

Genipa americana L.: Processing and effect on its bioactive compounds and antioxidant capacity

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Abstract

This research aimed to determine the effect of processing on the antioxidant capacity and bioactive compounds during the extraction of huito (*Genipa americana* L.) juice. Freshly extracted and pasteurized juice were characterized in terms of their bioactive compound content and antioxidant capacity, and compared to the unprocessed fruit. Furthermore, the stability of the juices was evaluated over time (0, 15, and 30 days) under ambient conditions following four treatments: SPSC: unpasteurized and preservative-free; PSC: pasteurized without preservative; SPCC: unpasteurized with preservative; and PCC: pasteurized with preservative. The juice pasteurized with a chemical preservative (PCC) remained stable without microbial growth. This sample was subsequently stored for 90 days, and the results indicated that the combination of chemical preservative, pasteurization, and the acidic pH of the juice constituted an effective barrier for its preservation. A randomized controlled trial was used to evaluate the effect of processing. The results showed losses of vitamin C, total polyphenolic compounds, genipin, and geniposide after processing; however, antioxidant capacity increased at each stage, likely due to the formation of new antioxidant molecules. Finally, the presence of minerals such as potassium, magnesium, phosphorus, and calcium were determined, suggesting that this fruit could be considered a nutraceutical and functional food.

INTRODUCTION

Most fruits and vegetables are seasonal and perishable, making them unavailable year-round. Therefore, various food preservation and/or processing methods are necessary. These processes have allowed us to make food available year-round and worldwide. Combined with good manufacturing practices, this reduces food loss, prevents disease, and even minimizes or eliminates toxins present in food, thus preventing food poisoning (1). Among the treatments are thermal ones such as pasteurization, used for more than two hundred years, preventing diseases transmitted by spoiled food caused by pathogenic microorganisms (*E. coli*, salmonella species, *Clostridium perfringens*, among others)(2).

In addition, these treatments can extend the shelf life of food. However, they alter the composition of the food, often resulting in losses of bioactive compounds. These losses will vary depending on the intensity and duration of the treatment.(3). Consumers believe that subjecting food to industrial processes makes it lose its "natural" quality, making it less healthy. However, these processes actually result in food that is more palatable, appealing, and, most importantly, safe(4). Currently, there is a need for the production of highly nutritious, minimally processed, sustainable, and environmentally friendly foods. (5). This is the case with native fruits produced in certain regions, some of which are little known for their nutritional value and have little to no commercialization beyond the local market. In Peru, there are countless fruit species that are poorly studied, among them the fruit of *Genipa americana* L., belonging to the Rubiaceae family, commonly known as huito, whose fruits are edible(6). It is globular in shape, grayish-yellow in color, with fleshy and juicy pulp; it is harvested in March and less frequently from April to August. Ramírez-Morales and Orozco-Cardona (7), They mention that the tree bears fruit year-round; a single tree can have fruit at different stages of growth, so flowers and fruit can be found throughout the year. In the Peruvian Amazon, this stage occurs between May and September. The harvesting of the ripe fruit is done manually and once a year; its maturation lasts from 6 to 9 months since not all the fruit ripens at the same time(8). The ripe huito fruit contains bioactive compounds such as polyphenols (flavonoids, tannins, coumarins) and iridoids (genipin, genipodium acid, among others). These latter compounds characterize this fruit. In folk medicine, it is attributed with pharmacological actions such as anti-inflammatory, antibacterial, antioxidant, analgesic, antiviral, and mutagenic properties. Genipin is one of the main compounds attributed with neuroprotective, anticancer, and anti-inflammatory activities(9). This fruit is also rich in vitamins such as vitamin C and minerals such as iron, which is why it is used by the surrounding communities to combat anemia in children. Regarding its antioxidant activity, this is present in the whole fruit, including the peel, mesocarp, endocarp, and seeds. This antioxidant activity has been associated with the presence of phenolic and iridoid compounds such as genipin, geniposide, genipin derivatives, genipin-1- β -gentiobioside, genipin in its geniposide aglycone form, and geniposidic acid 6'-O-p-coumaroyl.

