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ORIGINAL RESEARCH

Antioxidant capacity of the wild fruit pipisco (*Jaltomata procumbens)*, and its application in the preparation of a sauce

Capacidad antioxidante del fruto silvestre pipisco (*Jaltomata procumbens)*, y su aplicación en la preparación de una salsa

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ABSTRACT

In this work, the antioxidant capacity of the wild fruit (*Jaltomata procumbens*) commonly known as “pipisco” was determined. The evaluated samples were: a) Fresh pipisco, b) Pipisco subjected to heat treatment, c) A preparation of a sauce with fresh pipisco and d) Pipisco´s extracts. The antioxidant capacity was determined by spectrophotometric methods ABTS (2,2´-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt), DPPH (2,2-diphenyl-1-picryl-hydrazyl), TBARS (thiobarbituric acid reactive substances), total carotenoids and polyphenols. In the test of ABTS and DPPH there was evaluated the aptitudes of the sample to trapping the radicals previously mentioned, the reductions of the free radicals was 69.21% ± 0.92 and 95.08% ± 3.38 respectively. In the TBARS there decided a percentage of inhibition of lipoperoxidation of 40.24%. The results in the total carotenoids content were 6.88 ± 0.05 µg/g of sample. The concentration of the total polyphenols in the fruit was 0.2621 ± 1.074 mg equivalents of the gallic acid/g per fruit.

Keywords: antioxidant activity, *Jaltomata procumbens*, sauce, TBARS.

RESUMEN

En este trabajo se determinó la actividad antioxidante del fruto silvestre (*Jaltomata procumbens)*, conocido popularmente como “pipisco”. Las muestras evaluadas fueron: a) el pipisco en su estado natural, b) Sometido a un proceso de cocción, c) Una preparación en salsa con pipisco, y d) En extracto. La actividad antioxidante fue determinada mediante los métodos espectrofotométricos ABTS (ácido 2,2-azinobis-3-etilbenzotiazolin-6-sulfonico, sal diamónica), DPPH (2,2-difenil-1-picrilhidrazil), TBARS (sustancias reactivas al ácido tiobarbiturico), carotenos totales y polifenoles. En la prueba de ABTS y DPPH se evaluó la capacidad de las muestras para atrapar los radicales antes mencionados, la reducción de los radicales libres fue de 69.21% ± 0.92, y 95.08% ± 3.38, respectivamente. En la prueba de TBARS se determinó un porcentaje de inhibición de lipoperoxidación de 40.24%. Los resultados en el contenido total de carotenoides fue de 6.88 ± 0.05 µg/g de muestra. La concentración de polifenoles totales presentes en el fruto fue de 0.2621 ± 1.074 mg equivalentes al ácido gálico/g de fruta.

Palabras clave: actividad antioxidante, *Jaltomata procumbens*, salsa, TBARS.

1. INTRODUCTION

Figure 1. *Jaltomata procumbens* fruits

Solanaceae are one of the most economically important plant families on a global scale and Mexico is recognized as a country with a great biodiversity for this type of crops (Davis & Bye 1982; Benítez, 2010). Pipisco (*Jaltomata procumbens* Cav.J.L.Gentry*)* is a native plant of the south of Mexico, however, it grows from the south-west of the United States up to the Andes. It a plant that grow up erect with leafy green stem, which can reach 1.5 m height. It has simple sheets with smooth rim; flowers of whitish green or pale yellow; the fruit is a round juicy berry of bitter-sweet flavor. It has small seeds and possesses a characteristic purple flesh, which is consumed basically in fresh, however it is also used for the preparation of some traditional dishes. The season's fruit goes on July-October; they are often between the cornfields, although in spite of its tropical origins they tolerate some frosts (Saldivar *et al*., 2010). The pipisco in its maturity presents purple coloration possibly this coloration is attributed by anthocyanins, this type of metabolites has antioxidant activity that works as a free radical scavenger of oxygen (Garzon, 2008).

The free radicals are produced normally during the aerobic metabolism in the human being, they are present in diverse physiological processes, like a defense mechanism against infectious agents (Delgado *et al*., 2010; Hernández & McCord, 2007). They are integrated principally of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Halliwell, 2010). When high free radicals concentrations exist the oxidizing stress is originated, which is a condition that is evident in the organism, when the production of highly reactive substances overcomes the antioxidant capacity mechanism (Luna & Delgado, 2014).

