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RESEARCH ARTICLE

Biochemical and sensory properties of Juçara pulp submitted to gamma radiation

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There are no studies regarding the irradiation of juçara pulp with a view to their conservation. Therefore, this study pursued to test the conservation of juçara pulp submitted to gamma radiation at the doses 0.0 (control), 2.5, 5.0; 7.5 and 10.0 kGy, and analyzed in 1, 7, 14, 21 and 28 days post irradiation. The samples were kept at 6 °C. We evaluate the bioactive compounds (anthocyanin and total phenolic), the enzymatic activity polyphenol oxidase and peroxidase, and the sensorial quality (acceptance test with hedonic scale) of the samples. The results of the biochemical analyses were submitted to the regression test and the sensorial analysis to the Tukey's test at $p<0.05$. The samples remained within the prevailing legal standards regarding the microbiological quality. The anthocyanin levels decreased during the first week and its reduction was inversely proportional to the dose increments. The phenolic compounds showed high values in the first day, with a reduction in the later period and a constant average to the end. The activity of PPO and POD enzymes decreased in all periods, regardless of the radiation dose applied. The gamma radiation did not affect juçara pulp sensorial quality.

Highlighted Conclusions
The gamma radiation affects the conservation and biochemical characteristics of juçara pulp under refrigeration, but it does not affect its sensorial quality.

To date, research about juçara (*Euterpe edulis*) has focused on the palm production; however, its pulp is becoming increasingly popular. The palm fruit is rich in anthocyanins, natural pigments responsible for its purple color (Einbond et al. 2004, Schulz et al. 2016, Vieira et al. 2017). Anthocyanins are powerful antioxidants capable of inhibiting or decreasing the free radicals effects. Health benefits associated to its consumption consist in reduced risk of coronary artery disease, protection against obesity and hypoglycemia, memory enhancement and protection of brain tissue in fetuses (De Brito et al. 2007).

However, as well as açai (*Euterpe oleracea*) palm fruit, juçara is highly perishable, which difficult conservation of its pulp at room temperature (Neves et al. 2015). Microbial, enzymatic and chemical effects cause oxidation reactions, reduction of total anthocyanins and the pulp discoloration, which change the product properties leading to sensory impairment (Alexandre et al. 2004).

In this context, the use of radiation becomes interesting for fruit pulp processing such as juçara because it is a non-thermal technology, which has little effect on the physicochemical, nutritional and sensory characteristics of food, besides being efficient in removing or reducing the microbial contamination (Arvanitoyannis et al. 2009). Furthermore, juçara has the number of anthocyanin pigments as its main functional characteristic, which is degraded when the product is subjected to thermal processes, making food irradiation an attractive technique.

The efficacy of gamma radiation treatment has been described in several studies with fruit products, such as tamarind (*Tamarindus indica*) juice (Lee et al. 2009), kiwi (*Actinidia deliciosa*) fruit (Kim and Yook 2009) and acerola (*Malpighia emarginata*) pulp (Gonçalves et al. 2006).

This study aimed to evaluate the biochemical and sensory parameters of juçara pulp subjected to treatment with gamma radiation and stored under refrigeration.

MATERIAL AND METHODS

Raw material and Irradiation process. The juçara palm fruits (*Euterpe edulis*) was collected from Parque das Neblinas (23º40'26" S, 46º11'05" W, Mogi das Cruzes, SP, Brazil). The fruits were selected, washed and sanitized using a chlorine solution at 200 mg L^{-1} (S-Dichloro triazinatriona dihydrate Sodium) during 15 minutes. The samples were swollen by soaking them in water at 40 °C for 20 minutes in a stainless-steel tank. The pulp was extracted on a stainless steel pulper with water (2:1). Juçara pulp was packed in polyethylene bags (100 mL) and kept under freezing until the irradiation process.

The pulp irradiation was performed at the Nuclear and Energy Research Institute (IPEN, São Paulo, SP, Brazil), using doses ranging from 0.0 (control), 2.5±0.06, 5.0±0.20, 7.5±0.20 to 10±0.07 kGy. The irradiation process was carried out using a rate of 40.0 Gy min^{-1}. Inside the equipment the polystyrene bags were placed at 50 cm from the source guard. The control samples were kept under the same conditions of the others, but without undergoing the treatment.

