Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.105422

Papaya seeds (*Carica papaya* L. var. Formosa) in different ripening stages: unexplored agro-industrial residues as potential sources of proteins, fibers and oil as well as high antioxidant capacity

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Abstract

The use of whole fruits and vegetables, including the parts usually discarded during food processing, is an alternative to reduce the quantity of agro-industrial wastes. This study aimed to evaluate papaya seeds' nutritional and bioactive potential in two ripening stages. The seeds in the stages 0 and 5 of ripening were analyzed regarding their physicochemical composition, while the oil and the hydroethanolic extracts of the seeds were studied in respect of their fatty acid profile, total phenolic content, antioxidant and antimicrobial capacities. The seeds in both ripening stages show good nutritional quality, because they are sources of protein, fiber, and oil. The oil extracted from the seeds is majorly composed by oleic fatty acid (around 70%). The seeds extracts did not present antimicrobial activity against *Salmonella enteritidies*, *Escherichia coli* e *Staphylococcus aureus*. However, they presented high contents of total phenols (58.1 and 36.0 mg GAE/g dry extract for seeds in the ripening stages 0 and 5, respectively) and good antioxidant capacity, according to the FRAP and ABTS$^{\bullet+}$ assays. Papaya seeds provide nutrients and bioactive compounds and their use is a promising alternative to reduce the disposal of food wastes in the environment.

Keywords: Monounsaturated fat; seed oil; hydroethanolic extract; byproduct; waste.

Practical Application: Reducing waste, using byproducts, and natural food additives are important sustainability trends. This study proved that unripe and ripe papaya seeds have nutritional value, since they are good sources of fiber, protein, and oil. Their oils are rich sources of oleic fatty acid, so they are monounsaturated fats. Their hydroethanolic extracts are rich sources of phenolic compounds. In this context, the use of papaya seeds in the animal feed and food industries is a feasible possibility, because they are sources of nutrients and bioactive compounds, and such use allows for reducing the amount of food wastes that are discarded in the environment.

1 Introduction

Papaya (*Carica papaya* L.) is a tropical fruit, which is native from Central and South America and cultivated around the world (Food and Agriculture Organization, 2019). Papaya has spread to different areas of the planet, due to its ease of cultivation and production all year round. In 2019, the world production of papaya reached 13 million tons, with India, Brazil, Mexico, Indonesia, Dominican Republic and Nigeria being the largest producers (Food and Agriculture Organization, 2019). Brazil is the third largest world producer (1 million tons in 2019), second only to India (5.7 million tons in the same year) (Food and Agriculture Organization, 2019). The fruit is cultivated in all Brazilian states, but Bahia, Espirito Santo and Ceará stand out as the largest producers, accounting for 75% of the Brazilian harvest. The main varieties sold in Brazil are based on two groups: those of the Formosa group and those of the Solo group (also known as Hawaii papaya) (Empresa Brasileira de Pesquisa Agropecuária, 2022).

Great amounts of waste are generated as a result of the industrial processing of papaya, which aims to produce a range of products, such as candied fruit, raisins, nectars, jellies, juices, jams, papain and pectin. These agro-industrial wastes are peels and seeds, representing about 50% of the papaya weight, while the seeds alone account for about 14% (Martin et al., 1989; Venturini et al., 2012).

The reduction in the disposal of these agro-industrial wastes may be achieved by using the commonly discarded parts of fruits and vegetables, such as the seeds. The wastes often contain several nutritionally valuable compounds, including proteins, minerals, carbohydrates, fibers and bioactive compounds, and they have the potential to be converted into new value-added products, as long as adequate processes and technologies are applied (Laufenberg et al., 2003; Uchôa-Thomaz et al., 2014).

Considering the information presented above, the use of papaya seeds to obtain new products is a promising alternative, because it could add value to a by-product, provide new ingredients to the food industry, and diminish the disposal of agro-industrial wastes. Many studies have already evaluated different ways of extracting oil from papaya seeds (Anwar et al., 2019; Chielle et al., 2016a,

Received 20 Aug., 2022
Accepted 23 Oct., 2022
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2016b; Devi & Khanam, 2019; Samaram et al., 2014; Samaram et al., 2015). However, this study becomes a differential compared to others, as it compares different stages of seed maturation, which changes several bioactive characteristics. In this sense, this work brings innovation to the food technology field, because it addresses the use of papaya seeds of the variety Formosa, which is widely consumed in Brazil. Thus, this work aimed to evaluate papaya seeds' nutritional and bioactive potential (var. Formosa) in different ripening stages, in addition to studying the seed oil and the hydroethanolic seed extracts.