The antioxidants are compounds capable of inhibiting or slowing down the oxidation by the reception of free radicals (Krinsky, 1989). They have been classified in accordance of their chemical structure and biological function, dividing them in endogenous and exogenous. The endogenous antioxidants are a group of enzymes specialized in neutralizing the ROS by different mechanism, such is the case of the superoxide dismutase (SOD), the catalase (CAT) and the glutation peroxidase (GPX), for example (Martínez *et al*., 2002). The exogenous antioxidants are those compounds that are obtained from the diet, as selenium, zinc, riboflavin, retinol (vitamin A), ascorbic acid (vitamin E), carotenoids and flavonoids (Magalhães *et al*., 2008). Due the relevance of the biological antioxidant function, in the last years methods have developed to evaluate the antioxidant capacity, the most used methods are ABTS, DPPH based on the

generation of free radicals form certain organic molecules and TBARS oxidation of LDLs (Kuskoski *et al*., 2005). The aim of this research was to determine the content of bioactive compounds and antioxidant capacity of a) fresh pipisco, b) boiled, c) the final product of mixture prepared with onion, garlic, chili and pipisco (sauce) and d) extract pipisco; as well as evaluate the antioxidant capacity by influencing thermal processes.

2. MATERIALS AND METHODS

Mature fruits were collected in the community of Carmen Aztama, belonging to the municipality of San Luis Teolocholco, Tlaxcala México 19°13'54.5"N 98°13'10.5"W in September 2015. The selection criteria were: similar size and color, free of bruises and in complete ripeness.

2.1 Preparation of the samples for the experiments a-d

For the characterization of the antioxidant capacity of a sauce (sample c) prepared with pipisco, two alternative treatments were analyzed (samples b and d), plus a fresh fruit control (sample a) as follows: a) 100 g of fresh pipisco; b) 120 g of stew pipisco subjected to a heat treatment in 300 mL of water for 7 minutes at 90 °C; c) 150 g of fresh pipisco for the sauce, 60 g of onion (*Allium cepa),* 25 g of serrano chili (*Capsicum annuum*) and 5 g of garlic (*Allium sativum*). All this ingredients were roasted and ground d) Obtaining extracts: it was used 350 g of fresh pipisco lyophilized at -50 ^{0}C, 0.014 mbar of pressure for 72 hours, obtaining 36 g of lyophilized pipisco. For the extracts preparation sequential extractions are developed using 30 g of the biological sample, starting with 300 mL of *n*-hexane, dichloromethane, ethyl acetate, acetone, ethanol and methanol, bottled in an amber color jar for 72 hours to 25 °C for every dissolver and using the same biological sample. Subsequently it evaporates to dryness with a rotary evaporator in a temperature between 35 to 50 °C.

2.2 Neutralization of the ABTS radical cation

The procedure is described by (Kuskoski *et al*., 2005), which consists in producing the radical ABTS 7 Mm by the solution of ABTS 7 mM with potassium persulfate 2.45 mM (final concentration). It is mixed and incubated in the dark from 12-16 hours at 25 °C. Once formed the radical is diluted by ethanol until obtaining an absorbance value from 0.70 to 436 nm in a spectrophotometer Genesys 10 UV, Thermo Electron Co. U.S.A.).

25 g of the different samples were used (a, b and c). There was added a mixture of 20 mL of ethanol-acetone 1:1 under agitation for 1 hour to 25 °C. It was filtered with cotton and it was centrifuged at 12000 rpm for 5 minutes, finally taken 10 µL and are added 990 µL of the radical $ABTS^{\bullet+}$ previously obtained. For the sample d, it was used 10 µL of the extracts at final concentration of 10, 100 and 1000 mg/L, 990 µL of the solution of the $ABTS^{\bullet+}$ is added and it is monitored every minute for 6 minutes.