Once harvested, the fruits continue to undergo physiological changes, and if not properly treated, they will deteriorate very quickly. For this reason, processing is necessary to ensure their availability throughout the year, as well as the preservation of their bioactive compounds. One method is juice extraction, in which most of the bioactive components are dissolved. To demonstrate that juice extraction is a viable option, this study aimed to evaluate the processing effect of huito juice extraction on its bioactive compounds compared to the characteristics of the fruit in its natural state.

MATERIALS AND METHODS

Chemical reagents

All reagents used in the analyses were chemically pure; the preservative used, potassium sorbate, was food-grade. The genipin and geniposidic acid standards were 98% pure.

Raw material

Ripe huito fruits were collected at the Caña Brava Farm, Leoncio Prado hamlet, Yarinacocha district, Pucallpa city. Approximately 200 kg of selected ripe fruits were obtained and packed in wooden crates to prevent physical damage during transport. They were then washed, disinfected, and stored under refrigeration at 4°C until processing. Two extractions were performed, using 100 kg in each replicate.

Equipment

For the extraction of the juice from the fruit, an extruder press with a screw length of 24.5 cm and a motor speed of 18.00 rpm was used; for subsequent analyses, a Buchi brand rotary evaporator model R-210, an Ohaus brand analytical balance, an Agilent 7800/7900 ICP-mass (Inductively Coupled Plasma Mass Spectrometry), and a UNICO brand UV-Visible spectrophotometer model 2100 were used.

Methods:

Vitamin C was performed by the **Iodometric method**, recommended by Herrera (10); **Total polyphenols**: based on the Folin-Ciocalteu method described by Guzman (8), With a modification for juice analysis, a one-liter sample of juice was taken and concentrated in a rotary evaporator until a syrupy consistency was reached. The results were expressed as milligrams of gallic acid equivalent (mgGAE/100g of sample). **Antioxidant capacity**: using the ABTS method, described by Perez-Jimenez y Saura-Calixto(11). **Quantification of genipin and geniposidic acid**: method according to Chen(12); with modifications in sample preparation, as indicated by Guzman et al (8). **Mineral Quantification**: According to EPA Method 200.7. 1994 using ICP-Mass (Inductively Coupled Plasma Mass Spectrometry) equipment. **Microbiological analysis**: The International Commission on Microbiological Specifications for Foods (2000) method was followed. Thermophilic aerobic counts, mold and yeast counts, total coliforms, fecal coliforms, and viable mesophilic aerobes were analyzed. These analyses were performed after each operation to verify good manufacturing practices and evaluate the stability of the juices at 20°C.

Statistical analysis: A randomized controlled trial (RCT) was used to evaluate the effect of the process and/or treatment using the R v.3.5.2 program. (R Core Team 2018. R: A Language and Environment for Statistical Computing. R. Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>). A significance level of $p < 0.05$ was used, with five repetitions for each trial.

Treatment of fruits and extracted juice

The fruit was treated according to the scheme shown in Fig. 1. The total volume of juice obtained was subjected to four treatments: pasteurized juice without preservative (PSC), pasteurized juice with preservative (PCC), unpasteurized juice with preservative (SPCC), and unpasteurized juice without preservative (SPSC). 0.01% potassium sorbate was added, relative to the juice weight, to evaluate the barrier effect of heat and the chemical preservative on the microbial load and to determine if the bioactive components of the fruit naturally inactivate the microbial load without the need for heat or additives. Once the juice was obtained, it was packaged and stored at room temperature (20°C), with microbiological testing performed at the beginning (time zero), 15 days, and 30 days. The samples were evaluated for molds, yeasts, total coliforms, and viable mesophilic aerobic microorganisms, expressed as CFU/mL in each treatment. The juice with the best response was selected from the four groups and then stored for 90 days at 20°C; during this final stage, its stability over time without refrigeration was assessed.

RESULTS AND DISCUSSION

Obtaining huito juice

With the equipment used, a yield of 65.11% was obtained, Fig. 2, a high value compared to fruits such as orange, mandarin and grapefruit that are around 54; 45 and 41% respectively; Rojas and Shaikh-Qasem (13, 14) report a yield of 46.25% for orange juice that does not exceed the values obtained in our study.