Thus, the pulps were stored under refrigeration (6 °C, 90% relative humidity, away from light) and evaluated for their biochemical and sensory characteristics in 1, 7, 14, 21 and 28 days after processing. The microbiological analysis was performed only in frozen pulp to ensure its consumption.

The experimental design used in biochemical and sensory analysis was factorial randomized 5 x 5 (five doses of gamma radiation – 0.0, 2.5, 5.0, 7.5, 10.0 kGy – and five storage periods – 1, 7, 14, 21, 28 days), in triplicate.

Biochemical analyses. The total anthocyanins (ATC) were determined using the pH difference technique (AOAC 2005) and the results were expressed in mg equivalent of cyanidin 3-glucoside 100 g^{-1} juçara pulp. The phenolic compounds (PC) were measured by colorimetry with results in mg of gallic acid 100 g^{-1} juçara pulp (Singleton et al. 1999).

The preparation of the enzyme extract and determinations of polifenoloxidase (PPO) and peroxidase (POD) activities were adapted from the specific method (Cano et al. 1997) according to the experimental conditions. The juçara pulp was centrifuged at 8,000 g for 20 minutes at 4 °C (Eppendorf-5810-R, Hamburg, Germany). An extract was obtained from the homogenization of 2 mL of the supernatant added 10 mL of 0.2 M phosphate buffer solution (pH 7.0) and 0.1 g of polyvinyl pyrrolidone (PVPP) in Biomixer-QL-901 vortex equipment (São Paulo, SP, Brazil). For the PPO activity, 2 mL of the extract were homogenized with 4.0 mL of 0.11 M catechol solution in 0.05 M sodium phosphate buffer (pH 7.0) and immediately after we checked the absorbance at 420 nm in a Femto-432C spectrophotometer (São Paulo, SP, Brazil) at time zero and after 3 minutes. For the POD activity, we homogenized 0.1 mL of the extract with 5 mL of 0.05 M sodium phosphate buffer (pH 7.0), 0.1 mL of hydrogen peroxide 1.5% and 0.2 ml of 1% phenylenediamine. Immediately after, we checked the absorbance at 485 nm at time zero and after 3 minutes. The results were expressed in enzyme activity per minute per mL of sample (AE min^{-1} mL^{-1} sample).

Sensory analysis – acceptance test with hedonic scale. This experiment was evaluated and approved by the Research Ethics Committee of the Luiz de Queiroz College of Agriculture (University of São Paulo), with process number 64, in accordance with the Brazilian National Health Council Resolution 196/96.

The acceptance test with hedonic scale of seven points was used to the panelists indicate how they liked or disliked each sample for the attributes taste, aroma, color, texture, appearance and overall impression of the product (7 = really liked, 1 = dislike very much) (Meilgaard et al. 2006). The sensory analysis was performed by 36 untrained panelists, 83.4% female, selected according to their availability and their interest in evaluating the sensory difference between juçara pulp samples. Five samples were presented, corresponding to doses 0.0 (control), 2.5, 5.0, 7.5 and 10.0 kGy. The codified samples were served at 6 °C in plastic cups of 50 mL with the addition of 10% sugar (v/w) in individual cabins with white light.

Statistical analysis. The means of the biochemical analyses were adjusted to regression models following the coefficient of determination (R^2). The results of the sensory analysis were submitted to Analysis of Variance (ANOVA) for testing F. The statistical difference of means at a significance level of 5% ($p < 0.05$) was determined by the Tukey test, using the Statistical Analysis System Model 9.2.

RESULTS AND DISCUSSION

Biochemical analyses. In the first day, equivalent averages of anthocyanins (ATC) were observed among the samples, except for the pulp irradiated with 7.5 kGy, which showed lower values. During the storage period, all samples decreased the values on the seventh day, with subsequent trend to constancy until the end of the experiment. The samples irradiated with 5.0, 7.5 and 10.0 kGy were higher than the others from the second evaluation period. The use of 10.0 kGy was effective in retaining of ATC until the first week, because the control and the sample irradiated with 2.5.0 kGy lost almost all of pigments. On the other hand, after this period the values were very low; thus, the use of that dose is not justified it as a viable treatment (Figure 1).

