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Comparative analysis of fresh and processed mango (*Mangifera indica* L, cv. "Maria") pulps: influence of processing on the volatiles, bioactive compounds and antioxidant activity

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Abstract

The objective of this work was to evaluate the possible modifications due to industrial processing in the volatiles (HS-SPME/GC-MS), bioactive compounds (content total, and phenolic profile by UFLC-DAD) and antioxidant capacity (ABTS, DPPH, FRAP, ORAC) of fresh and processed mango pulp with addition of additives, pasteurized, and pasteurized with additives. The physicochemical characteristics of the samples were evaluated. All parameters were significantly ($p \leq 0.05$) affected by processing. According to the PCA analysis, the stability of the samples was influenced by the thermal processing and the work clearly demonstrated the separation between the analyzed samples as a function of the effect of thermal processing. It is concluded that industrial processing, although important for the preservation of useful life and maintenance of the microbiological quality of mango pulp, influence the functional and aromatic properties, causing a significant reduction in the volatile and bioactive compounds, which can negatively influence the acceptance of the processed product by consumers.

Keywords: thermal processing; additives; chemical composition; UFLC-DAD; GC-MS.

Practical Application: Due to the economic importance of mango industrialization, it is essential that the pulp produced maintain the functional and sensorial characteristics of the fruit preserved, aiming at quality maintenance. This research provides scientific information useful in choosing the type of treatment to be adopted in the industrialization process, allowing the search for alternative methods to avoid changes in the final product.

1 Introduction

The high production of mangoes, as well as fruits in general, makes it necessary to deploy technologies that extend its shelf life, reduce postharvest losses, and allow consumption of the fruit during all months of the year (Kaushik et al., 2016). This fact demonstrates that processed products are of great commercial importance, with frozen pulp being the main raw material for the processing of other products, such as juices, nectar, sweets, jams and jellies (Giarola et al., 2016).

To ensure the quality and safety of the processed products, thermal processing techniques, freezing and in some cases the addition of preservatives are applied. However, processing methods may produce undesirable effects on the sensory and nutritional properties of the final product, causing it to lose the inherent organoleptic characteristics of the fresh fruit (Liu et al., 2016). These changes in fruit products are negative factors, since they may affect consumer acceptance (Wibowo et al., 2015).

Scientific research has already been carried out analyzing the quality of mango and pulp (Benevides et al., 2008; Kaushik et al., 2018). In Brazil, although there is a wide production of mango fruits, and a large-scale processing industry of frozen pulp, no study has yet been undertaken to evaluate the possible changes due to industrial processing on volatile compounds, bioactive composition and antioxidant activity of mango pulp, comparing fresh pulp with pasteurized pulp with or without addition of an additive.

Thus, the objective of this work was to evaluate the possible changes resulting from the industrial processing of mango pulp of the "Maria" variety, on its volatile compounds, bioactive compounds and antioxidant activity, analyzing both fresh and processed pulps.

2 Materials and methods

2.1 Samples and extracts

Pulp samples of ripe mango variety "Maria", were collected from a fruit pulp industry, located in the city of Nossa Senhora de Socorro, Sergipe, in their fresh form (FP) and processed with additive (PA). The additives used were the preservative sodium benzoate (INS211), the antioxidant sodium metabisulfite (INS223) and the acidulant citric acid (INS330), however the industry did not specify the dosages used. To evaluate other forms of processing, in the laboratory, these two samples were divided and part of them was pasteurized (at 85 °C/4 min) according to the method used by

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Vásquez-Caicedo et al. (2007), giving rise to two more samples: pasteurized pulp (PP) and pasteurized pulp with additives (PPA), totaling four samples for analysis: FP, PA, PP and PPA.

The extracts were prepared based on the methods used by Ronchi et al. (2015) and Lim et al. (2019): using ultrasound (USC-1400A, Brazil) with frequency of 40 kHz, at 25 °C/20 min, 1.25 g of sample and 10 mL of ethanol (50%). Subsequently, it was centrifuged (5810R-Eppendorf-AG, Germany) at 20 °C/10 min, 10.000 rpm, and the supernatant was collected.

2.2 Physicochemical characterization

For the physicochemical characterization of the samples, the official methods recommended by the Association of Official Analytical Chemists (2000) were followed in triplicate: titratable acidity, pH, ash, lipids, proteins, moisture, water activity (AquaLab electronic meter 4TEV, USA), total soluble solids (DAS digital refractometer E-SCAN, USA), color (colorimeter CHORMA-METER/CR-400/Konica-Minolta, Japan).

2.3 Volatile profile (HS-SPME/GC-MS)

The extraction and identification of volatile compounds of the mango pulps were performed in triplicate using the optimized conditions of Wibowo et al. (2015), using a DVB/CAR/PDMS fiber (50/30 µm divinylbenzene-carboxen-polydimethylsiloxane) with headspace extraction, a gas chromatograph (Agilent-GC7890B, USA) coupled with a mass spectrometer (Agilent-MSD5977A, USA) having a HP5-MS (30 m × 0.25 mm, 0.25 µm, JW Scientific, USA) low polarity column, and the carrier gas was helium (99.99% pure). The identification of compounds was carried out by comparing their mass spectra, retention times and linear retention indices (LRI) with those obtained from authentic standards, and when it was not possible to have authentic standards, were identified by matching the mass spectra available the database of the National Institute of Standards and Technology (2011, version 2.0).