2 Material and methods

2.1 Materials

Unripe papaya seeds of the Formosa variety (Figure 1A-1C) in ripening stage 0 (fully grown fruits, with 100% green peels) were obtained from two confectioneries: "Doces Mineiro" (Uberaba, Minas Gerais, Brazil) and "Doces Colmeia Ltda" (Caldas, Minas Gerais, Brazil). Ripe papaya seeds of the same variety (Figure 1D-1F) in ripening stage 5 (fruits with 76% to 100% yellow peels) were obtained from Doceria Schmidt Ltda (Engenheiro Schmitt District, São José do Rio Preto, São Paulo, Brazil). All seeds were wastes from the production of candied and syrup fruits.

2.2 Obtaining the papaya seed flour

Unripe and ripe papaya seeds were washed to remove any pulp residue and then were dried in a dehydrator with forced air circulation (PE 60, Pardal Tec, Petrópolis, Brazil), at 65 °C for 18 h. After that, the dried seeds were grinded in a domestic blender (Viva Problend RI2134, Philips Walita, Rio de Janeiro, Brazil) and sieved (20 mesh sieves).

2.3 Physicochemical composition of the papaya seeds

Unripe and ripe papaya seeds were characterized regarding their moisture content, ash, proteins, fibers and lipids, according to the methods described by A.O.A.C (Association of Official Analytical Chemists, 2004). The carbohydrate content was determined by difference.

2.4 Extraction of the papaya seed oil

The papaya seed oil was extracted from the seed flour with hexane (1:5 w/v). The mixture was agitated at 600 rpm for 30 min, at room temperature. Then, the solution was vacuum filtered through a Whatman no. 3 paper and concentrated in a rotary evaporator (TE-019, Tecnal, Piracicaba, Brazil), at 25 °C. The obtained oil was stored in an amber glass vial at 4 °C for further analysis.

