stock at Bangabandhu Sheikh Mujibur Rahman Agricultural University.

2.2. Preparation of Jackfruit Seed Flour (JSF). The seeds were washed with clean water and sun-dried to reduce moisture properly. The seeds which were physically defective and damaged by mold or other insects were removed. The good seeds were then peeled to remove the white seed layer on top and soaked in 5% NaOH for half an hour to aid in the removal of the brown layer. The seeds were then washed under clean tap water to remove any remaining skin and NaOH. The clean seeds were sliced into approximately 3×5 mm size and dried in a cabinet dryer at 40°C for 24 hours. After the drying process was complete, the dried seeds were blended (Jencons Laboratory Blender) into powder and filtered through a 0.250 mm mesh sieve. The obtained powder was then packed in high-density polythene using vacuum packaging (4100050 Sealcom-V) and stored in a dark and dry place for further processing.

2.3. Preparation of Jackfruit Seed Protein Isolate (JSPI). An alkaline extraction method was adopted to extract protein from JSF following the method described in Ulloa et al. [30] with slight modifications. Flour was mixed with deionized water in a ratio of 1 : 10 and pH of the flour-water mixture was raised to 11 using 1 N NaOH. The slurry was stirred with a magnetic stirrer for an hour at room temperature. Then the solution was centrifuged at 4500 RCF (BioBase BKC-TH21) for 5 minutes. The supernatant was collected in a beaker and pH was set at 4 using 1 N HCl. The sediment was also collected for later use in mineral extraction. The supernatant solution was kept overnight at 4°C. Then, the solution was centrifuged again at 2500 RCF for 15 minutes, and this time, the protein precipitates were separated and washed using deionized water. The supernatant after centrifugation was saved for mineral extraction. Proteins were then resuspended, and pH was set to 7. The protein solution was then dried using a spray dryer (Yamato ADL-311S Spray Dryer) to get dry protein powder. The input temperature was 150°C, the output temperature was 70°C, and the nozzle air pressure was 0.2 MPa. The spray-dried powder, JSPI, was then stored at room temperature in a glass bottle.

2.4. Extraction of Minerals and Storage for Future Use. The mineral extraction method was adopted from Mitić et al. [46] with a slight modification. Extraction was carried out in two different processes: process 1 utilized solely the sediment starch after protein extraction, whereas in process 2, the residual supernatant water after the separation of protein was also used in addition to sediment starch. The procedural difference between the two processes is presented in Figure 1. For both processes, the starch sediment was mixed with deionized water in an approximate ratio of 1 : 10, and the pH was adjusted at 4 using 1 N HCl. The solution was stirred moderately for an hour. Then, it was allowed to rest overnight (this step is unnecessary for process 2). The clear supernatant on top of the starch precipitate was collected by siphoning and centrifuging at 4500 RCF for 10 minutes. In process 2, the residual supernatant after protein separation was mixed at this stage. The subsequent steps were followed similarly which started with filtration through Whatman filter paper 1 to reduce starch content. The filtered solution was then spray dried (Yamato ADL-311S Spray Dryer) at the conditions of inlet temperature 150°C, outlet temperature 70°C, and nozzle air pressure 0.2 MPa. The mineral concentrate was then collected by scrapping from the cyclone and wall of the drying chamber. The mineral concentrate was then kept in glass jars for further use and analysis.

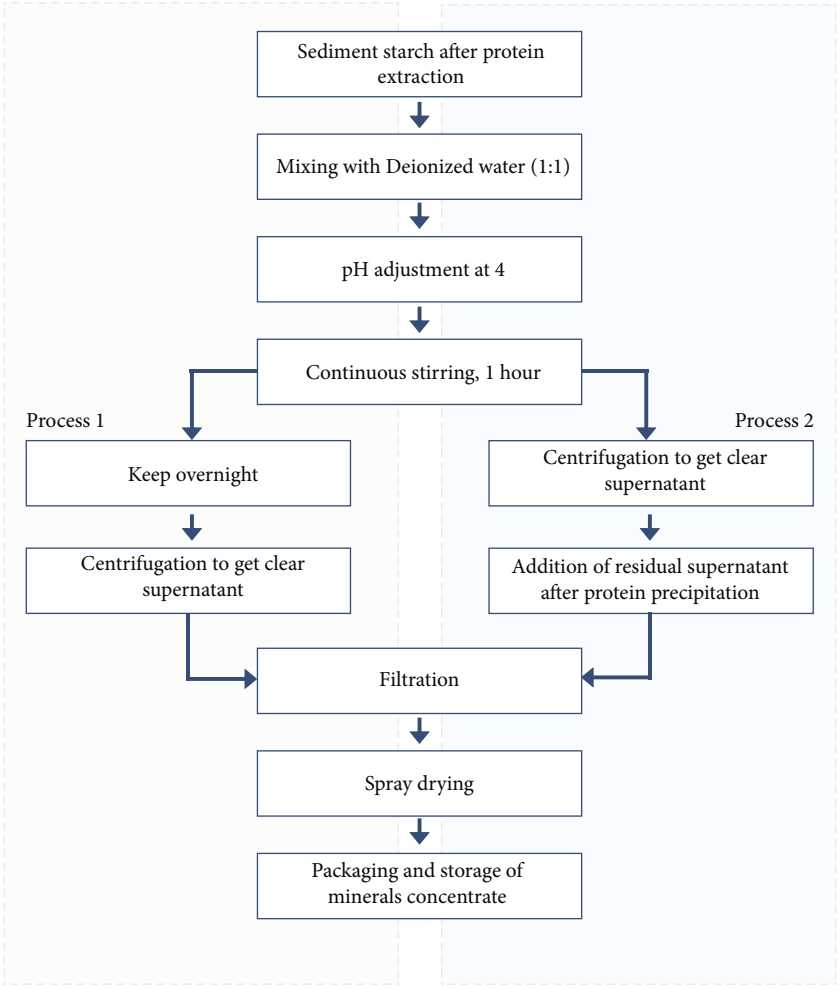

Figure 1: Extraction of minerals using process 1 and process 2.

