Physicochemical characterisation of gulupa (*Passiflora edulis Sims.* fo *edulis*) fruit from Colombia during the ripening

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Abbreviated running title: *Physicochemical chacterisation in gulupa fruit*

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Abstract

Gulupa (*Passiflora edulis* Sims.fo *edulis*) is a tropical fruit native to America. This study was undertaken to characterize the physicochemical properties of this fruit in three maturity stages. In all stages, the pH, °Brix, texture, and titratable acidity were determined. pH Value and solid soluble content increased during ripening and titratable acidity decreased during this process; in contrast, texture values did not show significance variance. It was confirmed the presence of cyanidin-3-*O*-β-D-glucopyranoside as major anthocyanin. The changes on colour were followed by tristimulus colorimetry using image analysis, a very useful new approach for the measurement of non-homogenous colours. By using PCA (Principal Component Analysis), clusters of data corresponding to each stage could be defined. Additionally, the volatile composition was followed by HS-SPME (Headspace-Solid Phase Microextraction) and GCMS analyses. The results showed an increase in the amount of volatile during fruit ripening, with aliphatic esters as major constituents.

Keywords:

Passiflora edulis Sims fo *edulis*

Ripening

Colour changes

Gulupa

Tropical fruit

Image analysis

1. Introduction

The fruit species belonging to family *Passifloraceae* are mostly native to tropical America, and they are characterized by their exotic and distinctive aroma. They are shrubs or herbs, mostly climbers with auxiliary tendrils (Dhawan, Dhawan & Sharma 2004). Among these species, *Passiflora edulis* sims. fo *edulis* commonly known as gulupa, chulupa or maracuyá púrpura, is a native species of the southern Andes, growing between 1600 and 2000 meters, in climates with average temperatures between 16 and 22 °C. The fruits are round-shape, with a diameter between 6 and 8 cm, and green to purple peelings at maturity (Fig. 1). Inside, they contain many seeds (as the other Passifloraceae species) surrounded by a gelatinous yellow pulp, that exhibited an intense aroma and sweet-acid taste. These fruits are considered as vitamin A, thiamine, riboflavin, niacin, calcium, phosphorus, and ascorbic acid source (Wenkam, 1990). Pulp is used to prepare juices and soft drinks. To the best of our knowledge there is not any chemical study concerning this fruit; however, the change of some physicochemical properties during ripening has been published (Pinzón, Fisher & Corredor, 2007).

Currently, Colombia is considered one of the main producer countries of tropical fresh fruit worldwide. This dynamic exportation of fruits is highly linked to the exotic fruit species, which are within the new preferences in the international markets due to they are innovative and exhibit excellent sensory, nutritional and/or nutraceuticals qualities. Tropical fruits belonging to the genus *Passiflora*, such as, the passion fruit (*Passiflora edulis* var. Flavicarpa), the purple passion fruit (*Passiflora edulis* Sims), granadilla (*Passiflora ligularis*), and gulupa (*Passiflora edulis* sims. fo *edulis*), are species widely

appreciated for their organoleptic properties and they have shown a positive growth rate in the export market in Colombia since 1995. For the case of gulupa, the main customers are Germany, the Netherlands, the UK and Belgium with sales of close to 1,700,000 USD in 2007 and up to 4,100,000 USD in 2008, being Germany the largest market taking approximately of 55% of annual production (Proexport, 2010). However, during the long shipping or air transportation periods of times, fruits undergo changes by accelerated ripening; they lose organoleptic quality generating economic losses for exporting companies up to 15% of the total volume shipped. Thus, the aim of this work was to chemically characterize gulupa fruits harvested in three maturity stages, with the future purpose of better determine the influence of some variables in the quality loss of fruit to be exported.

2. Materials and methods

2.1. Plant material

The fruits (exportation quality) from different cultivars were harvested at three different degrees of maturity, and classified according to the peel colour as unripe (stage I, green), turning (stage II, purple-green), and full ripe (stage III, purple). The fruits were supplied by OCATI S.A (Cota, Cundinamarca, Colombia) in three different harvesting times (june, august, and october 2009), to perform all of the analyses by triplicate. Each sample lot consisted of 80 fruits. A voucher specimen (COL 527652) was identified and deposited at the Instituto de Ciencias Naturales, Universidad Nacional de Colombia.

2.2. *Physicochemical characterization*

These analyses were carried out in the following way for each harvesting time and maturity stage: ten batches of fruits were used, each one containing one fruit visually presenting the same physical characteristics high quality appearance. Each batch was replicate, performance the triplicate. The composition of the fruits was determined following the procedure published by AOAC (2006).

2.2.1. *Titratable acidity and pH*

Titratable acidity was determined by standard procedures (AOAC, 2006) using 1 g of pulp, and the results expressed as percentage of citric acid. The pH of the pulp was determined by using a C6820 pHmeter (Schott Gerate).

2.2.2. *Total soluble solids*

Total soluble solids were determined by using an Atago refractometer (HRS-500) and the results were expressed as °Brix.

2.2.3. *Texture*

The texture of gulupa fruits was measured with a TA-TX PLUS texture analyser (Stable Micro Systems Ltda., United Kingdom). Compression tests were performed with a 75 mm compression plate (P/75) at 2.0 mm/s until a 25% strain was reached. Each fruit was compressed in the equatorial section. All experiments were conducted at 18 °C and results were expressed as kg.

