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Scientific Research

Nutraceutical Properties and Toxicity Studies of Flour Obtained from *Capsicum pubescens* Fruits and Its Comparison with "Locoto" Commercial Powder

Marisa Rivas[1,2], Dora Vignale[3], Roxana M. Ordoñez [1,4,5,6], I. Catiana Zampini[1,4,5,6], M. Rosa Alberto[1,4,5,6], Jorge E. Sayago[1,4,5,6], María I. Isla[1,4,5,6*]

[1]Instituto de Química del Noroeste Argentino (INQUINOA), CONICET, San Miguel de Tucumán, Argentina
[2]Cátedra de Biología Celular, Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, San Salvador de Jujuy, Argentina
[3]Cátedra de Botánica Sistemática y Fitogeografía, Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, San Salvador de Jujuy, Argentina
[4]Cátedra de Fitoquímica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina
[5]Cátedra de Elementos de Química Orgánica y Biológica, Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina
[6]Fundación Miguel Lillo, San Miguel de Tucumán, Argentina
Email: [*]misla@tucbbs.com.ar

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Abstract

The aim of this research was to evaluate the phytochemical profiles of flour from red, yellow and green fruits of *Capsicum pubescens* (commun name: locoto) and compare with "locoto" commercial powder (Lcp). Soluble protein (SP), total sugar (TS), free phenolic compounds (FPC), ascorbic acid (AA), total monomeric anthocyanin (TMA), condensed and hydrolizable tannin (CT, HT) and carotenoids (C) were determined by spectrophotometric methods. Multielemental composition by ICP-MS was also done. The flour showed low amounts of calories and high potassium and low sodium levels. Flour from "locoto" fruits contained mainly carotenoids (0.82 to 20 g Eβ-C/100g DW), polyphenolic compounds (681 - 822 mg GAE/100g DW) and ascorbic acid (100 - 199 mg AA/100g DW). The polyphenolic profile of Lcp was similar to flour obtained in our laboratory but the level of carotenoid was lower than that of flour. The fiber content of flour was around 3% in all cases. All preparations obtained from flour and Lcp showed antioxidant activity with SC_{50} values be-

[*]Corresponding author.

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tween 1.4 and 3 µg GAE/mL. Inhibitory capacity on Lipo-oxygenase enzyme was also demonstrated. Non mutagenic effect was detected. The "locoto" flour showed nutraceutical characteristics that are demanded by functional food and could be used as a dietary supplement.

Keywords

Locoto Fruit; Locoto Flour; *Capsicum pubescens*; Nutritional Properties; Functional Properties

1. Introduction

Capsicum is a genus in the Solanaceae family that is known to include species of economic importance which have been the basis for the human diet such as potato. *Capsicum* species' use dates back to pre-Columbian times, where their primary use was as a condiment. In traditional medicine, irritant laxative and expectorant properties are attributed to them. They are used to treat certain skin conditions such as rashes, wounds, and infected sores [1] [2]. Several studies consider that *Capsicum* has antioxidant properties and is a good source of vitamins A, C, E and polyphenolic compounds [3] [4]. Previous studies have reported anti-inflammatory, antimicrobial, hipoglucemic, antimicrobial and antitumoral activities [5]-[10]. It is also known that the capsaicinoids present in these species are alkaloids with specific pharmacological properties [11].

Capsicum pubescens known in Argentina as "locoto chili" is one of five species of the genus cultivated since pre-Columbian times, along with *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens*. The fruit is a subglobose berry (8 cm length). Its color varies from dark green, yellow to red. Its seeds are discoid and blackish brown. The fruits are eaten fresh or dried and grounded ("locoto" powder). At the present, the fruits are largely used in South America to make salad or soup [12]-[14].

However, there are few reports on the chemical and nutritional quality of *C. pubescens*. The objectives of this study were to evaluate phytochemical composition and nutraceutical properties of *C. pubescens* flour obtained from fruits by lyophilization process.

