Extraction, Purification and Spectroscopic Characterization of Ferulic Acid by Alkaline Hydrolysis from Brans of Assam, India

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Abstract: Ferulic acid is a hydroxycinnamic acid and it is one of the most significant natural phenolic acids generally found in the seeds as well as leaves both in its free form and covalently conjugated with the plant cell wall materials. The extraction of Ferulic acid has been found much attention now a days due to the fact that it exhibits wide variety of biological activities including antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis, and anticancer activities. Plant products such as orange peels, rice bran, sugarcane bagasse which are easily available in North Eastern region, India contains measurable amount of phenolic acids. The extraction procedure of phenolic acids from biomass is very complicated and proper methodology is yet to be developed. We studied the extraction and purification of phenolic acid viz. ferulic acid from rice bran and orange peels by solvent extraction method. The simple and efficient purification procedure for ferulic acid from the alkaline extracts was based on the solubility of ferulic acid in ethanol. The extracted phenolic acid was characterized by IR spectroscopy and UV Visible spectroscopy. The presence of phenolic acids was confirmed by Folin Ciocalteu phenol reagent method. Extraction of major phenolic compounds such as ferulic acid from agriculture crop residues is important for the development of value added products from renewable by products.

Key words: Ferulic acid, solvent extraction, FolinCiocalteu phenol reagent

1. Introduction

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a bioactive phenolic acid. Phenolic compounds are secondary plant metabolites which help the plant to maintain an intricate balance with the environment and it is naturally present in a plant material, including food products of plant origin [1]. Ferulic acid along with dihydroferulic acid, is a component of lignocellulose, helps to maintain cell wall rigidity by cross linking lignin and polysaccharides. It is generally found in seeds of many plants such as rice, wheat and oats. Ferulic acid exhibits biochemical role in the inhibition of seed germination, inhibition of indole-acetic acid and enzyme, inhibition of decarboxylation activity and other protective effect on micro-organisms and pets. It can be absorbed and easily metabolized in the human body. Ferulic acid has been shown to protect against DNA damage and other human disorders. Besides acting as an antioxidant, it reduces inflammation, microbial activity, also prevents allergy and cancer. Liver protective and antithrombotic nature, increase of sperm viability, antiviral and vasodilatory action are also some of the important biological activities. Ferulic acid reduces blood glucose level and it has an enormous possibility to become a anti diabetic drug. It can fight coronary disease, lower cholesterol in serum and liver, and increase sperm viability [2-5]. Ferulic acid exists as a phenolic cell wall constituent in a variety of monocotyledon plants and some dicotyledons such as spinach, beet and glasswort and a Caryophyllales [6]. Ferulic acid is an antioxidant present in many staple foods, such as fruits, vegetables, cereals, coffee. In addition, ferulic acid is a component of curcumin, the major yellow pigment which is found in turmeric and mustard. This pigment is extensively used as a food preservative and yellow agent for foods [7-9]. Due to the presence of a phenolic nucleus and an extended side chain in the structure ferulic acid readily forms a resonance stabilized phenoxy radical justifying its free radical scavenging effect. This enables ferulic acid to protect DNA and lipids against oxidation through reactive oxygen species (ROS). In most vegetables and fruits, ferulic acid is found in a conjugated form of hydroxyl acids like quinic acid (in coffee, cabbage, celery and carrots), glucaric and galataric acids (in citrus), tartaric acid (in grape), and malic acid or with mono/disaccharides like glucose (in apple and cabbage), digalactose (in spinach) and gentiobiose (in broccoli) [10]. In root vegetables and grains, ferulic acid mainly occurs in pectic or...
hemicellulosic polysaccharides through its dimers (di-ferulic acid), and/or esterified with arabinose and galactose residues [11].

