

# A rapid microtitre plate Folin-Ciocalteu method for the assessment of polyphenols

## Research Article

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Received 26 April 2012; Accepted 24 August 2012

**Abstract:** Several methods have been described for the determination of phenolic compounds in animal and plant products using the Folin-Ciocalteu (FC) assay. Most of these methods describe the use of this reagent and sodium carbonate in spectrophotometric methods. The macro FC assay was compared with two micro FC assays carried out on a microplate reader. Excellent correlation was obtained among the three assays with a molar extinction coefficient of  $5.228 \pm 0.187 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ . The micro assay may serve as a high throughput method for the rapid determination of polyphenols in various samples.

**Keywords:** UV-VIS spectrophotometry • Microplate reader • Methanolic extracts • Tannic acid

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## 1. Introduction

Phenolics represent a large number of secondary metabolites present in most fruits and vegetables. These are subsequently divided into the simple phenols or phenolic acids, with one phenol ring and polyphenols, with at least two phenol rings. Typical examples include resorcinol, phloroglucinol, some coumarins, stilbenes, flavonoids, lignans and tannins amongst others.

Several methods have been used for the determination of polyphenols in plants. Typically, high performance liquid chromatography [1,2] and spectrophotometric analysis are frequently quoted. More specifically the Folin-Ciocalteu method has been described by many authors [3]. Originally this method was developed for the colorimetric determination of tyrosine, a phenolic non-essential amino acid [4]. Thereafter, this method has been used for the determination of several compounds including plant phenolics, drugs [5-7], vitamin C [8] and other constituents in a wide range of samples ranging from plant extracts to urine and bee products, in particular. The spectrophotometer-cuvette method is also frequently quoted. Little reference is made to the microanalysis using the FC reagent. Although a

micromethod has been described for wine phenolics, plastic or glass cuvettes were used [9]. However, this method employs micro volumes for the FC reagent and sodium carbonate. A microplate method has been described specifically for the determination of total phenolic compounds in urine [10] and in food products [11]. The latter method gives a short reaction time of 3 minutes. In most experiments, either gallic acid or tannic acid was used as a phenolic standard.

The present study attempts to demonstrate a rapid method for the determination of polyphenols in a high throughput assay when compared to classical FC assays used.

## 2. Experimental Procedures

### 2.1 Materials

Tannic acid, methanol, Folin-Ciocalteu reagent and sodium carbonate were purchased from Sigma Chemical Co. (St., Louis, USA). Tannic acid was prepared in five 1 in 2 dilutions, from  $960 \mu\text{g ml}^{-1}$  down to  $60 \mu\text{g ml}^{-1}$ . The FC reagent was diluted 1:10 with de-ionised water, while sodium carbonate was prepared as a 1 M solution [12].

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