




Effect of Pasteurization on Chemical and Functional Properties of Xoconostle (*Opuntia joconostle*) Juice

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HIGHLIGHTS

- Pasteurization temperature had no adverse effect on the antioxidant activities of xoconostle (*Opuntia joconostle*) fruit.
- After the pasteurization process, betacyanins and betaxanthins contents increased to 0.112 and 0.096 µg/g, respectively.
- In spite of fresh xoconostle juice, the pasteurized juice revealed no anti-hyperglycemic properties in animal model.

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Acronyms and abbreviations

OGTT=Oral Glucose Tolerance Test
TA=Titrateable Acidity
TSS=Total Soluble Solids

ABSTRACT

Background: The xoconostle (*Opuntia joconostle* Web.) plant is produced mainly in the Central Highlands region of Mexico. The main aim of this research was to determine the effect of pasteurization on chemical and functional properties of xoconostle juice.

Methods: Total Soluble Solids (TSS), pH, Titrateable Acidity (TA), total phenolic and flavonoid content, betacyanins, betaxanthins, and reducing and non-reducing sugars contents were determined in both unpasteurized and pasteurized xoconostle juices. *In vivo* assay using Oral Glucose Tolerance Test (OGTT) was done in male rats to evaluate the anti-hyperglycemic effect of juice. Data were statistically analyzed using SigmaPlot.

Results: There was a meaningful increasing ($p<0.05$) in the pigment contents after the pasteurization process, as betacyanins and betaxanthins contents increased to 0.112 and 0.096 µg/g, respectively. In spite of pasteurized xoconostle, the unpasteurized group showed anti-hyperglycemic effects at 60 min of OGTT.

Conclusion: Pasteurization temperature had no adverse effect on the antioxidant activities of xoconostle fruit. Although fresh xoconostle juice revealed considerable anti-hyperglycemic properties in rats, this effect was not found in the pasteurized xoconostle juice.

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Introduction

The *Cactaceae* family plants grow in arid and semi-arid regions worldwide, especially in central Mexico.

Xoconostle are cacti species that produce acid and/or bittersweet fruits of the *Opuntia* genus (plants with flat

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cladodes), *Cylindropuntia* (columnar plants) with a species of *C. imbricata* (Haw.) F.M. Knuth, and in *Stenocereus* (columnar cacti with arboreal appearance) with only one species of *S. stellatus* (Pfeiff.) Ricco Bono.

Xoconostle (*Opuntia joconostle* Web.), produces a wide range of secondary metabolites involved in defense mechanism against biotic and abiotic stresses (Cabañas-García et al., 2020; Santos-Díaz and Camarena-Rangel, 2019). The pulp of xoconostle cultivars has soluble fiber and antioxidant compounds such as ascorbic acid, while the seeds are a source of fiber, phenolic compounds, flavonoids, and pigments (betacyanin's and betaxanthin's), which provide a good antioxidant capacity to pulp and seeds. Xoconostle fruit is a good source of bioactive compounds and nutrients that can be added to food products (Morales et al., 2012). Xoconostle peel (pericarp) is an industrial waste that contains gallic, vanillic, 4-hydroxybenzoic acids, catechin, epicatechin, and vanillin (Guzmán -Maldonado et al., 2010).

The xoconostle is produced mainly in the Central Highlands region of Mexico and is used in traditional medicine for various treatments (Dávila Hernández et al., 2019). It has been stated that the consumption of xoconostle can lower the concentration of glucose and increase the concentration of insulin in type 2 diabetes mellitus patients (Pimienta-Barrios et al., 2008). It is important to mention that in Mexico, the most frequent diabetes is type 2, which is associated with a limited activity of the pancreas, by which the health system has designed different strategies to treat this disease. However, some diabetic patients prefer medicinal plants to control glucose concentration in the blood (Aarland et al., 2015, 2017; Gili et al., 2007; Kaur et al., 2012).

