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# Comparative Analysis of Qualitative Attributes for Selection of Calabaza Genotypes in the Southeast United States

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**Abstract:** *Cucurbita moschata*, commonly known as winter squash, tropical squash, and calabaza, is native to Central America. This tropical squash thrives in tropical and subtropical climates, including parts of the southeastern U.S. and is very popular among people of Hispanic and West Indian heritage. Development of calabaza cultivars that meet consumer acceptability is a major goal in breeding programs. The current study aims to determine and compare the quality parameters of novel calabaza germplasm lines with that of commercially available cultivars of calabaza (La Estrella and Soler) and butternut squash (Whatman Butternut). All cultivars ranged greatly in quality parameters, with the most promising germplasms highlighted within the study including UFTP 8 and UFTP 24. The basis of this ranking was dependent on these germplasm lines' desirable attributes, including their color saturation (chroma (>80) (using the CIELAB scale)), °Brix (11.6 and 10.7 respectively), yeast fermentable extract (>67%), and firmness/hardness, (>3600). These findings show the potential to enhance consumer preferences for calabaza through breeding and provides a basis for commercial release of the superior germplasm identified.

**Keywords:** calabaza; germplasms; pumpkin; sensory quality; southeastern U.S.



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## 1. Introduction

The *Cucurbita* genus consists of four predominantly cultivated squash species, *Cucurbita maxima*, *C. pepo*, *C. argyrosperma*, and *C. moschata* [1]. Within *C. moschata*, there are four main groups (cheese, crookneck or cushaw, bell-shape, and calabaza) whose fruit may be consumed in either an immature (summer squash) or mature (winter squash) state [2–4]. These fruit-maturity states and shapes are major parameters that define both squash and pumpkins [2]. For this study, the objective was to understand the properties of novel germplasm lines and compare them to commercially available calabaza within the United States market.

In Florida, Puerto Rico, and Caribbean nations, cultivars of the calabaza varietal group (tropical pumpkin) such as La Estrella and Soler are commonly grown due to their resilience to the hot and humid tropical and subtropical climates [5–8]. The per capita annual consumption of calabaza in Puerto Rico is estimated at 7 kg and is the most important nonroot vegetable [9]. Calabaza is very popular in Jamaica and Haiti, and its soup is considered a national dish in both countries [10]. The most recent ethnic market analysis for calabaza in Florida was estimated at $5 million [11], while its total imports into the U.S. are estimated at $30 million [12]. Furthermore, according to the IRI's innovation report, as of the end of 2020, products with origin claims (e.g., "made in the USA" and "local") posted

the highest dollar sales growth among foods/beverages carrying a benefit descriptor [13]. With this influx of consumer demand for calabaza, additional research should be conducted to aid in supplying the demand.

The demand for calabaza is increasing due to growing populations of ethnic communities in the U.S. (Caribbean, Central American, South American, and Asian), who are familiar with the fruit [8,14–16]. These diverse communities include calabaza as an ingredient in many types of culturally relevant dishes year-round. There are many preparation methods, including boiling, steaming, microwaving, sous vide [17], stir frying [18], pickling [19], fermenting [20], drying [21], and eating raw [2]. Notably, fermentation (the metabolism of sugar by specific microorganisms) is a value-added process used to increase shelf life and modify the sensory properties of the final product. Squash can undergo fermentation to produce various end products, namely beer [22], wine [23], kimchi [24], water kefir [20], and vinegar [25]. Cultivars of *C. moschata* are known for high concentrations of micronutrients, including lutein, beta-carotene, total phenolics, manganese, magnesium, iron, potassium, calcium, copper, zinc, and sodium [26,27].

The objective of this study was to evaluate and compare quality attributes of new calabaza hybrids against reference commercial cultivars (Waltham Butternut squash, Soler, and La Estrella). For the selection of a calabaza cultivars for a retail market, sensory parameters of the fruit should be tested to understand consumers perception [28]. A consumer's perception is influenced by the following physical and chemical parameters: soluble solids, titratable acidity, soluble solids/titratable acidity ratio, L*a*b* value, texture, and enzymatic activity including polyphenol oxidase (PPO). Therefore, these measurements were taken to create a quality profile for each germplasm line. Additionally, flesh yield and yeast fermentable extract (YFE) were assessed for each germplasm line. An assessment of flesh yield allows for the determination of the profitability and ratio of flesh/waste for a germplasm line. YFE is a commonly used measurement for the assessment of the percentage of fermentable sugars within a produce item for fermentability. The design of the experiment allowed for direct comparisons among calabaza germplasm lines with the goal of identifying and addressing grower needs and constraints for calabaza as an emerging crop.

## 2. Materials and Methods

### *2.1. Calabaza Germplasm*

A total of eight winter squash genotypes, including five new calabaza hybrids and three commercial cultivars, were evaluated. These hybrids were either compact-by-compact, compact-by-long vine, or long vine-by-compact derived from inbred lines and self-landraces developed at the University of Florida and the University of Puerto Rico [5,6] (Table 1). Stephens (1994) found that trailing fruit vines of calabaza vary in length from the central root to the vine apex and demonstrate a potential average fruit yield of 20–25 tons per acre [29]. The La Estrella cultivar is a commercial compact plant hybrid released by the University of Florida in 2002 [6], while Soler is a popular open pollinated calabaza cultivar developed at the University of Puerto Rico [7]. The Waltham Butternut is an open pollinated cultivar of the bell-shaped varietal group widely grown in the continental United States [30].

### *2.2. Crop Management and Harvest*

Fifteen seeds each for the eight squash genotypes were sowed on 15 March 2022, in a greenhouse in plastic seedling trays containing potting mix and starter fertilizer in Gainesville, FL. At two weeks old, the seedlings of each germplasm line were transplanted on 31 March 2022 into a single plot in the field at the University of Florida, Plant Science Research and Education Unit in Citra, FL, USA. Weekly pest and fertilizer management was conducted following recommendations for pumpkins in the Vegetable Production Handbook of Florida [31].