2.5. Preparation of Yogurt. Initially, the experimental yogurts were formulated using 2%, 4%, 6%, 8%, 10%, and 12% JSPI and a fixed amount of mineral concentrate 747 mg/100 g. A control sample that contained ingredients like a commercial plain yogurt (without addition of extra JSPI and mineral concentrate) was also prepared. From the preliminary trials, it was observed that the samples containing 10% and 12% JSPI (of total raw material, by weight) failed to set yogurts with satisfactory texture; hence, they were not considered for further study. Therefore, the experimental yogurt samples were prepared with the addition of 2%, 4%, 6%, and 8% JSPI and mineral concentrate, along with a control yogurt sample without the addition of JSPI and mineral concentrates, as designed in Table 1.

According to Jackson and Lee [47], the sources of minerals and quantity have a significant effect on the sensory attributes of fortified dairy foods. Ocak and Köse [48] reported that certain minerals cause impediments in the fermentation process. Moreover, minerals have minimum and maximum intake levels. Maximum intake level is associated with potential toxicity and risk of developing noncommunicable chronic illnesses such as cardiac arrhythmias, delirium, and nephrolithiasis [49]. Therefore, the amount of mineral was incorporated based on the reported literal information. Achanta et al. [34] fortified the blend of pure minerals at about 25% of the recommended dietary allowance in the yogurts. Following their estimation, an amount of 250 mg mineral was supplemented per 100 g of yogurt samples in the current study. Considering the JSF mineral concentrate contained 33.45% mineral, which is discussed in Section 3.2, an amount of 747 mg mineral concentrate was added in each of the samples (Table 1).

The process for the preparation of yogurt was adopted from Parvin et al. [50] with subtle modifications. Milk was heated to a boil, and then sugar was added following the ratio of 12 g sugar for 100 ml of milk. The mixture was heated to evaporate and reduce the volume to one-third of the raw milk. After that, the mixture was cooled until the temperature decreased to 35°C. The starter culture was introduced following the back-slopping method, utilizing locally sourced, preexisting yogurt. The culture was added as 6 g/1000 ml of raw milk. The mixture was stirred, and then the enrichment of yogurt was done by the addition of extracted JSPI and mineral concentrate. The yogurt was then poured into serving cups and incubated at 40°C for six hours (Figure 2). After that, the yogurts were kept at 4°C inside a

Table 1: Formula for preparing protein- and mineral-enriched yogurt (% addition of JSPI and mineral concentrate).

Sample name	Added JSPI	Added mineral concentrate (mg/100 g yogurt)
S1	8%	747
S2	6%	747
S3	4%	747
S4	2%	747
S5 (control)	0	0

refrigerator and at room temperature to study shelf life in both conditions.

2.6. Sensory Evaluation. The evaluation was done on a nine-point hedonic scale as described by [51]. Different concentrations of added protein and mineral in yogurts were considered as treatments, and the experiment was carried out in a completely randomized design (CRD) environment.

Five yogurt samples were prepared and presented before a set of 20 semitrained panelists. The panel contained five academicians (3 males and 2 females) with ages between 28 and 50 years, six staff (3 males and 3 females) with ages between 28 and 35 years, and nine students with ages between 20 and 26 years (4 males and 5 females). They were explained about the test method, procedure, properties, and requirements prior to conducting the test. Prior to conducting the experiment, consents were taken from all the participants included in this study.

Samples were prepared in mass; each panelist was served using a spoon from that mass in separate plates, without mixing one another. Then, the samples coded using random three-digit numbers were presented before them along with a hedonic scale sheet to the degree of likeness in terms of color, taste, flavor, texture, and overall acceptability. Panelists were asked to evaluate the samples by scoring on a 9-point hedonic scale: 9 = like extremely; 8 = like very much; 7 = like moderately, 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.

2.7. Proximate Composition and Other Physicochemical Analysis

2.7.1. Determination of Protein Content. The protein content of JSPI and yogurt samples was determined by micro-kjeldahl method [52]. Protein determination using micro-kjeldahl method is comprised of three steps, namely, digestion, distillation, and titration. Using this method percentage of nitrogen is calculated which is then converted into protein content using a conversion factor. The conversion factor was considered as 6.38 for the estimation of protein in this study [53].

Equation (1) was used to calculate the protein content in yogurt [50].

$$\%\text{Protein Content} = \frac{(c-b)\times 14\times d\times 6.38}{a\times 1000}\times 100, \quad (1)$$

where a is the sample weight in g; b is the volume of NaOH to neutralize 25 ml of 0.1 N H_2SO_4; c is the volume of NaOH to neutralize 0.1 N H_2SO_4 in control or back titration; d is the strength of NaOH (normality of NaOH solution), and 14 is the molecular weight of nitrogen.

2.7.2. Determination of Moisture Content. The moisture content of the samples was determined using the oven drying method [52]. Clean Petri dishes were dried, cooled, and weighed. The samples were placed in the Petri dishes and weighed again to get sample weights. The Petri dishes were then placed into an oven dryer, already set at 105°C. The samples were allowed to dry for 24 hours to achieve complete drying. The dried samples were cooled in a desiccator and then weighed. The weight differences were calculated by subtracting the weight of the Petri dishes. The moisture content of each sample was calculated using the following equation:

$$\%\text{Moisture Content} = \frac{\text{Weight of Sample after drying (g)}}{\text{Weight of sample before drying (g)}}\times 100. \quad (2)$$

2.7.3. Determination of Ash Content. According to AOAC [52], the dried and ground samples were placed in dry, already-weighed porcelain crucibles and weighed again to get the sample weight. Then, the crucibles with samples were placed in a muffle furnace and burned at 600°C for 6 hours. After burning the samples to ash, the muffle furnace was switched off, and the crucibles were allowed to cool off for a few minutes and then placed in a desiccator to cool off completely. After cooling, the crucibles with the remaining ash were weighed and recorded. Ash content was calculated using the following equation:

$$\%\text{Ash Content} = \frac{\text{Weight of Ash (g)}}{\text{Weight of Sample (g)}}\times 100. \quad (3)$$

