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ORIGINAL ARTICLE

Development of a Pigmented Soju-Like Beverage Using Natural Colorants from Sweet Potatoes (*Ipomoea Batatas* L.)

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ABSTRACT
Purple-fleshed sweet potato (PFSP) is known for its high anthocyanin content, which not only enhances the color of food products and beverages but also provides nutrients, particularly antioxidants. Due to the safety of purple sweet potato's anthocyanin (PSPA), it is currently being positioned as an alternative to synthetic pigments. One of the widely known alcoholic beverages today is Soju, a Korean clear distilled liquor. Therefore, this study aimed to develop a pigmented soju-like beverage by utilizing yellow-fleshed sweet potato (YFSP) for alcohol production with PFSP as the color source. During the fermentation, the total soluble solids (TSS), pH, and alcohol content were analyzed. Additionally, total monomeric anthocyanin pigment (TMAP) content and sensory properties were measured during the steeping process. Results showed a decrease in TSS and pH of YFSP due to yeast activity, while alcohol content increased during fermentation. Steeping for 6 days resulted in higher TMAP, TSS, and alcohol content, lower pH values, and sensory attributes were more acceptable. In conclusion, the study successfully developed a naturally colored soju from sweet potatoes. These findings can contribute to the manufacture of soju with unique product qualities.

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1. Introduction

Sweet potato (Ipomea batatas L.), a plant belonging to the family Convolvulaceae, is a vegetable primarily known for its large, starchy, and sweet-tasting tuberous roots consumed by both humans and animals. It is also a valuable medicinal plant that exhibits anti-inflammatory activities (Mohanraj & Sivasankar, 2014). Generally, sweet potato is considered the world's sixth most important food crop after rice, wheat, Irish potatoes, maize, and cassava (International Potato Center-CIP, 2024). Earlier records indicate that this crop is a staple food source for many indigenous groups in Central and South America, Africa, and some parts of Oceania (Bovell-Benjamin, 2007). According to the Philippine Statistics Authority (2021), data showed that more than 546 thousand metric tons of sweet potatoes were produced in the Philippines in 2020, with about 84 thousand hectares of area harvested, and an average value of 1.43 million pesos a month from the year 2000 to 2022. The widespread production is attributed to the crop's ability to thrive in various environmental conditions and year-round growth. Furthermore, its economic significance is rooted in its nutritive values, as it contains significant amounts of macronutrients and micronutrients, and serves as a crucial raw material for starch production (Laveriano-Santos et al. 2022; Sawicka et al. 2014).

Sweet potatoes come in a wide range of colors which can be white, yellow, orange, or purple. Anthocyanin and beta-carotene, the compounds responsible for the colors, are known antioxidants that have health benefits. The purple-fleshed sweet potato variety contains anthocyanin which are water-soluble natural pigment from phenolic groups that give red and purple colors to many grains, fruits, and vegetables. It is also used as a nutraceutical ingredient due to its effectiveness against numerous non-communicable diseases such as cardiovascular and metabolic diseases (Mattioli et al., 2020). Owing to purple sweet potato anthocyanin's (PSPA) safety, bright color, and other physiological functions, it is widely used in the food industry (Nhut Pham et al., 2019; Khoo et al., 2017). Records also showcased the extensive use of PSPA in different beverages like wine, energy drinks, sweet drinks, and other alcoholic beverages with above 15% alcohol, due to its solubility in water and alcohol, its stability in most conditions, and its easy extraction (Morata et al., 2019). Following the increasing consumer awareness regarding some safety issues of synthetic pigments, PSPA and other natural edible pigments are now being favored by more consumers (Li et al., 2019).