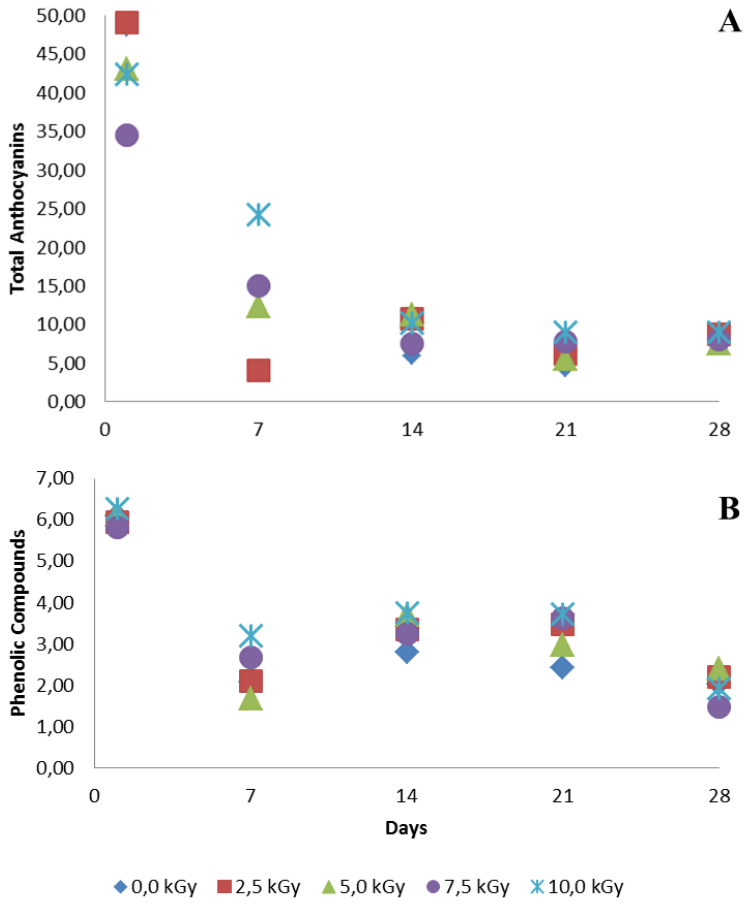

Figure 1. Total anthocyanins (mg of equivalent cyanidin 3-glucoside 100 g^{-1}) (A) and Phenolic compounds (mg gallic acid 100 g^{-1}) (B) of juçara pulp submitted to gamma radiation and stored for different periods (n=6). 0.0 kGy: Non-irradiated pulp (control); 2.5 kGy: Irradiated pulp with 2.5±0.06 kGy; 5.0 kGy: Irradiated pulp with 5.0±0.20 kGy; 7.5 kGy: Irradiated pulp with 7.5±0.20 kGy; 10.0 kGy: Irradiated pulp with 10.0±0.07 kGy.

The ATC levels decreased in all samples in the first seven days of storage. The reduction was inversely proportional to the increasing of the gamma radiation dose. The control sample (0.0 kGy) lost 92.3% of anthocyanins, the sample treated with 2.5 kGy lost 91.8%, followed by doses 5.0, 7.5 and 10.0 kGy that lost 71.5%, 56.4% and 42.9%, respectively. This analysis indicated that, during this period, high doses of radiation were capable of cease the anthocyanin losses in juçara pulp.

This was also observed in acerola pulp submitted to gamma radiation and stored up to 15 days at room temperature (Gonçalves et al. 2006). The anthocyanins structure was not affected because the absorbance spectrum had the same profile for all treatments, being that the sample irradiated with 4.0 kGy showed lower degradation kinetics of anthocyanins.

Due to radiolysis on the food components, the gamma radiation is characterized by liberation of free radicals in a system. The flavonoids group, which is part of the anthocyanins, presents chemical structures suitable to act as

an antioxidant because it can donate hydrogen or electrons to free radicals or capture and move them from their aromatic structure (Kuskoski et al. 2004). This characteristic may have caused the significant reduction of ATC levels in the juçara pulp, because the reaction of these pigments with the free radicals introduced caused self-oxidation of many molecules of anthocyanins in a chain reaction where one molecule of free radical causes destruction of a large number of anthocyanin molecules.