From the treatment of the extracted juice

Of the four treatments, it is observed that the PSC and PCC treatments, table 1, are the ones that present good stability under the conditions evaluated, therefore, the juice could be consumed with only the pasteurization treatment. Elias and Villareal (15, 16) mention that the combination of pasteurization and preservative are a good barrier that extends the shelf life of the product, ensuring its quality and safety. For this reason, the PCC sample was chosen for stability evaluation. The SPSC and SPCC treatments tested positive for mold and yeast growth, and further evaluations were discontinued.

Note

Values < 3 and < 10 indicate absence of microorganisms, according to MINSA/DIGESA (2008).

*The evaluation of total coliforms was intended to verify the use of good manufacturing practices in obtaining huito juice.

Where:

SPSC: unpasteurized without preservative; PSC: pasteurized without preservative; SPCC: unpasteurized with preservative; and PCC: pasteurized with preservative.

NE: Not Evaluated

The PCC treatment was the one stored until 90 days were up, the combination of the chemical preservative and pasteurization in addition to its acidic pH, made a perfect conservation barrier, so that microorganisms do not develop and the quality of the juice is not altered. Potassium sorbate acts better at acid pH (2.3–3.7) and will not generate toxic compounds during storage. Its use guarantees the non-growth of mold, preventing deterioration of the product and preserving its freshness(17). In addition, this demonstrates that the previous treatments used in its production were carried out adequately, applying good manufacturing practices.

Effect of processing on antioxidant capacity and polyphenolic compounds

The antioxidant capacity of a food is determined by the synergistic interactions between its different antioxidant compounds, such as polyphenols, vitamins, and carotenoids, present in it, as well as the specific mode of action of each of them(11); Under this premise, it means that being in a stressful environment,

such as the rupture of tissues caused by peeling and chopping the fruit, induces physiological changes(18), such as the increase in respiratory rate, which in turn stimulates other metabolic pathways in response to the damage exerted to avoid or counteract the oxidation produced by exposure to oxygen in the environment and radiation in the form of light, increasing the production of polyphenols due to the rupture of cell membranes causing the release of these compounds, exhibiting a significant antioxidant activity, dependent on the reactivity of the phenolic residue due to containing a hydroxyl group (OH) and the aromatic ring(19); Because of this, an increase in antioxidant capacity is observed immediately after juice extraction. Due to wound stress, the concentration of antioxidant compounds increases (Fig. 3). The processing is still very complex because, in addition, there are enzymes present in plant tissues, such as superoxide dismutase, catalase, and others found within cells. These enzymes are released due to mechanical damage, resulting in an increased antioxidant capacity(20), This process modifies the food matrix as a consequence of the rupture of cell walls, improving the availability of bioactive compounds(2).

In the pasteurization stage where the juice is exposed to heat (boiling temperature) for a very short time (30s), we observed a slight increase in antioxidant capacity; Vitamin C, polyphenols and carotenes (the latter not evaluated), are not the only components that exhibit antioxidant activity(21); During fruit processing, new compounds are formed by the action of heat as a result of the Maillard reaction, such as quinones, high molecular weight polymers such as melanoidins, which are what give colored products, as well as furan compounds, which give the caramel color, among others(22, 23) which are considered non-nutrient antioxidants that would counteract the loss of the main antioxidant components (19, 24). Similar results were obtained by Cano y Arnao(25) in the pasteurization of orange juice where compounds were released that accentuated the antioxidant properties of the product (26). As the concentration of Maillard products increases, so does the antioxidant capacity. Majzoobi, et al.(5) They obtained higher antioxidant capacity values by heating wheat germ to 180°C for 5 to 20 minutes.