One of the most selling spirits in the world is soju, a Korean clear distilled liquor made from materials such as rice, sweet potatoes, and tapioca (Park, 2021). This alcoholic beverage also means "burnt liquor" as it involves distillation at a high temperature after fermentation thereby resulting in an alcohol content of at least 15% while the average is 25%. Recent advancements in the industry have focused on adding flavors and colors to improve the sensory characteristics of alcoholic beverages (Tamayo-Sánchez et al. 2023; Spence, 2016; Lin et al. 2023). Although few studies directly utilized PFSPs in making alcoholic beverages (Ray et al., 2012; Lee et al., 2015), incorporating natural pigments from PFSPs through the steeping process into soju remains limited in this context. Therefore, the objective of this study was to explore the feasibility of developing a purple-pigmented soju-like beverage, by utilizing yellow-fleshed sweet potato (YFSP) for alcohol production and PFSP as the color source. The addition of color to the beverage is expected to positively influence the consumers' perception, making it a unique selling proposition.

2. Materials and methods

2.1 Materials

Purple-fleshed and yellow-fleshed sweet potatoes were provided by the Institute of Plant Breeding (IPB), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB). The enzymes, specifically including alpha-amylase (AA) and amyloglucosidase (AMG), were purchased from Sunson Industry Group Co., LTD, with enzyme activities of 180,000 u/mL and 300,000 u/mL, respectively. The yeast (scientific name: *Saccharomyces cerevisiae* BIOTECH 2055) for the fermentation process was acquired from the Food Microbiology Laboratory of the Institute of Food Science and Technology, CAFS, UPLB.

2.2 Liquefaction and Saccharification

To facilitate the conversion of complex sweet potato starch polymers into simpler structures, liquefaction and saccharification for starch were employed. A kilogram of YFSP was steamed, cooled, peeled, and grated manually. Distilled water was added to the grated samples following a 1:4 ratio to make a slurry. The slurry was then subjected to liquefaction and saccharification, following the procedures derived from preliminary experiments and the manufacturer's suggestion. During the liquefaction phase, the slurry was heated to approximately 50-60 ^{o}C, and about 4.5-5.5 mL of alpha-amylase was added upon achieving the desired temperature. This condition was maintained for an hour with constant stirring. During the saccharification phase, the pH of the slurry was adjusted to 4.5-5.0 by adding anhydrous citric acid, and around 19-20 mL of glucoamylase was added. The temperature was maintained again for an hour with continuous stirring. The TSS was measured after saccharification, and it was adjusted by adding a certain amount of refined sugar.

2.3 Alcoholic Fermentation

The YFSP slurry from the previous step was inoculated with *Saccharomyces cerevisiae* BIOTECH 2055, a yeast commonly used for alcoholic fermentation. The fermentation process was monitored for approximately 15 days, with sampling conducted at 3-day intervals, to evaluate the parameters specifically the pH, TSS, titratable acidity, and alcohol content. The fermentation was initially done under aerobic conditions to allow the growth of yeast, after which the process transitioned to anaerobic conditions to allow alcoholic fermentation to proceed. The fermentation was terminated upon reaching 6° Brix or when bubbling was no longer observed.

2.4 Distillation

The samples were initially filtered using a cheesecloth or 100-micron cloth mesh to remove the solids remaining from the fermentation process. The filtered samples were distilled using a single-stage distillation set-up and only the distillate from a specific fraction with the desirable quality for soju was collected.

2.5 Steeping

For this stage, steamed purple-fleshed sweet potato (PFSP) was blended with the soju following a 1:5 ratio (w/v). This ratio was derived from the related study conducted to explore which steeping ratio is best to apply in PFSP-steeped soju. Several samples were bottled and stored in a dark area at room temperature. The physicochemical and phytochemical properties of the samples were monitored for about 1 week every 3 days.

2.6 Analysis of Physicochemical Properties

Total soluble solids (TSS) content was measured using a digital refractometer (ATAGO, N1, Japan) expressed in ◦Brix. The pH of the samples was measured using a pH pen (K13, China). The alcohol content in % (v/v) was quantified using an alcohol refractometer for spirit alcohol volume %, after the distillation of samples. All measurements were done in triplicates.

