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Influence of Extraction Methods on Phenolic Compounds from Pulp and Peel of Genipap (*Genipa americana* L.) Fruit

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Abstract

Brazil has a great variety of fruits which are rich in bioactive compounds, such as the genipap fruit. Both the peel and the pulp of genipap have beneficial components for health, making the study of this fruit important for the proper use of its functionalities. The objective of this work was the extraction of bioactive compounds from the peel and pulp of genipap by different techniques. Extraction processes were carried out using different devices (orbital incubator shaker, ultrasonic bath, and ultrasonic probe) and at different temperatures (40, 60, 70, 80 and 90 °C). The best process for extracting phenolic compounds from the pulp of genipap fruit was with the ultrasonic probe at 40 °C, which indicated the efficiency of applying the sound waves directly to the sample. Regarding the peel, the best method for extracting phenolic compounds was using the orbital incubator shaker at 80 °C.

Keywords: Extraction methods; Genipap fruit; Phenolic compounds; Bioactive compounds; Antioxidant

1 Introduction

The Cerrado of Brazil holds a great biodiversity with diverse species of fruits, such as the genpap, which are little known or studied. In this biome, native and exotic fruits are found, which have great economic, nutritional and functional potential. Thus, the processing of non-conventional tropical fruits contributes to the local economy, as well as to the dissemination of fruits from the Brazilian biome (Luiz Cardoso Bailao et al., 2015).

Fruit processing produces residues with different characteristics that are commonly used for composting or production of animal feed. The potential of these residues, which have high antioxidant activity, carbohydrate and fibre contents, biosorption properties, amongst others, are not being exploited (Evangelista Vasconcelos Schiassi et al., 2018; Gupta & Verma, 2015). Thus, it is necessary to make a better use of the residues from fruits, such as genipap. The genipap peel represents about 12.5% of the total fruit mass but there are few studies in relation to its use (Chagas Barros et al., 2017). The genipap peel has fibre, minerals, carbohydrates and bioactive compounds, such as iridoids (cyclopentane-[C]-pyran skeleton and carbocyclic iridoids) (Nathia-Neves et al., 2018). Some studies have reported the use

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of unripe genipap fruit as source of iridoids and natural antioxidants. Genipin, genipinic acid, geniposide, sigmasterol, β-sitosterol, among others, were identified in genipap. Genipin has anti-inflammatory and anticarcinogenic actions, also acting in the control of cholesterol, while geniposide and geniposidic acid have a purgative activity (Belle et al., 2018; Nathia-Neves et al., 2017; Shanmugam et al., 2018). The literature shows that genipin and geniposide are in higher concentration in unripe genipap fruits (Belle et al., 2018). The degree of maturation of genipap influences the content of these bioactive compounds i.e., decreasing by about 90% with maturation (Belle et al., 2018). Bentes and Mercadante (2014) reported that the seeds presents greater concentration of genipin than the pulp of unripe genipap.

Several methods can be used to extract genipin and other bioactive compounds from genipap fruit. The selection of proper technologies depends on the compound of interest, the cost and the scale of production (Nathia-Neves et al., 2019). Some nonconventional extraction methods are reported in the literature that increase the extraction efficiency (Barba et al., 2016; Sagar et al., 2018). Nathia-Neves et al. (2019) carried out the extraction of genipin from pulp and peel of ripe genipap fruit by using pressurized liquid extraction at 50 ^{o}C, and reported the pulp and peel extracts contain genipin concentrations of 20.7 $\pm$ 0.9 mg/g and 6.18 $\pm$ 0.07 mg/g, respectively. Bentes and Mercadante (2014) reported a geniposidic acid concentration of 2.54 $\pm$ 0.05 mg/g in the methanolic extract of unripe genipap pulp after a stirring extraction at 22 ^{o}C. Mayela Ramos-de-la-Pena et al. (2014) reported a genipin concentration of 7.85 $\pm$ 0.33 mg/g after an ultrasound assisted extraction at 285 W and 24 kHz. Nathia-Neves et al. (2017) applied a pressurized extraction process with ethanol and reported that the endocarp gives the highest recovery of genipin (48.6 $\pm$ 0.6 mg/g raw material) and the extraction from the mesocarp allowed the greatest recovery of geniposide (59 $\pm$ 1 mg/g raw material). Enzyme-assisted extraction in liquid-liquid two-phase aqueous system was applied by Belle et al. (2018). Belle et al. (2018) tested different commercial enzymes for extract, and found that cellucast enzyme (10 %, 36 ^{o}C and pH 3.7) resulted in an extract with 196 mg/g of genipin. Madrona et al. (2019) reported that the optimal conditions for aqueous extraction of polyphenols from genipap fruit pulp were at 71 ^{o}C for 49 min by using a magnetic stirrer (400 rpm), fruit pulp:water ratio of 1:3 (w/w), which resulted in an extract containing 3.18 mg GAE/g.

