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Isolation and Identification of Lactic Acid Bacteria from Fermented Soybean Pulp and Its Application as Starter Culture for Soy Yogurt

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Food waste processing holds great potential in mitigating global food insecurity. Valorization of food waste is a sustainable practice that also lessens the effects of climate change. Isolation, identification, and characterization of lactic acid bacteria from fermented soya pulp was conducted. The isolated lactic acid bacteria species was utilized as starter culture to produce soy yogurt. This study was performed to identify LAB isolated from fermented soya pulp using 16s rRNA gene sequencing; to ferment soymilk using the LAB isolated from fermented soya pulp and develop soy yogurt; to determine the proximate nutritional composition of the soy yogurt through proximate analysis; and to evaluate the sensory characteristics of the yogurt such as color, aroma, texture, taste, and overall acceptability. The 16s rRNA gene sequencing procedure identified the isolated lactic acid bacteria species as *Pediococcus pentosaceus* Mees, 1934. Four yogurt samples were formulated using *P. pentosaceus* and a commercial starter culture. Proximate compositions such as titratable acidity, ash content, crude protein, and crude fat were determined. The soy-based yogurt drinks were similarly high in protein compared with the cow's milk counterpart. Notably, formulation 1 with the *P. pentosaceus* as the sole starter culture scored highest in crude protein. Significant difference in the average ash content between the four samples was observed. Post-hoc analyses using the Bonferroni correction revealed that formulation 1 with the isolated starter culture, *P. pentosaceus*, had the highest ash content. Sensory evaluation revealed that formulation 2 with both the isolated starter culture and commercial starter gained peak in color, taste, and overall acceptability. There was significant difference in the average aroma and texture scores between the four samples, favoring the cow's milk yogurt sample for the aroma and the soymilk yogurt with both *P. pentosaceus* and commercial starter culture for the texture. This study showed the potential of *P. pentosaceus* as a sole starter culture and in combination with commercial starter culture in producing functional foods from food waste such as soymilk-based yogurt. Refinement and standardization of the yogurt production protocol is recommended to produce the yogurt with better texture and taste. By so doing, product development from food waste can support sustainable food production and mitigate global food insecurity and climate change.

Keywords: isolation, identification, lactic acid bacteria, starter culture, soymilk yogurt, okara

I. Introduction

Global food insecurity is on the rise these days. According to the Food and Agriculture Organization (FAO), around 1.3 billion tons of food made for human consumption is wasted annually. This number is equivalent to more than one-third of the total food produced worldwide. These deficits would result to wastage of resources (FAO, 2019). One solution to this pressing problem is valorization of food waste. Food waste processing methods include isolating, characterizing, and identifying lactic acid bacteria to produce fermented functional food products.

Meanwhile, most of the soybean pulp products are outcomes of fermentation using various microorganisms. Fermented foods have better shelf life, bioactive molecules, vitamins, and enhanced availability of necessary contents (Rezac et al., 2018). Some also have therapeutic properties such as improving gut microbiota and gastrointestinal health and overcoming diabetes and cardiovascular diseases (Melini et al., 2019). In a study in 2024, the soya pulp was used as a culture medium for lactic acid bacteria to minimize improper disposal of soymilk by-product and to explore soya pulp potential as a culture medium to grow lactic acid bacteria and save the cost of buying the standard MRS medium (Retnowati et al., 2024).

Lactic acid bacteria have been reported to possess several health benefits such as improving gut health, modulating appetite, and managing weight, modulating the immune system, decreasing the severity of infection, showing antimicrobial effects on food-borne pathogens, lowering blood pressure, high radical scavenging activity, and improving cognitive health (Mathur et al., 2020). Because they are generally recognized as safe (GRAS), they are considered as one of the most important groups of microorganisms being utilized as starter cultures in producing functional food, exerting probiotic effects, and exhibiting food preservation potential (Frick et al., 2007). Due to their fermentative nature, they are used to produce dairy, meat, and vegetables fermented products (De Vuyst and Leroy, 2007). Lactic acid bacteria isolates can be identified from fermented foods which are being studied for the potential of

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antimicrobial activity against foodborne pathogens and non-resistance to antibiotics (Banik et al., 2023). By far, dairy, and fermented foods provide the main source of probiotics. But because of lactose intolerance and high cholesterol levels, non-dairy fermented products are now gaining more interests (Liu et al., 2022). Lactic acid bacteria can be used against food-borne pathogens using fermentates. Food-grade cultures and undefined fermentates of lactic acid bacteria are more consumer-friendly and do not require extensive labelling, in contrast to purified fermentates which require extensive labelling as additives (Field et al., 2015).