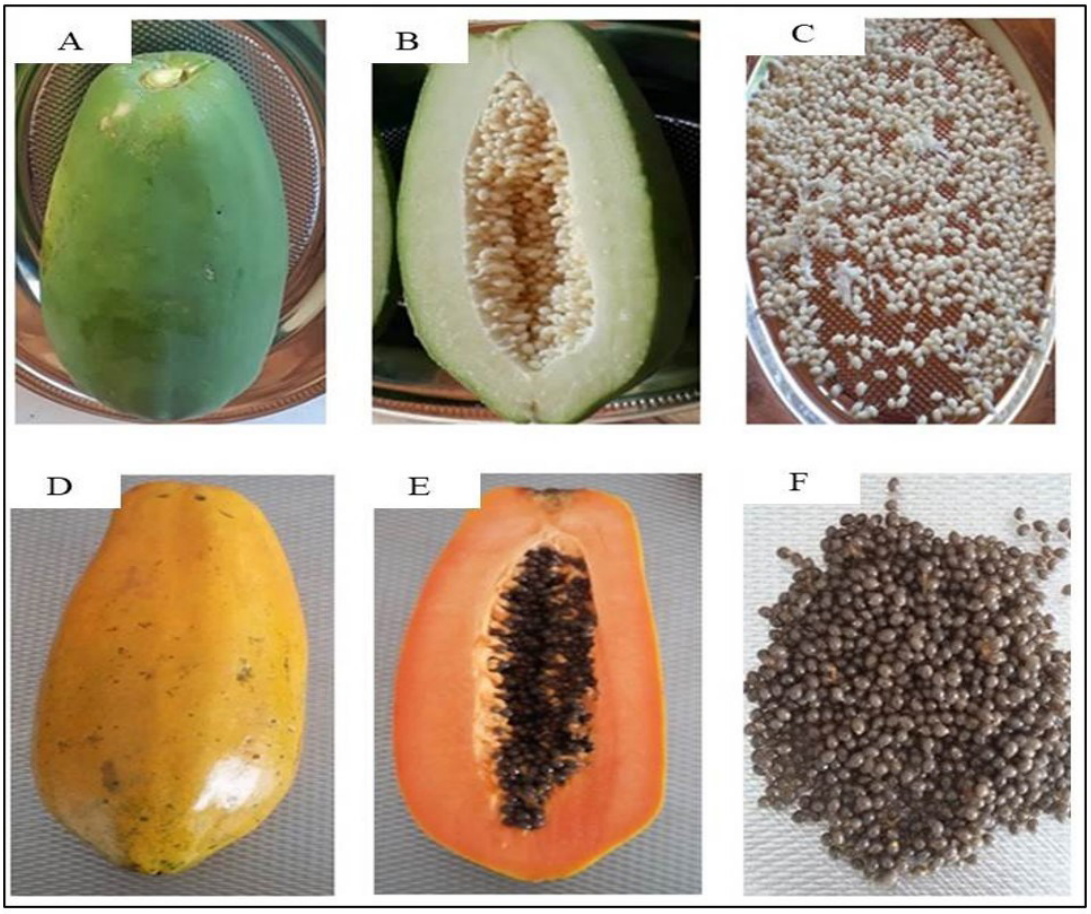

Figure 1. Appearance of the papayas and their seeds used in this study. In this picture, A (Unripe papaya); B (Longitudinal section of unripe papaya); C (Unripe Seeds); D (Ripe papaya); E (Longitudinal section of ripe papaya); F (Ripe seeds).

2.5 Fatty acid profile of the papaya seed oil

Initially, the fatty acids of the papaya seed oil were converted into methyl esters through transesterification reactions, as described by the AOCS method Ce2-66 (American Oil Chemists' Society, 1998). The fatty acid methyl esters were analyzed in a capillary gas chromatograph (Shimadzu, model 2010 AF, Kyoto, Japan), equipped with an automatic injector (Shimadzu, model AOC 20i, Kyoto, Japan) and a flame ionization detector (FID), following AOCS method Ce 1-62 (American Oil Chemists' Society, 1998). The analysis was carried out in the following conditions: SP-2560 capillary column 0.20 μm film thickness, 100 m length x 0.25 mm internal diameter (Supelco, Bellefonte, PA, USA), helium as carrier gas at 0.74 mL/s, injector temperature of 250 °C, FID temperature of 260 °C, and injection volume of 1 μL (split ratio of 100:1). The samples were maintained at 140 °C for 5 min, then heated up to 240 °C at 4 °C/min and kept at this temperature for 15 min.

Fatty acids were identified by comparison with external standards (Supelco, Bellefonte, PA, USA) and quantified by comparing the peak areas of each fatty acid of the sample to the peak area of the internal standard, methyl tridecanoate C13:0 from Sigma-Aldrich (Bellefonte, PA, USA), using the response correction factors of the flame ionization detector and the conversion of methyl esters of fatty acids to fatty acid (Instituto Adolfo Lutz, 2008).

2.6 Obtaining the hydroethanolic extracts of the papaya seeds

The residue from the extraction of the papaya seed oil was dried in a forced circulation oven at 65 °C for 18 h, to completely remove the hexane. Ethanol extraction conditions were defined based on preliminary tests. Next, the dried residue was mixed with ethanol 40% (v/v) in the proportion 1:10 (w/v) and the mixture was kept at 60 °C in a water bath (Solidsteel, Model SSD 10L, Piracicaba, Brazil), under agitation (600 rpm), for 45 min. After that, the extracts were centrifuged (5430R, Eppendorf, São Paulo, Brazil) at 5762 x g and 25 °C for 5 min and the supernatants were vacuum filtered through a Whatman no. 3 paper. The filtered extracts were concentrated in a rotary evaporator (TE-211, Tecnal, Piracicaba, Brazil) at 45 °C, until 40% of the initial volume.

2.7 Characterization of the concentrated extracts

The moisture content of the concentrated liquid extracts was determined using a moisture analyzer (MB 35, Ohaus, Ohio, USA) at 105 °C. The total soluble solids content was evaluated with a manual refractometer and expressed as °Brix. The pH of the extracts was measured with a pHmeter (MB-10, Marte, Piracicaba, Brazil).

Total phenolic compounds

The total phenolic content of the papaya seed extract was determined using the Folin-Ciocalteu method, as described by Singleton et al. (1999) and adapted by Souza et al. (2014). The liquid extract was diluted to different concentrations. An aliquot of 0.25 mL of each extract dilution was mixed with distilled water (2 mL) and the Folin-Ciocalteu reagent (0.25 mL). The samples were kept in the dark for 3 min, then mixed with 0.25 mL of sodium carbonate saturated solution (Na_2CO_3) and homogenized for 10 s. After that, the samples were maintained in a water bath at 37 °C for 30 min, in the absence of light, to complete the reaction. The absorbance of the samples was measured at 740 nm using a spectrophotometer UV-Vis (Genesys 10s, Thermo Scientific, Waltham, EUA). The results were calculated using a gallic acid calibration curve and expressed as mg gallic acid equivalent (GAE)/ g extract.