Since the beginning of the 21st century, interest in xoconostle has increased due to its nutritional and functional properties (Guzmán-Maldonado et al., 2010). Recently a lot of attention has been paid to the cacti derived from the genus *Opuntia* (Pérez-Alonso et al., 2015). In Mexico, *O. ficus indica* with young flat cladodes are used in human food, either directly as vegetable or processed type (Gallegos-Vázquez et al., 2014). Xoconostle fruits are known well for its culinary uses (González-Cruz et al., 2018). In addition, xoconostle is used in Mexican cuisine to prepare refreshing drinks, jams, fruits in syrup, dried and crystallized fruit, sauce, liquors, etc.

The short shelf life of the fresh juice does not make it viable as a pharmacological alternative, for which it is necessary to explore conservation techniques such as pasteurization that maintains the pharmacological effect of the juice. However, if pasteurization temperature can induce an adverse effect on the compound of this fruit or

not, is not still clear. Therefore, the main aim of this research was to determine the effect of pasteurization on chemical and functional properties of the xoconostle (*O. joconostle*) juice.

Materials and methods

Fruits

Acid xoconostle (*O. joconostle*) fruits (n=48) were used in this study. The fruits, weighting approximately 20 kg, were obtained at a local market in Texcoco. These fruits were harvested on September 2017 in Texcoco, Mexico State, Mexico.

Preparation of acid xoconostle juice

The selected fruits were weighed to calculate the yield, which is reported on fresh weight basis; washed and disinfected with chlorinated water. After that, the fruits were peeled manually with a knife, they were cut into small portions and afterward they were processed in a fruit extractor (Turmix), which separated the bagasse of the juice. The total juice obtained was divided into two batches, one of them was lyophilized directly and the other was pasteurized before lyophilizing. The lyophilized samples were kept at -20 °C until their use.

Pasteurization of the juice

For pasteurization, the Low Temperature Holding (LTH) process was used that is generally applied for pasteurization of acid juices in the food industry. To this purpose, the batches of the juices were placed in a water bath at a temperature range from of 67 to 70 °C for 30 min. Afterwards, the pasteurized samples were placed on ice for 15 min. An aliquot of the pasteurized juices was stored at -20 °C for further chemical assays and the rest was lyophilized for Oral Glucose Tolerance Test (OGTT).

Determination of pH

For the pH determination of the juices, 1:10 dilutions of each of the juices were made, aliquots of 10 ml of these dilutions were taken and their pH was measured with an OAKTON pH700 pH meter.

Determination of Total Soluble Solids (TSS)

The TSS in fresh and pasteurized juices was measured with a manual refractometer (Pocket Refractometer Pal-1, Atago, Tokyo, Japan) previously calibrated with distilled water. The results were expressed as degrees Brix (°Bx) as described previously by Raddatz-Mota et al. (2019).

Determination of Titratable Acidity (TA)

For the determination of TA, 10 ml aliquots of both fresh and pasteurized juices were taken. Then, 1:10 dilutions were made with distilled water, a 10 ml aliquot was taken in triplicate of the dilutions in an Erlenmeyer flask, and 3 drops of phenolphthalein were added as an indicator and it was titrated with 0.1 N NaOH until a pink color was obtained. TA was expressed as a percentage of malic acid (Raddatz-Mota et al., 2019).

Determination of total phenolic content

Total phenolic content of the juices was determined using the Folin-Ciocalteu reagent by the technique adapted by Aarland et al. (2017). An aliquot of 200 µl of both xoconostle juices extracts diluted in distilled water was used. This dilution was mixed with 1 ml of the Folin-Ciocalteu reagent (1:10 (v/v) with distilled water) and incubated for 1 min at room temperature. Subsequently, 0.8 ml of 7.5% sodium carbonate (w/v) was added. The reaction mixture was incubated for 1 h at room temperature, and subsequently the absorbance at 765 nm was determined. Total phenolic content was reported as Gallic Acid Equivalents (GAE) in 1 ml of each xoconostle juice.