At present, the usefulness of 16S rRNA gene sequence is expanded to food processing application where molecular characterization has recently become essential in classifying and identifying lactic acid bacteria (Ayivi and Ibrahim, 2022). This modern step of molecular identification for lactic acid bacteria isolated from soya milk or soya pulp can be done through 16S rRNA sequencing (Retnowati et al., 2024).

Several of studies have been published about yogurt production using lactic acid bacteria and cow's milk. However, in soymilk yogurt production, only very few research is available. Limited reviews mention only a few isolated lactic acid bacteria from fermented soybean pulp; mostly are from different fermented sources (Qi et al., 2021; Jiang et al., 2021). Of the few isolated lactic acid bacteria from fermented soybean pulp, *P. pentosaceus* was only used as a mixed starter culture for soymilk yogurt along with other lactic acid bacteria species. There are no published studies on using *P. pentosaceus* isolated from fermented soybean pulp as a pure starter culture for soymilk yogurt production. Hence, this study was conceived.

II. Materials and Methods

Place and Duration of Study

The molecular characterization of lactic acid bacteria samples was done by Macrogen Inc., 1007, 254 Beotkkot-ro, Geuncheon-gu, Seoul 08511, Rep. of Korea. The preparation of soy yogurt was conducted at the Isolation Room of the Department of Biology in Mountain View College of Seventh-day Adventists, Inc., College Heights, Mt. Nebo, Valencia City. Lyophilization or freeze-drying of the samples and proximate analysis were conducted at the Natural Science Research Institute (NSRI) and Center for Food Research, Innovation, and Extension (CFRIE) of Central Mindanao University, University Town, Musuan, Maramag, Bukidnon, Philippines. Sensory evaluation was performed by selected participants. The study started on February 5, 2025 until May 2025.

Molecular Characterization of LAB Isolated from Fermented Soya Pulp

Pure lactic acid bacteria that were previously isolated from fermented soya pulp and stored in 85% MRS broth and 15% Glycerol were sent to Kinovett Scientific Solutions, Co. Then, online request for services to Macrogen Inc. was made following the procedures prescribed by Kinovett Scientific Solutions, Co. The service requested was identification, specifically 16S rRNA gene sequencing. Kinovett Scientific Solutions, Co. facilitated the air mail of the samples to Macrogen Inc., a South Korean public biotechnology company. Macrogen communicated the progress, results, and payment invoice through emails.

Preparation and Allocation of Starter Culture

The method of Utami et al., (2020) was followed with some modifications. Frozen stock cultures of isolated lactic acid bacteria from fermented soya pulp was thawed and activated in 1 L 10% skim milk and 1% sucrose mixture. The mixture was incubated at 30°C for 18 hours. The 1L starter culture was allocated into three different formulations: each formulation received 100 mL of the starter culture per replicate. There were three replicates per formulation.

Preparation of Soy Yogurt

For this procedure, the method of Ugwona et al., (2018) was followed with some modifications. The following set-ups were made: Control (Soymilk + Yakult), Formulation 1 (Soymilk + Starter Culture), Formulation 2 (Soymilk + Yakult + Starter Culture), Formulation 3 (Cow's milk + Starter Culture). Three replicates were made per set-up. One point five liter (1.5 L) of soymilk was used in all soymilk set-ups. The Cow's milk was prepared by mixing 125 g of full cream powdered milk (Bear Brand) with 1 L of distilled water. The liquid Cow's milk and soymilk samples were separately pasteurized at 85°C for 15 mins and cooled at 44°C. After pasteurization, formulations 1-3 were inoculated with 100 mL (6.7%) starter culture per replicate at 44°C. Four (4) bottles of Yakult (80 mL per bottle) with live *Lacticaseibacillus paracasei* strain Shirota was used for each liter of soymilk in the control sample and formulation 2 sample. One-hundred milliliters (100 mL) of starter culture was mixed with soymilk in formulation 1 and another 100 mL of starter culture was added into the soymilk and Yakult mixture in formulation 2. One-hundred milliliters (100 mL) of starter culture was also mixed with Cow's milk in formulation 3. All the samples were fermented in a water bath at 44°C for 7h and then allowed to cool gradually.

