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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 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.
In a previous study of Dicko et al. (8), 7 were selected for the purpose of this study. Of the 50 samples originally studied by Dicko et al. (8), 7 were selected for the purpose of this study. Of the 50 samples originally studied by Dicko et al. (8), 7 were selected for the purpose of this study. Of the 50 samples originally studied by Dicko et al. (8), 7 were selected for the purpose of this study.
Phenolics present in the pericarp of sorghum flour (while another, close to 335 nm, is due to the flavonoids and at both wavelengths, the proportionality is linear (PA signals (at 335 and 475 nm) plotted versus phenolic content; the magnitude of the normalized PA signal.

The magnitude of 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 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 $n^{0.5}$. 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.

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**LITERATURE CITED**


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