Determination of total flavonoid

The colorimetric method of aluminum chloride was used as described previously by Chang et al. (2002) and Aarland et al. (2017). Briefly, an aliquot of 0.5 ml of both xoconostle juices was taken and mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride (w/v), 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min and the absorbance at 415 nm was determined. A calibration curve of quercetin of 10-100 µg/ml in methanol was prepared. The results were reported in µg of quercetin per ml of each xoconostle juice.

Determination of betacyanins and betaxanthins

For the determination of betacyanins and betaxanthins, an aliquot of 0.1 g of the juice sample was taken, including lyophilized, pasteurized, and unpasteurized juices. The aliquots were re-suspended in distilled water and 1 ml was taken (in triplicate) to perform the analyses for betacyanins and betaxanthins (García-Cruz et al., 2013). The results were reported in µg of betacyanins or betaxanthins per g of juice.

Determination of reducing and non-reducing sugars

Carbohydrates were determined by High Performance Liquid Chromatography (HPLC; Agilent 1260 Infinity;

USA). Before the determinations, each xoconostle extract was passed through a nylon filter with 0.45 µm pore size (Millex, Millipore, Bedford, USA). Twenty µl of the filtered juices were injected and analyses were performed with an Agilent Hi-Plex Ca²⁺ column (8% cross-linked, 7.7×300 mm, 8 µm). The mobile phase consisted of Milli-Q water and the isocratic flow was 0.6 ml/min; the temperature of the column was set at 70 °C. The results were expressed as ppm of sugar per g of dry weight of xoconostle juice (Aarland et al., 2015, 2017). For quantification of carbohydrates, standard curves of glucose, fructose, and sucrose were prepared.

In vivo analysis

-Ethics

All the animal experiments were ethically performed according to the statutes of the Institutional Committee for Care and Use of Laboratory Animals, based on the Official Mexican Standard (NOM-062-ZOO-1999).

-Animal experimental assay

To evaluate the functional anti-hyperglycemic activity of the lyophilized juices of xoconostle, Wistar male rats with 150-200 g in weight were used. All the animals (n=6 rats/group) were delivered by Metropolitan Autonomous University-Iztapalapa Bioterium.

The OGTT was performed at 14-h fast. To perform the OGTT curve, the rats were administered a glucose load of 2 g/kg orally and the blood glucose was quantified at time zero (before and after administration of glucose), 30, 60, 90, and 120 min (Oídor-Chan et al., 2016). A glucometer was used to record the measurements obtaining a small drop of blood by a small puncture in the caudal vein. For the study, the following groups were formed. Group 1 (Control): glucose 2 g/kg; Group 2: metformin (300 mg/kg); Group 3: lyophilized juice of unpasteurized xoconostle (100 mg/kg); and Group 4: lyophilized juice of pasteurized xoconostle (100 mg/kg). The metformin was administered 30 min before the glucose load and the lyophilized juices 15 min before glucose load; lyophilized juices were dissolved in purified water. Capillary glucose was expressed as the mean±standard error of the mean in mg/dl.

Sensory analysis

The sensory analyses were performed using hedonic scale with 30 trained consumers including 15 males and 15 females from the University community with an age ranged from 20 to 40 years old. The consumers evaluated the color, smell, and taste of the juices, and responded the questions.

Statistic analysis

The statistical program SigmaPlot v.12.0 was used to analyze the anti-hyperglycemic effect. To analyze the statistical differences in the OGTT, a two-way Analysis of Variance (ANOVA) was carried out, followed by a Duncan's post hoc test. The Areas Under the Curve (AUC) were analyzed with a one-way ANOVA followed by a Student-Newman-Keuls post hoc test. *P* values less than 0.05 were considered significant.