2.7.4. Determination of Fat Content. Fat contents of the samples were determined using the solvent extraction method [52]. Two grams of predried and ground sample was taken into a predried extraction thimble and weighed. Predried boiling flask was weighed. N-Hexane was used as solvent. The Soxhlet apparatus was assembled with a condenser and heating source. Extraction was carried out for 16 hours at 69°C. After the extraction was completed, the boiling flask was dried in a hot air oven at 105°C for 30 min, cooled in a desiccator, and weighed again. Fat in the samples was calculated by deducting the flask weight from the weight we got by weighing the flask with fat. Fat percentage was calculated using the following equation:

$$\%\text{Fat Content} = \frac{\text{Fat in Sample(g)}}{\text{Weight of Sample (g)}}\times 100. \quad (4)$$

2.7.5. Determination of Total Carbohydrate Content. Total carbohydrate content was calculated based on the substruction of other components from the total weight of the sample. In this approach, the constituents of food (protein, fat, ash, and moisture) were determined individually and then

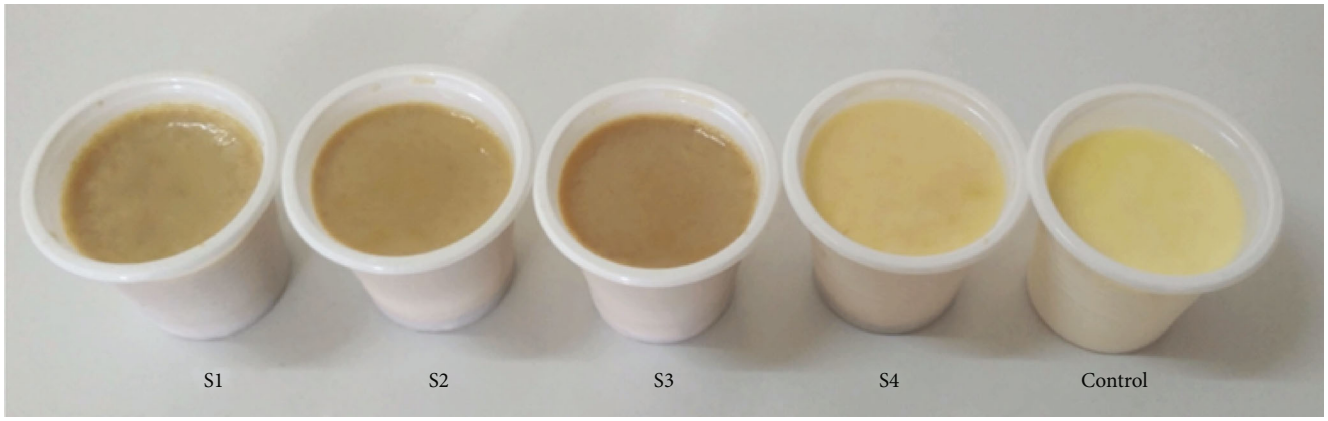

Figure 2: Prepared experimental (S1–S4) and control yogurts in the current study.

summed and subtracted from the total weight of the food [54]. Equation (5) is used for total carbohydrate calculation.

$$\text{Total Carbohydrate} = 100 - (\text{Protein} + \text{Fat} + \text{Ash} + \text{Moisture}). \quad (5)$$

2.7.6. Determination of Mineral Content. For the determination of sodium, potassium, phosphorous, calcium, iron, copper, magnesium, and zinc content in yogurt sample, the flame atomic absorption spectroscopy (FAAS) technique was used [55]. This analysis was performed using Thermo Scientific™ iCE™ 3000 Series AAS. Around 0.5 g of dried yogurt sample was digested with nitric acid and perchloric acid mixture, then filtered, and diluted before analysis. Standard was also prepared in known concentrations to derive standard curve to be used within the instrument. The instrument reading for each element was taken using specific lamps and settings.

2.7.7. Determination of pH. pH values of the prepared yogurt samples were determined by Hanna Instruments (HI 2211 pH/ORP meter). Before measurement, a two-point calibration was performed on the instrument using a buffer solution of pH = 4.0 and pH = 10.0. Electrode was dipped into the samples, and pH was recorded from the instrument display after holding the electrode for a while.

2.7.8. Determination of Total Soluble Solids (TSS). TSS of yogurt samples were determined by using the Hanna TSS meter (Model: HI 96801). The meter was calibrated using distilled water. Then, the samples were introduced to the instrument for measurement.

2.7.9. Determination of Energy Content. The calorie contents of the yogurt samples were determined by a bomb calorimeter (Parr Instrument Company, Model 1341). The dry and weighed sample was placed in a confined oxygen-filled vessel known as the bomb. The bomb was then placed in a pot, where water surrounds it. Temperature of water in the pot was continuously recorded using a digital thermometer. Then, the combustion was electrically triggered, and a change in water temperature was observed. Similar to the sample, a standard pellet of benzoic acid was used to calculate the energy equivalent of the instrument. Different minor correction factors were omitted for the sake of simplicity. Equation (6) was used to calculate the energy equivalent.

$$\text{Energy Equivalent}(W) = \frac{(H \times m1) + e1 + e3}{t1}, \quad (6)$$

where W is the energy equivalent of the calorimeter in calories per °C. H is the heat of combustion of the standard benzoic acid sample in calories per gram. $m1$ is the mass of the standard benzoic acid sample in grams. $t1$ is the net corrected temperature rise in °C. $e1$ is the correction in calories for heat of formation of nitric acid. $e3$ is the correction in calories for heat combustion of fuse wire.

