# STUDY ON FERMENTATION CONDITIONS FOR "YOGURT" PRODUCTION FROM SOYBEAN (*Glycine max* (L.) Merill) AND JACKFRUIT SEED (*Artocarpus heterophyllus* Lam.)

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**ABSTRACT**

Nowadays, consumers tend to consume plant-based products due to their high nutritional value and health benefits, making plant-based yogurt a potential product for development. In this study, milk liquid from soybean and jackfruit seed were used to completely replace animal milk in the yogurt fermentation process. This approach contributes to increasing the value of agricultural products and utilizing the abundant by-product of jackfruit seeds. The objective of this study was to develop a fermentation process for soybean and jackfruit seed "yogurt" by determining (i) the commercial bacterial strain, (ii) sugar content and the soybean-to-jackfruit seed ratio, (iii) fermentation temperature, time, and bacterial inoculum concentration. The results revealed that the commercial bacterial strain "Start for yogurt (SC)" effectively fermented the mixture, yielding yogurt with favorable physicochemical, microbiological, and sensory properties. The optimal conditions were identified as a soybean-to-jackfruit seed milk ratio of 6:4, 15% (w/v) sucrose addition, an inoculum concentration of 2.3% (v/v), and fermentation at 44.6 °C for 7.01 h.

*Keywords*: Fermentation, jackfruit seed, optimization, soybean, yogurt.

## 1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is considered a "superfood" due to its abundant and various nutritional components, including protein, lipids, vitamins, etc. The protein content accounts for high level in soybeans, ranging from 35.5% to 40%, and it is the highest quality protein among plant-based sources. Fat accounts for 20% of the dry matter of soybeans, of which approximately 80-93.6% are unsaturated fatty acids (UFAs), which have been proved to be beneficial for health, such as limiting the formation of bad cholesterol (LDL cholesterol) and preventing cardiovascular diseases [1]. In particular, soy isoflavones, which are present in high amounts (0.1-0.3%), are the richest source of these compounds in nature. They have antioxidating effects and help prevent diseases such as cardiovascular diseases, osteoporosis… and also benefit to skin care [2]. Soybeans also contain 4-6% raffinose and stachyose (other sugars account for negligible amount) which can cause bloating when digested but this can be solved by microbial metabolism through fermentation process [3].

Jackfruit (*Artocarpus heterophyllus* Lam.) is a kind of fruit tree widely cultivated in many tropical regions on Earth. Only 30-35% of a jackfruit's weight is typically consumed as food. The seeds of jackfruit constitute a significant portion of the total weight of the fruit (18-20%) [4], but they are not commonly consumed or used as raw material in industries, especially in the food production field. Research has shown that jackfruit seeds have a rich nutritional composition, essential for the body, including protein (12.45%), carbohydrates (70.76%), and vitamins. Additionally, ash contains various minerals (2.46%), and fat (0.77%) also constitutes a small proportion of the jackfruit seed composition [5]. Carbohydrates are the most abundant component, primarily in the form of starch which serves the energy metabolism, nonetheless, a high starch content can cause indigestion. Fiber and resistant starch play a crucial role in enhancing gut probiotics by serving as essential prebiotics for bacterial activity. These prebiotics, along with bioactive compounds (saponins, lignans, etc.) in jackfruit seeds, help reduce the risk of cardiovascular diseases, aging, high cholesterol, and more [6].

Nowadays, yogurt has become one of the most popular fermented foods worldwide, known for its delicious taste, nutritional value, and health benefits. As society develops, the trend of consuming natural products is attaining popularity due to their abundance in nutrients, safety and bringing well-being. In response to this demand, plant-based yogurt and yogurt alternatives to dairy products have been widely researched. Several researches have been conducted regarding the production of plant-based yogurt such as Tran Thi Dinh *et al.* or Kumar & Mishra [7, 8]. However, these studies, along with many others, still contain dairy in their ingredients, while 2-3% of the global population is allergic to cow's milk, and it has been shown that yogurt still include lactose, with levels only 30% lower than in milk [9, 10]. Moreover, concerns about dairy products are increasing because of the presence of antibiotic residues and growth hormones [7]. This causes safety risks and discomfort for a significant number of consumers. Nevertheless, completely removing dairy from the ingredients results in a reduction in product quality, requiring the use of more additives. Therefore, this study combined soy milk with jackfruit seed extract to ferment vegan "yogurt", leveraging the abundant prebiotics in jackfruit seeds to support bacterial growth during the fermentation process. Additionally, the starch content in jackfruit seeds has the potential to stabilize the structure and enhance the sensory qualities of the "yogurt", similar to the findings of Jimoh & Kolapo [11]. This study aimed to find a suitable commercial starter culture, stabilize the production process, and optimize the conditions of fermentation in order to create acceptable-quality plant-based "yogurt".

## 2. MATERIALS AND METHODS

### 2.1. Materials and chemicals

Soybeans were purchased from a grocery store in Can Tho City. They were small-sized beans with a bright yellow color, not damaged, and possessing a characteristic pungent odor.

Jackfruit seeds (Thai Changai variety) were obtained from a produce store in Can Tho City. The seeds were intact, ungerminated, and free from mold.

The freeze-dried bacterial cultures were collected from the following suppliers:

– "Yogurt with Probiotic" starter culture from Yogourmet (France) includes: *B. longum, L. rhamnosus, L. casei, L. helveticus, L. bulgaricus, L. acidophilus,* and *S. thermophilus.* Coded "MET".