Allocation of Samples for Proximate Analysis and Nutrition Facts Analysis

Fresh soy yogurt samples were brought to the Center for Food Research, Innovation, and Extension (CFRIE) of Central Mindanao University for immediate analysis of titratable acidity and ash content. Freeze-drying at -86 °C for 42 hours was performed using the lyophilizer at the Tuklas Lunas Development Center (TLDC). Freeze drying was done for the analysis of percentage crude fat and crude protein.

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Sensory Evaluation

Ten (10) semi-trained panelists performed the sensory evaluation assessing the attributes of the yogurt such as: color, aroma, texture, taste, and overall acceptability. Their feedback was recorded using a hedonic scale, ranging from 1 (extremely disliked) to 9 (extremely liked). Labels of the finish product were concealed from the panelists to prevent bias (Asghar et al., 2022).

Statistical Analysis

Data were analyzed using Microsoft Excel (2016). Analysis of Variance (ANOVA) was used to compare means at $p < 0.05$ level of significance.

III. Results and Discussion

Identification of the LAB Isolated from Fermented Soya Pulp through 16s rRNA Sequencing

Macrogen Inc. identified the lactic acid bacteria species that was isolated from the fermented soya pulp as *Pediococcus pentosaceus* Mees, 1934 (Figure 1). *Pediococcus* is a genus of Gram-positive lactic acid bacteria, previously placed within the family of Streptococcaceae (Chen et al., 2020), and recently classified under the family of Lactobacillaceae (d'Acierno et al., 2025). *P. pentosaceus* is one of the 12 species of *Pediococcus* that have been described; and along with *P. acidilactici*, are associated with dairy products such as cheese, fermentation of milk, and cheese manufacture (Holland et al., 2010). *Pediococcus* species usually occur in pairs or tetrads, and divide along two planes of symmetry. They are coccus shaped microbes, non-motile, and non-spore forming. The end product of their metabolism is lactic acid, making them acid tolerant, and thus classifying them under lactic acid bacteria. They are also responsible for the fermentation of cabbage, making it sauerkraut, together with *Leuconostoc* and *Lactobacillus* (Macrogen, 2025). Previous studies confirmed that *P. pentosaceus* occurs naturally in spontaneously fermented products that could have a considerable role in quality, safety, and efficiency when put together with other bacteria in a mixed fermenter (Gong and Qi, 2020; Montemurro et al., 2020; Xu et al., 2021). Like most lactic acid bacteria, *P. pentosaceus* are anaerobic and ferment sugars. They can be found in plant materials, ripened cheese, and a variety of processed meats (Macrogen, 2025).

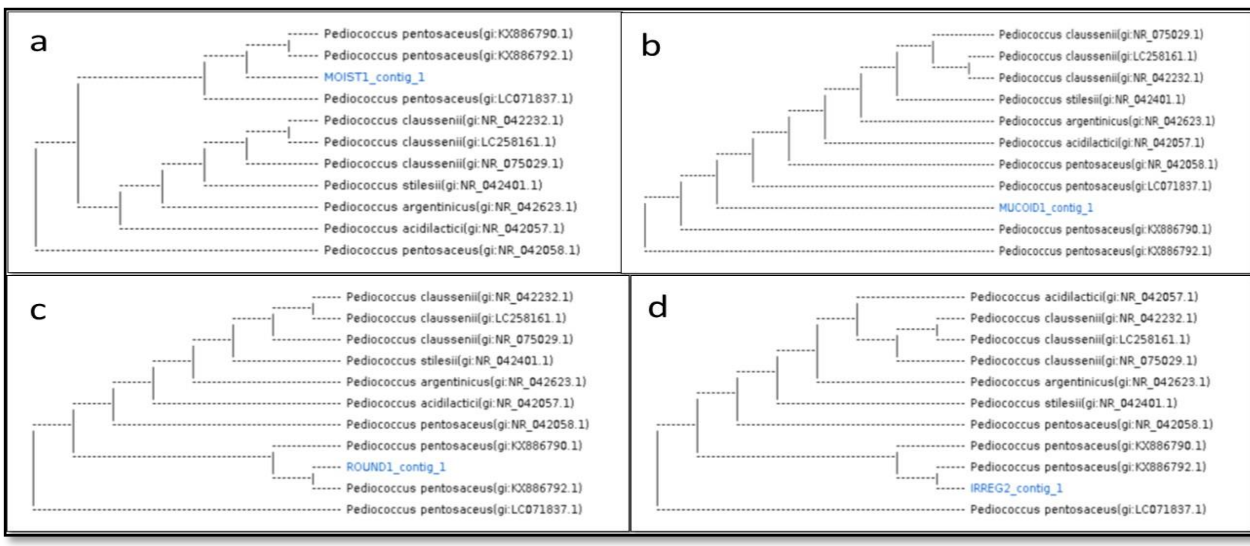

Figure 1. Molecular identification of the isolated lactic acid bacteria from fermented soya pulp using 16S rRNA gene sequencing. a) Sample from moist colony. b) Sample from mucoid colony. c) Sample from round colony. d) Sample from irregularly shape colony.

