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Chemical composition of juice and antihyperglycemic studies in seed of the prehispanic fruit tunillo (*Stenocereus stellatus*) collected in Oaxaca, Mexico

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Tunillo, or pitaya of august (*Stenocereus stellatus*) is a prehispanic fruit, endemic to the Mixteca region in Mexico and to which a lot of medicinal properties have been associated. However, there are few scientific studies regarding its characterization and use. For these reasons, in this study we carried out a chemical characterization of the juice of four-color variants as well as determine the antihyperglycemic capacity of seed. Physical and chemical characterization were carried out in juice of fruits of *Stenocereus* after a preselection based on pulp color. Total soluble solids, pH, titratable acidity, organic acid and betalains were quantified in juice and antihyperglycemic capacity was measured in seed. Physicochemical parameters in juice were similar in the 4 variants; regarding the content of pigments, the red variant showed the highest values as well as the highest organic acids. However, the white, orange, and red variants showed better antihyperglycemic capacity. Red tunillo is the best candidate for obtaining pigments and its higher organic acids content correlates with its lower acceptance by the local population. The seeds of the white, orange, and red colors showed promising anhyperglycemic capacity, which suggest that they should be considered for the development of antidiabetic treatments. These results contribute to the use of compex matrices considered waste products of the fruits. This would undoubtedly increase their commercial value.

Keywords: Antihyperglycemic, Betalains, Juice, *Stenocereus stellatus*, Tropical fruits, Tunillo

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Sweet xoconostle, august pitaya or tunillo (*Stenocereus stellatus*) is a cactus species of prehispanic origin, native to arid and semi-arid areas[1]. This plant is being mentioned in the codex Martín de la Cruz, the first pharmacology book on American origin. This fruit does not require a large economic investment for its harvesting and exploitation. On the other hand, it can be an option of reforestation and soil recovery and in turn represent an alternative and energy food, endemic to Mexico. In the Mixteca zone, multiple traditional uses can be found for this plant, as well as gastronomical and medicinal uses. It can be considered functional food that protects against chronic diseasses thanks to its capacity to reduce the propagation of free radicals within organisms, due to its high content of total phenolics and ascorbic acid[2].

There is a high variety of pulp color, phenomenon that is being studied by many researchers. There are red, orange, white and purple fruits and thanks to their beautiful tonality the ancient inhabitants used them as natural colorants. There is a very popular anecdote that when the maids would show up with red lips, everyone knew it was thanks to tunillo fruit she had just savoured. Over the years and with evolution of technology, synthetic colorants have displaced the natural ones leaving the use of the tunillos only to the artisan market. This change is mainly due to low costs of synthetic pigments, their greater availability, etc. However, in recent years, modern societies concerned about the side effects associated with the use of synthetic colorants and the possible health

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repercussions of populations that consume this type of pigments, have begun to reverse this trend by returning to the use of colorants based on natural matrices[3].

Currently, there is a growing search for new sources for obtaining natural colorants such as betalains, anthocyanins, chlorophylls, etc. Betalains are plant pigments, which give color to the flowers that belong to the order of the Cariophyllales, which includes among other species the cacti. Betalains have only two colors in nature: yellow (betaxanthines) and violet (betacyanines)[4]. They have been used in the form of concentrated or lyophilized beet extracts in the food industry to modify the color of a wide variety of products such as yoghurts, creams, or ice cream. Although beets have traditionally been a good source of betalains, their extracts have an unpleasant earthy flavor due to the presence of geosmin (produced by certain microorganisms in soils), this is one of the reasons why there has been a search for other sources of these pigments. In this regard, tunillos are a promising source of betalains. On the other hand, little is known about the antihyperglycemic potential associated with these fruits by the Mixteca population and there is also a lack of scientific information about these properties. Given the above we proposed a study of tunillo from the point of view of the chemical characterization of the juice and evaluation of antihyperglycemic capacity associated with the seed.