2.4 Phenolic profile (UFLC-DAD)

The identification and quantification of the phenolic profile was performed in triplicate with diluted extracts (1:8), following the method described by Rajan et al. (2019) using a liquid chromatograph (UFLC-DAD, Shimadzu, Kyoto, Japan), equipped with an automatic sampler (SIL-20AT), a degasser (DGU-20A3), with two quaternary pumps (LC-20AD), connected to a system diode array detection (SPD-M20A), and a Kinetex-C18 column (250 cm × 4.60 mm, 5 µm-Phenomenex, California, USA). Chromatograms were recorded and evaluated by Shimadzu Technologies 'LC' Solution Software (1.24-SP2).

2.5 Determination of ascorbic acid (AA), carotenoids (CC), total phenolic (TPC) and flavonoid (TFC) content, free radical capture activities (ABTS and DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC)

Measurements were performed on a 96-well microplate spectrophotometer (SpectraMax-M2, Molecular Devices, USA). All determinations were performed in triplicate:

The AA was determined by the colorimetric method described by Benassi & Antunes (1988). The CC was determined according to the method described by Lichtenthaler (1987), and the reading with wavelengths of 663/647/470 nm. The TPC was determined by the method described by Singleton & Rossi (1965), using gallic acid (GAE) as the reference standard and a calibration curve (0-500 mg/L), and the reading with wavelength of 765 nm. The TFC was determined according to the method described by González-Aguilar et al. (2007), using quercetin (QE) as the reference standard and a calibration curve (0.025-0.6 mg/mL), and the reading with wavelength of 415 nm.

The ABTS was determined by the method described by Re et al. (1999) and read with wavelength of 734 nm. The DPPH was determined by the method described by Kim et al. (2002), and the reading with wavelength of 517 nm. The FRAP was determined by the method described by Thaipong et al. (2006), and the reading with wavelength of 593 nm. The ORAC was determined by the method described by Albarici et al. (2009), and the reading with wavelengths of 485/520 nm. Calibration curves were constructed using the analytical standard Trolox: ABTS (5-500 mg/L), DPPH (0-4.5 mMol/L), FRAP (0-160 mg/L) and ORAC (0-4.5 mMol/L).

2.6 Statistical analysis

All the results were expressed as mean ± standard deviation. Data were submitted to analysis of variance (ANOVA) and Tukey's test to determine significant differences ($p \leq 0.05$) between the samples; the multivariate statistical approach was also used, applying the Principal Component Analysis (PCA) test, of the Biplot type, using software for Windows SPSS 20.0 (Science, Chicago, USA).

3 Results and discussion

3.1 Physicochemical characterization

The results on physicochemical composition of the fresh and processed mango pulps are presented in Table 1.

No significant differences ($p \leq 0.05$) were found between the samples in their water activity. The values found were similar to those obtained by Grizotto et al. (2005), who reported 0.995 of water activity (a_w) in mango pulp. The results obtained demonstrate that the mango pulp has a high water content, being a highly perishable product, requiring the application of conservation methods to maintain its stability. The results found by Brunini et al. (2002), demonstrated that the homogenized mango pulp, frozen and stored at -18 °C, maintained the aspects of coloration, flavor and vitamin C content acceptable for up to 20 weeks, suggesting that this is the ideal shelf life for the product. However, this period can be extended if other conservation methods are applied.

Regarding the color analysis, the samples differed significantly ($p \leq 0.05$) from each other. In relation to the parameter L* that indicates luminosity, the samples that most differentiated were PA that obtained the lowest value, and PPA which had the highest value. The parameter h* that defines the average tone presented significant differences ($p \leq 0.05$) between the FP sample,

Table 1. Physicochemical composition of fresh and industrialized mango pulps.

Characteristics	Samples			
	FP	PA	PP	PPA
Water activity	0.99 ± 0.00[a]	0.99 ± 0.00[a]	0.99 ± 0.00[a]	0.99 ± 0.00[a]
Color	h* 78.36 ± 0.29[b]	h* 78.86 ± 0.11[b]	h* 79.50 ± 0.62[a]	h* 79.61 ± 0.28[a]
	L* 28.41 ± 2.61[b]	L* 22.27 ± 1.65[c]	L* 28.75 ± 0.14[b]	L* 33.27 ± 2.84[a]
	a* 5.25 ± 0.11[b]	a* 5.44 ± 0.07[b]	a* 5.06 ± 0.33[b]	a* 6.15 ± 0.64[a]
	b* 25.30 ± 1.33[b]	b* 27.64 ± 0.30[b]	b* 34.40 ± 0.04[a]	b* 36.76 ± 3.01[a]
	c* 25.83 ± 1.33[b]	c* 28.17 ± 0.30[b]	c* 34.73 ± 0.13[a]	c* 36.80 ± 1.33[a]
pH	3.26 ± 0.01[b]	3.38 ± 0.01[a]	3.21 ± 0.01[c]	3.28 ± 0.01[b]
Soluble solids (SS °Brix)	11.85 ± 0.01[a]	10.20 ± 0.06[b]	11.84 ± 0.15[a]	11.85 ± 0.05[a]
Moisture (g/100 g)	87.70 ± 0.02[b]	88.75 ± 0.13[a]	87.62 ± 0.08[b]	87.52 ± 0.08[b]
Lipids (g/100 g)	0.25 ± 0.03[a]	0.07 ± 0.01[b]	0.14 ± 0.02[c]	0.25 ± 0.00[a]
Protein (g/100 g)	0.55 ± 0.04[a]	0.30 ± 0.01[b]	0.56 ± 0.03[a]	0.42 ± 0.04[c]
Ash (g/100 g)	0.29 ± 0.02[b]	0.28 ± 0.03[b]	0.33 ± 0.01[a]	0.32 ± 0.01[a]
Acidity (AT g/100 g)	0.23 ± 0.00[b]	0.18 ± 0.00[c]	0.24 ± 0.00[a]	0.23 ± 0.00[b]
Ratio (SS/AT)	51.52 ± 0.01[b]	56.66 ± 0.01[a]	49.33 ± 0.01[c]	51,52 ± 0.01[b]

