March, 2004

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Mamoudou H. DICKO, Université de Ouagadougou
Otto DOKA
Dane D. BICANIC
Maja SLINGERLAND

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Photoacoustic Approach to Direct Determination of the Total Phenolic Content in Red Sorghum Flours

OTTO DÓKA,† DANE D. BICANIC,*‡‡ MAMOUDOU H. DICKO,§ and MAJA A. SLINGERLAND†,

Department of Physics, Faculty of Agriculture and Food Sciences, University of West Hungary, P.O.B. 90, 9201 Mosonmagyaróvár, Hungary. E-mail: dokao@mtk.nyme.hu Fax: 36-96-566 620, Laser Photoacoustic Laboratory, Biophysics Division, Department of Agrotechnology and Food Sciences, Wageningen University and Research Centre, Dreijenlaan 3, 6700 HA Wageningen, The Netherlands, Université de Ouagadougou, Laboratoire de Biochimie, UFR-SVT, CRSBAN, 03 BP. 7021, Ouagadougou 03, Burkina Faso, and Department of Plant Sciences, Crop and Weed Ecology Group, Wageningen University and Research Centre, Haarweg 333, 6709 RZ Wageningen, The Netherlands.

Photoacoustic (PA) spectroscopy in the ultraviolet and visible was demonstrated to be a suitable tool for direct determination of total phenolic content in red sorghum flours. The PA spectra obtained feature two characteristic peaks: the first centered at 285 nm is due to the aromatic amino acids, while the second one at 335 nm is associated with the total phenolic content. The outcome of the PA study was compared with the results obtained by a conventional, tedious Folin–Ciocalteau chemical method. Statistical analysis indicates no significant difference between the two methods used in this study.

KEYWORDS: Total phenolic content; sorghum; photoacoustics

INTRODUCTION

Sorghum [Sorghum bicolor (L.) Moench], the worldwide production of which amounted to some 57 million MT (1) in 2000, is used for both animal feed (USA) and human nutrition (Africa, Asia). It is the staple cereal in sub-Saharan Africa and India. For instance, in Burkina Faso, it represents between 48 and 57% of the total cereal production, with the average annual consumption of 200 kg per capita (2).

Sorghum grain contains varying concentrations of phenolics that may be determinants of nutritional quality in foods for both human and animals (3). When interacting with proteins, phenolics may hinder digestibility and palatability (4). The tannins (proanthocyanidins), a special group of high molecular weight phenolic compounds, may inhibit hydrolytic enzymes and also link with macronutrients, notably protein and carbohydrate, to form indigestible complexes. On the other hand, vicinal hydroxyl groups of phenolic compounds may chelate metal ions and reduce their bioavailability (5).

Before attempts can be made to determine, as well as to improve, the nutritional quality of sorghum grain (either through the breeding or food processing), its content in phenolics may be assessed. Different methods used to determine the total phenolic content are all laborious, time consuming, and rather costly, mainly because preparatory steps are needed prior to the actual measurement. Furthermore, current methods require liquid–solid extraction, implying different extraction yield. An inexpensive test capable of rapidly determining total phenolic compounds is needed in plant breeding (6). The major reason for this is the fact that, following the screening of a large number of varieties, only a few will be selected for breeding trials.

The objective of the present study was to explore the potential of the new candidate method, the photoacoustic spectroscopy (PAS) to directly determine phenolic content in red sorghum flours. The results obtained were compared to data acquired from the same samples by means of the adapted Folin–Ciocalteau (FC) approach (7) as adapted by Dicko (8).

MATERIALS AND METHODS

Different red sorghum flours investigated in this study were the specimens previously prepared by Dicko et al. (8) that were kept stored at −80 °C until the photoacoustic analysis. A unique number code (Tables 1 and 2) was assigned to each of the samples. The values of total phenolic content in these samples reported in Table 1 were originally determined by means of the adapted FC method by Dicko et al. (8).

Briefly, grains were surface-sterilized by washing (5 min) and stirring them in a 5% (v/v) aqueous solution of sodium hypochlorite. The grains were initially dried (ventilation at 20–25 °C) to reach 12–14% (w/w) moisture content and then ground (microanalytical mill from Fritsch, Marius Instruments, The Netherlands) into flour to pass a mesh screen.
(during 20 min) with 1.5 mL of 1% HCl in methanol at 25 °C. The suspension was then centrifuged (5000 g) and the supernatant was collected. The residue was re-extracted with HCl/methanol as described above, and the two supernatants were pooled. Total phenolic compounds were determined using the FC method adapted to a 96-well plate assay. The 25 μL of FC reagent (50% v/v) was added to a 10-μL extract. After a 5 min incubation period, 25 μL of 20% aqueous solution of sodium carbonate and water was added to a mixture to make total volume of 200 μL. A blank for each sorghum sample was prepared by replacing FC reagent with water. Gallic acid was used as a standard, and results were expressed as the gallic acid equivalent per gram of flour (w/w). After 30 min, the absorbance at 760 nm was measured using a multiwell plate reader (EAR 400, Labinstruments, Australia). As to the standards, freshly prepared samples were used consistently.

