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Short Communication

Determination of ferulic acid and related compounds by thin layer chromatography

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The analysis of certain phenolic compounds from plants, and their chemical transformation with microorganisms or isolated enzymes, has application in the food and pharmaceutical industry. The rapid quantitative estimation of ferulic acid by thin layer chromatography is described by measurement of the area of the obtained spots. Furthermore, the qualitative analysis of a number of related phenolics, some of which are transformation products of ferulic acid, is achieved on thin layer chromatographic plates by the use of different spray reagents.

Key words: Phenolic compounds, quantitative, qualitative, ferulic acid, thin layer chromatography.

INTRODUCTION

Various phenolic compounds have attracted the attention of food and medical scientists because of their fragrant aroma, antioxidant and anti-inflammatory properties. Vanillin made by enzymatic means from ferulic acid would qualify as natural and be in great demand in the food industry. Baker's yeast can convert ferulic acid to 4–vinyl guaiacol in high yield which cannot be done readily by chemical means (Huang et al., 1993). A white rot fungus isolated from decaying wood has the ability to metabolise ferulic acid, transforming it to 4-vinyl guaiacol, which was further metabolized to give acetovanillone (unpublished data), a product with potential applications in the pharmaceutical industry (Engels et al., 1992; Lafeber et al., 1999; van den Worm et al., 2001). These results illustrate the importance of certain transformation products of ferulic acid and their potential applications.

In most studies involving the analysis of plant phenolic acids, these acids are released by alkali treatment and the hydrolysates acidified and extracted with a suitable organic solvent at an appropriately low pH. Visualization of certain phenolic monomers can be achieved by a brief exposure to UV light after separation on thin layer chromatography (TLC) plates (absorbance at 254 nm and fluorescence at 366 nm) and by the colour developed after spraying with various spray reagents (Mabinya et al., 2002).

The rapid quantitative and qualitative analysis by TLC from crude extracts offers a practical and simple procedure for many applications. For example, ferulic acid is found in a bound form in maize hulls and its successful extraction and enzymatic modification has important commercial applications. Therefore, the ability to screen many microbial transformations simply and cheaply and obtain quantitative and qualitative results rapidly has a significant practical value.

MATERIALS AND METHODS

Quantitative analysis of ferulic acid by TLC

TLC was carried out on Merck silica gel 60 F_{254} plates (20 cm x 20 cm). Aliquots of a standard ferulic acid solution in methanol ranging from 5 – 25 µg were applied as spots at the origin on a plate and developed with chloroform : methanol : formic acid (85:15:1) in a pre-saturated chromatographic chamber. Developed plates were dried in a stream of hot air (hair dryer) and visualized at 254 nm and 366 nm UV light. Ferulic acid absorbs strongly at 254 nm on these plates and fluoresces blue at 366 nm. The spots were carefully circled with a pencil and the Scion image program (*www.scioncorp.com*) was used to determine the area of spots on a digital image of a developed chromatoplate. The average of three estimates of the area of each spot from 5 - 20 µg ferulic acid was then used to draw the standard curves in Figure 1.

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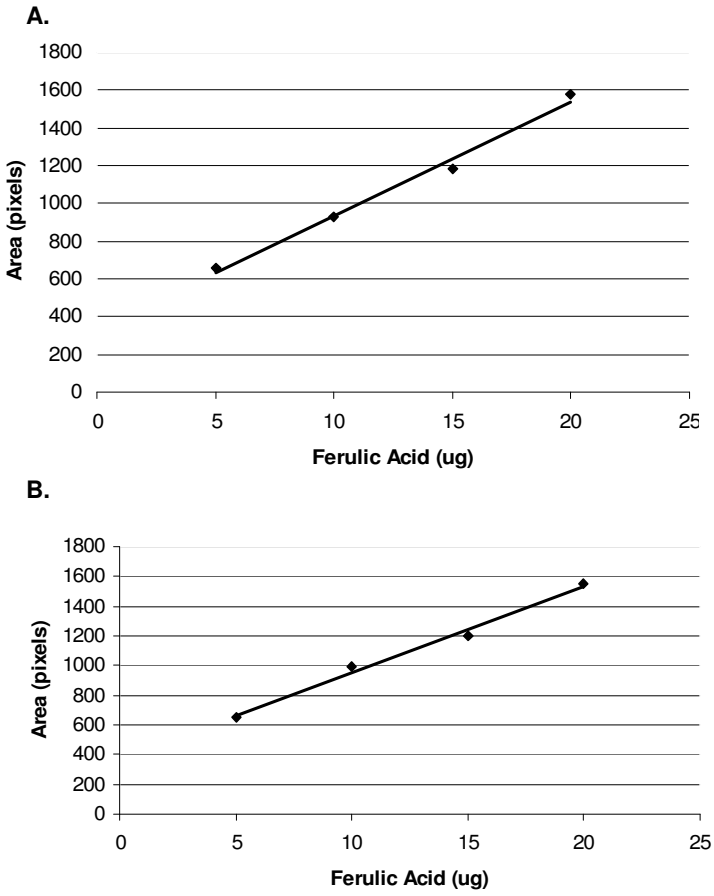

Figure 1. Two standard curves (A) for ferulic acid from two different chromatoplates using data from the Scion image program.