The ultrasonic assisted extraction process has been investigated mainly to extract compounds of interest from a solid matrix, since the bubbles which are created by sound waves promote the breaking of the cell walls, resulting in better extraction (Gonzalez-Centeno et al., 2015). Basically, there are two types of ultrasound equipment, the ultrasonic bath and the ultrasonic probe. In the ultrasonic bath, the waves are emitted at the bottom of the tank reaching the raw material placed inside the equipment,whilst the ultrasonic probe is used directly in the sample and, thus, gives higher efficiency than the ultrasound bath (Dolatowski & Stasiak, 2012).

Therefore, the objective of this work was to evaluate the effects of different extraction methods, as well as the influence of temperature on the extraction of bioactive compounds from the pulp and peel of genipap fruit, by using incubator shaker, ultrasonic bath, and ultrasonic probe.

2 Materials and Methods

The unripe genipap fruits were purchased in a local market of Uberlândia city (Minas Gerais state, Brazil), peeled, cut in half, vacuum-packed (peels and pulps were individually packed) and frozen in the Food Engineering Laboratory of the Federal Universidad of Uberlândia, campus Patos de Minas (Minas Gerais state, Brazil), as shown in Figure 1.

Extractions of the bioactive compounds were performed for the pulp and peel of genipap by using different equipment: orbital incubator shaker at 120 rpm (SolabSL-223, Piracicaba, Brazil), ultrasonic bath at 40 kHz (Unique, USC-1400, Indaiatuba, Brazil) and ultrasonic probe at amplitude 70 %, 20 kHz, 500 W (COLE-PARMER, EW-04711-30 Ultrasonic homogeniser, Vernon Hills, USA), as well as at different temperatures (40, 60, 70, 80 and 90 ^{o}C). This temper-

ature range was chosen based on the values suggested in the literature for extraction of bioactive compounds from natural sources (Bindes et al., 2019). Water was used for the extraction at a ratio of 5:1 by mass of raw material, and for an extraction time of 45 min, as suggested by Madrona et al. (2019). Water was chosen in order to favour the extraction of phenolic compounds and in order to use an environmentally- and health-friendly solvent.

A kinetic study was carried out to evaluate the concentration of phenolic compounds in relation to the extraction time. Extractions of genipap pulp were made using the incubator shaker at 60 ^{o}C for a total time of 180 min.

The moisture, ash, protein, lipid and dietary fibre contents were determined according to AOAC International (2010). The carbohydrate levels were calculated using the formula: 100 - (% ash + % lipids + % protein + % total dietary fibre).

Total phenolic analyses were performed using the Folin-Ciocalteau method proposed by Singleton and Rossi (1965). Hence, 0.125 mL of extract, 0.125 mL of Folin-Ciocalteau reagent (Sigma-Aldrich) and 2.25 mL of 2.8% sodium carbonate (Dinâmica, PA) solution were added into a test tube. The contents were mixed and held for 30 min at room temperature (25 ^{o}C), protected from light. Then, the total phenolic concentration was determined with a spectrophotometer (Ionlab, IL-226, Araucária, Brazil) at wavelength of 725 nm. Gallic acid (Sigma-Aldrich, 98.5%) was used as the standard acid, and the concentration was expressed in milligrams of gallic acid equivalents per gram of fresh genipap pulp (mg GAE/g).

For the analysis of the antioxidant activity of the peel and pulp extracts of genipap *in natura,* the procedure was performed according to the Avila et al. (2018) adapted method. Thus, 0.1 mL of sample and 2.46 mL of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich) at a concentration of 2.4 mg/100 mL were added to a test tube of 50% ethanol (Dinâmica, 99.5%). The tubes were shaken and stored in the dark at room temperature for 50 min. The absorbance was then read in a spectrophotometer Ionlab IL-226 at wavelength of 515 nm. The analysis was performed in triplicate, with the ability to sequester the radical expressed as the percentage of decrease of the absorbance in relation to the control (0.1 mL of water with 2.46 mL of DPPH). The percentage of reduced DPPH (% DPPH) was calculated using Equation 1.

$$\%DPPH = (\frac{ABS_C - ABS_A}{ABS_C}) \times 100 \qquad (1)$$

where % DPPH is the percentage of reduced DPPH, ABSC is the absorbance of the control and ABSA is the absorbance of the sample.

