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Research Article

Microbial Fermentation of Banana Pseudo-Stem Core Juice Enriched with Honey and Whey for Quality and Nutritional Improvement

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ABSTRACT

An experiment was under taken to improve the nutritional quality of banana pseudo-stem core juice by enriched with honey (5%) and liquid whey (10%) through yeast (*Saccharomyces ellipsoideus*, NCIM-3200) and lactic acid bacteria (*Lactobacillus plantarum*, MTCC 6161) fermentation. The results of the study indicated that the pseudo-stem core juice enriched with 5% honey fermented by yeast showed more reduction in terms of pH (4.12), total soluble solids (5.33° Brix) and total sugar (7.16%) with highest alcohol production (7.05%) and sensory score value compared to other treatments. Similarly, pseudo-stem core juice enriched with 10% whey fermented by lactic acid bacteria showed more reduction in pH (3.61), total soluble solids (12.98°Brix) with highest titrable acidity (0.76%), lactic acid bacterial population (7.45 log cfu/ml) and sensory score value compared to other treatments. The results clearly indicated that the microbial fermentation of pseudo-stem core juice enriched with honey and whey improves the quality and nutrition with respect to chemical composition and acceptability for consumption.

Keywords: Banana pseudo-stem core juice; Whey; Honey; *Saccharomyces*; *Lactobacillus plantarum* ellipsoideus

INTRODUCTION

Banana crop produces large quantity of biomass (12 tonnes/ha) apart from fruit, e.g. pseudo-stem, leaves, suckers. These are considered to be absolute waste after the harvest of fruit. Farmers are spending about 8000 to 10000 Rupees/hectare for the disposal of pseudo-stem. Pseudo-stem is trunk of banana plant made of tightly packed overlapping leaf sheath. It consists of outer non-edible hard and soft fiber and inner edible fibrous stalk known as centre core. Banana pseudo-stem core is rich in fiber, potassium and vitamin B6 which help in treating ulcers. It is also used in development of a sport drink and provides many health benefits such as preventing kidney stones, treating diarrhea, dysentery, diabetes, pain and snakebite and also found to possess antioxidant activity [1].

Lactic acid fermentation is one of the oldest methods of preserving fruits and vegetables which contributes desirable physical and flavour characteristics. There are several reports on alcoholic and non-alcoholic beverages or wine preparation from different fruits like apple, plum, apricot, pomegranate, strawberry, guava, jamun, sapota, litchi, amla, orange, carambola etc. Shriniketan studied on optimization of different fermentation factors for the development of quality fermented beverages from banana pseudo-stem core juice using yeast and lactic acid bacteria. Ravi et al. developed value-added plantain stem juice enriched with grape juice which had enhanced nutritive value and increased viscosity, pH and sugar components compared to standard juices. Bornare and Khan developed blended RTS beverage from banana pseudo-stem juice and papaya with increased sensory quality and a shelf life of 90 days.

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Honey with fruits and vegetables gained lot of importance with respect to nutrition and health point of view. Hence, 5 per cent of honey is explored by blending with center core juice in the present study. Similarly, whey is one of the important nutritious by-products obtained from the dairy industry milk processing waste composed of lactose (5%), water (93%), proteins (0.85%), minerals (0.53%) and a minimum amount of fat (0.36%) as reported by Pescuma et al. Shukla et al. reported on possibility of utilizing the milk whey in the fruit beverage preparation. Hence, this can be explored by blending with pseudo-stem juice for the nutritional improvement of fermented banana centre core juice beverage [2].

MATERIALS AND METHODS

Collection of banana pseudo-stem

Collected banana pseudo-stem required for the experiment from the department of horticulture, university of agricultural sciences, GKVK, Bengaluru. The edible inner central core of pseudo-stem was selected for study.

Preparation of pseudo-stem core juice

The procedure for the preparation of banana pseudo-stem core juice was followed as per Bornare and Khan [3]. The selected core was cut into pieces and grounded in grinder/mixer to get pulp. Juice was extracted. Total Soluble Solid (TSS) was adjusted to 20°Brix by adding sugar. Juice was pasteurized at 72°C for 15 seconds. The experiment set up consisted of 9 treatments with 3 replications under CRD design (Table 1) [4].

Table 1: Treatment details.