2. Materials and Methods

2.1. Plant Material

The fruits of *C. pubescens* (**Figure 1**) yellow, red and green were purchased in local markets in the province of Jujuy, Argentina. A voucher of each sample was deposited in the Muestrario de Plantas útiles de la Cátedra de Botánica Sistemática, Fitogeografía de la Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy (M-CBSF-073-076). The "locoto" commercial powder was also purchased in local markets in the province of Jujuy, Argentina (**Figure 1**).

2.2. Flour Preparation

Ripe fruits were lyophilized and them was ground (**Figure 1**) to obtain flour from "yellow locoto (YL); red locoto (RL) and green locoto (GL)".

2.3. Phytochemical Extraction from "Locoto Flour" and "Locoto Commercial Powder"

Acetone-water extract (AWE): Flour or commercial powder (1 g) was extracted with 12.5 mL acetone: water (70:30, v/v) in an ultrasonic bath for 30 min at room temperature and then centrifuged at 9,000 *x g* during 10 min. The pellet was extracted exhaustively with the same solvent system. All organic extracts were combined and the acetone was evaporated, then the final volume was adjusted to 5 mL. The aqueous fraction was subjected to acid hydrolysis by adding sulfuric acid (2N) and heating at 100˚C during 26 h.

Ethanolic extract (EE): Flour or commercial powder (1 g) was extracted with 12.5 mL ethanol 96% in an ultrasonic bath for 30 min at room temperature and then centrifuged at 9000 *x g* during 10 min. The suspension obtained was filtered and the remaining solids were extracted exhaustively with the same solvent system. All organic extracts were combined and named extract enriched in phenolic compounds.

(a) (b) (c) (d)

Figure 1. Flour from "locoto" fruits and "locoto" fruits: (a) green; (b) red; (c) yellow; (d) "locoto" commercial powder.

2.4. Phytochemical Determinations

2.4.1. Sugar

Sugar determination: The phenol-sulphuric acid method [15] was used to determine total neutral sugars in ethanolic preparations. Reducing sugars were measured using the Somogyi-Nelson method [16] [17]. Results were expressed as g of glucose (GE)/100g dry weight (DW).

2.4.2. Protein

Soluble protein concentration in all preparations was determined by the method of Bradford [18] using bovine serum albumin (BSA) as standard. Results were expressed as mg of BSA/100g dry weight (mg BSA/100g DW).

2.4.3. Total Polyphenols and Non-Flavonoid Phenols

Total phenolic content of the samples was determined using the Folin-Ciocalteu reagent [19]. Results were expressed in mg of gallic acid equivalents per 100 g dry weight (mg GAE/100g DW). Non-flavonoid phenols were measured by determination of total phenol content remaining after precipitation of the flavonoids with acidic formaldehyde [20]. Results are expressed in mg GAE/100g DW.

2.4.4. Flavonoids

The $AlCl_3$ method [21] was used for the determination of the flavonoids content of the flour extracts. 0.5 mL of ethanolic 2% $AlCl_3.6H_2O$ was added to equal volumes of each extract. The mixture was shaken and the absorbance read at 420 nm after 60 min incubation at room temperature. Flavonoid content was expressed as mg quercetin equivalents per 100 g dry weight (mg QE/100g DW).

2.4.5. Condensed Tannins

The total condensed tannins content was determined with 4-dimethylaminocinnamaldehyde (DMAC) according to Prior *et al*. [22]. 450 µL of DMAC solution (0.1% in acidified ethanol) were added to 150 µL of AWE. The absorbance was measured at 640 nm after 20 min at 25˚C. Data were expressed as mg of procyanidin B2 equivalents per 100 g dry weight (mg PB2E/100g DW).