2.7 Total Monomeric Anthocyanin Pigment (TMAP) Content

The Total monomeric anthocyanin pigment (TMAP) content was measured using the pH differential method as described by Giusti & Wrostald (2001). Expressed as cyanidin-3- glucoside (cyd-3-glu) equivalents, TMAP was calculated using the following equation:

$$\text{TMAP (mg/L)} = \frac{A * MW * DF * 10\text{^}3}{\varepsilon * 1}$$

where A = (A530nm – A700nm) pH 1.0 – (A530nm – A700nm) pH 4.5; MW (molecular weight of cyd-3- glu) = 449.2 g/mol; DF = dilution factor; ε = 26,900 molar extinction coefficients in L x mol-1 x cm-1 for cyd-3-glu; 10^3 = factor for conversion from g to mg; and 1 = path length in cm.

2.8 Sensory evaluation

Generally, Qualitative Descriptive Analysis (QDA) using a 15-point hedonic scale was used for the evaluation of the final soju product. Participants who met certain criteria, specifically those who are of legal age, fond of drinking soju or other related alcoholic beverages, and currently don't have severe medical conditions, were chosen to partake in the evaluation session following also the set ethical guidelines. A total of 16 panelists, which were students from the University of the Philippines Los Baños, evaluated the soju-like beverage. The soju sensory attributes, specifically color, aroma, sweetness, sourness, bitterness, astringency, body, and overall acceptability, were evaluated by the panelists (Choi et al., 2022). Ethical clearance was obtained through the National Ethical Committee, Philippine National Health Research System.

Criteria used for the sensory evaluation:

Color (0– 2.5: very light pink; 2.5-5.0: light pink; 5.0-7.5: pink 7.5-10.0: light violet; 10.0-12.5: violet 12.5-15.0 very dark violet)

Aroma, Alcohol, Sweetness, Bitterness, Astringency, Body, Flavor (0– 2.5: Not Perceptible; 2.5 – 5.0: Poorly Perceptible; 5.0 – 7.5: Fairly Perceptible; 7.5 – 10.0: Moderately Perceptible: 10.0 – 12.5: Perceptible; 12.5 – 15.0 = Highly Perceptible)

General Acceptability (0 – 2.5: Not Acceptable; 2.5 – 5.0: Poorly Acceptable; 5.0 – 7.5: Fairly Acceptable; 7.5 – 10.0: Moderately Acceptable: 10.0 – 12.5: Acceptable; 12.5 – 15.0 = Highly Acceptable).

2.9 Statistical analysis

All experiments and analyses were run in triplicates. Descriptive analysis was done from the data which includes mean, standard deviation, and standard error. One-way ANOVA followed by Tukey's post hoc test was used to analyze the changes in the physicochemical properties during fermentation and the steeping periods of PFSP in soju. Lastly, an independent sample t-test was used for the analysis of sensory evaluation data. Conclusions at each statistical analysis done were made with a statistical significance of 5%.

3. Results and Discussion

3.1 Changes of YFSP during the Fermentation Process.

Alcohol content increases as yeasts metabolize sugars to ethanol during alcoholic fermentation. Results showed that the highest alcohol content of 15% was observed during the 12^{th} and 15^{th} day, the terminal periods of the fermentation (Figure 1). At this phase, the alcohol content did not increase further, possibly due to the ethanol tolerance of some yeast strains within this range of alcohol concentration. It was reported that the growth of yeasts was completely inhibited using 16% ethanol (Varize et al., 2022)

