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Physico-chemical properties, probiotic stability and sensory characteristics of *Lactobacillus plantarum* S20 – supplemented passion fruit (*Passiflora edulis* f. *flavicarpa* Deg.) juice powder

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Abstract

The study aimed to develop a non-dairy-based probiotic-supplemented product using an underutilized crop in the Philippines such as the yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Deg.). The physico-chemical properties (moisture content, water activity, pH, and total soluble solids), probiotics stability at different storage temperatures (4°C, 25°C, and 37°C), and the sensory characteristics of *Lactobacillus plantarum* S20-supplemented passion fruit juice powder was evaluated. Passion fruit juice powder and *L. plantarum* S20 were first prepared using low-temperature spray drying utilizing maltodextrin as a carrier, with yield as 42.97% and 21.17%, respectively. Spray drying of probiotics culture also resulted in 42.68% log survivability. The formulated juice powder had a final moisture content of 1.729±0.38% and water activity of 0.398±0.0051, and with recommended dilution with water, had a final pH and total soluble solids of 3.40±0.10 and 12.00±0.00° Brix, respectively. Results also showed that storage of the formulated juice powder at 4°C yielded the highest probiotic stability, maintaining a viable log count of 4.27 per g, while storage at 37°C showed no microbial growth. Sensory evaluation of probiotic-supplemented passion fruit juice against a non-probiotic-supplemented one revealed significant difference in terms of color, sweetness, and sourness, while no significant difference was observed in terms of aroma, mouthfeel, and general acceptability.

1. Introduction

Probiotics are live beneficial microorganisms which, when taken in adequate amounts, offer health benefits beyond normal nutrition (Song *et al*., 2012). For a food product to be considered probiotic, 10^6-10^7 CFU/g of the probiotic culture must be maintained until the end of the product's shelf life (Goderska, 2012). Probiotic food products are usually seen in the form of dairy products such as milk, cheese, and yoghurt which might confer a problem to individuals who are lactose intolerant. Moreover, vegetarianism is becoming prevalent these days. Non-dairy probiotic food products are acceptable alternatives for individuals who are either vegetarians or lactose intolerant (Lee and Salminen, 2009). The production of probiotic food requires a starter culture. Initially, commercial starter cultures are present in a liquid form. This is inconvenient because of the high costs utilized in bulk culture preparation and the risks of bacteriophage infection (Desmond *et al*., 2002). Because of the recent advancements made in biotechnology, concentrated starter cultures in frozen and freeze-dried forms were made available. Spray drying has been extensively used in the production of powdered foods. Spray drying addresses the problems involved in the storage, maintenance, and handling of liquid cultures. This method uses high temperatures, thereby instantly producing the powdered product as it is dried (Goderksa, 2012).

Passion fruit (*Passiflora edulis*) is a perennial crop which grows mostly as a vine with a shallow root system, growing up to a length range of 15-20 feet. Its leaves are trilobate, long and deep green, with a glossy upper surface and matte on its lower surface. The fruit is circular or oval shaped and is filled with membranous sacs containing the seeds. It is also filled with aril (or pulp) and juice. Passion fruits are mostly harvested in the tropical and sub-tropical parts of the world. There are an estimated 500 species of the genus *Passiflora* in the family *Passifloraceae,* with two species gaining commercial importance: the standard yellow (*Passiflora edulis* f. *flavicarpa* Deg.) and the purple (*Passiflora edulis* f. *edulis*). The yellow passion fruit is more acidic,

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with a pH of 2.8 while the purple passion fruit has a pH of 4.2. In terms of starch content, the former has 0.06% starch, while the latter has 0.74% starch. The amylose content of the yellow variety (8.7%) is also comparatively higher than the purple one (5.8%) (Zas and John, 2016). The purple passion fruit is mainly grown for the processing of juice due to its desirable and unique flavor. The flavor could be attributed to the several aromatic compounds present which deteriorates with high temperatures (Strohalm *et al.*, 2007). Passion fruit is considered as underutilized and is a commonly neglected crop in the Philippines. Processing this fruit into value-added products will boost its economic value. Consequently, it will also boost utilization and further production studies.