2.4.6. Hydrolized Tannins

The AWE (2 mL) was first hydrolyzed with 4 mL of 2 N H_2SO_4 at 100˚C for 26 h and the gallic acid released was determined with the rhodanine method [23]. The hydrolyzed AWE (HAWE) and non hidrolyzed AWE were dried under nitrogen and resuspended in 200 µL of 0.2 N H_2SO_4. Two hundred µL of 0.2 N H_2SO_4 and 300 µL rhodanine (0.667% methanol) were added to the diluted extracts. After 5 min, 200 µL of 0.5 N potassium hydroxide and 4 mL distilled water were added and the absorbance at 520 nm was determined. Gallotannin concentrations were expressed as mg gallic acid equivalents per 100 g dry weight (mg GAE/100g DW).

$$\textbf{Gallotanins}\ (\textbf{mg GAE}) = \textbf{GHAWE} - \textbf{GAWE}$$

where GHAWE is the amount of gallic acid present in HAWE and GAWE is the amount of gallic acid present in the sample without hydrolysis.

2.4.7. Total Monomeric Anthocyanins (TMA)

Flour or commercial powder (1 g) was extracted with 5 mL 1% HCl in methanol overnight at 5˚C and then filtered through Whatman N 1 filter paper and the remaining solids were extracted three times with the same solvent. All extracts were combined, vacuum-concentrated and resuspended with 5 mL MILLIQ water to obtain the total monomeric anthocyanin extract (TMA).

Total anthocyanins were evaluated by the pH differential method [24]. The TMA in 25 mM potassium chloride solution (pH 4.5) and 400 mM sodium acetate buffer (pH 1.0) were measured simultaneously at 520 nm and 700 nm, respectively. The content of total anthocyanins was expressed as mg cyanidin-3-glucoside equivalents per 100 g of dry weight (mg C3-G E/100g DW).

2.4.8. Total Carotenoids

Samples or commercial powder (1 g flour) were extracted with 10 mL of hexane:acetone:ethanol (50:25:25, v/v/v). After centrifugation at 13,000 *x g* for 10 min at 4˚C, the top hexane layer was recovered and the absorbance was measured at 450 nm. Total carotenoid content was calculated as mg of *β*-carotene equivalents per 100 g of dry weight (g *β*-CE/100g DW) [25].

2.4.9. Ascorbic Acid

Flour or commercial powder (0.5 g) were extracted with 1.35 mL of H_3PO_4 2% according to Barros *et al*. [26]. After centrifuging at 12,000 *x g* for 10 min, the supernatant was reserved to determine the ascorbic acid content using 2.6 dichloroindophenol sodium salt hydrate (IDF). Different dilutions were added to 125 µL of sodium acetate buffer 400 mM, pH 4; 40 µL of IDF and distilled water until 1 mL. After mixing, the absorbance was measured at 515 nm. Vitamin C was calculated and expressed as mg L-ascorbic acid per 100 g dry weight (mg L-AA/100g DW).

2.4.10. Crude Fiber

Fiber content was determined according to Jaafar *et al*. (2009) [27].

2.4.11. Elemental Analyses

The analysis was carried out by quadrupole inductively plasma mass spectrometry (Q-ICPMS). A Thermo-Elemental X7 series (Thermo Fisher Scientific, Bremen, Germany), equipped with an ASX-100 autosampler model (CETAC Technologies, Omaha, NE), was used (Instituto Superior de Investigación Desarrollo y Servicios en Alimentos , ISIDSA).

2.5. Measurement of Antioxidant Capacity

2.5.1. ABTS Free Radical Scavenging Activity

The antioxidant capacity assay was carried out by the improved $ABTS^{\bullet+}$ method [28]. One milliliter of $ABTS^{\bullet+}$ solution, radical catión 2,2-azinobis-(3-etilbenzotioazolín-6-sulfónico), was added to extractions or commercial antioxidant (hidroxitolueno butilado, BHT, ascorbic acid, AA and quercetina, Q) and mixed thoroughly. Absorbance was recorded at 734 nm during 6 min. The concentration of "locoto" extract required to scavenge 50% of $ABTS^{\bullet+}$ (SC_{50} values) was calculated as µg GAE/mL

2.5.2. *β*-Carotene Bleaching Assay

Antioxidant activity was determined using the system linoleic acid-*β*-Carotene [29]. The initial absorbance at 470 nm was registered at zero time (t_0) and during 120 min. Antioxidant activity (AA%) was calculated as percent inhibition relative to control without "locoto" extract. IC_{50} values denote the µg GAE/mL required to inhibit 50% *β*-carotene bleaching.