	Treatments
Yeast fermentation	T1: Pseudo Stem Core Juice (PSCJ) control
	T2: PSCJ+*Saccharomyces cerevisiae* var. ellipsoideus
	T3: PSCJ+5% honey+*Saccharomyces cerevisiae* var. ellipsoideus
	T4: PSCJ+10% whey+*Saccharomyces cerevisiae* var. ellipsoideus
	T5: PSCJ+5% honey+10% whey+*S. cerevisiae* var. ellipsoideus
LAB fermentation	T6: PSCJ+LAB (*Lactobacillus plantarum*)
	T7: PSCJ+5% honey+LAB (*Lactobacillus plantarum*)
	T8: PSCJ+10% whey+LAB (*Lactobacillus plantarum*)
	T9: PSCJ+5% honey+10% whey+LAB (*Lactobacillus plantarum*)

Preparation of yeast and LAB starter culturesss

The purified and authenticated yeast culture *Saccharomyces cerevisiae* var. ellipsoideus (NCIM-3200) was maintained on yeast extract peptone dextrose agar media. A loopful of culture was inoculated into sterilized pseudo stem core juice and incubated at 28°C. After incubation, when yeast population reached upto 106 cfu/ml, it was used as a starter culture for the experiment [5].

Similarly, the purified and authenticated Lactic Acid Bacteria (LAB) *viz., Lactobacillus plantarum* (MTCC-6161) was maintained on MRS agar media. A loopful of culture was inoculated into sterilized pseudo stem core juice and incubated at 35°C. After incubation, when LAB population reached upto 106 cfu/ml, it was used as a starter culture for the experiment.

Biochemical analysis

The biochemical analysis of the fermented blended pseudo-stem core juice by yeast and lactic acid bacteria as influenced by blending with honey and whey were analyzed as per the standard procedures for pH, total soluble solids, titrable acidity, total sugars, vitamin C and alcohol estimation [6].

The pH of the yeast and LAB fermented blended pseudo-stem core juice sample was analyzed using digital pH meter (digital pH meter type MK-VI). Standard buffer solutions of pH 4.0, 7.0 and 9.2 were used to calibrate the instrument.

Total Soluble Solids (TSS) of the fermented samples of different treatments were measured with the help of "ERMA" hand refract meter having a range of 0 to 35°Brix at room temperature. One drop of each sample was placed on the prism of refractometer and noted the value coinciding the shadow. Titrable acidity of the samples was determined as per the procedure followed by Srivastava and Kumar. Alcohol content was estimated calorimetrically [7].

Microbial analysis

The assessment of microbial population of the fermented samples was done by employing dilution plating method and the results were expressed in terms of logarithms of colony forming units/ml of the sample (log cfu/ml).

Sensory analysis

The developed fermented beverages from core juice were evaluated by selected five panel members. He donic scale of 20 scores was considered to evaluate the product based on the appearance, color, aroma, taste and acceptability. The data obtained from the experiments were subjected to statistical analysis to evaluate treatment effects [8].

Statistical analysis

Analysis was carried out by completely randomized design using WASP-1 tool. Critical difference values were used to locate significant mean difference.

RESULTS AND DISCUSSION

Biochemical quality

The results pertaining to changes in pH, TSS, titrable acidity of yeast and LAB fermented pseudo-stem core juice as influenced by honey and whey enrichment are presented in Table 2 [9].

Table 2: Treatment details.

Treatments		pH	TSS (°Brix)	Titrable acidity (%)
Yeast fermentation	T1-PSCJ (control)	5.22^{a}	20.00^{a}	0.05^{f}
	T2-PSCJ+yeast	4.12^{b}	6.97^{ef}	0.36^{d}
	T3-PSCJ+honey (5 ml) +yeast	4.12^{b}	5.33^{ef}	0.31^{e}
	T4-PSCJ+whey (10 ml) +yeast	4.22^{b}	7.10^{ef}	0.34^{dc}
	T5-PSCJ+honey (5 ml) +whey (10 ml)+yeast	4.20^{b}	7.40^{ef}	0.37^{d}
LAB fermentation	T6-PSCJ+LAB	4.08^{b}	14.77^{ef}	0.37^{d}
	T7-PSCJ+honey (5 ml) +LAB	3.53^{b}	15.90^{ef}	0.68^{d}
	T8-PSCJ+whey (10 ml) +LAB	3.61^{c}	12.98^{ef}	0.76^{a}
	T9-PSCJ+honey (5 ml) +whey (10 ml)+LAB	3.46^{c}	14.47^{ef}	0.96^{a}