The chemically synthesized Ferulic acid is structurally confirmed by spectroscopic techniques, depicted the presence of an unsaturated side chain in ferulic acid, and also existence of both cis and trans isomeric forms. The distinct structural motifs are responsible for biological activity of Ferulic acid and its ester derivatives due to the presence of electron-donating substituent groups in the aromatic ring (hydroxyl and/or methoxyl). The acid or ester group linked to unsaturated C=C double bond contributes additional sites of attack for free radicals. The carboxylic group acts as an anchor to the lipid bilayer and the ester group, thus contributing to the significant lipophilicity of these molecules. The double bond present in the side chain is subjected to cis–trans isomerization and the resonance stabilized phenoxy radical accounts for its effective antioxidant activity. The chemical structure of ferulic acid and its cis-trans isomerism is shown Figure 1 and Figure 2.

Figure 1: Structure of ferulic acid

Figure 2: Schematic representation of two different isomeric forms of Ferulic acid

The pharmaceutical and food industries has been emphasizing on the need of developing a fast and efficient extraction method for the extraction and purification of ferulic acid from the biomass as it is very complex due the complex nature of the cell walls of biomass. Ferulic acid is insoluble in water at room temperature but it is soluble in hot water, ethyl acetate, ethanol and ethyl ether. It was observed that ethanol (60%) is suitable for the efficient extraction of ferulic acid [12]. The purification of ferulic acid from alkaline extracts was based on the solubility of ferulic acid in ethanol. The solubility of pure ferulic acid was determined using different ethanol concentrations. Attempts to precipitate ferulic acid from the concentrated extract after vacuum evaporation were unsuccessful due to the presence of a lipophilic brown substance. This is the main concern in alkaline extracts of crop residues, because the purification procedure of ferulic acid from this brown substance uses activated charcoal and resin exchange chromatography. The oily substance was precipitated from the extracts by adding anhydrous ethanol to an ethanol concentration of 30%. Since ferulic acid is soluble in 30% aqueous ethanol at room temperature and the lipophilic brown substance remained insoluble, separation was readily achieved by centrifugation [4, 6, 12]. The purpose of the present study was to examine the extraction process of ferulic acid from brans of North East India. In this study, we have reported the extraction and purification of ferulic acid from rice bran and orange peels of North East India and characterized the extracted ferulic acid with the help of IR spectroscopy and UV-Visible spectroscopy.

2. Materials and Methods

Orange peels are removed from fresh oranges which are procured from local market and the chemicals used for this study namely were petroleum ether, sodium hydroxide, ethanol, hydrochloric acid, methanol, chloroform procured from Qualigens, India (Mumbai) which were of analytical grade. Rice bran was procured from nearest rice mill and which was oven dried.

2.1 Extraction of Ferulic Acid

Finely grounded bran was refluxed with petroleum ether for 30 min. Filter the solution and the residue was treated with 1M NaOH with a solid to liquid ratio of 1:10 (g/ml) for 4h at 40°C. Filter the solution and the filtrate is neutralized with 6M HCl to pH 6.0. Concentrate the solution and add 3 volume of ethanol to precipitate hemicellulose. Filter the solution and evaporate ethanol and precipitate lignin at pH 1.5 by using 0.1M H₂SO₄. Extract the filtrate with chloroform (3 volume) and methanol. Dried the methanol layer under vacuum to get the ferulic acid.

2.2 Determination of total phenolic compounds in extracted sample

Folin-ciocalteu phenol reagent method is used for analysis of total polyphenols in extracted ferulic acid samples. To a 1 mL extract solution in water, 1 mL folin-ciocalteu reagent (FCPR) and 2 mL sodium carbonate (350 g sodium carbonate dissolved in 1000 mL water) reagent were added and made up the volume to 6 mL by adding 2 mL distilled water. Shaken well and stand for 30 min to develop colour [13]. The absorbance was measured at 725 nm. The formation of blue colour and UV spectra shows the presence of phenolic in the sample. The extracted samples were analyzed in IR spectrometer for the determination of functional groups of the ferulic acid. Small amount of the samples was withdrawn.
from the extracted mixture and analyzed in a IR spectrometer (Bruker, Germany)

3. Result and Discussion

The extracted solution of ferulic acid is shown in Fig 3. The yields of phenolic compounds from rice brans and orange peels by non-pressurised alkaline hydrolysis are shown in Table 1.