FP: Fresh pulp; PA: Fresh pulp processed with additive; PP: Pasteurized pulp; PPA: Pasteurized pulp with additives. Mean values ± standard deviation of triplicate determinations (n=3). The mean values followed by same letters in the same line do not differ by the Tukey test at 5% of error probability, whereas the different letters represent significant differences (p<0,05) among them.

with PP and PPA samples. On parameter c*, which represents saturation, the PF sample also differed (p≤0.05) with PP and PPA samples. According to the results of a* that represents red and b* representing yellow, the predominance of yellow color was verified, being that this color is ideal for its mango pulp appearance and preparation of by-products, where the sample with the highest value was PPA and the lowest was FP sample. The results were slightly higher than those to the values obtained by Sánchez Riaño et al. (2018) evaluating the incidence of hydrocolloid type on quality parameters in mango leathers Yulima variety: h* 54.55-60.31, L* 38.53-45.41, a* 10.08-13.06, b* 14.16-24.48 and c* 17.39-28.18.

For the pH, the FP sample differed significantly (p≤0.05) between PA and PP samples. An increase in the pH value was observed in the pulps in which additive was added, and the PA sample was the only one that was within the standard range (3.30-4.50) established by the Identity and Quality Standard (PIQ) for mango pulps (Brasil, 2000). The lowest pH was found in the PP sample. This demonstrates that the different processing influences the pH of the mango pulp.

Regarding the soluble solids content, the only sample PA differed significantly (p≤0.05) from all others. The results obtained, except for the sample PA agreed with the minimum value established by the PIQ for the mango pulp, being 11.0 °Brix (Brasil, 2000). It demonstrates that the presence of the additives influenced a decrease in soluble solids content in the PA sample, which becomes a negative factor, since the content of soluble solids is an indicator of the sweetness of fruits and an important parameter in pulp processing, as it reduces the need to add sugar at the time of consumption or reprocessing, reducing costs and increasing quality.

For the moisture content, the PA sample differed significantly (p≤0.05) from the others, being also the one that obtained a higher value. In the samples that received heat treatment, the moisture decreased a little in comparison with the FP sample, however this decrease was not significant. The results obtained for moisture contents were superior to the minimum value (86.5%) established by PIQ for mango pulp (Brasil, 2000). The analysis of moisture content in fruit pulp is important, because during the storage period, the pulp loses water to the environment, and may influence other aspects of the product, including the soluble solids content, as observed by Brunini et al. (2002).

In lipid contents of the pulps, there was no significant difference (p≤0.05) between FP and PPA, but the PA and PP samples had significant difference and obtained results lower than those determined in the Brazilian Table of Food Composition (Universidade Estadual de Campinas, 2011), which is 0.20%. It demonstrates that addition of the additive and pasteurization, when applied alone, resulted in a significant decrease in lipid content. The lipids exhibit differences in their solubility and their functional properties, so it is possible that conservation methods affected their solubility, and for this reason the values obtained in PA and PP were low. Younis et al. (2011) reported that the addition of SO_2 caused the reduction of the lipid constituents of the mango pulp.

For proteins, the samples of FP and PP did not differ significantly (p≤0.05) from each other. It was found that the protein content in the PA sample was reduced with the addition of the additive as compared to the FP sample. However, it was slightly increased in the PPA sample receiving additive and pasteurization. These changes may have occurred due to exposure to external agents such as heat and the use of additives, which may have altered protein structures. The results obtained were close to those recommended value by Universidade Estadual de Campinas (2011) that establishes 0.4 g/100 g as the ideal. Younis et al. (2011) reported that in mango pulp the protein content decreased with the use of sodium benzoate in combination with other preservatives.

As for ash analysis, the FP sample did not differ significantly ($p \leq 0.05$) from the PA, but differed significantly from the PP and PPA samples. It was observed that the pulps that were thermally treated had higher results, which may have occurred due to the concentration of the inorganic components in the pasteurization process. However, the results obtained were lower than the recommended value by Universidade Estadual de Campinas (2011) that establishes 0.4 g/100 g as the ideal. Since it represents the total mineral content of foods and implies in their nutritional value, it can affect quality.

For acidity, it was observed that the samples differed significantly ($p \leq 0.05$), and the sample PA presented lower acidity. The acidity values obtained were lower than the minimum value established (0.32 g/100 g) by PIQ for mango pulps (Brasil, 2000). Acidity is well accepted in fruit products, and its determination in food is very important, considering its effects on conservation.