2.3 Free radicals neutralization by DPPH

It is used a free radical DPPH solution, at a concentration of 133.3 µM dissolved in ethanol. It is added 200 µL of ethanol in Elisa plaques that are used as diluent, in other pot were added 150 µL of DPPH solution and 50 µL of the prepared sample to analyze a, b and c. For the sample d, it is used 50 µL of each extract so the final concentrations are of 10, 100 y 1000 mg/L. 150 µL of solution of DPPH are added, is incubated for 30 minutes at 37 ºC with orbital agitation. The lectures are made at λ=517 nm in a microplaque reader (Bio-Tek EL800, U.S.A.), the percentage of activity is calculated in base on the initial concentration of the free radical DPPH, using the DPPH radical reduction percentage formula= [(C-E)/C] x 100, where:

C= control absorbance

E= Absorbance of the evaluated sample.

2.4 Lipid peroxidation induced by iron rat brain homogenate (TBARS)

Is worked with rat male from Wistar strain, using 2 g of brain. The brain tissue of rat is quickly dissected and waste of blood is removed with a saline solution 0.15 M, it gets the homogenized with a glass Potter-Elvehjem homogeniser using 10 mL of phosphate buffer saline (PBS) 0.1 m (0.2 g of KCl, 0.2 g of KH_2PO_4, 8 g of NaCl, and 2.16 g of $NaHPO_4.7H_2O$/L) per gram of brain, at pH 7.4 and temperature of 5 °C per gram of brain. The homogenate is centrifuged at 3000 rpm for 10 minutes, take the supernatant and determines the content of protein by the method described by (Lowry *et al*., 1951). Adjusting to 2.66 mg of protein/mL with phosphate buffer, using bovine serum albumin as standard. The supernatant (375 µL, 1 mg of protein) is placed in 1.5 mL Eppendorf tubes, add 50 µL of EDTA, you add 25 µL PBS and 25 µL of the sample a, b and c prepared with the method described above, for the sample d add 50 µL of each extract to generate in concentrations of work 10, 100 and 1000 mg/L, incubated at 37 °C for 30 minutes. The

peroxidation is initiated with the addition of 50 µL of $FeSO_4$ freshly prepared (final concentration 10 µM) (Rainov, 2000) is continued incubation for one more hour. After the tubes are immersed in an ice bath and add 0.5 mL of reagent TBA (1% of thiobarbituric acid in 0.05 N NaOH and 30% trichloroacetic acid 1:1) the mixture is heated to 94 °C for 30 minutes, then centrifuge at 12000 rpm for 10 minutes. The assessment of the scope of the lipid peroxidation was determined for each sample according to the production of MDA (malondihaldehyde) of supernatant by the development of the TBARS assay described by (Kibanova *et al*., 2009). Take 200 µL of supernatant and the optical density is determined at λ=540 nm in a microplate reader. The targets for each sample are prepared and valued in the same way to exclude any external contribution. The result of TBARS were expressed as equivalent of MDA using standard 1,1,3,3-tetramethoxypropane (Esterbauer & Cheeseman 1990). The percentage of inhibition is defined as the decrease in the formation of TBARS due to the chelation of the extracts and is calculated as follows:

% inhibition = [(C-E)/C] x 100 where:

C= absorbance of control

E= absorbance of the sample to assess.

2.5 Quantification of total carotenoids

Used 2 g of the sample a, b and c, add 10 mL of hexane/acetone/ethanol (50:25:25, v/v), and is under orbital shaker for 1 hour at 25°C, centrifuge for 5 minutes at 6500 rpm. Finally left to rest at 5 °C for 1 hour. The upper phase is separated out and dilute to mark to 25 mL with *n*-hexane. Take an aliquot of 1 mL and measured the absorbance six times in a Genesys 10 UV to 450 nm, used the extinction coefficient of β-carotene ε= 2505 and is reported as µg/g of sample according to the formula:

x (µg)= * and (mL) 106/ to 1cm 1% 100

x (µg)= x (µg)/g shows

where: x= is the concentration of carotenoids in the sample. And: volume of solution that gives the reading. To*: the extinction coefficient of β-carotene.

