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Research Article

A SIMPLE METHOD FOR THE EXTRACTION OF PHENOLIC COMPOUND (ELLAGIC ACID) FROM STRAWBERRY USING ULTRASOUND AND ANALYZE IT BY HPLC

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ABSTRACT

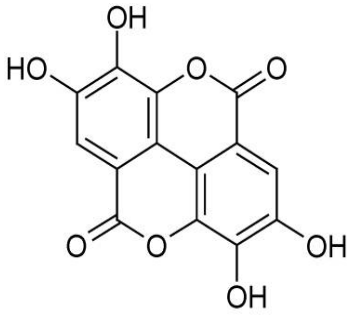
Ultrasound-assisted extraction was used for the determination of phenolic compound (Ellagic acid) present in strawberries. The sample immersed in an aqueous solution containing 5-sulphosalicylic acid (15% w/v)was sonicated for 2 min (duty cycle 0.2 s), output amplitude 20% of the nominal amplitude of the converter , applied power 100W with the probe placed (1 cm)from the bottom of the water bath and (5cm) from the walls of the precipitate glass. Subsequent separation was carried out by liquid chromatography (HPLC) with photodiode array UV detection. This proposed method, is much faster and produces less analyte degradation than methods as solid–liquid, subcritical water and microwave-assisted extraction.

Keywords: Ellagic acid , Strawberry, Ultrasound irradiation, HPLC.

1. INTRODUCTION

Ellagic acid (C14H6O8) is a naturally occurring polyphenolic compound. It is found in different fruits, berries and nuts such as pomegranates[1,2,3] , grapes, raspberries, blackberries, strawberries and walnuts, etc.[4] It has antibacterial and antiviral properties.[5,6] Recent studies have indicated that ellagic acid may have anticarcinogenic effects against liver, esophageal, prostate, and colorectal cancer cell lines.[7,8,9,10] Other studies have reported that ellagic acid is a potent antioxidant[5,11]. It is a cancer inhibitor which has the ability to cause apoptosis or normal cell death in cancer[12]. Ellagic acid has also been said to reduce heart disease, birth defects, liver problems, and to promote wound healing[16]. There are also reports that it may help the liver to break down or remove some cancer-causing substances from the blood[17]. Several studies have found that ellagic acid can inhibit the growth of skin, esophagus, lung and other tumors caused by carcinogens[18,19]. Recently Italian researchers found that ellagic acid seemed to reduce the side effects of chemotherapy in men with advanced prostate cancer, although it did not help to slow disease progression or improve survival[20]. However, futher studies would be needed to confirm these results and to determine if other results apply to humans.

Strawberries (*Fragaria x ananassa*) are an excellent source of ellagic acid it contained high ellagic acid levels (greater than 40 mg/100 g) and considered to be one of the most potent sources of ellagic acid[32], which has been shown to provide many health benefits. Ellagic acid has been determined earlier in plant products using High Pressure Thin-layer Chromatography (HPTLC)[13]. High Pressure Liquid Chromatography (HPLC)[14,15], Gas Chromatography (GC) and other.



Ellagic acid

HPLC with direct injection is the technique usually applied for beverages and other liquid samples[22]. Filtration of the samples is the only pre-treatment needed. Solid foods require an appropriate prior extraction[23,24], for which solvents such as ethanol, acetone or methanol are used[25], or a water–methanol mixture which contains both hydrochloric acid and an antioxidant[21]. The extraction of phenolic compounds requires special care, because they are easily oxidized and rapidly degraded by light.Different techniquesas supercritical fluid extraction (SFE)using
either pure or modified CO_2[26] and microwave-assisted extraction[27] have been applied. These techniques offer a better control over the extraction conditions and allow the extraction to be performed in shorter times and in a more selective way. Ultrasonicradiation is a powerful aid to accelerate of various steps of the analytical process. This energy is of great help in samples as itfacilitates and accelerates operations such as the extraction oforganic and inorganic compounds[28,29] homogenization[30] and various others[31]. Ultrasound-assisted leaching is an effective way to extract analytes from different matrices in shorter time than other extraction techniques.