– "Alsa-mon yaourt Maison" starter culture from Alsa (France) includes: *S. thermophilus* and *L. bulgaricus.* Coded "AL".

– "Vegan Yogurt Starter" starter culture from Hanh Phuc Co., Ltd. (Vietnam) includes: *Lactobacillus, Bifidobacteria, B. subtilis,* and *S. thermophilus.* Coded "HP".

– "Start for Yogurt" starter culture from Shangchuan (China) includes: *L. bulgaricus, S. thermophilus, B. lactis, B. longum, L. acidophilus,* and *L. casei.* Coded "SC".

Chemicals: NaOH 0.1 N solution (Cemaco, Vietnam), phenolphthalein, absolute ethanol (Xilong Scientific, China), agar and sucrose (Bien Hoa, Vietnam). MRS broth medium (GM369, Himedia, India).

### 2.2. Methods

#### *2.2.1. Production of soybean and jackfruit seed "yogurt"*

*Preparation of soy milk and jackfruit seed milk:* The soybean was cleaned and soaked in water (ratio 1:2.5 w/v) for 6-7 h at room temperature. After soaking, the soybeans were blanched in a 0.25% $NaHCO_3$ solution at 80 °C, then finely ground with water (ratio 1:5 w/v) and filtered to obtain soy milk with pH ranged from 6.5 to 6.8 [12]. For jackfruit seeds, after collection, the seeds were washed to remove impurities and soaked in water for 5-6 h. After soaking, the seeds were rinsed with clean water, blanched in boiling water for 2-3 min, and peeled. Next, the seeds were finely ground in clean water (ratio 1:3 w/v) and filtered twice to obtain a milky white liquid [13], pH value was at closely 6.5.

*Preparation of commercial bacterial cultures:* Dry yogurt starter cultures were activated at 43 °C and proliferated on a shaker in MRS broth at 37 °C for 48 h.

*Preparation of starter culture on soy milk and jackfruit seed milk:* The filtered soy milk and jackfruit seed milk were mixed in a 1:1 ratio (v/v), added with 15% (w/v) sucrose, and heated to 95 °C for approximately 10 min. The milk mixture was then cooled to 43 °C and inoculated with starter cultures at a concentration of 10% (v/v). The mixture was incubated at 40-45 °C for 8 h and then ready to be used.

*2.2.2. Selection of suitable commercial starters for "yogurt" fermentation from soy milk and jackfruit seed*

The filtered soy milk was supplemented with 15% sucrose (w/v) and 0.35% agar (w/v) [3, 8]. It was then heated to a temperature of 92-95 °C, continuously stirred for approximately 10 minutes to cook the soy milk and dissolve the agar. Next, jackfruit seed milk was added at the ratio 5:5 (v/v) and stirred for 3 minutes. After that, the milk mixture was cooled to 43 °C, and the starter culture was inoculated at a concentration of 5% (v/v), with a lactic acid bacteria count of $10^6$ CFU/mL. The jars of milk mixture were finally incubated at 41 °C for 8 h, then stored in the refrigerator (2-6 °C) for 12 h before measuring the parameters [11]. The evaluation parameters included pH, level of increase in total titratable acidity (Δacid, g/L), firmness (gf), water holding capacity (WHC, %), lactic acid bacteria count (logCFU/mL), and sensory quality (texture, color, aroma, and taste).

*2.2.3. Investigation of the effect of supplementary component ratios on "yogurt" quality*

After filtering, soy milk and jackfruit seed milk were blended at various ratios (5:5, 6:4, 7:3, 8:2 v/v), added sucrose at different concentrations (10%, 15%, 20% w/v), and 0.35% agar (w/v). The mixture was heated for approximately 10 min at a temperature of 92-95 °C, then cooled, inoculated with the starter culture, incubated and stored, and the evaluation parameters were the same as described in section 2.2.2.

*2.2.4. Optimization of fermentation conditions using the response surface methodology (RSM)*

A Box-Behnken design was applied to minimize experimental trials while ensuring the necessary precision for optimizing fermentation conditions. The factors which were studied included fermentation temperature (37-45 °C), fermentation time (6-10 h), and bacterial concentration (1-10% v/v), each tested at three levels (-1, 0, 1). The central values for these factors were based on the previous studies [3, 7]. The pH value of the samples was selected as the response variable. Data were analyzed using Design-Expert 7.0.0 software, with ANOVA performed at a significance level of 5% ($\alpha = 0.05$).

"Yogurt" samples were prepared following the method described in section 2.2.2, using the starter culture, sugar content, and soybean-jackfruit seed milk ratios determined from the previous experiments.

*2.2.5. Analytical methods*

pH measurement: pH was measured using a Horiba pH meter (pH1100, Japan). Brix measurement: brix value was determined using an Atago refractometer (Master-2α, Japan). Determination of total titratable acidity (TTA): acidity was measured by titration with 0.1N NaOH solution [14]. Water holding capacity (WHC, %): WHC was analyzed using a refrigerated centrifuge (Hettich, Germany). "Yogurt" samples (20-25 g) were centrifuged at 5,000 rpm at 4 °C for 10 minutes [15]. Firmness measurement (gf): Firmness was measured using a TA-XT Plus texture analyzer (Stable Micro System Ltd, UK) with a cylindrical 35 mm diameter acrylic probe at a test speed of 1.00 mm/s [16]. Lactic acid bacteria count (logCFU/mL) was determined using the colony-counting method on MRS agar medium [17].