2.2.4. Anthocyanin content

Fruit peel (1.4 kg/3 kg fruit, 0.245 kg/0.507 kg fruit, and 0.220 kg/0.409 kg fruit at ripe, turning, and unripe maturity stages, respectively) were separately ground in a blender and extracted overnight with 900 mL of methanol-acetic acid (19:1 v/v) at room temperature, in the dark. After the solvent was removed under vacuum, the residue was applied to a 20.0 x 4.5 cm Amberlite XAD-7 resin open column (Aldrich Chemical Company, Milwaukee, WI, USA). The column was rinsed with water, and the adsorbed compounds were eluted with 1 L of methanol-acetic acid (19:1, v/v), according to the procedure described by Degenhardt, Knapp & Winterhalter (2000). The eluate was concentrated under vacuum, and the residue was freeze-dried. The final product was 11.2 g, 2.1 g, and 1.1 g of anthocyanin-rich extract (ARE) of ripe, turning, and unripe fruits, respectively. HPLC analyses were performed as it was published by Osorio et al. (2010). The concentration of anthocyanins in the ARE extracts was determined by the spectrophotometric pH-differential method (Giusti & Wrolstad, 2001). Dilutions were prepared in 0.025 M potassium chloride and in 0.4 M sodium acetate, adjusted respectively to pH 1.0 and 4.5 with HCl. The absorbance of each dilution was measured at 520 and 700 nm against a distilled water blank using a Thermo Scientific Evolution 300 UV-Vis spectrophotometer (Thermo Electron Corporation, Madison, WI). The total monomeric anthocyanin content was calculated as cyanidin-3-glucoside equivalents (in mg) per 100 mg of dry matter (ε value of cyanidin-3-glucoside dissolved in 0.1% HCl in methanol was 26900 L cm^{-1} mol^{-1} and the molar mass is 449.2 g mol^{-1}).

2.2.5. Colour measurement

The colour of the fruits (five) at different maturity stages was assessed by digital image measurements. The DigiEye imaging system (Luo, Cui, & Li, 2001) was used to capture digital images. The latter system includes a digital camera (Samsung A503, 5.2 Megapixels), a computer (provided with appropriate software), a colour sensor for calibrating displays, and an illumination cabinet designed by VeriVide Ltd. Digital images were taken in order to obtain the total appearance (non-homogeneous colour peel) of the fruits. In these measurements, the samples were illuminated by a diffuse D_{65} emulator. A pressed barium sulphate plate was used for calibration purposes. For each image, a 180 x180 pixels fixed area was cut. The chromatic heterogeneity of the fruit was measured through the pixel proportion that deviate more than ten percent of the average image intensity. The DigiFood® software (Heredia et al, 2006) was used for image processing. In the CIELAB ($L^*a^*b^*$) colour space, L^* defines the lightness (taking values ranging from 0 (black) to 100 (white)) and coordinates a^* and b^* define the red-green and yellow-blue axes, respectively. From these coordinates, other colour parameters, namely chroma and hue, are defined within the space. The hue angle (h_{ab}) is the qualitative attribute of colour according to which each one have been traditionally regarded as bluish, yellowish, reddish, etc. Chroma (C^*_{ab}) is the attribute that allows to assess the degree of difference of any given hue relative to a grey colour with the same lightness, being considered the quantitative attribute of colourfulness.

2.3. Volatile analyses

The volatile compounds released from the headspace of gulupa fruit pulp were analysed by HS-SPME (Headspace-Solid Phase Microextraction) (Carasek & Pawliszyn, 2006). The pulp of 10 fruits (at each maturity stage and harvesting time) was mixed and a portion of 10 grams of fruit pulp, were equilibrated during 1 in a 20 mL sealed vial at 40 °C. The headspace was collected on a DVB/CAR/PDMS fibre (70 mm thickness, Supelco) during one hour, and then directly injected (desorption time was set at 5 min.) into an gas chromatograph Shimadzu GC-17A coupled to a selective mass detector QP5050 operated in splitless mode. Mass spectra were recorded in electronic impact (EI) ionization mode at 70 eV and were scanned in the range *m/z* 40-350 amu. A FFAP fused silica column (J&W Scientific, 30 m x 0.32 mm i.d., 0.25μm film thickness) was used. The column oven was programmed from 50 (after 4 min) to 250 °C at 4 °C/min and the final temperature was held for 5 min; the injector temperature was maintained at 250 °C; carrier gas was 1.5 mL of He/min; and make up gas was nitrogen at 30 mL/min flow rate. All measurements were performed by triplicate.

Linear retention indices were calculated according to the Kovats method using a mixture of normal paraffin C_6-C_{28} as external references. Mass spectral identification was completed by comparing spectra with commercial mass spectral databases WILEY and EPA/NIH and by comparison with published data or with data from authentic reference standards (Barrios & Morales, 2005).

2.4. Aroma profile analysis

Sensory experiments were performed at 20 ± 1 °C in a sensory room with single cabins. Pulp of gulupa at each maturity stage was placed in glass vessels which were closed with ground glass lids. Then, the samples were presented to a well trained sensory panel consisting of 8 members. The assessors were asked to orthonasally evaluate the intensity of five odour qualities in the overall aroma of the gulupa fruit on a five point scale from 0 (not detectable) to 5 (intensely detectable). The odour qualities were compared with aqueous solutions of the following reference odorants: acetic acid (acid), (*Z*)-3-hexenal (green-grassy), ethyl butanoate (fruity), 3-sulfanyl-1-hexanol (sulphury), 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (caramel-sweet). The concentrations of the reference odorant solutions amounted to 10 times the respective odour threshold. The data were analysed by variance and regression analysis and average values were compared using Tukey's test with a probability $p \leq 0.05$.

2.5. Statistical analysis

Principal Component Analyses (PCA) were applied to analyze the sets of data obtained during the physicochemical characterization of gulupa fruits at different maturity stage and harvesting time. PCA analysis was applied within MATLAB environment (The MATHWORKS, USA). Similarity maps of images were drawn using the component scores. Interpretation of components was obtained by looking at the linear combination coefficients, called loadings.