Then a linear curve was drawn of the antioxidant capacity of the extract against its concentration. Linear regression of the data gavethe regression equation that was used to calculate the EC_{50} (Brand-williams et al., 1995). Analyses were performed in triplicate. All the analyses were compared with the control extract, which was obtained from the peel and pulp of the genipap without the use of heat, that is the extraction process was done at room temperature (25 ^{o}C). The free radical scavenging activity was expressed as the concentration required to inhibit 50% of free radicals (EC_{50}). To obtain the EC_{50} values (concentration of the extract necessary to reduce 50% of the DPPH radical) of the extracts, the antioxidant activity in different concentrations was calculated using Equation 2.

$$EC_{50} = \frac{concentrationofsample(mg/mL) \times 50\%}{\%reduceofDPPHofthesample} \qquad (2)$$

For chromatographic analysis of some samples, a Shimadzu HPLC chromatograph (LC-20A Prominence, Barueri, Brazil) equipped with a Discovery HS C18 column at 280 and 320 nm at a temperature of 40 ^{o}C was used. Following the methodology of Ribeiro et al. (2015), the mobile phase was 2 % (v/v) acetic acid in water (eluent A) and 0.5 % acetic acid and water in acetonitrile (50:50 v/v, eluent B): gradient from 10 to 24 % B (20 min), from 24 to 30 % B (20 min), from 30 to 55 % B (20 min), from 55 to 100 % B (15 min), 100 % B isocratic (8 min), from 100 to 10 % B (2 min). Total run time was 90 min. The injection volume for all samples was 10 μL at flow rate of 0.7 mL/min. Genipin (Sigma-Aldrich), geniposidic acid (Sigma-Aldrich) and gallic acid (Sigma-Aldrich) were used as standards.

Extractions and analyses were carried out in triplicate. Statistical analyses were performed using

the Statcamp software, version 3.5.152.34 build 4 (Statcamp, Campinas, São Paulo, Brazil) at a 5% level of significance. The effects of the main factor on the content of phenolic compounds and antioxidant activity were determined by analysis of variance (ANOVA) and, if necessary, by Tukey's test, according to the following model (Equation 3):

$$Y_i = \beta_0 + \beta_1 x_i + \varepsilon_i \quad (3)$$

where, Y_i is the response variable in the i-th observation; x_i represents the value of the explanatory variable temperature; ε_i is a random variable that represents the experimental error; β_0 and β_1 are the parameters of the model, which were estimated, and which defined the regression line.

3 Results and Discussion

Table 1 presents the composition of peel and pulp of genipap fruit. These results were in agreement with that found in the literature (Bentes & Mercadante, 2014; Nathia-Neves et al., 2017; Nathia-Neves et al., 2020). The genipap is a juicy fruit, with a relatively high moisture percentage. Genipap fruits can be considered as a source of healthful carbohydrates. The genipap peel presented higher content of fibre and lipids than the genipap pulp, which could be used as a source of bioactive compounds instead of being discharged. Concentrations of total phenolic compounds in the extracts of genipap pulp and peel with all the proposed extraction methods and at temperatures varying from 40 to 90 ^{o}C are presented in Tables 2 and 3.

The increase in temperature had a negative influence on the content of phenolic compounds from genipap pulp using the shaker and ultrasonic probe (Table 2). These results may have been due to the agitation provided by the shaker, which in excess can promote oxidation due to the contact of the compounds with oxygen. In addition to the agitation, excessive temperatures can degrade sensitive compounds and the ultrasonic probe has a heater at its end which together with the temperature of the system may have negatively influenced the concentration of phenolic compounds (Das & Eun, 2018; Vinatoru et al., 2017). At 40 ^{o}C, the extracts obtained with ultrasonic probe and shaker presented higher contents of phenolic compounds than the extract obtained without mixing at room temperature (control). This behaviour was likely due to the effect of mixing and heating extraction technique. Due to the cavitational effect provided by ultrasonic waves, there was a greater heat and mass transfer through disruption of plant cell walls. Thus, ultrasound is commonly used in the extraction of phenolic compounds, such as those present in genipap (Barba et al., 2016).