Sensory Evaluation: Sensory characteristics were scored (1-5 points) based on 4 criteria (texture, color, aroma, and taste) by 9 trained panelists specializing in food and biotechnology. The scoring descriptions for "yogurt" are as follows: Texture (5- firm, uniform, and smooth; 4- firm but less uniform and smooth; 3- weak firmness, uneven with lumps; 2- poor firmness, excessive syneresis; 1- no firmness, heavy syneresis); Color (5- milky white; 4- milky white with a slight yellow hue; 3- ivory white with a light yellow hue; 2- ivory white with a deep yellow hue; 1- unusual color); Aroma (5- characteristic yogurt aroma; 4- mild yogurt aroma with hints of jackfruit seed and/or soybean; 3- distinct aroma of soybean and/or jackfruit seed; 2- unpleasant odor of soybean and/or jackfruit seed; 1- unpleasant or off-putting odor); Taste (5- harmonious sweet-sour balance, lingering and appealing aftertaste; 4- fairly balanced sweet-sour taste, light and pleasant aftertaste; 3- very sour or overly sweet; 2- bland or unusual taste; 1- unpleasant or off-putting taste).

*2.2.6. Statistical analysis*

All experiments were conducted in three replicates, and the data are presented as mean ±SD. Statistical analysis was performed using Statgraphics Centurion XV software (Statpoint Technologies Inc., USA). Optimization data were analyzed with Design-Expert 7.0.0 software (Stat-Ease, Inc., USA).

## 3. RESULT AND DISCUSSION

### 3.1. Selection of suitable commercial starters for "yogurt" fermentation from soy milk and jackfruit seed

The effects of starter cultures on the physicochemical and microbiological properties of "yogurt" from soybean and jackfruit seed are shown in Table 1. The statistical analysis of the result indicated that the average pH values of "yogurt" from soybean and jackfruit seed across different starter cultures showed no significant differences (p>0.05) under other fixed fermentation conditions (temperature, incubation time, and bacterial concentration). The pH values ranged from 4.52 to 5.07 and resembled previous related studies. Tran Thi Dinh *et al.* [7] reported pH values of 4.5-4.9 for soybean yogurt fermented with LAB strains isolated from soybeans after 8 h, while Li *et al.* observed pH values of 4.6-5.1 using some lactic acid bacteria [18]. The initial total titratable acidity (TTA) was 1.08±0.23 g/L (calculated as lactic acid). After fermentation, the TTA considerably increased in all samples, with no significant overall differences between starter cultures (p>0.05). However, a notable difference was observed between SC (4.62 g/L) and AL (2.85 g/L).

The type of microorganism plays an important role in the conversion of sugars into lactic acid due to differences in their characteristic metabolic pathways. Even though all cultures were inoculated with the same initial count of $10^6$ CFU/mL and at a concentration of 5% (v/v), there were significant differences in the bacteria species composition among cultures. The data showed the lowest Δacid in culture AL, which comprises only two basic species, *S. thermophilus* and *L. bulgaricus*. Neither of these species is capable of starch hydrolysis [19]. *S. thermophilus* produces invertase enzymes that efficiently metabolize sucrose and can also metabolize raffinose to produce lactic acid, yet it shows negligible capacity to metabolize stachyose [20]. Regarding *L. bulgaricus,* it demonstrates very limited capacity to metabolize sucrose, D-raffinose, and stachyose [21]. With the sucrose supplementation fixed at 15% (w/v) and considering that soybeans naturally contain 4-6% dry weight of sugars such as stachyose and raffinose [22], the limited metabolic capacity of these two species resulted in the lowest total acid production among the four cultures tested.

*Table 1.* Effects of starter cultures on the physicochemical and microbiological properties of the soybean and jackfruit seed "yogurt"

| Starter | pH | Δacid (g/L) | LAB Count (logCFU/mL) | Firmness (gf) | WHC (%) |
|---|---|---|---|---|---|
| AL | 5.07±0.57[a] | 2.85±0.34[b] | 8.24±0.47[b] | 64.22±7.60[a] | 68.72±2.11[a] |
| HP | 4.98±0.53[a] | 3.78±0.78[ab] | 9.31±0.06[a] | 68.66±4.89[a] | 62.74±0.67[b] |
| MET | 5.08±0.15[a] | 3.66±0.99[ab] | 8.73±0.65[ab] | 58.9±5.27[ab] | 60.53±0.79[b] |
| SC | 4.52±0.16[a] | 4.62±0.35[a] | 9.22±0.39[a] | 49.74±2.38[b] | 52.50±1.80[c] |

*Different letters in superscript indicate statistically significant differences (p<0.05)*

