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Research Article

Effect of Preprocessing Storage Temperature and Time on the Physicochemical Properties of Winter Melon Juice

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Numerous studies demonstrated that winter melons (*Benincasa hispida*) have a long storage life at 20°C without quality and flavor degradation in fruit. However, fruit for processing are frequently handled under refrigerated conditions or exposed to a warehouse without air conditioning. Therefore, this research aimed to evaluate whether a short high- and low-temperature storage of fruit, prior to processing, changes the flavor and nutritional profiles of winter melon juice. Weight loss of 1.71% was recorded subsequent to 20 days of 10°C storage, with 5.15% weight loss at 30°C. Sugar content significantly decreased during storage at 10°C and 30°C, while the soluble solids content slightly increased. Several specific phenolic compounds were detected, and the total concentration of phenolics increased over the storage time at both temperatures. The concentration of sulfur compounds, as well as hexanal and total volatiles that are principally responsible for off-flavor reduced significantly during storage and the reduction was greater at 10°C than at 30°C. The results indicate that preprocessing fruit storage at 10 or 30°C for 20 days will not harm the quality and flavor of winter melon juice. However, longer storage time caused water-soaked spots at 10°C and dry rot at 30°C.

1. Introduction

Winter melon (*Benincasa hispida* (Thunb.) Cogn; synonym: *Benincasa cerifera* Savi) is a vine crop whose fruit is a gourd with low sugar content (<3%). The fruit has been widely planted in many southeast Asian countries, including China, India, and Thailand [1]. The crop exhibits tremendously high yields, and robust pest and disease resistance [2]. The crop can also be found in south Florida and similar climatic areas of the United States [3]. It is highly suitable for cultivation in Florida, where one acre can yield as much as 18 tons of fruit annually [4].

The mature fruit is reported to be abundant in B1 and C vitamins [4, 5] and possesses high concentrations of dietary fiber [6]. The concentration of dietary fiber in winter melon juice averages 27.5% of the dry weight [7], which contributes to its functional properties. Winter melon juice also possesses high concentrations of organic acids, amino acids (particularly phenolic amino acids, up to $40\,mg{\cdot}L^{-1}$, depending on variety) and minerals [1] as well as nucleosides [7], which contribute to antioxidant activity, anti-inflammatory properties, and reduced memory loss in humans [8, 9]. Additionally, previous research showed that winter melon is a good candidate for low sugar juice, alone or blended, with a sugar content of less than 2%, total soluble solids less than 3%, and is physically stable with little sedimentation [10]. Thus, the crop could be a candidate for the development of low sugar, low calorie food products.

Flavor is understood to be due to a perception of retronasal olfaction and taste, while volatiles contribute to the aromatic sensation [11]. In excess of 15 individual volatile chemicals have been detected in certain varieties of winter melon fruit and its beverages, primarily alcohols and hydrocarbons [1]. The high concentration of sulfur volatile compounds, similar to numerous other vegetables, particularly cucurbits, raises the risk of unpleasant taste in the resulting juice [12].

Winter melons can be stored for more than 4–6 months without quality or flavor deterioration even when kept at 20°C [13, 14]. Cold storage is the primary method for extending the life of fruit and vegetables. Temperatures in cold storage usually range from 0 to 4°C. However, similar to many other chilling sensitive fruit and vegetables [15, 16], winter melons suffer chilling injury when storage temperatures are below 13°C [17, 18]. However, fruit for processing are frequently handled under less ideal conditions, such as a refrigerator or warehouse without air conditioning. The objective of this study was to investigate the changes of juice physicochemical properties, including soluble solids content, sugars, titratable acidity, volatiles, phenolic amino acids, and hydroxycinnamic acids, of the winter melon fruit stored at 10°C and 30°C.