Another factor that may have contributed to the anthocyanins degradation was the presence of browning enzymes, especially peroxidase. Its activity can discolor carotenoids and anthocyanins, besides to catalyzing the non-enzymatic degradation of unsaturated fatty acids, resulting in the formation of volatile compounds (oxidized flavor) (Eskin and Shahidi 2013).

The same pattern demonstrated for anthocyanins was observed to the Phenolic compounds (PC) contents: high values on the first assessment day with a decreasing in the subsequent period with averages tending to be constant until the end of the experiment. The samples treated with doses 7.5 and 10.0 kGy showed the higher values of PC up to 21 days of storage.

Between the first and second periods, the decrease in PC values was approximately four times for non-irradiated samples, three times for the samples treated with 2.5 kGy and 3.7 times for those with 5.0 kGy. Similar to the evaluation of anthocyanins, pulps irradiated with 7.5 and 10.0 kGy showed more balanced compound degradation in comparison to the others, with a total decline of 2.2 times and 3.3 times, respectively.

The degradation of PC after the first week of storage is related to the same causes reported for the anthocyanins degradation in the irradiated pulp. The phenolic compounds or polyphenols, products of secondary metabolism of plants, are into the flavonoids group, which is the most important group of polyphenols with ability to capture free radicals (antioxidant activity) in the medium (Sánchez-Moreno 2002). Besides, the radiolysis of phenolic acids promotes an extensive hydroxylation resulting in the addition of radiolytic-OH radicals formed in water and in the aromatic ring (Breitfellner et al. 2002).

Another possible cause for the decrease in the levels of PC in irradiated juçara pulp is the existence of a large quantity of browning enzymes in fruits of *Euterpe* palms. Polyphenoloxidase darkens the product, oxidizing phenolic compounds with consequent formation of quinones, which are condensed and produce dark insoluble pigments (melanin). The O-quinone formed can interact with amino and thiol groups, reducing the availability of PC (Eskin and Shahidi 2013).

The ionizing radiation of yerba *matè* (*Ilex paraguariensis*) had not impact on the loss of PC content, but there was a small increase in the number of these compounds in the beverage subjected to doses 3.0 and 7.0 kGy compared to control. At 10.0 kGy, there was a slight reduction in the quantity of PC (Furgeri et al. 2009).

All treatments showed a reduction in the Polyphenoloxidase (PPO) activity (AE min^{-1} mL^{-1}) during the storage. The pulp irradiated with 10.0 kGy showed the greatest oscillation of AE min^{-1} mL^{-1} of PPO during the period. These results indicated that the enzyme decreased in all times evaluated, regardless of the radiation dose (Figure 2A). The averages of PPO AE min^{-1} ml^{-1} between 21 and 28 days for the 2.5 kGy, and 5.0 kGy samples indicated a slight increase resulting in a regeneration of the enzyme. The increase of PPO activity caused by the irradiation is probably due to an enzyme activation rather than new synthesis. At the same time, the PPO activation is consequence of an increase of the enzyme extraction due to the damage of radiation in the cell wall or to some changes in its active center (Thomas and Nair 1971). The PPO activity of fresh kale juice after irradiation up to 5.0 kGy was evaluated (Kim et al. 2007); that gamma radiation did not affect the PPO activity, which was gradually decreasing during storage without significant differences among the irradiated and control samples.

In the control sample, the Peroxidase (POD) activity showed a clear trend to increase from day 21 of storage. However, in all evaluation periods, it presented lower values of AE min^{-1} mL^{-1} POD compared to irradiated pulps. The data for the first and seventh days of evaluation showed the greatest reduction of AE min^{-1} mL^{-1} POD in the control sample (30.16%). Among the irradiated pulps, the treatment that most stopped the POD action was 2.5 kGy (18.55%), followed by 5.0 kGy (15.78%), 10.0 kGy (7.45%) and 7.5 kGy (3.18%).