Conversely, the SC and MET cultures produced higher levels of total acid compared to the other two cultures. This can be attributed to the presence of additional LAB species in SC, including *B. lactis*, *B. longum*, *L. acidophilus*, and *L. casei*, alongside *L. bulgaricus* and *S. thermophilus*. Specifically, *B. lactis* and *B. longum* are classified as heterofermentative, obligate anaerobic bacteria which generate lactic acid and other by-products. Both species possess the ability to metabolize sucrose, raffinose, and stachyose through enzymes such as invertase, sucrose phosphorylase, and α-galactosidase. Additionally, certain strains within these species have been found to produce intracellular amylase, enabling starch hydrolysis into simpler carbohydrates for metabolism in starch-rich environments [23, 24]. *L. acidophilus* and *L. casei*, both homofermentative bacteria, can metabolize sucrose via sucrase and sucrose phosphorylase enzymes [25]. They also efficiently convert raffinose and stachyose into lactic acid and other by-products. Furthermore, *L. acidophilus* has been shown to secrete extracellular amylase for starch hydrolysis, with increased amylase yielding observed at temperatures above 32 °C but below the species' maximum tolerance [26]. Research indicates that a minority of *L. casei* strains (<10%) possess the amylase gene, enabling starch metabolism into acids [25]. In the MET culture, in addition to *B. longum*, *L. acidophilus*, and *L. casei*, it includes two additional LAB species, *L. rhamnosus* and *L. helveticus*, both of which are homofermentative [27]. While neither species can effectively hydrolyze starch due to the absence of endogenous amylase, they efficiently metabolize sucrose, raffinose, and stachyose, facilitated by enzymes such as sucrose phosphorylase and α-galactosidase [28, 29]. The HP culture contains three primary strains (*Lactobacillus*, *Bifidobacteria*, and *S. thermophilus*) as in SC and MET, along with an additional strain, *B. subtilis*. This bacterium produces high-activity amylase, breaking down starch into simpler substrates, which are then metabolized by the other species to generate organic acids [30].

In terms of microbiology, the results indicated no statistically significant differences ($p>0.05$) in the lactic acid bacterial count. From the initial count at 6 logCFU/mL, their counts rised to a range of 8.24 to 9.31 logCFU/mL. However, the AL culture exhibited the lowest bacterial count, which was significantly different from most of the other strains. The AL culture, consisting of only two basic species (*S. thermophilus* and *L. bulgaricus*), had limited substrate metabolism capacity (as discussed earlier), leading to insufficient nutrient availability for substantial biomass increase compared to the other cultures.

The texture analysis revealed significant differences ($p<0.05$) in firmness across the samples. The HP, AL, and MET strains had similar firmness values of 68.66 gf, 64.22 gf and 58.90 gf, respectively, with no statistical differences, while the SC culture exhibited the lowest firmness at 49.74 gf. Regarding water-holding capacity (WHC), species variation also led to statistically significant differences ($p<0.05$), with the AL culture showing the highest WHC (68.72%) and the SC culture the lowest (52.50%). Yogurt texture depends on factors such as dry matter content, stabilizers, starter concentration, incubation temperature, acid levels, etc. [15]. Comparing the firmness of these samples to animal milk-based yogurt, the differences are not substantial. For instance, fruit jam yogurt with 0.4% gelatin reported by Nguyen Minh Thuy *et al.* had a firmness of 20.11 gf [17], while yogurt fermented with *L. plantarum* using 0.07% gelatin and modified starch ranged from 68.5 to 98.0 gf [31]. However, plant-based yogurts showed greater variability. Lin *et al.* reported that 100% soy yogurt exhibited a firmness of approximately 200 gf, which decreased significantly when soy milk was partially replaced with cow milk [32]. This study, however, did not address the impact of sugar content on firmness. Notably, the WHC of plant-based yogurt was about 50%, comparable to the SC strain evaluated in this research.

The "yogurt" samples were evaluated for sensory attributes and the results are presented in Fig. 1. The sensory evaluation results indicated that bacterial starter culture significantly influenced the sensory quality of soy bean and jackfruit seed "yogurt", resulting in statistically significant differences ($p<0.05$) across all 4 criteria, including texture, color, aroma, and taste.

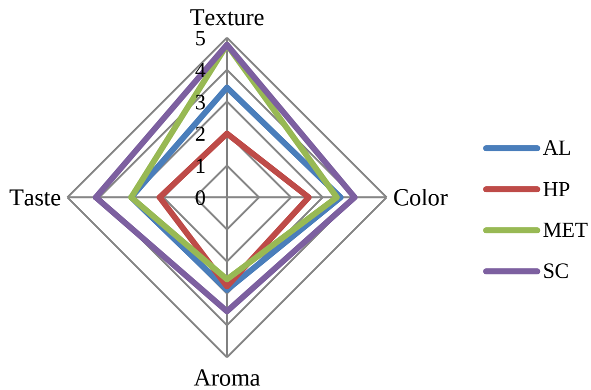


*Figure 1.* Effect of starter cultures on the sensory quality of the soybean and jackfruit seed "yogurt"

In terms of texture, most samples exhibited favorable attributes such as minimal syneresis, smoothness, and absence of clumping. This is attributed to the exopolysaccharides (EPS) produced by most species in the four cultures, enhancing rheological properties and providing a creamy mouthfeel [7]. Acid content and pH levels also affected texture by influencing the isoelectric point of soy proteins (pH 4.5-4.7) [3]. Despite HP's optimal acid content and pH, the protease activity of *B. subtilis* in this culture created voids and reduced smoothness. Stabilizers, particularly 0.35% agar added to all samples, contributed to improving texture and water-holding capacity. This aligns with previous studies, such as Kumar and Mishra, which demonstrated similar outcomes using 0.4% stabilizers [8]. Additionally, starch from the raw materials also played a role in stabilizing the "yogurt" structure [11]. Color was largely influenced by the raw ingredients and heat treatment, which were consistent across all samples, resulting in similar color evaluations. Regarding aroma, all samples had the characteristic scents of soy and jackfruit but lacked a pronounced yogurt-like aroma. However, SC received the highest aroma scores, while HP scored lowest due to *B. subtilis* producing sulfur-based compounds (DMDS, DMTS) that contribute to unpleasant odors [33]. For taste, sucrose in the raw materials served both as a substrate for bacterial metabolism and as a sweetener in the unfermented mixture. SC was rated highest for its balanced sweet-and-sour taste, as 5 out of 6 species in this culture efficiently metabolized sucrose into organic acids, creating a harmonious flavor profile. Conversely, the AL, HP, and MET cultures, which contained strains with limited substrate utilization capabilities, produced overly sweet "yogurt".