And the gross heat of combustion was calculated by following:

$$\text{Gross heat of combustion}(\text{Hg}) = \frac{(W \times \text{t2}) - e1 - e2 - e3}{m2}, \quad (7)$$

where W is the energy equivalent of the calorimeter, determined under standardization. $m2$ is the mass of the sample (g). $t2$ is the temperature rise in sample combustion (°C). $e1$ is the correction in calories for heat of formation of nitric acid. $e2$ is the correction in calories for the heat of formation of sulfuric acid. $e3$ is the correction in calories for heat combustion of fuse wire.

2.8. Shelf-Life Study of Yogurt. Shelf life of all yogurt samples was studied in two different conditions: household refrigerated condition (4°C) and room temperature. During the study, the organoleptic properties (appearance, surface structure, and flavor) of the samples were checked periodically. The study was conducted for 15 days for refrigerated storage and 6 days for room temperature storage, starting from the day of preparation of the samples.

2.9. Statistical Analysis. The collected responses in triplicates were statistically analyzed using SPSS 25 (IBM, USA) software with a 5% confidence level. One-way ANOVA and

Duncan's multiple range test were performed to analyze the derived results.

3. Results and Discussion

3.1. Protein Content in JSPI. Figure 3(a) illustrates the amounts of protein present in JSPI and JSF. The protein content of the protein isolate made from JSF was 78.47%. This protein content is comparable with other studies conducted for exploring plant protein sources, such as Chandi and Sogi [56], who concentrated rice bran protein with 60% protein content, and Sogi et al. [57], who developed tomato seed protein isolates with 71.32% protein content. On the other hand, Joshi et al. [58] estimated that lentil protein isolates contain roughly 90% protein. The methods adopted for protein isolation, type of protein in the sources, and intracomponent linkages in the sources from which the proteins are being isolated may be the causes of the variance in protein contents in the isolates.

3.2. Mineral Content in the Mineral Concentrate. As presented in Figure 3(b), the mineral concentrate derived from process 1 was found to have higher ash content (33.45%) than the one derived from process 2 (17.63%). The result is in agreement with Nakagawa and Tanaka [59]. They patented a method for the preparation of milk mineral concentrate and found the ash content of produced mineral concentrate to vary between 20 to 35%. The variation of mineral content (correspondence to ash content) of the two processes is clearly an effect of the procedural variation. In process 2, the residual supernatant after protein precipitation was added, which was a diluted source of mineral. This addition of this supernatant contributed to increase the bulk of the raw material but added little to the final mineral concentrate. Another determining factor for this variation is the extraction time. In process 1, the starch sediment and water mixture were allowed to rest overnight after one hour of stirring, whereas in process 2, the starch and water mixture was instantly centrifuged after stirring to a get clear supernatant. According to Zhu et al. [60], longer extraction time yields more minerals in the concentrate. Mitić et al. [46] extracted minerals from garden sage (*Salvia officinalis* L.) using water as a solvent and studied the effect of different parameters: time, temperature, and solid-to-liquid ratio on extraction efficiency. They also reported that finding higher temperature and longer time was suitable for better extraction yield. For higher mineral extraction performance, we have used process 1 to extract the mineral and for further study onwards.

The mineral contents in the mineral concentrate are presented in Table 2. The major minerals found in the concentrate are Ca, Mg, P, K, Na, and Fe which are estimated as 202.20 mg, 230.01 mg, 193.51 mg, 2820.60 mg 478.42 mg, and 132.51 mg, respectively, per 100 g of concentrate sample. The mineral K was the highest content in the concentrate, valued at 2820.60 mg/100 g. Besides, good amounts of Cu and Zn were also present in the sample. The mineral contents in the concentrate were much higher (10 to 15 times) than the original contents in the JSF. The mineral contents of JSF measured in this study, presented in Table 2, are comparable with the reported values. For example, Ajayi [21] reported 19.00 mg Ca, 24.00 mg Mg, 247.00 mg K, 39.80 mg Na, 14.90 mg Fe, 4.10 mg Zn, and 2.20 mg Cu contents per 100 g of flour. The current study measured the mineral contents of JSF as Ca 18.31 mg, Mg 22.20 mg, P 17.51 mg, K 226.40 mg, Na 42.01 mg, Cu 3.20 mg, Fe 12.40 mg, and Zn 5.20 mg per 100 g of flour. These estimated values show good agreement with the previous reports of Swami et al. [61] as well.

3.3. Sensory Evaluation of Yogurts. The results from the sensory evaluation were analyzed and summarized in Table 3. It is suggested from the results that the addition of up to 6% of JSPI retained the acceptable sensory attributes. The organoleptic observation suggests that the fortification of protein and minerals minutely decreased the sensory scores, and this declination did reflect a statistically significant difference within this range of fortification. A further increase of JSPI (8%) in the formulations led to a cracked or wrinkled yogurt surface, resulting in a bad texture rating. Besides, this increment enhanced the grassy smell in the yogurt, which was not liked by all. The color of each yogurt sample was similarly attractive to the panelists. Addition of protein and mineral darkened the color of yogurt to a brownish shade but was still appealing to the evaluators.

3.4. Proximate Compositions and Physiochemical Properties of Yogurt. The yogurt samples were studied for physicochemical properties, and the results are presented in Table 4.

3.4.1. Protein Content. As expected, the protein content increased with the addition of JSPI in the yogurt formulations (Table 4). The change in protein content was also found statistically significant among all the fortification levels. In the control sample, the protein content was estimated as 3.13%, which increased to a maximum value of 9.42% in sample S1 (prepared by adding 8% JSPI). The protein content of plain (control) yogurt prepared in this study agrees with the literal value. In a comparative study, Kiros et al. [62] prepared a control yogurt and found that the protein content was 3.14%. Another recent study by Damayanti et al. [63] studied the effect of the addition of red beans, dates, and milk on yogurt and found protein content to vary from 2.30% to 3.63% which is consistent to the results of our control yogurt. Based on the statistical analysis of sensory evaluation scores (Table 3) for the prepared yogurt, it was observed that sample S2, fortified with maximum of 6% JSPI for protein content, had no significant impact on sensory attributes and contained 7.71% protein. Beyond this level of fortification, the panelists assigned less favorable scores.

