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PHYSICOCHEMICAL PROPERTIES OF GULUPA FRUITS (*Passiflora edulis* Sims) DURING PRE AND POSTHARVEST

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Key words: Tropical fruits, carbohydrates, organic acids, nonlinear models

ABSTRACT

The so-called “high-andean fruits” constitute an ample group of species typically found in elevated tropical areas. Regarded as pleasant products, they are currently receiving growing acceptance, not only as supplementary sources of healthy metabolites, but also due to their variety, colorfulness and dietary contribution. Provided that the short postharvest life of these fruits - which include gulupa (*Passiflora edulis* Sims) - affects their quality, the physiological processes that allow understanding their metabolism and preserving their quality had not been studied yet. In this context, the current research work addressed the evolution of the factors that determine gulupa fruit quality under the conditions of the Colombian Lower Montane rain forest (LM-rf). After labeling the flowers, fruit age was measured as days after flowering (DAF), in order to conduct weekly destructive samplings that allowed pre and postharvest monitoring and analysis. The results showed that the total soluble solid content (TSS) of the fruit juice increased constantly, while pH showed a stable trajectory and Titrable Acidity (TA) started decreasing when the fruit reached the ninth week of age; hence, the Maturity Index (TSS/TA) increased since 63 DAF. Since 91 DAF, sucrose levels were found to decline, while those of glucose and fructose increased. Citric acid was found to be dominant in the fruit pulp, and tended to decline since 91 DAF. Malic and ascorbic acids followed a similar but weaker trend. Non-linear models were adjusted to describe TSS and TA variations.

PROPIEDADES FISICOQUÍMICAS DE FRUTOS DE GULUPA (*Passiflora edulis* Sims) EN PRECOSECHA Y POSCOSECHA

Palabras clave: Frutos tropicales, carbohidratos, ácidos orgánicos, modelos no lineales.

RESUMEN

En el trópico alto, se ha reconocido un amplio grupo de especies frutales que se denominan “frutales alto-andinos”, considerados frutos agradables, que ~~y~~ gozan de una creciente acogida por su variedad, colorido y aporte dietario, así como por ser fuente suplementaria de metabolitos que ayudan a mantener una buena salud. Estos frutos tienen corta vida ~~en~~ de poscosecha, con un rápido deterioro en esta fase, lo cual incide en la conservación de su calidad. La gulupa (*Passiflora edulis* Sims) forma parte de dicho grupo y en ella se desconocen los procesos fisiológicos que permiten entender su metabolismo, con el fin preservar la calidad del fruto. Se estudió la evolución de las variables que determinan la calidad del fruto en condiciones del bosque húmedo montano bajo (bh-MB) colombiano. Se marcaron flores y se contabilizó la edad del fruto como días después de floración (DDF), para realizar muestreos destructivos semanales, que permitieran el seguimiento y realización de los análisis en precosecha y poscosecha. Los resultados mostraron que el contenido de sólidos solubles totales (SST) del jugo se incrementó constantemente, el pH presentó una evolución con trayectoria estable y la acidez titulable (AT) disminuyó a partir de la novena semana de edad del fruto por lo que el Índice de Madurez (SST/AT) aumentó a partir de los 63 DDF. Se redujo la sacarosa después de los 91 DDF, mientras que la glucosa y la fructosa aumentaron en los días siguientes. El ácido cítrico resultó ser predominante en la pulpa del fruto, con tendencia a disminuir después de los 91 DDF; lo mismo sucedió, pero con menor intensidad, con los ácidos ascórbico, málico y oxálico. Se ajustaron modelos no lineales que lograron describir las variaciones de los SST y la AT.

INTRODUCTION

The consumption of fruits and vegetables is currently increasing globally, not only because of the vitamins, minerals and fiber they contribute, but also due to their nutraceutical properties. This has also been motivated by a growing concern about a balanced diet containing lesser amounts of carbohydrates, oils and fats, and a greater contribution of vegetables and fruits; by the lower caloric needs of modern life, featured by increasing comfort and sedentarism; and by a higher consciousness of the importance of nutrition for health and longevity (López, 2003).

Generally regarded as pleasant foods, the so-called "high-andean fruits" are highly demanded by the consumer. Indeed, a broad group of these species – which includes "gulupa", showing commercial development possibilities, has been identified in the Andean region. However, their short postharvest life and rapid physiological deterioration processes at this stage negatively affect their quality. This makes it necessary to conduct studies on the physical, physiological, and biochemical progress of these fruits, in order to make better use of their properties, cope with the lack of technological offer during postharvest, and contribute to the improvement of the manipulation techniques that can be achieved through a better understanding of the fruit ripening biochemistry (Jaramillo *et al*., 2000).

The evolution of fruit components, during ripening and postharvest, has been studied in numerous species from different perspectives. Among them we can count tree tomato (*Cyphomandra betacea* Cav Sendt), blackberry (*Rubus* spp.), guava (*Psidium guajava* L.), pineapple, (*Ananas comosus* L.) pitaya (*Hylocereus undatus* (Haworth) Britton & Rose), melon (*Cucumis melo* L.), yellow passion fruit (*Passiflora edulis* Sims var. flavicarpa), black zapote ~~negro~~ (*Diospyros digna* Jacq), lulo (*Solanum quitoense* Lam) and rambutan (*Nephelium lappacerum* L.), among others. All these studies have intended to understand fruit behavior during postharvest, in order to maintain consumption quality and make good use of their nutraceutical properties (Araújo *et al.,* 1997; Silva and Mercadante, 2002; Ordóñez *et al.,* 2005; Villanueva *et al.,* 2004; Arellano *et al.,* 2005; Kafkas *et al.,* 2006; Menéndez *et al.,* 2006; Márquez *et al.,* 2007; Saradhuldhat and Paull, 2007; Centurión *et al.,* 2008; Yingsanga *et al.,* 2008). Nonetheless, there are still many fruits with economic potential in tropical areas about which little information is available, resulting in deficient postharvest management and elevated losses at this step of the productive chain.