The processing method affects the polyphenol content of the extracted fruit, specifically its concentration in the juice compared to the initial content in the fruit. This is due to the preparation of the fruit for extraction. During this process, the fruit is peeled, cut, and pressed. The pressing, being the most severe action, causes rupture of the cell structure, thus releasing the compounds within the plant cells(27). This explains the increased polyphenol content observed in the juice. After heat treatment, a 15% decrease was observed, likely due to the oxidation of these compounds. Peeling and cutting increase the product's metabolic activity, primarily affecting the phytochemicals present and consequently altering its properties(28, 29). The degradation or loss of these polyphenolic compounds, such as flavonoids, may be due to varying degrees of sensitivity to heat treatment, which depends on their structure and the presence or absence of oxygen. Polyphenol oxidase, in the presence of oxygen, can catalyze the oxidation of some polyphenols. Therefore, when the fruit was exposed to oxygen during chopping and extraction, these compounds were likely affected, with the oxidation catalyzed by the heat.(19, 30). Thermal processes have a significant influence on the availability of polyphenols in food, directly related to the magnitude and duration of heat exposure. As a consequence of this treatment, the juice darkened; freshly extracted, the juice was pale yellow, but after pasteurization, it turned a caramel color, possibly due to the degradation of phenols by the enzyme polyphenol oxidase (PPO)(28). In this regard, Ribeiro(31), It suggests that the combination of time and temperature should be carefully considered in heat treatments applied to food in order to guarantee microbiological safety, the preservation of sensory attributes and, above all, the stability of its bioactive compounds.

Effect of processing on bioactive compounds: Vitamin C, genipin and geniposidic acid

The vitamin C content in the huito fruit is very low and is affected by processing, causing a considerable loss due to the juice's exposure to atmospheric oxygen(32), Fig. 4, pasteurization, also affected its content, decreasing significantly with respect to the initial content (of the fruit in its natural state); Villareal et al(15) They mention that ascorbic acid retention is greater with less heat treatment, both in terms of time and temperature. Likewise, vitamin C is unstable and highly sensitive to temperature and storage conditions(33). Therefore, vitamin C is a technological control parameter during food processing (34). Even environmental factors influence the synthesis and stability of this vitamin; when fruits are stored for a long time, whether at room temperature or refrigerated, vitamin C degrades and therefore its concentration decreases(31).

According to the results in Fig. 4, a content of 0.72 and 1.64 mg/100 mg for genipin and geniposidic acid respectively was determined in the fruit in natura, these values are low compared to those reported by Neri-Numa(35) 5.03 for genipin and 5.51 for geniposidic acid, this may be due to the different climatic and geographical conditions where the fruit develops(36), to the extraction method used and especially to the specific state of ripeness of the fruit(37)

Huito fruit has a low concentration of genipin because this compound is concentrated in the endocarp, the seed area of the fruit when it is unripe or green, and during ripening it decreases even further when glycosylated, transforming into genipin and geniposide acid. The endocarp area becomes gummy, making it difficult to separate the seeds. For this reason, the fruit was processed with the endocarp and mesocarp together, in order to obtain the highest concentration of genipin and genipin acid in the juice. Therefore, the mechanical action exerted on the fruit in preparation for extraction (peeling, chopping) causes the molecular structure to be destroyed, causing the expulsion of these bioactive compounds that are found within the organelles of plant cells such as vacuoles, which within their various functions is to store bioactive compounds; due to this effect, we observe an increase in the concentration of iridoids at this stage. In turn, it is facilitated by the mechanical energy that is converted into heat, due to the friction of the fruit as it is transported inside the chamber with great shear stress produced by the feeder screw at the time of juice extraction(38). During the pasteurization process, heat causes the loss or degradation of these metabolites; approximately 78% of genipin and 15% of geniposidic acid are lost(39); due to its sensitivity to oxidation (21).

Finally, the PCC juice was evaluated for its mineral composition, obtaining the results shown in the table 2. According to the mineral profile present in huito juice, it is rich in potassium, magnesium, phosphorus, sodium, and calcium, and other minerals in smaller but no less important proportions. Ávila(40) in his study on the availability of phytometals reported that the pulp of the fresh fruit of *Genipa americana* contains Na (0.67), P (43.05), Mg (7.05), B (1.04), Ca (33.25), Zn (3.94) and Fe (3.68) expressed as mg/100g. Values compared with those obtained in the juice are lower than the results of this research.

CONCLUSIONS

Processing affected the vitamin C content, with an approximate loss of 40% during the juice extraction stages, confirming the high sensitivity of this vitamin before, during, and after extraction. Polyphenolic compounds also showed high sensitivity to processing, with a 47% reduction, pasteurization being the most significant factor in their degradation. The iridoids evaluated were similarly sensitive to pasteurization, confirming their low thermal stability. However, antioxidant capacity increased during processing: the mechanical action of cutting and chopping the fruit releases numerous compounds with antioxidant activity, and pasteurization generates products that further contribute to this capacity.