2. Materials and Methods

2.1. Fruit and Juice Processing. "Large Round" winter melon (average weight: 10.2 kg) fruit were harvested randomly from a commercial farm block located in Loxahatchee, FL (26°80′N, 80°33′W) in Oct. 2017. In total, 105 melons were used, including: day 0 + (days 10, 20, and 30) × (10°C and 30°C), 7 treatment combinations × 5 melons per replication. During storage, samples were taken to determine the concentration of volatiles, phenolic compounds, acids, sugars, and soluble solids content (SSC) at day 0, day 10, and day 20. The melons at day 30 sampling were deteriorated, thus the chemical compounds were not determined. Juice samples were extracted from fruits by standard processing at PepsiCo, Inc (Barrington, IL). Briefly, after cleaning using a fruit-specific cleansing agent (Fruit Cleaner 395, JBT Food Tech., Lakeland, FL), melons were rinsed, and the fruit flesh was collected after removing the peel (approximately 5 mm), and the core tissues (septum, endocarp and seeds). A juicer (Model 6001; Acme Juicer Mfg Co, Sierra Madre, CA) was used for juice extraction. The juice was then passed through a 0.51 mm screen size pressure filtration finisher, and finally pasteurized at 90°C for 10 s with a flow rate 1.2 $L \cdot min^{-1}$ using an ultrahigh-temperature (UHT)/high-temperature-short-time (HTST) Lab 25EHV Hybrid pasteurizer (Microthermics, Inc., Raleigh, NC, USA). The pH of the juice was adjusted to 4.0 using citric acid, poured into 1.89 L plastic jugs, and kept at −20°C for further analysis. Fruit deterioration (dry rot spot, stem scaring, and water-soaked spots/soft rot spots) was visually observed during storage.

2.2. Free Sugars and Titratable Acidity. Roughly, 20 g of juice samples were centrifuged for 15 min at a speed of 15,000 × g. Supernatants were then subjected to analysis for identification of individual sugars, and measurement of soluble solids content (SSC) and titratable acidity (TA). To determine the individual sugars, the samples were prepared by filtering the supernatant through a 0.45 μm GD/X Nylon syringe filter. The sugar content was qualified using high-performance liquid chromatography (HPLC) equipped with a refractive index detector and a column (Waters Sugar Pak, PerkinElmer, Norwalk, CT, USA) [10]. The mobile phase was ethylenediaminetetraacetic acid disodium calcium salt (0.1 $mmol \cdot L^{-1}$) with a 0.5 $mL \cdot min^{-1}$ flow rate at 90°C. SSC was measured by using a digital refractometer (Atago RX-5000α, Tokyo, Japan), and TA was determined by an autotitrator (Brinkman, Titrando 808, Riverview, FL, USA).

2.3. Phenolic and Amino Acid Analysis. The phenolic amino acids and hydroxycinnamic acids in the juice were analyzed by an HPLC (Waters 2695 Alliance, Waters, Medford, MA, USA) connected to a PDA detector (Waters 996, Waters) and a single-quadrupole mass spectrometer (Waters/Micromass ZQ, Waters) equipped with an electrospray ionization source. The detailed preparation procedure and equipment settings were described previously [7].

2.4. Volatile Components. The preparation process and volatile analysis methods of winter melon juice were described in a previous report [19]. In brief, samples were extracted using a 2-cm solid phase microextraction (SPME) fiber (50/30 μm DVB/Carboxen/PDMS; Supelco, Bellefonte, PA), and a nonpolar DB-5 column equipped with gas chromatography (GC) were applied to separate the volatile compounds. Those volatile compounds were detected by a mass spectrometry (MS) detector, which were further identified by matching their spectra with those from the Mass Spectral Library (NIST 14; WebBook, SRD69) and the standards of authentic volatile compounds. The concentration of each volatile compound was calculated by using its standard curve.

2.5. Weight Loss. The weight of each winter melon was measured on sampling days. Values were described as percentage of weight loss per original fruit weight. At least 5 fruits were weighed each time.

2.6. Statistical Analysis. Experimental results were evaluated using analysis of variance (ANOVA) employing SPSS (V. 17, Experian QAS, Boston, MA). A Duncan's multiple range test was used to determine mean separation ($p < 5\%$). All experiments were replicated at least three times.

3. Results and Discussion

3.1. Sugars and Titratable Acidity. The concentrations of both sugars and acids in the winter melon juice samples were relatively low. The content of glucose and fructose was 0.65–0.76% and 0.64–0.83% (w/w), respectively (Figure 1). The total sugar content was about 1.5% in all juice samples, compared to more than 10% total sugar contents in most other fruit juices [20]. The SSC and TA were 2.4–2.6% and 0.35–0.40%, respectively (Figure 1). Glucose and fructose decreased significantly during storage at both 10°C and 30°C, but soluble solids content (SSC) increased (Figure 1). Galactose, an indicator of rhamnogalacturonan concentration,