Non-pressurized alkaline hydrolysis has been used for total release of ferulic acid by breaking the ester bonds in the lignin/phenolics carbohydrate complexes in agricultural crop residues. The alkaline hydrolysis used in this study was for the quantification of ferulic acid in the biomass. The results indicated that the content of ferulic acid in orange peels was much lower than in rice bran. The influence of NaOH concentration, reaction temperature on ferulic acid extraction is shown in Table 2. The concentration of ferulic acid increase with increase in reaction temperature and reaction time. High concentration of ferulic acid was achieved at the center point level. This suggested that the range of the involved parameters for subsequent of optimization needs to be increased more than the initial selected parameters. The solutions after the purification for the analysis of ferulic acid is shown in Figure 3. Figure 4 shows the determination of ferulic acid by Folin Ciocalteu Phenol reagent method. The absorbance was measured at 725 nm. The formation of blue colour and UV spectra shows the presence of phenolic in the sample.

The purification of ferulic acid from alkaline extracts was based on the solubility of ferulic acid in ethanol. Ferulic acid is insoluble in water at room temperature but is soluble in hot water, ethanol, ethyl acetate and ethyl ether. Ethanol (60%) was reported to be suitable for the extraction of ferulic acid and our strategy was focused on its solubility in aqueous ethanol [2,14-15].

Table 2: Influence of NaOH concentration, reaction temperature on ferulic acid

<table>
<thead>
<tr>
<th>Concentration of NaOH (M)</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Concentration of ferulic acid (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>80</td>
<td>2</td>
<td>2.490</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>1</td>
<td>2.176</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>2</td>
<td>2.534</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>1</td>
<td>2.396</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>2</td>
<td>2.799</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
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</tr>
<tr>
<td>1</td>
<td>120</td>
<td>2</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>1.5</td>
<td>2.591</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>1.5</td>
<td>2.533</td>
</tr>
</tbody>
</table>

In purifying ferulic acid, hemicellulose was precipitated from the neutralized extracts by diluting it 3-fold with 95% ethanol. Attempts to precipitate ferulic acid from the concentrated extract after vacuum evaporation were unsuccessful due to the presence of a lipophilic brown substance.

Table 1: Yields of phenolic compounds extracted from Orange peels and rice bran by non-pressurised alkaline solution.

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Biomass</th>
<th>Mass (g)</th>
<th>Extraction Solvents, temperature</th>
<th>Yield Ferulic acid (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non pressurised</td>
<td>Orange</td>
<td>5.0</td>
<td>0.5M NaOH, 50° C</td>
<td>251± 10</td>
</tr>
<tr>
<td>Non pressurised</td>
<td>Rice</td>
<td>5.0</td>
<td>0.5M NaOH, 50° C</td>
<td>391± 50</td>
</tr>
</tbody>
</table>

Figure 5. Determination of ferulic acid by FCPR method

The FT-IR spectra of isolated ferulic acid from orange peels and rice bran are shown in Figure 5 & 6. Isolated ferulic acid from orange peels and rice bran produced a similar spectrum confirming its high purity. The ferulic acid gave signals at 3321 cm⁻¹ and 3326 cm⁻¹ for –OH group for rice bran and orange peels respectively. Similar to the carbonyl functional groups of ferulic acid clear signals at 1656 cm⁻¹ are shown for both the samples. The C–H bending is observed at about 1450 cm⁻¹.
4. Conclusion

The extraction of ferulic acid from rice bran and orange peels were done by using sodium hydroxide and hemicelluloses was removed by using ethanol as a solvent. The waxy materials from the biomass was removed by using petroleum ether prior to the NaOH extraction which enhances the quality of the extracted ferulic acid. Purification of ferulic acid from alkaline extracts is greatly simplified by exploiting its solubility. The total ferulic acid was determined by FCPR method and IR spectroscopy confirms the presence of functional groups in the extracted ferulic acid. This method can be extended for further study leading to process development for commercial exploration of the product.

5. References