Pediococcus pentosaceus has many health benefits such as probiotic effects, rich in antioxidants, lowering cholesterol, and boosting the immune system. Recently, *P. pentosaceus'* potential as an ingredient in probiotic fermentation products increasingly attracts more attention (Qi et al., 2021). A study in 2022 stated that *P. pentosaceus* is increasingly studied in the dairy industry due to its promising qualities as a starter culture bacterium. The study explored the physiological, survivability, and genomic properties of *P. pentosaceus*. The strain revealed amazing tolerance in low pH and high concentrations of bile salts. It also showed 71% and 84% activity against pathogens, strong biofilm formation, and notable resistance to antibiotics. The whole genome sequence also showed various genes responsible for stress tolerance, adhesion, biofilm formation, as well as acids and bile salts tolerance (Mgomi et al., 2022).

The use of *P. pentosaceus* from different sources is widespread in several applications such as antioxidant and fatty acid profiling of fermented goat, camel, and cow's milk (Balakrishnan and Agrawal, 2014); as an adjuvant therapeutic option for patients with ulcerative colitis (UC) (Bamba et al., 2018); anti-listerial activity (Jang et al., 2014); as starter culture in sourdough bread-making

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(Plessas et al., 2020); as a heat stable, bacteriocin-producing and vancomycin-sensitive lactic culture from beans (Venkateshwari et al., 2010); and producing key odorants and non-volatile metabolites in broccoli juices (Xu et al., 2021). However, in soymilk yogurt production, only very few researches have been published; and those studies only mention the use of *P. pentosaceus* as a mixed starter culture for soymilk yogurt along with other lactic acid bacteria species. In the two reviews read about *P. pentosaceus*, there was no mention about it being isolated from the fermented soybean pulp, but from other different fermented sources (Qi et al., 2021; Jiang et al., 2021). There are also no published studies on using *P. pentosaceus* isolated from fermented soybean pulp as a pure starter culture for soymilk yogurt production. Hence, this study was conceived.

Proximate Nutritional Composition of the Soy Yogurt

There were four proximate compositions determined by the Center for Food Research, Innovation, and Extension (CFRIE) of the Central Mindanao University. One was a physico-chemical component called, titratable acidity; and chemical components such as ash, crude protein, and crude fat. All were determined in percentage form (Table 1).

Table 1. Mean percentage and standard deviation values of the proximate nutritional compositions of the soy yogurt drink samples.

Proximate Composition	Control (%)	F1 (%)	F2 (%)	F3 (%)
Titratable Acidity	0.73±0.009	0.5±0.02	0.61±0.02	0.43±0.01
Ash Content	5.63±0.08[a]	6.13±0.10[bd]	5.89±0.82[f]	3.37±0.24[ceg]
Crude Protein	15.4±0.25	19.63±0.09	18.8±0.73	15.45±0.12
Crude Fat	1.73±0.08	7.61±0.50	4.59±0.22	15.02±0.15

*mean value of the same letter are not significantly different at P value 0.05.

The titratable acidity, crude protein, and crude fat of the samples were comparably similar. The soymilk based samples however showed high fermentation activity indicated in the titratable acidity. This meant that soymilk based samples produced more lactic acid compared to the control. Meanwhile, the soy-based yogurt drinks were similarly high in protein compared with the cow's milk counterpart. Notably, formulation 1 with the *P. pentosaceus* as the sole starter culture scored highest in crude protein. This meant that soy-based yogurts can be good protein source alternatives for cow's milk yogurt. Additionally, the results revealed that *P. pentosaceus* can be used solely as a starter culture to develop equally protein rich, plant-based alternative functional food from food waste.