Overall, based on physicochemical, microbiological, and sensory properties, SC was identified as the most suitable culture for further experiments.

### 3.2. The effect of supplementary component ratios on "yogurt" quality

The results in Table 2 show that the mean pH values among the treatments were not statistically significantly different (p>0.05), ranging from 4.77 to 5.14. This range aligns with similar studies, such as Li *et al.* [18], where the final pH of fermented soy yogurt ranged between 4.6 and 5.1, and the range of 4.5 to 4.9 reported by Tran Thi Dinh *et al.* [7]. This pH range corresponds to the point at which bacterial strains begin to stagnate in activity and are inhibited by their own metabolic products, particularly organic acids like lactic acid. The correlation between the increase in acid content (Δacid) and pH value is evident in the formulated "yogurt" with 15% sugar and 6:4 (S:J) ratio, as this sample had the lowest pH and the highest increase in acid content, showing a statistically significant difference compared to most other samples (p<0.05).

*Table 2.* Effect of sugar content and soy-jackfruit seed milk ratio on the physicochemical and microbiological properties of the soybean and jackfruit seed "yogurt"

| Sugar (% w/v) | S:J (v/v) | pH | Δacid (g/L) | LAB Count (logCFU/mL) | Firmness (gf) | WHC (%) |
|---|---|---|---|---|---|---|
| 10 | 5:5 | $4.84{\pm}0.07^{ab}$ | $4.00{\pm}0.18^{bcd}$ | $8.17{\pm}0.21^{d}$ | $53.13{\pm}0.68^{e}$ | $56.91{\pm}0.23^{c}$ |
| 10 | 6:4 | $4.96{\pm}0.06^{ab}$ | $4.66{\pm}0.85^{ab}$ | $9.01{\pm}0.46^{b}$ | $73.02{\pm}0.91^{c}$ | $52.02{\pm}3.02^{f}$ |
| 10 | 7:3 | $4.91{\pm}0.15^{ab}$ | $4.30{\pm}0.18^{bc}$ | $8.46{\pm}0.46^{cd}$ | $56.66{\pm}0.59^{d}$ | $56.91{\pm}0.23^{c}$ |
| 10 | 8:2 | $5.01{\pm}0.24^{ab}$ | $3.10{\pm}0.18^{e}$ | $8.87{\pm}0.13^{bc}$ | $91.59{\pm}0.76^{a}$ | $52.94{\pm}0.56^{ef}$ |
| 15 | 5:5 | $4.89{\pm}0.13^{ab}$ | $4.50{\pm}0.26^{bc}$ | $8.42{\pm}0.10^{cd}$ | $45.80{\pm}3.13^{f}$ | $54.31{\pm}0.74^{de}$ |
| 15 | 6:4 | $4.77{\pm}0.13^{b}$ | $5.25{\pm}0.32^{a}$ | $9.88{\pm}0.60^{a}$ | $50.32{\pm}2.39^{e}$ | $54.67{\pm}0.76^{de}$ |
| 15 | 7:3 | $5.00{\pm}0.26^{ab}$ | $3.78{\pm}0.58^{cde}$ | $8.93{\pm}0.07^{b}$ | $51.49{\pm}0.67^{e}$ | $55.89{\pm}0.84^{cd}$ |
| 15 | 8:2 | $5.10{\pm}0.30^{ab}$ | $3.16{\pm}0.10^{e}$ | $8.98{\pm}0.04^{b}$ | $73.31{\pm}1.18^{c}$ | $59.73{\pm}1.57^{b}$ |
| 20 | 5:5 | $4.97{\pm}0.22^{ab}$ | $3.76{\pm}0.10^{cde}$ | $7.38{\pm}0.10^{e}$ | $36.54{\pm}0.91^{g}$ | $54.93{\pm}1.93^{cde}$ |
| 20 | 6:4 | $5.12{\pm}0.05^{a}$ | $3.28{\pm}0.65^{de}$ | $8.67{\pm}0.13^{bc}$ | $25.22{\pm}4.72^{h}$ | $54.29{\pm}0.91^{de}$ |
| 20 | 7:3 | $5.02{\pm}0.27^{ab}$ | $4.25{\pm}0.76^{bc}$ | $8.78{\pm}0.08^{bc}$ | $51.67{\pm}1.52^{e}$ | $53.38{\pm}1.16^{ef}$ |
| 20 | 8:2 | $5.14{\pm}0.32^{a}$ | $3.16{\pm}0.10^{e}$ | $9.10{\pm}0.06^{b}$ | $87.34{\pm}1.19^{b}$ | $67.72{\pm}0.60^{a}$ |

*S:J represents the ratio of soy milk to jackfruit seed milk. Different letters in superscript indicate statistically significant differences (p<0.05)*