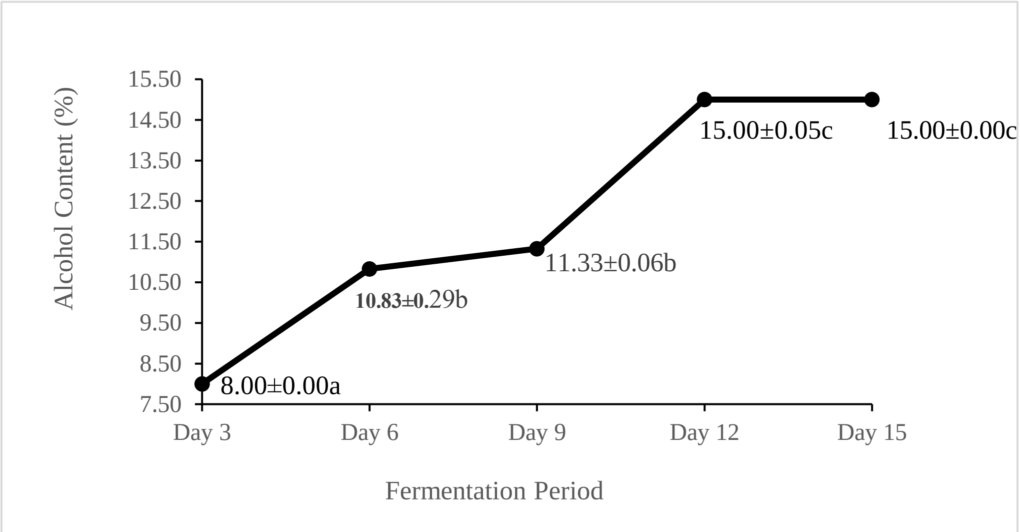

Fig. 1. Alcohol content (% v/v) of YFSP slurry samples during fermentation. Results display mean values ± standard deviations (n = 3) values. [abc] Mean values that have different letters indicate a significant difference (p≤0.05).

Total soluble solids (TSS) content is commonly used to assess the quality and sweetness, and the fermentation period of fruit-derived beverages, especially wine. It was observed that the TSS gradually decreased until Day 15 (Figure 2). During fermentation, yeast consumes and utilizes the available sugars thereby decreasing the TSS values (Jagadeesh et al., 2022). The drastic decrease in TSS between days 3 and 6 must be due to the high yeast activity initially. As the fermentation progresses, the decrease in TSS values becomes more incremental which is possibly due to increasing ethanol concentration thereby affecting the yeast activity (Kasture & Kadam 2018).

Furthermore, it was also observed that the pH values of the fermented samples gradually decreased from day 3 up to day 15 of fermentation (Figure 3). This may be attributed to the production of organic acids, such as lactic and succinic acid, as by-products of alcoholic fermentation (Pan et al., 2023). It can also be due to the increasing amount of alcohol in the samples during fermentation like glycerol and ethanol which caused a decline in pH. Significant differences in pH values were observed from the 3rd to 9th days, the periods when yeast activity is presumed to be highest. Afterward, the decrease in pH became considerably smaller, possibly due to the lowering of yeast activity.

3.2 Physicochemical Properties of PFSP in Soju During Steeping

Table 1 shows the changes in the TSS, alcohol content, and pH of PFSP-steeped soju for 6 days of steeping. The TSS content of the soju steeped with PFSP consistently increased but a significant difference ($p<0.05$) was only seen between day 3 and day 6. The observation agrees with the study of Son et al. (2009) where TSS increased as more interaction of sugars with water and ethanol occurred. Similarly, the alcohol content of the PFSP-steeped soju sample gradually increased from day 0 until day 6 of steeping and was found to be significantly different from each other ($p<0.05$). This is consistent with the results of Flowers (2014) in his study on increasing the alcohol content of alcoholic beverages. More so, the pH values of the steeping samples were generally found to have a decreasing trend albeit not significantly. The decrease in pH after steeping on day 6 can be attributed to a higher amount of anthocyanin being added to the sample. Dangles & Fenger (2018) have stated that anthocyanins express much richer chemical reactivity than the other flavonoid classes owing to their pyrylium nucleus and for instance, anthocyanins are weak diacids.

