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Photoacoustic Analysis of Blue Corn Pigments in Nixtamalized Flours[1]

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Anthocyanins are natural pigments with antioxidant properties, and recently they have received more attention because these pigments are consumed in nutritional diets with therapeutic effects. In addition, anthocyanins are important in the treatment of diseases caused by oxidation of free radicals in live systems, which could be the cause of chronic diseases, like cancer. Anthocyanins, found in flowers and some fruits, are also present in blue Mexican corn (*Zea mays L.*). In this work, photoacoustic spectroscopic, chemical and spectrophotometric analyses of nixtamalized blue corn flours are presented. Different $Ca(OH)_2$ concentrations were used in the fractioned nixtamalization process, and the total anthocyanin concentrations of these flours were obtained by chemical extraction of these pigments and compared with relative intensity of optical absorption obtained by photoacoustic spectroscopy.

KEY WORDS: anthocyanins; blue corn; nixtamalization; photoacoustic spectroscopy.

1. INTRODUCTION

Anthocyanis are natural pigments with antioxidant properties, and recently they have received more attention because these pigments are consumed in nutritional diets with therapeutic effects. In addition, they are important in the treatment of diseases caused by oxidation of free radicals in live systems, which could be the cause of chronic diseases, like cancer [1–4]; on

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the other hand, these natural pigments could replace the use of synthetic colorants. Pigments in blue corn are attributed to the presence of anthocyanins found mainly in the pericarp and/or the aleuronic layer. These natural pigments are considered to be safe for human consumption, and they could be used as effective food additives. Several authors [5, 6] have reported the use, in the U.S.A. and Mexico, of blue corn in different foods such as tortillas, chips, and snacks. In the present paper, we performed a study of nixtamalized blue corn flours obtained from four nixtamalization processes. The changes in total anthocyanins for each process, with respect to the raw corn, was studied by the chemical extraction of these pigments, together with spectroscopic analysis, and then compared with the relative intensity of the optical absorption spectra, at a fixed wavelength, obtained from photoacoustic spectroscopy (PAS).

2. EXPERIMENTAL

2.1. Materials and Methods

Extracts of anthocyanins were obtained from raw blue corn, traditional nixtamalization flour (TNF), and the fractional nixtamalization process (FNP) as described in [7]. According to the method of Salinas [8], 20 g of flour were weighed out and 100 mL of dissolvent were then added (methanol-acetic acid-water; 10:1:9), carrying out four successive extractions at a low temperature (4°C) and agitating for 24 h in each case. At the start of each extraction, the mixture was adjusted to pH 3, with 1N of HCl. At the end of each extraction, the solvent was separated from the flour and its volume was measured. An aliquot of 3 m of the extract was centrifuged at 10,000 rpm for 20 min at room temperature to eliminate turbidity. The absorbance of this centrifuged aliquot was measured using a Milton Roy spectrophotometer at 520 nm. A calibration curve for the calculation of total anthocyanins was prepared from chlorinated pelargonidin (Sigma Chemical Co., St. Louis, Missouri) in concentrations from 0 to 25 ppm.

2.2. PAS

The optical absorption spectra were obtained in the range of 300–800 nm by using a homemade photoacoustic (PA) spectrometer. The experimental setup consisted of a 1000 W xenon lamp (Oriel), a variable frequency mechanical chopper, set at 17 Hz, a monochromator, and an air-filled brass cell with a condenser microphone. The PA signal from the microphone provided the input to the signal channel lock-in amplifier (SR-850), which

is interfaced to a personal computer, displaying the wavelength-dependent signal amplitude and phase simultaneously. In order to take into account the xenon lamp emission spectrum, the PA signal was normalized to the signal obtained from charcoal powder [9].

3. RESULTS AND DISCUSSIONS

3.1. Total Anthocyanin Quantitation

Table I shows the different nixtamalization processes to obtain the blue corn flours. In the case of treatment M this corresponds to raw corn. As was expected for the M, sample, we observed the highest proportion of total anthocyanins, 27.20 mg/100 g of flours, when compared with those treated with $Ca(OH)_2$ (A, B, C, and T). This denotes the high susceptibility of anthocyanins to high values of pH, present in the nixtamalization process. These pigments are highly sensitive to pH and temperature [10]. The anthocyanin content of the raw corn sample is similar to the values reported by Salinas [8]. When it is compared to the total content of anthocyanins, from fractional nixtamalization treatment at the higher $Ca(OH)_2$ concentrations, we can see a slight diminution of anthocyanins from B to A treatments (Table I).