2. Experimental

2.1. Instruments and apparatus

Ultrasonic irradiationwas applied by means of a Branson 450sonifier (20 kHz, 100 W) equipped with a cylindrical titanium alloy probe (2.54 cm diameter) which was immersed in a water bath in which a precipitate glass with the sample was placed.
The HPLC analysis was carried out on a Shimadzu The extract was directly loaded on to the Sep-PakODS(5 µm, 250 mm × 4.6 mm) column classLC-VP HPLCsystem with class LC-VP5 , a pump (LC-10Advp), an autosampler (SIL- 10AD) and a diode-array detector (SPD-M10Avp).

2.2. Extraction and hydrolysis

Five g of strawberry were placed in a precipitate glass and 10ml of an aqueous 15% sulphosalicylic acid solution were added for simultaneousextraction and hydrolysis. This unit was immersed ina water bath and sonicated for 2min (duty cycle 0.2 s, outputamplitude 20% of the nominal amplitude of the converter, applied power 100W with the probe placed (1 cm) from the bottom of the water bath and(5 cm) from the walls of the precipitate glass. After complete extraction, the extract was filtered through a filter paper(ashless filter paper, 12.5 cm) ,(1ml) of extract was then diluted withmethanol–water (10:90, v/v) adjusted to pH 3 with acetic acid and filtered through a 0.45_m filter that was compatiblewith organic solvents prior to injection into the HPLC–DAD system.

2.3. Chromatography

The HPLC separation was performed using acetonitrile–watergradient. The mobile phase consisted of: water (0.1%TFA, v/v) (A), and Acetonitrile (B), the gradient program was as follows:0–25 min, 32% B, 25–35 min,35% B. Sep-Pak ODS(5 µm , 250 mm × 4.6 mm) column, flow rate 1 ml/min; The chromatograms were acquired at 375 nm.

Chemicals

Standard of ellagic acids was purchased fromRoth (Karlsruhe, Germany). Compound was dissolvedin methanol to obtain a stock solution (0.5mg/mL). All solvents used were of analytical or HPLCgrade (Merck, Darmstadt, Germany)