2.6 Determination of total polyphenols

Used 500 mg of the sample a, b and c, is extracted with 40 mL of methanol/water (50:50 v/v) to 25 °C for 1 hour; saves the supernatant. The residual material is extracted with 40 mL of

acetone/water (70/30 v/v) to 25 °C for 1 hour; the supernatants are combined and concentrated in a rotary evaporator; the concentrate is dissolved in 10 mL of ethanol and the residue is dissolved in 10 mL of distilled water. Take 1 mL of the solution above both ethanolic aqueous solutions as and dilute to mark to 2.5 mL with HCl to 0.3%. Take an aliquot of 50 µL and adds in 1 mL of Na_2CO_3 to 2%; added 50 µL of Folin-Ciocalteau reagent diluted in water (1:1). After 30 minutes the absorbance is measured with the spectrophotometer at 750 nm. The concentration of polyphenols is calculated using gallic acid as standard and the results are expressed as mg gallic acid per gram of fresh weight. (Mendez *et al*., 2013).

3. RESULTS AND DISCUSSION

3.1 ABTS radical scavenging activity

The antioxidant capacity of a food depends on the concentration and nature of the antioxidants present in these. The radical cation $ABTS^{\bullet+}$, it is widely used to measure the antioxidant capacity in fruits, as well as to measure phenolic compounds. The results obtained in this test are shown in table 1.

Table 1. Results of antioxidant activity of the radical cation ABTS

Sample	% reduction of radical $ABTS^{\bullet+}$	TEAC 1 minute	TEAC 6 minute
a	69.21 ± 0.92	12.44 ± 0.53	13.87 ± 0.87
b	36.60 ± 2.11	6.23 ± 1.87	5.62 ± 1.98
c	51.73 ± 0.08	7.98 ± 0.33	10.2± 0.05

(a) fresh pipisco
(b) pipisco boiled
(c) preparation in sauce with pipisco
Data were expressed as the means ± standard error of mean (SEM, n=3)
TEAC (antioxidant activity equivalent to Trolox)

Given the differences described in the times of measurement, you can compare the values of TEAC obtained in the different samples. In this study, we observed variations in the percentage of inhibition of the radical of a sample to another, showing that high temperatures affect the antioxidant capacity of a food. The results obtained in the sample d, were in a final concentration

of 1000 mg/L using 990 µL of ABTS with a absorbance of 0.700 to 0.702 more 10 µL of the sample) (Table 2).

Table 2. Evaluation of the antioxidant capacity of sample of the ABTS$^{\bullet+}$ in the group d

Solvent	% reduction	TEAC
n-Hexane	20.12 ± 1.55	3.57 ± 1.32
Dichloromethane	53.32 ± 0.87	10.54 ± 0.72
Ethyl acetate	40.60 ± 1.23	7.87 ± 1.32
Acetone	94.21 ± 0.83	19.12 ± 0.98
Ethanol	96.34 ± 1.02	19.57 ± 1.53
Methanol	72.70 ± 3.01	14.61 ± 2.65

Data were expressed as the means ± standard error of mean (SEM, n=3)
TEAC (antioxidant activity equivalent to Trolox)

Pipisco extracts presented greater activity in acetone and ethanol solvent, the content metabolites of high polarity can be responsible for example metabolites of type polyphenolics. The antioxidant activity in the radical reduction of ABTS is 20% lower that the tree tomato (*Cyphomandra betacea*) and 40% higher that the aguaymato (*Physalis peruviana*) both fruits of the same family, and, (Repo & Encina, 2008). This test is specific to evaluate phenolic compounds as antioxidants. Nevertheless, developed other general tests as the defense evaluation the radical DPPH.

3.2 Neutralization of free radicals by DPPH

Assessed the antioxidant capacity contained extracts of pipisco to reduce the radical before mentioned, the percentage reduction was quantified by measuring the decrease in the absorbance at 517 nm. The capacity of the radical reduction can be attributed to the donation of atoms of hydrogen. The results obtained in the samples a, b and c are represented in table 3.

Table 3. Reduction of radical DPPH.