In this study, spray drying would be used as the drying method to convert the microbial culture (*L. plantarum* S20) and passion fruit (*Passiflora edulis* f. *flavicarpa*) into a powdered form. This study aims to test the stability of spray dried *L. plantarum* S20 on passion fruit juice powder. Specifically, the study aimed to: (1) determine the viability of *L. plantarum* S20 after spray drying; (2) determine the drying yield of *L. plantarum* S20 cells and passion fruit slurry after their respective spray drying processes; (3) test for certain physico-chemical properties (water activity, moisture content, total soluble solids, and pH) of the passion fruit juice powder incorporated with spray dried *L. plantarum* S20; (4) determine the effect of storage conditions with respect to varying temperatures (4, 25 and 37°C) on the survivability of *L. plantarum* S20 incorporated in passion fruit powder; and (5) conduct a sensory test that would compare the attributes between two treatments: passion fruit juice with and without spray dried *L. plantarum* S20 cells.

2. Materials and methods

2.1 Microbial culture

Pure culture of *L. plantarum* S20 was obtained from University of the Philippines Los Baños – National Institute of Molecular Biology and Biotechnology (UPLB-BIOTECH). The microbial culture was grown in de Man, Rogosa, and Sharpe (MRS) broth added with 30% (v/v) glycerol and stored at a temperature of 4°C.

2.2 Spray drying of microbial culture

L. plantarum S20 culture was incubated at 37°C for 18 hours in MRS Broth to achieve a targeted 10^{11} CFU/g microbial cell count. The cell suspension was aseptically transferred to centrifuge tubes and was subjected to centrifugation at 4300 x *g* for 15 mins at 4°C. Next, 1 ml of 0.85% saline solution was used to wash the remaining filtrate twice. Afterward, 30% (v/v) of 50% maltodextrin solution was added as the encapsulating agent. The spray drying of the cell suspension with maltodextrin and saline solution was done using an industrial-scale model YK-100 Low-Temperature Spray Dryer (True Ten, Taiwan) at the Fermentation Engineering Pilot Plant of UPLB-BIOTECH. The spray dryer operates with a single fluid atomizer and a co-current regime at a flow rate of 0.67 L/hr. The prepared solution was fed into a spray dryer at an inlet temperature of 42°C and an outlet temperature of 35°C. The resulting powder was collected at the bottom of the cyclone separator and stored in sterilized vials. The drying yield of the spray dried microbial culture was computed using Equation 1:

$$\text{drying yield (\%)} = \frac{\text{weight of spray dried product, g}}{\text{weight of feed solution, g}} \text{x } 100 \quad (1)$$

2.3 Determination of viability of spray dried cells

Standard microbial enumeration using MRS agar was done to determine the viability of the cells. Percent log survivability of the microbial cells was then computed using Equation 2, with No = cell count before spray drying, and N = cell count after spray drying.

$$\%\ \log \text{survivability} = \frac{\log(N)}{\log(No)} \text{ x } 100 \quad (2)$$

2.4 Spray drying of passion fruit

Ripe yellow passion fruits (*Passiflora edulis* f. *flavicarpa* Deg.) were obtained from a local market in Lucban, Quezon Province, Philippines. The passion fruits were processed into puree using standard protocols. The passion fruit slurry was diluted with water in a 1:3 ratio (passion fruit puree: water). The resulting solution was added with 25% (w/v) of 50% maltodextrin. Next, the slurry was fed into an industrial-scale model YK-100 Low-Temperature Spray Dryer (True Ten, Taiwan) at the Fermentation Engineering Pilot Plant of UPLB-BIOTECH. The spray dryer operates with a single fluid atomizer and a co-current regime at a flow rate of 0.67 L/h. The inlet temperature used was 55°C and an outlet temperature of 40°C. The resulting powder was collected at the bottom of the cyclone and stored in a sterilized jar. Drying yield was computed using Equation 1.

2.5 Formulation of passion fruit juice powder with spray dried Lactobacillus plantarum S20

The formulation of the powder mix was based on the experimental trials which when diluted with 100 mL of distilled water would yield total soluble solid (TSS) of 12.00 and pH between 3.2 to 3.5. The formulation included 2.5 g of passion fruit powder, 9.9 g of sugar, and 0.05 g of citric acid. The spray dried probiotic culture was added equivalent to 10% to the total weight

of the powder solids.

2.6 Probiotic stability at different storage temperatures

The powdered juice added with spray dried *L. plantarum* cells were stored in sealed and sterilized polypropylene bags. Stability of the bacteria in the passion fruit juice powder was tested in different storage temperatures: 4°C, 25°C and 37°C. Standard microbial enumeration in MRS agar was done every week for four consecutive weeks.

2.7 Physico-chemical analysis of the formulated juice powder

The resulting passion fruit juice powder incorporated with spray dried *L. plantarum* was tested for the following parameters: (1) pH using Milwaukee pH600 pocket-size pH pen, (2) Total Soluble Solids (TSS) using ATAGO Master-M (0-33%) hand-held refractometer, (3) moisture content using oven drying method (AOAC, 2007), and (4) water activity using Novasina LabSwift table-top water activity meter. All measurements were done in triplicate.