Declarations

Conflicts of Interest:

The authors declare no conflict of interest.

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Tables

Tables 1 is available in the supplementary files section

Table 2 is not available with this version

Figures

Figure1. Experimental scheme of the huito juice treatment

Raw material selection - Sorting Washing - Disinfectia peeling and cutting the fruit	Juice Extraction	Estandarizacian	Treatment	Storage 90 days at room Temperature	Characterization of the final product
Huito fruit		Preservative free (SC) With preservative (CC)	without pasteurization (SPSC) pasteurized (PSC) without pasteurization (SPCC) pasteurized (PCC)	Selected product	Characterized product
parameters to be evaluated during processing	Yield (%) Bioactive compounds VC, PT, CA, G y AG		Molds, yeasts, Total coliforms, Viable Mesophilic aerobic Microorganisms Bioactive compounds VC, PT, CA, G y AG	Molds, Yeasts, Total coliforms, Viable Mesophilic aerobic Microorganisms	Minerals VC, PT, CA, G y AG

Figure 1

Experimental scheme of the huito juice treatment

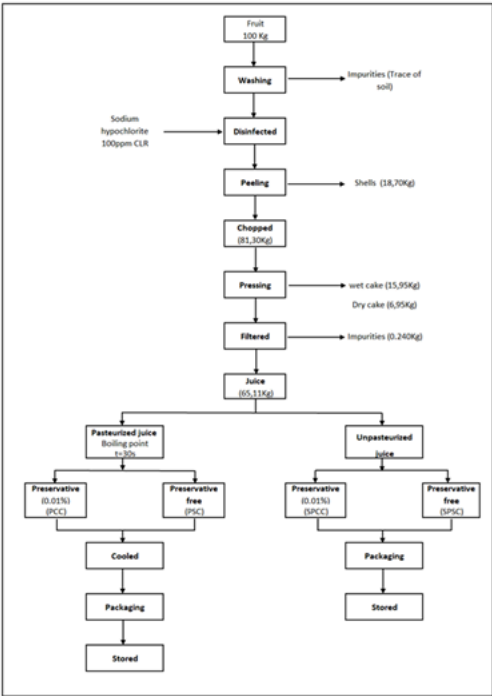


Figure 2

Flow chart of Genipa americana fruit processing and its mass balance

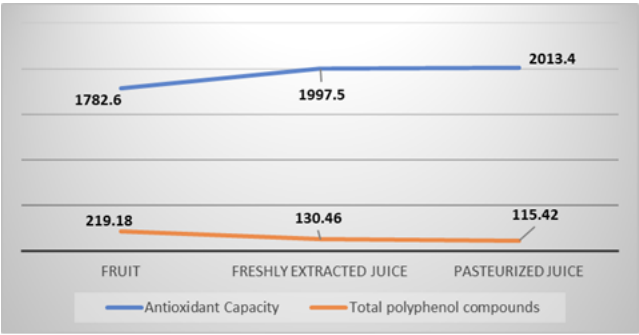


Figure 3. Variation in the content of polyphenolic compounds and antioxidant capacity

Figure 3

Variation in the content of polyphenolic compounds and antioxidant capacity

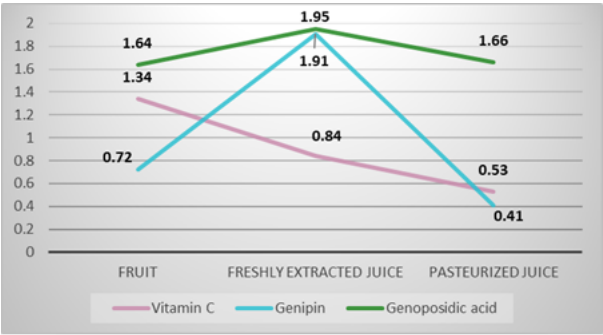


Figure 4. Variation of bioactive compounds during processing

Figure 4

Variation of bioactive compounds during processing

Supplementary Files

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- [Table1.Microbiologicalevaluationofsamplesvertime2.png](#)