Dhawan *et al*. (2004) conducted a review of the genus *Passiflora,* in which they addressed the morphology, microscopy, traditional uses, phytoconstituents, pharmacological data and medicinal and toxicological applications of these plants. Regarding *Passiflora edulis* Sims, they mention, in the fruits, the presence of glycosides, phenols, alkaloids, carotenoids, L-ascorbic acid, anthocyanins, lactones, aromas, volatile oils, amino acids, carbohydrates, minerals, enzymes and triterpenes. In turn, Pruthi (1963) established that ripe purple passion fruits (*Passiflora edulis* Sims) harvested from the same orchard every two weeks showed highly significant differences regarding all physicochemical characteristics, except for reducing sugars. This author asserts that the chemical composition of this fruit is certainly changeable as affected by variety, ripening stage, plant condition, harvest time, climate and cultivation site, among others.

After a fruit has been harvested, it stops receiving the normal water, mineral and organic molecules (*e.g.,* sugars and hormones) supply. Nevertheless, most fruit tissues are capable of transforming available components through physiological processes that can be beneficial or harmful to product quality. Furthermore, the intensity of these processes determines the fruit's postharvest life span. Growth and physiological ripening are completed when the fruit remains attached to the plant, but organoleptic ripening and further senescence may continue after harvest (Wills et. al., 1984; Haard, 1985).

In one study on gulupa fruits, from the municipality of *Venecia* (Cundinamarca, Colombia), Pinzón *et al*. (2007) characterized the product through a seven-degree color scale, of which they recommend stage 3 as the optimum harvest moment. Shiomi *et al*. (1996b) propose harvesting at the half ripe stage and, to place the fruit under low-temperature, which allows long-distance commercialization, including exportation. In this case, ethylene application, in the place of destination, several days after harvest could be used to reach the fruit skin color that is required for final consumption commercialization.

In gulupa, total soluble solids (TSS) have been observed to increase constantly until reaching their maximum value (15.91 ºBrix) at ripening stage 3 (Pinzón *et al*., 2007). In Kenya, Shiomi *et al*. (1996a) found this value at 80 DAF, which was reported by Rodríguez and García (2010), in Colombia, to take place at 17 DAF. According to the former authors, TSS content in the juice of this fruit increases constantly since 20 DAF and until ripening, to reach values between 14 and 17% during postharvest, after which it declines (Shiomi *et al*., 1996a). Sugars, especially sucrose, are transported to the cells of the plant's organs for the synthesis of cellulose, starch and other polysaccharides. Among the latter, celluloses, hemicelluloses, pentoses and other pectic substances constitute the structural materials that wrap starch, fructans, sucrose and hydrolysis product reserves (Wills *et al*., 1984; Hodge and Osman, 1985; Belitz and Grosch, 1997; Primo, 1998).

Several authors have observed how in gulupa juice pH follows an increasing trend, shifting from 2.99 to 3.6, depending on the ripening stage. Contrarily, acidity (measured as percentage of citric acid) starts declining 60 days after anthesis and reaches values of 3.92 at the moment of harvest (Shiomi *et al.,* 1996a; Pinzón *et al.,* 2007; Rodríguez and García, 2010). In yellow passion fruit, pH remains constant and acidity declines (Menéndez *et al.,* 2006).

Fruit acidity usually originates in the organic acids that are chiefly stored in vacuoles (Leshem *et al*., 1986); corresponding, basically, to citric and malic acids, those making up the mature fruit's taste and aroma increase during ripening (Srivasta, 2002). Their accumulation might result from the capture of intermediate components of the tricarboxylic acid cycle during the process of CO_2 fixation in the darkness; from amino acid dis-amination and probably from their mobilization from other parts of the plant. The levels of these components usually decline during ripening, probably due to their utilization in respiration (Leshem *et al*., 1986). In climacteric fruits most of the stored starch turns into sugars during ripening. The enzymatic processes associated to ripening lead to increased sugar levels. After harvest, ripe fruits sweeten due to the presence of sucrose and other sugars, obtained from starch reserves. In general, fruits and vegetables store more glucose and fructose than sucrose. Plant tissues also present other sugars such as xilose, mannose, arabinose, galactose, maltose, sorbose, octulose and celobiose. Depending on the species, the variety and the degree of ripeness, sugar concentrations vary from 5 to 18% (Wills *et al*., 1984; Haard, 1985; Primo, 1998).

In gulupa fruits harvested when unripe (*i.e.,* just initiating to show purple color), sucrose content is 2.5 times that of glucose or fructose, but this proportion is gradually inverted later on (Shiomi *et al.,* 1996a; Pinzón *et al.,* 2007; Rodríguez and García, 2010; Cruz *et al.,* 2010). During ripening, glucose and sucrose levels are similar (Shiomi *et al.,* 1996b). In yellow passion fruits, Menéndez *et al*. (2006) observed an increase in TSS due to the hydrolysis of structural polysaccharides, while sucrose content dropped and glucose and fructose increased, especially the former.

During the ripening of gulupa fruits, the organic acids detected by Shiomi *et al*. (1996b) were citric and malic ones, the concentration of the former being eight times higher than that of the latter, thus confirming a previous report on yellow passion fruit (Menéndez *et al*., 2006). Contrasting with the results of the former authors, Cruz *et al*. (2010) studied several ripening stages in yellow passion fruit, recording elevated levels of citric and malic acids at 0% ripening, while at 50 and 100% ripening oxalic and ascorbic acids were dominant. Just as well, they found that TA in "gulupa" peaked at 60 DAF, followed by a rapid decrement, thus indicating that consumption quality is not obtained before 60 DAF. In this same fruit they found the ripening index to range from 2.08 to 4.34, corresponding to stages zero and six, respectively. These values are similar to those reported by Pinzón *et al*. (2007).

In this context, the objective of the present study was to observe the evolution of the physicochemical factors determining gulupa fruit quality under conditions of the Colombian Lower Montane rain forest (LM-rf), thus opening the possibility to make better use of the properties of this crop.