3.4.2. Moisture Content. The moisture content of the yogurt samples ranged between 73.66% and 78.62%. The values are comparable with a previous study done by Aportela-Palacios et al. [64]. They found that the moisture contents ranged between 77% and 81% in the fiber-supplemented yogurts. Parvin et al. [50] prepared yogurt with wood apple powder and found that the moisture ranged from 71.42% to 73.14%, which is also quite similar to our results. A recent study conducted by Aamir et al. [65] on yogurt fortification

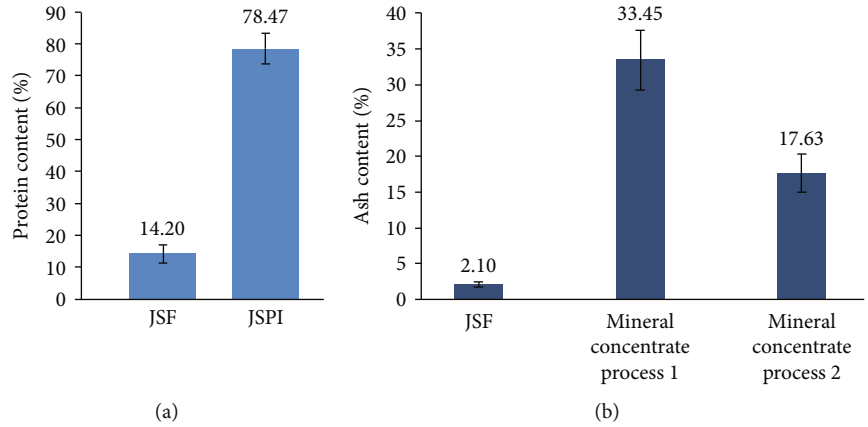

Figure 3: (a) Protein content of JSF and JSPI. (b) Ash content of JSF and mineral concentrates.

Table 2: Mineral content in the concentrate and yogurt samples (mg/100 g).

Minerals	JSF	Mineral concentrate	Yogurt samples				
			S1	S2	S3	S4	S5
Ca	18.31 ± 0.01	202.20 ± 1.12	219.50 ± 0.06^{b}	214.40 ± 0.01^{d}	221.50 ± 0.02^{a}	218.70 ± 0.07^{c}	206.10 ± 0.01^{e}
Mg	22.20 ± 0.04	230.01 ± 1.48	25.20 ± 0.01^{b}	26.20 ± 0.01^{a}	23.90 ± 0.01^{d}	24.60 ± 0.01^{c}	11.50 ± 0.01^{e}
P	17.51 ± 0.07	193.51 ± 0.19	142.00 ± 0.02^{d}	144.41 ± 0.01^{c}	145.50 ± 0.01^{b}	151.00 ± 0.05^{a}	130.20 ± 0.01^{e}
K	226.40 ± 0.10	2820.60 ± 1.65	295.39 ± 0.06^{a}	288.61 ± 0.02^{d}	292.30 ± 0.01^{c}	293.20 ± 0.01^{b}	150.40 ± 0.01^{e}
Na	42.01 ± 0.01	478.42 ± 1.19	65.00 ± 0.01^{c}	68.21 ± 0.01^{a}	63.00 ± 0.01^{d}	66.41 ± 0.04^{b}	37.00 ± 0.01^{e}
Cu	3.20 ± 0.01	390.01 ± 1.46	1.80 ± 0.01^{c}	1.90 ± 0.01^{b}	2.10 ± 0.01^{a}	1.70 ± 0.01^{d}	0.04 ± 0.01^{e}
Fe	12.40 ± 0.01	132.51 ± 1.76	6.30 ± 0.01^{b}	5.80 ± 0.01^{d}	6.80 ± 0.01^{a}	6.00 ± 0.01^{c}	0.05 ± 0.01^{e}
Zn	5.20 ± 0.01	64.51 ± 1.21	3.50 ± 0.01^{c}	3.90 ± 0.01^{a}	3.70 ± 0.01^{b}	3.40 ± 0.01^{d}	0.30 ± 0.01^{e}

Mean ± standard deviation values; different lowercase letters denote a significant difference between the columns at a 5% level. Here, S1 = 8% protein+mineral; S2 = 6% protein+mineral; S3 = 4% protein+mineral; S4 = 2% protein+mineral; S5 = control yogurt, no added protein and mineral.

Table 3: Sensory assessment scores of protein and mineral-enriched yogurts.

Sample name	Color	Flavor	Texture	Taste	Overall acceptability
S1	7.13 ± 0.35^{a}	6.70 ± 0.41^{b}	6.18 ± 0.33^{b}	7.02 ± 0.28^{b}	6.70 ± 0.25^{b}
S2	7.15 ± 0.27^{a}	7.28 ± 0.25^{a}	7.43 ± 0.31^{a}	7.35 ± 0.42^{a}	7.20 ± 0.36^{a}
S3	7.10 ± 0.28^{a}	7.30 ± 0.25^{a}	7.47 ± 0.37^{a}	7.38 ± 0.49^{a}	7.30 ± 0.36^{a}
S4	7.27 ± 0.40^{a}	7.32 ± 0.25^{a}	7.52 ± 0.33^{a}	7.42 ± 0.43^{a}	7.35 ± 0.35^{a}
S5 (control)	7.16 ± 0.35^{a}	7.34 ± 0.26^{a}	7.55 ± 0.35^{a}	7.47 ± 0.43^{a}	7.35 ± 0.37^{a}

Mean ± standard deviation values; different lower-case letters denote a significant difference between the rows at a 5% level. Here, S1 = 8% protein+mineral; S2 = 6% protein+mineral; S3 = 4% protein+mineral; S4 = 2% protein+mineral; S5 = control yogurt, no added protein and mineral.

with ginger (*Zingiber officinalis* Roscoe) reported that the fortified yogurt had around 79.16% moisture, consistent with our result. Notably, the moisture content slightly decreased in the yogurt product with increased JSPI in the formulation (Table 4), and this change is statistically significant. This decrease in moisture is attributed to an increase in total solid content resulting from fortification with JSPI.

