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Studies on the Lactic-fermentation of Sugar Apple (*Annona squamosa* L.) Puree

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ABSTRACT

Sugar apple (*Annona squamosa* L.) is a fruit tree of economic importance in Taiwan, with the fruit primarily consumed fresh. Overproduction in recent years, coupled with short shelf life and other practical issues, make practical the research into uses for sugar apples in formats other than fresh. This study presents an exploration of lactic-fermented sugar apple products. The product has a unique sweet aroma and a test panel found its texture to be appealing. For this study, sugar apple puree was used as the substrate for fermentation using mixed starters in a ratio 1:1 or 1:1:1. The high total soluble solid nature (20.5 ± 4.33 °Brix) of sugar apple puree as compared to that of mixed fruit juice (lower than 10 °Brix) indicates its potential to be used in fermentation. Following fermentation, the properties and effects of different starter inoculations were recorded and discussed. Fermentation achieved a pH value of 3.8 after 60 hr. The performance of fermented product in the DPPH (α,α-diphenyl-β- picrylhydrazyl) decreased from 92% to 78% after 48 hr, followed by a stationary state. Fermented sugar apple puree and fresh sugar apple juice blended in a ratio of 2:8 delivered the highest DPPH scavenging efficiency (88%) and iron chelating ability (49%). The relatively high values of these properties offer the potential for sugar apple juice to be further developed as a novel functional food. Such a development would surely help to ease recent overproduction problems.

Key words: Annonaceae, DPPH scavenging, *Lactobacillus*, fermentation

INTRODUCTION

Sugar apple (*Annona squamosa* L.), a member of the Annonaceae family, is a tropical and subtropical fruit tree that is widely distributed in Asia, Africa and the Americas[(1)]. The sugar apple is an economically important fruit tree in Taiwan, especially along the east coast. The 5,600 hectares cultivated in sugar apple in Taiwan makes the island the largest commercial producer, followed by Spain, Australia and Peru[(2)]. Taiwan's sugar apple crop, approximately 55,000 metric tons (mt) in 2002, implies a potential export value of around US $58 million per year[(3)].

Due to its high respiration rate, sugar apples have an extremely limited post-harvest shelf life, making handling, storage and distribution key issues for growers[(4)] and impeding the development of export sales. Compounding sales channel issues, Taiwan has increasingly been over producing sugar apples[(3)]. Therefore, developing new processing techniques to overcome short shelf-life problems would both open new business opportunities for Taiwan sugar apples and help ease supply pressures. In recent decades, *Lactobacillus* (L.)-led fermentations have been employed widely in food production, with evidence demonstrating its functionality in promoting gastro-intestinal health[(5,6,7,8)]. Fermented fruit products have also been identified in previous studies as having antioxidant properties[(5,6,9)].

In this study, a new fermented sugar apple product prototype was studied and its chemical changes, fermentation condition and antioxidant characteristics were investigated. This study is hoped to contribute substantively toward solving the problem of sugar apple overproduction in Taiwan.

MATERIALS AND METHODS

Ripe sugar apples (*Annona squamosa* L.), freshly detached from the tree in the early morning, were provided by the Taitung County Bureau of Agriculture. Immediately after arrival at the laboratory, samples were processed and deseeded by hand using sterile plastic examination gloves. *L. delbrueckii* subsp. *lactis* ATCC 7830, *L. paracasei* subsp. *paracasei* ATCC 25598 and *L. casei* subsp. *casei* ATCC 393 were purchased from American Type Culture Collection, USA.

I. *Preparation of the Sugar Apple Fermentation Substrate*

Fifty milliliter of sterile water was added to 1,500 g of deseeded sugar apples. The solution was then blended into a puree.

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II. *Fermentation of Sugar Apple Puree*

L. delbrueckii subsp. *lactis* ATCC 7830 (A), *L. paracasei* subsp. *paracasei* ATCC 25598 (B) and *L. casei* subsp. *casei* ATCC 393 (C) were selected as fermentation process starters based on the previous experience of Chen (2002)[9]. The starters were cultured in MRS broth for 24 hr at 37°C and reached an OD_{600} 1.0. They were either inoculated singly or co-inoculated using A+B or A+C or B+C in a 1:1 ratio or A+B+C in a 1:1:1 ratio, with 5 mL inoculated into 150 mL of sugar apple puree. The fermentation process was carried at 37°C for 70 hr as suggested by Chen *et al.*[6]. Fermented sugar apple puree with viable bacterial count (VBC) greater than 10^4 CFU mL^{-1} was heated for 90°C for 1 min.