The mechanical agitation provided greater contact of the solvent with the solid phase, contributing efficiency of the extraction (Bergman et al., 2017). This could explain the higher value obtained when using the incubator shaker at low temperature (40 ^{o}C) for the genipap peel extraction when compared to the control. In addition, the incubator shaker provided uniformly distributed heating due to its temperature-controlled chamber with fully agitated flask, in which diffusion of the solvent into the sample could be increased and thereby improved mass transfer in the extraction system, and consequently contributed to the outcome.

At almost all temperatures, the use of the ultrasonic bath gave lower extraction of compounds from the pulp than the other equipment. This could be because of the relatively high frequency of the ultrasonic bath (40kHz) or due to loss in the energy distribution in the bath, since the frequency generator is mounted in the bottom of the tank, thus the size of the equipment and the position of the sample inside the bath could influence or diminish the extraction efficiency. On the other hand, the ultrasonic probe has the advantage of transmitting the energy directly to the sample which contributes to better extraction (Luque-Garcia & de Castro, 2003).

Therefore, the best extraction of phenolic compound gave 11.05 ± 0.08 mg GAE/g of genipap pulp when using the ultrasonic probe at 40 ^{o}C. This technique promoted an efficient extraction even at low temperatures due to the cavitation phenomenon, as reported by Barba et al. (2016). Genipap has about 12% of peel, which is often discarded. However, this residue contains components of technological and biological interest and nutritional and economic benefits (Singh et

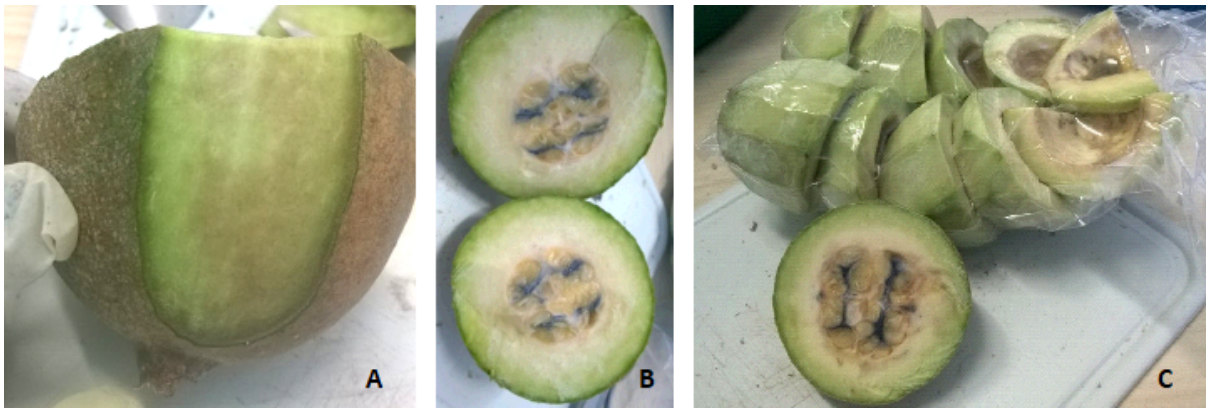

Figure 1: A) genipap fruit peel removed; B) fruit cut in half and C) genipap pulp vacuum packed and frozen