Overall, the factors of sugar content and the ratio of soy milk to jackfruit seed milk (S:J) had a significant impact, resulting in notable differences in Δacid among the formulae. At the same sugar contents of 10% and 15%, differences in Δacid were relatively distinct. A trend was observed starting at the 5:5 ratio, where Δacid values increased from 4.00 g/L (10% sugar) and 4.50 g/L (15% sugar) to a peak at the 6:4 ratio (4.66 g/L and 5.25 g/L, respectively). At this ratio, the total acid content closely aligned with the findings of Gan *et al.* [34], which reported a maximum total acid content of 6.3 g/L or Lin *et al.* reported the highest titration acidity at approximately 5 g/L during soybean fermentation [32]. Beyond the 6:4 ratio, Δacid values decreased. At a sugar concentration of 20%, Δacid values showed no consistent trend, with the lowest value recorded at the 8:2 ratio. High sugar levels create an osmotic pressure, which inhibits intracellular enzyme activity, thereby reducing substrate metabolism [35]. However, Kim *et al.* noted that at moderate osmotic pressures, fermentation is prolonged, enabling bacteria to metabolize and produce more products [36]. When examining the same S:J ratio, changes in sugar content did not result in significant differences at ratios of 5:5, 7:3, and 8:2. However, at the 6:4 ratio, Δacid at 20% sugar was notably lower than that at the other two sugar contents.

Statistical results indicated that sugar content and the S:J ratio significantly affected lactic acid bacteria (LAB) counts among the samples (p<0.05). The composition of the ingredient mixture including nutrients, acids, and inhibitors, plays a crucial role in the growth, development, and metabolic products of LAB [35, 32]. Sugar serves as the primary nutrient for LAB; however, excessive sugar levels can create high osmotic pressure, causing intracellular dehydration and negatively affecting the bacteria. Akin *et al.* reported that LAB counts peaked at a sugar content of 15-18% and dropped to its lowest at 21% (w/v) [37]. Similarly, in this soy and jackfruit seed "yogurt", LAB counts were generally highest at 15% sugar content and decreased slightly at other levels. Notably, in the 7:3 and particularly the 8:2 ratios, LAB counts showed minimal decline at 10% and 20% sugar content compared to other ratios. This could be attributed to the plentiful presence of antioxidants (isoflavones, saponins) and lipids in soybeans, which support bacterial survival and growth [35]. Increasing the soybean proportion resulted in enhancing these components, creating favorable conditions for maintaining and developing bacterial biomass. Consequently, at the same sugar level, the 5:5 ratio consistently exhibited the lowest LAB counts among the four S:J ratios. Furthermore, besides prebiotics, jackfruit seeds also contain certain antimicrobial components (jacalin, artocarpin) [38], which may limit bacterial growth and reduce the effectiveness of prebiotics.

The structural characteristics of yogurt depend on various factors. Beyond stabilizers (discussed in section 3.1), the composition and solid content in the ingredient mixture significantly affect product structure, leading to statistically significant differences among formulae ($p<0.05$). Data in Table 2 indicate that at all three sugar levels, "yogurt" firmness varied distinctly across S:J ratios, with the highest value observed at 8:2, decreasing toward 5:5. Ratios with higher soybean proportion showed greater firmness due to increased antioxidants and lipids, which promoted bacterial activity, acid production, and protein coagulation, enhancing structural firmness. However, the firmness of the "yogurt" at the increasing ratios of jackfruit seed extract showed significant similarity to related studies mentioned in section 3.1, ranging from 50 to 70 gf. Regarding the same S:J ratios, higher sugar levels reduced "yogurt" firmness. Sugar binds water, reducing free water and increasing water retention, resulting in a softer and smoother texture [37]. Water holding capacity (WHC) indicated dominant values compared to relevant studies such as 40.24% [7] or approximately 52% [32] and showed significant differences among treatments ($p<0.05$). At 15% and 10% sugar levels, the 8:2 ratio generally experienced the highest WHC, which declined as soybean content decreased. Higher soybean content improved WHC and stabilized gel strength in yogurt [39]. However, the WHC at the S:J ratio of 5:5 exhibited notable stability and relatively high values, which could be attributed to the gel-forming and water-holding capacity of the protein-starch complex in the product. Regarding 10% sugar, WHC showed irregular trends, fluctuated in the medium to low range. At the same S:J ratio, increasing sugar content generally caused slight fluctuations in WHC across all four ratios. Sugar's strong water-binding ability enhances the water-holding capacity of the product, most notably at the 8:2 S:J ratio.

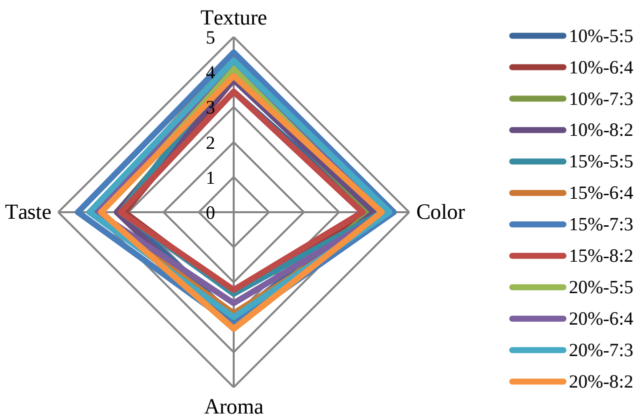


*Figure 2.* Effect of sugar content and soy-jackfruit seed milk ratio on the sensory quality of "yogurt" from soybean and jackfruit seed

Statistical analysis showed that sugar content and the ratio of soy milk to jackfruit seed milk (S:J) significantly influenced the sensory quality of soy and jackfruit seed "yogurt", with notable differences ($p<0.05$) in structure, aroma, and taste. However, color showed no significant difference ($p>0.05$). Results are illustrated in Fig. 2. The color of the "yogurt" was primarily influenced by the raw materials and initial processing (seed variety, heating conditions, etc.). As all samples were undergone identical processing, color differences were negligible, indicating a stable and optimized production process. For other criteria, "yogurt" texture showed minimal variation among samples, but higher sugar levels (15% and 20%) resulted in better ratings due to reduced syneresis and a softer texture. Aroma was generally rated average, with no distinct yogurt-specific scent or significant differences between samples. Regarding taste, samples with 15% and 20% sugar were rated higher for their balanced sweet-sour flavor, especially at S:J ratios of 6:4 and 7:3. In contrast, 10% sugar formulae received lower and similar scores across all ratios.