- Initial TSS maintained: Yeast fermentation-20°Brix, LAB fermentation-16°Brix.
- Yeast- var. ellipsoideus (NCIM-3200); LAB- (MTCC6161).
- Values are presented as mean.
- The same lowercase letters within a column are not significantly different at p<0:05.

pH

After 6 days of incubation, yeast fermented beverages showed reduction in pH. However, there was no significant difference between the treatment with respect to change in pH. Similar results of reduction in pH were also reported by Maragatham and Panneer Selvam in papaya wine due to blending. Similarly, LAB fermentation of enriched or non-enriched pseudo-stem core juice also showed reduction in pH that ranged between 3.46 and 4.08. The variation in pH among different treatments with yeast and LAB fermentation might be due to change in the ability of microorganism to convert sugar into alcohol or/and acids [10].

Reduction of pH in both yeast and lactic acid fermentation is a clear indication of utilization of sugars. The reduction in pH was found to be more in lactic acid bacteria fermented juices compared yeast fermented juices. This may be due to the efficient consumption of blended substrates and production of acids during LAB fermentation. These results support the work of. In beet juice fermentation by *Lactobacillus plantarum* resulted in decrease of pH from 6.3 to 4.1 after 3 days of fermentation. Similarly, Sabokbar and Khodaiyan reported the reduction in pH from 4.23 to 3.40 in LAB fermented beverage of pomegranate juice when blended with whey [11].

TSS

The initial TSS content of enriched pseudo-stem core juice for yeasts and lactic acid fermentation were 20°Brix and 16°Brix respectively. Yeast fermentation of pseudo-stem core juice had resulted in reduction of TSS content that varied from 5.33 to 7.40°Brix between the treatments. The highest reduction

(5.33°Brix) was observed in the pseudo-stem core juice enriched with honey (T3). Result indicated that addition of honey has influenced the efficiency of yeast in utilization of sugar. This finding is similar to the result reported by Chaudhary et al. in yeast fermented grape and jamun blended juice and Ribeiro et al. in sugarcane and pineapple fermented blend juice. LAB fermentation of enriched and non-enriched pseudo-stem core juice had resulted in reduction of TSS content that varied from 12.98 to 15.90°Brix between treatments. The highest reduction was observed in whey enriched (12.98°Brix) fermented juice (T8) followed by non-enriched treatment (T6) recorded with 14.77° Brix. Least reduction was observed in the treatment with honey blending (T7). This indicated that addition of whey had influenced the efficiency of LAB in utilization of sugar. The reduction of TSS is a clear indication of consumption of sugar by yeasts and lactic acid bacteria. Variation in TSS reduction between yeasts and lactic acid bacteria may be due to change in sugar conversion and fermentation efficiency. Results revealed that yeasts are more efficient in sugar conversion than LAB. This finding supports the results of Priya et al. in tomato fermented juice [12].

Titrable acidity

After 6 days of yeasts and lactic acid bacterial fermentation of enriched pseudo-stem core juice results in significant increase in titrable acidity from 0.05 to 0.96 per cent between the treatments. Among yeasts fermented beverage, both honey and whey enriched pseudo-stem core juice (T5) showed highest titrable acidity (0.37%). The findings are similar to results of study conducted in blended Kinnow fermented beverage. Lactic acid bacteria fermented pseudo-stem core juice blended with honey and whey (T9) showed maximum titrable acidity (0.96%). There was a significant difference in titrable acidity between enriched and non-enriched LAB fermented beverages. These findings support the work of Sasi Kumar reported in fermented whey blended aloe vera juice with increase in acidity up to 0.525%. Similarly, in the study conducted by Shukla M, et al. There was increase in titrable acidity (0.92%) in the fermented whey and pineapple blended beverage developed a probiotic cabbage juice using lactic acid bacteria resulted in increase in amount of titrable acidity which was expressed as lactic acid. The results clearly indicated that pseudo-stem core juice enriched with honey and whey by yeast and lactic acid bacteria fermentation significantly enhanced the titrable acidity of the pseudo-stem core juice. Increase in titrable acidity of fermented beverages might be to the production organic acid (lactic acid) during fermentation. Acids present in fermented juices had added up sourness and acidity taste.