There was significant difference in the average ash content between the four samples. Post-hoc analyses using the Bonferroni correction revealed that the amount of ash in the control group scored significantly lower compared with the ash of formulation 1. The ash of the control group scored significantly higher than formulation 3. The ash of formulation 1 also scored significantly higher than formulation 3; and the ash of formulation 2 also scored significantly higher than formulation 3. Notably, formulation 1 with the *P. pentosaceus* as the sole starter culture scored highest in the ash content. This may suggest higher mineral content that is naturally present in formulation 1 (Figure 2).

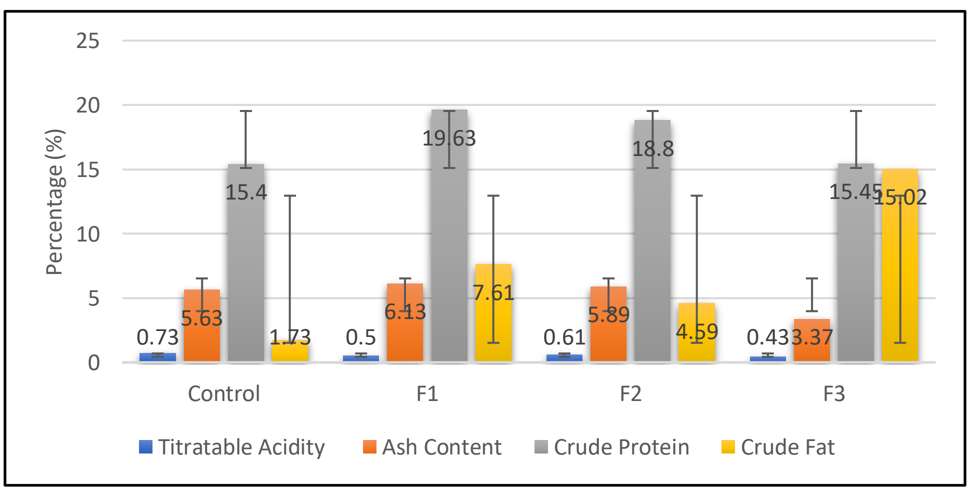

Figure 2. Proximate nutritional components of the soymilk-based yogurt drink samples: control, formulation 1, and formulation 2; in comparison with formulation 3 as the cow's milk-based yogurt drink counterpart. All the results are presented as mean ± standard deviation of the mean (n=3).

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Sensory Characteristics of the Yogurt

A 9-point hedonic scale sensory evaluation of the formulated yogurt drink samples was used. There were five sensory parameters considered: color, aroma, texture, taste, and overall acceptability (Table 2).

The color, taste, and overall acceptability of the yogurt drink samples were comparably similar. However, formulation 2 (*P. pentosaceus* with commercial starter culture) scored highest in color, taste, and overall acceptability. Although using different lactic acid bacteria species, this result is similar with Olusola and Adepoju (2022), where the soy yogurt fermented by both the isolated lactic acid bacteria starter culture and the commercial starter culture scored higher in sensory evaluation in contrast to the soy yogurt solely fermented by the isolated lactic acid bacteria.

Table 2. Mean and standard deviation values of the proximate nutritional compositions of the soy yogurt drink samples.

Sensory Parameters	Control	F1	F2	F3
Color	6.5±0.97	6.5±1.18	7.3±1.16	6.6±1.65
Aroma	6.9±0.74^{a}	5.5±0.97^{b}	6.8±1.55^{a}	7±1.25^{a}
Texture	5.2±0.42^{a}	6.1±1.29^{a}	6.3±1.06^{b}	5±0.82^{c}
Taste	6.3±0.67	6±0.47	7.5±0.53	4.6±0.52
Over-All Acceptability	7.1±0.88	5.8±0.79	7.2±0.79	3.1±1.10

There was significant difference in the average aroma scores between the four samples. Post-hoc analyses using the Bonferroni correction revealed that the aroma of the control group scored significantly higher compared with the aroma of formulation 1 (Tables 3 and 4).

Table 3. Mean and variance values of the aroma scores in the yogurt drink samples.

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	10	69	6.9	0.544444
F1	10	55	5.5	0.944444
F2	10	68	6.8	2.4
F3	10	70	7	1.555556

Table 4. ANOVA of the aroma scores in the yogurt drink samples.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.9	3	4.966667	3.64898	0.02143	2.86626555
Within Groups	49	36	1.361111			
Total	63.9	39				

There was also significant difference in the average texture scores between the four samples. Post-hoc analyses using the Bonferroni correction revealed that the texture of the control group scored significantly lower compared with the texture of formulation 2; and the texture of formulation 2 scored significantly higher than formulation 3 (Tables 5 and 6).