Regarding the ratio parameter, it was observed that the samples differed significantly ($p \leq 0.05$). The values obtained were higher than those found by Benevides et al. (2008) who found 27.94-43.18. The PIQ does not present minimum or maximum values for this parameter, but according to the values required for the contents of soluble solids and for acidity, this relationship can be obtained, finding a value of 34.37, indicating then that the results obtained in this research may mean a greater need to add sugar to the final product.

In general, the differences presented in the physicochemical characterization of the samples in relation to the data found in the literature are probably related to the types of products compared, variety studied, culture conditions and analytical techniques used.

3.2 Volatiles profile

The list of volatile compounds found in the fresh and processed mango samples is presented in Table 2.

In general, 53 compounds were found. The main class identified was of terpenes, which are generally reported as the major compounds in the mango pulp, followed by aromatic hydrocarbons, esters, lactones, alcohols and others.

It was observed that sixteen compounds (2, 4, 5, 6, 9, 10, 17, 23, 27, 33, 38, 41, 49, 50, 52, 53) that were present in the FP pulp were lost, while thirteen new compounds (13, 19, 21, 22, 24, 25, 29, 30, 31, 32, 36, 39, 40) were found in the PA sample. In the PP sample seventeen compounds (2, 5, 6, 9,10, 23, 26, 27, 33, 38, 41, 44, 46, 49, 50, 52, 53) that were present in the FP sample were not found, while two new compounds (30, 37) were found. In the PPA sample, eighteen compounds (2, 5, 9, 10, 23, 27, 33, 35, 38, 41, 44, 46, 47, 49, 50, 51, 52, 53) that were present in the FP pulp were lost and a new compound was found (25).

Among the samples analyzed, the ones that underwent thermal treatment presented lower amounts of compounds, being this factor due to the process of pasteurization, that brought about a decrease in the number of compounds resulting from the heating effect and the consequent volatilization of some highly volatile compounds. The reduction in the number of identified terpenes and even in some cases the increase in specific terpenes, such as β-pinene, can be explained by the fact that the thermal processing, under acidic conditions, can cause the conversion of the terpenes to other terpenes, however, many of these compounds cannot be detected in the chromatographic run because of their low volatility, as reported by Biasoto et al. (2015). In summary, an explanation for the reduction or increase of the terpenes in the thermally treated mango pulp would be due to the sensitivity of these compounds, which may degrade to other compounds during thermal processing, depending on their polarity.

The major compound present in all samples of mango pulp was β-pinene, which contributes to the aroma of mango with odor notes of woody, pine, resin and turpentine, with a higher concentration in PP sample. The second compound with higher concentration was caryophyllene, which offers sweet, woody and spicy odor notes, and its concentration was higher in the PA sample. The third compound found in higher concentration in FP sample was the (R)-α-pinene, which offers odor notes of herbal of pine and resin.

In addition to these three compounds, other terpenes were highlighted in relation to their concentrations in this work: humulene, trans-β-ocimene, β-eudesmene, β-cis-ocimene, α-gurjunene, D-limonene, β-phellandrene e (-)-β-pinene, demonstrating that the compounds that most contribute to the characteristic aroma of the mango have odor notes ranging from sweet, citrus, balsamic, earthy, woody, herbs, and mint.

Franco et al. (2004), identified that car-3-ene was the major component of the Haden and Keitt cultivars, while the Tommy-Atkins variety showed a predominance of car-3-ene and α-pinene. These data demonstrate, that the volatile compounds found in greater amount in mango pulp may change depending on the mango variety, as well as location, climate and the fruit cultivation techniques.

3.3 Phenolic profile

The individual phenolic compounds found in fresh and processed mango pulp samples are shown in Table 3.

Overall, eight compounds were identified. The main class identified was of phenolic acids, which have high antioxidant activity and comprise four of the identified compounds. The other four compounds were catechins, which are flavonoid-derived compounds and are among the main constituents of the mango phenolic composition.

All samples differed significantly ($p \leq 0.05$) from each other, where the FP sample had the highest concentration of compounds (except for epicatechin gallate which was higher in PA sample), which also differed from the other samples in terms of the number of compounds identified, since the eight identified compounds were present in this sample, and in the PA, PP and PPA samples only seven compounds could be identified.

The PA sample had the lowest concentrations of compounds, demonstrating that the addition of the additive affected negatively on the preservation of the bioactive compounds in the mango pulps. The effect of pasteurization was also negative, as shown in the PP sample which also had the lowest concentrations of

Table 2. Volatile compounds in the fresh and industrialized mango pulps.