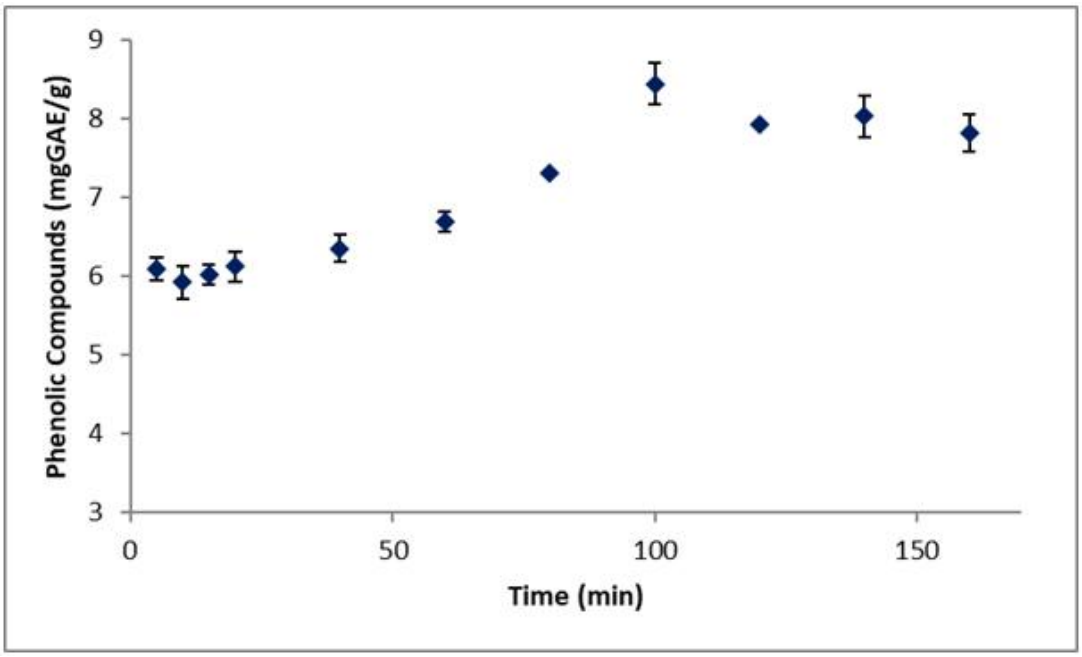

Figure 2: Kinetic extraction of the phenolic compounds from genipap pulp at 60 ^{o}C using the incubator shaker equipment

Table 1: Composition of the peel and pulp of genipap fruit

Physical and chemical characteristics	Peel	Pulp
Moisture (%)	69.60 ± 1.34^{Y}	76.60 ± 1.86^{X}
Ash (%) *	3.65 ± 0.02^{Y}	4.50 ± 0.21^{X}
Proteins (%) *	4.06 ± 0.09^{X}	4.05 ± 0.01^{X}
Carbohydrates (%) *	65.00 ± 1.20^{Y}	76.40 ± 0.62^{X}
Lipids (%) *	1.94 ± 0.04^{X}	1.50 ± 0.01^{Y}
Total fibre (%) *	25.35 ± 1.05^{X}	13.55 ± 0.39^{Y}

* dry basis.
Treatment means followed by different superscript letters (X – Y) in the rows differ from each other at 5% level of significance, according to the Tukey test.

Table 2: Content of total phenolic compounds (mgGAE/g) in the extract of genipap pulp after extractions by orbital incubator shaker, ultrasound bath and ultrasound probe at temperatures ranging from 40 to 90^{o}C

	Extraction equipment		
Extraction temperature (^{o}C)	**Orbital Incubator Shaker**	**Ultrasound Bath**	**Ultrasound Probe**
40	$9.37 \pm 0.08^{a,B}$	$4.34 \pm 0.23^{b,C}$	$11.05 \pm 0.08^{a,A}$
60	$7.90 \pm 0.20^{b,B}$	$4.34 \pm 0.58^{b,C}$	$8.31 \pm 0.08^{c,A}$
70	$7.50 \pm 0.02^{c,A}$	$5.12 \pm 0.76^{b,B}$	$9.31 \pm 0.02^{b,A}$
80	$6.31 \pm 0.03^{d,C}$	$8.73 \pm 0.05^{a,B}$	$9.51 \pm 0.01^{b,A}$
90	$6.05 \pm 0.07^{d,C}$	$7.50 \pm 0.16^{a,B}$	$8.81 \pm 0.06^{c,A}$
Control[1]	$8.08 \pm 0.07^{b,A}$	$8.08 \pm 0.07^{a,A}$	$8.08 \pm 0.07^{c,A}$

* Treatment means followed by different superscript uppercase letters (A – C) in the rows, and different superscript lowercase letters (a - d) in the columns differ from each other at 5% level of significance according to the Tukey test.
[1]Extract obtained from the sample without mixing and at room temperature (25 ^{o}C).