**Table 1.** Pedigree of calabaza hybrids and cultivars used in the study.

| Entry | Pedigree | Parental Internode |
|---|---|---|
| Waltham Butternut | Open pollinated | Medium vine |
| UFTP 8 | E-5 x TP331 | long x short |
| UFTP 22 | TP331 x TP411 | short x short |
| UFTP 24 | G38-2-38 x JP5 | short x long |
| UFTP 38 | TP331 x Fairytale | short x long |
| UFTP 42 | Soler * x TP331 | long x short |
| Soler | Open pollinated | long |
| La Estrella | G38-2-38 x La Primera | short x long |

* Open pollinated cultivar developed at the University of Puerto Rico.

On 1 July 2022 (91 growing days), the mature tropical pumpkin genotypes were manually harvested and labeled. Fruit maturity was determined by a combination of expected rind color and rind firmness by using the thumb-pricking technique. If light pressure from a thumbnail on the skin of the fruit was able to leave a slight indent that then fully recovered instantaneously or pierced through the skin, the fruit was deemed ripe for harvest. Conversely, if the light pressure from a thumbnail on the skin leaves an indent that does not recover, the fruit was not harvested. The fruit were wiped clean and labeled with cultivar numbers directly on the fruit.

### *2.3. Curing and Storage*

A representative sample of 10–15 calabaza fruits per genotype were used for analysis. By using the United States Department of Agriculture (USDA) recommended storage conditions for curing and long-term storage [32], after harvest, calabaza were stored in a 75 °F/24 °C and 65% relative humidity (RH) room to cure for 7 days. This was performed to ensure that the respiration rate of the pumpkins did not escalate, resulting in weight loss and fruit degradation [33]. The cured pumpkins were transferred into a permanent storage cooling room at 10 °C and 60% RH room as described by Gross (2016) for 7 days until processing [32].

### *2.4. Processing*

Each fruit was weighed before and after deseeding. All flesh was cubed by using a 1/2-inch cuber (Nemco N55450, Hicksville, OH, USA) to achieve 1/2-inch (12.7 mm) samples. A uniform sample of each germplasm line was created by combining all cubes from 10–15 calabaza for random analysis. For texture analysis, "ideal" cubes (squares that were 1/2-inch by 1/2-inch by 1/2-inch) were separated from the miscellaneously shaped samples. The "ideal" cubes were used for texture analysis to ensure as much uniformity in pressure of the sample. Miscellaneously shaped samples were used for all other analyses, as they involved blending. All samples were stored in single-layer vacuum-sealed bags at 4 °C for up to 1 week until analyzed.

### *2.5. Flesh Yield*

Following the determination of starting weight, each fruit was initially weighed. Each fruit was cut in half and labeled on both halves before deseeding and peeling. After deseeding and peeling, the two halves were reweighed together to determine the fruit yield shown in Equation (1). This was done to all 10–15 *C. moschata* fruit for the eight cultivars of interest. We have

$$Flesh\ \% = \frac{Final\ Weight}{Initial\ Weight} \times 100, \quad (1)$$

where the edible portion of the fruit was calculated as a ratio with the final weight being the edible flesh after processing and initial weight being the weight of one unadulterated tropical squash.

### 2.6. Color

The color of the pumpkin flesh was evaluated immediately after being cut in half (within 5 min) by using a tristimulus color analyzer (Konica Minolta, C4-400, Tokyo, Japan) equipped with an 8 mm diameter measuring area following the methods of Itle, with slight variation [34]. This color system measures the L* (100 = white; 0 = black), a* (+, red; −, green), and b* (+, yellow; −, blue) values of a sample. These can be used to calculate chroma (C*) (Equation (2)) and hue angle (h*) (Equation (3)), which are used to compare and contrast color samples for food [35]. Chroma represents the colorfulness of a sample and differentiates between this color and a grey color of the same lightness. We have

$$C^* = \sqrt{a^2 + b^2}, \tag{2}$$

where a* is equal to a, and b* is equal to b, which encompasses the range of color values. We have

$$h^* = \tan^{-1}\left(\frac{b}{a}\right), \tag{3}$$

where a and b are the range of color values. Hue angle is used to differentiate a sample's traditional color (e.g., reddish, blueish, etc.) in reference to a gray color with the same lightness (L*). An angle of 0 or 360 represents red, while angles of 90, 180, and 270 represent yellow, green, and blue hues, respectively [36].

Fruits were cut vertically, and three measurements were taken per fruit, with five fruits representing each germplasm line. The three locations of flesh measurement are shown in Figure 1, being right below the stem (1), at the fruit equator (2), and right above the base (3), avoiding the seed cavity and peel (≈10 mm) for the most comprehensive sampling per fruit [34].

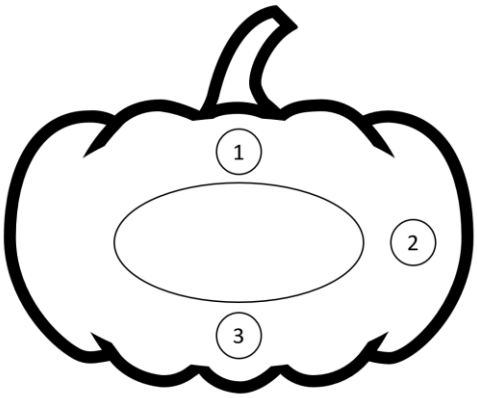


**Figure 1.** L*a*b* measurement location on calabaza.

### 2.7. Peroxidase (POD)

The POD of the flesh was determined by following the methods of Sampedro and Zhou, with slight modifications [37,38]. Samples (20 g) were blended with 20 mL of 4% polyvinyl polypyrrolidone (PVPP) in 0.2 M aqueous phosphate buffer (pH = 6.5) for 2.5 min. Blended samples were held at 4 °C for 1.5 h. The samples were then centrifuged at 12,000 rpm by using a centrifuge (Beckman J2-21, Palo Alto, Santa Clara, CA, USA) for 20 min at 4 °C. The supernatant was vacuum filtered by using 9.0 mm filter paper and gathered in a test tube for POD activity.

A 0.1 mL aliquot of the sample extract was added to 0.2 mL of 1.5% $H_2O_2$ and 3.0 mL 1.0% ($v/v$) guaiacol (dissolved in 0.2 M phosphate buffer, pH 6.5) in a test tube. The contents were vortexed for 5 s to achieve an even mixture and placed in a 30 °C water bath for 20 s. The sample was poured into a quartz cuvette and the absorbance was measured by using a UV spectrophotometer (Shimadzu, UV-1900, Kyoto, Japan) every half-second for 120 s.