3. Results and discussion

3.1. Physicochemical characterization

The pH and total soluble solids content of gulupa increased slowly whereas titratable acidity decreased at different maturity stages, with values significantly different between them (Table 1). The first finding is attributed to the hydrolysis of starch to sugars, according to the behaviour of carbohydrates during fruit ripening. The acidity varies due to the consumption of organic acids because of fruit respiration in this process. In most of the fruits during the ripening process usually occurs a softening and in the case of the gulupa the peel surface suffers a wrinkling; however, in the conditions of this study (fruits were ripened in the tree), external texture values of gulupa showed an almost constant value with no significant differences between the three stages. In a previous study (Pinzón, Fisher & Corredor, 2007), it was reported a firmness loss of 12% between green and ripe fruits, however the harvesting conditions were no specified.

3.2. Anthocyanin content

Fruit peel colour is a major criterion used to judge maturity of gulupa. The fruit usually harvested at unripe stage is green with scattered pink spots. After harvest, the purple colour continues to develop quickly until cover most of the peel (Figure 1). Thus, the anthocyanin content in the three stages were determined by using pH-differential method. It was no found any measurable content in the stage I; in contrast, the anthocyanin content increased

in stage II, and in stage III the amount was three-times higher than stage II. The HPLC-PDA analysis of AREs at three maturity stages revealed the presence of one major anthocyanin with λ_{max} at 511 nm. The presence of characteristic fragments of the anthocyanidins in the ESI-MS spectra evidenced that this compound was a derivative of cyanidin (*m/z* 287). Finally, this compound was identified as cyanidin-3-*O*-β-D-glucopyranoside by its chromatographic properties with samples belonging to our lab (Osorio et al., 2010).

3.3. Colour analysis

The results obtained by tristimulus colorimetry showed a decrease in lightness (L^*) and chroma (C^*_{ab}) during ripening in agreement with the development of purple (dark) colour. The hue (h_{ab}) also diminished from stage I (118, 113, and 123, for each harvesting time) to stage III (43.8, 41.1, and 24.0, respectively), according with the change of colour from yellowish-green to dark-purple. The representation of data obtained by image analysis in the a^*b^* diagram of fruits from three different harvesting times (Figure 2) showed a dispersion in agreement with the chromatic hetereogenity. However, the colour at different maturity stage could be well-differenciate. The most noticeable changes were detected in the a^* value, which increased significantly during ripening. This is in accordance with the replacement of the green colour with purple colour, which increased during this period.

3.4. Sensory and volatile analysis

To get a preliminary idea of the changes in the overall fruit aroma during ripening, an aroma profile analysis was performed at the three maturity stages (Figure 3). It was found that unripe fruits were characterised by predominant grassy and acid notes. During ripening a fruity, sweet, and sulphury odour notes were developed, but the grassy odour note was less intense.

The volatile compounds were analysed by HS-SPME finding an increase in the amount of volatile during ripening (Figure 4). The ethyl octanoate, hexyl butanoate, hexyl hexanoate, and ethyl butanoate esters were found in significant amount in the stages II and III, indicating that their biogenesis is activated during fruit ripening (Jiménez, Sierra & Osorio, 2010). These compounds have been detected in other passifloraceas, such as yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) (Carasek, E., & Pawliszyn, 2006) and purple passion fruit (*Passiflora edulis* Sims) (Parliment, T., 1972; Brat, et. al. 2000).

3.5. Principal component analysis (PCA)

To obtain a broad view on the physicochemical changes that occurs during gulupa ripening, the data set of pH, solid soluble content, texture, and titratable acidity was analysed by PCA (Figure 5A). In this analysis, the samples were relatively indiscriminant (for samples in the same maturity stage) from one another and the distance between samples types increased across the first component with different maturity stages. The two first principal components accounted the 82% of total variance. Next, this analysis was further used to study the distances between the different parameters and allows getting

more knowledge on the relationship between these different parameters. As it was expected, the texture neither exhibited any influence over the maturity stages nor correlated with other variables, since its values were constant through ripening of gulupa. In contrast, the titratable acidity showed a high value over the second principal component, and a negative correlation to °Brix value. The physicochemical properties having the major values over principal component were °Brix and pH, namely the most important variables during the ripening of the fruit.

The PCA analysis showed that samples at each maturity stage were clearly separated on the basis of their physicochemical properties and the tendency of these data was to group in clusters (Figure 5B). The discard of data was performed by using the Q and T^2 Hotelling graphics (Figure 6). It was found that most of the data were adjusted to the model, as can be seen in the above-mentioned figure, with only a few data ruled out. However, the data of two harvesting times were close in comparison with the third one (fruit collected in august), which data were quite far from the others. The weather conditions (drought) caused that physicochemical data were deviated from the trend. The clusters obtained allow to classify each maturity stage according to their physicochemical properties and confirmed that they are statistical differentiable.

4. Conclusions

The results showed that ripening in gulupa fruit is a process with stages well-differentiated in their physicochemical properties. During this process the volatile compounds is increased, as well as, the anthocyanin content. These findings allow growers

to have a tool to select and monitoring the fruits before packaging for exportation. The use of image analysis was appropriate to give a real interpretation of the hetereogenity chromaticity exhibited by gulupa fruits. A further study on the aroma active volatiles may be important in terms of determine parameters to ultimate fruit quality.

Acknowledgements

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Figure captions

Fig. 1. Ripe gulupa (*Passiflora edulis sims.* fo *edulis*) fruit.

Fig. 2. Localisation area of gulupa peel color on the (*a*b**) diagram at three different harvesting times a) june b) august, and c) december 2009, at the three maturity stages.

Fig. 3. Aroma profiles at different maturity stages, unripe, turning, and full ripe.

Fig. 4. GCMS analyses of volatile compounds from gulupa obtained by HS-SPME in different stages, a) unripe, b) turning, and c) ripe.

