

Extraction of Condensed Tannins from Mexican Plant Sources

Ramiro García^a, Antonio Aguilera^a, Juan C. Contreras-Esquivel^b,
Raúl Rodríguez^b, and Cristóbal N. Aguilar^{b,*}

^a Food Science and Nutrition Department, Universidad Autónoma Agraria
"Antonio Narro", Buenavista, Saltillo, Coahuila, México

^b Food Research Department, School of Chemistry, Universidad Autónoma de Coahuila,
Saltillo, Coahuila, México. Fax: +52 (844) 415-9534. E-mail: cag13761@mail.uadec.mx

* Author for correspondence and reprint requests

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Contents of total polyphenols, condensed tannins and proanthocyanidins, and their stability to various pH values and temperatures were studied in Mexican blueberry, cuatecomate fruit, garambullo fruit, aubergine, coffee pulp and residues of black grapes. Several aqueous extracts, obtained through a one-pass-extraction process, were analyzed using liquid chromatography in order to quantify the condensed tannin (proanthocyanidin) content responsible for their antioxidant activity and colour. All tested samples included high proanthocyanidin contents demonstrating that these Mexican fruits and vegetables are good sources of natural antioxidants, and they all could be considered as excellent functional foods due to their bioactivity measured as the condensed tannin level.

Key words: Extraction, Proanthocyanidins, Mexican Plants

Introduction

Polyphenols are secondary plant metabolites and constitute a wide and complex group of phytochemicals that exhibit antioxidant action and consequently good physiological effects (Bravo, 1998; Martinez-Valverde *et al.*, 2000). Their ability to delay lipid oxidation in food stuffs and biological membranes, in addition to their propensity to act as a prophylactic agent, has motivated research in food science and biomedicine (Farombi *et al.*, 2000). Considering the bioactivity of polyphenols and their presence in a wide range of fruits and vegetables, these foods are considered as functional foods (McDonald *et al.*, 2001).

Phenolic substances including phenolic acids derived from gallotannins, ellagitannins, condensed tannins and complex tannins have been isolated from a variety of sources: rosemary and sage (Wu *et al.*, 1982; Cuvelier *et al.*, 1994; Okamura *et al.*, 1994; Lu and Foo, 2001), oregano, thyme and pepper (Nakatani, 1992; Vekiari *et al.*, 1993).

The antioxidant activity is a direct function of the polyphenolic concentration. Melo (2002) reported 2.734 mg of total phenolics (catechin equivalents) per 100 g of dried sample (DS) in an aqueous coriander extract (*Coriandrum sativum*) exhibiting considerable antioxidant activity. Only six anthocyanidins play an important role in the

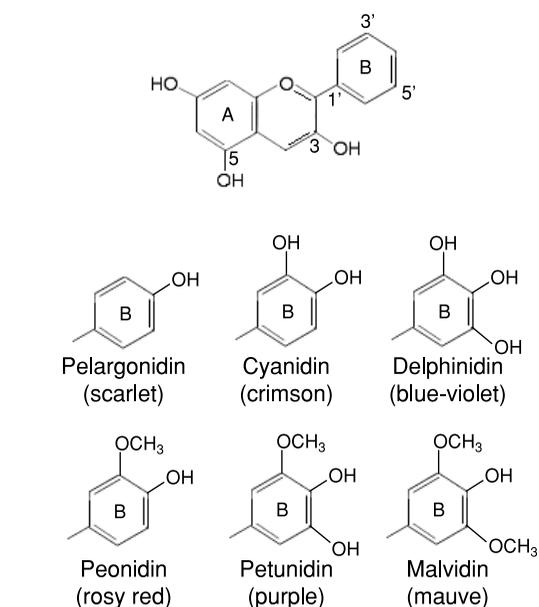


Fig. 1. Anthocyanidins: Basic and hydroxylated and methoxylated structures.

food industry: cyanidin, pelargonidin, delphinidin, peonidin, petunidin and malvidin (Fig. 1).

In Mexico, a great variety of native vegetables and fruits has not been chemically studied in detail. By this reason their potential as sources of

natural pigments and antioxidants is unknown. The main objective of this study was to evaluate the contents of total polyphenols, condensed tannins and proanthocyanidins, and their stability to various pH values and temperatures in Mexican blueberry, cuatecomate fruit, garambullo fruit, aubergine, coffee pulp and residues of black grapes.

