JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 2133−2136 **2133**

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 hydrolic 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 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

* To whom correspondence should be addressed. Fax: 31-317-482725. E-mail: dane.bicanic@wur.nl.

[†] University of West Hungary.

[‡] Department of Agrotechnology and Food Sciences, Wageningen University and Research Centre.

[§] Université de Ouagadougou.

[||] Department of Plant Sciences, Wageningen University and Research Centre.

10.1021/jf030421a CCC: $27.50
Published on Web 03/20/2004

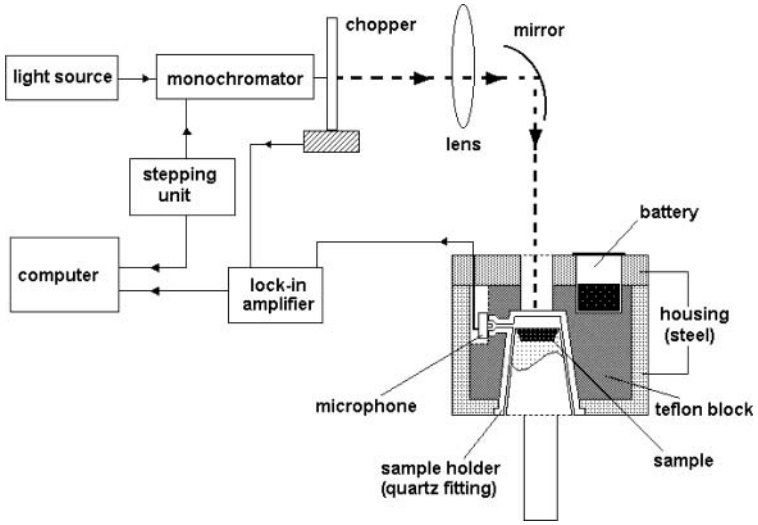

Figure 1. The experimental setup used in the PA study on red sorghum flours.

Table 1. Seven Red Sorghum Flours and Their Total Phenolic Content Determined by the Adapted FDC Method (*8*)

code	variety	gen type[a]	origin	harvest year	Ra[b]	color	total phenolic[c] (mg/g)
7	Framida	IL	South Africa	1998	KC	red	27.18
18	Magadji1-509	LR	Burkina Faso	1996	GC	red	8.99
33	Kapla-57	LR	Burkina Faso	1997	G	red	15.37
34	IRAT	IL	Cameroon	1998	C	red	13.88
38	G 1296	LR	Burkina Faso	1996	GC	red	26.42
40	Zouobdo rose-781	LR	Burkina Faso	1998	G	red	16.86
41	Sariaso 808	LR	Burkina Faso	1997	G	red	11.42

[a] Gen type, genetic type; IL, inbred line; LR, land race. [b] Ra, race with C, Caudatum; G, Guinea; GC, Caudatum–Guinea; KC, Kafir–Caudatum. [c] These data have been collected during the previous study conducted by Dicko et al. (*8*).

Table 2. Total Phenolic Content (expressed in mg/g) Determined by PA and FC Methods. Data[a] Refer to Results Obatined by the Modified FC Method during Previous Study of Dicko et al. (*8*)

code	phenolic content as determined by PA at 475 nm	PA at 335 nm	FC method[a]
sample18	7.769	9.995	8.99
sample41	7.947	7.076	11.42
sample34	20.324	17.582	13.88
sample33	15.587	12.468	15.37
sample40	15.030	20.179	16.86
sample38	28.777	27.638	26.42
sample7	24.122	25.178	27.18
mean	17.078	17.159	17.16
sum of X	119.551	120.119	120.12
sum of X^2	2415.402	2419.664	2361.156
SD	7.891	7.728	7.069
variance	62.268	59.735	49.982
SEM	2.9824	2.921	2.672
CV	46.203	45.039	41.199
deg of freedom	6	6	6

[a] These data were collected during previous study of Dicko et al. (*8*).

(size 0.5 mm). Care was exercised to avoid overheating when milling at 4 °C. To prevent endogenous enzymatic or nonenzymatic reactions, flours were stored at −80 °C prior to analysis (*8*).

Adapted FC Method. The adapted FC method as used by Dicko et al. (8) to quantify total phenolic compounds is as follows: The phenolic compounds in 50 mg of sorghum flour were extracted by continuously stirring (during 20 min) with 1.5 mL of 1% HCl in methanol at 25 °C. The suspension was then centrifuged (5000*g*, 10 min, 25 °C), 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.

Photoacoustic 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, Cermax 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 (*12*) 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.