2.6. Anti-Inflammatory Activity of "Locoto" Flour

Lipoxygenase Enzyme Assay

Lipoxygenase (LOX) activity was determined using a spectrophotometric method, based on the enzymatic oxi-

dation of linoleic acid to the corresponding hydroperoxide [30]. The reaction mixture contained substrate (50 µM linoleic acid in 0.2 M borate buffer pH 9), enzyme (0.9 nM soy LOX-1) and different extract concentrations. The assay to obtain the 100% of LOX activity was performed with the vehicle as solvent control. Absorption at 234 nm was recorded as a function of time during 4 min. The concentration of "locoto" extract that produce 50% inhibition of hydroperoxide-release (IC_{50}) was calculated from the concentration-inhibition response curve by regression analysis. Caffeic acid was used as reference anti-inflammatory compound.

2.7. Mutagenicity Assay

The mutagenicity assay with *S. typhimurium* was performed as described by Maron and Ames (1983) [31]. The experiments were performed with and without an exogenous metabolic system, the S9 fraction (Moltox-Molecular Toxicology Inc.). Three different concentrations (between 25 to 100 µg GAE/plate) of phenolic extract obtained from flour were evaluated in this assay. One hundred microliters of an overnight culture of bacteria (cultivated for 16 h at 37˚C, approximate cell density of 2 - 5 × 10^8 cells/mL), the different concentrations of extract and 500 µL of sodium phosphate buffer (0.2 M, pH 7.4 for assay without S9-mix) or 500 µL of S9-mix were added to 2 mL aliquots of top agar (containing traces of D-biotin and L-histidine). The resulting complete mixture was poured on minimal agar plates. The plates were incubated at 37˚C for 48 h (−S9) or 72 h (+S9) and the revertant bacterial colonies of each plate were counted. An extract was considered mutagenic if the number of revertants per plate was more than twice the number of colonies produced on the solvent control plates (spontaneous revertant frequency). Samples were tested in duplicate with two replicates. The positive controls employed were 4-nitro-o-phenylenediamine (4-NPD), 10 µg/plate and 2-aminofluorene (2-AF), 10 µg/plate.

To discriminate citotoxicity, the number of surviving cells was determined by plating appropriate dilutions of treated bacterial suspension onto complete agar plates.

3. Results

3.1. Phytochemical of "Locoto" Fruits Flour

Flour was obtained from each fruits and characterized.

Carbohydrates mainly reducing sugar were the major component of flour. Sugar content was similar in all "locoto". The protein content was low (between 0.14 and 0.41%) (**Table 1**). According to these results, the "locoto" flour showed low calories and could be incorporated in a hipocaloric diet. The commercial powder of locoto showed lower sugar content and higher protein content than "locoto" flour obtained in our laboratory. The mineral elements composition of "locoto" flour was determined. The "locoto" flour was high in potassium (2820 - 4010 mg K/100g) and low in sodium (no detected). The green and red flours had the high contents of calcium, and magnesium (**Table 2**). Therefore, the daily mineral requirements could be cover partially with "locoto" fruits flour.

The total polyphenol content of flour extracted with 70% aqueous/acetone was around 0.68 - 0.82 g GAE/100 g DW) (**Table 3**). Vasco *et al*. (2008) [32] classified the fruits according to their dry matter polyphenolic content in low (<100 mg GAE/100g), medium (100 - 500 mg GAE/100g) or high (>500 mg GAE/100g) categories, category 1, 2 and 3, respectively. In all cases "locoto" flour would be considered to be in the category 3, the same as analyzed commercial powder.

Phenolic non flavonoids were the dominant phenolics in the flour (456 - 487 mg GAE/100g DW) followed by flavonoid phenolic (216 - 344 mg QE/100g DW).