III. *Determination of Total Soluble Solids (TSS)*

Fermented sugar apple puree was filtered with an 11 cm diameter Whatman #1 filter (Post Apple Scientific, Inc., USA). TSS was determined using a Hand Refractometer (ATAGO, Japan) and data was collected in triplicate. The recorded data were present as °Brix.

IV. *Titratable Acid Determination*

Following the analysis methodology recommended by AOAC[10], we found malic acid to be the major titratable component of sugar apple acids[11]. Fermented puree was filtered with a Whatman #1 filter, and 20 g of filtered juice was titrated using 0.1 M NaOH at room temperature to a pH value of 8.1.

V. *Sugar Determination*

Fermented puree was centrifuged under 7,500 ×g at 4°C for 20 min, the supernatant was then filtered with a 0.45 μm pore size membrane (Millipore, USA) and injected into the HPLC (Intelligent HPLC System LC-800 series, JASCO, Japan) using mobile phase CH_3CN/H_2O (85/15) with injection volume 10 μL. The HPLC was equipped with a system controller (model 801-SC); pump (model PU-980); column: LichroCart RP18, 125 × 4 mm, 5 μm, Merck, Germany; column oven: Model TU-100; and detector: UV Detector, model 870-UV, JASCO, Japan.

VI. *pH Determination*

Fermented puree was filtered with a Whatman #1 filter and measured by a pH Vision 6071 microcomputer (Jenco Electronics Ltd., Taiwan, ROC).

VII. *Antioxidant Ability*

Differing ratios (7:3, 6:4, 5:5; 4:6, 3:7 and 2:8) of fermented sugar apple puree to fresh sugar apple juice were used to gauge antioxidant ability and iron chelating effect. The DPPH scavenging ability of fermented sugar apple juice was determined by adding 0.5 mM DPPH in methanol to 2 mL of fermented sugar apple juice, which was stirred well and incubated in the dark for 30 min. A spectrophotometer (Hitachi U-2000, Japan) was used and the absorbance determined to be 517 nm. The scavenging effect was calculated as follows:

Scavenging effect (%) = [1–(absorbance at 517 nm)/absorbance at 517 nm of sample without adding fermented sugar apple juice]×100

VIII. *Iron Chelating Effect*

Fermented samples (1.0 mL) were added to 3.7 mL of methanol, 0.1 mL of 2 mM $FeCl_2$, after 30 sec, 0.2 mL of 5 mM Ferrozine was added and reacted for 10 min. Spectrophotometer (Hitachi U-2000, Japan) was used to determine the absorption at 562 nm. The iron chelating effect was calculated using the following formula:

Iron chelating effect (%) = [1–(absorbance at 562 nm)/absorbance at 562 nm of sample without adding fermented sugar apple juice]×100

IX. *Sensory Testing for Fermented Sugar Apple Product Acceptance*

Fermented sugar apple puree was mixed with fresh sugar apple juice in various ratios and then evaluated by 50 students from the Department of Horticulture, National Taiwan University. They were requested to rate the solutions in terms of color, aroma, sweetness, tartness and overall acceptability along a scale of 1 to 7, with 1 indicating the lowest and 7 indicating the highest levels of acceptance.

RESULTS AND DISCUSSION

I. *The Fermentation Substrate and Its Characteristics*

After washing and deseeding, the sugar apple flesh was blended into a puree. Table 1 outlines the basic characteristics of the sugar apple puree. The viable bacterial

Table 1. The composition of sugar apple puree (mean ± S.D., triplicate)

No.	Measurement	Description
1.	Viable bacteria count (VBC)	4.5×10^3 CFU/mL
2.	Titratable acid (malic acid)	0.25 ± 0.023%
3.	pH value	4.09 ± 0.63
4.	Total solid soluble (TSS)	20.5 ± 4.33 °Brix
5.	Fructose	3.25% f.w.[a]
6.	Sucrose	7.8% f.w.
7.	Glucose	5.12% f.w.