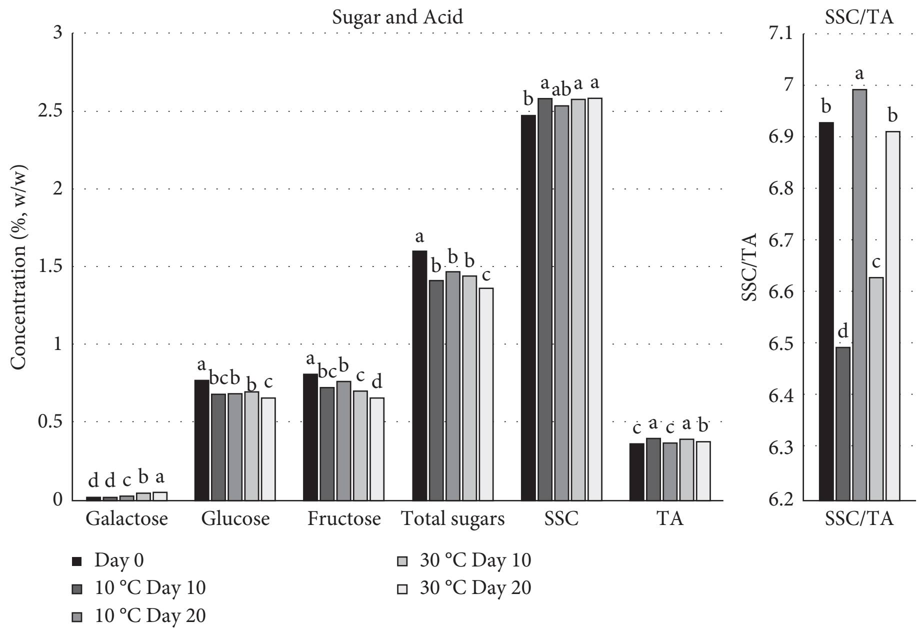

Figure 1: Sugar and acid concentration (%, w/w), and SSC/TA in winter melon juices stored under different conditions. (SSC: soluble solids content; TA: titratable acidity; mean values followed by different letters in the same column indicate significant differences using *t*-test ($p < 0.05$)).

increased over time and did so more quickly at 30°C than at 10°C (Figure 1). According to previous research, the major natural sugars in winter melon juice were glucose and fructose, and the levels of them were reported to decline along with maturity from 0.9% to 0.5%, and 0.8% to 0.5%, respectively [4, 5]. A significant decrease in sugar content was also observed for sweetcorn during storage with a greater decline at higher temperatures [21]. The decrease in the concentration of glucose and fructose at 30°C may have been because a significant increase in the biosynthesis of anthocyanins required more monosaccharides to form Cy3G and its derivative [21]. Sugar content is often equal to SSC in many fruits, and producers often use SSC as a rough estimate of sweetness [7]. The SSC of winter melon is relatively low compared to other melons and to orange juice [22, 23]. The SSC increased during storage while sugars decreased, however, indicating that an increased amount of soluble organic matter formed during storage [24]. The TA value was significantly reduced after 20 days storage compared to day 10, and due to this, the SSC/TA ratio significantly increased (Figure 1).

3.2. Phenolic and Amino Acid Compositions. Seven phenolic compounds (tyrosine (Tyr); phenylalanine (Phe); tryptophan (Trp1, 2, and 3); *p*-coumaric acid (*p*-CA); and 4-hydroxy-3-methoxy cinnamic acid (HCA)) were identified and quantified in the winter melon juice samples (Table 1). Tryptophan (Trp1) showed the highest concentration (between 1.030 and 2.081 mg·L^{-1}) in the juice samples (Table 1). The concentration of total phenolic compounds ranged from 3.376 to 4.231 mg·L^{-1}. The content fluctuated over storage time and there was no consistent pattern at 10°C storage. However, the total phenolic compounds content increased over storage at 30°C. Tryptophan (Trp1) produced a singular UV spectrum and there were two additional peaks (Trp2 and Trp3) with nearly identical UV spectra and produced results similar to the MS of tryptophan [7]. An increase in total phenolic compounds during storage was also observed with jujube fruit [25]. The essential amino acid phenylalanine, significantly reduced after 10 days storage at both 10 and 30°C, and this may have been due to conversion to cinnamic acid, which is an important precursor compound in the metabolism of phenylpropanoids, including flavonoids and capsaicinoids, by the enzyme phenylalanine ammonia lyase (PAL) [26]. Tyrosine and *p*-CA, showing high antioxidant activity, can be synthesized from tyrosine by the enzyme tyrosine ammonia lyase (TAL) in the natural phenolic biosynthesis pathway [27]. Tryptophan, an essential amino acid and a precursor to serotonin, also has an important role in the biosynthesis of proteins and possesses strong antioxidant and anti-insomnia/depression activities [28]. The results of the current research support the clinical use of winter melon in modern medicine.