Table 1. Physicochemical properties of PFSP-steeped soju at different steeping periods.

STEEPING PERIOD (day/s)	TSS (◦Brix)	ALCOHOL CONTENT (% v/v)	pH
0	5.70 ± 0.10^{a}	10.83 ± 0.29^{a}	4.70 ± 0.00^{a}
3	5.80 ± 0.00^{a}	12.67 ± 0.58^{b}	4.70 ± 0.10^{a}
6	6.13 ± 0.06^{b}	17.00 ± 1.00^{c}	4.60 ± 0.00^{a}

Results display mean values ± standard deviations (*n* = 3) values. [abc] Mean values within a column that have different letters indicate a significant difference (p≤0.05)

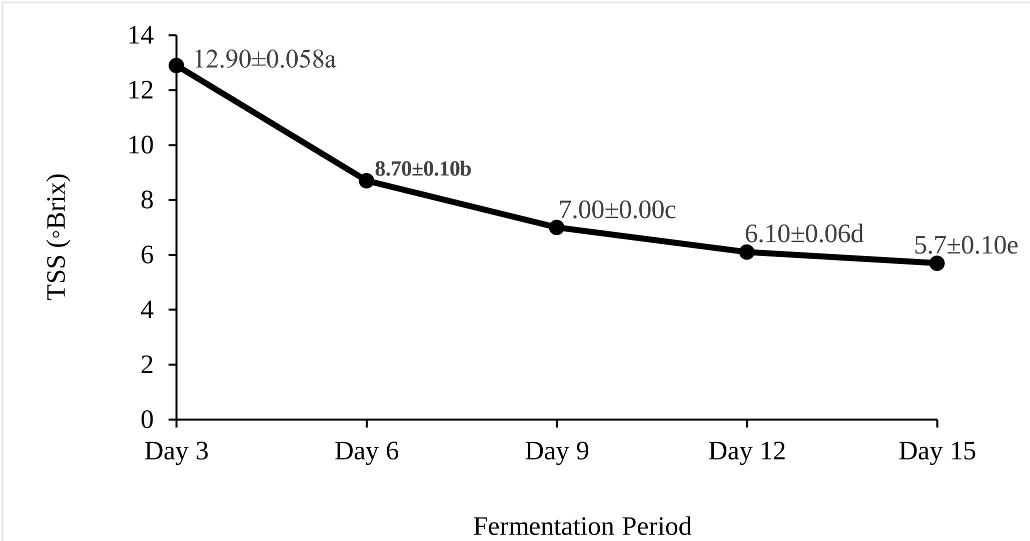

Fig. 2. Total soluble solids (◦Brix) of YFSP slurry samples during fermentation. Results display mean values ± standard deviations (n = 3) values. [abc] Mean values that have different letters indicate a significant difference ($p \le 0.05$).