MATERIALS AND METHODS

An experimental crop was planted in the municipality of *Rionegro,* department of Antioquia (Colombia), at *La Selva* Research Center, which belongs to *Corporación Colombiana de Investigación Agropecuaria – Corpoica.* The experimental site is located at 2090 masl, registering the following yearly average environmental conditions: temperature, 17 ºC; precipitation, 1917 mm; relative humidity (RH), 78%; sunshine, 1726 hours/year; and evapotranspiration, 1202 mm. The ecological life zone corresponds to a LM-rf. The instrumental work was conducted at the Laboratory of Food Science of *Universidad Nacional de Colombia*, Medellín campus; and at the Quality Analysis Laboratory of *Corpoica*.

The studied plant material included 10 gulupa accessions from the departments of Antioquia, Putumayo and Nariño (Colombia), obtained from the Germplasm Bank of the Colombian Nation, which is managed by *Corpoica*. According to Ortiz *et al*. (2012), the material in question exhibits low genetic variability as determined through AFLPs (Amplified Fragment Length Polymorphisms) and SSRs (Simple Sequence Repeats). The fruits were obtained by selecting flowers at the homogamous phase, with and without herkogamy, and these were marked with colored threads, according to Ángel *et al*. (2011). These stages were taken as day zero of fruit age, based on which sampling time was determined as DAF.

All analyses included destructive samplings every seven days, starting at 49 DAF and continuing until 112 DAF. For the study of the physiological evolution of the fruits during postharvest, they were gathered at 91 DAF to conduct measurements every seven days until

21 days after harvest, thus corresponding to 98, 105 and 112 DAF. This allowed comparing fruits ripening on and off the vine. The latter were stored at 20 ºC and 70% RH. The sampling unit, at each age, was composed by were 10 fruits taken at random from the studied materials, thus defining balanced and independent samples. The fruits were transported in Styrofoam boxes containing dry ice at an approximate inner temperature of 4 ºC. Juice was obtained by cutting the fruits along their equatorial zone and separating the shell from the pulp with a spatula. The seeds were then set apart from the pulp, which was sieved through a piece of tulle cloth. The juice was kept in an (externally) ice-cooled recipient. In order to measure sugars and organic acids, the juice of 10 fruits was mixed to obtain three sub-samples for analysis. The following parameters were studied:

Physicochemical properties

Total soluble solids (TSS). They were determined under Colombian Technical Norm NTC 4624 (Icontec, 1999). One drop of the juice, obtained from the fruit pulp, was placed on the prism of the refractometer (Milton Roy Company®). The measurement was read in ºBrix. Temperature correction was carried out at 20 ºC.

pH. It was assessed in a Schott Gerate® pH meter model C6820.

(TA). It was determined with a pH meter (AOAC, 2005). The result was expressed as percentage of citric acid.

Soluble sugars.

Sucrose, glucose and fructose contents were determined through high pressure liquid chromatography (HPLC), following a modified version of the protocol of Eyeghé *et al*. (2012). The aqueous extract was filtered (0.45 µm pore size) and diluted in supra-pure water before injecting it into the chromatographer. The measurement was conducted on a Shimadzu® chromatographer model LC-20AD, equipped with a SIL-20A/HT autoinjector, a CBM-20A communication module and a Refractive Index Detector (RID). Sugar quantification was carried out on a BIORAD (Aminex HPX-87H, 300 mm X 7.8 mm) column. Five mM sulphuric acid was used as mobile phase. At 20 ºC and under isocratic conditions, the flow rate of the mobile phase was 0.6 mL min^{-1}. Compound identification and quantification was done by preparing calibration curves for peak areas.

Organic acids

Citric and malic acid content determination followed methodology by Kelebeck *et al.* (2009), resorting to HPLC analysis. The aqueous extract was filtered (0.45 µm pore size) and treated by several dilutions in supra-pure water before its injection into the chromatographer. This procedure made use of a Shimadzu® chromatographer model LC-20AD equipped with a SIL-20A/HT autoinjector and a CBM-20A communication module, together with a (PDA) SPD-M20A detector calibrated at 210 nm. Analytical conditions were 0.3 mL min^{-1} flow, 0.045 NH_2SO_4 eluent with 6% acetonitrile (v/v). Identification and quantification was done by preparing calibration curves for peak areas.

The content of ascorbic acid was determined by HPLC, according to Kelebek *et al*. (2009). The aqueous extract was filtered (0.45 µm pore size) and treated by several dilutions in supra-pure water before its injection into the chromatographer, using a Shimadzu® chromatographer model LC-20AD equipped with a SIL-20A/HT autoinjector and a CBM-20A communication module, together with a (PDA) SPD-M20A detector calibrated at 245 nm. Ascorbic acid quantification was done in a C-8 (5 µm, 250 mm x 4.6 mm) column. Mobile phase was 0.1% formic acid. The flow rate of the mobile phase was 0.8 mL min^{-1}, at 35 ºC and under isocratic conditions. Compound identification and quantification was done by preparing a calibration curve for peak areas.

Statistical analysis

TSS and acidity progress were explained by adjusting the non-linear models reported by Kiviste *et al*. (2002): allometric, exponential, Terazaki, Korf, Gemesi, Korsun, Gram, Sloboda, Verhulst-logistica, Wingert, Pearl-reed, Simek, Moiseev III, Monomolecular-Weber, Todorovic III, Var der vliet, Kovessy, Thomasius I, Thomasius II, Bass, Gompertz, Gompertz-wenk, Mitscherlich I, Bertalanffy, Weibull II, Weibull II and Mitscherlich III. DAF were used as predictive variable. For each response, the model that offered better adjustment (*i.e.,* more homogeneous distribution of residuals, higher prediction determination coefficient [R^2pred] and lower Mean Square Error and statistic PRESS values) was selected. Equivalent samples coming from fruits on and off the vine were compared in terms of fruit components and chemical parameters through a t test that allowed the comparison of mean values.