3.3. Volatile Profile. Seven volatile compounds were identified by GC-MS, and the main volatile compound found in the winter melon juice was hexanal with a concentration between 1180.10 and 3310.06 μg·L^{-1} (Table 2). Three sulfur compounds: methanethiol, dimethyl sulfide, and dimethyl disulfide were identified and quantified in juice samples (Table 2). Volatile compounds such as (E)-2-hexenal, hexanal, and n-hexyl formate produce fatty, green, and buttery odors [29]. Lipoxygenase activity results in the production of hexanal, and sensory perception of off-flavor may correlate well with the presence of hexanal [30]. Hexanal was the compound with the highest concentration

Table 1: Phenolic amino acid concentrations (mg·L^{-1}) in winter melon juices stored under different conditions.

Phenolics	Day 0	10°C day 10	10°C day 20	30°C day 10	30°C day 20
Tyr	0.405a	0.400a	0.362b	0.387ab	0.410a
Phe	0.494a	0.409a	0.642a	0.425a	0.502a
Trp1	1.110c	1.030c	1.602b	1.490b	2.081a
p-CA	0.006c	0.008b	0.010a	0.007bc	0.006c
HCA	0.009	0.006	0.007	0.008	0.011
Trp2	0.351a	0.389a	0.235b	0.367a	0.342a
Trp3	1.001bc	1.213a	0.614d	1.094ab	0.879c
Total	3.376d	3.455c	3.472c	3.778b	4.231a

Tyr: Tyrosine; Phe: Phenylalanine; Trp: Tryptophan; *p*-CA: *p*-Coumaric acid; and HCA: 4-hydroxy-3-methoxy cinnamic acid. Mean values followed by different letters within a row indicate significant differences using *t*-test ($p < 0.05$).

Table 2: Aroma volatiles (μg/L^{-1}) in winter melon juices stored under different conditions.

Volatiles	RI	Day 0	10°C day 10	10°C day 20	30°C day 10	30°C day 20
Methanethiol	509	2.50a	1.65a	1.64a	1.78a	2.31a
Dimethyl sulfide	544	0.08b	0.18a	0.07b	0.06b	0.05b
Ethyl acetate	600	6.40a	7.11a	4.58a	4.82a	4.19a
Dimethyl disulfide	723	1.32a	1.14ab	0.83b	1.40a	0.82b
Hexanal	772	3310.06a	2703.34a	1180.10c	2489.31ab	1700.12b
a-pinene	908	5.62a	3.91ab	3.39b	2.82b	4.35ab
D-limonene	996	9.99b	33.37a	5.99b	22.68a	10.14b
Total sulfur		3.90a	2.97ab	2.54b	3.24a	3.18a
Total		3335.97a	2750.70a	1196.60c	2522.87ab	1721.98b

RI: Retention Index. Mean values followed by different letters in the same row indicate significant differences using *t*-test ($p < 0.05$).

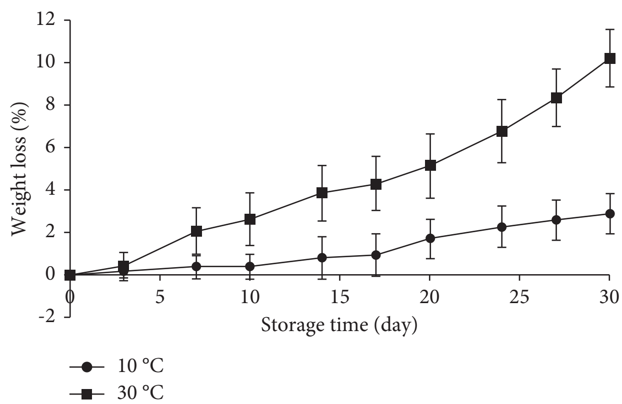

Figure 2: Weight loss of winter melon fruit stored at 10 and 30°C.