Enzyme activity of POD (Abs/min) was determined by using the slope from the linear portion of the reaction curve at Δ470 nm.

### *2.8. Texture (Double Compression)*

The texture profile of the flesh was determined by using a double compression test following Marian [39] with minor changes. The flesh collected for analysis was only from the midsection (labeled II) of the pumpkin at a distance of 4 mm from the skin for uniformity, as shown in Figure 2. The other sections of the calabaza fruit (labeled I and III) were not utilized for this analysis. Due to the curvature of the calabaza, the axis of the square samples was perpendicular to the surface of the skin.

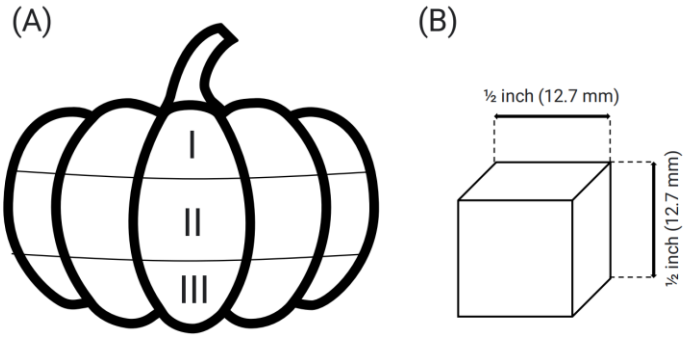


**Figure 2.** Calabaza sampling location (**A**) and texture analysis sample size (**B**).

Ideal cubes (1/2-inch or 12.7 mm) with vertical fiber grain orientation underwent a double compression test. A Texture Technologies TA.XT Plus Connect (Hamilton, MA, USA) texture analyzer outfitted with a 25 mm diameter cylindrical probe was used. The speed of the measuring head in each cycle was 0.83 mm·min$^{-1}$. The probe descended until 50% of the sample height (6.35 mm) compression was achieved. The time interval between the first and second compression was 5 s. Analysis was performed on 12 cubes from each representative randomized germplasm sample. Parameters measured for double compression include firmness/hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience.

### *2.9. Total Soluble Solids ($^{o}$Brix)*

$^{o}$Brix for calabaza fruit samples (200 g) was determined by blending the flesh until homogeneous. Samples were then centrifuged at 12,000 rpm by using a centrifuge (Beckman J2-21, Palo Alto, CA, USA) for 20 min at 20 °C. The liquid component and water-soluble constituents of the calabaza flesh were collected as a supernatant. This was vacuum filtered by using 9.0 mm filter paper and measured by using a benchtop refractometer with water bath temperature correction set for 20 °C (LeicaAbbe Mark ii refractometer, model 13104800, Buffalo, NY, USA) [40].

### *2.10. Titratable Acidity (TA) and pH*

Titratable acidity was determined by following Papanov and AOAC methods with slight modifications [41,42]. A total of 200 g of calabaza was blended until homogeneous. Samples were then centrifuged at 12,000 rpm by using a centrifuge (Beckman J2-21, Palo Alto, CA, USA) for 20 min at 20 °C. The supernatant (collected in Section 2.9) was vacuum filtered by using 9.0 mm filter paper and collected. The titratable acidity was expressed as a percentage of malic acid. This was determined through titration of 25 mL of pumpkin juice plus three drops of 1% phenolphthalein in 95% ethanol as an indicator with 0.1 M NaOH to a pH value of 8.1. The pH was determined by using a pH probe (Fisher Scientific, Accumet AB15 basic, Walthan, MA, USA) after calibrating with buffer solutions of pH 4.0, 7.0, and

10.0 following the operational instructions of the instrument. Malic acid was calculated by using Equation (4) based on the TA%. We have

$$Malic\ Acid\left(\frac{g}{100g\ sample}\right) = \left(\frac{100}{D{\cdot}Vs}\right){\cdot}(Vt{\cdot}N{\cdot}Eq.\ of\ malic\ acid), \quad (4)$$

where the malic acid weight per 100 g sample was determined by using the density (D) of each individual germplasm juice (g/L), volume of sample size in mL (Vs), volume of titrate size in mL (Vt), normality (N), and equivalence (Eq.) of malic acid.

### *2.11. Yeast Fermentable Extract (YFE)*

Yeast fermentable extract was determined by following the American Society of Brewing Chemists (ASBC) WORT 5 method [43]. A 600 g sample of calabaza was blended until homogeneous. Samples were then centrifuged at 12,000 rpm by using a centrifuge (Beckman J2-21, Palo Alto, CA, USA) for 20 min at 20 °C. The supernatant (100 mL) was added to 2 g of lager yeast (*Saccharomyces pastorianus*) and three drops of defoamer (Atmos 300 K) in 250 mL Erlenmeyer flasks for fermentation. Flasks were placed into a shaker water bath to ferment for 48 h at 25 °C. Fermented samples (50 mL) were added to 0.5 g of sparkaloid (clarification agent) and filtered by using 9.0 mm filter paper. This filtrate was further filtered by using 0.45 µm syringe filter until 12 mL of sample was collected. Samples were placed in a sonicator (Branson ultrasonic cleaner B-52 240 Watts, Brookfield, WI, USA) for 30 s to remove any $CO_2$ bubbles and run through an alcohol and extract meter (ALEX 500–Anton-Paar, Houston, TX, USA) for a beer sample. The final °Brix measurement was determined, allowing for the calculation of the yeast fermentable extract by using Equation (5),

$$YFE = \left(\frac{Inital\ ^{\circ}Brix - Final\ ^{\circ}Brix}{Inital\ ^{\circ}Brix}\right) \times 100, \quad (5)$$

where the initial °Brix is the soluble solids content before fermentation and the final °Brix is the soluble solids content after fermentation with ethanol concentration taken into account.

### *2.12. Statistical Analysis*

All tested parameters were analyzed by using a one-way ANOVA with Duncan's separation in Statistical Analysis Systems™ 9.4 (SAS Inst. Inc., Cary, NC, USA) to distinguish significant differences at $\alpha$ = 0.05 between each calabaza germplasm for the following parameters: L*, a*, b*, chroma, hue angle, pH, TA, malic acid, °Brix, POD, YFE, firmness/hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. Each parameter's mean was then used to run a Pearson correlation in Microsoft Excel to describe the relationship between all tested parameters.