RESULTS AND DISCUSSION

Physicochemical properties.

Juice TSS content was observed to increase steadily until reaching final values of 16 ºBrix (Figure 1a), thus coinciding with reports for gulupa by Shiomi *et al.* (1996a), Medina *et al.* (2000), Pinzón *et al.* (2007), Rodríguez and García (2010), Cruz *et al.* (2010), Jiménez *et al.* (2011) and Orjuela *et al.* (2011), and for yellow passion fruit by Menéndez *et al*. (2006). These authors cite 13 – 17 ºBrix values in fruits harvested when ripe, based on which Jiménez *et al.* (2011) considered that TSS is an important parameter when it comes to determining the ripening stage. It can also be observed that TSS contents in gulupa show intermediate values between those found in yellow passion fruit and granadilla (*Passiflora ligularis* Juss). The observed increment can be attributed to hydrolysis of polysaccharides such as starch and pectins, and other oligosaccharides that are present in the cell wall. The degradation of these compounds reaches their simplest components, which dissolve in the aqueous phase and become part of the fruit juice (Menéndez *et al.,* 2006). Just as well, the enzymatic processes associated to ripening lead to increased sugar contents. In this way, ripe fruits sweeten after harvest due to the presence of sucrose and other sugars resulting from starch reserves and from inter-conversion of the released sugars (Wills *et al*., 1984; Haard, 1985; Leshem *et al.,* 1986; Primo, 1998).

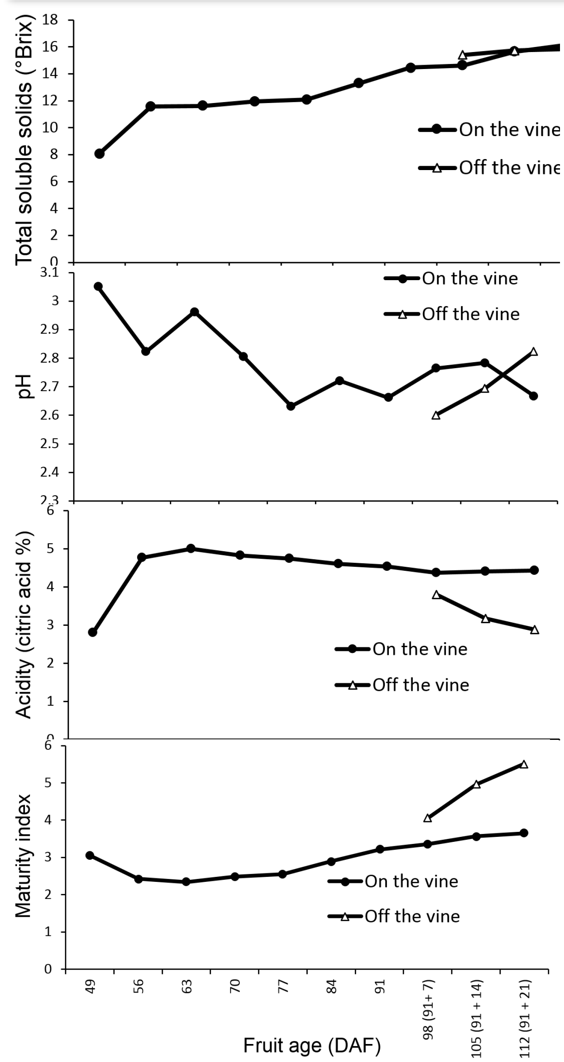

Figure 1. Evolution of TSS (a), pH (b), TA (c), and MI (d) in gulupa fruits (*Passiflora edulis* Sims) during their development and postharvest. Bars indicate standard deviation.

In gulupa, Pinzón *et al.* (2007) recorded the maximum TSS value (15.9 ºBrix) at color stage 3 (according to the scale they developed), whereas Shiomi *et al.* (1996a) observed a peak for this parameter at 80 DAF, and so did Rodríguez and García (2010) at 70 DAF. Villanueva *et al.* (1999) found that TSS content in yellow passion fruit tended to increase, as it went from a 9-11 ºBrix range at 42 DAF, to a 14-16 ºBrix range at around 70 to 84 DAF. In the current study, maximum TSS values were observed at 112 DAF in fruits on the vine (16.1 ºBrix), and at 21 DAF in fruits off the vine (15.9 ºBrix). The latter record is higher than those reported by Flórez *et al.* (2012) for different regions of Colombia. In other passifloras such as purple and yellow passion fruit, Arjona *et al.* (1991) have reported respective TSS concentrations of 12.9 and 15.2ºBrix, which are lower than those we found in gulupa. In turn, Pruthi (1963) quantified TSS values of 14.4ºBrix and 18.7ºBrix in unripe and ripe purple passion fruits, respectively, the latter being higher than the one found in the current study.

Postharvest TSS results are in agreement with those of Sierra *et al.* (2011), who reported a slight increase at this stage. Contrarily, Shiomi *et al.* (1996a) and Flórez *et al.* (2012) found TSS to decrease. According to Orjuela *et al.* (2011), increments in this parameter can be attributed to fruit carbohydrate reserve preservation. No differences were observed in this regard between fruits on and off the vine (Table 1), which guarantees fruit quality. TSS evolution over time was described through Korsun's model, with an R^2 pred value of 0.37 (Table 2 and Figure 2a).

Table 1. Mean comparisons of gulupa (*Pasiflora edulis* Sims) physicochemical parameters measured on juice samples obtained from same – aged fruits on and off the vine.