All flours contained mainly carotenoids being red "locoto" flour that which contained higher carotenoid level (20 g Eβ-C/100g DW).

The ascorbic acid was an abundant component in all flour the same as in the commercial powder. The values were higher than the reported for fruits like kiwi [33]. The ascorbic acid daily intake recommended in packaged food is 45 mg. Hence, the consumption of 25 to 50 g of "locoto" flour may be enough to cover these requirements in ascorbic acid. Tannin content was low in all flours.

The Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20 December 2006, states that in the EU, a food product can only be declared a source of fiber if it contains 3 g of fiber per 100 g as a minimum. In this sense, the insoluble fiber content of green, red and yellow "locoto" flour was 3.1; 3.5 and 3.7 g/100g DW, respectively. Thus, it could be considered a "source of fiber", since its contents surpass 3 g/100g.

Table 1. Macronutrients in flour of "locoto" fruits Flour of green "locoto" (GL), yellow "locoto" (YL), red "locoto" (RL), Lcp: "locoto" commercial powder.

Flour	Total soluble sugar g GE/100g DW	Reducing sugar g GE/100 g DW	Soluble proteing BSA/100g DW
GL	14.01 ± 1.5	15.55 ± 1.2	0.16 ± 0.01
YL	14.52 ± 2.2	13.45 ± 1.0	0.14 ± 0.01
RL	11.06 ± 2.8	10.12 ± 0.8	0.20 ± 0.02
Lcp	8.76 ± 1.7	6.54 ± 2.3	0.41 ± 0.02

Table 2. Minerals in flour of "locoto" fruits.

flour	Minerals (mg/100g flour)					
	Na	Ca	Fe	Mg	K	P
GL	ND	100 ± 10	4.6 ± 0.3	194 ± 3	4020 ± 70	191 ± 1
YL	ND	80 ± 10	4.8 ± 0.1	172 ± 5	2820 ± 80	397 ± 1
RL	ND	130 ± 20	4.0 ± 0.2	190 ± 2	3430 ± 10	359 ± 1

ND: non detected.

Table 3. Phytochemicals in "locoto" fruits flour and "locoto" commercial powder (Lcp).

Phytochemicals Content	GL	YL	RL	Lcp
Total phenolics	712.00 ± 20	681.60 ± 15	822.00 ± 22	724.00 ± 20
Phenolic non flavonoids	455.96 ± 12	465.48 ± 23	487.37 ± 15	379.63 ± 13
Phenolic flavonoids	256.00 ± 0.4	216.00 ± 0.8	334.63 ± 0.7	344.37 ± 2.3
Flavones and flavonols	90.00 ± 11	34.00 ± 0.1	85.00 ± 0.9	86.34 ± 10
Condensed Tannins	8.39 ± 0.4	6.27 ± 0.5	7.77 ± 0.8	9.00 ± 0.7
Hydrolizable Tannins	4.00 ± 0.2	16 ± 0.10	4.00 ± 0.21	3.58 ± 0.70
Anthocyanins	0.03± 0.01	1.45 ± 0.02	0.65 ± 0.11	1.04 ± 0.66
Ascorbic acid	100.12 ± 15	198.78 ± 20	151.34 ± 12	223.00 ± 25
Carotenoids	0.82 ± 0.06	2.64 ± 0.88	20.09 ± 2.11	0.12 ± 0.09

Data expressed as mg GAE/100g DW (total, flavonoids and non-flavonoids phenolics); mg QE/100g DW (flavones and flavonols); mg procyanidin B2/100g (condensed tannins); mg EAG/100g (hydrolized tannins); mg C3GE/100g (anthocianin); mg AA/100g (ascorbic acid) and g E β-C/100g (carotenoids).