Results

The examined xoconostle fruits had 84% pulp, 26% peel, and approximately 130 ml of juice. Table 1 shows chemical parameters of fresh and pasteurized acid xoconostle juice. No significant differences ($p>0.05$) was found between the pasteurized and unpasteurized juices regarding TSS, TA, SST/AT ratio, pH, total phenols, and total flavonoids parameters. Also, no undesirable changes were observed in the sensory analysis by the consumer panel in all cases. However, there were significant differences ($p<0.05$) in sugars and pigments levels between pasteurized and unpasteurized juices.

Lyophilized juice of unpasteurized xoconostle showed anti-hyperglycemic effects at 60 min of OGTT. By the other hand, lyophilized juice of pasteurized xoconostle showed no anti-hyperglycemic effects at any time (Figure 1). The same trend was observed when treatment was administered 15 min before the glucose load (Figure 2). The anti-hyperglycemic effect was more intense in the unpasteurized juice when it was administered 15 min before glucose load, resulting in a preload effect.

Discussion

In the current study, the percentage of xoconostle peel

and pulp was similar with previous reports by Guzmán-Maldonado et al. (2010) and García-Saavedra et al. (2019). The SST/AT ratio is an indicator of the fruit's ripeness and its flavor. Our results coincide with the findings of the sensory analysis carried out by the consumer panel. There were no differences in pH, total phenolics, and total flavonoid content after pasteurization of xoconostle juices. Similar results were obtained Cortez-García et al. (2015) who showed that steaming and microwaving did not affect the antioxidant properties of xoconostle. Ornelas-Paz et al. (2010) reported an increase in phenolic content in Mexican peppers after boiling. Inversely, Perla et al. (2012) showed a decrease in phenols and flavonoids of potato tubers after boiling. These authors suggest that the heating process can affect the phenolic content in different ways. The increase in sugars after pasteurization may be due to hydrolysis of part of the mucilage to become sucrose.

Our results regarding the anti-hyperglycemic activity of the lyophilized juice of unpasteurized xoconostle were in accordance with Cassiana-Paíz et al. (2010) who found glucose-lowering properties of xoconostle in diabetic rats. However, we found that when the juice was pasteurized, not such effect was observed. This may be due to the fact that the sugar content is varied in the pasteurization process and high temperatures promote the conversion of mucilage to sucrose (Cassian-Paíz et al., 2010). Another hypothesis relation with that antidiabetic effect of xoconostle may be related with the high fiber content and the antioxidant effect (Morales et al., 2012). The present investigation indicated that the anti-hyperglycemic effect of unpasteurized xoconostle juice was increased when it was administered 15 min before glucose load (in comparison with 60 min). This finding suggests that *O. joconostle* juice should be administered before meals to obtain the best anti-hyperglycemic effect.

Table 1: Chemical parameters of fresh (unpasteurized) and pasteurized xoconostle juice

| Parameters | Unpasteurized | Pasteurized |
|----------------------------------------|---------------|----------------------------|
| Total Soluble Solids (TSS; °Bx) | 4.19±0.46 | 4.26±0.50 |
| Titrateable Acidity (TA; % malic acid) | 0.182±0.01 | 0.167±0.0 |
| TSS/TA | 23.018±1.95 | 25.55±2.53 |
| pH | 3.01±0.02 | 3.09±0.02 |
| Total phenols (mgEAG/ml) | 9.146±0.81 | 9.842±0.72 |
| Total flavonoids (mgEQ/ml) | 0.169±0.02 | 0.153±0.00 |
| Sucrose (ppm) | 482±25 | 858±22 * |
| Glucose (ppm) | 7753±60 | 5892±55 * |
| Fructose (ppm) | 2320±30 | 1555±20 * |
| Betacyanins (µg/g) | 0.0700±0.003 | 0.112±0.007 * |
| Betaxanthins (µg/g) | 0.0050±0.004 | 0.096±0.009 ⁴ * |

* Significant differences due to pasteurization, mean comparison ($p<0.05$)