among all compounds identified in winter melon juice (Table 2). The concentration of hexanal was significantly reduced by 64.3% and 48.6% after 20 days storage at 10°C and 30°C, respectively (Table 2). Similarly, it was significantly reduced in apple juice stored at 30°C [31]. The C6 volatile compounds significantly decreased during storage in nectarines as well [32]. Sulfur compounds typically have a larger impact on flavor impression in vegetables than most other types of compounds [33]. Many sulfur compounds are formed by the Maillard reaction through heat processing [33]. Four main sulfur compounds (methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide) were previously identified in winter melon juice [7]. The concentration of total sulfur compounds was significantly reduced about 35% after 20 days storage at 10°C (Table 2), probably due to the reduced enzymatic activity [32]. The total volatile compounds were significantly reduced after 20 days storage at both 10°C and 30°C (Table 2). Likewise, a decrease in total volatile abundance in tomato was observed under low temperature storage [34].

3.4. Water Loss and Deterioration of the Fruit. There was 1.71% and 5.15% weight loss after 20 days storage at 10°C and 30°C, respectively (Figure 2). After 30 days storage, the weight loss was 2.90% and 10.21% for the fruit stored at 10°C and 30°C, respectively (Figure 2). The melons showed significantly higher weight loss under higher temperatures, which could be attributed to higher transpiration and respiration rates at higher temperatures [35]. After 25 days storage, 10°C stored fruit showed water-soaked spots on the fruit surface which developed into soft rot spots and stem

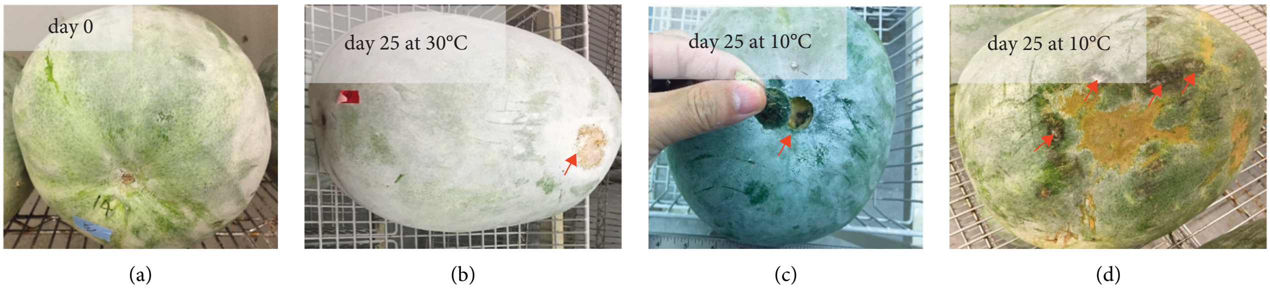

Figure 3: Appearance of winter melon fruit stored under different conditions (a) day 0; (b) day 25 at 30°C; (c) and (d) day 25 at 10°C. Red arrows indicate the dry rot spot, stem scaring, and water-soaked spots/soft rot spots, respectively.

scaring (Figure 3). Soga et al. [18] reported that when storage temperature was lower than 13°C, chilling injury occurred in winter melons. The chilling injury caused similar damage to the surface of cucumber fruit [36]. The pitting was manifested as soft rot due to the presence of fungi [37]. Winter melons stored at 30°C showed larger dry rot spots with a diameter larger than 5 cm (Figure 3).

4. Conclusions

The concentration in winter melons of fructose and glucose significantly decreased when kept at both storage temperatures, but the concentration of soluble solids raised in winter melon juice. Galactose increased through the storage period and the increase was higher at 30°C than 10°C. The concentration of titratable acid continually decreased during storage, particularly in melons stored at 10°C. Seven phenolics were detected and the concentration of total phenolics content increased during storage. Total sulfur compounds, total volatiles, and specifically hexanal were significantly reduced during storage. The weight loss of the fruit was 2.90% and 10.21% after 30 days storage at 10°C and 30°C, respectively. The results indicate that winter melon stored for 20 days at 10°C and 30°C did not cause physicochemical quality deterioration. However, after 25 days storage, chilling injury and associated wet decay occurred at 10°C storage, and dry rot occurred at 30°C storage. The juice from 30°C stored fruit possessed better overall quality with a higher Brix/TA ratio, a higher phenolic compound content, and a lower concentration of flavor damaging sulfur compounds.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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