3.2. Antioxidant Activity of "Locoto" Fruits Flour

The antioxidant activity of extracts obtained from "locoto" flour was analyzed. All preparations exhibited ABTS reducing capacity (**Figure 2**) with SC_{50} values between 1.7 to 3 µg GAE/mL for ABTS. The extract obtained from yellow "locoto" was more active as antioxidant followed by green and red "locoto" flour. The extract enriched with polyphenolic compounds extracted from commercial powder was more active than "locoto" flour obtained by lyophilization (SC_{50} values of 1.35 µg GAE/mL for ABTS). In general, all the preparations obtained from flour showed higher antioxidant capacity than natural and synthetic antioxidants (Q: SC_{50}= 18 µg/mL, AA:SC_{50} = 54 µg/mL and BHT: SC_{50} = 55 µg/mL).

Furthermore, all extracts were able to protect lipids from oxidation with IC_{50} values similar to BHT (IC_{50} = 4 µg/mL) and lower than Q (IC_{50} = 20 µg/mL) and AA (IC_{50} = 52 µg/mL). The commercial powder (IC_{50}= 4.5 µg/mL) was more active than green "locoto" flour (IC_{50} = 9.5 µg/mL) followed by red and yellow "locoto" flour (14.6 and 15.4 µg/mL, respectively, (**Figure 3**).

3.3. Anti-Inflammatory Activity of Flour from "Locoto" Fruits and Commercial Powder

Inhibitors of the 5-LOX pathway have a therapeutic potential in a variety of inflammatory and allergic diseases as well as in cancer therapy. The polyphenolic extract obtained from "locoto" flour showed an inhibitory effect on LOX activity with an IC_{50} value of 1 to 4 mg/mL (**Figure 4**) with the following potential: red "locoto" flour > "locoto" powder > green "locoto" flour > yellow "locoto" flour (1.47; 2.33; 3.44; 3.81 mg DW/mL, respectively).

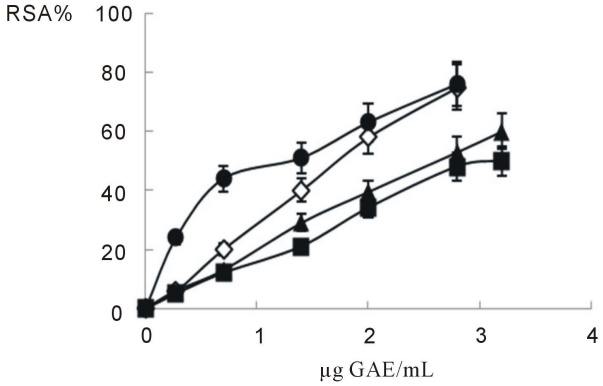

Figure 2. Percentage of ABTS free radical scavenging activity (RSA %). Flour. -◊- yellow "locoto" (YL) -■- green "locoto" (GL) -▲- red "locoto" (RL) -●- "locoto"commercial powder (Lcp).

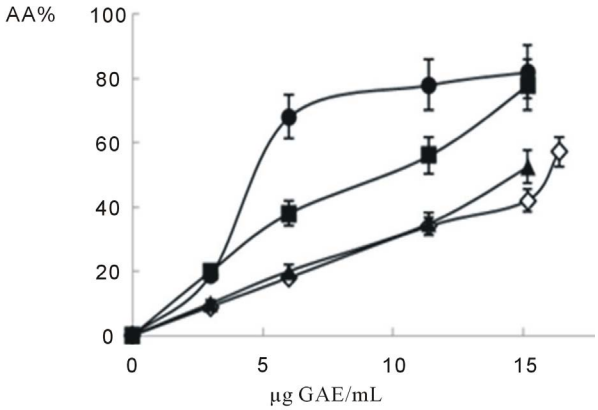

Figure 3. Percentage of radical antioxidant activity (AA%) relative to control using the *β* carotene bleaching assay. -◊- yellow "locoto" (YL) -■- green "locoto" (GL) -▲- red "locoto" (RL) -●- "locoto" commercial powder (Lcp).