Fruit age (DAF) on and off the vine	Average pH		Average TSS (ºBrix)		Average acidity (% of citric acid)		Average maturity index	
	On the vine	Off the vine	On the vine	Off the vine	On the vine	Off the vine	On the vine	Off the vine
98 (91+7)	2.74a*	2.60 b	14.6 a	15.4 a	4.36 a	3.80 b	3.35 a	4.05 b
105 (91+14)	2.78 a	2.70 b	15.6 a	15.7 a	4.41 a	3.18 b	3.56 a	4.94 b
112 (91+21)	2.67 a	2.82 b	16.1 a	15.8 a	4.43 a	2.87 b	3.65 a	5.52 b

**Different letters on the same row indicate significant differences at α=0.01, according to the T-test.*

pH was observed to remain steady within an approximate 2.5 - 3.0 range (Figure 1b), as also indicated by Menéndez *et al.* (2006) in studying yellow passion fruit. In gulupa, other researchers have detected an increasing trend for this parameter, which was observed to shift from 2.5 to 3.6, depending on the ripening stage (Shiomi *et al.,* 1996a; Medina *et al.,* 2000; Pinzón *et al.,* 2007; Sierra *et al.,* 2011; Jiménez *et al.,* 2011; Flórez *et al.,* 2012 and Orjuela *et al.,* 2011). In the current study, pH oscillated between 2.6 and 2.8, thus falling within the reference framework provided by previous reports. The observed pH behavior corresponds to the explanation suggested by Menéndez *et al.* (2006) for yellow passion fruit, in the sense that there probably exists a self-regulation system for this parameter, operating by buffering citric acid which, indeed, tends to be transformed into its corresponding salt, thus remaining steady. Pruthi (1963) has recorded pH values ranging from 2.8 to 3.3 in purple and yellow passion fruit, which are certainly higher than those found in the present study. The comparison of means between fruits on and off the vine, revealed differences in pH values (Table 1), which were generally observed to be higher in the former than in the latter.

TA started to decline after week nine of fruit age (Figure 1c), as was also indicated by Leshem *et al.* (1986), Shiomi *et al.* (1996a), Rodríguez and García (2010) and Jiménez *et al.* (2011). Shiomi *et al.* (1996a) found the highest record for this parameter at 60 DAF, followed by a decrement. This indicates that, coinciding with the findings of the present study (wherein TA peaked at 63 DAF), fruit quality improves after reaching this turning point. The behavior of this parameter is explained by Wills *et al.* (1984) as a consequence of the consumption of organic acids during fruit respiration, or by their transformation into sugars, which allows considering them as one more of the energetic reserves of the plant. According to this, organic acid content is expected to decline during ripening, as it was observed in the current study.

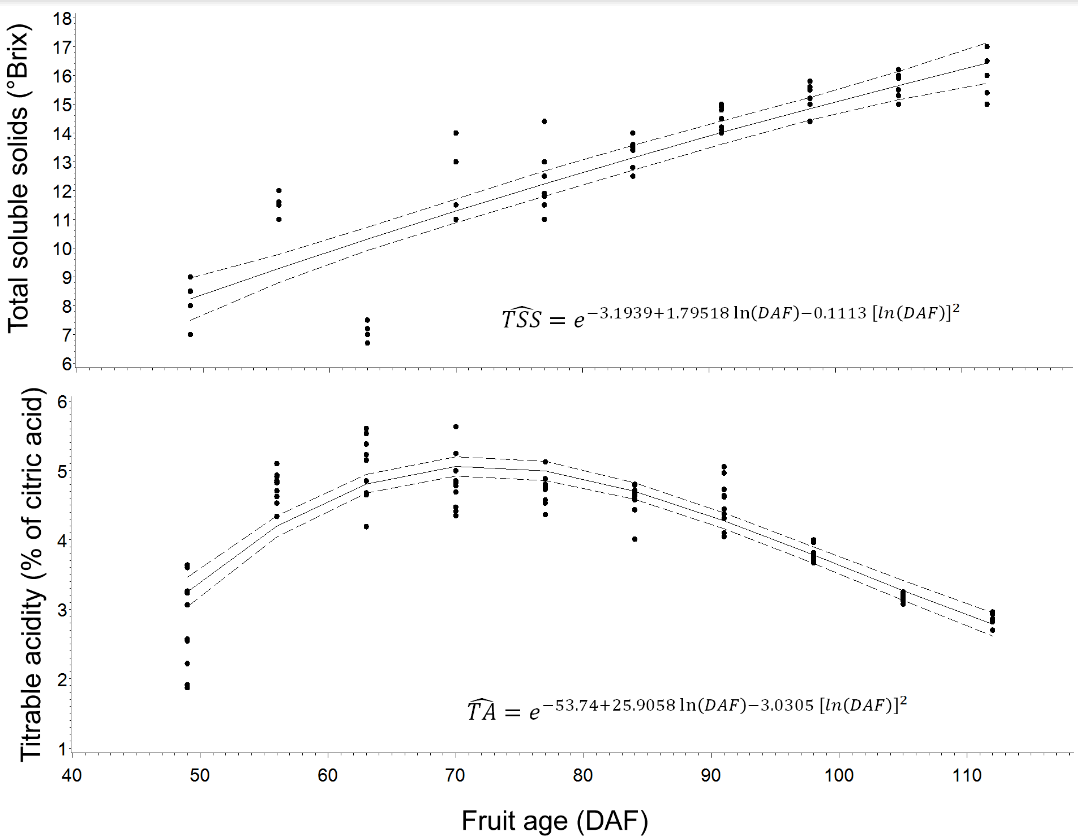

Figure 2. Evolution of TSS (a) and TA (b) in gulupa pulp (*Passiflora edulis* Sims), according to fruit age. The solid line corresponds to the function estimated by Korsun's model. The dotted lines represent the 95% confidence limits for the expected values. The circles correspond to observed measurements.

Table 2. Estimators of the parameters of Korsun's model $Y = e^{a+b\ln(DDF)-c\,[\ln(DDF)]^2}$ and goodness of fit statistics used for the chemical analysis of gulupa (*Passiflora edulis* Sims) fruits.

