SUPPLEMENTARY MATERIAL

Thermal properties of tannins extracted from *Anacardium occidentale* L. using TGA and FTIR Spectroscopy.

Vinod Viswanath^{1,2}, Vincent Vineeth Leo¹, Sabna Prabha S¹, Prabhakumari.C¹, V P Potty¹ and Jisha M.S.^{2*}

¹*Cashew Export Promotion Council Laboratory and Technical Division, Kollam, Kerala, India.*

²*School of Bioscience, Mahatma Gandhi University, Kottayam, Kerala*

Abstract

The chemical nature of the polyphenols of cashew kernel testa has been determined. Testa contains tannins, which present large molecular complexity and have an ancient use as tanning agents. The use of tannins extracted from cashew testa, considered in many places as a waste, grant an extra value to the cashew. In this work we have analyzed through High Performance Liquid Chromatography (HPLC), Infrared spectroscopy (FT-IR) and Thermo Gravimetric analysis (TGA) the average molecular weight, main functional groups and thermal properties of tannins extracted from *Anacardium occidentale* L. The results of these analyses are compared with the commercial grade tannic acid. The FTIR spectra showed bands characteristic of C=C, C-C, OH bonds. This important bioactive compound present in the cashew nut kernel testa- was suggested as interesting economical source antioxidants for use in food and nutraceutical industry.

Keywords: *Anacardium occidentale* L; Cashew testa; FTIR; HPLC; Tannic acid; TGA

Experimental

Chemicals

Tannic acid supplied by Sigma-Aldrich and all other solvent and chemicals were used in the analytical grade.

Sample collection

Cashew testa samples were collected from cashew processing unit, Kollam. Testa were grinded and collected as a powdered form. The powder form of cashew testa later used for the determination of poly phenolic compounds. Then the raw material was sun-dried for 2–3 days. It was stored in bags under adequate ventilation.

Extraction and Quantification of tannin.

The powdered cashew testa sample (1g) was extracted with 100ml of 80% of HPLC grade methanol for 30 minutes in a hot plate and filtered through whatmann No.1 filter paper. Dried extract was dissolved in 80% of HPLC grade methanol. The final concentration was 1mg/ml. Quantification of tannin was done by Folin Ciocalteu method (Makkar et al.1993)

HPLC

The molecular weight of tannins was obtained by high pressure liquid chromatography (HPLC). The Sample was loaded at a concentration of 1 mg/ml to the HPLC (Waters, USA). Sample was diluted in solvent containing Acetonitrile:Water (50:50). Injection volume was 50 μ l. Absorbance was monitored at 265 nm and flow rate maintained at 1 ml/min. The sample was applied to symmetry column 184.6x 250mm and eluted with a linear gradient of acetonitrile containing 0.1 % TFA (trifluoroacetic acid). The sample and standard tannic acid acid were run identically.

FTIR

FTIR analysis was carried out for the Structural characteristics of the compounds from cashew industry waste cashew testa. The extracted sample was subjected to FT-IR spectroscopic analysis (Shimadzu, Japan), equipped with KBr beam splitter with DTGS (Deuterated triglycine sulphate) detector (7800-350cm⁻¹).The parameters used in FT- IR analysis were: spectral range 4000-400cm⁻¹, resolution 4cm.⁻¹

Thermo Gravimetric Analyzer (TGA)

Thermal-oxidative stability studies were carried out using thermoanalytical measurements (TG) using a SHIMADZU TGA-50H thermogravimetric analyser. Thermogravimetric (TG) measurements were performed at a scanning rate of 10° C/min. Samples of approximately 5 mg were heated from 5° C to 600° C. The measurements were carried out under nitrogen atmosphere (20 ml/min).

Reference:

Makkar HPS, Blummel M, Borowy NK, Becker K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. Journal of the Science of Food and Agriculture.61:161-165.

Table S1: Infrared position of tannins and tannic acid

Group	Vibration	Approach frequency/experimental (cm ⁻¹)
Arom-CH ₂ -OH	v OH	3400/3387
Arom-CH ₂ -OH	va CH ₂	2920/2924
Arom-C-O	v C-O	1606/1651
Arom-CH ₂ -OH	δ CH ₂	1444/1445
Arom-O	va C-O	1149/1193
Arom-O	v C-O	869

The figure displays two chromatograms side-by-side, both plotting Absorbance Units (AU) on the y-axis against time in Minutes on the x-axis (ranging from 0.00 to 10.00).

Left Chromatogram: Shows a major peak at 2.240 minutes (AU ≈ 0.65) and two minor peaks at 3.168 minutes (AU ≈ 0.02) and 4.651 minutes (AU ≈ 0.01).

Right Chromatogram: Shows a major peak at 2.322 minutes (AU ≈ 3.3) and four minor peaks at 2.852 minutes (AU ≈ 0.2), 3.152 minutes (AU ≈ 0.1), 4.397 minutes (AU ≈ 0.05), and 4.681 minutes (AU ≈ 0.05).

S1 (b)

Figure 1 displays two infrared (IR) spectra. The left plot shows the IR spectrum of poly(2,2,2-trifluoroethyl methacrylate) (PTEMA), with the y-axis representing transmittance (%) from 80 to 90 and the x-axis representing wavenumber (1/cm) from 4000 to 1000. The right plot shows the IR spectrum of poly(2,2,2-trifluoroethyl methacrylate-co-methyl methacrylate) (PTEMA-co-PMMA), with the y-axis representing transmittance (%) from 70 to 90 and the x-axis representing wavenumber (1/cm) from 4000 to 1000. Both plots show characteristic absorption bands, with the PTEMA-co-PMMA spectrum exhibiting additional peaks compared to the PTEMA spectrum.

Figure S3: TGA of cashew testa showing percentage of mass loss.

Figure S4: TGA of Tannic acid showing percentage of mass loss.