The flour of treatment C, from the fractional nixtamalization process, had a total anthocyanin content slightly higher than that obtained from the traditional nixtamalization process (T), and higher than the A and B treatments. Sequential fractional nixtamalization treatment reduced

Table I. Different Nixtamalization Processes Used to Obtain Corn Blue Flours, along with Their Total Anthocyanin Content

Sample	Process	$Ca(OH)_2$(%)	Time Process (min)	Temp. Process (°C)	Total anthocyanins (mg/100 g)	Total anthocyanins with respect to crude corn (%)
A	Sequential Frac. Nixt.	1.5	45	90	3.91 ± 0.01	14.0
B	Sequential Frac. Nixt.	1.0	45	90	3.97 ± 0.03	14.8
C	Sequential Frac. Nixt.	0.5	45	90	5.33 ± 0.11	19.4
T	Traditional Nixt.	1.0	40	90	4.94 ± 0.04	18.2
M	Raw corn	0.0	0.0	0.0	27.20 ± 0.08	100

the anthocyanin concentration in the flours when the $Ca(OH)_2$ concentration was increased; this reduction was more important when $Ca(OH)_2$ was increased from 0.5 to 1.0%. When the total anthocyanin content from B and T flours is compared, in which we used the same concentration of $Ca(OH)_2$, it was observed that the sequential fractional nixtamalization process is more aggressive to anthocyanins than the traditional nixtamalization. This is probably due to various factors such as the fact that, in the fractional nixtamalization process, the endosperm brand and embryo are separately nixtamalized, facilitating the contact of the lime with the anthocyanins, and for this reason, greater anthocyanin losses are expected when compared with the traditional nixtamalization process.

In the traditional process, the whole grains are nixtamalized, protecting the grain external layers, which would result in minor anthocyanin losses. Also, the time difference (5 min) between the B and T processes could result in an increase of these losses. Finally, the anthocyanin losses, during the nixtamalization process, also could occur from the pH effects on these compounds. Brovillard [11] indicated that alkaline pH destroys the benzopiril ring changing anthocyanins from red to pale yellow color.

3.2. PAS Results

The PAS technique was employed to obtain the optical absorption spectra of chlorinated pelargonidin, cyanidin, malvidin, and nixtamalized blue corn flour samples. Figure 1 shows the PA spectra of pelargonidin, cyanidin, and malvidin standard anthocyanins. By taking the derivative of the pelargonidin PA absorption spectrum as a function of the wavelength (not shown here), we obtained the point where a decrease of the PA signal amplitude is initiated, which corresponds to 520 nm. Pelargonidin was used for the calibration curve to determine the total anthocyanins by the chemical extraction procedure. With regard to cyanidin and malvidin anthocyanins, a wide optical absorption band was observed that decreased starting from 569 and 576 nm, respectively.

Figure 2 shows the PA optical absorption spectra of blue corn flours obtained from different nixtamalization processes. The solid and dashed lines correspond to sample M (raw corn) and traditional nixtamalization processes (T), respectively. For the case of the fractional nixtamalization process, the diamond, circle, and triangle symbols correspond to C, A, and B samples respectively. For the case of raw corn (solid line), three main absorption bands that contribute to the photoacoustic signal were observed. The first one is localized around 327 nm and the second and third at 558 nm and 678 nm, respectively. We can see that these absorption

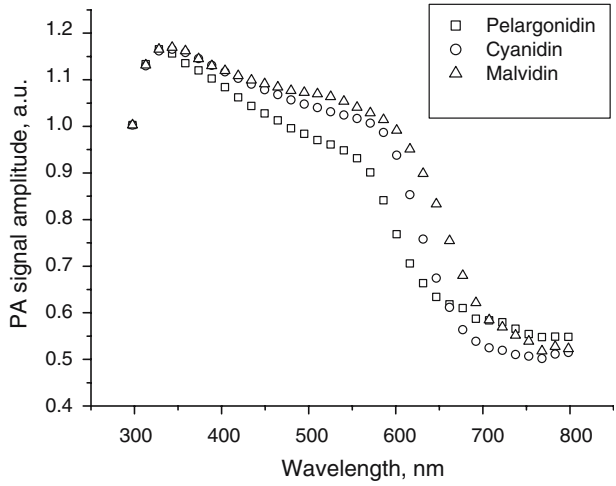

Fig. 1. PA optical absorption spectra of pelargonidin, cyaniding, and malvidin.

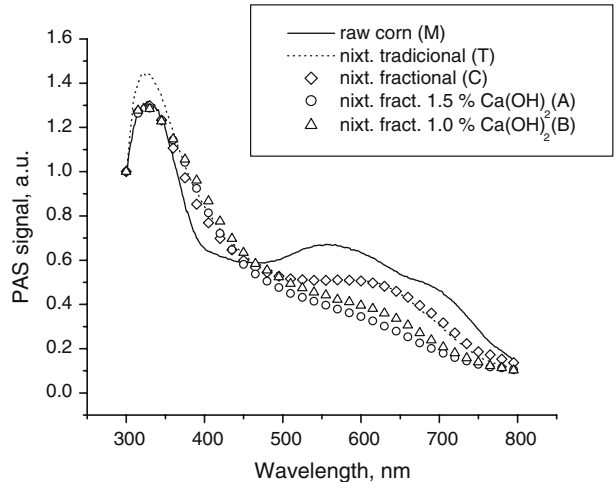

Fig. 2. PA optical absorption spectra of blue corn flour samples.

centers correspond to the presence of anthocyanins such as pelargonidin, cyaniding, and malvidin as shown in Fig. 1.