[a]f.w.: fresh weight.

count found in the sugar apple puree was relatively low compared to values obtained for mixed vegetable juice (Chen, 2002). This may be due to the low pH of sugar apple juice (pH 4.0) relative to mixed vegetable juices, which typically have pH values above 4.6 (e.g., carrot juice has an average pH value of 6.0)[9]. Titratable acid was expressed as malic acid equivalents and had a value of 0.25%. This was similar to those reported by Chen and Chen[10] (0.247%), but higher than that reported by Chen[9], who recorded a value of 0.15% for mixed vegetable juice. No heat treatment was necessary to inactivate microbes in the puree prior to fermentation. In TSS, sugar apple puree has a TSS of 20 °Brix, higher than that of both mixed vegetable juice (5 °Brix)[9] and carrot juice (8 °Brix)[12]. Due to the considerably high TSS content in the puree, no further substrate was added for the fermentation. The log phase of *L. delbrueckii* subsp. *lactis* ATCC 7830, *L. paracasei* subsp. *paracasei* ATCC 25598 and *L. casei* ATCC 393 were obtained after 8~15 hr under 37°C (Figure 1). They were added individually, or in different combinations, to fermented sugar apple puree to initiate fermentation.

II. *Mixed Starter Fermentation*

Sugar apple puree was inoculated with single or with 1:1 or 1:1:1 combinations of above-mentioned *Lactobacillus*. The fermentation process was carried out at 37°C and the fermentate was sampled to determine changes occurring at different times throughout the process. Total soluble solid changes in the fermentation are illustrated in Figure 2, which shows that all starter combinations decreased in the total soluble solid from 20.5 °Brix to 15 °Brix within 60 hr. The titratable acid changes showed a fermentation end-point at 60 hr fermentation in 37°C (Figure 3). Starter *L. paracasei* subsp. *paracasei* ATCC 25598 produced the most acid, with more than 1% titratable acid obtained after 60 hr. In pH changes, fermentation started by *L. paracasei* subsp. *paracasei* ATCC 25598 resulted in the lowest pH value at 36 hr with a pH value of 2.8, which escalated to pH 4.0 at 60 hr. Fermentation led by *L. delbrueckii* subsp. *lactis* (ATCC 7830) produces a gradual decrease in pH value, reaching around 3.9 after 30 hr. Fermentation led by all other combinations of *Lactobacillus* rose after 60 hr, reaching pH 3.8 after 60 hr. This observation is similar to that of Chen[9]. With the depletion of the substrate and growth of *Lactobacillus*, metabolite waste accumulation may cause pH levels to increase by the 60 hr marker. *L. paracasei* subsp. *paracasei* ATCC 25598 was found to have a pH value of 3.8 at 70 hr (Figure 4). *Lactobacillus* had plate counts of $8.5 \times 10^8 \sim 9.2 \times 10^9$ CFU mL^{-1} after 12 hr, followed by a stationary phase (data not shown). In terms of growth rates, *L. paracasei* subsp. *paracasei* ATCC 25598 was found to grow the fastest, followed by *L. paracasei* subsp. *paracasei* ATCC 25598 and *L. casei* subsp. *casei* ATCC 393 (combination of B+C) (data not shown).

The DPPH scavenging effect of fermented sugar apple puree started by different starter combinations decreased from 92% to 78% over a 48 hr period, followed by a stationary state. *L. casei* ATCC 393 was observed to have the highest DPPH scavenging effect of the various starter combinations (Figure 5). In order to obtain better fermented product properties, fermentation led by *L. casei* ATCC 393 was further mixed with fresh juice and evaluated based on its DPPH scavenging ability. Results showed that fermented