## 3. Results and Discussion

### *3.1. Flesh Yield*

Flesh yield for the calabaza, varied greatly among the squash genotype (Table 2), and are markedly distinguishable from the previously reported values on either extreme [6]. Soler was much larger on average than any of the other cultivars (3.5 kg) while the Waltham Butternut cultivar was much smaller on average (0.72 kg). Generally, fruit size in *Curcurbita* is dependent on genotype but can also be influenced by the environment [44,45]. In certain markets, smaller squash fruits are preferred by consumers due to convenience of handling, dish preparation, and less labor [46]. Therefore, small fruit calabaza cultivars might be more desirable for a fresh market within the U.S. compared to large fruit cultivars [47].

**Table 2.** Yield table for calabaza germplasms used in the study.

| Cultivar | Avg. Total Fruit Weight (kg) | Avg. Fruit Flesh (kg) | Flesh Yield (%) |
|---|---|---|---|
| Waltham Butternut | $0.72 \pm 0.18^{d}$ | $0.54 \pm 0.14^{d}$ | $75.17 \pm 3.46^{c}$ |
| UFTP 8 | $1.62 \pm 0.33^{c}$ | $1.24 \pm 0.27^{c}$ | $76.55 \pm 3.36^{bc}$ |
| UFTP 22 | $1.70 \pm 0.35^{c}$ | $1.36 \pm 0.29^{c}$ | $80.18 \pm 2.28^{a}$ |
| UFTP 24 | $2.51 \pm 1.03^{b}$ | $1.99 \pm 0.90^{b}$ | $78.36 \pm 3.77^{abc}$ |
| UFTP 38 | $2.75 \pm 0.75^{b}$ | $2.14 \pm 0.63^{b}$ | $77.52 \pm 3.51^{abc}$ |
| UFTP 42 | $2.58 \pm 0.77^{b}$ | $2.11 \pm 0.79^{b}$ | $79.88 \pm 6.63^{ab}$ |
| Soler | $3.50 \pm 0.75^{a}$ | $2.77 \pm 0.64^{a}$ | $78.96 \pm 3.02^{ab}$ |
| La Estrella | $1.82 \pm 0.78^{c}$ | $1.38 \pm 0.62^{c}$ | $75.21 \pm 4.43^{c}$ |

$n$ = 10–15 squash of each cultivar were measured to determine average values. Note. Letters compare means in the same column. Different letters correspond to significant change ($p < 0.05$) by Duncan's multiple range test.

### *3.2. Fruit Shape, Growth Habit, and Flesh Color*

The calabaza genotypes varied in fruit shape, growth habit, and flesh color (Table 3). The fruit shapes observed varied from globe shaped (UFTP 8, UFTP 22) to round shaped (UFTP 24, La Estrella) to oblate shaped (UFTP 38, UFTP 42, Soler) and bell shaped (Waltham Butternut). Calabaza fruit shapes are dependent on the parent lineage [48] and could be a factor affecting consumer preference [47]. Flesh yield for the calabaza, shown in Table 2, ranges greatly from previously seen values on either extreme [6]. The UFTP 45 cultivar was much larger on average than any of the other cultivars (3.44 kg) while the Waltham Butternut squash was much smaller on average (0.72 kg). It should be noted that convenience is also one of the major contributing factors in purchasing power due to many variables including but not limited to saving time, reduced cost, less skill required for complex dishes, and less labor [46]. Therefore, smaller calabaza might be better for a fresh market within the U.S. compared to larger fruit.

The flesh color was determined by using the Hunter lab L*a*b* color scale. Statistically significant differences were observed among the squash genotypes in flesh color attributes, including lightness (L*), red/green (a*), and blue/yellow (b*) and are presented in Table 3. Interestingly, the values observed for the Waltham Butternut cultivar are different from values of those previously reported in literature. For example, Mashiane (2021) reported average L*, a*, b* values of 33.22, −7.45, and 10.61 in butternut, respectively [18]. However, the findings in the current study are within the range of those found in literature for other *C. moschata* cultivars [34,49,50]. Silva (2019) found that cultivars with high a* and high b* (for an intense orange color) were preferred by panelists [17]. Using this preference trend, UFTP 8, UFTP 22, and UFTP 24 would have the highest consumer color preference. The chroma and hue angle was determined by using Equations (2) and (3), respectively (Table 4). These additional color indicators are also within ranges seen for various calabaza germplasm lines [8,51]. Chroma value (color purity) was statistically the highest for UFTP 8 with UFTP 24 being the only other cultivar that was not statistically significantly different. This color intensity would further support these germplasm lines' higher consumer acceptability, as multiple studies have shown that appearance is a very important quality that consumers consider when buying a food [17,52].

**Table 3.** Color (L*, a*, b*), chroma, hue angle, pictures of vine growth and cross section of calabaza genotypes grown at the Plant Science Research and Education Unit in Citra, Florida. 2022.

| Cultivar | L* | a* | b* | Chroma | Hue Angle | Illustration (Fruit and Vine) |
|---|---|---|---|---|---|---|
| Waltham Butternut | 69.13 ± 2.02 $^{bc}$ | 22.37 ± 1.75 $^{b}$ | 70.21 ± 2.49 $^{c}$ | 73.93 ± 2.81 $^{c}$ | 72.41 ± 0.91 $^{cd}$ |  |
| UFTP 8 | 67.90 ± 3.03 $^{cd}$ | 25.23 ± 2.23 $^{a}$ | 77.40 ± 2.69 $^{ab}$ | 81.44 ± 2.52 $^{a}$ | 71.93 ± 1.70 $^{d}$ |  |
| UFTP 22 | 69.93 ± 2.55 $^{ab}$ | 22.72 ± 3.42 $^{b}$ | 74.79 ± 2.37 $^{b}$ | 78.21 ± 2.98 $^{b}$ | 73.15 ± 2.11 $^{cd}$ |  |
| UFTP 24 | 68.63 ± 2.15 $^{bc}$ | 20.10 ± 2.43 $^{c}$ | 77.93 ± 4.58 $^{a}$ | 80.52 ± 4.41 $^{ab}$ | 75.50 ± 1.96 $^{b}$ |  |
| UFTP 38 | 66.41 ± 1.84 $^{d}$ | 22.20 ± 3.19 $^{b}$ | 59.92 ± 4.04 $^{e}$ | 63.93 ± 4.64 $^{e}$ | 69.74 ± 2.01 $^{e}$ |  |
| UFTP 42 | 69.70 ± 2.75 $^{abc}$ | 20.66 ± 1.98 $^{b}$ | 70.21 ± 3.82 $^{c}$ | 73.20 ± 4.03 $^{c}$ | 73.62 ± 1.18 $^{c}$ |  |
| Soler | 71.18 ± 2.42 $^{a}$ | 14.85 ± 2.19 $^{d}$ | 67.51 ± 3.63 $^{d}$ | 69.14 ± 3.90 $^{d}$ | 77.63 ± 1.36 $^{a}$ |  |
| La Estrella | 71.17 ± 1.73 $^{a}$ | 19.00 ± 3.80 $^{c}$ | 76.06 ± 3.86 $^{ab}$ | 78.49 ± 3.70 $^{b}$ | 75.96 ± 2.91 $^{b}$ |  |