3.4.3. Ash Content. Table 4 illustrates that the ash content of the enriched yogurt samples markedly increased up to 1.43% from its approximate original content of 0.80% found in the control sample. This change is statistically significant. Since the quantity of mineral concentrate remained consistent for fortification, the control sample had only 0.80% ash content, while the fortified yogurt displayed ash contents ranging from 1.39% to 1.43%, almost doubling the original levels. These results clearly indicate that the addition of mineral concentrate derived from jackfruit seeds led to the observed increase in ash content. In comparison to our findings, the ash content of our control yogurt (0.80%) is slightly higher than that reported by Farinde et al. [66] at 0.60%, similar to the results of Parvin et al. [50] at 0.70% and El-

Table 4: Proximate composition and physicochemical parameters of prepared yogurt.

	S1	S2	S3	S4	S5 (control)
Protein (%)	9.42 ± 0.07^{a}	7.71 ± 0.09^{b}	6.19 ± 0.07^{c}	4.57 ± 0.05^{d}	3.13 ± 0.03^{e}
Moisture (%)	73.66 ± 1.09^{d}	75.45 ± 0.78^{c}	76.85 ± 0.94^{bc}	77.48 ± 0.69^{ab}	78.62 ± 1.07^{a}
Ash (%)	1.43 ± 0.05^{a}	1.41 ± 0.04^{a}	1.39 ± 0.02^{a}	1.40 ± 0.04^{a}	0.80 ± 0.03^{b}
Fat (%)	3.67 ± 0.09^{a}	3.69 ± 0.08^{a}	3.66 ± 0.07^{a}	3.64 ± 0.04^{a}	3.63 ± 0.04^{a}
Carbohydrate (%)	12.51 ± 0.08^{d}	12.62 ± 0.06^{d}	12.80 ± 0.05^{c}	13.29 ± 0.08^{b}	13.81 ± 0.04^{a}
pH	4.78 ± 0.01^{a}	4.76 ± 0.02^{ab}	4.75 ± 0.01^{b}	4.72 ± 0.01^{c}	4.71 ± 0.01^{c}
TSS (%)	24.34 ± 0.05^{a}	23.55 ± 0.05^{b}	21.85 ± 0.09^{c}	20.82 ± 0.08^{d}	20.78 ± 0.04^{d}
Energy (Kcal/100 g)	76.54 ± 0.56^{a}	73.03 ± 0.25^{b}	70.91 ± 0.24^{c}	62.37 ± 0.29^{d}	57.67 ± 0.37^{e}

Mean ± standard deviation values; different lower-case letters denote a significant difference between the columns at a 5% level. Here, S1 = 8% protein +mineral; S2 = 6% protein+mineral; S3 = 4% protein+mineral; S4 = 2% protein+mineral; S5 = control yogurt, no added protein and mineral.

Nawasany et al. [67] at 0.68% to 0.73%, and more in line with the findings of Bhat et al. [68] at 0.77%.

3.4.4. Fat Content. The fat contents of the protein-enriched yogurt samples varied so slightly that the change was rendered statistically insignificant, ranging between 3.63% and 3.69% (Table 4). Sodini et al. [45] fortified yogurt with commercial whey protein concentrates and found that the fat contents ranged between 2.10% and 3.70%. In one recent study, Afiyah et al. [69] found the fat content of control yogurt to be 3.15%. The addition of JSPI in the formulation clearly did not contribute to the increment of fat in the fortified yogurt samples, so the changes are also statistically insignificant.

3.4.5. Carbohydrate Content. The carbohydrate content of the yogurt samples ranged from 12.01% to 13.81%. Normally, the carbohydrate content of yogurt depends on the amount of added sugar in the formulation. In the current study, the amount of sugar (sucrose) was constant for all samples; still, the carbohydrate content showed a subtle trend of decreasing with the increase of JSPI in yogurt. Parvin et al. [50] found similar data for carbohydrate content, ranging from 12.72% to 18.83%, whereas Hossain et al. [70] found carbohydrate content ranging from 16.60% to 18.91%, which is higher than what was found in the study. As mentioned above, the variation of total carbohydrate content in different studies is principally due to the variation of the amount of added sugar for the desire of sweetness in the final product, as well as the presence of sugar in the milk in yogurt preparation. However, in our case, the increase in protein content and ash content appeared to contribute to the reduction in carbohydrate content, as evident from our calculation method, where carbohydrate levels were determined through subtraction.

3.4.6. Mineral Content. The mineral content of the yogurt samples is presented in Table 2. The control sample (S5), which was prepared with no additional mineral concentrate, contained 206.10 mg, 11.50, 130.20, 150.40, 37.00, 0.04, 0.05, 0.30 mg of Ca, Mg, P, K, Na, Cu, Fe, and Zn, respectively, per 100 g yogurt. Calcium is known as "milk mineral" as the presence of this mineral in milk is relatively high [71]. Yogurt as a milk product is also supposed to have a large calcium content. According to Tamime and Robinson [72], full cream milk yogurt has a calcium of 200 mg/100 g, which is comparable to our control yogurt.

Ocak and Rajendram [73] reported whole milk plain yogurt having 11 mg/100 g of magnesium. We found the similar amount of magnesium per 100 g of control yogurt. The phosphorous content is comparable with the findings reported by Tamime and Robinson [72] and McCance and Widdowson [74], where they found a similar amount of phosphorous (170 mg/100 g) in control yogurt. The estimated potassium (K) content in the control yogurt is also supported by the findings of Tamime and Robinson [72], who showed 140 mg of potassium in 100 g of yogurt. The sodium (Na) content of our control sample corresponds to Luis et al. [75] and Hernandez and Park [76]. They reported the sodium content of yogurt to be 46.20 mg/100 g and found 47.50 mg/100 g, respectively. The copper (Cu) content of control yogurt, determined in this study, is similar to Luis et al. [75] and Musaiger et al. [77] who found 0.03 mg/100 g and 0.04 mg/100 g of copper in plain yogurt samples. The iron (Fe) content of our control yogurt is alike to what Luis et al. [75] and Hernandez and Park [76] found, where they stated an iron content of 0.03 mg/100 g and 0.21 mg/100 g, respectively, analyzing the plain yogurts prepared by cow milk. The zinc (Zn) content of the control is similar to the study of Luis et al. [75] in which they found almost the same data of 0.31 mg of zinc per 100 g of yogurt.