Overall, the 15% sugar (w/v) and 6:4 S:J (v/v) formula demonstrated optimal physicochemical and microbiological properties, aligning with similar products, and ranked among the best in sensory quality, making it the suitable choice for subsequent experiment.

### 3.3 Optimization of fermentation conditions using the response surface methodology

In this experiment section, pH was selected as the target variable for the optimization model. This parameter plays a critical role in the growth of lactic acid bacteria, cellular stress levels, and indirectly influences the production of their metabolites (EPS, organic acids, flavor compounds, etc.) [35, 40]. Additionally, the studies mentioned in the previous sections also highlighted significant differences in pH when conducted under varying temperatures, durations, and bacterial concentrations. The optimized fermentation parameters affecting pH are presented in Table 3. ANOVA analysis and regression modeling for the target variable Y (pH) yielded the following second-order polynomial equation representing the dependence of "yogurt" pH on three fermentation parameters: $pH = 4.42 + 0.031B - 0.150C + 0.042AB - 0.110B^2 + 0.084C^2$ with $R^2 = 0.9725$. Where A, B and C are the actual level of factors shown in Table 3. Those represent temperature, time and bacterial concentration, respectively.

The ANOVA results from Table 4 for the second-order regression model indicate that the model is statistically significant ($p<0.05$), confirming that the factors in the model significantly influence the target variable Y (pH). Additionally, the non-significant Lack of Fit ($p>0.05$) suggests that the model aligns well with the experimental data, with no substantial deviation, thereby enhancing the reliability of the model's applicability to the fermentation process under study. The F-value for the bacterial starter concentration (C) is the highest among the factors analyzed (F-value = 161.68), highlighting its dominant impact on pH variation in the soybean and jackfruit seed "yogurt" product. Fermentation time (B) also had a significant impact on pH ($p<0.05$), whereas temperature was observed to have no substantial effect on the target variable ($p>0.05$). These findings underscore the critical role of optimizing the bacterial starter concentration fermentation time to achieve the desired pH in the fermentation process. The interaction effects of temperature and bacterial concentration, and fermentation time and bacterial concentration on pH variations are illustrated in Fig. 3.

*Table 3.* Experimental results of optimized fermentation parameters affecting pH

| Fermentation temperature (ºC) | Fermentation time (h) | Bacterial concentration (% v/v) | pH value |
|---|---|---|---|
| 41 | 8 | 5.5 | 4.47 |
| 45 | 6 | 5.5 | 4.25 |
| 37 | 6 | 5.5 | 4.37 |
| 45 | 10 | 5.5 | 4.40 |
| 41 | 6 | 10 | 4.27 |
| 41 | 8 | 5.5 | 4.43 |
| 45 | 8 | 1 | 4.68 |
| 41 | 6 | 1 | 4.47 |
| 41 | 10 | 1 | 4.60 |
| 41 | 10 | 10 | 4.27 |
| 41 | 8 | 5.5 | 4.38 |
| 37 | 10 | 5.5 | 4.35 |
| 45 | 8 | 10 | 4.37 |
| 41 | 8 | 5.5 | 4.41 |
| 41 | 8 | 5.5 | 4.45 |
| 37 | 8 | 1 | 4.74 |
| 37 | 8 | 10 | 4.35 |

*Table 4.* Analysis of variance (ANOVA) test for Box-Behnken factorial design

| Source | Sum of Squares | df | Mean Square | F-value | P-value Prob > F |
|---|---|---|---|---|---|
| Model | 0.29 | 9 | 0.032 | 27.51 | 0.0001 (significant) |
| A-Temperature (°C) | 1.512E-003 | 1 | 1.512E-003 | 1.29 | 0.2929 |
| B-Time (h) | 7.813E-003 | 1 | 7.813E-003 | 6.68 | 0.0362 |
| C-Bacteria (%) | 0.19 | 1 | 0.19 | 161.68 | < 0.0001 |
| AB | 7.225E-003 | 1 | 7.225E-003 | 6.18 | 0.0419 |
| AC | 1.600E-003 | 1 | 1.600E-003 | 1.37 | 0.2804 |
| BC | 4.225E-003 | 1 | 4.225E-003 | 3.61 | 0.0991 |
| $A^2$ | 2.901E-003 | 1 | 2.901E-003 | 2.48 | 0.1593 |
| $B^2$ | 0.050 | 1 | 0.050 | 42.57 | 0.0003 |
| $C^2$ | 0.030 | 1 | 0.030 | 25.25 | 0.0015 |
| Residual | 8.187E-003 | 7 | 1.170E-003 | | |
| Lack of fit | 3.737E-003 | 3 | 1.246E-003 | 1.12 | 0.4401 (not significant) |
| Pure Error | 4.450E-003 | 4 | 1.113E-003 | | |
| Cor Total | 0.30 | 16 | | | |