Note. Five squash was sampled per genotype, with three measurements taken per squash. Letters compare means in the same column. Different letters correspond to significant change ($p < 0.05$) by Duncan's multiple range test.

**Table 4.** Pearson correlation analysis for all sensory based parameters.

| Parameter | °Brix | pH | Malic Acid | SSC/TA | YFE | L* | a* | b* | Chro. | Hue | Hard. | Adhes. | Spring | Cohesiv. | Gum. | Chew. | Resilien. | POD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| °Brix | 1.00 | −0.43 | 0.77 | −0.35 | 0.23 | 0.02 | 0.62 | 0.73 | 0.80 | −0.1 | 0.88 | 0.01 | 0.06 | 0.68 | 0.83 | 0.87 | 0.75 | 0.21 |
| pH | −0.43 | 1.00 | −0.73 | 0.63 | 0.49 | −0.27 | 0.11 | −0.10 | −0.08 | −0.13 | −0.11 | −0.04 | −0.09 | 0.16 | 0.04 | −0.01 | 0.11 | −0.28 |
| Malic Acid | 0.77 | −0.73 | 1.00 | −0.84 | −0.09 | 0.36 | 0.24 | 0.38 | 0.41 | 0.01 | 0.49 | 0.00 | 0.17 | 0.10 | 0.32 | 0.40 | 0.18 | 0.34 |
| SSC/TA | −0.35 | 0.63 | −0.84 | 1.00 | 0.18 | −0.71 | 0.25 | −0.16 | −0.12 | −0.36 | −0.11 | 0.01 | −0.14 | 0.33 | 0.10 | 0.04 | 0.24 | −0.51 |
| YFE | 0.23 | 0.49 | −0.09 | 0.18 | 1.00 | −0.18 | 0.25 | 0.41 | 0.44 | 0.02 | 0.55 | −0.42 | −0.57 | 0.39 | 0.56 | 0.40 | 0.44 | 0.32 |
| L* | 0.02 | −0.27 | 0.36 | −0.71 | −0.18 | 1.00 | −0.67 | 0.34 | 0.24 | 0.84 | 0.04 | 0.06 | 0.00 | −0.25 | −0.15 | −0.15 | −0.19 | 0.73 |
| a* | 0.62 | 0.11 | 0.24 | 0.25 | 0.25 | −0.67 | 1.00 | 0.19 | 0.33 | −0.8 | 0.50 | 0.11 | 0.26 | 0.61 | 0.62 | 0.70 | 0.60 | −0.51 |
| b* | 0.73 | −0.10 | 0.38 | −0.16 | 0.41 | 0.34 | 0.19 | 1.00 | 0.99 | 0.39 | 0.89 | 0.06 | −0.15 | 0.74 | 0.84 | 0.81 | 0.81 | 0.57 |
| Chroma | 0.80 | −0.08 | 0.41 | −0.12 | 0.44 | 0.24 | 0.33 | 0.99 | 1.00 | 0.25 | 0.93 | 0.07 | −0.12 | 0.80 | 0.90 | 0.88 | 0.87 | 0.48 |
| Hue | −0.17 | −0.13 | 0.01 | −0.36 | 0.02 | 0.84 | −0.83 | 0.39 | 0.25 | 1.00 | 0.03 | −0.04 | −0.31 | −0.18 | −0.12 | −0.20 | −0.13 | 0.81 |
| Hard | 0.88 | −0.11 | 0.49 | −0.11 | 0.55 | 0.04 | 0.50 | 0.89 | 0.93 | 0.03 | 1.00 | −0.19 | −0.27 | 0.83 | 0.97 | 0.91 | 0.89 | 0.39 |
| Adhes. | 0.01 | −0.04 | 0.00 | 0.01 | −0.42 | 0.06 | 0.11 | 0.06 | 0.07 | −0.04 | −0.19 | 1.00 | 0.85 | 0.01 | −0.12 | 0.11 | −0.01 | −0.29 |
| Spring. | 0.06 | −0.09 | 0.17 | −0.14 | −0.57 | 0.00 | 0.26 | −0.15 | −0.12 | −0.31 | −0.27 | 0.85 | 1.00 | −0.03 | −0.19 | 0.09 | −0.07 | −0.55 |
| Cohes. | 0.68 | 0.16 | 0.10 | 0.33 | 0.39 | −0.25 | 0.61 | 0.74 | 0.80 | −0.18 | 0.83 | 0.01 | −0.03 | 1.00 | 0.93 | 0.93 | 0.99 | −0.06 |
| Gum. | 0.83 | 0.04 | 0.32 | 0.37 | 0.56 | −0.15 | 0.62 | 0.84 | 0.90 | −0.12 | 0.97 | −0.12 | −0.19 | 0.93 | 1.00 | 0.96 | 0.96 | 0.19 |
| Chewi. | 0.87 | −0.01 | 0.40 | 0.52 | 0.40 | −0.15 | 0.70 | 0.81 | 0.88 | −0.20 | 0.91 | 0.11 | 0.09 | 0.93 | 0.96 | 1.00 | 0.96 | 0.05 |
| Resilien. | 0.75 | 0.11 | 0.18 | 0.24 | 0.44 | −0.19 | 0.60 | 0.81 | 0.87 | −0.13 | 0.89 | −0.01 | −0.07 | 0.99 | 0.96 | 0.96 | 1.00 | 0.04 |
| POD | 0.21 | −0.28 | 0.34 | −0.51 | 0.32 | 0.73 | −0.5 | 0.57 | 0.48 | 0.81 | 0.39 | −0.29 | −0.55 | −0.06 | 0.19 | 0.05 | 0.04 | 1.00 |

[1] Heat mapped according to statistical significance of the Pearson coefficient with dark red indicating a strong negative correlation (≤−0.9) and dark green indicating a strong positive correlation (≥0.9). [2] Many physiochemical attributes were abbreviated including Hue (Hue angle), Hard (harndness), Adhes. (Adhesiveness), Spring. (Springiness), Cohes. (Cohesiveness), Gum. (Gumminess), Chewi. (Chewiness), and Resilien. (Resilience). [3] Color values (L*, a*, and b*) are denoted with an asterisk.