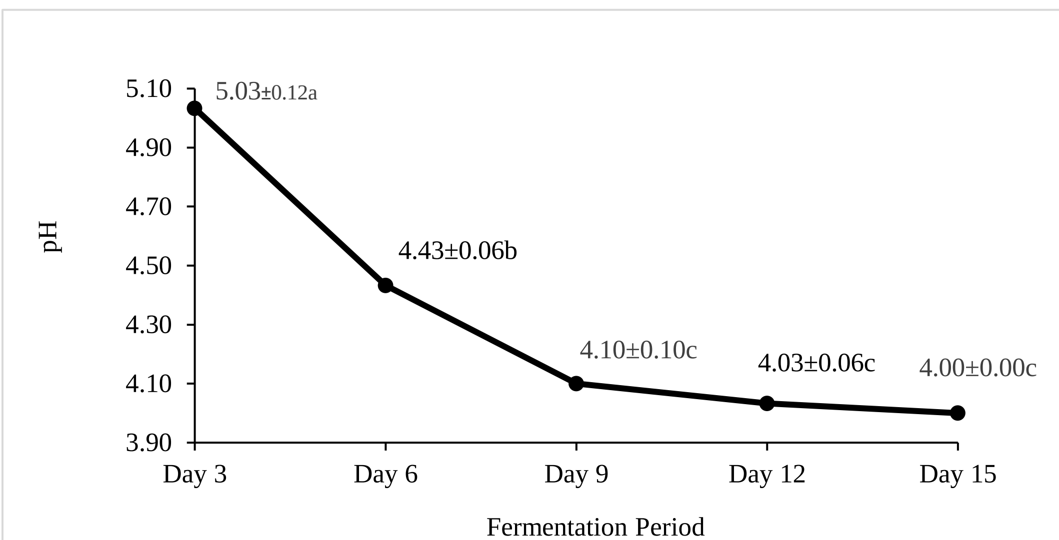

Fig. 3. Levels of pH of YFSP slurry samples during fermentation. Results display mean values ± standard deviations (n = 3) values. [abc] Mean values that have different letters indicate a significant difference ($p \le 0.05$).

3.3 TMAP Content During Steeping of PFSP in Soju

Table 2 summarizes the TMAP content of the steeping samples. The highest TMAP value was obtained on the 6th day which may be attributed to the length of the steeping period, as more anthocyanins were extracted over time. Additionally, the high alcohol content on the 6th day of steeping may have facilitated the extraction of more anthocyanins from the PFSP, as these compounds are also soluble in ethanol aside from water (Khoo et al., 2017; Enaru et al. 2021). Furthermore, this observation may also be due to the low pH value measured on the 6th day. This finding aligns with the previous studies by Chen et al. (2019) and Enaru et al. (2021) on the stability of anthocyanins, where it was found to be more stable at lower pH values. However, the study by Ray et al. (2012) on the direct use of PFSPs in winemaking demonstrated a reduction in anthocyanin content from the must stage to when the ethanol content in the wine increased. The results showed that the steeping process of steamed PFSP to soju has better anthocyanin retention than direct utilization.

Table 2. TMAP (mg/L) of PFSP-steeped soju at different steeping periods.

STEEPING PERIOD (day/s)	TMAP (mg/L)
0	120.25±2.45^{a}
3	122.52±1.57^{a}
6	136.48±0.66^{b}

Results display mean values ± standard deviations (*n* = 3) values. ^{abc} Mean values within a column that have different letters indicate a significant difference (p≤0.05)

Figure 4 shows the resulting product of steeping PFSP in soju, wherein the color was observed to fall within the reddish-purple spectrum. This shade can be due to the low pH or acidic conditions. According to Khoo et al. (2017), anthocyanins have a basic structure of flavylium cation, which imparts an ionic nature to this compound, rendering it highly reactive to varying pH levels. The study by Countinho et al. (2004) also revealed the red-colored pigments of anthocyanins are predominantly in the form of flavylium cations, which intensifies the red hue at acidic conditions. Anthocyanins project a purple hue at neutral pH conditions (Chen et al., 2019). However, in basic conditions, flavylium cations undergo deprotonation, where quinoidal bases favor alcoholic solutions, shifting the purplish red color to a bluish-violet color.

3.4 Sensory Characteristics of Soju Steeped with PFSP

For the sensory evaluation, the samples steeped for 3 days (S3D) and 6 days (S6D) were compared while using a commercially available soju (Brand: So Nice) as a reference, and the results are summarized in Table 3. This was conducted to assess whether the steeping period had a substantial effect on the sensory qualities of the soju. Based on the results, significant differences were observed in some of the sensory attributes of the two samples. Both treatments were found to be generally acceptable, but the S6D had a higher mean score and was more acceptable.