The current study successfully enriched the yogurts with minerals by adding JSF mineral concentrate to the formulations. As shown in Table 2, the mineral content of the enriched samples is higher than that of the control sample. For example, the calcium content of the enriched samples increased to a range of 218.70 mg to 221.50 mg per 100 g of yogurt, compared to its original value of 206.10 mg (control sample). Similarly, the magnesium content of the enriched samples increased to a range of 23.90 mg to 26.20 mg per 100 g of yogurt, compared to its original value of 11.50 mg in the control sample. The greatest increase in mineral content was observed in potassium, which increased to a range of 288.61 mg to 295.39 mg per 100 g of yogurt, compared to its original value of 150.40 mg in the control sample. This boost is logical because of the highest content of this mineral in the concentrate that has been added. The other minerals were also enriched proportionately due to adding the

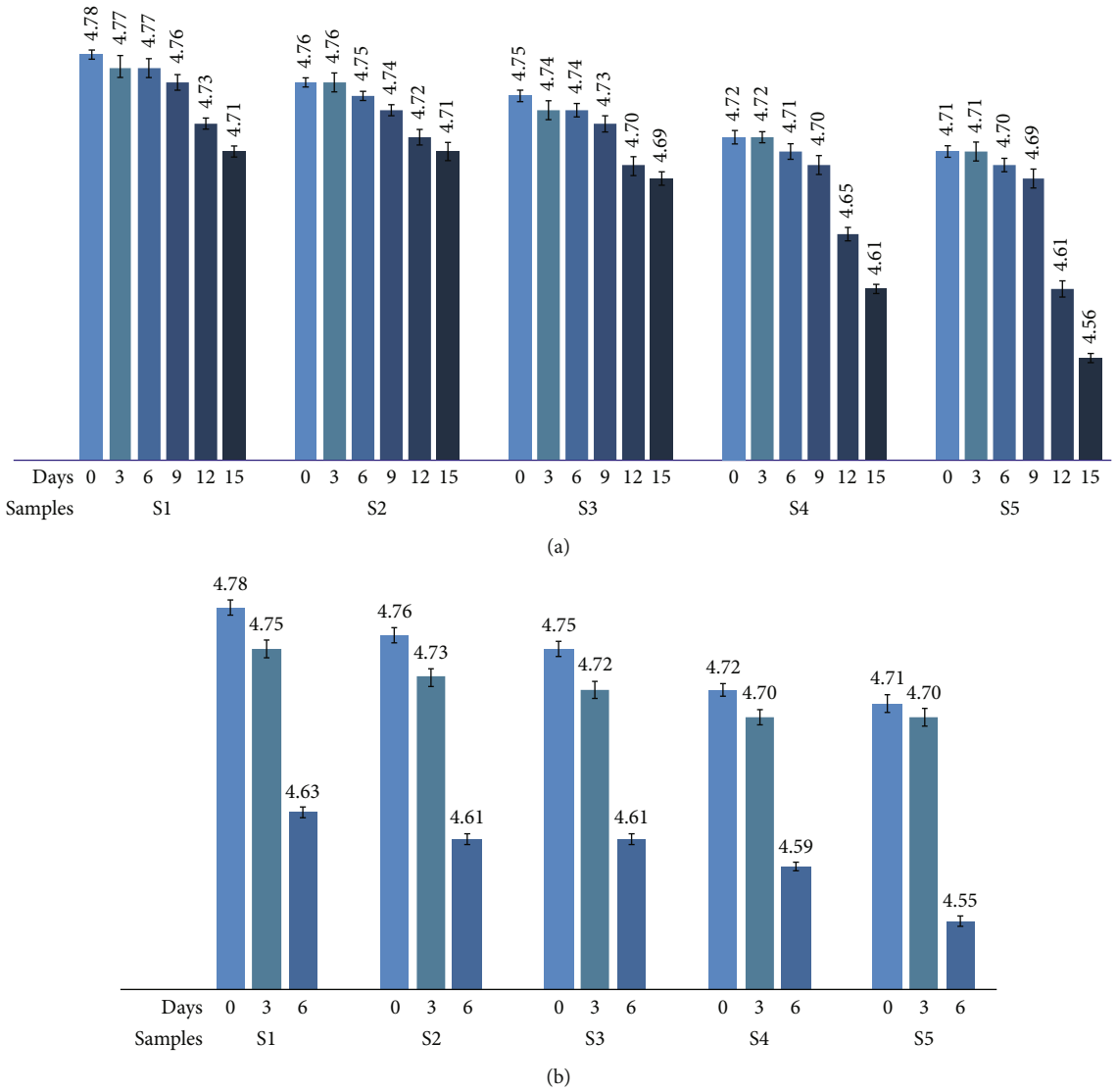

Figure 4: pH changes during storage of yogurts. (a) Storage at refrigeration temperature and (b) storage at room temperature. Here, S1 = 8% protein+mineral; S2 = 6% protein+mineral; S3 = 4% protein+mineral; S4 = 2% protein+mineral; S5 = control yogurt.

mineral concentrate (as shown in Table 2). The mineral contents in 100 g enriched yogurt meet from 20% to 100% of the recommended dietary allowance (RDA) of different minerals set by NUH [78] and National Health and Medical Research Council, Australian Government Department of Health and Ageing, and New Zealand Ministry of Health [79].