Materials and methods

Documentation of the study topic. It was carried out by consulting official biographical sources in Mexico.

Biological material. Tunillo fruits were harvested and collected in orchards of San Juan Joluxtla, municipality of Chazumba, State of Oaxaca, Mexico The harvested fruits, which had a thick red and green peel and thorns, were transported to Mexico City to the Universidad Autónoma Metropolitana and the Universidad Autónoma de la Ciudad de México and stored at 11°C until further use.

Obtaining the juice. Each fruit was considered an experimental unit and was minimally processed. All fruits were weighed, washed and dried; the thorns and peels were removed. Afterwards, they were cut into small pieces and ground in a food processor. The juice obtained was filtered through a fabric called organza, to remove impurities and seeds and its pH was adjusted to 5.8 to preserve the structure of betalains. The juice of each fruit was kept at -20°C until its use in the biochemical assays.

Determination of Brix degrees (°Brix). A portable refractometer was used to determine the total soluble solids (TSS). A drop of distilled water was added for calibration and afterwards a drop of tunillo juice was placed and the TSS were measured. The results were expressed in °Brix.

Determination of pH. A simple potentiometer was used to determine the pH of tunillo juice.

Analysis of the sugar content. The analysis of content of sugars (glucose, fructose and sucrose) was carried out by HPLC (Agilent Technology model 1260, equipped with a quaternary pump). 1 mL of fresh juice was passed through 0.45 μm nylon filters (Millex, Millipore, Bedford, USA). The filtrate (20 μL) or glucose, fructose and sucrose standards were injected into the HPLC system mentioned above with a refractive index detector. The column used was Hi-Plex Ca Agilent (8% crosslinked, 7.7 x 300 mm, 8 μm) using HPLC grade water in isocratic mode as the mobile phase. The flow was 0.6 mL min^{-1} and the temperature of the column was set at 75°C. The results were expressed as ppm of sugar per gram of dry weight[4]

Analysis of organic acids. For the determination of organic acids (citric and malic acids), 1 mL of the fresh juice was filtered through 0.45 μm nylon filters (Millex, Millipore, Bedford, USA). 20 μL of filtered juice or the organic acid standards were injected in HPLC (Agilent Technology model 1260, equipped with a quaternary pump), with a multiple wavelength detector (MWD). The column used was an X-Terra MS C_{18} column, 5 μm (4.6 x 250 mm), and the mobile phase consisted of phosphate buffer (50 mM, pH 2.8) in isocratic mode. The flow was adjusted to 0.7 mL min^{-1}. The results were recorded at 210 nm, finally interpolated in a standard curve of citric and malic acids, respectively. The results were expressed in parts per million fresh juice[5-8].

Betalain quantification. The quantification of betalains was carried out by spectrophotometry using the empirical equation proposed by Castellanos-Santiago and Yahia (2008): BC = (100) (A (DF) (MW) Vd) / (εLWd), where BC represents the content of betacyanins and betaxanthines in mg kg^{-1}, A corresponds to the absorbance at 538 nm for betacyanins and 483 for betaxanthines, DF is the dilution factor, MW is the molecular weight (550 g mol^{-1}) of the betacyanines (betanin) (308 g mol^{-1}) The molar extinction coefficient ε=60000 L / (mol cm) for betaxanthines (indixanthin) ε=48000 L / (mol cm).[9]