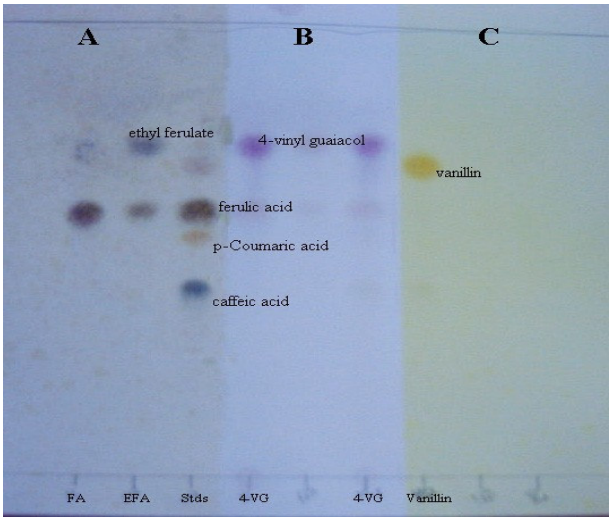

Figure 2. Thin layer chromatoplate of ferulic acid and metabolites after spraying different sections with three different spray reagents (Section A with 1% ferric chloride, Section B with 1% ethanolic vanillin followed immediately with 10% ethanolic H_2SO_4, and Section C with 2, 4 Dinitrophenylhydrazine).

Qualitative analysis of ferulic acid and related phenolics.

Standard solutions (1 µg/µL) of ferulic acid, *p*-coumaric acid, caffeic acid, ethyl ferulate and vanillin were prepared in methanol. 4-Vinyl guaiacol was isolated in the laboratory from the transformation products of ferulic acid by Bakers yeast cells (Huang et al., 1993). Each compound was applied as a spot to a silica gel 60 F_{254} plate, developed and dried as described above. For qualitative identification of the various compounds, different sections of the plate were sprayed with three different spray reagents and dried in a stream of hot air to give distinctive colours for each of the different compounds (Figure 2).

RESULTS AND DISCUSSION

The standard curves shown in Figure 1 are good enough to determine and estimate the production of ferulic acid from microbial or enzymatic treatment of plant material such as maize hulls. This procedure makes it possible to screen many attempted microbial transformations of ferulic acid for the liberation of ferulic acid by enzymatic means.

Figure 2 illustrates that all the applied compounds are resolved with the exception of ethyl ferulate and 4-vinyl guaiacol. However, these two compounds can be distinguished by spraying with 1% ferric chloride (Figure 2 section A) or 1% ethanolic vanillin followed immediately by 10% ethanolic H_2SO_4 (Figure 2 section B). Carbonyl containing compounds, such as vanillin, can be visualized by spraying with 2,4-dinitrophenylhydrazine (2,4-DNPH) (Figure 2 section C).

Many studies have attempted to produce vanillin from ferulic acid by enzymatic means. We have found that a number of yeasts produce 4-vinyl guaiacol from ferulic acid in good yield (Huang et al., 1993). In addition, a white rot fungus isolated from decaying wood produces4-vinyl guaiacol from ferulic acid and metabolises it further to acetovanillone, which can be visualized by 2,4- DNPH on TLC plates. Acetovanillone (apocynin) is a constituent of root extracts of the medicinal herb, *Picrorhiza kurroa*, and it possesses anti-inflammatory and cartilage protecting properties (Engels et al., 1992; Lafeber et al., 1999; van den Worm et al., 2001).

The results described illustrate that TLC can be used for the quantitative analysis of ferulic acid. The commercial production of ferulic acid in good yield from the bound form found in plant material, such as maize hulls, by enzymatic means has been investigated in many studies and still remains a difficult challenge. TLC separation of crude extracts and visualization by UV light or various spray reagents offers a rapid method for the

routine high-throughput detection and determination of any ferulic acid in such studies. In addition, the use of certain spray reagents would allow selective screening for the production of higher value compounds, such as vanillin or acetovanillone. The procedures described are ideal for low budget research projects and the results obtained can be complemented and confirmed by more complex techniques such as high performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS).

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