	Classes	Compound	Samples (% Area)					Odor note
			LRI*	FP	PA	PP	PPA	
1	Terpene	(R)-α-pinene	1010	11.43	5.43	7.55	7.29	Herbal, pine, resin, turpentine
2	Aromatic	methylbenzene	1059	0.13	0	0	0	Solvent, resin
3	Terpene	camphene	1076	0.29	0.08	0.19	0.20	Fresh, pine
4	Terpene	(-)-β-pinene	1157	2.15	0	1.04	0.91	pine, resin, turpentine
5	Aromatic	ethybenzene	1122	0.06	0	0	0	Plastic, solvent
6	Aromatic	m-xylene	1118	0.08	0	0	0.11	plastic, green, pungent
7	Terpene	β-pinene	1157	50.25	50.36	71.30	69.72	Woody, pine, resin, turpentine
8	Terpene	α-terpinene	1188	0.13	0.32	0.18	0.16	Lemon, chemical
9	Aromatic	*o*-xylene	1178	0.01	0	0	0	Fatty, geranium
10	Aromatic	*p*-xylene	1178	0.01	0	0	0	Fatty, geranium
11	Terpene	D-limonene	1181	0.91	1.48	1.09	1.02	Citrus, lime, mint
12	Terpene	β-phellandrene	1172	0.79	1.75	1.08	0.89	Herbaceous, turpentine, terpenic, minty
13	Ester	butyl butanoate	1226	0	0.11	0	0	Sweet, fruit, fresh
14	Terpene	trans-β-ocimene	1266	2.44	4.13	2.17	2.95	Sweet, woody and herbal
15	Terpene	γ-terpinene	1245	0.37	0.56	0.24	0.34	Herbaceous, citrus
16	Terpene	β-cis-ocimene	1250	1.19	2.68	1.13	1.40	Sweet, woody and herbal
17	Terpene	*p*-cymene	1291	0.10	0	0.07	0.11	Citrus
18	Terpene	terpinolene	1287	0.29	0.32	0.26	0.30	Sweet, piney, woody, oily
19	Others	bicyclo[4.2.0]oct-1-ene, 7-exo-ethenyl-	1310	0	0.05	0	0	-
20	Terpene	neo-allo-ocimene	1390	0.47	1.04	0.36	0.33	Herbal, green, mango leaf
21	Others	3,4-dimethyl-2,4,6-octatriene	1402	0	0.11	0	0	-
22	Ester	butyl caproate	1410	0	0.06	0	0	Solvent, lemon
23	Aromatic	1-phenyl-1-butene	1421	0.05	0	0	0	-
24	Others	2,6-dimethyl-1,3,5,7-octatetraene, E,E-	1469	0	0.11	0	0	-
25	Ester	2-butoxyethyl acetate	1483	0	0.47	0	0.11	Resin
26	Terpene	copaene	1515	0.07	0.21	0	0.09	Sweet, woody
27	Alcohol	1-hexanol, 2-ethyl-	1512	0.74	0	0	0	Green
28	Terpene	α-gurjunene	1519	1.38	2.61	1.14	0.6	Woody, earthy, balsamic
29	Terpene	α-elemene	1615	0	0.06	0	0	Green, woody
30	Others	cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-	1601	0	0.08	0.09	0	-
31	Terpene	isocaryophillene	1591	0	0.04	0	0	Woody
32	Terpene	α-bergamotene	1577	0	0.19	0	0	Woody
33	Terpene	6-epi-shyobunol	1612	0.08	0	0	0	-
34	Terpene	caryophyllene	1608	5.94	14.44	6.61	7.18	Sweet, woody, spicy and tenacious
35	Terpene	terpinen-4-ol	1611	0.20	0.39	0.16	0	Sweet, piney, oily
36	Terpene	bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene)-, [1S-(1R*,9S*)]-	1619	0	0.08	0	0	-
37	Terpene	myrcenol	1625	0	0	0.07	0	Green, resinous, balsamic
38	Lactona	butyrolactone	1673	0.15	0	0	0	Caramel, sweet
39	Terpene	farnesene, epoxide, E	1672	0	0.19	0	0	-
40	Terpene	γ-gurjunene	1675	0	0.05	0	0	Woody, earthy, balsamic
41	Terpene	γ-chlorobutyrophenone	1679	0.10	0	0	0	-
42	Terpene	humulene	1680	2.97	4.82	1.92	3.37	Sweet, woody and hay
43	Terpene	4,5-di-epi-aristolochene	1665	0.516	0.638	0.263	0.307	-
44	Terpene	(+)-ledene/ guaia-1(10),11-diene	1672	0.09	0.13	0	0	-
45	Terpene	α-terpineol	1690	0.15	0.31	0.52	0.79	Sweet, piney, oily
46	Terpene	eremophilene	1732	0.01	0.12	0	0	-
47	Terpene	β-eudesmene	1728	3.95	4.66	2.19	0	Nutty
48	Terpene	α-selinene	1750	0.24	0.33	0.15	1.72	Floral, nutty
49	Aromatic	naphthalene	1744	0.73	0	0	0	Fresh, pungent
50	Others	2-oxazolidinone, 3-amino-5-(4-morpholinylmethyl)-	1745	0.92	0	0	0	-
51	Terpene	(-)-α-panasinsen	1840	0.14	0.14	0.07	0	-
52	Ester	pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	1881	0.93	0	0	0	Cheese, acid, unpleasant
53	Others	2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	2370	0.23	0	0	0	-

FP: Fresh pulp; PA: Fresh pulp processed with additive; PP: Pasteurized pulp; PPA: Pasteurized pulp with additives; *Retention indexes calculated and not found in the database above for comparison.

Table 3. Concentrations of polyphenolic compounds present in fresh and industrialized mango pulps.