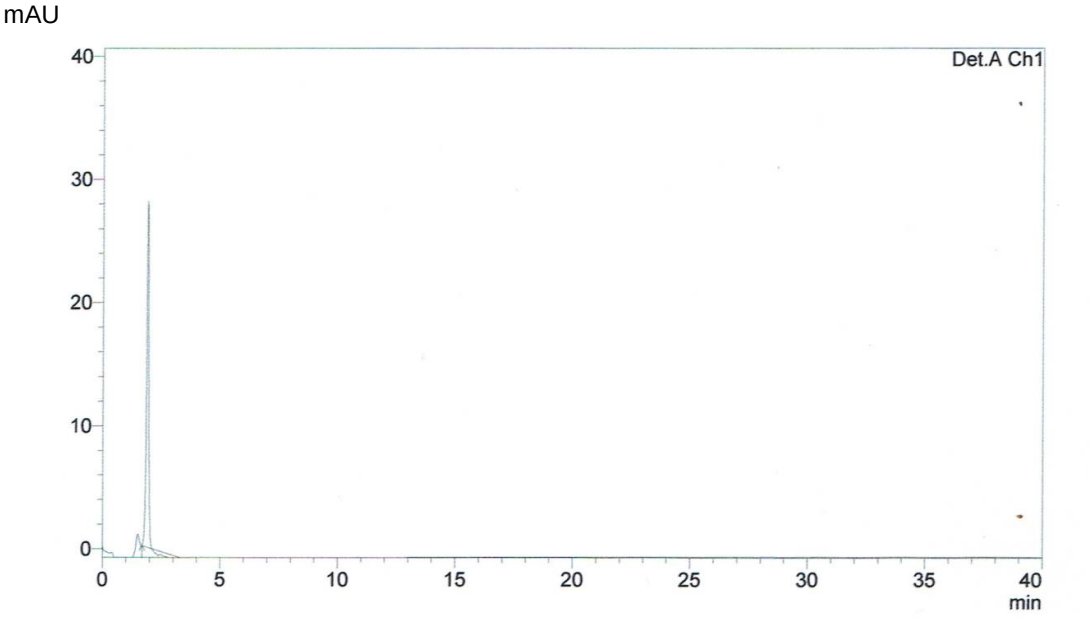


Fig. 1: Chromatogram of Ellagic acid mobile phase : water (0.1%TFA, v/v) (A) , and Acetonitrile (B), The gradient program was as follows:0–25 min, 32% B, 25–35 min,35% B. Sep-Pak ODS (5 µm , 250 mm × 4.6 mm) column, flow rate 1 ml/min; The wave length 375 nm

3. RESULTS AND DISCUSSION

The research presented here is based on the application ofultrasound to leach and hydrolyzeellagic acid compound. The composition and flow rate of the mobile phase were optimized using Different water(containing 0.1%THF)–Acetonitrile mixtures (90:10, 75:25,68:32,65:35,50:50) and different gradients were tested on the(ODS)column. The best separation was achieved using the gradientprogram given in Section 2.3in the experimental. The influence of the flow rate wasstudied in the range 0.3–1.2 ml/min. Flow rates given in Section2.3were selected as the values providing separation in a shortertime.Two injection volume were tested (10 and 20 ul). An injection loop of 10 ul was selected in order to minimise peak overlapping. Three extractants were studied in order to select the most suitable in this case: a 5% Sulphosalicylic acid, 10% Sulphosalicylic acid and 15% Sulphosalicylic acid were tested. Maximumefficiency was observed when the latter extractant was used.

A multivariate optimization approach was used for the extraction–hydrolysis stepdue to the interrelation between the variablesinfluencing them. The variables optimized in this stepwere the probe position (distance to the glass container andheight from the bottom of the water bath), radiation amplitude,percent of duty cycle of ultrasound exposure, sonication time, volume and concentration of sulphsalicylicacid inthe extractant.

The sonication time andthe acid concentration of the extractant were significant factorsin the ranges studied for some analytes. The upper value testedfor the sonication time (2 min) and the intermediate value testedfor the acid concentration of the extractant (15%sulphsalicylic acid) in the range studied were selected for subsequent experiments. Thus, the lower valuestested for the extractant volume, height of the probe and radiationamplitude were selected for subsequent experiments due totheir negative effects. Likewise, the upper values tested for thedistance between the probe and the glass container were selectedfor subsequent experiments due to their positive effect.

Lowervalues for the duty cycle, sonication time and acid concentration in the extractant were tested using a two-level full factor design involving eight randomised runs plus three center points. In thiscase, the duty cycle was not a significant factor in the rangestudied for all analytes. However, the upper value tested (0.2 s) was selected due to their positive effect.