Antioxidant capacity: by 2,2'-azinobis-(3-ethylenebenzothiazoline)-6-sulfonic acid assay (ABTS$^{\bullet+}$ assay)

The scavenging activity of the papaya seed extracts against $ABTS^{\bullet+}$ radicals was measured according to Rufino et al. (2007). The $ABTS^{\bullet+}$ radical solution was obtained by mixing 5 mL of an $ABTS^{\bullet+}$ stock solution at 7 mmol/L with 88 μL of a potassium persulfate solution at 140 mmol/L. The mixture was kept in the dark at room temperature for 16 h, to stabilize and complete the reaction. After that, the solution was diluted with ethanol until an absorbance of 0.70 ± 0.05 at 734 nm was reached. The papaya seed extracts were diluted to different concentrations and then 30 μL of each dilution was mixed with 3 mL of the $ABTS^{\bullet+}$ radical solution. The samples were homogenized for 10 s and maintained in the dark for 6 min to complete the reaction. At the end of this time, the absorbance of the solutions was measured at 734 nm using a spectrophotometer UV-Vis (Genesys 10s, Thermo Scientific, Waltham, EUA), with ethanol as blank. The results were calculated using a Trolox calibration curve and expressed as μM Trolox/ g extract.

Antioxidant capacity: by ferric reducing antioxidant power assay (FRAP assay)

The ferric reducing antioxidant power of the papaya seed extracts was determined in accordance with the method described by Rufino et al. (2006). The FRAP reagent was obtained by mixing 25 mL of acetate buffer at 0.3 mol/L, 2.5 mL of TPTZ (2, 4, 6-tripyridyl-s-triazine, T1253, Sigma, St. Louis, EUA) solution at 10 mmol/L and 2.5 mL of an iron chloride hexahydrate (Dinâmica Indaiatuba, Brazil) solution at 20 mmol/L. The papaya seed extracts were diluted to different concentrations and 90 μL of each dilution was mixed with 270 μL of distilled water and 2.7 mL of the FRAP reagent. The samples were homogenized for 10 s and then kept in the dark for 30 min, at 37 °C. After that, the absorbance of the solutions was measured at 595 nm in a spectrophotometer UV-Vis (Genesys 10s, Thermo Scientific, Waltham, EUA), using the FRAP reagent as blank. The results were calculated using an iron sulfate calibration curve and expressed as μM iron sulfate/g extract.

Antimicrobial activity

The antimicrobial activity of the papaya seed extracts against the bacteria *Salmonella enteritidis* (ATCC13076), *Escherichia coli* (INCQS0017) and *Staphylococcus aureus* (ATCC25923) was evaluated by the disk diffusion method (Clinical and Laboratory

Standards Institute, 2018). Tetracycline and gentamicin were the positive controls, while distilled water and BHI broth were the negative controls. Initially, each bacterium was cultivated in BHI broth for 18 h, at 37 °C. At the end of this time, the concentration of the bacterial suspensions was adjusted to the 0.5 McFarland standard, according to which an absorbance between 0.08 and 0.1 equates to 1-2 x 10^8 CFU/mL. Next, Petri dishes containing Muller Hinton agar were inoculated with the standardized bacterial suspensions and left to dry at room temperature. Then, filter paper discs containing 20 μL of extract, antibiotics and control were placed on the agar surface and from each treatment it was poured onto the discs. After incubation at 35 °C for 24 h, the diameters of the inhibition halos formed around the discs were measured.

2.8 Statistical analysis

Data were analyzed using Statistica software (STATISTICA, version 10, StatSoft. Inc., Tulsa, USA). ANOVA and post-hoc Tukey's tests were performed, with a significance level of 0.05.