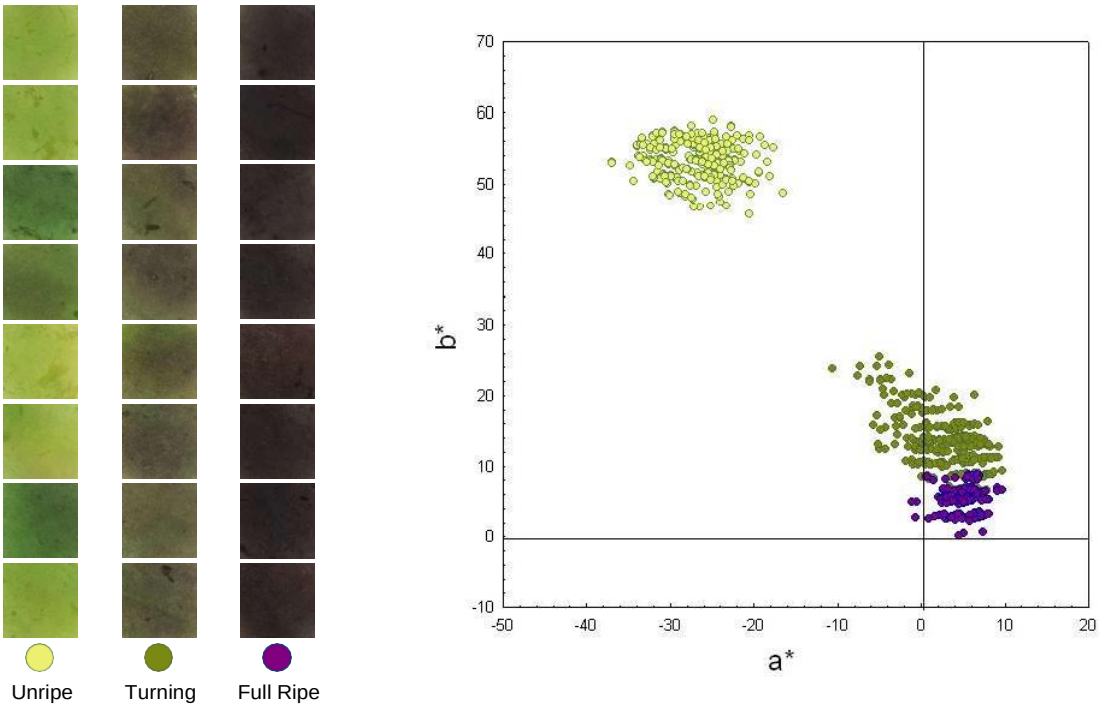
Fig. 5. A) Principle component analysis results of different physicochemical parameters measured at different maturity stages in all of the harvesting times , (□) june, (○) august, and (▲) october of 2009. B) Principal component analysis results of distinct maturity stages, unripe (U), turning (T), and ripe (R) as measured in the three harvesting times.

Fig. 6. Q and T^2 Hotelling graphics for discard ruled out data.

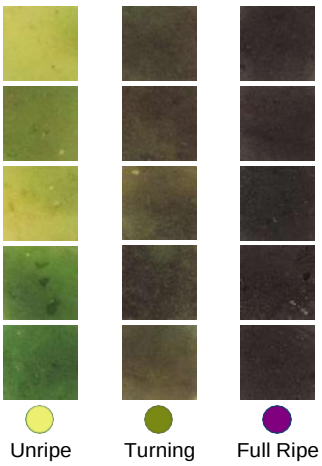
Figure 1

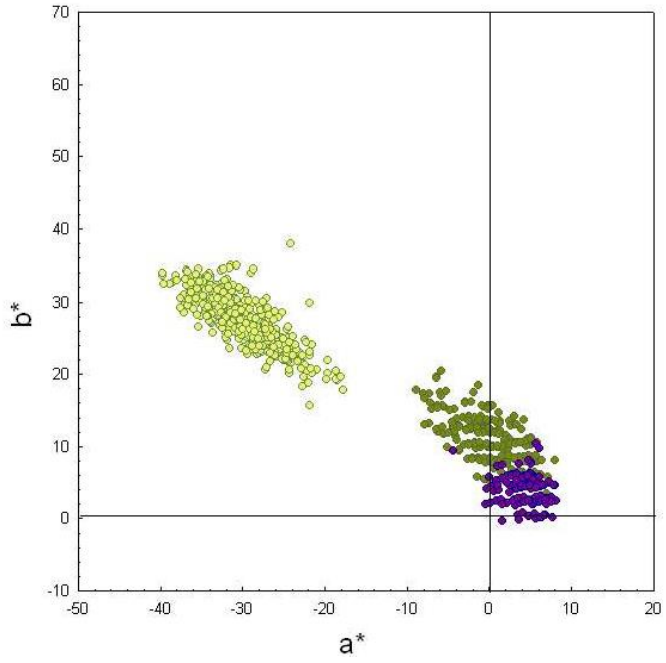
Figure 2

A


Unripe
Turning
Full Ripe
b*
a*
70
60
50
40
30
20
10
0
-10
-50
-40
-30
-20
-10
0
10
20

B


Unripe
Turning
Full Ripe


b*
a*
70
60
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40
30
20
10
0
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-50
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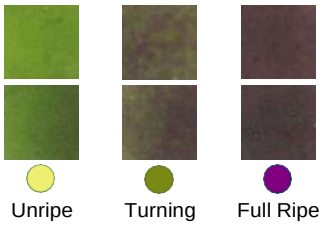
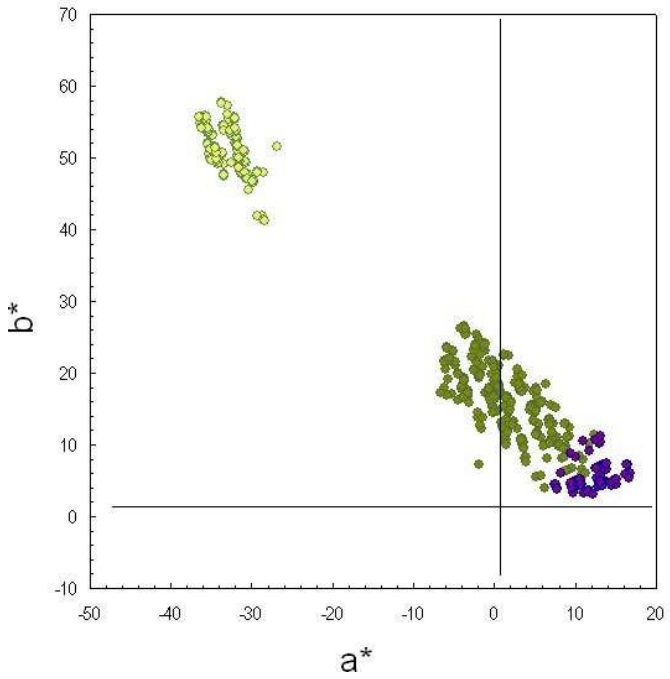
-30
-20
-10
0
10
20

C


Unripe
Turning
Full Ripe


b*
a*
70
60
50
40
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-30
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-10
0
10
20

Figure 3.