**Photocoustic Method.** The PA method involves the exposure of a condensed phase sample to the periodically modulated radiation. The fraction of the energy absorbed by the sample is converted to heat as a result of which sample’s temperature oscillates at a frequency equal to that of the modulation itself. Generated thermal waves reach the surface of the sample, causing the periodic heating and cooling of the surrounding gas layer. The expansions and contractions of the gas give rise to acoustic waves, which are eventually detected as the voltage (called PA signal) by a suitable microphone. The PA spectrum is usually obtained by measuring the magnitude of the PA signal while varying the wavelength of the incident radiation. Optical and thermal parameters of both the sample and contacting gas play a decisive role in the generation process of the PA signal. To eliminate the effect of the wavelength-dependent power output of the excitation source on the magnitude of the PA signal, this latter is usually normalized to a PA signal obtained from a strongly absorbing reference such as carbon black (9–11).

In general, PAS offers several advantages above other analytical techniques: It is nondestructive, requires no pre-preparation of the sample, and it is applicable to "difficult to study" specimens such as powders as well as optically opaque and gelatinous samples.

The PA spectrometer (Figure 1) used in this study comprised a 300 W Xe lamp (ILC Technology, Cernmax XL, 300 UV), a monochromator (Jobin-Yvon, H-10, spectral resolution 16 nm), a modulator, and a homemade PA cell. After passing through the monochromator, the collimated beam of mechanically chopped (16 Hz) radiation was collected by a quartz lens and focused into the PA cell loaded with the sample under investigation. Of the 50 samples originally studied by Dicko et al. (8), 7 were selected for the purpose of this study.

At 467 nm, the wavelength corresponding to the lamp’s maximal emission, the actual power reaching the PA cell is estimated to be 5 mw. The light enters the PA cell (1/2) through a 1/2 inch diameter quartz window; the coupling between the microphone and the sample volume was achieved by means of a 3-mm long thin capillary (inner diameter 300 μm). The PA signal was processed by a dual phase lock-in amplifier (Stanford SR530) connected to the computer.
The normalized PA spectra within spectral range extending between 250 and 550 nm obtained from all test samples (Figure 2) show two characteristic bands. The first one (centered at 285 nm) is due to aromatic amino acids in sorghum flour (9, 13), while another, close to 335 nm, is due to the flavonoids and phenolics present in the pericarp of sorghum flour (14, 15). The PA signal decreases with increasing wavelength across the entire spectral range studied. On the basis of this fact, an attempt was made to establish a correlation between phenolic content (determined by a modified FC analysis) of sorghum flour and the magnitude of the normalized PA signal. Figure 3 displays PA signals (at 335 and 475 nm) plotted versus phenolic content; at both wavelengths, the proportionality is linear ($R = 0.90$ and $R = 0.91$ respectively; symbol $R$ refers to the correlation coefficient). The calibration curves at 335 and 475 nm satisfy equations $y = 0.00393796x + 0.17781026$ and $y = 0.00319608x + 0.06747758$, respectively. In these equations, $y$ is the magnitude of normalized PA signal (dimensionless quantity), while $x$ is the concentration expressed in mg/g.

By use of the experimentally obtained values for normalized PA signals, total phenolic content (Table 2) was calculated from two above-mentioned equations. As 7 mg/g appears to be the lowest phenolic content still measurable by PA technique, one can state that above this concentration, results obtained by PA and FC methods are practically the same. To confirm such a statement, PA and FC methods were statistically compared in terms of variance and expected value using $F$-(Fisher) and $t$-tests (Student), respectively.

In the expression for the sample variance

$$\sum_{i=1}^{n} (X_i - \bar{X})^2/(n - 1)$$

$X_i$ is the total phenolic content of sorghum samples, and $\bar{X}$ is the arithmetic average of $X_i$, while $n$ refers to the number of samples. The standard error of the mean (SEM) is calculated as the standard deviation (square root of the sample variance) divided by $n^{1/2}$. The coefficient of variation (CV), actually a measure of precision, is expressed as the percentage of the standard deviation of the mean. In this case, there are $n - 1$ degrees of freedom because statistical parameters were calculated using $X_i$ and $\bar{X}$. Because the average is no longer independent of $X_i$, it is necessary to subtract 1 from the number of samples $n$.

The $F$-test for the variances between data shown in columns 2 and 4 of Table 2 reveals no significant difference. The same conclusion applies to data shown in columns 3 and 4. In both cases, calculated $t$ values as the outcome of $t$-tests are smaller than the critical $t$ value, thereby providing the evidence that there is a probability of 95% that two methods will produce the same results.

The agreement between the results obtained by PA and FC methods is good for red sorghum flour with the total phenolic content exceeding 6 mg/g. At present, the lowest detectable concentration of total phenolics in red sorghum flour is estimated to be 2.25 mg/g. However, additional sensitivity enhancement for PA method is anticipated if instead of the currently used Xe lamp, the more powerful source (for example a laser) is used for the excitation. However, the most pronounced advantage of the PA approach above the conventional FC method is its unique ability to study powdered samples directly (i.e., simply as they are); this greatly reduces the time needed to complete the analysis.

ACKNOWLEDGMENT

We thank the Centre International de Recherche Agronomique pour le Développement (CIRAD) Ouagadougou (Burkina Faso) and Montpellier (France) and the Centre National de Recherche Scientifique et Technique (Burkina Faso) for providing different varieties of sorghum [Sorghum bicolor (L.) Mounch].

LITERATURE CITED


Received for review June 4, 2003. Revised manuscript received December 22, 2003. Accepted December 30, 2003.

JF030421A