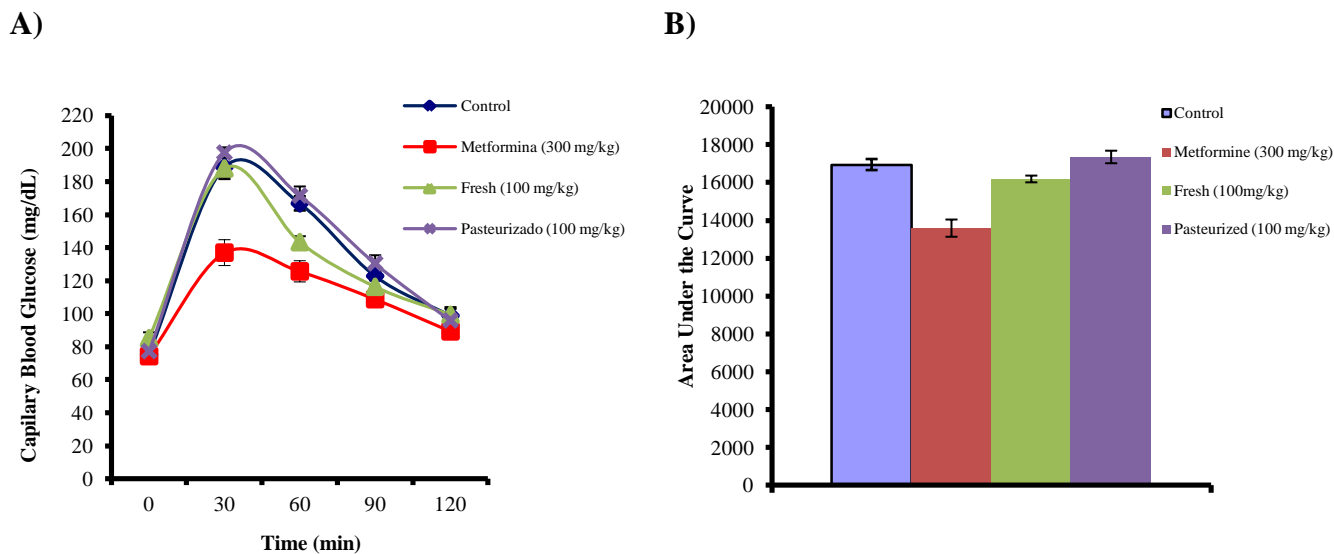


Figure 1: A) Oral Glucose Tolerance Test (OGTT) in male Wistar rat model. Lyophilized juice of unpasteurized group showed anti-hyperglycemic effects at 60 min of OGTT compared to the control group. B) Area Under the Curve (AUC) of the OGTT. Lyophilized juice of unpasteurized and pasteurized xoconostle show no statistically significant differences compared to the control.