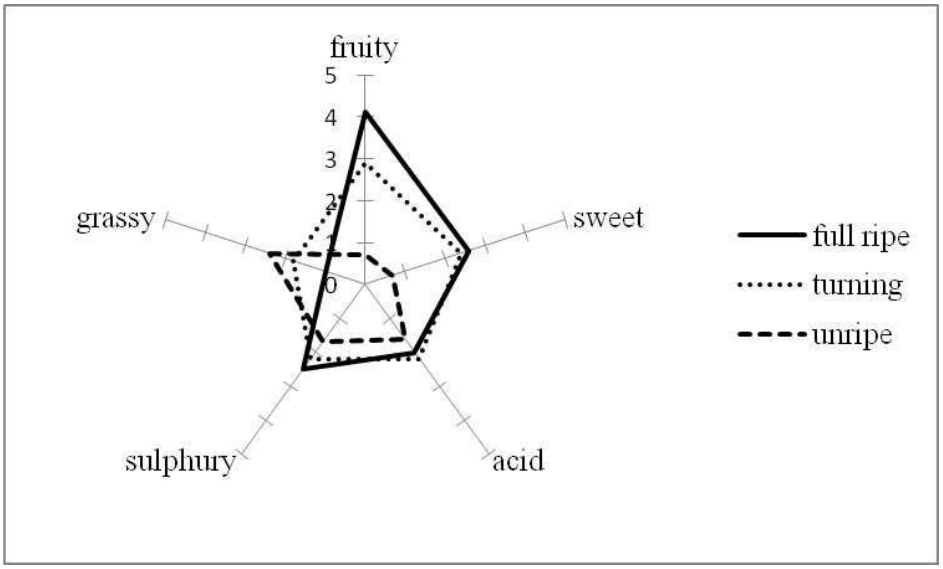
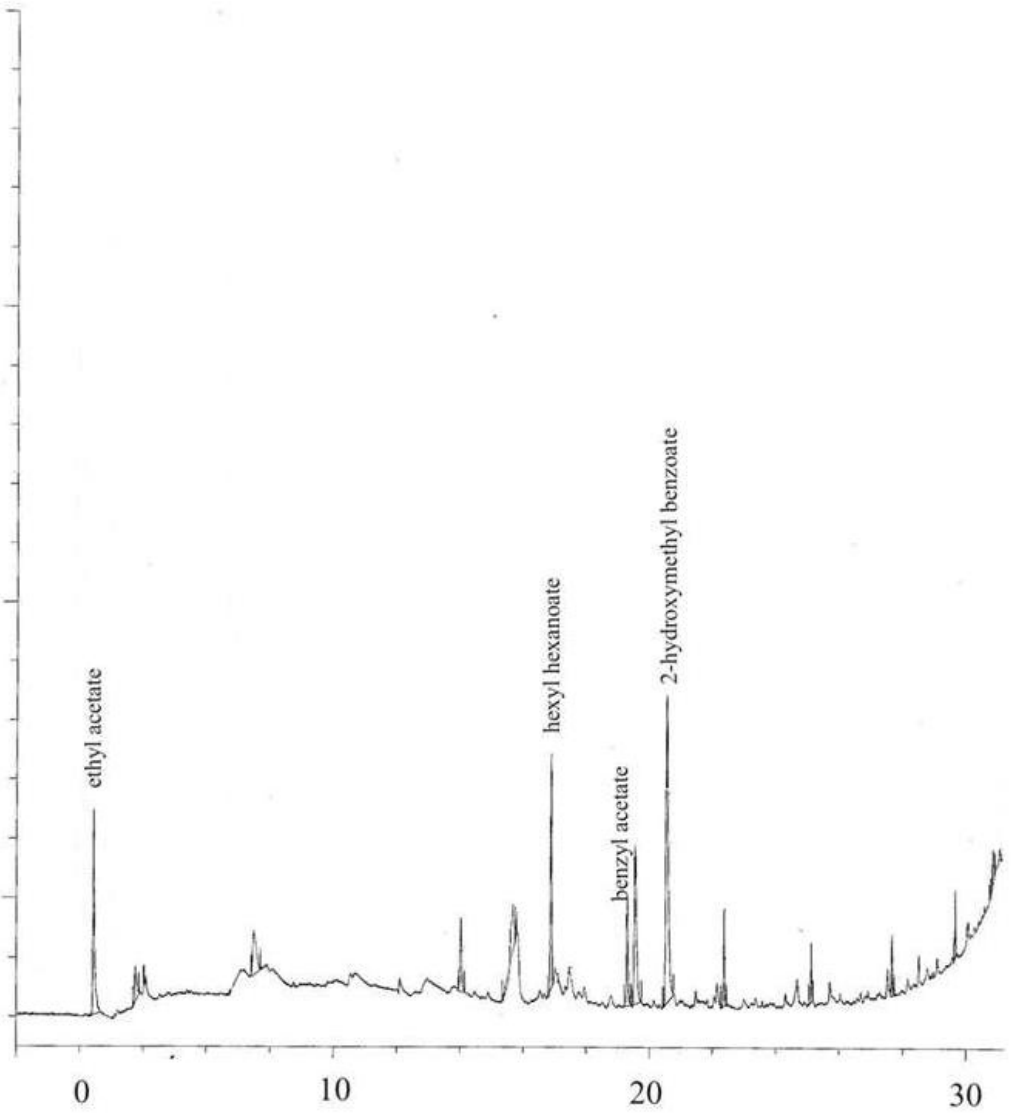

fruity
5
4
3
2
1
0
sweet
acid
sulphury
grassy
full ripe
turning
unripe

Figure 4.