Variable	Model parameters	*PRESS* $=\sum_{i=1}^{n}\left(\frac{r_i}{1-h_{ii}}\right)^2$	*Mean Square Error*	R^2*pred* $=1-\frac{PRESS}{\sum_{i=1}^{n}(y_i-\bar{y})^2}$
TSS	a=-4.9386 b=2.7609 c=0.2386	83.4165	0.94442	0.84961
Acidity	a=-53.7400 b=25.9058 c=3.0305	18.9604	0.42476	0.75492

The fruits off the vine registered lower TA values than those on the vine (Table 1), thus corresponding with previous reports by Sierra *et al*. (2011), who observed fluctuations between 4.4% and 3.1% from days 7 to 21 after harvest; and by Shiomi *et al*. (1996a), who found lower values but still following a decreasing trend. Similarly, Flórez *et al*. (2012) concluded that postharvest acidity in gulupa declines with fruit age. In turn, Jiménez *et al*. (2011) consider this to be an important parameter for determining ripeness in gulupa, because of the higher acid contents recorded during this stage. The behavior of acidity over time was described through Korsun's model, with an R^2 prediction value of 0.75 (Table 2 and Figure 2b).

In studying yellow passion fruit, Villanueva *et al.* (1999) found that juice acidity increased until 55 DAF, as it went from an initial 5.0% – 5.4% range to a final 5.9% – 6.2% range. Thereafter, it followed an opposite trend, until reaching a 4.5 – 4.8% range by 84 DAF, which parallels the findings of the current study, wherein fruit acidity peaked at 63 DAF (5.0%) and then went down to 4.4%, around harvest time. This indicates that gulupa is less acid than passion fruit, as also reported by Pruthi (1963) and Villanueva *et al.* (1999), who observed values of 3.6% and 3.1% in unripe and ripe fruits, respectively. On the other hand, and showing consistency with García (2008), the acidity of gulupa was observed to be higher than that of other passifloras such as granadilla.

The maturity index (MI) was higher in fruits off the vine than in those on the vine (Table 1) at all evaluated stages of fruit development. This parameter went from 2.3 at 63 DAF to 3.6 in ripe fruits (Figure 1d). This is consistent with the findings of Pinzón *et al.* (2007), who found the MI of gulupa to vary from 2.1 to 4.3 (corresponding to stages 0 and 6, respectively, of the ripening scale they developed). A similar behavior was found by García (2008) in granadilla, which revealed a steady increase in this parameter, and by Villanueva *et al.* (1999) in yellow passion fruit. The sugar to acid balance observed in the current study confers gulupa an acid taste. Postharvest and preharvest behaviors were similar, but the former showed higher values, as also found by Flórez *et al.* (2012) and Orjuela *et al*. (2011). This is explained by changes resulting from the metabolic activity associated to fruit ripening. In this sense, Pruthi (1963) has found that the MI of purple passion fruit ranges from 3.4 to 7.7, and that in yellow passion fruit it goes from 1.9 to 3.0. According to these data, purple passion fruit is similar in taste to gulupa, while yellow passion fruit comes to be more acid, thus confirming a previous report by Villanueva *et al.* (1999), who calculated the MI of this fruit in 3.4 (ripe fruits) and 3.7 (postharvest). Defined as the TSS/TA ratio, the MI should increase over time, as it was observed, indeed, in this study.

Soluble sugars.

Sucrose peaked at 84 DAF (Figure 3a), close to the time of harvest, with an average value of 7,170 mg/100 g of fresh fruit (f.f.), which confirms the notion expressed by Haard (1985), Belitz and Grosch (1997), Srivastava (2002) and Leshem *et al.* (1986), in the sense that during ripening the fruit increases its sugar levels due to starch hydrolysis mediated by the action of invertase, or to additional supply from other parts of the plant. After the mentioned peak, sucrose declined, coinciding with an increase in reducing sugars, which is a normal event within the ripening process, during which sucrose is hydrolyzed to glucose and fructose (Haard, 1985; Belitz and Grosch, 1997 and Srivastava, 2002). In turn, this is related to the rise of TSS and the MI, coupled to a decrement in acidity. Along these lines, Haard (1985) and Belitz and Grosch (1997) utter that sucrose content varies depending on the tissue and the degree of ripeness of the fruit. Also in this sense, Wills *et al*. (1984), Haard (1985) and Primo (1998) state that the enzymatic processes associated to ripening increase the sugar levels of the fruit, which is the reason why ripe fruits sweeten after harvest, as they produce sucrose and other sugars from their starch reserves.

In unripe gulupa fruits harvested when they start showing purple hues, Shiomi *et al.* (1996b) found sucrose levels to be 2.5 times higher than those of glucose or fructose, resembling the results of the present study. On the other hand, the sucrose content decreased during maturation, with gradual increase of glucose and fructose (Shiomi *et al.,* 1996b; Cruz *et al.,* 2010). According to the work of Menéndez *et al*. (2006), the progress of sucrose in yellow passion fruit is similar to the one it shows in gulupa. These authors found that sucrose decreased while fructose, and particularly glucose, increased. Provided that Arjona *et al.* (1991) have reported higher levels of sucrose in yellow than in purple passion fruit, these concentrations are believed to be variety-dependent.

Flórez *et al.* (2012) found sucrose to be the most abundant sugar in 100% ripe gulupa fruits, with contents ranging from 5,424 to 6.590 mg/100 g of pulp. This result can be compared to the one obtained in the present study until 84 DAF (close to harvest), with a value of 7,170 mg/100 g of f.f. Since then, sucrose dropped to 423 mg/100 g of f.f., to finally reach concentrations below those of glucose or fructose, as confirmed by Menéndez *et al.* (2006) in yellow passion fruit. Sucrose content variation across different research works seems to be determined by environmental factors (*e.g.,* temperature) (Flórez *et al.,* 2012).

During postharvest, sucrose content showed a constant decrement (Figure 3a), to finally reach lower levels than the other reducing sugars. Contrarily, postharvest sucrose levels have been observed to be higher than those of the other reducing sugars both in gulupa (Shiomi *et al.,* 1996b) and purple passion fruit (Pruthi, 1963).