Table 3: Content of total phenolic compounds (mgGAE/g) in the extract of genipap peel after extractions by orbital incubator shaker, ultrasound bath and ultrasound probe at temperatures ranging from 40 to 90 ^{o}C

Extraction temperature (^{o}C)	**Orbital Incubator Shaker**	**Ultrasound Bath**	**Ultrasound Probe**
40	$4.87\pm0.21^{e,C}$	$6.99\pm0.04^{a,B}$	$8.03\pm0.04^{a,A}$
60	$7.27\pm0.26^{c,A}$	$6.28\pm0.27^{b,A}$	$6.60\pm0.27^{c,A}$
70	$7.44\pm0.04^{c,A}$	$5.63\pm0.17^{cd,C}$	$6.50\pm0.17^{c,B}$
80	$9.71\pm0,03^{a,A}$	$5.31\pm0.03^{d,C}$	$7.34\pm0.03^{b,B}$
90	$8.95\pm0,03^{b,A}$	$4.27\pm0.02^{e,C}$	$6.45\pm0.02^{c,B}$
Control	$5.90\pm0.07^{d,A}$	$5.90\pm0.07^{bc,A}$	$5.90\pm0.07^{d,A}$

* Treatment means followed by different superscript uppercase letters (A – C) in the rows, and different superscript lowercase letters (a – e) in the columns differ from each other at 5% level of significance according to the Tukey test.

al., 2018). Table 3 presents the phenolic compound contents extracted from the genipap peel. The increase in the temperature gave higher extraction of the compounds when using the incubator shaker. Since higher temperatures could decrease the viscosity of the solvent extract, thus it contributed to the transfer of mass and consequently the improvementof the extraction (Magalhaes et al., 2018). However, high temperatures can degrade the compounds in the plant matrix, justifying the decrease in the phenolic content at 90 ^{o}C for all three extraction methods. Therefore, the best extraction of phenolic compounds was 9.71 ± 0.03 mg GAE/g of genipap peel when using the incubator shaker at 80 ^{o}C.

The values found in this study were similar to those found by Porto and Cardoso (2014) in which were 8.57 ± 0.05 mg GAE/g using the dry whole genipap. Nathia-Neves et al. (2017) obtained 7.4 ± 0.2 mg GAE/g using pressurized liquid extraction at 80 ^{o}C with ethanol for the genipap pulp *in natura* and obtained 2.38 ± 0.02 mg GAE/g using pressurized liquid extraction at 80 ^{o}C for the genipap peel *in natura*. Madrona et al. (2019) reported 3.18 ± 0.12 mg GAE/g from the aqueous extract of genipap fruit obtained by ultrasound assisted at 71 ^{o}C. Terra et al. (2019) reported 1.50 mg GAE/g by performing an aqueous extraction at 60 ^{o}C of genipap fruit.

Comparing the results using the ultrasonic bath, as the temperature increased the content of phenolic compounds decreased in the extract of the peel (Table 3) of genipap, whereas the reverse occured with the pulp (Table 2). The differences between the results for pulp and peel could be explained by differences in the structure of the matrix, since in the peel there is twice as much fibre, 25.35 ± 1.05 % (Table 1), when compared to the pulp. In this way, the effect of agitation and/or temperature can be altered by the different structures in the peel and the pulp matrix, and therefore influencing the extraction efficiency of the process (Mayela Ramos-de-la-Pena et al., 2014).

In relation to the ultrasonic probe, the extracts obtained from the pulp presented a phenolic profile similar to the peel. Thus, as the temperature increased the content of the phenolic compounds decreased. This may have been due to the instability of the compounds at high temperatures, since the probe causes greater heating at its extremity, and so there may have been greater degradation of the phenolics (Arruda et al., 2017; Nathia-Neves et al., 2017).

Some compounds present in fruits have antioxidant properties that inhibit reactions that promote the oxidation of molecules or cellular structures. The main compounds that have this potential are vitamins C and E, carotenoids, minerals and phenolic compounds and their derivatives (Shahidi & Ambigaipalan, 2015). When fruits containing these components are consumed

they contribute beneficially to health, helping to prevent cardiovascular and degenerative diseases. Thus, it is important to study these components and verifying the best way to preserve their bioactive activity (Singh et al., 2018).

Among the evaluation methods of antioxidant activity, DPPH stands out. This technique has as principle the reaction of the antioxidant compounds with 2,2-diphenyl-1-picrylhydrazila (DPPH) by converting it to diphenylpicrylhydrazine and changing the colour of the solution, indicating the degree of activity (Arruda et al., 2017). The percentages of antioxidant activity (%DPPH) of each condition are found in Table 4 for the pulp and Table 5 for the peel.

For the shaker and the ultrasonic bath, the increase in temperature positively influenced the antioxidant activity. These results were observed in both pulp and peel. In general, the results were satisfactory, since almost all the conditions resulted in higher antioxidant percentages than the control extract, indicating the efficiency of the extraction process of each method. In addition, all values were above 50%, showing the potential of genipap in relation to beneficial health properties.