A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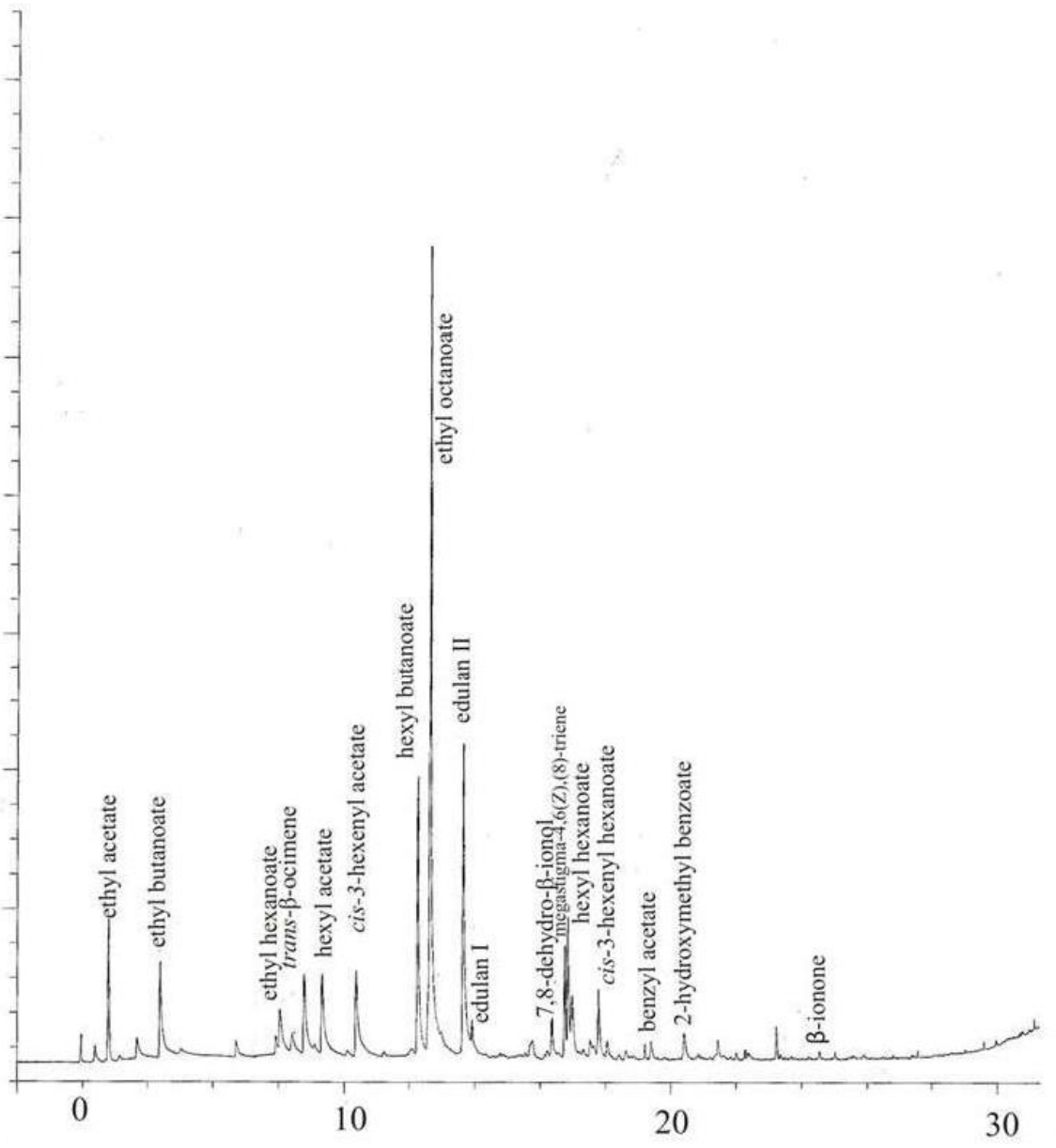
benzyl acetate
2-hydroxymethyl benzoate
0
10
20
30