3 Results and discussion

3.1 Physicochemical composition of the papaya seeds

Table 1 shows the centesimal composition of the papaya seeds in two different ripening stages. The moisture content decreased as the ripening stage of the seeds changed from 0 to 5, which was expected, because the synthesis of new compounds during ripening results in water loss. Regarding the quantity of ashes, the values obtained in this study were similar to the reported in the literature. For instance, Silva et al. (2007) found an ash content of 7.1% in papaya seeds in the ripening stage 0, while Rosário (2019) and Azevedo & Campagnol (2014) observed that papaya seeds in the ripening stage 5 presented 8.1% and 6.4% of ashes, respectively. These values are higher than the reported for other types of seeds, such as watermelon seeds (3%), guava seeds (1%) and pumpkin seeds (4.2%) (Severino et al., 2019; Silveira et al., 2017; Tabiri et al., 2016) revealing the potential usage of unripe and ripe papaya seeds as mineral sources.

It can be noticed that the protein content of the seeds did not change after ripening. In fact, this parameter is usually stable during seeds ripening (Sartori et al., 2002). The values found here are similar to the obtained by Azevedo & Campagnol (2014) for the same material (25%) and show that papaya seeds are good sources of proteins, which can be extracted and used for animal and human feeding.

Table 1. Centesimal composition in dry base of the papaya seeds.

Parameters (dry base %)	Unripe Seeds	Ripe Seeds	p
Moisture	6.8 ± 0.1	4.4 ± 0.1	0.001
Ashes	8.4 ± 0.3	7.2 ± 0.1	0.031
Proteins	26.1 ± 0.1	26.9 ± 0.5	0.234
Raw fiber	27.2 ± 0.3	33.5 ± 0.2	0.001
Lipids	20.5 ± 0.3	28.6 ± 0.3	0.001
Carbohydrates	17.8 ± 0.3	3.8 ± 0.6	0.007

± Means followed by the standard deviation; means on the same line with $p \leq 0.05$ differ from each other at the 5% probability level by the ANOVA test.

The Fruit ripening resulted in decreased content of carbohydrates and increased contents of fibers and lipids. As can be observed in Table 1, unripe and ripe papaya seeds present significant amounts of fibers. These compounds are important for human health, because their consumption is related to the improvement of gastrointestinal disorders. In this specific case, fibers. Intestinal constipation, because it has particular qualities such as water retention and increased fecal volume. With the increased volume of the fecal bolus due to the hydrophilic capacity, the stool softens, thus stimulating intestinal peristaltic movements and promoting a greater frequency of bowel movements, the excretion of bile salts and fats, it has similar effects to drugs that increase evacuation (Lacerda & Pacheco, 2006).

The consumption of fibers is also associated with reduction of obesity, due to their ability to increase satiety by forming gels (Farias et al., 2018); control of the blood glucose level by reducing the glucose absorption; and reduction of blood cholesterol, because fibers can bind to bile acids and diminish their reabsorption power (Bertonhi & Dias, 2018). The Food and Drug Administration (FDA) recommends a daily fiber intake of about 25 g, of which 25% must be soluble fibers (U.S. Food and Drug Administration, 2018).

Recent studies have shown the advantages of incorporating fibers into food products. Mesquita et al. (2017) obtained strawberry jams with increased contents of fibers by adding papaya seed flour to the formulations. In another work, (Santos et al., 2018) incorporated a flour obtained from papaya by-products into loaf bread, which resulted in increased nutritional value and sensory acceptance of the product.

Regarding the lipid content, the values found in the present study are higher than the observed in other types of seeds. Superior to the seed oil of the Count fruit (7.52%) (Favaro et al., 2021) and the oil of the grape seeds (12%) (Silva, 2019). Content similar to yellow passion fruit seed oil (28%) (Araújo et al., 2019) and yellow melon seeds (25%) (Malacrida et al., 2007).

Despite the ripe seeds presenting more lipids than unripe ones, the seeds in both ripening stages may be considered valuable lipid sources. The information presented here reveals the importance of using papaya seeds to enrich food, resulting in products with improved nutritional value and potential for preventing the development of diseases. Moreover, since the seeds are by-products of papaya processing, their incorporation into foods, drugs and cosmetics is economically feasible.

3.2 Fatty acid profile of the papaya seed oil

Table 2 presents the fatty acid profile of the oils extracted from papaya seeds in two ripening stages. Saturated, monounsaturated and polyunsaturated fatty acids were identified and quantified, the main of them in both seed oils being oleic, palmitic, linoleic and stearic acids. Other fatty acids were observed in minor amounts. The values presented here are in accordance with the literature (Malacrida et al., 2011; Senrayan & Venkatachalam, 2018).

The oils extracted from unripe and ripe papaya seeds are composed mostly by monounsaturated fatty acids, which account for more than 70% of the total fatty acid content in both oils.