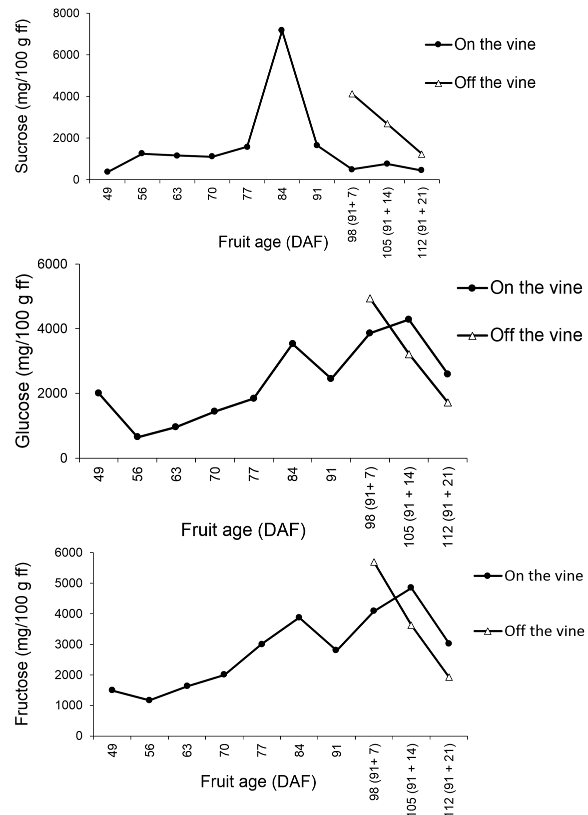

Figure 3. Changes in sucrose (a), glucose (b) and fructose (c) contents in the pulp of gulupa fruits (*Passiflora edulis* Sims) during their development and postharvest.

During preharvest, glucose levels showed two peaks (Figure 3b). The first one reached 3,530 mg/100 g of f.f., corresponding to the highest sucrose record. The second glucose peak started at 91 DAF, with an average concentration of 4.282 mg/100 g of f.f., thus opposing the trend exhibited by sucrose and paralleling that of fructose, as also reported by Arjona *et al.* (1991) in passion fruit. Glucose increase corresponded to a similar shift in both soluble solids and the MI, while acidity declined. Therefore, the ripening process can be said to increase fruit sweetness and reduce acidity. Around 105 DAF, glucose content went down, which indicates that fruit ripening on the plant enters in senescence at this age. As a consequence, harvest should take place around 84 DAF, and consumption within the next 14 days, in order to take advantage of the organoleptic quality of the fruit.

In the work of Flórez *et al.* (2012), glucose contents in 100% ripe fruits of gulupa oscillated between 1,162 and 1,184 mg/100 g of pulp, which is below the value recorded in the present study by 84 DAF (3,530 mg/100 g of f.f.). Thereafter, glucose kept increasing until 105 DAF (4,282 mg/100 g of f.f.), which confirms the aforementioned statement about harvest and consumption moments. As reported by the cited authors, our final glucose concentrations were below those of fructose, thus revealing a decreasing trend during postharvest and also confirming a previous report by Shiomi *et al.* (1996b).

Fructose showed an analog behavior to that of glucose (Figure 3c), as also stated by Shiomi *et al.* (1996b) in the sense that, during ripening, glucose and fructose contents are similar. The current results also correspond with those of Flórez *et al.* (2012), who observed the levels of fructose to be higher in ripe fruits. Contrarily, Menéndez *et al*. (2006) noted that in yellow passion fruit glucose rosed higher than fructose. Again paralleling glucose, fructose peaked by 105 DAF (4,837 mg/100 g of f.f., thus confirming the harvest and consumption recommendation made above for the former sugar), its increment accompanying a concomitant shift in TSS and the MI, as well as an acidity drop. Finally, fructose also declined during postharvest, as reported by Shiomi *et al.* (1996b), too. The reductions observed in all fruit sugars are associated to respiratory processes (Wills *et al*., 1984).

Organic acids.

At early fruit ripening stages, citric acid showed two concentration peaks, the first one by 77 DAF, with 0.097 mg/100 g of f.f., and the second one by 98 DAF, with 0.064 g/100 g of f.f. (Figure 4a). This confirms a previous report by Shiomi *et al.* (1996b), who observed citric acid concentration to be eight times higher than that of malic acid. In consequence, the former compound can be said to be dominant in gulupa, as it is in yellow passion fruit (Menéndez *et al.,* 2006) and purple passion fruit (Pruthi, 1963). General acid content varies depending on the place of origin and the ripening stage of the studied material (Flórez *et al.*, 2012). Regarding gulupa, these authors found citric acid values ranging between 2.8 and 5.4 g/100 g of pulp, which overcomes the records of the current study (about 0.064 g/100 g of f.f.). In yellow passion fruit, Cruz *et al.* (2010) observed elevated levels of citric and malic acids at the ripening, that they defined as 0% of maturation, which parallels the results of the present study.

During postharvest, citric acid declined in similar ways in both fruits on and off the vine. The behavior exhibited by the organic acids in the current study corresponds to the opinion of Wills *et al*. (1984) in the sense that, as they are respirable substrates and gulupa is a climacteric fruit (Flórez *et al*., 2012), they are likely to be used in respiration or to be transformed into sugars. Paralleling the results of the present study, citric acid levels in yellow passion fruits (stored without changing the surrounding environmental conditions) have been observed to undergo a slight drop with respect to the moment of harvest (Villanueva *et al*., 1999), which is attributed by these authors to the ripening process.