3.4.7. pH. pH, a measure of acidity or alkalinity, is a crucial factor in determining the quality of yogurt. During the preparation of yogurt, the pH of milk curd, which is initially neutral at 7, falls below 7 after the addition of starter culture due to the conversion of lactose in milk into lactic acid. An acidic pH is desirable for both digestion and storage. The pH of the yogurt samples in this study ranged between 4.65 and 4.78 (Table 4). According to Lee and Lucey [80], the typical pH of yogurt is 4.6. In a study by Brückner-Gühmann et al. [81], the enrichment of yogurt with oat protein fractions resulted in similar findings, where the increase in protein concentration led to an insignificant change in pH. Santillán-Urquiza et al. [33] fortified yogurt with nano and microsized calcium, iron, and zinc and found a pH range of 4.68 to 4.77. In the current study, the reduction of pH was slightly less in the enriched yogurts compared to the control (pH 4.71). This may be due to the fact that the addition of JSPI and mineral concentrate in the formulation increased the solid content and decreased the bacterial activity in the production of lactic acid [82].

3.4.8. Total Soluble Solids (TSS). TSS, or total solids, are another important parameter for determining the quality of yogurt, specifically for the thickness and cohesiveness of the particles in its texture. The TSS of our yogurt ranged from 20.78% in the plain yogurt to 24.34% in the enriched yogurt and showed an increasing trend with the increase of protein concentration (Table 4). Hossain et al. [70] used different fruit juices at different concentrations and found TSS to be within 25.33% to 27.17%. Similarly, Parvin et al. [50]

prepared yogurt using wood apple powder and found TSS range from 25.87% to 27.55%. Both studies had slightly higher TSS values than our results. Santillán-Urquiza et al. [33] found total solids within the range of 15.98% to 17.98%, which is slightly lower than what was found in this study.

3.4.9. Energy Content. The energy content of the protein and mineral-enriched yogurt samples in this study ranged from 57.67 to 76.54 kcal/100 g, as shown in Table 4. These values are consistent with those found by Weerathilake et al. [83] in their analysis of various types of low-fat and whole-milk yogurt (54-79 kcal/100 g). A similar result was reported by Chandan and Shah [84] for fruit-flavored whole milk yogurt (61 kcal/100 g). The increase in energy content of the enriched yogurts can be attributed to the additional protein content.

3.5. Shelf-Life Study of Yogurts. The shelf-life study of yogurt was done in refrigerated and room conditions. At refrigeration temperature, the yogurt samples were unfit for consumption after 12 days of storage. On the 10th day, all the samples were still acceptable, except for a slight off-flavor detected by sniffing. On the 11th day, all samples were rated as unpleasant, except sample S1 (with 8% JSPI) which was rated as slightly unpleasant with a slight grassy smell. In the shelf-life study at room temperature, all samples were edible for up to three days. The control and the less enriched samples (2% and 4% JSPI added) became unacceptable by the 4th day of storage. All the samples were rated as unpleasant at the end of the 6th day of storage.

It is commonly assumed that, in both storage temperatures, the samples with protein enrichment remained edible for a longer period. This is logical as yogurts prepared with just milk and sugar are more susceptible to microbial spoilage than those prepared with added JSPI and minerals. The added solid content in the latter restricts the growth of spoilage microorganisms and slows down chemical reactions.

3.5.1. Changes in pH during Shelf-Life Study of Yogurt. Lactic acid bacteria (LAB) are the predominant microbe in yogurt. During the storage period of yogurt, LAB may still exercise some metabolic activity, which is associated with lactose consumption, and end up contributing to the lactic acid and galactose production that reduces the pH of yogurt [82, 85]. Kailasapathy and Sultana [86] reported that β-galactosidase, an enzyme produced by *Lactobacilli*, stays active even at temperatures of 0-5°C.

The reduction in pH during storage, at both temperatures, occurred due to the continuity of lactic acid production in yogurt (Figures 4(a) and 4(b)). The figures exhibited that the yogurts with higher protein content (S1) showed a lower trend of pH reduction compared to the control yogurt. Furthermore, excessive acid production showed a negative relation with the shelf life of yogurt. Therefore, the yogurt samples, especially the samples with no or minimum added protein, initiated sensory dissatisfaction marked with acidic flavor. A drastic reduction of pH was seen after 12 days of storage at refrigerated temperature (Figure 4(a)) and after 6 days of storage at room temperature (Figure 4(b)). Mataragas et al. [87] reported a similar case during the study of the shelf life of yogurts mixed with fruit. In their storage study at refrigerated temperature, the pH remained almost constant for up to 14 days and slightly decreased after this period. However, they found a drastic declination of pH from the 2nd day of storage at 20°C and reported that changes in pH during storage are related to storage temperature, initial acidity of yogurt, and acidifying power of culture used. Room temperature influences the increased activity of microbes in yogurt and changes in pH faster in contrast with low-temperature storage.

4. Conclusions

The enrichment of food products, such as yogurt, with essential nutrients can play a crucial role in addressing widespread malnutrition. This is particularly effective as yogurt is a widely consumed food across all age groups. The protein isolate obtained from jackfruit seed flour via an alkaline extraction method has a protein content of 78.47%, while the mineral concentrate derived from the same seed flour has an ash content of 33.45%.

In this study, yogurts were enriched with varying amounts of extracted protein and a fixed quantity of concentrated minerals. The results indicate that the enriched yogurts possess significantly higher nutritional value compared to the control yogurt that was not subjected to fortification. The most satisfactory outcome was achieved by incorporating 6% of JSPI into the yogurt, which resulted in a higher protein content while maintaining acceptable sensory properties. Additionally, a consistent rate of 747 mg JSF mineral concentrate per 100 g of yogurt was found to be optimal for successful enrichment. Fortifying mineral concentrate in yogurts significantly increased the mineral components but remained within the acceptable range of human recommended dietary allowance (RDA). The proposed methods and findings from this study would be useful for the protein and mineral-enriched food products as well as for the related research in the subject.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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