Material and Methods

Raw materials

Mexican blueberry and “cuatecomate fruits” were purchased at a local market of Mexico City; garambullo fruits, aubergine and residues of black grapes were collected at a local market of Saltillo, Coahuila, México, and the coffee pulp was collected in Orizaba, Veracruz, México. Samples were transported immediately after collection under freezing conditions to Food Research Department of Universidad Autónoma de Coahuila, Saltillo, México, where the study was performed. After copious washing in running water, the samples were dried with forced circulating air at 45 °C for 48 h. The dried products were ground into a fine powder, passed through an 80-mesh sieve and kept frozen at -18 °C in black polyethylene bags during the study.

Extraction procedure

The polyphenolic extracts were obtained through a one-pass-extraction process, using a multiple reflux system where the powdered samples (10 g) were individually submitted to extraction with 70% acetone (40 ml) for 60 min, under agitation at 60 °C. Then the mixtures were centrifuged at 3000 × *g* for 10 min. Supernatants were concentrated in a condenser and aqueous extracts were obtained and separated in a chromatographic column packed with amberlite and collected in a FPLC system (ActaPrime, Pharmacia-Amersham, Uppsala, Sweden). In this step, sugars, pigments and other contaminants were removed. Aqueous extracts were transferred into amber flasks, flushed with nitrogen and stored in a freezer at -18 °C until used for analysis.

Total phenolic compounds

The content of total phenolic compounds in samples obtained through column chromatography was measured with the Folin-Ciocalteu reagent (Merck), according to the procedure de-

scribed by Wettasinghe and Shahidi (1999) using catechin as a standard. Suitable aliquots of the fractions were taken in test tubes. Then, 0.5 ml of Folin-Ciocalteu reagent and 1 ml of saturated sodium carbonate solution were added sequentially in each tube. The total volume of the system was adjusted to 10 ml with distilled water. The tubes were vortexed, placed in a dark box for 60 min and the absorbance was recorded at 725 nm. The results were expressed as mg of total phenolics per 100 g of DS.

Condensed tannins

The concentration of condensed tannins in samples was evaluated using the spectrophotometric method reported by Waterman and Mole (1994).

HPLC assay for proanthocyanidins

Condensed tannins were quantified by HPLC using the methodology reported by Strack and Wray (1989). A photodiode array detector was used to determine the proanthocyanidin content. Petunidin and pelargonidin (Sigma-Aldrich) were used as standards.

Stability of extracts

Condensed tannin extracts from each Mexican plant were equilibrated to several pH values using buffers in the range from pH 2 to 12. Also, these extracts were incubated at several temperatures ranging from 10 to 80 °C. Apparent changes were visually detected after 30 min under those conditions.

Results and Discussion

In this study the extraction, quantification and stability of condensed tannins present in seven Mexican native fruits were studied. Fig. 2 shows the total tannin contents. Mexican berry has more than 8 mg of total tannins per 100 g of DS and coffee pulp contains a content of total tannins higher than 5 mg per 100 g of DS; the rest of the samples has a tannin content of ca. 3.5 mg per 100 g of DS. All samples are excellent sources of this kind of polyphenols.

Fig. 3 presents the proanthocyanidin content in all tested samples. Grape cuticle and seed samples showed high tannin contents, representing almost 100% of their total tannin content. For garambullo, coffee pulp and Mexican berry the proanthocyanidin contents were higher than 1% (rep-