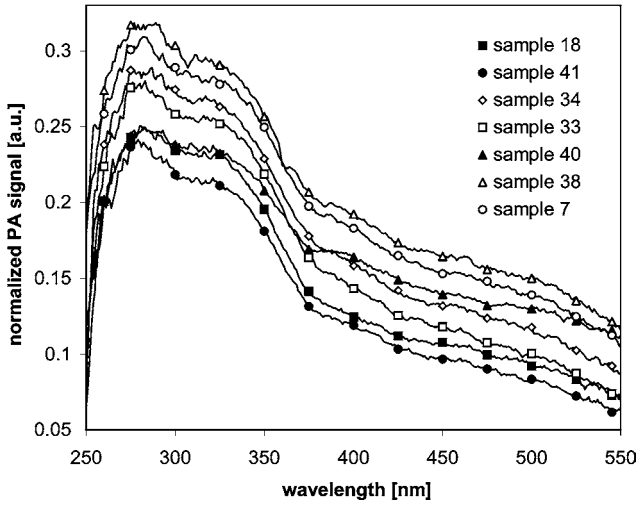

Figure 2. The normalized PA spectra of seven different red sorghum flours.

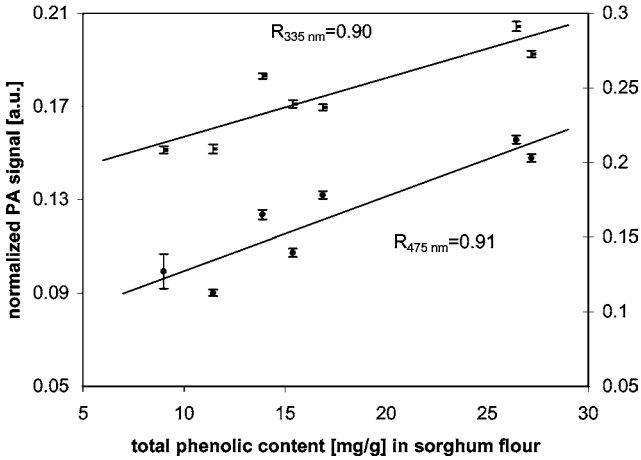

Figure 3. The magnitude of the normalized PA signal plotted versus the total phenolic content. The left and the right *y*-axes refer to the calibration curves recorded at 475 and 335 nm, respectively. In both cases, the vertical bars represent the extent of the experimental error based on 512 successive readings of the PA signal taken from the lock-in amplifier.

RESULTS AND DISCUSSION

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 obatined by PA and FC methods are practicaly the same. To confirm such a statement, PA and FC methods were statisticaly 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 sorgum 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

(1) Food and agricultural organisation (FAO), 2003. FAOSTAT Agricultural database 2003. http://apps.fao.org/.

(2) Maatman, A. *Si le fleuve se tord, que le crocodile se torde.* Ph.D. Thesis, **2000**, Centre for Development Studies, University of Groningen, The Netherlands.

(3) Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds, nature, occurrence, dietary intake, and effects on nutrition and health. *J. Sci. Food Agric.* **2000**, *80*, 1094−1117.

(4) Haslam, E.; Lilley, T. H. Natural astringency in foodstuffs, a molecular interpretation. *Crit. Rev. Food Sci. Nutr.* **1988**, *27*, 1−40.

(5) Slabbert, N. Complexation of condensed tannins with metal ions. In *Plant Polyphenols: Synthesis, Properties, Significance.* Hemmingway, R. W., Laks, P. E., Eds.; Plenum Press: New York, 1992, 421−436.

(6) Cai, T.; Ejeta, G.; Butler, L. G. Screening for grain polyphenol variants from high-tannin sorghum somaclones. *Theor. Appl. Genet.* **1995**, *90*, 211−220.

(7) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152−177.

(8) Dicko, M. H.; Hilhorst, R.; Gruppen, H.; Traore, A. S.; Laane, C.; van Berkel, W. J. H.; Voragen, A. G. J. Comparison of content in phenolic compounds, polyphenol oxidase, and peroxidase in grains of 50 sorghum varieties from Burkina Faso. *J. Agric. Food Chem.* **2002**, *50*, 3780−3788.

(9) Rosencwaig, A. In *Photoacoustic and Photoacoustic Spectroscopy.* John Wiley and Sons: New York, 1980.

(10) Yasa, Z. A.; Jackson, W. B.; Amer, N. M. Photothermal spectroscopy of scettering media. *Appl. Opt.* **1982**, *21*, 21−31.

(11) Tam, A. C. Applications of photoacoustic sensing techniques. Photothermal spectroscopy of scettering media. *Rev. Mod. Phys.* **1986**, *58*, 381−431.

(12) Dóka, O.; Kaszás, Gy.; Lörincz, A. High sensitivity cell for measurement of photoacoustic spectra of powders. *Acta Ovariensis* **1991**, *34*, 39−46.

(13) Dóka, O.; Szöllösy, L.; Novák, Á. Photoacoustic spectra of skim milk powder and of milk protein concentrate. *Acta Alimentaria* **1994**, *23*, 325−330.

(14) Favier, J. P.; Buijs, J.; Mikós, A.; Lörincz, A.; Bicanic, D. Photoacoustic characterization of different food samples. *Z. Lebensm.-Unters. Forsch.* **1994**, *199*, 59−63.

(15) Dóka, O.; Sikolya, L.; Szöllösy, L. Comparative study of grain and milling products of amaranth and wheat: Photoacoustic measurements in ultraviolet and visible range. *Acta Agron. Óvariensis* **1996**, *39*, 53−58.

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

JF030421A