Results pertaining to variation in total sugar and alcohol production in the yeast and LAB fermented pseudo-stem core juice as influenced by honey and whey enrichment is shown in Figure 1.

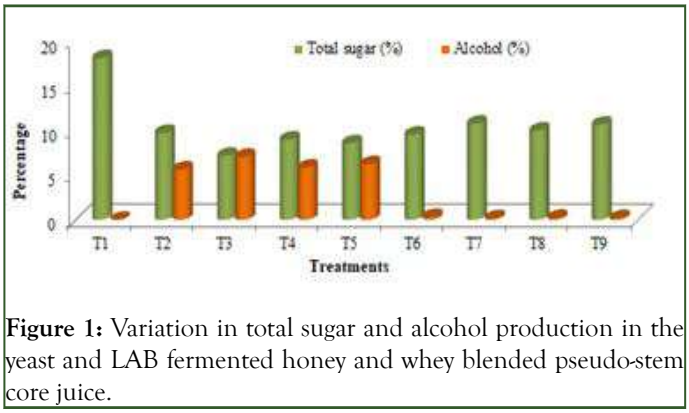

Figure 1: Variation in total sugar and alcohol production in the yeast and LAB fermented honey and whey blended pseudo-stem core juice.

T1-PSCJ (control)

T2-PSCJ+yeast

T3-PSCJ+honey (ml)+yeast

T4-PSCJ+whey (10 ml)+yeast

T5-PSCJ+honey (5 ml)+whey (10 ml)+yeast

T6-PSCJ+LAB

T7-PSCJ+honey (5 ml)+LAB

T8-PSCJ+whey (10 ml)+LAB

T9-PSCJ+honey (5 ml)+whey (10 ml)+LAB

Total sugar

The initial total sugar content of enriched pseudo-stem core juice was 18.09%. The results revealed that the total sugar content significantly reduced to 7.16 and 10.76 per cent after 6 days of fermentation by yeast and lactic acid bacteria respectively. Yeast fermentation of pseudo-stem core juice enriched with honey (T3) showed lowest total sugar content (7.16%) and showed significant difference from non-enriched (9.72%) beverage and enriched (9.45%) beverages. This indicates that fermentation efficiency of yeast has been influenced by honey blending which support the work reported that total sugar and reducing sugar of plum wine ameliorated with honey was 1.24% and 0.32% respectively. The decreasing trend of total sugar is also reported in papaya fermented beverage.

Alcohol

The yeast fermentation of enriched pseudo-stem core juices results in alcohol production ranged from 5.62% to 7.05% between treatments. The fermentation of pseudo-stem core juice enriched with honey (T3) showed highest alcohol content (7.05%) followed by combined blending with honey and whey (T5) recording 6.23 percent. The results indicated that addition of honey influence on enhancing alcohol content. Added advantage was with addition of 5% honey which produced highest alcohol (7.05%). Yeast fermentation with increased fermentative activity and level of sugar utilization produced maximum alcohol. The addition of 5 percent honey which produced maximum alcohol (7.05%), this may be due to higher release of sugar from carbohydrates of honey by enzymatic

activities of yeast. Fermented grape and jamun juice blend but similar to the result reported in beverage of sugarcane and pineapple beverage. LAB fermentation results in negligible amount of alcohol production were seen which ranged between 0.17% and 0.32%. The inoculums *Lactobacillus plantarum* is a hetero fermentative. Hence, alcohol has been produced by them as a primary metabolite other than lactic acid. Results pertaining to effect of yeast and LAB fermentation of pseudo-stem core juice enriched with honey and whey on vitamin C is presented in Table 3.

Table 3: Effect of supplementation of honey and whey to pseudo-stem core juices during fermentation on vitamin C content.