Sample	% reduction of radical DPPH
a	95.08% ± 3.38
b	9.48% ± 2.63
c	78.53% ± 0.58

(a) fresh pipisco
(b) pipisco boiled
(c) preparation in sauce with pipisco
Data were expressed as the means ± standard error of mean (SEM, n=3)

It is noteworthy that the sample a has an almost total inhibition of radical to the 30 minutes of reaction, similar to the one described in a study that evaluated by this method, the phenolic compounds in four different genotypes of "tomatillo" *(Physalis ixocarpa)*, the results stand out in two of the samples approximate values to a 90% followed by a 55% and 28% of inhibition of the radical (González *et al*., 2010). In Figure 2 presents the results of the sample d. The extracts to 10 mg/L of *n*-hexane and acetone were the most active in catch the radical (3.43 ± 1.22 and 3.60 ± 0.88) respectively while the least assets were extracts from dichloromethane and ethanol (1.76 ± 1.83 and 2.06 ± 1.05) the extracts which were well below the limits of detection were the ethyl acetate and methanol.

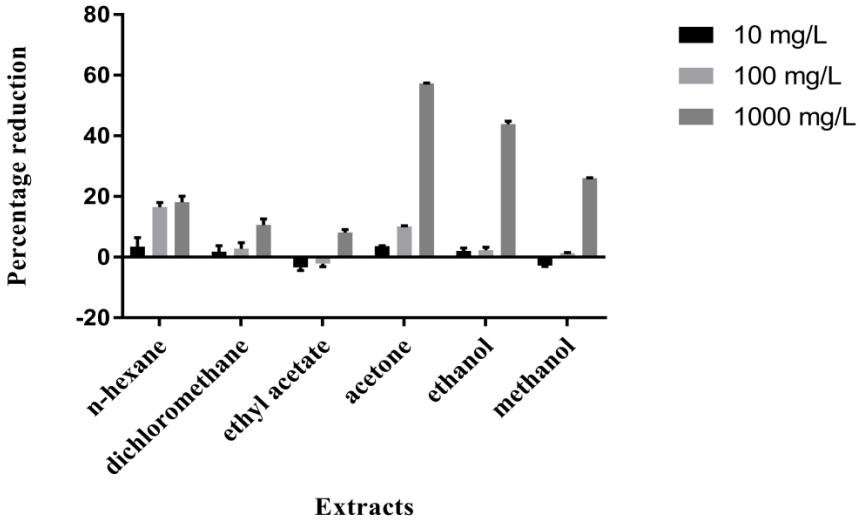

Figure 2. Percentage reduction of radical DPPH in extracts

We calculated the mean inhibitory concentration IC_{50} of the extracts of acetone and ethanol, obtaining 884.33 and 607.39 mg/L respectively.

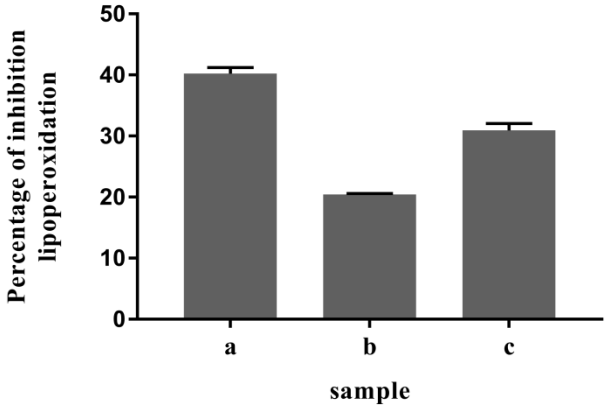

Figure 3. Percentage of inhibition of lipoperoxidation of radical TBARS
(a) fresh pipisco
(b) pipisco boiled
(c) preparation in sauce with pipisco
Data were expressed as the means ± standard error of mean (SEM, n=3)

The increased levels of lipoperoxidation have been correlated with cancer, atherosclerosis and the disease of Alzheimer and aging processes (Dorado *et al*., 2003). The MDA is one of the end products of lipid peroxidation of the cell membrane, (Pham *et al*., 2008) this reacts with the TBA to form a chromophore that was easily measured by spectrophotometry. The results of the inhibition of lipoperoxidation of samples a, b and c in the rat brain, are presented in Fig. 3.

The polar extracts had a better result in the inhibition of the peroxidation of lipids, being the most active in this test, the acetone extract (12.94 ± 2.23) to 100 mg/L and the extract of ethanol (2.51 ± 1.43) to 10 mg/L, as shown in figure 3. In the same way is only calculated the IC_{50}, the extracts more active, resulting in 548.80 mg/L for the acetone extract and 612.39 mg/L for the ethanol extract. The other extracts were not evaluated with regard to the calculation of the IC_{50} due to its poor performance as inhibitors of the antioxidant activity.