As presented in Figure 4b, malic acid exhibited a similar behavior to that of citric acid, but with lower concentrations all along the fruit development process (about 0.011 g/100 g of f.f.). This corresponds with the findings of Shiomi *et al*. (1996b), but contrasts with those of Flórez *et al*. (2012), in which malic acid is said to have increased within a broader range during ripening (0.35 to 0.66 g/100 g of pulp). In purple passion fruit, malic acid has also registered lower levels than citric acid (Pruthi, 1963). The decreasing trend exhibited by malic acid during postharvest has also been reported by Shiomi *et al*. (1996b) and, as well as that of other compounds, attributed to respiratory processes (Wills *et al*., 1984). Oxalic acid behavior was similar to those of citric and malic acids (Figure 4c), but showing values that were up to 77 times lower than those of citric acid, and 13 times lower than those of malic acid. The contents of this acid in gulupa fruits coming from contrasting environments ranged between 20.9 and 131.2 mg/100 g of pulp (Flórez *et al.,* 2012), which overcomes the findings of the present study. This compound was observed to decrease during postharvest both in fruits on and off the vine, although the latter registered somewhat lower values.

The progress of ascorbic acid through the fruit ripening process accompanied that of the other studied organic acids (citric, malic and oxalic) (Figure 4d), exhibiting higher levels than those of malic and oxalic acids, but lower than those of citric acid. Ranging between 42.6 and 43.5 mg/100 g of f.f., ascorbic acid peaked at 77 and 91 DAF, thus confirming again the aforementioned recommendations about the right time for the harvest and consumption of gulupa. Contrasting with the other organic acids, which we found in lower amounts than those recorded for this same fruit by Flórez *et al.* (2012), our assessment of ascorbic acid at the moment of harvest (42.6 mg/100 g of f.f.) overcame the ones presented by the mentioned authors (8.5 to 32.81 mg/100 g of pulp in 100% ripe fruits), as well as that of Medina *et al.* (2000) (28.5 mg/100 g of f.f.). Considered to be good sources of vitamin C, the passifloras, including gulupa, have good antioxidant power (Bliss, 2007).

At postharvest, ascorbic acid levels decreased for seven days in row since the moment of harvest, to increase thereafter until day 21, thus paralleling the behavior described by Flórez *et al. (*2012) in this same fruit. From their assessment of this parameter, in yellow passion fruit, Vasco *et al*. (2008) consider that their measurement (30 – 40 mg/100 g of fruit dry weight) is certainly elevated; but they mention how other passifloras such as curuba (*Passiflora mollisima* Bailey) contain even more ascorbic acid, whereas granadilla produces less. Pruthi (1963) reports ascorbic acid levels ranging between 19.9 % and 33.7% (from unripe to ripe fruits, respectively). These values are lower than those of gulupa, which can be influenced by the environment where it is grown. Contrasting with the results of the current study, Shiomi *et al.* (1996 b) and Flórez *et al.* (2012) have observed that the dominant acids in completely ripe fruits are oxalic and ascorbic ones.

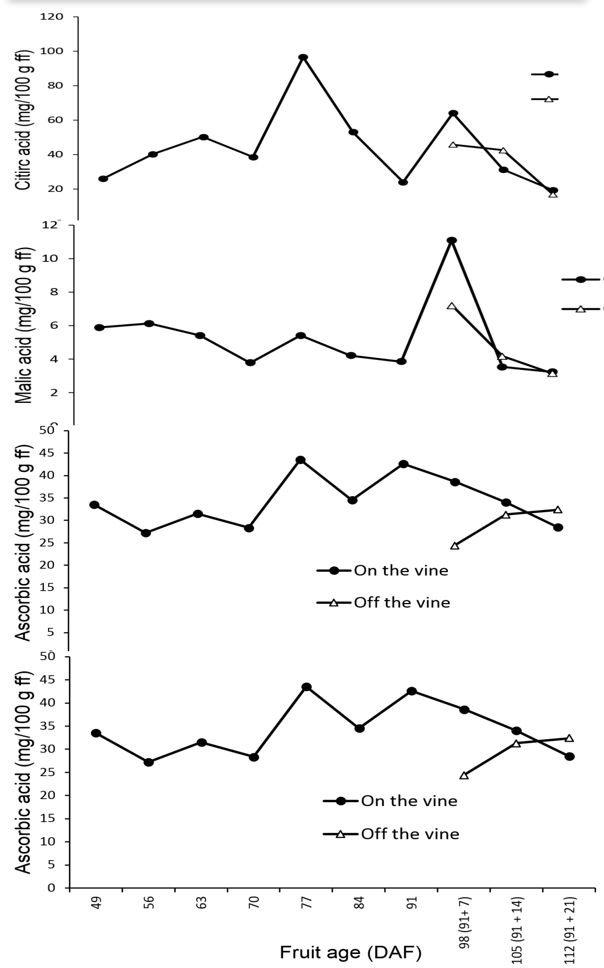

Figure 4. Changes in the pulp of gulupa fruits (*Passiflora edulis* Sims) during their development and postharvest as assessed for citric (a), malic (b), oxalic (c), and ascorbic (d) acids.

CONCLUSIONS

The parameters assessed in this research study during ripening and postharvest of the gulupa fruit constitute important information for the development of quality standards and regulations, intended to help growers and consumers to obtain the best possible advantage from the commercialization of this product.

TSSs are higher in gulupa than in other passifloras, which, together with the level of organic acids, confer this fruit its characteristic taste and make it a good alternative to be consumed fresh or in juice. Although yellow passion fruit and gulupa share the same scientific name, they have different pH and acidity attributes, which are certainly lower in gulupa.

The chemical assessment of the gulupa fruit indicates that fructose is the dominant sugar, while the main organic acid is the citric one. Its elevated concentration of ascorbic acid points at this crop as a good source of vitamin C, which might be fruitfully used as a commercial strategy after establishing the moment when the highest levels are attained.

The non-linear models adjusted in the current study account quite well for the progress of some of the studied physicochemical parameters, which, in as much, as they can be predicted by the models in question, and might be useful for monitoring final fruit quality.

Under the conditions of the current study, harvesting the fruits at about 91 DAF guarantees that the quality attributes of the product are adequately expressed during ripening.

Additional experimentation should be carried out with the aim of exactly establishing how the *genotype + environment + genotype x environment* productive function influences the concentration of important metabolites, enhancing the nutraceutical quality of the fruit, which are certainly of commercial interest.

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