B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ethyl hexanoate
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hexyl butanoate
ethyl octanoate
edulan II
edulan I
7,8-dehydro-β-ionol
megastigma-4,6(Z),(8)-triene
hexyl hexanoate
cis-3-hexenyl hexanoate
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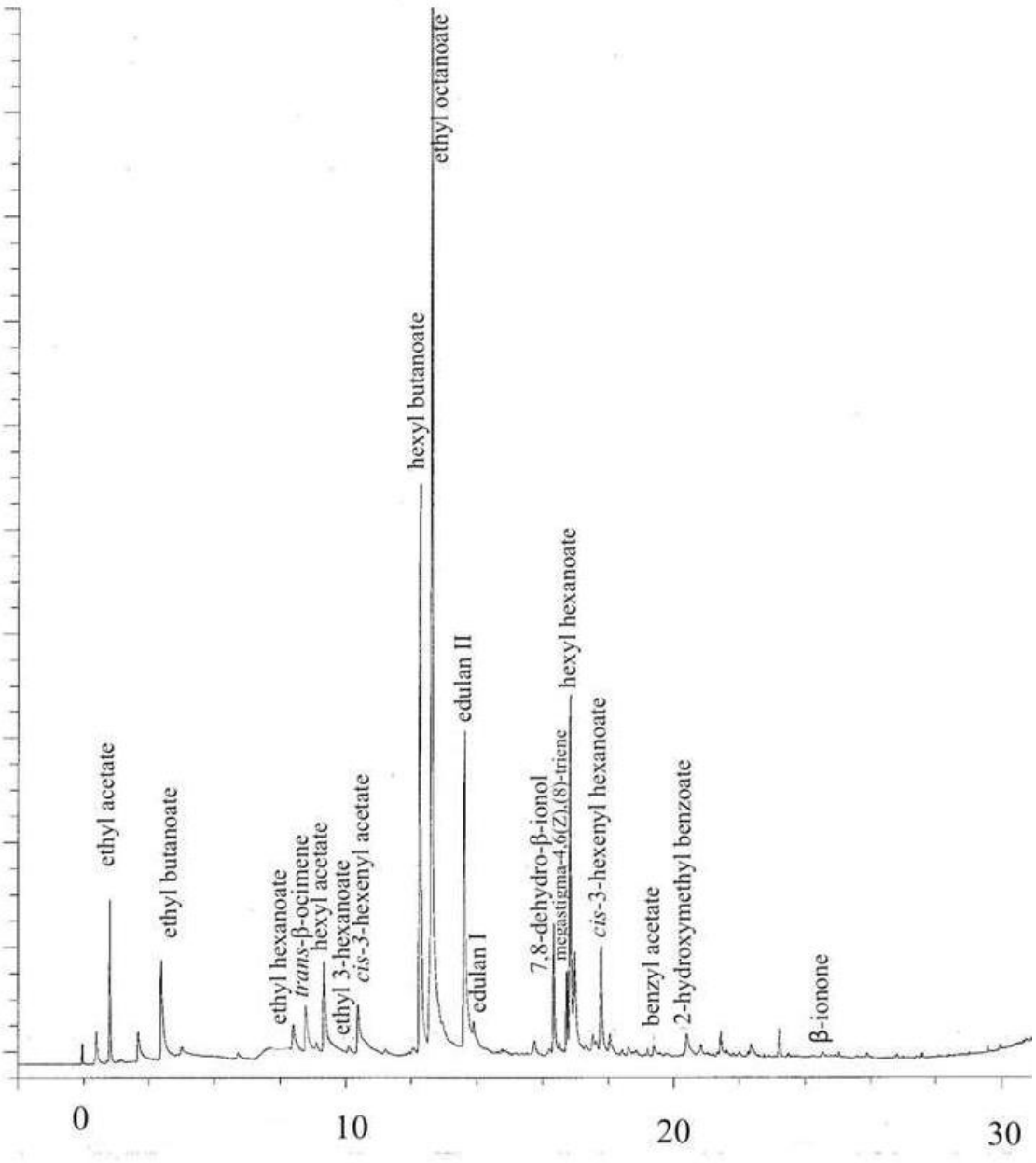
2-hydroxymethyl benzoate
β-ionone
0
10
20
30

C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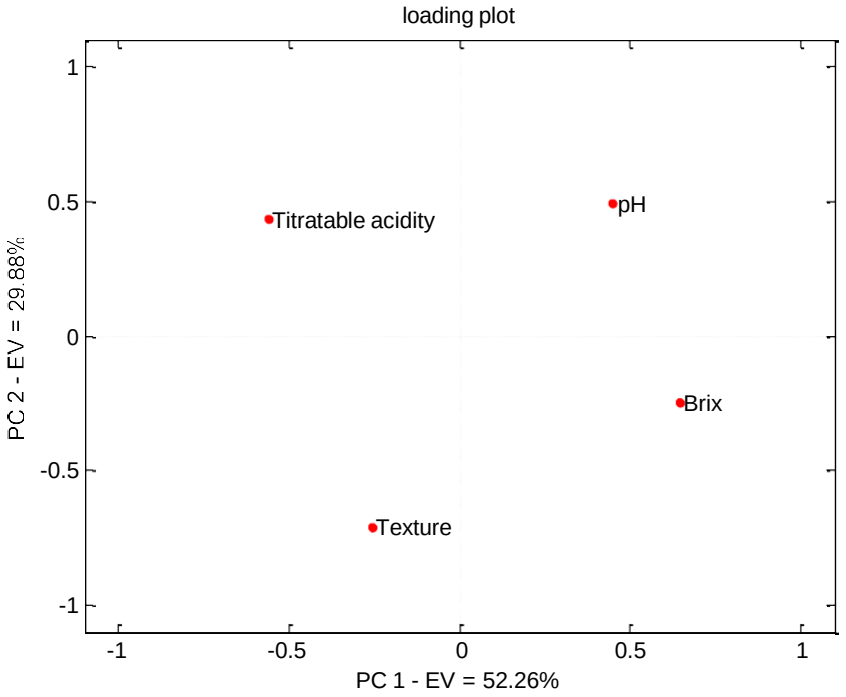
ethyl butanoate
ethyl hexanoate
trans-β-ocimene
hexyl acetate
ethyl 3-hexanoate
cis-3-hexenyl acetate
hexyl butanoate
ethyl octanoate
edulan II
edulan I
7,8-dehydro-β-ionol
megastigma-4,6(Z),(8)-triene
hexyl hexanoate
cis-3-hexenyl hexanoate
benzyl acetate
2-hydroxymethyl benzoate
β-ionone
0
10
20
30

Figure 5.


loading plot
Titratable acidity
pH
Brix
Texture
PC 2 - EV = 29.88%
PC 1 - EV = 52.26%