Analysis of the antihyperglycemis effect. Male Wistar rats weighing 150-200 g were used. The Oral Glucose Tolerance Test (OGTT) was performed after a 14-h fast. After fasting, the basal glucose was measured (Time 0) and the rats were weighed. The capillary glucose measurements were made with the help of a glucometer (Accu-chek® Roche) obtaining a small drop of blood by means of a small puncture in the caudal vein. After measuring the basal capillary glucose and weighing the rats, they received a load of glucose (anhydrous dextrose) orally (2 g kg^{-1}) with the aid of a metal cannula for rats. Glucose was measured at 30, 60, 90 and 120 min. The experimental groups were as follows: Group 1: Control (Glucose 2g kg^{-1}), Group 2: Metformin (300 mg kg^{-1}), Group 3: lyophilized peels from each of the fruits studied (25 mg kg^{-1}), Group 4: lyophilized peels from each of the fruits studied (100 mg kg^{-1}) and Group 5: lyophilized peels from each of the fruits studied (200 mg kg^{-1}). The lyophilisate was prepared with the different color varieties of tunillo, by separating the seeds from the pulp in a blender. Metformin was administered 30 min before glucose administration and the lyophilisate 15 min before glucose administration. Capillary glucose was expressed as the mean ± the standard error of the mean (SEM) in mg dL^{-1}. Finally, the statistical program SigmaPlot 12.0 was used for further analysis.

Results and discussion

Documentation of the study topic. Mexico has a wide geographic distribution, which can be seen in each of the states that make up its territory. Thus, located geographically in the northeastern part of the state of Oaxaca, we locate the Oaxacan Mixteca which borders the states of Puebla and Guerrero and is divided into upper Mixteca, lower Mixteca and Mixteca from the coast. The first is made up of the districts of Coixtlahuacan, Nochixtlan, Teposcolula and Tlaxiaco; the second includes Huajuapan, Juxtlahuacan and Silacayoapan and the last Mixteca refers to the nearby communities established on the coast.

Due to the altitudes that vary between 1,200 and 2,300 meters above sea level, the Mixteca region is characterized by a desert and semi-desert climate with abundant vegetation, temperatures range between 20º to 25ºC in the low Mixteca and between 12º to 18ºC in the high valleys. There are records of extreme temperatures that go up to 5º C in Coixtlahuaca, Juxtlahuaca, Nochixtlán, Teposcolula and Tlaxiaco, and up to 37ºC in districts of Silacayoapan and Huajuapan. According to the mentioned characteristics, the Mixteca provides the adequate conditions for the distribution of a great variety of wild vegetation, where species adapted to dry and extreme ecosystems predominate; in these conditions the tunillo *Stenocereus stellatus* is born.

Stenocereus stellatus is a cactus species that belongs to the order Caryophyllales, Family: *Cactaceae* and Genus Stenocereus[1]. This species grows very well in arid and semi-arid areas, with a low rainfall index of between 40 to 700 mm and with drought times ranging from 6 to 12 months. Thus, *Stenocereus stellatus* has been reported mainly in the borders of the states of Guerrero, Puebla, Morelos and Oaxaca.

This columnar cactaceous presents a height that varies from 2 to 4 meters, in its stems they present from 8 to 12 "ribs" and as physical characteristic they present greater number of thorns than the "pitaya de May''. It has been recorded that the flower has the characteristic of being tubular, in the form of a narrow pink bell, having the tip of the column of the stem of the cactus as its origin.

The fruit of *S. stellatus* is commonly referred to as "xoconostle" "pitaya of August" or "sweet tunillo" among the inhabitants of the region where the species is native[10] (Fig. 1). The fruits weigh between 65 and 150 g, have a spherical shape and green or red tones, the pulp of the fruits is variable in color, having red, yellow, purple and white tones, with a delicious bittersweet taste.

Chemical description and potential for obtaining pigments. As can be observed the red tunillo has higher content of pigments, which makes it the best candidate to obtain betalains. The purple and orange tunillo showed different coloration due to the ratio of betacyanins and betaxanthines, this condition