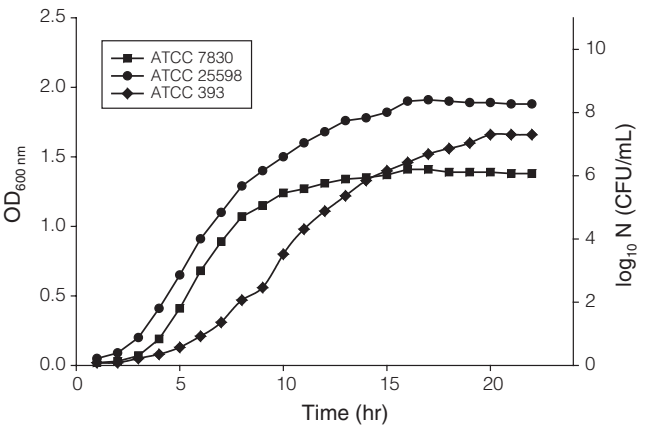

Figure 1. Growth curve for *Lactobacillus* sp. in MRS broth.

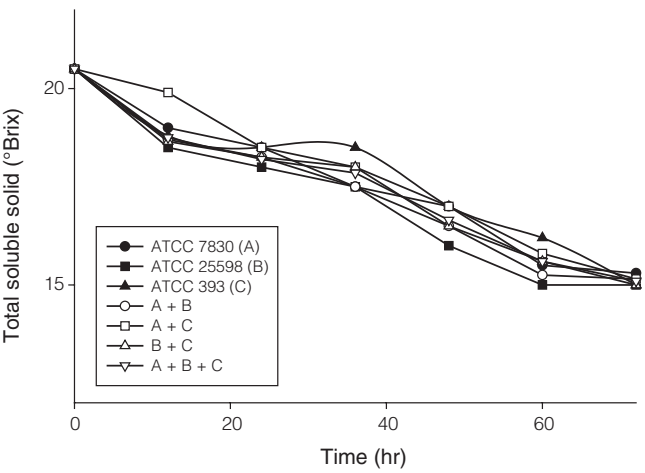

Figure 2. Total soluble solid changes in fermentation by mixed starters.

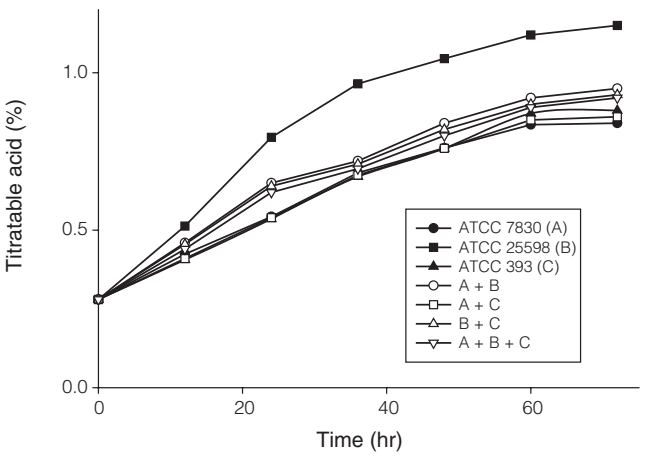

Figure 3. Titratable acid changes of fermentation in mixed starters.

sugar apple puree and fresh sugar apple juice mix in a 2:8 ratio delivered the highest DPPH scavenging efficiency (88%) and iron chelating capacity (49%) (Table 2). Neither fermented-juice-to-fresh-juice ratios of 0:10 or 10:0 gave satisfactory DPPH scavenging and iron chelating effects, demonstrating the synergistic effects realized by combining the 2 juice products. The starter used in this study also was different to those used by Chen (2002)[9], who found *L. casei* subsp. *casei* to be the optimal fermentation starter in carrot juice.

Our fermented product acceptance survey showed that the fermented juice mix with a fresh juice mixture ratio of 3:7 gained the highest in overall performance (Table 3). This may reflect the enhanced sweetness / reduced tartness resulting from the addition of fresh sugar apple juice. This preference test also revealed that most people do not appreciate the texture of fermented sugar apple puree due to its high stone cell content. However, processing the fruit to remove stone cells both reduces the juice's unique aroma and raises production costs. The addition of fresh juice both enhances the functional properties of the product and, most important in terms of processing, contributes to the modification of product texture.