Color. The initial product of distillation before steeping was a clear distilled spirit as depicted in Figure 5. When the 2 treatments were compared, S3D appeared to have a darker violet shade than S6D. This assessment was done using the provided color guide (Figure 6). A significant difference in their mean scores was also observed, which could be associated with the pH values of each sample. However, anthocyanins are greatly affected by factors such as light and oxygen, which may also contribute to the color variation observed. Moreover, color is considered a significant sensory cue that plays a crucial role in shaping consumers' expectations of the taste and flavor of a food product (Spence, 2015). Further research also suggests that the human experience of taste and flavor is vastly influenced by the expectations formed before tasting a product. These expectations are guided by extrinsic factors such as labeling, branding, and packaging, as well as intrinsic factors such as smell and appearance. In a study by Velasco et al. (2015) as cited by Spence (2019), participants from various continents associated drinks with red, blue, and purple colors as being sweet. Therefore, the addition of color can be intentionally designed to give consumers preconceived notions regarding the taste and flavor of the soju.

Aroma. The distinct smell or aroma of steamed purple sweet potato was more prominent in S6D compared to S3D. This can be attributed to the longer extraction period of volatile compounds that provide the aroma of steamed sweet potatoes such as phenylacetaldehyde, which gives a floral odor, methional, which gives a potato odor, maltol, which gives caramelized odor, and methyl geranate, which gives a sweet odor (Nakamura et al., 2013) These volatile compounds start to be exhibited after treating sweet potatoes with heat (Wang et al., 2000).

Sweetness. The S3D was perceived by the panelists to be sweeter than the S6D. The perceived sweetness is due to the breaking down of complex sugar compounds during steeping. Considering the TSS after steeping, S6D was initially expected to be sweeter as it exhibited a higher TSS value towards the end. However, the higher alcohol content observed on day 6 may have affected the perception of sweetness, as Mughal (2021) has stated that alcohol decreases tongue sensitivity to sweetness.

Alcohol. Accordingly, S6D was perceived to have stronger or higher alcohol than S3D wherein the mean score difference was found to be statistically significant. This is in line with the alcohol content measurement of using an alcohol refractometer. S6D had 17-18% while S3D had 13-16% alcohol before the sensory evaluation.

Astringency, Bitterness, and Body. In terms of these attributes, S6D received higher mean scores than S3D albeit the difference was not significant. Beecher et al. (2008) stated that astringency is more pronounced in foods with lower pH levels, which was observed for both samples. Moreover, bitterness is mainly elicited by phenolic compounds and microbial metabolites produced during fermentation such as tannins, bitter peptides, and alcohols (Luo et al., 2020). Thus, S6D was more bitter as it had a higher alcohol content. Subsequently, the body of alcoholic beverages is the pressure or the viscosity of the beverage felt by the tongue. According to Yanniotis et al. (2007), the viscosity of wines is primarily affected by the dry extract and alcohol content, which may elucidate the higher body mean score of S6D. However, TSS may also be correlated with the body as it measures the solids dissolved from purple sweet potato due to steeping.

Flavor: This parameter is associated with the taste and aroma of the steamed purple sweet potato perceived in the soju. Results revealed that S6D received a higher score in aroma and flavor compared to S3D. However, it should be emphasized that not only volatile compounds can affect the aroma, but also furans, sugars, and terpenes from sweet potatoes (Zhang et al., 2023).

Lastly, a commercially available soju, branded as So Nice Original Soju, was also evaluated alongside the two treatments. So Nice Soju, with a 17% alcohol content, achieved a higher score in terms of alcohol, bitterness, astringency, and body compared to the steeped samples. This can be due to purer alcohol and clearer quality of commercialized soju, as they undergo modern production methods such as continuous distillation and filtration (Choi et al., 2022). Nonetheless, it was reported that despite grains like rice being the main raw material used in soju production, the increasing demand fueled the development of soju also from sweet potatoes (Oke & Workneh, 2019).

Table 3. Mean scores of the sensory attributes of PFSP-steeped soju samples steeped for 3 days and 6 days.