2.8 Sensory evaluation

The intensity of different sensory attributes namely, color, aroma, mouthfeel, sweetness, sourness, as well as the general acceptability of the samples was determined by quality scoring using a 15-cm line scale. These attributes were evaluated by 25 selected panelists who already took basic courses in sensory evaluation of food. The suggested number of medium-trained panelist for quality scoring is 8-12, or five (5) highly trained panelist (Kilcast, 2010). The number of panelists was increased to 25 to make the responses approach normality based on the law of large numbers. For the statistical analysis, randomized complete block design (RCBD) was used as the experimental design. Analysis of variance (ANOVA) was analyzed using the Statistical Tools for Agricultural Research (STAR) Software Version 2.0.1 (IRRI – BBI, 2019). The significance of differences was defined as $P < 0.05$.

3. Results and discussion

3.1 Spray drying yield

The drying yield percentage of the passion fruit and *L. plantarum* cells is shown in Table 1. The liquid passion fruit was incorporated with 25% maltodextrin while the probiotic cells were incorporated with 30% maltodextrin. The amount of maltodextrin added to the inlet suspension is relatively close to the drying yield percentage acquired in *L. plantarum* cells. Since the passion fruit slurry is quite sticky in nature, more particles adhered to the encapsulating agent, attaining a higher drying yield.

Table 1. Spray drying yield of microbial cells and passion fruit slurry.

Sample	Drying Yield, %
Passion Fruit	42.97
L. plantarum cells	21.17

3.2 Viability of Lactobacillus plantarum S20 after spray drying

The logarithmic survivability of the *L. plantarum* S20 cells after spray drying is shown in Table 2. Spray drying uses high temperatures which may denature proteins, making the microorganism unable to perform its metabolic processes properly. Moreover, the high pressure utilized in spray drying could have damaged the cells resulting in low cell viability. However, the presence of surviving cells suggests that the carrier, maltodextrin, offered a certain degree of protection for the cells. Maltodextrin, and combinations with other wall material such as inulin and oligofructose, had shown satisfactory recovery of probiotic cells after spray drying, resulting in microbial counts of up to 10^{10} CFU/g (Paim *et al*., 2016).

Table 2. Survivability *L. plantarum* S20 cells after spray drying.

Parameter	Value
Initial Cell Count, log CFU/mL	11.2
Final Cell Count, log CFU/mL	4.78
Log Survivability, %	42.7

3.3 Physico-chemical properties of the passion fruit juice powder with probiotics.

Table 3 summarizes the physicochemical properties of the formulated juice powder. The average pH of the formulated passion fruit juice is 3.4. With this pH value, the probiotic bacteria *L. plantarum* would still be able to proliferate since it could tolerate lower pH levels of up to 3.2 (Zago *et al*., 2011), with some studies showing growth of *L. plantarum* at a pH as low as 2.5 (Guo *et al*., 2017). The pH of the raw yellow passion fruit (*Passiflora edulis f. flavicarpa* Deg) is 2.8 (Zas and John, 2016). The observed water activity of the formulated juice powder was 0.398. Water activity is a measure of stability in foods since it indicates the amount of available water for microbial growth and chemical reactions. For most food systems, the critical water activity below which no microorganisms can grow commonly ranges from 0.6-0.7, with critical water activity for growth of pathogenic bacteria set at 0.85-0.86. In relation, the water activity of the powdered product is far from the critical water activity, hence it could be a measure to state that the final product is safe in terms of the growth of pathogenic bacteria. However,

Table 3. Physicochemical properties of passion fruit juice powder with spray dried *L. plantarum* S20.

Sample	Physicochemical Properties			
	pH*	TSS, °Brix*	%MC	Water Activity (A_w)
Passion fruit juice with spray dried *L. plantarum* S20	3.40±0.1	12.00±0.0	1.729±0.38	0.398±0.0051
Passion fruit juice without spray dried *L. plantarum* S20	3.32±0.2	12.00±0.0	1.690±0.51	0.385±0.0040

* Preparations with 10% solution (w/v)

since the water activity value is low, it plays a factor in the decrease in the growth of spray dried *L. plantarum* S20 after spray drying and storage. Moisture content is the amount of free water in each system. Knowledge of moisture content is important because it is a factor in dictating product stability during storage. Moreover, low moisture content is economical since the weight of water contributes to the total weight of the product. Less moisture could make transportation costs of products cheaper for manufacturers.