The variations of the values of antioxidant activity in relation to the content of phenolic compounds can be related to the fact that there are several compounds present in the matrix can exhibit antioxidant ability. The efficacy of iridoid-rich extracts from genipap fruits, as peroxyl radical scavengers points to their potential to prevent and/or treat oxidative stress-related diseases. However, phenolic acids, tannins, and phytochemicals can be found in fruits, which also have an antioxidant activity. In addition, phenolic compounds may act in synergy with other active components, which are affected by the processing conditions of the fruit such as high temperatures. Therefore, heating may result in an increase in antioxidant activity depending on how the compound is present in a raw material (Neri-Numa et al., 2020).

Thus, the best values of antioxidant activity of the analysed extracts of the peel were given at 90 ^{o}C; however, using the ultrasonic bath gave the highest value, being 87.54 ± 0.51%, with an EC_{50} equal to 114.23 ± 0.68 mg/mL. The EC_{50} represents the equivalent concentration in mg required to reduce the DPPH reagent by 50%. Therefore, the lower this value the higher the antioxidant potential of the raw material. Regarding the pulp (Table 4), the highest percentage of activity was given using the ultrasonic probe at 70 ^{o}C, with a value of 79.79 ± 0.11% (EC_{50} = 125.33 ± 0.18 mg/mL). In studies conducted by Silva and Jorge (2019) EC_{50} values of the extracts of some fruits were: lemon 93.25 ± 1.13 mg/mL;, orange 40.58 ± 0.13 mg/mL; kinkan 115.59 ± 0.63 mg/mL; and for passion fruit 108 ± 1.58 mg/mL (Silva et al., 2015).

As reported by several authors, the degree of maturation, harvest season and condition, geographic origin, storage process and other factors may influence the content of the compounds present in fruits affecting their bioactivity (Bindes et al., 2019). There is a wide variety of phenolic compounds with antioxidant capacity, and flavonoids (flavones, flavanones, isoflavones, flavonols, flavanols and anthocyanins) which are known to capture and neutralize oxidizing species such as hydroxyl radical, superoxide anion (O2-) or peroxide radical. These phenolic compounds and flavonoids can act synergistically with other antioxidants such as vitamins C and E (Cushnie & Lamb, 2011).

We determined the extraction kinetic curve of the phenolic compounds at 60 ^{o}C using the incubator shaker (Figure 2). An increase of the compounds with time was observed reaching 8.44 mg GAE/g of genipap pulp. At 180 min, the content of phenolic compounds was reduced to 7.93 mg GAE/g. This was explained by the fact that the process reached the maximum point of extraction with thermal degradation of the compounds occurring after that time. Vega Arroy et al. (2017) and Vinatoru et al. (2017) reported that long extraction times caused the degradation of phenolic compounds, since light, oxygen and high temperatures have a great influence on the process and may contribute negatively to the levels of these compounds.

The consumption of foods such as genipap, containing significant amounts of bioactive compounds contributes to health, and may have an effect against chronic and degenerative diseases. Thus, the identification and quantification of these compounds becomes important,

Table 4: Antioxidant activity (%DPPH) of genipap pulp extract

Extraction temperature (^{o}C)	**Orbital Incubator Shaker**	**Ultrasound Bath**	**Ultrasound Probe**
40	$35.14\pm0.22^{e,C}$	$40.09\pm0.22^{e,B}$	$73.56\pm0.11^{d,A}$
60	$68.92\pm0.09^{b,C}$	$73.07\pm0.26^{a,B}$	$76.99\pm0.01^{bc,A}$
70	$43.49\pm0.37^{d,C}$	$57.03\pm0.22^{d,B}$	$79.79\pm0.11^{a,A}$
80	$68.13\pm0.28^{b,B}$	$57.27\pm0.11^{cd,C}$	$77.48\pm0.22^{b,A}$
90	$76.12\pm0.11^{a,A}$	$62.62\pm0.22^{b,B}$	$75.88\pm0.22^{c,A}$
Control	$58.02\pm1.21^{c,A}$	$58.02\pm1.21^{c,A}$	$58.02\pm1.21^{e,A}$

* Treatment means followed by different superscript uppercase letters (A – C) in the rows, and different superscript lowercase letters (a – e) in the columns differ from each other at 5% level of significance according to the Tukey test.