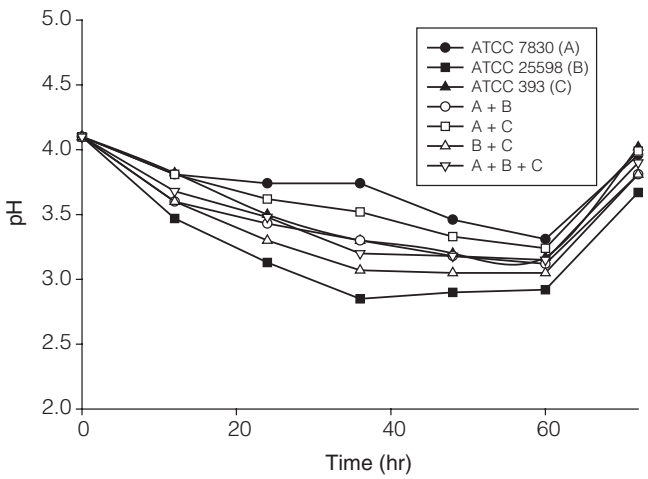

Figure 4. Change in pH value during fermentation by different *Lactobacillus* combination.

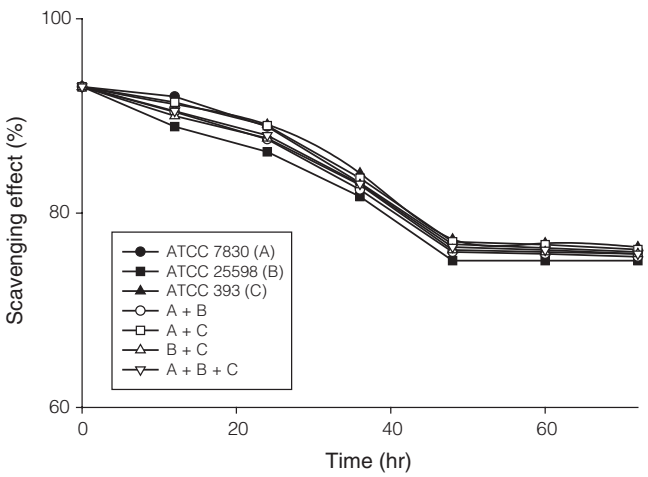

Figure 5. DPPH scavenging effect changes in mixed starters.

Table 2. Antioxidant characteristics and iron chelating effects of fermented sugar apple puree / fresh sugar apple juice mixtures in 6 different ratios (mean ± S.D., triplicate)

	Ratio of fermented sugar apple to fresh sugar apple juice (x:x)	DPPH scavenging ability (%)	Iron chelating ability (%)
I	10:0	79.32 ± 7.20	40.7 ± 3.43
II	7:3	82.09 ± 13.2	40.08 ± 7.34
III	6:4	83.51 ± 7.43	42.16 ± 4.76
IV	5:5	79.0 ± 11.7	43.72 ± 5.8
V	4:6	85.80 ± 6.44	44.76 ± 6.77
VI	3:7	87.74 ± 5.87	46.16 ± 8.09
VII	2:8	88.68 ± 12.04	48.96 ± 11.3
VIII	0:10	77.33 ± 4.75	39.4 ± 7.1

Table 3. Consumption preference data on fermented sugar apple puree / fresh sugar apple juice mixtures of different ratios

	Ratio of fermented/ fresh juice	Color	Aroma	Sweetness	Tartness	Overall acceptance
I	10:0	4.53	4.12	3.12	3.2	3.07
II	7:3	4.60	4.08	3.94	4.84	3.32
III	6:4	4.66	4.16	4.40	4.58	3.42
IV	5:5	4.68	4.72	4.80	4.48	3.58
V	4:6	4.80	4.76	5.16	4.18	4.34
VI	3:7	4.74	5.16	5.38	4.02	4.92
VII	2:8	4.68	4.96	5.48	3.76	4.52
VIII	0:10	4.33	4.83	5.71	3.30	4.38

The high level of antioxidants in the fermented product is a promising characteristic deserving further exploration in terms of sugar apple juice potentially being a functional food. Also, our panel's overall acceptance of the product's general food properties was high. The findings of this study are hoped to suggest a possible new outlet by which sugar apple overproduction and post-harvest problems in Taiwan can be resolved. Other processing methods are worth further study and development now that this basic research into the value of sugar apple products has achieved initial favorable results.

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