The POD action over the phenolic compounds and anthocyanins content could have caused the lower enzyme activity observed to control samples during the first week of refrigerated storage. Peroxidases are able to oxidize different compounds, such as mono and dihidroxyphenols and phenolic compounds (flavonoids) in their presence, generating free radicals. In the absence of peroxides, peroxidases can catalyze the oxidation of some substrates with assistance of molecular oxygen or the environment, introducing a hydroxyl into several aromatic compounds (Eskin and Shahidi 2013).

The high enzymatic activity of the irradiated samples can also be attributed to POD sensitivity to heat, associated to its ability to regenerate after thermal denaturation. The regeneration of the activity happens after few hours at room temperature and a longer rest period under refrigeration or freezing (Eskin and Shahidi 2013).

This action is also pH dependent and values between 5.0 and 8.0 recover its activity. The increase in AE min^{-1} mL^{-1} POD after 21 days of storage could be due to this fact, because the samples pH was close to pH 5.0 in that time.

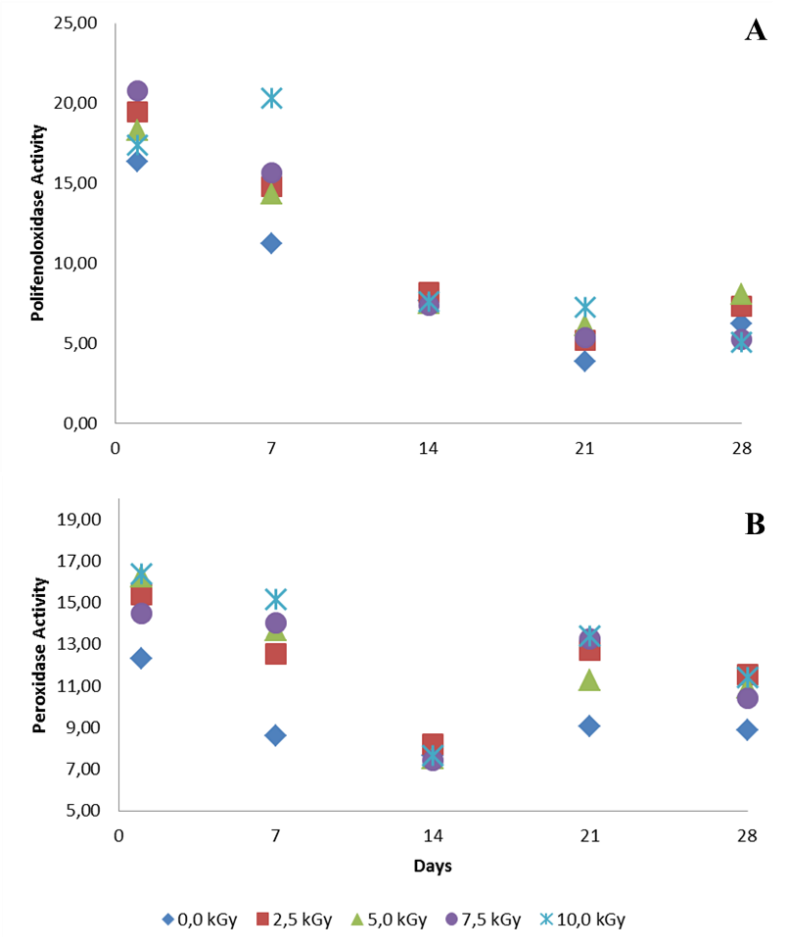

Figure 2. A: Polifenoloxidase (PPO) activity (AE min^{-1} mL^{-1}) and B: Peroxidase (POD) activity (AE min^{-1} mL^{-1}) of juçara pulps submitted to gamma radiation and stored for different periods (n=6). 0.0 kGy: Non-irradiated pulp (control); 2.5 kGy: Irradiated pulp with 2.5±0.06 kGy; 5.0 kGy: Irradiated pulp with 5.0±0.20 kGy; 7.5 kGy: Irradiated pulp with 7.5±0.20 kGy; 10.0 kGy: Irradiated pulp with 10.0±0.07 kGy.