Color parameters (a*b* and chroma) were shown to be positively correlated with °Brix, and firmness/hardness while lightness saturation parameters (L* and hue angle) were positively correlated with peroxidase as shown in Table 4. Helyes found that the relationship for color parameters (a*,b*, and chroma) could be explained through the ripeness of the fruit, as color and °Brix concentration naturally change throughout the fruit's development process [53]. The ripening process could also explain the positive correlation of chroma, a*, and b* with firmness/hardness, and many other texture parameters, as fruits rapidly become softer during the ripening process [54]. The L* value correlates with the lightness or darkness of a sample, with a positive L* value being lighter and a negative L* value being darker. Lightness (L*) and hue angle was shown to be positively correlated with POD. This is to be expected, as POD is responsible for enzymatic browning on fruit. Therefore, when the concentration of the enzyme increases, color change will be evident, mainly changing to brown. [55]. It should be noted that POD was negatively correlated with a*, (+, red; −, green), which is most likely due to the ripeness of the fruit being more of a red color than a green color. With POD making the overall color of the sample more brown, this would decrease the red hue, resulting in an inverse relationship between these two attributes.

### *3.3. Peroxidase Enzyme (POD)*

Among the calabaza genotypes, there was a narrow range (1.5–1.8 abs/min) of observed POD activity, as seen in Table 5. The enzyme activity observed for these calabaza genotypes were relatively high when compared to values found in literature (0.9–1.1 abs/min) [38,56]. This could be due to plant stress [57], level of fruit maturity [58], and days in storage [59]. Peroxidase enzyme, unlike polyphenol oxidase, is primarily known for its browning effect on color [60,61]. This relationship is supported with the finding shown in Table 4, where the primarily strong correlations present were for color-based parameters as previously discussed. It should be noted that POD was also negatively correlated with "springiness", a*, and SSC/TA. Springiness is defined as the ability of a food sample to recover its original height after being compressed between the time of the first and second compression [62]. Previous research on *C. maxima* shows that springiness is related to the moisture and fibrousness of the squash sample, which is also strongly correlated with ripeness [63]. As previously described (Section 2.6), a* is the color range from red (+) to green (−). With a* values being an indicator for fruit ripeness within the *C. moschata* species, a negative correlation with POD would support the fact that the higher the concentration present, the lower the a* value and therefore undesirable fruit ripeness.

**Table 5.** Titratable acidity (TA) (g/L), malic acid (g/100 g), °Brix/soluble solids content (g/L), SSC/TA ratio, peroxidase (POD) (abs/min), and yeast fermentable extract (YFE) for calabaza germplasms. Plant Science Research and Education Unit in Citra, Florida. 2022.

| Cultivar | Malic Acid (mg/100 g) | pH | °Brix (g/100 g) | SSC/TA | POD (Abs/Min) | YFE % |
|---|---|---|---|---|---|---|
| Waltham Butternut | 0.17 ± 0.01 $^{a}$ | 5.98 ± 0.01 $^{f}$ | 11.9 ± 0.12 $^{b}$ | 66.43 ± 1.58 $^{e}$ | 1.71 ± 0.09 $^{a}$ | 66.19 ± 0.65 $^{bc}$ |
| UFTP 8 | 0.09 ± 0.01 $^{d}$ | 6.58 ± 0.01 $^{a}$ | 11.6 ± 0.12 $^{c}$ | 127.83 ± 7.56 $^{bc}$ | 1.59 ± 0.11 $^{a}$ | 78.47 ± 0.67 $^{a}$ |
| UFTP 22 | 0.15 ± 0.01 $^{b}$ | 6.12 ± 0.02 $^{e}$ | 12.2 ± 0.15 $^{a}$ | 76.66 ± 0.72 $^{de}$ | 1.49 ± 0.17 $^{ab}$ | 60.80 ± 0.06 $^{d}$ |
| UFTP 24 | 0.08 ± 0.01 $^{e}$ | 6.16 ± 0.02 $^{d}$ | 10.7 ± 0.15 $^{e}$ | 134.29 ± 7.10 $^{ab}$ | 1.70 ± 0.16 $^{a}$ | 67.19 ± 0.27 $^{b}$ |
| UFTP 38 | 0.05 ± 0.01 $^{g}$ | 6.34 ± 0.01 $^{c}$ | 7.3 ± 0.01 $^{g}$ | 141.46 ± 12.8 $^{a}$ | 1.26 ± 0.03 $^{b}$ | 62.19 ± 0.19 $^{d}$ |
| UFTP 42 | 0.06 ± 0.01 $^{f}$ | 6.40 ± 0.01 $^{b}$ | 8.2 ± 0.06 $^{f}$ | 122.91 ± 4.22 $^{c}$ | 1.55 ± 0.10 $^{a}$ | 60.53 ± 0.04 $^{d}$ |
| Soler | 0.07 ± 0.01 $^{f}$ | 6.36 ± 0.01 $^{c}$ | 6.2 ± 0.06 $^{h}$ | 87.34 ± 1.65 $^{d}$ | 1.76 ± 0.09 $^{a}$ | 67.81 ± 2.41 $^{b}$ |
| La Estrella | 0.13 ± 0.01 $^{c}$ | 6.17 ± 0.02 $^{d}$ | 11.2 ± 0.15 $^{d}$ | 81.78 ± 3.71 $^{d}$ | 1.76 ± 0.27 $^{a}$ | 64.72 ± 0.51 $^{c}$ |

Note. A total of 10–15 squash for each genotype were measured to determine average values. Letters compare means in the same column. Different letters correspond to significant change ($p < 0.05$) by Duncan's multiple range test.