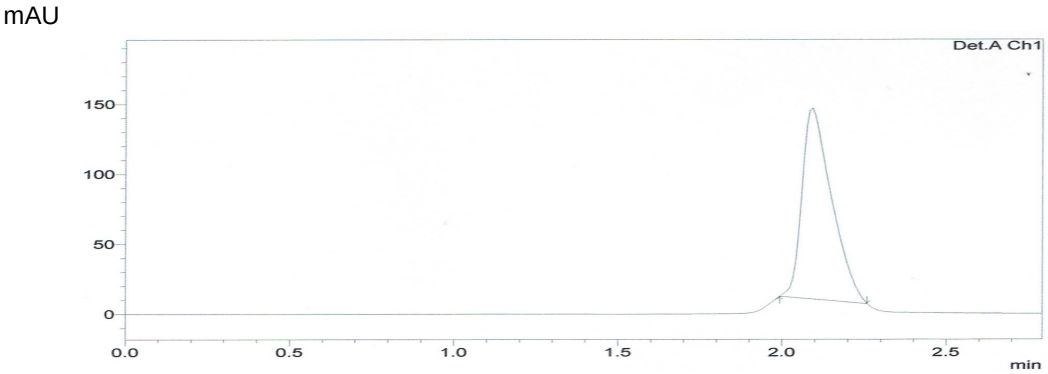


Fig. 2: chromatogram of Ellagic acid standard, mobile phase : water (0.1%TFA, v/v) (A) , and Acetonitrile (B),The gradient program was as follows: 0–25 min, 32% B, 25–35 min,35% B. Sep-Pak ODS (5 µm , 250 mm × 4.6 mm) column, flow rate 1 ml/min; The wave length 375 nm

The detector was operated at 357 nm wavelength whichcorresponded to the experimentally found maximum absorption of the ellagic acid standard. An aliquot of the extract were filtered through a 0.45 µm syringe filter prior to HPLC-UV analysis. Ellagic acid was separatedusing an ODS column (5 µm, 250 mm × 4.6 mm). The solvent flow rate was1 mL/min and the mobile phase was composed of solvent (A) water (0.1% TFA, v/v) and solvent (B)acetonitrile.Peak was identified by comparing itsretention time Fig(3) with those of standard Fig(2) and the concentration was calculated from the calibration curve Fig(4) where Y=40X + 267 was the equation and R^2 =0.996. The analysis was carried out in triplicate.

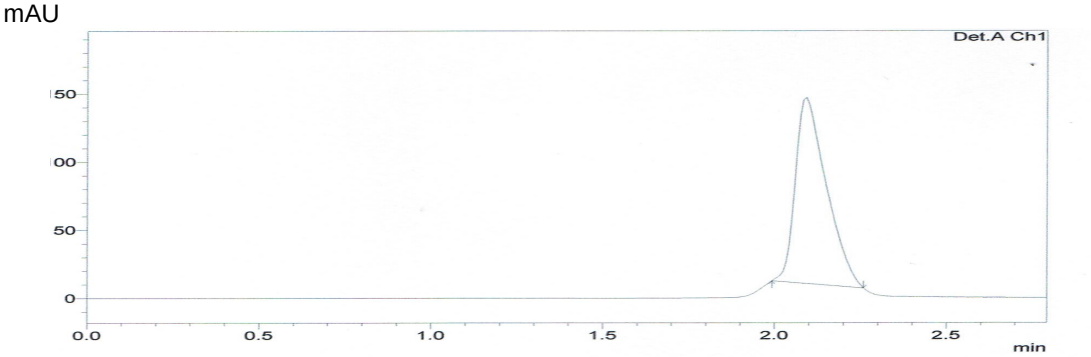


Fig . 3: chromatogram of Ellagic acid sample, mobile phase : water (0.1%TFA, v/v) (A) , and acetonitrile (B),The gradient program was as follows: 0–25 min, 32% B, 25–35 min,35% B. Sep-Pak ODS (5 µm , 250 mm × 4.6 mm) column, flow rate 1 ml/min; The wave length 375 nm

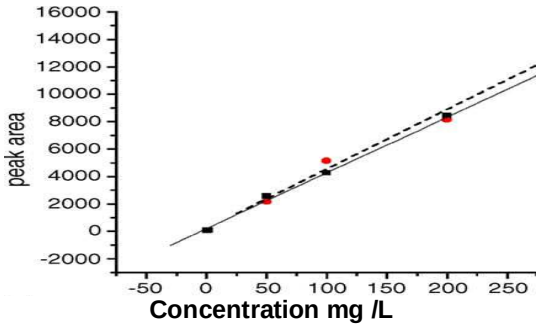


Fig. 4: Calibration curve of Ellagic Acid

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