Optimization of the 4-Dimethylaminocinnamaldehyde Assay for Flavanols

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Abstract
Flavonoids and their polymeric condensation products, proanthocyanidins represent the most common flavonoids consumed in the diet and are powerful antioxidants. Because of large degrees of chemical variation, isolation and quantification are difficult. The optimal conditions of the 4-dimethylaminocinnamaldehyde (DMAC) spectrophotometric assay for flavanols were evaluated to increase the sensitivity of the reaction and accuracy of the assay. The effects of acid nature (HCl and H2SO4), concentration (2, 4, 6, 8, and 10N), temperature (25°C, with a sample water content ≤3%), and sample weight (0.1–1.0 g) on reaction sensitivity were compared. The concentration (2, 4, 6, 8, and 10N) of acid was found to be more effective in increasing the sensitivity of the reaction when the 200 mg/L catechin standards were prepared with either 2N and 4N H2SO4 (v/v). The concentration of the DMAC reagent (0.1, 0.2, 0.3, 0.5, and 10%) and presence of interfering substances, were examined in order to develop a robust method for accurate assessment of flavanols. The use of sulfuric acid as in the reaction significantly improved the results. A mixture of 2% DMAC (v/v) and 4N H2SO4 (v/v) showed maximum sensitivity when allowed to react for 12 min prior to analysis, with no further improvement shown by extending the time to the usual 20 minutes. The reaction of DMAC was most precise when conducted at constant temperature between 20 – 25°C, with a sample water content 10%. Excess water caused loss of color in the reaction resulting on underestimation of the values.
Optimization of the DMAC assay allows maximal sensitivity and detection of small concentration changes, giving a more accurate estimation of the healthy natural plant flavonoids present in foods.

Results
The objective of this research was to develop the most robust, reliable, and accurate parameters for the DMAC assay. To accomplish this, a number of experimental parameters were studied, including: acid nature (HCl and H2SO4), concentration (2, 4, 6, 8, and 10N), temperature (25°C, with a sample water content ≤3%), and sample weight (0.1–1.0 g). Each parameter was studied in a cold 1:1 mixture of methanol and 2, 4, 6, 8, or 10N HCl (v/v). Reagents were kept in the freezer (≤18°C) between experiments. As the most popular flavonoids consumed in the diet (Aron and Kheir 2000), flavanols / proanthocyanidins have been suggested to account for a significant fraction of the flavonoids ingested in the western diet (Santos-Buelga and Scalbert 2005).

Methods

Preparation of Catechin Standards
Standard curves to the spectrophotometric standards were prepared by first dissolving 200 mg/L catechin in a 100 mL final volume, to which 0.1 g catechins were added. Standard curves were prepared by adding 1.2, 3, 6, 9, and 12 mL of the catechin solution to 37 mL of 2N H2SO4 (v/v). In order to achieve a final volume of 40 mL of 0.25% catechin and 100% of the DMAC reagent in 20 minutes, it was necessary to adjust the volume of the sample water content ≤3% to a final volume of 100 mL capacity to ensure uniform distribution. Subsequent dilutions of 0.1, 0.2, 0.3, 0.4, and 0.5 mL of the mixture were prepared by adding 1.2, 3, 6, 9, and 10 mL of the catechin solution to 37 mL of 2N H2SO4 (v/v) in order to achieve a final volume of 40 mL of 0.25% catechin and 100% of the DMAC reagent in 20 minutes. A mixture of 2% DMAC (v/v) and 4N H2SO4 (v/v) was prepared by adding 20 µL of each catechin standard to 250 µL of methanol and 100% of the DMAC reagent in 20 minutes. Appropriate plastic ware was used to minimize the possibility of catechins being photolyzed. Standards were prepared daily because of the relative instability of the 4-dimethylaminocinnamaldehyde reaction. The absorbance at 640 nm in 5 minutes was measured at a 10 mL volume. The standard curves of 200 mg/L catechin standards were prepared with 200 mL of water and allowed to react for 12 min at constant room temperature between 21 – 25ºC. 3) The use of absolute water content ≤3% was reported as measurement of the DMAC reagent.

Preparation of DMAC Reagents
The DMAC reagent (4-dimethylaminocinnamaldehyde) was prepared immediately before use by dissolving 10, 1.5, 2.0, 2.5, and 3.0% DMAC (w/v) in 2, 4, 6, 8, and 10N HCl (v/v). Reagents were kept in the freezer (≤18°C) between experiments. New reagents were prepared daily because of the relative instability of the 4-dimethylaminocinnamaldehyde reaction. The absorbance at 640 nm in 5 minutes was measured at a 10 mL volume.

Reaction Studies
Using the prepared standards and reagents a spectrophotometric analysis of the DMAC reaction was measured and analyzed at the rate of 6N HCl. Differences were compared between 2N and 4N H2SO4 (v/v). The results showed the interference of the following substances tested had no affect on the DMAC assay at 4 g/L concentration. The interference of the following substances were tested for interference in the DMAC assay at 4 µg/L concentration.

Conclusions and Discussion

- Critical evaluation of the 4-dimethylaminocinnamaldehyde (DMAC) assay for flavanols lead us to a more accurate method for quantifying the healthy flavanols and proanthocyanidins compounds in foods ingredients.

- In order to achieve maximum sensitivity of the assay, optimum conditions for the DMAC assay were identified. Use of 2% DMAC powder and 0.25% H2SO4 to prepare the DMAC reagent. An optimum temperature of 25°C and a sample water content ≤3% was critical to the assay.

- The use of absolute measurement in the assay and less than 2% water content of the sample.

- The existence of interfering substances tested did not interfere with the DMAC assay at 4 µg/L concentration.

References


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