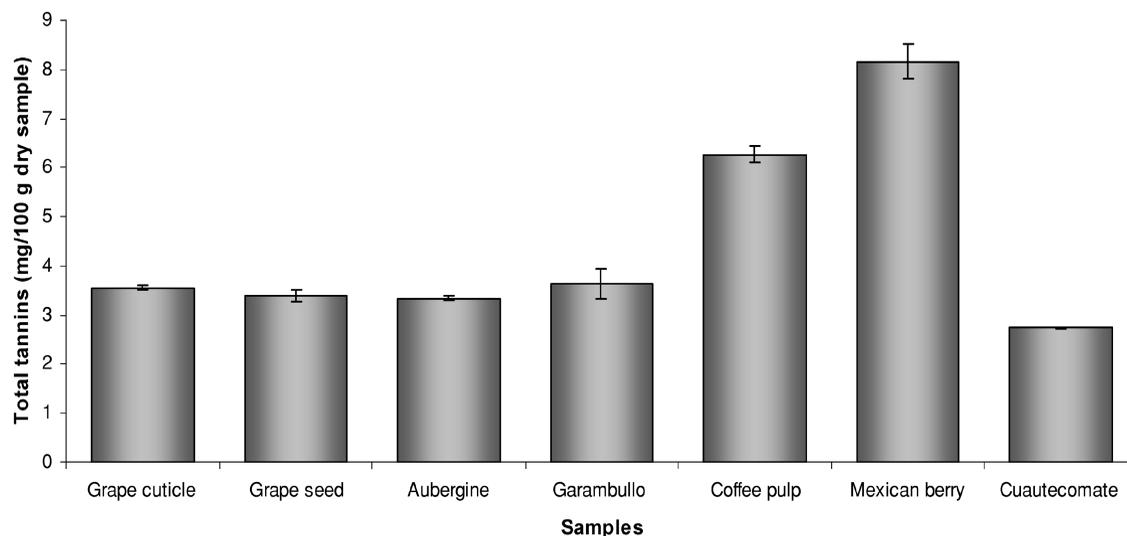


Fig. 2. Total tannin content in Mexican fruits.

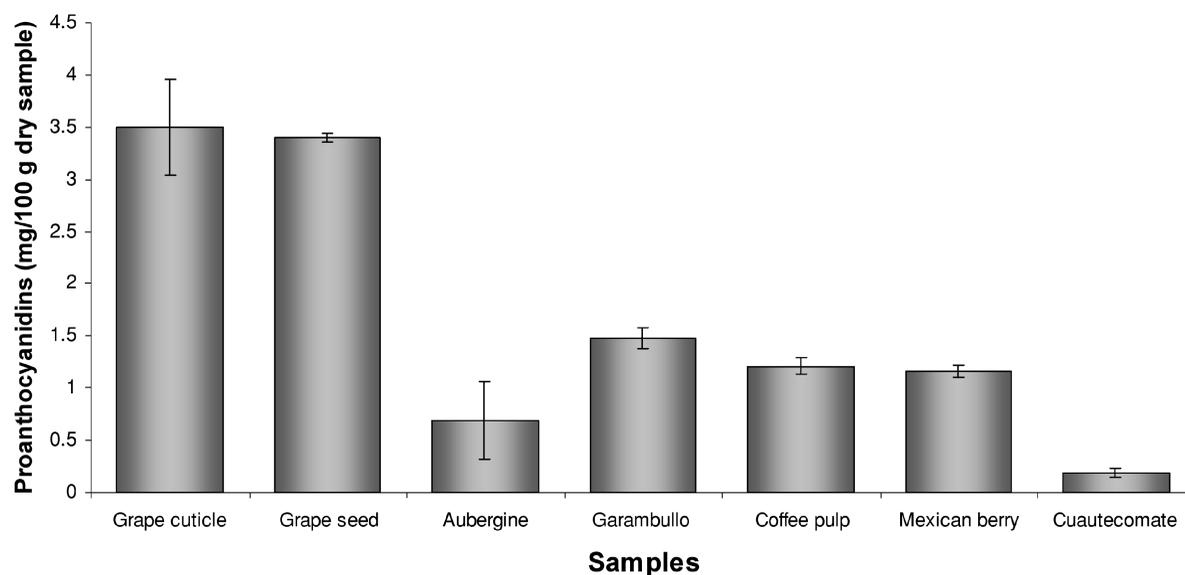


Fig. 3. Proanthocyanidin content in Mexican fruits.

representing 40, 20 and 28%, respectively, of their total tannin content). Aubergine and cuaatecomate fruits showed the lowest proanthocyanidin contents (lower than 0.5%), representing 18 and 9% of their total tannin content).

Condensed tannin contents were confirmed through the proanthocyanidin content evaluation by HPLC using petunidin and pelargonidin as standard molecules. Table I presents the proantho-

cyanidin percentage in all tested samples. Results demonstrate that the proanthocyanidin contents are according to the results obtained by the HCl/butanol method for condensed tannins (Waterman and Mole, 1994).

All samples were stable to pH values in the range 4 to 8 and to various temperatures from 10 to 70 °C. Out of these ranges extracts were oxidized to a brown colour.

Table I. Percent of proanthocyanidins of total tannins present in Mexican vegetables and fruits.

Sample	%
Grape cuticle	98
Grape seed	100
Aubergine	21
Garambullo	43
Coffee pulp	33
Mexican berry	28
Cuautecomate	7

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