Treatments		Vitamin C (mg/100 ml)
Yeast fermentation	T1-PSCJ (control)	0.11e
	T2-PSCJ+yeast	0.14d
	T3-PSCJ+honey(5 ml)+yeast	0.15d
	T4-PSCJ+whey (10 ml)+yeast	0.17c
	T5-PSCJ+honey (5 ml)+whey (10 ml)+yeast	0.18c
LAB fermentation	T6-PSCJ+LAB	0.35b
	T7-PSCJ+honey(5 ml)+LAB	0.34b
	T8- PSCJ+whey(10 ml)+LAB	0.37a
	T9- PSCJ+honey (5 ml)+whey (10 ml)+LAB	0.38a

- Initial TSS maintained: Yeast fermentation-20°Brix, LAB fermentation-16°Brix.
- Yeast- var. ellipsoideus (NCIM-3200); LAB- (MTCC-6161).
- Values are presented as mean.
- The same lowercase letters within a column are not significantly different at p<0:05.

Vitamin C

The initial vitamin C content of pseudo-stem core juice was 0.11 mg/100 ml. The yeast fermented pseudo-stem core juice enriched with honey and whey (T5) showed highest vitamin C content (0.18 mg/100 ml) compared to non-enriched beverages (0.14 mg/100 ml) which are significantly differ indicating addition of honey and whey very much influenced on vitamin C content enhancement. Similarly, LAB fermentation of pseudo-stem core juice enriched with honey and whey (T9) showed highest vitamin C content (0.38 mg/100 ml) compared to non-enriched beverage T6 (0.35 mg/100 ml) and honey enriched treatment T7 (0.34 mg/100 ml) which significantly differ indicating that addition of whey influenced the enhancement of vitamin C content. The results clearly indicated that addition of whey to pseudo-stem core juice and fermented by yeast and bacteria significantly enhanced the vitamin C content, whereas honey did not influence on vitamin C content.

Microbiological quality

Results pertaining to effect of yeast and LAB fermentation of pseudo-stem core juice enriched with honey and whey on yeast and LAB counts are presented in Figure 2.

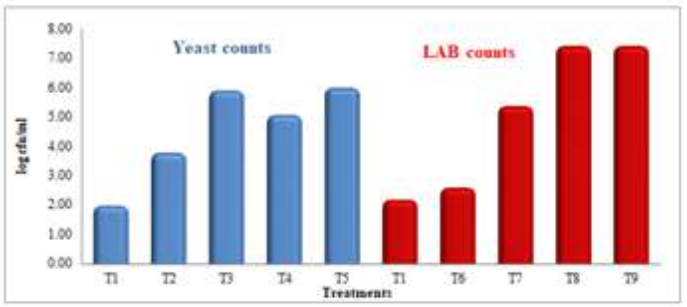

Figure 2: Yeast and bacterial population in the pseudo-stem core juice as influenced by blending with honey and whey fermented by yeast and lactic acid bacteria.

T1-PSCJ (control)

T2-PSCJ+yeast

T3-PSCJ+honey (5 ml)+yeast

T4-PSCJ+whey (10 ml)+yeast

T5-PSCJ+honey (5 ml)+whey (10 ml)+yeast

T6-PSCJ+LAB

T7-PSCJ+honey (5 ml)+LAB

T8-PSCJ+whey (10 ml)+LAB

T9-PSCJ+honey (5 ml)+whey (10 ml)+LAB

Microbial population of yeast and lactic acid bacteria

After 6 days of yeast and LAB fermentation of pseudo-stem core juice enriched with honey and whey fermented beverage samples were assessed for yeast and lactic acid bacterial population. Results showed that yeast population in fermented beverage was low compared to lactic acid bacterial population. Pseudo-stem core juice when enriched with honey alone (T3) as well as in combination with whey (T5) resulted in maximum yeast counts 5.93 log cfu/ml and 6.02 log cfu/ml respectively. This indicated that honey and whey influenced the fermentative activity of yeast in pseudo stem core juice. Similarly, Chaudhary et al. reported on increased yeast in fermented grape and jamun blended beverage. LAB fermentation of pseudo-stem core juice enriched with 10 ml whey (T8) recorded highest population of 7.45 log cfu/ml which is less than the population recorded in fermented mixed juice of watermelon and tomato beverage juice as reported by Sivudu et al. Results clearly showed the influence of whey on LAB population which supports the work of Shukla et al. pineapple enriched with whey fermented by LAB which recorded higher population (8.38×10^8 cfu/ml) after 24 hrs of fermentation.