Compounds	Chemical class	Samples (mg/100 g)				
		RT (min.)	FP	PA	PP	PPA
Gallic acid	Phenolic acid	6.171	23.74 ± 0.01[a]	7.98 ± 0.01[b]	14.34 ± 0.17[c]	16.71 ± 0.02[d]
Pyrocatechol	Flavonoid	11.404	<LQ	<LQ	<LQ	<LQ
Chlorogenic acid	Phenolic acid	12.779	5.30 ± 0.06[a]	3.35 ± 0.01[d]	3.73 ± 0.00[c]	4.25 ± 0.03[b]
Catechin	Flavonoid	12.779	14.96 ± 0.02[a]	6.93 ± 0.09[d]	8.67 ± 0.04[c]	10.74 ± 0.04[b]
Vanillic acid	Phenolic acid	16.499	<LQ	<LQ	<LQ	<LQ
Epigallocatechin gallate	Flavonoid	16.965	16.83 ± 0.01[a]	16.07 ± 0.00[d]	16.15 ± 0.02[c]	16.46 ± 0.00[b]
Epicatechin gallate	Flavonoid	18.234	16.61 ± 0.01[b]	16.92 ± 0.02[a]	15.50 ± 0.04[c]	15.38 ± 0.01[d]
Ferulic acid	Phenolic acid	20.573	76.30 ± 0.12	ND	ND	ND

FP: Fresh pulp; PA: Fresh pulp processed with additive; PP: Pasteurized pulp; PPA: Pasteurized pulp with additives. Mean values ± standard deviation of triplicate determinations (n=3). The mean values followed by same letters in the same line do not differ by the Tukey test at 5% of error probability, whereas the different letters represent significant differences (p<0,05) among them. LQ: limit of quantification; ND: not identified.

compounds. However, by combining additive with pasteurization in the PPA sample, it was observed that the concentration of the compounds was closer to the FP sample, although they were significantly (p≤0.05) different.

These results showed that all conservation methods applied to the samples caused the loss of ferulic acid, which was the compound with the highest concentration in the FP sample, and it is an important phenolic acid that acts as an efficient antioxidant present in fruits and vegetables, having several therapeutic effects (Itagaki et al., 2009). Kim et al. (2007) identified ferulic acid as a minority compound, evaluating changes in the phytochemical composition of green and ripe mango "Tommy Atkins".

Gallic acid was the second compound found in highest concentration in the FP sample, and is an important antioxidant, has anti-inflammatory, antimicrobial, anti-tumor and neuroprotective activity, and has also been cited among the major phenolic acids that are present in the bioactive composition of mangoes (Kim et al., 2007). Its lowest concentration was determined in the PA sample.

The epigallocatechin gallate was the third highest concentration compound in the FP sample, followed by epicatechin gallate and catechin, which make up the catechins identified in this work. Catechins represent an important group of flavonols that have various activities such as antioxidant, antiviral, hypoglycemic, anticancer, and are naturally present in fruits, vegetables, legumes and herbs (Nagao et al., 2005).

Pyrocatechol and vanilic acid, which are important antioxidant compounds commonly detected in fruits, were also identified in the samples, but were found below the limit of quantification. Sauthier et al. (2019) also identified vanilic acid below the limit of quantification in dried pulp and mango peel samples, and although they also studied fruits from mangoes grown in Brazil, obtained higher concentrations of phenolic acids in dry pulp for chlorogenic acid (2.18-32.02 mg/100 g), acid gallic (1.79-20.43 mg/100 g) and ellagic acid (2.10-4.80 mg/100 g); however, for flavonoids, higher values were obtained for catechin (7.71-40.56 mg/100 g) and rutin (0.81-2.35 mg/100 g).

The differences between the results obtained in this research and those found in the literature demonstrate that the varieties studied, as well as the geographical location and the type of processing of the sample, have a great influence on the content of bioactive compounds, resulting in differences in the composition of mango pulps.

3.4 Total bioactive compounds and antioxidant capacity

The results obtained in the analysis of bioactive compounds and antioxidant capacity of fresh and processed mango pulp can be visualized in Table 4.

Regarding the AA, there were significant differences (p≤0.05) among all the samples, where the PP sample had a lower value. As the PP sample had a lower value, it was found that pasteurization causes AA reduction, as this compound has a high sensitivity to heat. Santhirasegaram et al. (2013) recorded the degradation of 65% of the AA in the thermally treated juice samples of mango "Chokanan". On the other hand, the results showed that the addition of the additive was able to preserve and increase AA, both in the sample PA and in PPA.

In the CC, there were significant differences (p≤0.05) between the PP and PPA samples with FP and PA samples. The results show that the addition of additive was able to preserve the CC. However, pasteurization alone caused the reduction. Cortés et al. (2006) in their study with orange juice, also observed that the thermal treatment causes degradation of the carotenoids. The addition of additive and pasteurization, when combined, were able to preserve and increase the CC in the product.

For the TPC, there were significant differences (p≤0.05) between FP with PA and PP samples. The results showed that all the conservation methods used in the evaluated samples resulted in the reduction of the TPC, being pasteurization the worst method. The treatment method applied in the industrialization of mango pulp should preserve as much as possible the TPC in the final product, since are natural substances contained in fruits, and can significantly influence the antioxidant capacity, which has potential for the health protection against diseases (Ma et al., 2011).

Regarding the TFC obtained in this study, there were significant differences (p≤0.05) between the FP sample and the other three samples. In this case, the FP sample obtained a

Table 4. Total content of bioactive compounds and antioxidant activity of fresh and industrialized mango pulps.