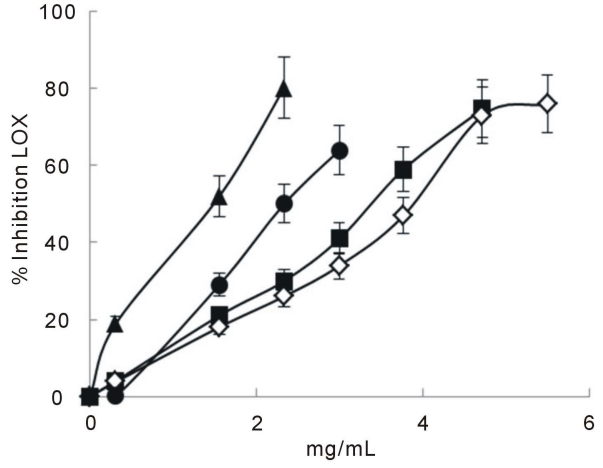

Figure 4. Effect of polyphenolic extract from "locoto" flour on LOX activity. -◊- yellow "locoto" (YL) -■- green "locoto" (GL) -▲- red "locoto" (RL) -●- "locoto" commercial powder (Lcp).

Table 4. Mutagenicity of "locoto" flour on *Salmonella* microsome assay (TA98 and TA100 strains).

	(µg GAE/ plate)	TA 98		TA100	
		–S9	+S9	–S9	+S9
C(–)		15 ± 2	35± 8	125 ± 9	179 ± 11
C(+)		3065 ± 184	830 ± 65	2498 ± 208	1210 ± 87
	25	15 ± 0	46 ± 0	107 ± 2	142± 4
GL	50	14 ± 2	31 ± 5	104 ± 8	137± 9
	100	18 ± 7	32 ± 6	92 ± 4	136± 5
	25	14 ± 2	39 ± 2	140 ± 14	140 ± 14
YL	50	21 ± 4	31 ± 3	118 ± 26	174 ± 10
	100	20 ± 4	35 ± 2	153 ± 24	173 ± 4
	25	20± 2	38 ± 5	108 ± 19	142 ± 46
RL	50	16 ± 5	29 ± 3	133 ± 30	148 ± 2
	100	16 ± 3	42 ± 0	131± 17	149 ± 4

[1]The number of spontaneous revertants was determined with the corresponding solvents (C–). [2]Positive controls (C+) employed were 4-nitro-o-phenylendiamine (4-NPD) at 20 µg/plate and 2-aminofluorene (2-AF) at 10 µg/plate, without and with S9 mix, respectively. With metabolic activation (+S9), data are means ± SD of three plates at two separate experiments. Values followed by the same letter are not significantly different (Tukey's HSD, $P \leq 0.05$).

3.4. Mutagenic Activity of Flour from "Locoto" Fruits

In light of the potential nutritional and functional applications of "locoto" flour, it is important to show that the products obtained from it are safe to consume. The current study reports the results of a toxicology evaluation including *in vitro* mutagenicity studies (**Table 4**). In the Ames test, it was shown that in the presence of different doses of extracts enriched with phenolic compounds, the mutation frequencies did not change significantly when compared to spontaneous ones, both on TA98 and TA100 strains, either in the presence or absence of metabolic activation (S9 mixture). These results indicate the inexistence of compounds in "locoto" flour that cause base substitution (detected in TA100) and frameshift (detected in TA98) mutations and neither do extracts present pro-mutagenic compounds. The absence of such effect by "locoto" against *S. typhimurium* bacterial strains is a positive step towards determining its safe use in traditional consumption.

4. Conclusions

The "locoto" fruit flour can play an important economic role, either in the international market or locally in certain countries of tropical America for its nutraceutical characteristics that are demanded by functional food. The lyophilized form obtained from them could be used as dietary supplement (antioxidant and anti-inflam- matory) for its content of bioactive compounds and low sugar content and used in hipocaloric diet.

The bioactive food components could be administered in encapsulated forms to overcome the drawbacks of their instability, alleviate unpleasant tastes or flavors, and improve the bioavailability and half-life of the bioactive compound *in vivo*.

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