Table 5: Antioxidant activity (%DPPH) of genipap peel extract

Extraction temperature (^{o}C)	**Orbital Incubator Shaker**	**Ultrasound Bath**	**Ultrasound Probe**
40	$50.79\pm0.46^{f,C}$	$65.89\pm0.11^{c,B}$	$80.03\pm0.22^{c,A}$
60	$67.67\pm0.37^{c,B}$	$85.53\pm0.26^{b,A}$	$84.58\pm0.11^{a,A}$
70	$64.94\pm0.33^{d,B}$	$63.42\pm0.22^{d,C}$	$83.30\pm0.11^{b,A}$
80	$71.56\pm0.22^{b,B}$	$62.86\pm0.33^{d,C}$	$83.08\pm0.22^{b,A}$
90	$81.53\pm0.28^{a,C}$	$87.54\pm0.51^{a,A}$	$85.30\pm0.00^{a,B}$
Control	$58.02 \pm 1.21^{e,A}$	$58.02 \pm 1.21^{e,A}$	$58.02 \pm 1.21^{d,A}$

* Treatment means followed by different superscript uppercase letters (A – C) in the rows, and different superscript lowercase letters (a – f) in the columns differ from each other at 5% level of significance according to the Tukey test.

Table 6: Iridoid content present in unripe genipap

Sample	Genipin (mg/g)	Geniposidic Acid (mg/g)	Gallic Acid (mg/g)
Shaker Peel, 80 ^{o}C	nd*	22.752 ± 0.455^{a}	0.642 ± 0.012^{a}
Control Peel, 25 ^{o}C	nd*	2.796 ± 0.060^{c}	0.626 ± 0.013^{a}
Control Pulp, 25 ^{o}C	nd*	15.080 ± 0.302^{b}	0.660 ± 0.013^{a}

* Treatment means followed by different superscript letters (a – c) in the columns differ from each other at 5% level of significance according to the Tukey test. nd = not detected

since it can show which bioactive components are present in the fruit under the conditions of analysis (Alves et al., 2017). Table 6 presents the contents of the iridoids, i.e., genipin, geniposidic acid and gallic acid found in unripe genipap under three different extraction conditions. These values were in accordance with those found in the literature. Nathia-Neves et al. (2017) found genipin concentration of 20.7 ± 0.9 mg/g of ripe genipap pulp and 6.18 ± 0.07 mg/g of ripe genipap peel using pressurized liquid extraction at 50 ^{o}C. However, Bentes and Mercadante (2014) did not find the presence of genipin in the unripe genipap pulp, but found geniposidic acid in the concentration of 2.54 ± 0.05 mg/g of unripe genipap pulp when using stirring extraction with a methanolic solution at 22 ^{o}C.

In accordance with Nathia-Neves et al. (2018), genipin and geniposide were at higher concentration in unripe genipap fruits. Thus, the degree of maturation of genipap influences the content of bioactive compounds, since the geniposide is present in a larger quantity in unripe fruits, which decreases by about 90% with maturation. This is due to the increase in the levels of enzymes responsible for glycosylation of the iridoids with maturation (Bentes & Mercadante, 2014). As presented in Figure 1 the genipap fruits we used were in the unripe stage of maturation, with the characteristic blue colour in the pulp after air exposure.

Genipin is the compound present in genipap which reacts with primary amines of amino acids, peptides and proteins in the presence of oxygen to form blue pigments. In this way, genipin has an antimicrobial, anti-inflammatory and anticarcinogenic action. Gallic acid has antioxidant, antimicrobial and antimutagenic properties. However, genipap has been little studied with respect to its edible parts and degree of maturation (Nathia-Neves et al., 2018).

In addition, the solvent used for extraction may influence the content of compounds, as genipin is more polar it tends to be more soluble in polar solvents like water. However, the geniposidic acid is a precursor of the geniposide. Thus, these three compounds were found in greater quantity in green genipap. However, the geniposide was the most bioactive compound (Nathia-Neves et al., 2017).

4 Conclusion

The best method for extracting phenolic compounds from the genipap pulp *in natura* in this work was the ultrasonic probe at 40 ^{o}C due to its propensity to promote greater mass transfer, and having a direct contact with the sample, which provided greater efficiency. Regarding the peel, the best extraction method of phenolic compounds was using the incubator shaker at 80 ^{o}C. The highest percentage of antioxidant activity occurred the ultrasonic bath was used at 90 ^{o}C. Higher antioxidant activities were obtained at higher temperatures.

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