The PA optical absorption spectra of samples C (fractional nixtamalized process) and T (traditional nixtamalization process) were also obtained. For these two samples we observed two absorption peaks around 324 and 617 nm, which correspond to the optical absorption of

some anthocyanins such as malvidin, pelargonidin, and cyanidin (see Fig. 1). For samples A (fractional nixtamalization process with 1.5% $Ca(OH)_2$) and B (fractional nixtamalization process with 1.0% $Ca(OH)_2$), we obtain approximately the same optical absorption spectra but with less intensity when compared with the other samples. This reflects the fact that these samples have lower total anthocyanin content as can be seen from Table I.

From the total anthocyanins, shown in Table I, we observe that the raw corn has the highest anthocyanin content. Samples C, T, B, and A show a successive decrease in their total anthocyanin content according to Table I. The PA signal amplitude decreases in the same order for these samples as we can see in Fig. 2. On the other hand, the PA signal amplitude, at 520 nm, preserved the same order with regard to the total anthocyanin content for each sample as shown in Table I. When the percentage of the total anthocyanin content in each sample with respect to the raw corn (M) (last column of Table I) and the ratio of the PA signal amplitude, for each sample, divided by the PA signal amplitude of raw corn, at 520 nm, are considered, we observe a linear relationship between these two percentages (see Fig. 3). This linear relationship can be explained by the fact that the PA signal depends on the quantity of the optical absorption centers in the sample; then, the samples with higher anthocyanin content are expected to give a higher PA signal.

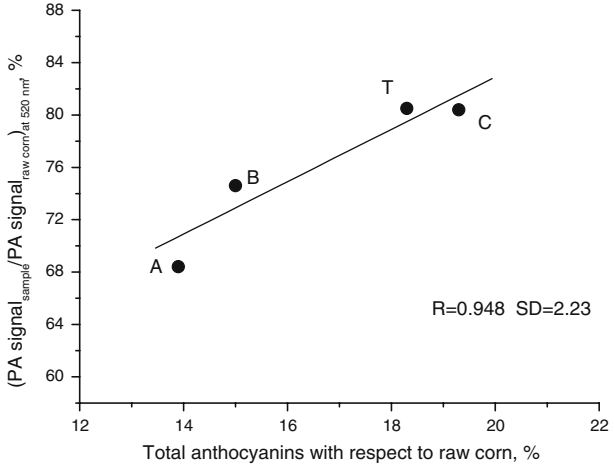

Fig. 3. Linear relationship between PA signal amplitude at 520 nm, normalized to raw corn (%) versus total anthocyanins from chemical extraction (%). Regression coefficient value (R) and standard deviation (SD) are included.

4. CONCLUSIONS

In this work, we have presented a chemical and photoacoustic analysis of blue corn flours obtained by the sequential fractional nixtamalization process, with different concentrations of calcium hydroxide, and also obtained by the traditional nixtamalization method. The total anthocyanin amounts in these flours were quantified by chemical extraction and the spectrophotometric method by using standard pelargonidin. By using PAS, it was possible to obtain optical absorption spectra of the studied flours, obtained from the different nixtamalization treatments. These results were compared with the chemical results, and we observed a linear relationship between the PA signal, at 520 nm, of each photoacoustic spectrum with the percentages of the total anthocyanins of the flours, from chemical extraction, as shown in Table I.

5. ACKNOWLEDGMENTS

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REFERENCES

1. L. S. Lee, E. U. Chang, J. W. Rihim, B. S. Ko, and S. W.Choi, *J. Food Sci. Nutr.* **2**:83b (1997).
2. M. Karaivanova, D. Dreska, and R. Ovcharov, *Eksp. Med. Morfol.* **29:**19 (1990).
3. J. Bonser, D. L. Madhavi, K. Singletary, and M. A. Smith, *Planta Med*. **62:**212 (1996).
4. J. B. Harborne and R. Self, *Phytochemistry* **26**:2417 (1987).
5. B. A. Cevallos-Casals and L. Cisneros-Zeballos, *Food Chem.* **86**:69 (2004).
6. J. A. Maga and M. B. Liu, *Dev. Food Sci. Amsterdam* **32:**983 (1993).
7. A. Cortes-Gomez, E. San Martín-Martínez, F. Martínez-Bustos, and G. M. Vazquez-Carrillo, *J. Food Eng.* **66**:273 (2005).
8. Y. M. Salinas, *Antocianinas en el grano de maíces criollos Mexicanos* (Tesis Doctoral, Colegio de Graduados, Institución de Enseñanza e Investigación en Ciencia Agrícola (IRGPFV), Montecillos, Texcoco, Edo. de México, 2000).
9. J. L. Jimenez-Perez, J. Jimenez-Perez, A. Bracamontes Cruz, A. Cruz-Orea, and J. G. Mendoza Alvarez, *Int. J. Thermophys.* **25**:503 (2004).
10. D. Markakis, "Anthocyanins as Food Additives," in *Anthocyanins as Food Colors* (Academic Press, New York, 1982), p. 245.
11. R. Brovillard, "Chemical Structure of Anthocyanins," in *Anthocyanins as Food Colors* (Academic Press, New York, 1982), p. 1.