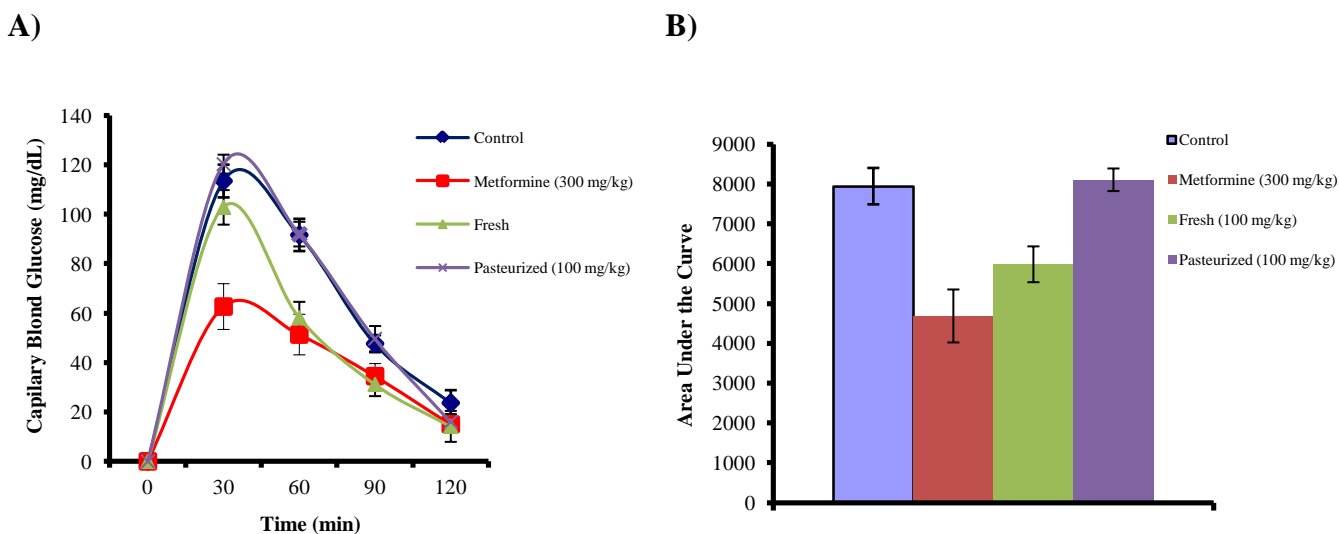


Figure 2: A) Changes in capillary blood glucose of the Oral Glucose Tolerance Test (OGTT). Lyophilized juice of unpasteurized xoconostle showed anti-hyperglycemic effects at 60 and 90 min of the OGTT. B) Area Under the Curve (AUC) of the OGTT. Lyophilized juice of unpasteurized xoconostle showed a lower AUC statistically significant compared to the control group.

The findings of this study showed that the levels of betacyanins and betaxanthins in unpasteurized xoconostle juice were 0.07 and 0.005 $\mu\text{g/g}$, respectively.

There was a meaningful increasing in the pigment contents of the xoconostle fruits after the pasteurization process, as betacyanins as well as betaxanthins contents

increased to 0.112 and 0.096 µg/g, respectively. Similarly, Cejudo-Bastante et al. (2016) reported an increase in betaxanthin content in yellow pitaya peels, from 25 µg/g to 125 µg/g after heat treatment at 80 °C. In contrast, Ramos et al. (2017) performed experiments with different heating methods, indicating a decrease in betacyanin and betaxanthin contents in beetroot. Considering this controversy, it seems that probably the effect of heating on pigment levels may be related to the type of the plants and their individual chemical characteristics.

The glucose and fructose levels of xoconostle found in the current research were 7753 and 2320 ppm, respectively. In comparison, Alvarez et al. (2005) showed that glucose and fructose contents in lemon were 5570 ppm and 5560 ppm, respectively. The anti-hyperglycemic effect of *O. joconostle* documented by Cassiana-Paíz et al. (2010) was reported only in fresh juice, and there are no evidences indicating that this effect is maintained after pasteurization. According to another investigation, the regular use of *O. joconostle* peel can be useful in the control of glucose level in individuals with type 2 diabetes (Pimienta-Barrios et al., 2008).

Conclusion

In conclusion, the results of this study showed that pasteurization temperature had no adverse effect on the antioxidant activities of xoconostle (*O. joconostle*) fruit. Although fresh xoconostle juice revealed considerable anti-hyperglycemic properties in animal model, this effect was not found in the pasteurized juice. Therefore, another preservative method should be investigated to maintain effectively the anti-hyperglycemic characteristics of this fruit.

Author contributions

J.A.M-E. and F.D.L-S. designed the study; S.I.G-C., F.R-C., J.I.M-G., F.C-S., and V.H.O-C. conducted the experimental work; R.C.A. and J.A.M-E. performed the analyses of data; S.I.G-C., J.A.M-E. and R.C.A. wrote the manuscript. All the authors read and approved the final manuscript.

Conflicts of interest

The authors have no conflict of interest.

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