Table 2. Fatty acid profiles (in mass %) of the oils extracted from papaya seeds in two different ripening stages.

Fatty acid	Unripe seeds	Ripe seeds	P
C14:0 – myristic	0.20 ± 0.02	0.12 ± 0.01	0.037
C16:0 – palmitic	15.5 ± 0.7	13.0 ± 0.6	0.059
C16:1 ω7 – palmitoleic	0.33 ± 0.02	0.24 ± 0.03	0.091
C17:0 - margaric	0.10 ± 0.01	ND	0.002
C18:0 – stearic	4.24 ± 0.03	3.55 ± 0.08	0.008
C18:1 ω9 – oleic	70.35 ± 0.07	72.40 ± 1.2	0.210
C18:2 ω6 – linoleic	7.30 ± 0.3	8.87 ± 1.4	0.266
C20:0 – arachidic	0.38 ± 0.01	0.33 ± 0.02	0.067
C20:1ω11 – gadoleic	0.46 ± 0.02	0.40 ± 0.04	0.178
C18:3 ω3 – linolenic	0.78 ± 0.01	0.77 ± 0.5	0.979
C22:0 – behenic	0.24 ± 0.01	0.23 ± 0.01	0.423
C24:0 – lignoceric	0.09 ± 0.01	0.05 ± 0.07	0.508
SFA	20.79	17.31	-
MUFA	71.14	73.03	-
PUFA	8.04	9.64	-

ND: not detected. ± Means followed by the standard deviation; means on the same line with $p \leq 0.05$ differ from each other at the 5% probability level by the ANOVA test. SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table 3. Characterization of the concentrated extracts from papaya seeds in two different ripening stages.

Parameter	Unripe seeds	Ripe seeds	p
pH	5.0 ± 0.1	5.1 ± 0.0	0.013
Soluble solids content (° Brix)	5.2 ± 0.1	2.1 ± 0.1	0.002
Moisture content (%)	94.8 ± 0.1	97.8 ± 0.2	0.001
Total phenolics (mg GAE/g extract)	58.1 ± 0.9	36.0 ± 0.6	0.003
ABTS (μM trolox⁄g extract)	899.1 ± 1.8	789.8 ± 1.9	0.001
FRAP (μM iron sulfate⁄g extract)	338.8 ± 3.0	357.0 ± 2.0	0.002

± Means followed by the standard deviation; means on the same line with $p \leq 0.05$ differ from each other at the 5% probability level by the ANOVA test.

In addition, the content of the main fatty acids observed in the papaya seed oil did not change significantly after ripening. Considering that the oils extracted from the papaya seeds are rich in oleic acid, they are a suitable alternative to the use, either for direct consumption or industrial usage, of other oils that present similar or lower levels of this fatty acid, such as olive (75.7%), canola (65.7%), avocado pulp (60.7%), and sunflower (26.2%) oils (Alves et al., 2019).

As stated before, the papaya seeds are by-products, and their reuse provide a high-quality vegetable oil, which is source of nutrients and bioactive compounds, besides contributing to reduce volumes of wastes from the food industry (Senrayan & Venkatachalam, 2018). In addition, the composition of these oils allows for their use in the production of different materials, including emollients and biodiesel (Khalaf et al., 2019).

3.3 Characterization of the concentrated extract

According to Table 3, the concentrated extracts obtained from the papaya seeds present high moisture contents and this content increased with the fruit ripening, while the soluble solids content (°Brix) decreased. There is an increase in moisture content as the fruit ripens, as well as a reduction in soluble solids (°Brix). This reduction may be associated with the depolymerization of pectic substances, which are structural polysaccharides of the plant cell wall, which undergo changes during fruit ripening influencing the solubilization and polymerization of other compounds during ripening, causing changes in their solubility, which are less soluble to the extraction solvent (Brummell & Labavitch, 1997).

Phenolic compounds constitute a broad category of bioactives produced by the secondary metabolism of plants, which are necessary for their development and reproduction. These compounds also play an important role in protecting the plant from infections, injuries, climatic factors, among others, besides being well known for their antioxidant capacity (Naczk & Shahidi, 2004; Shahidi et al., 1992). Phenolic compounds are associated to health benefits like preventing obesity, ageing and diabetes, improving the immune response and reducing the risk of cardiovascular diseases (Wijesooriya et al., 2019).