Sensory quality

Table 4 shows the changes in sensory attributes of yeast and LAB fermented pseudo-stem core juice enriched with honey and whey with respect to overall acceptability. The sensory score value with respect to overall acceptability for yeast and LAB fermented beverages as influenced by honey and whey were in the range between 10.25-16.5/20.0 between treatments. The highest score was obtained by LAB fermented beverage enriched with whey (T8) with the score 16.50. The organoleptic evaluation of yeast fermented product of pseudo-stem core juice enriched with honey and LAB fermented product enriched with whey showed highly acceptability and acceptance for consumption. Higher values with respect to overall acceptability may be due to yeast and LAB fermentation and blending with honey may be helping in acidity modulation and also alcohol production for higher acceptability for consumption. These finding are similar to the result reported by Prathima et al. In blended Kinnow fermented beverage and in Kokum juice blended with honey as reported by Latha et al. The results of the sensory evaluation of yeast and LAB fermented honey and whey enriched pseudo-stem core juices clearly indicated that pseudo-stem core juice enriched with honey fermented by yeast (T3) showed highest score with all sensory attributes and overall acceptability (16.0/20.0) for consumption. Similarly, pseudo-stem core juice enriched with whey fermented by LAB (T8) showed highest score with all sensory attributes and overall acceptability (16.5/20.0) for consumption.

Table 4: Sensory attributes of yeast and LAB fermented pseudo-stem core juice as influenced by blending with honey and whey.

Treatments		Appearance (2)	Colour (2)	Aroma (2)	Bouquet (2)	Acidity (2)	Sweetness (2)	Body (2)	Astringency (2)	Flavour (2)	Quality (2)	Overall acceptability (20)
Yeast fermentation	T1	1	1	1	1	1	1.25	1	1	1	1	10.25
	T2	1.5	1.5	1.5	1.25	1	1.25	1.25	1.25	1.25	1.25	13
	T3	1.75	1.75	1.75	1.5	1.5	1.5	1.5	1.5	1.75	1.5	16
	T4	1.25	1.25	1	1	1	1	1.5	1	1	1.25	11.75
	T5	1.25	1.25	1.5	1.25	1.25	1.25	1.5	1.25	1.5	1.25	13.5
LAB fermentation	T6	1.25	1.25	1	1	1.25	1.5	1.25	1.25	1.25	1	12
	T7	1.25	1.25	1.5	1.5	1.5	1.75	1.25	1	1.5	1.25	13.75
	T8	1.75	1.75	1.75	1.5	1.75	1.5	1.75	1.5	1.75	1.5	16.5
	T9	1.5	1.5	1.5	1.25	1.5	1.5	1.25	1.5	1.5	1.25	14.25

T1-PSCJ (control)

T2-PSCJ+yeast

T3-PSCJ+honey (5 ml)+yeast

T4-PSCJ+whey (10 ml)+yeast

T5-PSCJ+honey (5 ml)+whey (10 ml)+yeast

T6-PSCJ+LAB

T7-PSCJ+honey (5ml)+LAB

T8-PSCJ+whey (10 ml)+LAB

T9-PSCJ+honey (5 ml)+whey (10 ml)+LAB

- Initial TSS maintained: Yeast fermentation-20°Brix, LAB fermentation-16°Brix.
- Yeast- var. ellipsoideus (NCIM-3200); LAB- (MTCC-6161).

CONCLUSION

The initial vitamin C content of pseudo-stem core juice was 0.11 mg/100 ml. The yeast fermented pseudo-stem core juice enriched with honey and whey (T5) showed highest vitamin C content (0.18 mg/100 ml) compared to non-enriched beverages (0.14 mg/100 ml) which are significantly differ indicating addition of honey and whey very much influenced on vitamin C content enhancement. Similarly, LAB fermentation of pseudo-stem core juice enriched with honey and whey (T9) showed highest vitamin C content (0.38 mg/100 ml) compared to non-enriched beverage T6 (0.35 mg/100 ml) and honey enriched treatment T7 (0.34 mg/100 ml) which significantly differ indicating that addition of whey influenced the enhancement of vitamin C content. The results clearly indicated that addition of whey to pseudo-stem core juice and fermented by yeast and bacteria significantly enhanced the vitamin C content, whereas honey did not influence on vitamin C content.

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