The PPO and POD activities of endives (*Cichorium intybus*) subjected to gamma irradiation (3.0 kGy) and stored at 10°C showed that the irradiation limited the activation of POD and partially inhibited the PPO activity (Hanotel et al. 1995). The PPO and phenolic concentration tended to decrease during storage, while the POD activity remained at a high level. These results are similar to those found in this work, because the PPO activity increased after the first week of storage and the POD for the control sample showed lower values. According to the authors, like the PPO is a membrane bound enzyme, the inactivation observed after irradiation may be due to physical and chemical changes in membranes, induced by radiation. The radiation process results in changes in cellular lipid membranes, facilitated by the contact between PPO and its substrates.

Sensory analysis. The samples of juçara pulp frozen were provided according of legal standards by the RDC No. 12 of ANVISA (ANVISA 2001) regarding to coliforms and *Salmonella* spp., indicating that the product presented satisfactory sanitary conditions at the time of the sensory analysis.

The acceptance testing of radiated juçara pulp was performed only on the first day after processing due the biochemical changes observed after one week of storage. The results showed no statistical difference ($p<0.05$) between the radiation dose and the control sample for all selected attributes (taste, color, appearance, texture, flavor and overall impression). Thus, there was no effect of irradiation on the acceptability of the pulp.

For taste, the mean value was 3.4, classified as "slightly disliked"; the parameters color and appearance were placed in a better position at "moderately good" with mean values of 5.8 and 5.4 respectively. Panelists showed indifference ("Neither liked nor disliked") to give the attributes texture, aroma and overall average values 4.4, 4.4 and 4.3, respectively (Table 1).

Table 1. Acceptance test with hedonic scale (7=liked very much, 1=disliked very much) for different attributes of juçara pulp submitted to gamma radiation (mean values, ± SD, n = 36).

Dose (kGy)	Taste	Color	Appearance	Texture	Flavor	Overall impression
0.0	3.47±1.8	5.50±1.5	5.06±1.4	4.35±1.6	4.21±1.9	4.29±1.5
2.5	3.53±1.9	5.70±1.1	5.21±1.3	4.35±1.5	4.38±1.6	4.32±1.4
5.0	3.32±1.9	5.77±1.1	5.50±1.2	4.29±1.9	4.38±1.8	4.06±1.6
7.5	3.42±1.7	6.06±1.0	5.68±1.1	4.68±1.5	4.71±1.5	4.29±1.4
10.0	3.24±1.9	5.88±0.9	5.53±1.2	4.50±1.6	4.29±1.6	4.35±1.7

SD = standard deviation of the mean, n = number of replicates.

The results of sensory evaluation of juçara pulp are similar to those achieved by other studies. The overall acceptability (color, texture and taste) of dried apricots subjected to gamma radiation found no statistical difference in sensory attributes between the irradiated and control samples in the first day (Hussain et al. 2011). A Yerba mate beverage irradiated up to 10.0 kGy showed no significant difference in the sensory analysis between the irradiated and non-irradiated beverages (Furgeri et al. 2009).

The panelists were asked about what they sensorially appreciated most and least in the samples. The most part of the negative responses was related to bitter taste, fermented or sour; as positive responses, the appearance (purple color) (Table 2). It could be related to the habit to not consume juçara pulp as it was presented; the acai pulp – a product closest to juçara – is usually commercialized together guarana syrup, sugar, fruits, and other ingredients in the South and Southeast regions of Brazil. These components mask the original *Euterpe* fruits taste, which is slightly sour and earthy.

Table 2. General evaluation of the irradiated juçara pulp samples ("most liked" and "least liked").

Most liked	Least liked
Taste: sweet	Taste: salty, fermented, sour, acid, residual mouth
Appearance: good, purple color	-
Texture: Creamy	Texture: sandy, grainy
-	Aroma: olive, acid

The sensory acceptance of kiwi fruits treated with gamma radiation, using a five-point hedonic scale and trained panelists, showed that the irradiated treatments to 3.0 kGy had higher acceptability for the attributes sweetness, taste and overall acceptability (Kim and Yook 2009). The panelists classified the taste as sour. This characteristic was also raised by the panelists of juçara pulp (Table 2).

In conclusion, the irradiation does not affect the sensory characteristics of juçara pulp kept under refrigeration for 24 h. However, pulp degradation in a week of cold storage is also connected to this temperature, which helped in the degradation of anthocyanin and phenolic compounds, as well as in the high enzymatic activity observed during the first days of storage.

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