3.4 Probiotic stability at different storage temperatures

The viability of the spray dried probiotic cells in passion fruit juice powder was evaluated in different storage temperatures: 4°C, 25°C, and 37°C. The log reduction of the microbial cells per week at varying temperatures are listed in Table 4. The passion fruit added with spray dried *L. plantarum* S20 placed at 4°C had the best performance among the treatments, maintaining microbial count of up to 4.27 log CFU/gram or 91.63% log survivability after the test period. Moreover, it was observed that the powdered product stored at 25°C had growth in the first two weeks but stopped growing starting from the third week, while it was noted that the product stored at 37°C showed no observed growth after one week of storage. Possible reasons behind the cease in growth of *L. plantarum* are the presence of light during storage and the lack of pre-treatment prior to spray drying. Studies have been conducted wherein they were able to successfully maintain probiotic cell count even at room temperature. The probiotic powders were stored at room temperature, but light source was absent (Barbosa *et al*., 2012) and sub-lethal thermal shock was applied to the cells prior to spray drying (Anekella and Orsat, 2013). Meanwhile, having the combination of heat and low water activity resulted in the very poor performance observed in the cell growth at 37°C.

Table 4. Microbial change in passion fruit juice powder at 4, 25 and 37°C storage temperatures.

Storage Temperature (°C)	Time (Weeks)	Cell Count (Log CFU/g)	Log Survivability (%)
4	0	4.66	---
	1	4.58	98.28
	2	4.46	95.71
	3	4.29	92.06
	4	4.27	91.63
25	0	4.66	---
	1	3.94	84.55
	2	1.91	40.99
	3	0	0
	4	0	0
37	0	4.66	---
	1	0	0
	2	0	0
	3	0	0
	4	0	0

3.5 Sensory evaluation

Sensory evaluation is a tool used to determine human responses to food while minimizing biases and other information that influences perceptions (Lawless and Hildegarde, 2010). In this study, two treatments were tested: passion fruit juice without spray dried *L. plantarum* S20 and passion fruit juice with spray dried *L. plantarum* S20 based on six attributes. These attributes are color, aroma, mouthfeel, sweetness, sourness, and general acceptability. The sensory means scores of the two treatments are displayed in Table 5.

3.5.1 Aroma and mouthfeel

No significant difference was observed between the two preparations in terms of aroma and mouthfeel, suggesting that the added spray dried probiotic culture does not affect these sensory attributes. This can also be an indication that the incorporated microorganisms are

Table 5. Comparison of the sensory attributes of passion fruit juice with and without spray dried *L. plantarum* S20.

Treatments	Aroma	Color	Mouthfeel	Sweetness	Sourness	General Acceptability
Passion fruit juice without spray dried *L. plantarum* S20	8.41^a	4.13^b	11.78^a	8.25^a	7.67^a	10.16^a
Passion fruit juice with spray dried *L. plantarum* S20	8.71^a	6.60^a	10.92^a	9.89^b	6.42^b	10.93^a

* Means with the same letter in the same column are not significantly different from each other at $P<0.05$

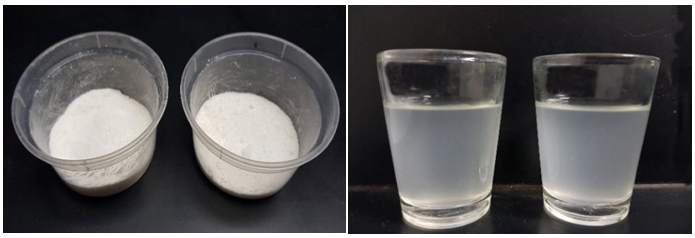
** Range of Scale:

Aroma: 0-weak to 15-strong; Color: 0- light to 15-dark; Mouthfeel: 0-gritty to 15-smooth; Sweetness: 0-bland to 15-sweet; Sourness: 0-weak to 15-strong; General Acceptability: 0-unacceptable to 15-Highly acceptable

non-fermenting with limited activities due to the low water activity (Troller and Christian, 1978) of the powdered product and thus, do not produce fermentation by-products which may contribute to the overall aroma and mouthfeel. The primary aroma of the passion fruit juice can be attributed to the volatile compounds ethyl butanoate and ethyl hexanoate, which shows the highest odoriferous importance for organic passion fruit (Janzantti and Monteiro, 2014).

3.5.2 Color

This sensory attribute aimed to test for the lightness or darkness of the two samples. The means scores of the two treatments were significantly different from each other with the probiotic-supplemented product having a higher mean score, indicating that it is darker in color. The incorporation of the spray dried microbial cells made the color of the juice darker and opaquer which could be attributed to the added solids. The image of the juice samples applied with and without spray dried microbial cells could be seen in Figure 1.