Parameter	Samples			
	FP	PA	PP	PPA
AA (mg/100 g)	9.56 ± 1.18[c]	12.66 ± 1.18[b]	3.62 ± 0.89[d]	16.53 ± 0.89[a]
CC (µg β-carotene/100 mL)	103.50 ± 0.13[b]	*105.70* ± 0.34[b]	85.16 ± 0.38[c]	133.40 ± 0.41[a]
TPC (mg GAE/100 g)	1550.22 ± 7.11[a]	1393.77 ± 7.11[b]	1384.30 ± 8.21[b]	1545.74 ± 4.10[a]
TFC (mg QE/ 100 g)	371.36 ± 5.31[a]	320.30± 3.47[b]	311.07 ± 7.24[b]	292.50 ± 3.48[c]
ABTS (mMol/100 g)	0.34 ± 0.03[b]	0.11 ± 0.01[c]	0.46 ± 0.01[a]	0.46 ± 0.01[a]
DPPH (mMol/100 g)	29.46 ± 0.25[a]	14.99 ± 0.42b[b]	14.31 ± 0.18[b]	15.56 ± 0.47[c]
FRAP (mMol/100 g)	0.31 ± 0.02[a]	0.15 ± 0.04[b]	0.27 ± 0.04[c]	0.16 ± 0.03[b]
ORAC (mMol/100 g)	1.07 ± 0.03[a]	1.06 ± 0.02[a]	0.96 ± 0.01[b]	1.05 ± 0.02[a]

FP: Fresh pulp; PA: Fresh pulp processed with additive; PP: Pasteurized pulp; PPA: Pasteurized pulp with additives; AA: Ascorbic acid; CC: Carotenoids content; TPC: Total phenolics content; TFC: Total flavonoids content. Mean values ± standard deviation of triplicate determinations (n=3). The mean values followed by same letters in the same line do not differ by the Tukey test at 5% of error probability, whereas the different letters represent significant differences (p<0,05) among them.

highest value and the PPA sample had a lower result. The results showed that all conservation methods reduced the TFC. This is a negative factor, since flavonoids represent the largest group and are more widely distributed in fruits, among the phenolic compounds, being generally very effective antioxidants. Ma et. al. (2011) when analyzing eight mango varieties have shown that flavonoids are the main phenolic compounds present in mango fruits and therefore these need to be preserved.

In the ABTS assay there were significant differences ($p \leq 0.05$) between the FP sample and the other samples. However, there were no significant difference between the PP and PPA samples. The results obtained, except for the PA sample, were superior to those found by Siddiq et al. (2013), who reported values of 0.22 mMol Trolox/100 g in fresh mango, and express that the addition of the additive resulted in a decrease the antioxidant capacity compared to the FP sample. However, pasteurization was not only able to preserve, but also increased the antioxidant capacity.

Regarding the DPPH assay, there were significant ($p \leq 0.05$), differences between the samples, where the FP sample was the one that obtained the highest result. The sample PA, was similar to PP. Siddiq et al. (2013) reported that the treatments evaluated in their work on the mango samples had an increase in the antioxidant capacity for DPPH. However, the results obtained in this work lead us to conclude that none of the conservation methods applied to the pulps was able to obtain satisfactory results in relation to the analysis of the antioxidant capacity by the DPPH assay.

For the FRAP assay, there were also significant differences ($p \leq 0.05$), where the FP sample was different from the others. The obtained results show that none of the preservation methods applied to pulps were able to obtain satisfactory results in comparison with the fresh pulp. When analyzing the antioxidant capacity by the FRAP assay, where the major loss was characterized in the samples that had addition of additive, since the differences between the samples were significant. The results obtained were superior to those found by Siddiq et al. (2013) who reported 0.07 mMol Trolox/100 g in the fresh manga pulp, and the values between 0.08-0.39 mMol Trolox/100 g in the samples treated with pasteurization.

Regarding the ORAC assay, there was a significant difference ($p \leq 0.05$) in the PP sample, which was of the lowest value. The results obtained were larger than the values found by Robles-Sánchez et al. (2011) who reported the values of 0.85 mMol Trolox/100 g in fresh mango and 0.79 mMol Trolox/100 g in minimally processed mango. The results obtained indicate that in the samples in which the additive was added, the antioxidant capacity did not significantly reduce in comparison to the FP sample. However, pasteurization when applied alone caused a significant reduction in antioxidant capacity.

The differences presented by the methods of assessing the antioxidant capacity can be justified by the mechanism of action involved, since each assay has peculiarities, as well as by the composition of the reaction medium (Sucupira et al., 2012). Thus, the conservation methods applied to the samples may have influenced in different ways on the results obtained in each test of antioxidant capacity.

As in other studies, there was a correlation between the content of bioactive compounds and the antioxidant capacity (Ma et al., 2011), since in most cases, the FP and PPA samples that showed a higher content of bioactive compounds also showed greater antioxidant capacity. It was found that the heat treatment decreased the content of bioactive components. Moreover, the differences between the results obtained in this study and the data found in the literature show that the mango variety and the type of product compared, influence the content of bioactive compounds, and consequently on the antioxidant activity.

3.5 Principal Composition Analysis (PCA)

To summarize the set of measured results as well as to facilitate the understanding of the behavior of the data, the PCA was applied. From the correlation matrix, PCA was used to determine the relationship between the five classes of volatile compounds, the volatiles compounds with the higher concentrations, physicochemical and bioactive constituents, phenolic profile, and the antioxidant capacity in the different samples of fresh and processed mango pulp, resulting in 70 main components.