Table 5. Mean and ANOVA values of the texture scores in the yogurt drink samples.

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	10	52	5.2	0.177778
T1	10	61	6.1	1.655556
T2	10	63	6.3	1.122222
T3	10	50	5	0.666667

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Table 6. Mean and ANOVA values of the texture scores in the yogurt drink samples.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12.5	3	4.166667	4.601227	0.00795	2.866266
Within Groups	32.6	36	0.905556			
Total	45.1	39				

Over-all, there were significant differences in the aroma and texture of the yogurt drink samples. Formulation 1 (*P. pentosaceus*) scored lowest in odor, while formulation 3 (cow's milk) revealed the most favored aroma. The lower preference in aroma for soy-based yogurt may be caused by its naturally, slightly beany or grassy smell. Meanwhile, formulation 2 (*P. pentosaceus* and commercial starter culture) scored highest in texture followed by formulation 1 (*P. pentosaceus*) (Figure 3). This result is similar with Olusola and Adepoju (2022).

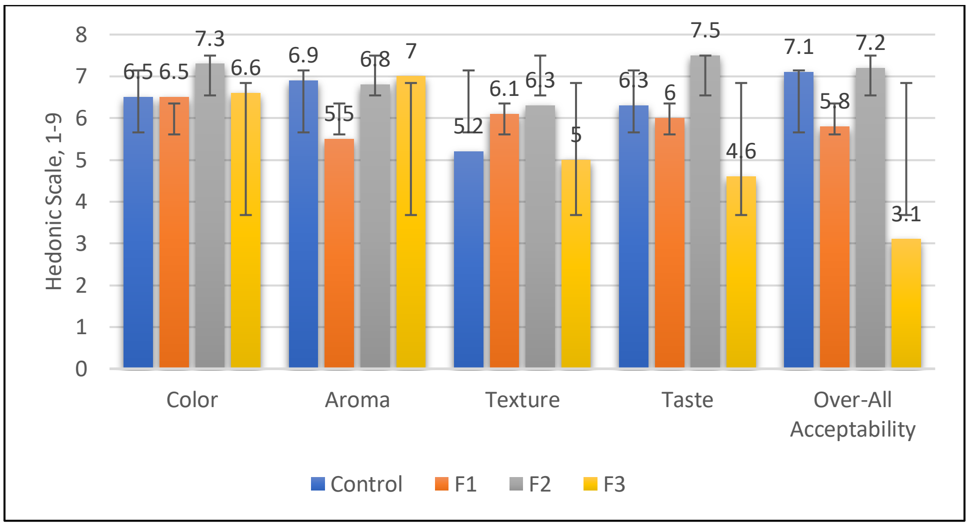

Figure 3. Sensory evaluation on attributes: color, aroma, texture, taste, and over-all acceptability of soymilk-based and cow's milk-based yogurt drinks. All the results are presented as mean ± standard deviation of the mean (n=10).

IV. Conclusion and Recommendation

Valorization of food waste using the naturally fermented soybean pulp as the prebiotic ingredient in isolating lactic acid bacteria with a probiotic potential was conducted prior to this study. Results revealed that the isolated lactic acid bacterium was identified as *Pediococcus pentosaceus* Mees, 1934. This species was utilized as a starter culture in producing the soymilk yogurt drinks in this study. Proximate analysis showed that formulation 1 with the *P. pentosaceus* as the sole starter culture scored highest in crude protein and ash content. It also scored second highest in crude fat. There was significant difference in the average ash content between the four samples. The color, taste, and overall acceptability of the yogurt drink samples were comparably similar. But formulation 2 scored highest in color, taste, and overall acceptability. Significant differences were observed in the average aroma and textures scores.

This study showed the potential of *P. pentosaceus* as a sole starter culture and in combination with commercial starter culture in producing functional foods from food waste such as soymilk-based yogurt. Refinement of the methods and ingredients used in the process is recommended to produce the yogurt with the desired texture and taste. It is also recommended to include the determination of moisture, pH, total soluble solids for the sweetness, and further nutritional facts analysis of the product. Further, increasing the participants for the sensory analysis is also recommended to improve the product's overall acceptability for commercial production. Bile tolerance test and antimicrobial screening against foodborne pathogens and non-resistance to antibiotics are also suggested to explore more of this species characteristics and usability. By so doing, product development from food waste can support sustainable food production and mitigate global food insecurity and climate change.

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