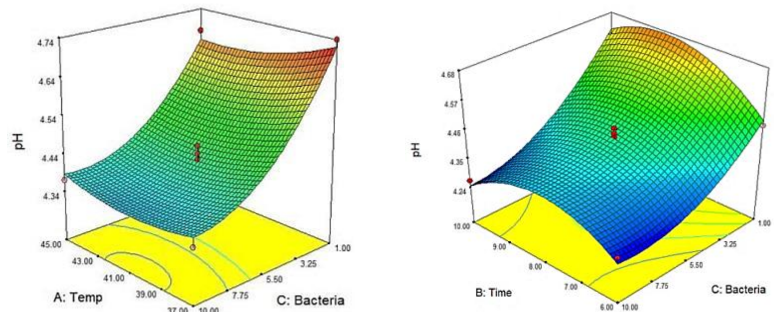


*Figure 3.* Interaction effects of temperature and bacterial concentration, and fermentation time and bacterial concentration on pH variations

The interaction between the factor pairs A x C and B x C are presented from left to right in Fig. 3. The AC plot showed that as bacterial concentration increases, the pH tended to decrease to a certain minimum value, while temperature did not significantly affect pH. The BC plot also highlighted the notable effect of bacterial concentration on pH when interacting with fermentation time. Specifically, an increase in bacterial concentration led to a continuous decrease in pH, whereas changes in fermentation time, either increasing or decreasing, also resulted in a continuous decrease in pH.

The data analysis confirms the model's validity, with approximately 25 optimal solutions proposed by Design-Expert software to achieve the target pH value of 4.5. The top three solutions with the highest suitability and significant differences were selected for validation experiments, and the results are presented in Table 5. The results indicate that all three proposed solutions yielded actual pH values that did not significantly differ from the theoretical pH values. A sensory evaluation was conducted to identify the solution with the best sensory quality. While no significant differences were observed in the texture and aroma (p>0.05), solutions (2) and (3) outperformed solution (1) in terms of color and taste.

*Table 5.* Experimental results of the proposed solutions

| Solution | Temperature (°C) | Time (h) | Bacterial concentration (%) | Target pH | Actual pH | Sensory evaluation | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Texture | Color | Aroma | Taste |
| 1 | 41.23 | 8.50 | 3.77 | 4.5 | 4.46±0.03 | 4.48±0.51$^a$ | 4.00±0.39$^b$ | 3.59±0.75$^a$ | 3.78±0.75$^b$ |
| 2 | 44.60 | 7.01 | 2.30 | 4.5 | 4.54±0.08 | 4.41±0.57$^a$ | 4.59±0.50$^a$ | 3.96±0.71$^a$ | 4.52±0.51$^a$ |
| 3 | 37.35 | 8.85 | 4.14 | 4.5 | 4.63±0.04 | 4.33±0.78$^a$ | 4.33±0.83$^a$ | 3.67±1.18$^a$ | 4.04±1.26$^{ab}$ |

Overall, considering the requirements for time and starter culture concentration, Solution (2) [44.6 °C - 7.01 h - 2.3% starter culture] proved to be the most effective and demonstrated superior sensory quality compared to the other solutions. Therefore, it was selected as the optimal set of parameters for achieving the target pH in the fermentation process of soybean and jackfruit seed “yogurt”.

## 4. CONCLUSION

This study identified the commercial starter culture “SC” as effective for fermenting soybean and jackfruit seed “yogurt” with optimal physico-chemical, microbiological, and sensory qualities. The recommended conditions include a ratio of soy milk to jackfruit seed milk of 6:4 (v/v), 15% (w/v) sucrose, 2.3% (v/v) starter culture, fermentation at 44.6 °C for 7.01 h. These findings provide a foundation for further product development research.

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## TÓM TẮT

### NGHIÊN CỨU ĐIỀU KIỆN LÊN MEN "SỮA CHUA" TỪ ĐẬU NÀNH (*Glycine max* (L.) Merill) VÀ HẠT MÍT (*Artocarpus heterophyllus* Lam.)

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Ngày nay, người tiêu dùng có xu hướng sử dụng các sản phẩm có nguồn gốc thực vật do giá trị dinh dưỡng cao và tốt cho sức khỏe, sữa chua thực vật cũng là một sản phẩm có tiềm năng phát triển lớn. Trong nghiên cứu này, sữa (dịch trích) đậu nành và hạt mít đã được sử dụng để thay thế hoàn toàn sữa động vật trong quá trình lên men sữa chua. Từ đó góp phần nâng cao giá trị nông sản và tận dụng được nguồn phụ phẩm dồi dào là hạt mít. Mục tiêu của nghiên cứu nhằm xây dựng quy trình lên men sữa chua đậu nành và hạt mít thông qua việc xác định (i) giống vi khuẩn thương mại, (ii) hàm lượng đường và tỷ lệ đậu nành và hạt mít, (iii) nhiệt độ, thời gian lên men và nồng độ giống chủng. Kết quả nghiên cứu cho thấy giống vi khuẩn thương mại "Start for yogurt (SC)" có khả năng lên men hiệu quả cho ra sản phẩm sữa chua có chất lượng hóa lý, vi sinh và cảm quan tốt khi tỷ lệ sữa đậu nành và hạt mít là 6:4, bổ sung 15% (w/v) đường sucrose, tỷ lệ giống vi khuẩn 2,3% (v/v) và lên men ở 44,6 $^{o}$C trong 7,01 giờ.

*Từ khóa:* Đậu nành, hạt mít, lên men, tối ưu hóa, sữa chua.