Expressive levels of total phenolics were found in the extracts of papaya seeds at both ripening stages. but the unripe seeds presented higher values than ripe ones. Lower contents (26.6 mg GAE/g extract) were reported by Ovando-Martinez et al. (2018) for the same material. A significant reduction of the total phenolics of the extracts occurred as the seeds ripened. which may be explained by the conversion of phenolics into insoluble compounds that were not extracted by the hydroethanolic solvent (Naczk & Shahidi, 2006). Another possible reason is related to the interaction of the Folin-Ciocalteu reagent with reducing substances. such as proteins, ascorbic acid and sugars which may have led to an overestimated phenolic content in the extract of the unripe seeds due to their higher soluble solids content (Ikawa et al., 2003). In addition, the increased levels of lipids and fibers in ripe seeds may have contributed to lower phenolics biosynthesis (Ovando-Martinez et al., 2018).

Antioxidant capacity

The ABTS assay is based on the reduction of the $ABTS^{\bullet+}$ radical to ABTS by an antioxidant compound. resulting in color changes that can be measured by colorimetric methods. Since the results are usually expressed as μM trolox per gram of sample. the higher the obtained value. the higher the antioxidant capacity of the material under study. The extracts of both seeds presented high antioxidant activity against $ABTS^{\bullet+}$ radicals. Considering that phenolics are the main antioxidants in the extracts. the lower amount of these compounds in ripe seed extract explains its lower antioxidant capacity in comparison to unripe seed extract. The values obtained in the present work are higher than the reported in the literature (623 ± 2 and 2.1 ± 0.3 μM trolox/g dry extract) for papaya seed extracts (Sofi et al., 2016; Zhou et al., 2011).

Regarding the FRAP assay, its principle lays on the reduction of the ferric complex to ferrous complex, producing a blue color whose intensity is proportional to the antioxidant capacity of the sample. In this case, the ripe seed extract showed greater

antioxidant capacity when compared to unripe seed extract, indicating that the first possess better reduction power against the ferric complex, despite its lower level of phenolics and scavenging activity against $ABTS^{\bullet+}$ radicals. The value obtained by Zhou et al. (2011) (1027 ± 18 μM iron sulfate/g dry extract) for the same material is higher than those found in this work. The differences between results of the present study and literature data may be explained by varying cultivation conditions of the papaya fruits, such as climate, fertilizing, composition and type of soil, precipitation frequency and plant nutrition (Bezerra et al., 2013).

In a recent study, Cruz et al. (2019) observed that the addition of papaya seeds to papaya jam increased the antioxidant capacity of the product in comparison to the control jam. In this sense the incorporation of papaya seeds and their extracts into a variety of foods and vegetable oils could be useful to avoid degradation by oxidation enhancing the shelf life of such products (Jorge & Malacrida, 2008; Sofi et al., 2016).

Considering that the production of extracts from the papaya seeds generates another residue, which is discarded, it could be worth investigating if this residue is nutritionally and toxicologically safe to get further usage, e.g., in animal food, in order to reduce almost completely the discard of papaya wastes in the environment.

Antimicrobial activity

The disk diffusion test was performed to assess the capacity of the papaya seed extracts to inhibit the growth of pathogenic bacteria. It is based on diffusion of the extract from paper disks to the agar. Inhibition halos are formed around the disks if the extract presents antimicrobial activity against the tested microorganism and the diameters of the halos are then measured and expressed in mm. The results obtained for the papaya seed extracts are shown in Figure 2.

Inhibition halos were not formed around the disks containing the papaya seed extracts, indicating that they do not possess antimicrobial activity against any of the tested bacteria. Only the positive control drugs (tetracycline and gentamicin) were able to inhibit the growth of the three microorganisms. Comparison of these results with literature data is limited due to the absence of studies reporting the antimicrobial activity of extracts obtained from papaya seeds of the Formosa variety.

However, other authors showed that ethanolic extracts from papaya seeds of the Sekaki variety and aqueous extracts from papaya seeds of the Red Lady variety presented antimicrobial activity against *Salmonella enteritidis*, *Escherichia coli*, *Vibrio vulnificus*. *Proteus mirabilis*, *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* (Muhamad et al., 2017; Wijesooriya et al., 2019). An explanation for the results obtained in the present work is that the composition of plants and fruits varies according to their cultivation conditions as stated before possibly contributing to the lack of antimicrobial activity of the papaya seed extracts. Also, the quantity of antimicrobial compounds in the extracts was probably below the Minimum Inhibitory Concentration (MIC) for the evaluated bacteria. In this sense, further studies are needed to investigate the effect of different extraction parameters (solvent type, extract:solvent ratio, temperature and time) on the concentration of antimicrobial compounds in the extracts.