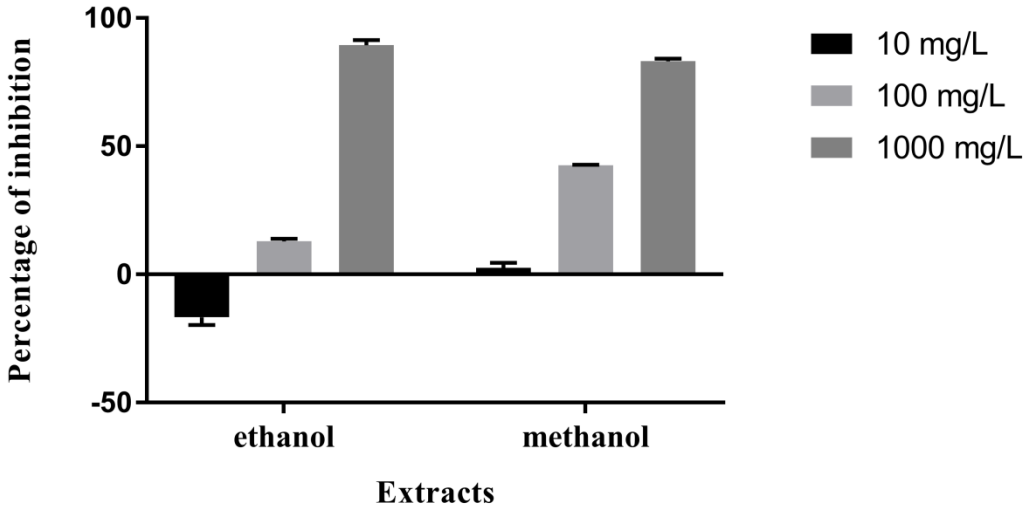

Figure 4. Percentage of inhibition of lipoperoxidation in extracts

3.3 Quantification of total carotenoids

The antioxidant capacity is directly related with the pigment content of the fruit. In the tomato (*Lycopersicon solanaceae)*, red color is attributed to the lycopene (Yaping *et al*., 2002; Zapata *et al*. 2007) identified the carotenoids present in the tomato, where the lycopene was the antioxidant with greater presence since it reported 18.93 ± 9.68 µg /g, and 0.44 ± 0.25 µg /g of β-carotene. The results that were obtained in the test of total carotenes were 6.03 ± 0.39, 5.46 ± 0.55 and 6.68 ± 0.05 µg/g in samples a, b and c respectively; compared with what is reported in the evaluation of β-carotene of two varieties of tomatillo, (*Physalis philadelphic)* and *(Physalis ixocarpa)* were high. The sample evaluated in this study were, in fresh (0.21 ± 0.03 µg/mg), (0.02 ± 0.001 µg/mg) and boiled (0.21 ± 0.01 µg/mg), (0.02 ± 0.001 µg/mg) (Elizalde & Hernández, 2007).

The concentrations of the biological compounds change depending on the cooking process, demonstrating in this study the influence of temperature and the decrease of the biological activity

of these compounds. This explains the decrease of total carotenes in the sample b. It was also noted during the conduct of the tests that the pipisco arrived to decrease or intensify their coloring, due to its content of anthocyanins, which are thermolabile and soluble in water, which causes them to be unstable (Garzón, 2008).

In this study, it was demonstrated that fresh pipisco, boiled and in sauce have a significant antioxidant capacity, that is the reason why is good choice of consumption. It was also observed an increase in the antioxidant activity in function of the incubation time to increase the antioxidant activity in neutralization tests of the DPPH, which can be regarded as a protective effect of prolonged antioxidants in against the danger of the material food oxidative. The sauce that was prepared with pipisco, is a sample of the combination of foods with high antioxidant capacity (onion and chili) although being subjected to high temperatures (roast) and mechanical processes (ground), there is no significant loss of antioxidants, so the pipisco can be a complement to some preparations as was done in ancient times.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare

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