PCA results for the four samples in triplicate (12 samples x 70 variables = 840 data points) are shown in Figure 1 which

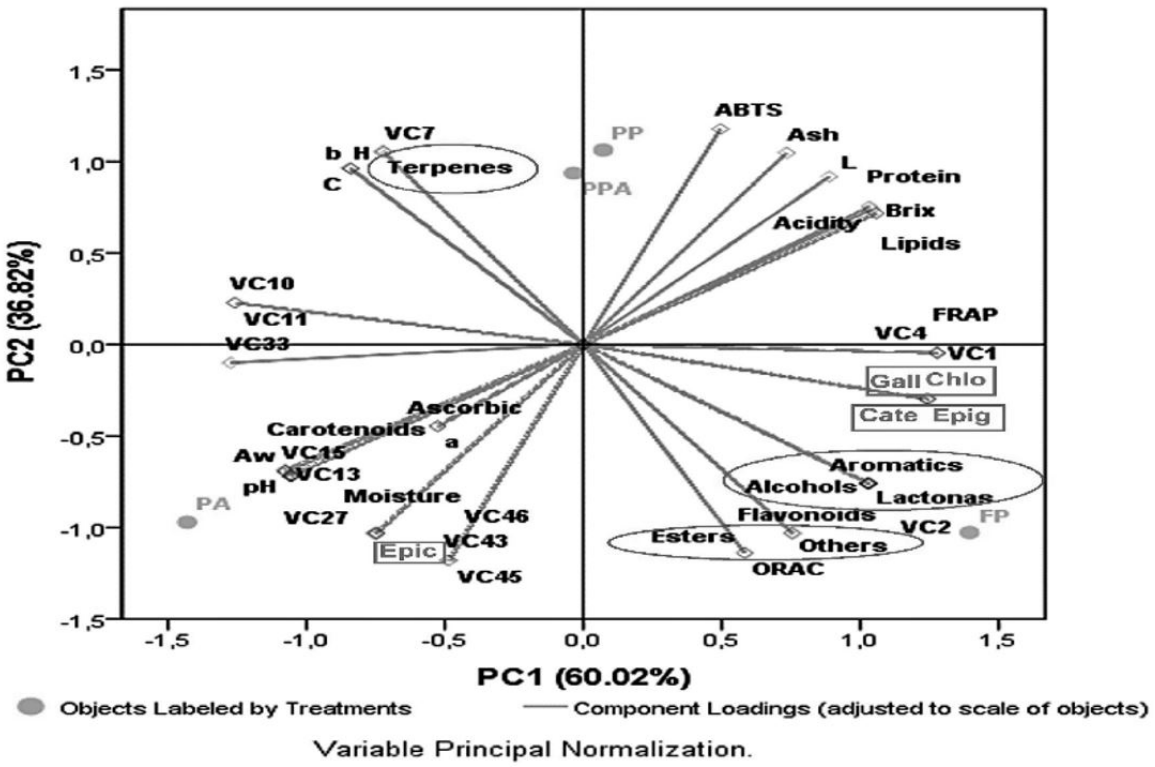

Figure 1. Principal component analysis (PCA) for physicochemical constituents, bioactive compounds, antioxidant activity and volatile profiles fresh and processed mango pulps. VC1: (R)-α-pinene; VC2: methylbenzene; VC4: (-)-ß-pinene; VC7: ß-pinene; VC10: D-limonene; VC11: ß-Phellandrene; VC13: trans-ß-ocimene; VC15: ß-cis-ocimene; VC27: α-gurjunene; VC33: caryophyllene; VC43: (+)-ledene; VC45: eremophilene; VC46: ß-eudesmene.

indicated two main components (PC1-PC2) that explained a total of 96.84% of the cumulative variance. According to the trend of separation PC1 and PC2 explained 60.02% and 36.82% of the analyzed data, respectively. The graph plotted with the load values (>0.70) indicated that the variables presented the large contribution for the separation of the treatments. For PC1, the variables were aw, pH, °Brix, lipids, protein, acidity, aromatics, alcohols, lactones, VC1, VC4, VC2, VC13, VC15, VC33, VC10, VC11, chlorogenic acid, catechin, epigallocatechin gallate, DPPH and FRAP; while in PC2, the variables of higher contribution were the colorimetric coordinates (H, L, b, C), moisture, ash, terpenes, esters, others volatiles, VC7, VC27, VC46, VC43, VC45, total flavonoids, epicatechin gallate, ABTS and ORAC.

According to the PCA graph, the PC1 component separated the different pulp samples into two groups, the fresh pulp from the other pulps with additives, being influenced mainly by the different volatile chemical classes, antioxidant capacity and the bioactive compounds, allocated to the positive side of PC1, while compounds with larger area coverage and terpene class contributed to the negative charges of component 1. As for PC2, it was the main axis for the division between the samples that did not undergo thermal treatment and those that underwent. The pulps that did not undergo heat treatment and the pasteurized pulp were distributed in the scores negative and positive, respectively. Based on the observed results, with the separations obtained in PC1 and PC2, the stability of the samples was influenced mainly by the thermal processing that is associated with a decrease in the main compounds identified in FP.

4 Conclusion

According to the results obtained in this study, it is concluded that processing significantly affects the functional and aromatic characteristics of the mango pulp. All parameters evaluated were affected, and the results showed a significant reduction in volatile composition, bioactive compounds and antioxidant capacity in the samples. Although pasteurization is an important conservation method in the production of mango products, it was the method that most affected the volatile and bioactive compounds in the samples.

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