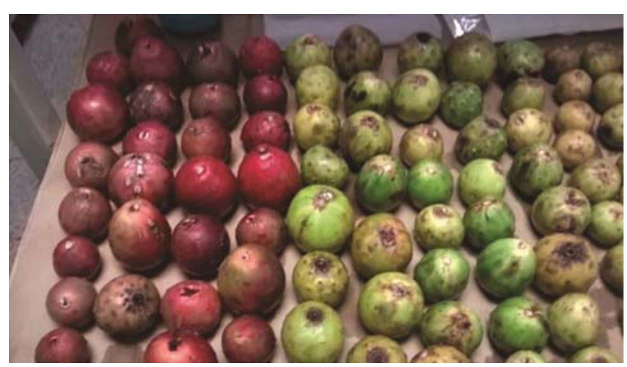

Fig. 1 — "Xoconochtle" "pitaya of August" or "sweet tunillo" collected in the Mixteca zone from Oaxaca in the San Juan Joluxtla región.

can be uses for the pigment's purification (Table 1), also showed the highest amount of citric and malic acid comparate with tunillo white, which coincides with the acidity reported by consumers local. As expected in the white tunillo we did not find a significant quantity of these pigments. In general, the red tunillo showed a higher number of quantifiable metabolites, which agrees with some authors who reported that this variety is the least domesticated of the 4 tunillo color studied, therefore the acid taste and the large number of metabolites of defense. Fructose showed the highest concentrations among the sugars and saccharose was also detected, although at very low concentrations. The saccharose concentration increases with fruit ripening due to the degradation of the mucilage. In relation to the organic acids, higher contents of malic acid were recorded; this coincides with the metabolism of crassulacean acid metabolism.

Anti hyperglycemic potential. According to the inhibitants of the Mixteca, the seeds are responsible for the hypoglycaemic effect, but there is no evidence in the laboratory. Fig. 2 & Fig. 3 show the results at the different concentrations and the total area. Some of the main results are: That the white tunillo seed shows activity like metformin at the concentration of 200 mg kg^{-1} as well as the red tunillo, although the

Table 1 — Physical properties and chemical composition of tunillo juice

	Tunillo			
Parameters	White	Orange	Red	Purple
Physicochemical parameters				
Weight	58.52±15.31^{a}	61.68±18.06^{a}	67.93±28.00^{a}	72.90±8.70^{a}
pH	4±0.14^{a}	3.92±00.17^{a}	3.90±00.40^{a}	4.35±0.20^{a}
°Brix	8.86±2.00^{a}	9.69±01.00^{a}	9.00±01.00^{a}	9.00±1.00^{a}
Chemical composition				
a) Organic acids				
Malic	4.95±1.96^{a}	7.57±01.76^{b}	8.45±2.58^{b}	6.85±0.73^{b}
Citric	1.02±0.60^{a}	2.18±00.72^{b}	3.32±1.84^{b}	0.99±0.09^{a}
b) Sugars				
Glucose	24,196±6,797^{a}	27,742±3,510^{a}	39,856±3,374^{b}	27,776±2,638^{a}
Fructose	33,109±7,522^{a}	32,146±4,998^{a}	41,738±3,506^{b}	48,127±1,896^{c}
c) Betalains				
Total b Betalains	0	32.23±19.75^{a}	134.36±46.66^{b}	43.19±13.83^{a}
Betacyanins	0	05.38±06.25^{a}	62.43±29.67^{b}	31.83±06.41^{c}
Betaxanthins	0	26.85±15.44^{a}	71.92±25.98^{b}	11.36±04.42^{a}
Ratio (Betacyanins/Betaxanthins)	0	0.2	0.86	2.80

Weight (g), volume (mL), organic acids (mg L^{-1}), glucose, fructose (mg L^{-1}), total betalains (mg g^{-1} fresh weight), betacyanines (mg betanine L^{-1}), betaxanthines (mg indixanthine L^{-1}). Data were analyzed by hypothesis testing (mean-two independent samples) with a level of significance α = 0.05. Equal letters in the same row do not present significant difference.