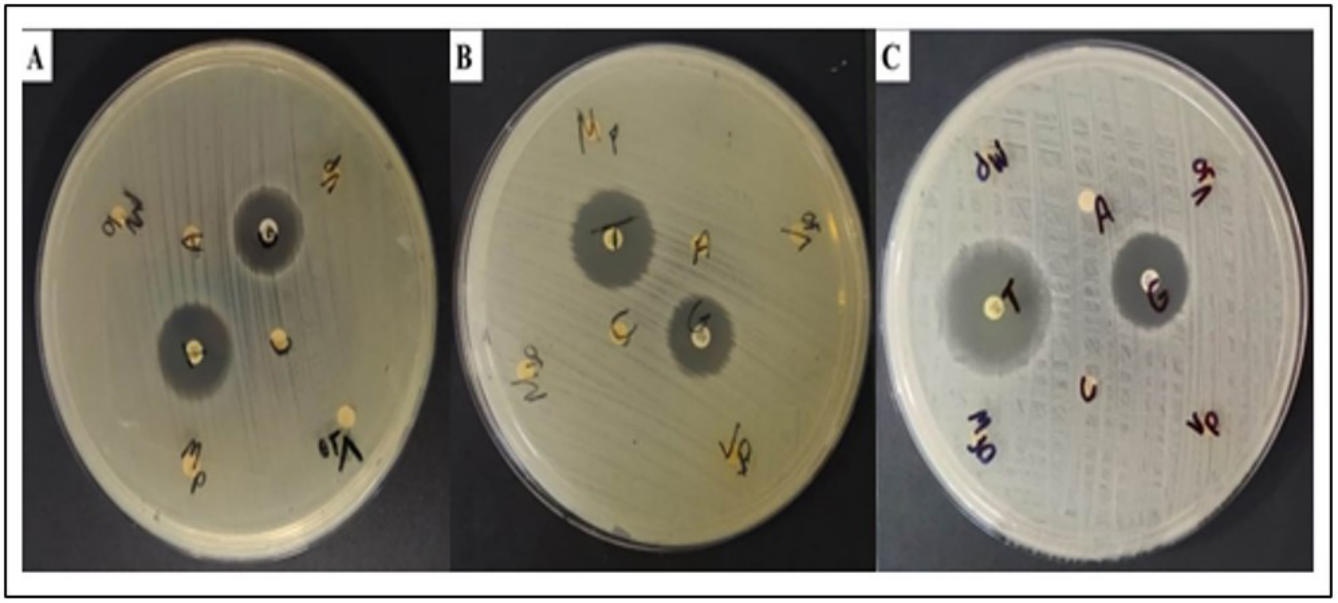

Figure 2. Antimicrobial activity of the papaya seed extracts in two different ripening stages against (A) *Salmonella enteritidis*. (B) *Escherichia coli* and (C) *Staphylococcus aureus*. T (tetracycline); G (gentamicin); A (distilled water); C (BHI broth); VP (extract from papaya seeds at ripening stage 0); V10 (extract from papaya seeds at ripening stage 0 diluted 10x); MP (extract from papaya seeds at ripening stage 5); M10 (extract from papaya seeds at ripening stage 5 diluted 10x).

4 Conclusion

Based on the results found in this study, the papaya seeds in the ripening stages 0 and 5 may be considered promising ingredients for the enrichment of food products because they are a good source of proteins, minerals, fibers, and lipids. In addition, the papaya seed oil is a great source of oleic acid which is a monounsaturated fatty acid and may be an alternative to the commonly used vegetable oils. The hydroethanolic extract obtained from the seeds is rich in phenolic compounds being suitable for application in foods as an antioxidant. The ripening of the papaya fruits has little influence on the composition and properties of the seed of their oil and hydroethanolic extract except for the phenolic content and antioxidant capacity of the latter. The use of papaya seeds in the food industry is a feasible possibility, because they are sources of nutrients and bioactive compounds and such use allows for reducing the amount of food wastes that are discarded in the environment.

Acknowledgements

Authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship granted to Mércia da Silva Mesquita (Code 001). Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research fellowship granted to Carmen S. Favaro-Trindade (#305115/2018-9).

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