PARAMETER	**MEAN SCORES**	
	3 Days	**6 Days**
Color	12.90±1.72[a]	12.19±2.40[b]
Aroma	7.39±3.09[a]	7.56±3.44[a]
Alcohol	6.99±3.47[a]	8.58±3.05[b]
Sweetness	4.08±2.85[a]	3.33±2.27[a]
Bitterness	4.96±2.87[a]	5.91±2.57[a]
Astringency	4.91±2.35[a]	5.85±2.42[a]
Body	6.74±2.54[a]	6.75±3.15[a]
Flavor	6.69±2.96[a]	6.97±2.65[a]
General Acceptability	7.60±3.02[a]	8.50±3.23[a]

Results display mean values ± standard deviations (*n* = 3) values. [abc] Mean values within a row that have different letters indicate a significant difference ($p \leq 0.05$).

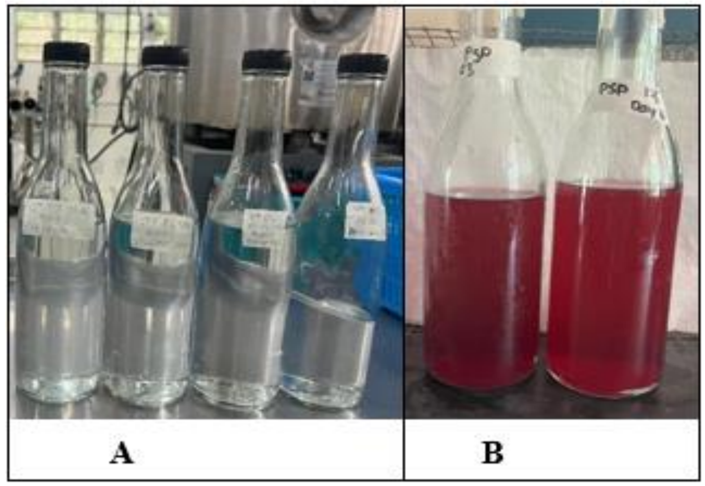

Fig. 4. The YFSP soju before and after steeping PFSP. A (the soju distillates recovered after distillation); B (from L to R: PFSP-steeped soju for 3 days and 6 days).

Conclusion

Recognizing the potential of purple-fleshed sweet potato (PFSP) as a natural food colorant, this study aimed to investigate the feasibility of developing a purple-pigmented soju using yellow-fleshed sweet potato (YFSP) for alcohol production while PFSP as the color source. This study closely monitored and evaluated the effects of processing on the important physicochemical and phytochemical properties of the soju.

During the fermentation of YFSP, corresponding changes in the total soluble solids, pH, and alcohol content were observed mainly due to the activity of yeast. To incorporate the purple color, a steeping process was employed by blending steamed PFSP with soju from YFSP for 6 days. This process showed that steeping at these conditions resulted in higher TMAP, TSS, and alcohol content, and lower pH values. Furthermore, sensory evaluation results showed that steeping for 6 days is generally more acceptable than steeping for 3 days.

The results of this study proved that it is possible to develop a pigmented soju-like beverage using PFSP as a natural color source. This inventive step is aimed at being a unique selling proposition for alcoholic drinks, especially soju, as color greatly influences the consumers' outlook on food products. This technology further showcases the potential of PFSP as a versatile root crop that can promote and increase its utilization in the food industry. It is worth mentioning also that the outcome of this study is only a minimum viable product that needs further improvements. Exploring the use of modern distillation technologies to prevent losses of other flavor and volatile compounds is recommended. Changes in the color of the product during storage must also be observed as anthocyanins are highly reactive to external factors such as light. Lastly, conducting market studies, such as focus group discussions and surveys, will provide valuable insights from the target consumers of soju and other alcoholic beverages regarding this technology.

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Conflict of interest

The authors declare that there is no conflicts of interest.

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