B

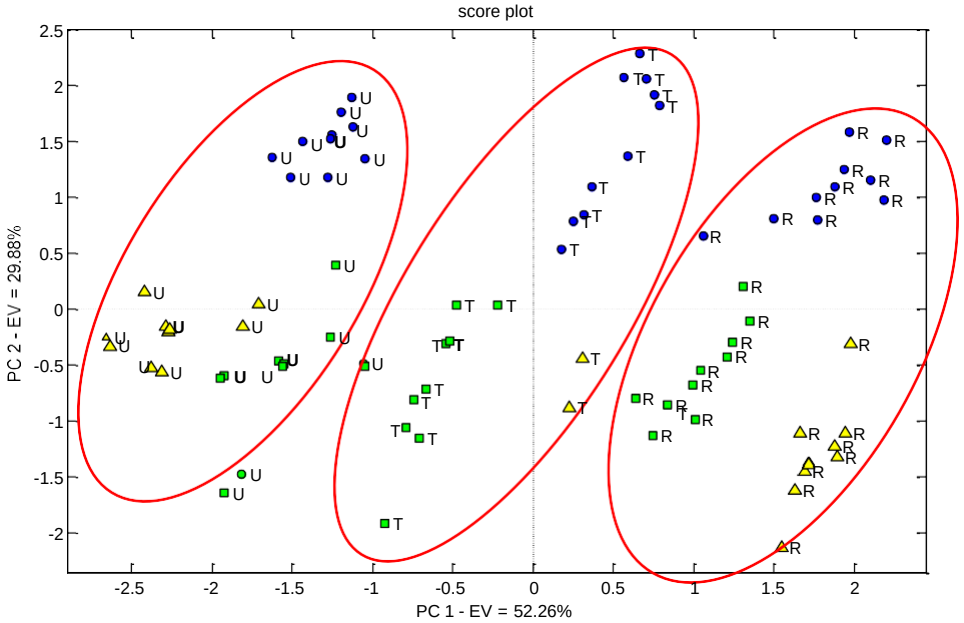

score plot
PC 2 - EV = 29.88%
PC 1 - EV = 52.26%

Figure 6.

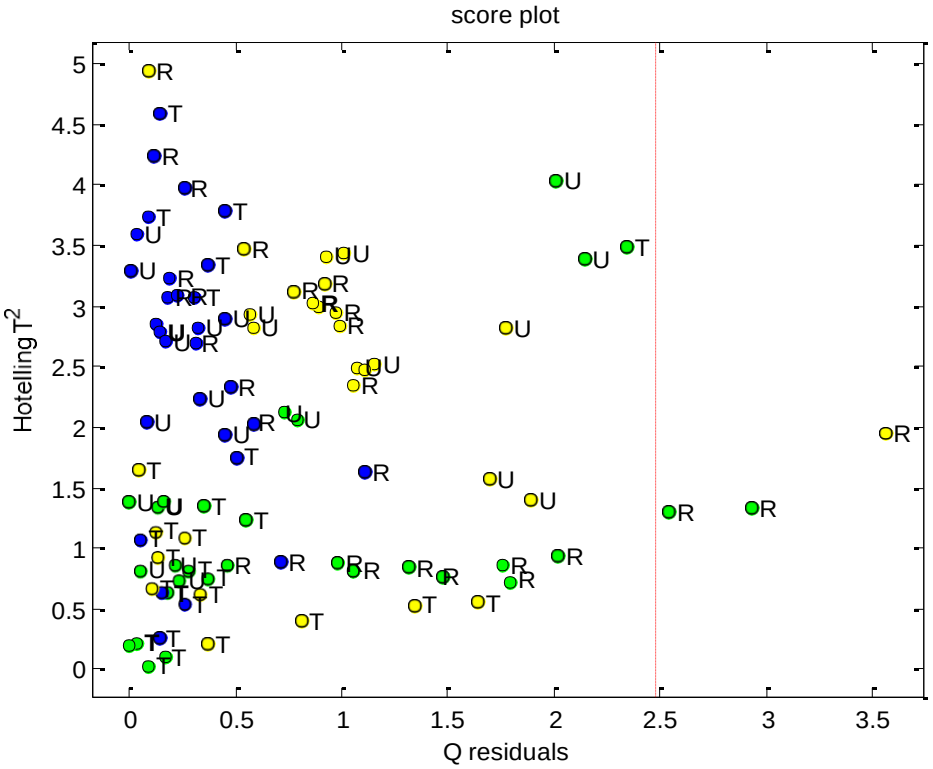


Table 1

Physicochemical characterization of gulupa (*Passiflora edulis sims.* fo *edulis*) fruit at different maturity stages

Property		Stage I (unripe)	Stage II (turning)	Stage III (full ripe)
Moisture content (% wet basis) [a]		85.3 ± 0.1	83.2 ± 0.1	82.1 ± 0.1
Total soluble solids (°Brix)		13.4 ± 0.7	15.5 ± 0.8	17.3 ± 0.4
pH		2.33 ± 0.14	2.51 ± 0.02	2.67 ± 0.15
Acidity (% citric acid)		4.61 ± 0.40	3.66 ± 0.40	2.65 ± 0.66
Texture (Kg)		19.93 ± 2.89	19.30 ± 2.87	19.08 ± 2.60
Anthocyanin content (g cy-3-glu equiv./kg fruit)		-	0.45 ± 0.04	1.70 ± 0.20
Proteins (%)[a]		0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
Lipids (%)[a]		0	0	0
Crude fibre (%)[a]		0.1	0.1	0.1
Carbohydrates (%)[a]		13.2 ± 0.1	15.5 ± 0.1	16.5 ± 0.1
Ash (%)[a]		0.6	0.5	0.5
Colour parameters				
	L^*	55.5 ± 11.8	34.8 ± 4.1	20.1 ± 3.9
	a^*	-18.9 ± 5.4	-0.4 ± 2.4	4.4 ± 2.8
	b^*	40.5 ± 10.3	14.6 ± 3.6	3.0 ± 1.2
	C^*_{ab}	45.2 ± 9.4	14.91 ± 3.6	5.5 ± 2.8
	h_{ab}	116.1 ± 8.7	88.8 ± 9.6	38.2 ± 7.8

All data are the mean of ten measurements ± standard deviation, (n = 100) $p < 0.0001$; - = not detected. [a] only three measurements.