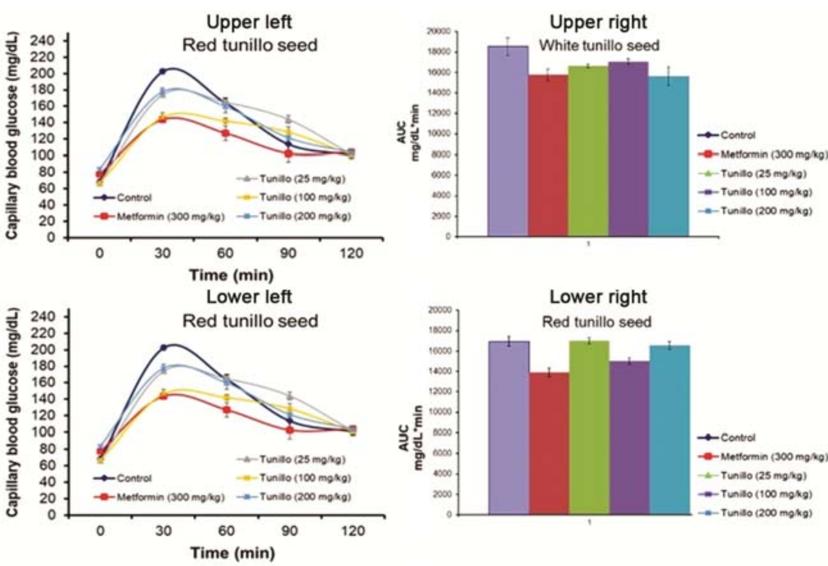

Fig. 2 — Antihyperglycemic activity of white and red tunillo seeds; Upper left, Fig. 2 Upper right, Fig. 2 Lower left, Fig. 2 Lower right

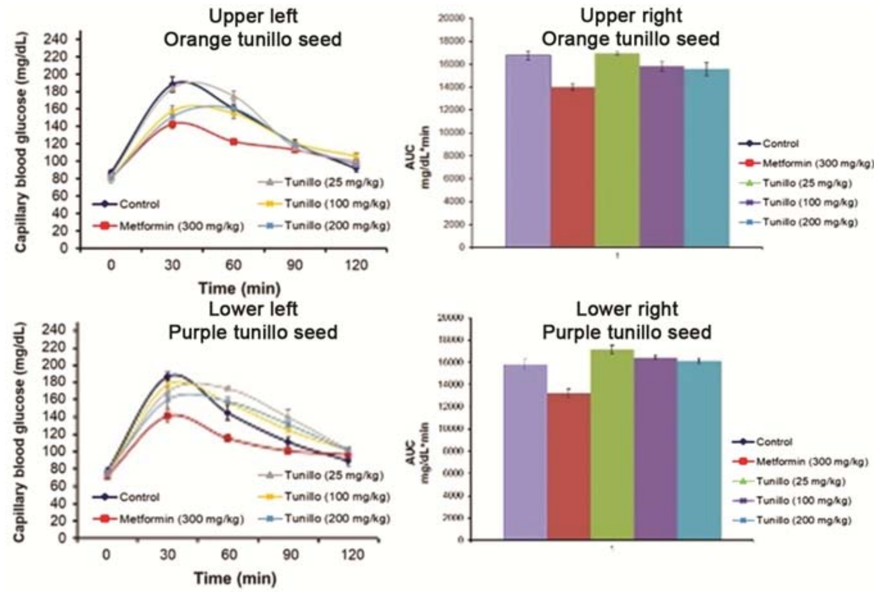

Fig. 3 — Antihyperglycemic activity of orange and purple tunillo seeds; Upper left, Fig. 3 Upper right, Fig. 3 Lower left, Fig. 3 Lower right

latter one at the concentration of 100 mg kg^{-1}; the orange tunillo showed activity in some of the times, but in general no difference was observed with the control, while the purple tunillo did not have any effect.

Conclusions

The red tunillo is the variety that contains the largest number of defense compounds derived from secondary metabolism. The seeds of white, orange and red tunillo can be a good alternative for the search of alternative treatments for diabetes. These results contribute to the use of complex matrices considered as waste from fruits and also give more results in the area of tropical fruit.

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