### *3.4. °Brix*

The °Brix values across the calabaza genotypes ranged between 6.2 and 12.2, with a mean of 9.9 (Table 5). The observed values are within the range present in existing literature (6.3–15), affirming that the °Brix measured is within an acceptable range [26,64,65]. Notably, UFTP 22 had the highest °Brix value (12.2) which may indicate this germplasm line has a higher perceived sweetness for consumers as °Brix is considered a reasonable measure of sugar content and the overall evaluation of fruit quality [66]. It should be noted that variation in °Brix is primarily genetic [67]; however, several other factors can influence this trait, including growing conditions [68], and optical activity, such as pectin, amino acids, fiber, and organic acids compounds impacting measurement [69,70].

Correlations between °Brix and many other tested parameters are shown in Table 4, with positive correlations including TA, a*, b*, chroma, firmness/hardness, cohesiveness, gumminess, chewiness, and resilience. °Brix concentration in relation to organic acid concentration has been used as an indicator for fruit and vegetable ripeness for quite some time [71]. This would therefore support the strong correlation (0.78) between °Brix and TA.

### *3.5. Titratable Acidity (Malic Acid), and pH*

Previous studies have shown that the butternut squash contains malic, citric, fumaric, ascorbic, and gallic acid [49,72,73]; however, the most prevalent acid among these was malic acid [38,41]. The reported malic acid concentration for *C. moschata* ranges from 0.16–0.28 mg/100 g (Table 5) [38]. This range is within that observed (0.05–0.17 mg/100 g) in the current study (Table 5). The germplasm line UFTP 22 and cultivars Waltham Butternut and La Estrella were the three highest malic acid concentrations, 0.15, 0.17, and 0.13, respectively. To account for the range and lower concentrations within this study, previous research has shown that acid concentrations are expected to be influenced by the cultivar genetics [74] and growing region [73]. These higher TA concentrations strongly negatively impact the germplasm lines SSC/TA ratio (−0.84). However, the SSC/TA ratio is not a catch-all for consumer acceptability, as it has been shown to be cultivar-dependent [75].

Fruit pH is an important fruit-quality parameter due to influences on color [76], microbial growth [77], and change during storage [72]. Previous studies in *C. moschata* showed a pH range between 5.3–7.79. The values for pH observed in the current study (5.98–6.58) are within the range (5.3–7.79) of those previously reported for *C. moschata* [26,38,41,50].

Previous literature suggests that titratable acidity does not directly correlate to the pH of the sample in all cases [78]. Papnov et al. (2021) previously showed that pH and TA (malic acid equivalent) had no correlation (0.04) while Zinash et al. showed a strong negative correlation (−0.86) between the two measurements [41,79]. Based on the data presented in Table 4, TA (as malic acid equivalent) and pH were strongly negatively

correlated for the fruits in this study. Acids in fruits of some species, such as tomatoes, have been shown to decrease acid concentration through respiration only [80]. This could mean that the germplasm lines, which are currently being compared, could have varying degrees of ripeness. It should be noted that TA and pH should have a negative correlation as the increase of organic acids would lead to a decrease in pH values. Therefore, the variation in concentrations and inversely proportional relationship between pH and TA is to be expected. Additionally, pH was shown to be positively correlated with the SSC/TA ratio which follows the same logical reasoning as the negative correlation between pH and TA.

### *3.6. Yeast Fermentable Extract (YFE)*

The YFE for the calabaza genotypes showed an initial extract range (9.92 ± 2.24) and a final extract range (1.92 ± 0.68) yielding a fermentable extract range of 60.5–78.5% (Table 5). The concentration of YFE for *C. moschata* has not been previously reported. However, this measurement is important to quantify as it reflects the amount of simple (fermentable) sugars affecting the consumer's perceived sweetness [81,82]. Furthermore, YFE has been shown to be a reliable indicator for fermentability of produce [83]. It should be taken into account that yeast strain, fermentation temperature, enzymes, and yeast nutrients have all been shown to impact fermentation [84]. Interestingly, UFTP 22 had the highest °Brix values (12.2) but had one of the lowest YFE% of 60.8. Comparing this to UFTP 8 which had the highest YFE% of 78.5 with a relatively high °Brix value of 11.6. This is supported by the correlation between °Brix and YFE (0.23) not being significant. This distinction between °Brix and YFE is important to assess as it can demonstrate the difference in market value potential between these germplasm lines for application-based purposes as described in the introduction. When comparing YFE to the °Brix values within a germplasm line, one can extrapolate that the UFTP 8 contains the highest concentration of simple sugars and is therefore likely to be most efficient for fermentation. The opposite is true for UFTP 42 and UFTP 22.

### *3.7. Texture Profile*

The texture profile for each calabaza germplasm line was determined with average values and statistical differences for each textural attribute presented in Table 6. All textural measurements (firmness/hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience) showed some statistically significant differences between germplasm lines tested, but this range of values was not wide. This is most likely due to the genotypes' similarity to one another but could also be due to storage time [85], and sampling location [38]. Firmness/hardness had statistically significant extremes with UFTP 8 having the highest firmness/hardness value (40,323 g) and UFTP 38 having the lowest firmness/hardness value (22,027 g). Gumminess was one of the most variable textural parameters with Soler having the lowest gumminess (ease of swallowing) but not being statistically significantly different than UFTP 38, and UFTP 42. As previously described, firmness/hardness is a strong indicator of fruit ripeness [54]; however, variation in texture parameters is to be expected based on genetics [61,63].

Concentration of compositional elements varied slightly, as seen in Table 5, but these ranges did not seem to impact or strongly correlate with textural parameters–excluding °Brix and YFE, as seen in Table 4. YFE is a measurement of the amount of fermentable sugars present within a sample while °Brix is a measurement of the total amount of soluble solids (starch, sugar, and fiber) suspended in the blended sample. Therefore, it would be expected to see some similarities between °Brix and YFE correlations, as YFE is part of the °Brix measurement.