(A) (B)

Figure 1. Color comparison of the two treatments. (A) powdered juice product; (B) prepared passion fruit juice. Per image: (left) preparation with *L. plantarum* S20; (right) preparation without *L. plantarum* S20.

3.5.3 Sweetness

Based on the results displayed in Table 5, the mean scores of the two treatments were significantly different from each other. The treatment incorporated with spray dried *L. plantarum* S20 cells turned out to be sweeter than the treatment without the spray dried cells. Maltodextrin was the encapsulating agent used during spray drying. Maltodextrin has a very low sweetness level graded as 0.05 times that of sucrose (Ashurst, 2016). Although it does not practically add sweetness to the product, maltodextrin offers a "bodying effect" or the apparent increase in the viscosity of the resulting juice drink (Kearsley and Dziedzic, 1995). For solutions with suprathreshold levels of sugar, it was observed that the more viscous solutions were perceived as sweeter (Rao, 2007).

3.5.4 Sourness

The two treatments were significantly different in terms of sourness. The judges perceived the treatment without added spray dried *L. plantarum* S20 cells to be sourer than the treatment added with spray dried cells. The addition of maltodextrin could have made the treatment added with spray dried cells sweeter, thus, masking their perception of the sourness.

3.5.5 General acceptability

The general acceptability accounts for the overall liking of the judges of the samples. Based on the results listed in Table 5, there was no significant difference in the general acceptability mean scores of the two treatments. Hence, the juice sample added with spray dried *L. plantarum* cells is comparable to regular passion fruit juice. Both treatments have mean scores that are closer to the right end of the scale, indicating that the passion fruit juice is acceptable by the judges.

3.5.6 Correlation of sensory attributes to the general acceptability

The attribute that greatly contributed to the final product's general acceptability was tested using the correlation analysis. The sweetness attribute had the highest correlation coefficient among other sensory attributes, followed by color, aroma, and sourness, respectively, and has the significant effect on the general acceptability, as shown by a P-value of 0.034. Mouthfeel, on the other hand, has a negative correlation with general acceptability, indicating that the panelists perceived the juice drink with increased smoothness as less acceptable compared to the juice drink with perceived texture which may be contributed by suspended particles in the preparation. The correlation coefficient and corresponding p-values of the sensory attributes compared to the general acceptability are presented in Table 6.

Table 6. Correlation of sensory attributes to general acceptability

Sensory Attribute Compared to General Acceptability	Pearson's Correlation Coefficient[1]	P-Value[2]
Color	0.1917	0.182
Aroma	0.176	0.221
Mouthfeel	-0.0581	0.689
Sweetness	0.3005	0.034
Sourness	0.1659	0.25

[1]N = 50 observations

[2]Level of significance is tested at P = 0.05.

4. Conclusion

This study aimed to produce a probiotic-supplemented passion fruit juice powder and test the stability of *L. plantarum* S20 in the juice powder. Low-temperature spray drying with maltodextrin as carrier

resulted in powdered passion fruit juice and *L. plantarum* S20 powder with recovery of 42.97% and 21.17%, respectively. Spray drying of probiotic culture also resulted in 42.68% log survivability. The formulated juice powder had a final moisture content of 1.729±0.38% and water activity of 0.398±0.0051, and with recommended dilution with water, had a final pH and total soluble solids of 3.40±0.10 and 12.00±0.00° Brix, respectively. The stability of *L. plantarum* in the final juice powder was evaluated at different storage temperatures (4°C, 25°C and 37°C) for four weeks. Among the treatments, only the sample placed in 4°C yielded the highest probiotic stability, maintaining a viable log count of 4.27 per g, while storage at 37°C showed no microbial growth. Sensory evaluation of probiotic-supplemented passion fruit juice against a non-probiotic-supplemented one revealed significant difference in terms of color, sweetness, and sourness, while no significant difference was observed in terms of aroma, mouthfeel, and general acceptability. For future research, it is highly recommended that pre-treatments like thermal shock be applied to the cells to maintain high cell viability. The combination of two types of probiotic cultures exhibiting synergistic effects could also be done to increase cell counts even after spray drying. During storage of the probiotic powders, the absence of light could be tested to determine whether higher cell counts would be attained. Moreover, addition of other encapsulating agents like trehalose and cornstarch could be used to test whether a higher spray drying yield and cell viability could be achieved.

Conflict of Interest

The authors declare no conflict of interest.

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