**Table 6.** Firmness/hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience for each calabaza germplasm by using a double compression texture test (TA.XT). Plant Science Research and Education Unit in Citra, Florida. 2022.

| Cultivar | Firmness/ Hardness (g) | Adhesiveness (g/Sec) | Springiness | Cohesiveness | Gumminess | Chewiness | Resilience |
|---|---|---|---|---|---|---|---|
| Waltham Butternut | 33,009 ± 16,040 $^{bc}$ | −47.74 ± 56.6 $^{bc}$ | 0.52 ± 0.18 $^{cd}$ | 0.21 ± 0.018 $^{d}$ | 9237 ± 10,579 $^{bcd}$ | 4885 ± 5837 $^{bc}$ | 0.11 ± 0.11 $^{bc}$ |
| UFTP 8 | 40,323 ± 6993 $^{a}$ | −47.99 ± 25.2 $^{a}$ | 0.51 ± 0.12 $^{d}$ | 0.38 ± 0.012 $^{a}$ | 15,792 ± 6661 $^{a}$ | 8154 ± 3701 $^{a}$ | 0.20 ± 0.07 $^{a}$ |
| UFTP 22 | 32,841 ± 5455 $^{bc}$ | −16.58 ± 17.2 $^{a}$ | 0.65 ± 0.09 $^{a}$ | 0.32 ± 0.09 $^{abc}$ | 10,730 ± 4405 $^{bc}$ | 7104 ± 3189 $^{ab}$ | 0.16 ± 0.05 $^{ab}$ |
| UFTP 24 | 35,974 ± 5061 $^{ab}$ | −37.71 ± 36.4 $^{abc}$ | 0.50 ± 0.09 $^{d}$ | 0.35 ± 0.09 $^{ab}$ | 12,725 ± 4782 $^{ab}$ | 6420 ± 2629 $^{ab}$ | 0.18 ± 0.05 $^{a}$ |
| UFTP 38 | 22,027 ± 5103 $^{d}$ | −47.99 ± 19.0 $^{bc}$ | 0.55 ± 0.09 $^{bc}$ | 0.24 ± 0.09 $^{cd}$ | 5628 ± 3168 $^{de}$ | 3207 ± 2123 $^{cd}$ | 0.11 ± 0.04 $^{bc}$ |
| UFTP 42 | 26,515 ± 6910 $^{cd}$ | −25.38 ± 29.8 $^{ab}$ | 0.58 ± 0.12 $^{b}$ | 0.25 ± 0.12 $^{bcd}$ | 7234 ± 5435 $^{cde}$ | 4360 ± 3630 $^{bcd}$ | 0.12 ± 0.06 $^{bc}$ |
| Soler | 22,541 ± 3943 $^{d}$ | −41.81 ± 12.35 $^{abc}$ | 0.52 ± 0.05 $^{cd}$ | 0.16 ± 0.05 $^{d}$ | 3665 ± 1436 $^{e}$ | 1859 ± 646.6 $^{d}$ | 0.07 ± 0.02 $^{c}$ |
| La Estrella | 36,432 ± 4524 $^{ab}$ | −61.81 ± 21.3 $^{c}$ | 0.49 ± 0.11 $^{d}$ | 0.32 ± 0.011 $^{abc}$ | 11,595 ± 4685 $^{abc}$ | 5793 ± 2570 $^{abc}$ | 0.16 ± 0.07 $^{ab}$ |

Note. $n$ = 12 for the number of measurements from a representative sample of 10–15 tropical squash. Letters compare means in the same column. Different letters correspond to significant change ($p < 0.05$) by Duncan's multiple range test.

ºBrix was positively correlated with all textural measurements except adhesiveness and springiness, which have almost no correlation at (0.01 and 0.06, respectively). Adhesiveness is the amount of force required to remove the food sample from the probe it was in contact with, also known as "stickiness" [86]. Both adhesiveness and springiness are related to the amount of moisture within a food sample [63], which could decrease the concentration of sugars and other soluble solids impacting texture. As previously discussed, firmness/hardness and many other texture parameters are impacted during the ripening process of fruits, which corresponds to an increase in ºBrix [54], supporting the strong correlations between ºBrix and all other textural measurements.

YFE was positively correlated with firmness/hardness and gumminess, as seen in Table 4. Gumminess is determined by multiplying the firmness/hardness of a sample by its cohesiveness [84]. Cohesiveness is a food sample's ability to resist a second deformation compared to the first deformation due to compression [86]. With both textural parameters being positively correlated and related to firmness/hardness, it can be reasonably assumed that as the fruit ripens, the sugar concentration and firmness/hardness parameters would increase. Additionally, springiness was negatively correlated with YFE. As previously described, both adhesiveness and springiness are related to the amount of moisture within a food sample [63], which could decrease the concentration of sugars and other soluble solids within a sample. As YFE is only the measurement of fermentable sugars, it would be more impacted by the dilution of sugars than ºBrix, resulting in this negative correlation.

## 4. Conclusions

The current study found significant differences among the eight calabaza genotypes tested which most notably includes, L*, a*, b*, hue angle, chroma, malic acid, ºBrix, YFE, and firmness/hardness. UFFP 22 had the highest ºBrix (12.23), while UFTP 8 had the highest YFE (78.5) and pH (6.58). The Waltham Butternut had the highest TA expressed as malic acid (0.11) concentrations, while Soler and La Estrella had the highest POD enzyme activity (1.76 abs/min). Generally, calabaza genotypes UFTP8 and UFTP 22 had superior quality attributes (high ºBrix, high chroma, and low POD) compared with the commercially available cultivars tested (Waltham Butternut, La Estrella, and Soler). While UFTP 38 and Soler had lower-quality attributes (low ºBrix, low chroma, and high POD), two of the germplasm lines grown in Florida were shown to have superior attributes based on color, sweetness, and texture compared to commercially available cultivars. Furthermore, ºBrix was shown to have strongly positive correlations among other tested parameters, including TA, malic acid concentration, b*, chroma, firmness/hardness, cohesiveness, gumminess, chewiness, and resilience. Chroma was also strongly positively correlated with many parameters including ºBrix, firmness/hardness, cohesiveness, gumminess, chewiness, and resilience. For growers, the correlations which ºBrix and chroma have with other quality attributes could be taken to account for much of the quality variability within a squash

germplasm. These genotypes may provide diversity in the calabaza cultivars available for commercial production in the tropics and subtropics.

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