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# Preparation of ferulic acid from corn bran: Its improved extraction and purification by membrane separation

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## A B S T R A C T

Corn bran contains a high amount of ferulic acid. However, the separation and purification of ferulic acid from corn bran still encounter technical problems. In this research, ferulic acid was obtained from corn bran via membrane separation from hydrolysate treated with an alkaline-ethanol aqueous solution. The technology was optimised as follows. One weight of corn bran was extracted using 0.25 mol/L NaOH in 50% ethanol aqueous solution at 75 °C for 2 h. Filtrates were ultrafiltrated at room temperature using a membrane with 5000 Da molecular cut-off. Permeates with 91.8% recovery of ferulic acid were concentrated by nanofiltration using a membrane with 150 Da molecular cut-off. Ferulic acid crystal (8.47 g/kg corn bran) with 84.45% purity was obtained after the pH of the concentrate was adjusted to 2.0. The reducing sugars released from the alkaline-hydrolysed residue increased by 54.5% compared with the untreated corn bran after xylanase hydrolysis for 8 h.

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**Keywords:** Corn bran; Ferulic acid; Alkaline-ethanol solution; Ultrafiltration; Nanofiltration; Reducing sugars

## 1. Introduction

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is widely used in the food, medical and cosmetic industries. This compound has several physiological functions, including antioxidant, antimicrobial, anti-inflammatory, anticancer and free radical scavenging activities. It also prevents coronary disease, lowers serum and liver cholesterol and increases sperm viability (Ou and Kwok, 2004; Ou et al., 2007; Belén et al., 2009; Eun et al., 2012; Jonas et al., 2013).

Ferulic acid in brans is usually linked to cell wall polymers by ester bond through their carboxylic acid group with the hydroxyl of the  $\alpha$ -L-arabinosyl side chains of xylans (Ishii, 1994; Buanaína, 2009). The highest content of ferulic acid can be found in corn bran (Zhao and Moghadasian, 2008). Corn (*Zea mays*) is a major crop worldwide. The annual production of corn in the USA is  $329 \times 10^6$  t, 11% of which is allocated for the manufacture of food products (Rose and Inglett, 2010a,b). The milling process during the processing of corn produces

60–70 g/kg of corn bran. However, the produced corn bran has low value and is often used for animal feed alone or in combination with corn germ cake or meal (Rose and Inglett, 2010a,b).

Ester-bound ferulic acid can be easily released by alkaline treatment (Mussatto et al., 2007; Torre et al., 2008), but the purification of ferulic acid from hydrolysate remains a problem because alkaline hydrolysate is brown and contains other components (Luo and Ou, 2007; Zhao et al., 2011). Our previous research (Ou et al., 2007) successfully purified ferulic acid in alkaline hydrolysate from sugarcane bagasse by activated charcoal adsorption/anion macroporous resin exchange chromatography. However, this method cannot be applied to aqueous alkaline hydrolysate from corn bran. The polysaccharides in hydrolysate must be removed for resin separation because viscous polysaccharides adhere to the resin and greatly influence separation. Given that approximately 70% of corn bran is composed of hemicelluloses (Rose and Inglett, 2010a,b), a very viscous solution is formed after

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alkaline treatment. Hence, the removal of polysaccharides by ultrafiltration is impossible because they block the membrane.

During alkaline hydrolysis of sugarcane bagasse, brown compounds, mainly lignin monomers, adsorb ferulic acid and coumaric acid, thus influencing the separation and purification of phenolic acids (Zhao et al., 2011). However, corn bran contains less lignin (~10 g/kg) than other brans and straws. The influence of lignin monomers on the purification of ferulic acid would be much alleviated. In this research, an alkaline-ethanol aqueous solution was used to release the bound ferulic acid, resulting in a less viscous hydrolysate with low hemicellulose content. Ferulic acid was separated sequentially by ultrafiltration, nanofiltration and crystallisation. Charcoal adsorption, resin adsorption and elution were omitted in this technology, which significantly simplified the separation process.

## 2. Materials and methods

### 2.1. Materials and chemical reagents

Corn bran was provided by Baolingbao Biology Company Ltd. (Yucheng, Shandong Province, China). Before extraction of ferulic acid, the bran was oven dried at 105 °C for 6 h and then ground to pass a 45-mesh sieve.

Ferulic acid was purchased from Sigma–Aldrich Company (St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade methanol and acetic acid were purchased from J.T. Baker (USA). All other reagents were of analytical grade.

### 2.2. Extraction of ferulic acid by alkaline hydrolysis

Different concentrations (on the basis of the total volume of water and ethanol) of sodium hydroxide were prepared in water and then mixed with different volumes of ethanol. Based on our previous research (unpublished), an orthogonal experiment design with different NaOH concentrations (0.2, 0.5, 0.75 and 1.0 mol/L), ethanol concentrations (30%, 50%, 70% and 90% v/v) solid-to-liquid ratios (1:5, 1:10, 1:15 and 1:20), extraction temperatures (65, 75, 85 and 95 °C) and extraction times (1.5, 2.0, 2.5 and 3.0 h) was used to obtain optimal extraction parameters. Triplicates of corn bran were suspended in 50 mL of extraction solution in a 250 mL Erlenmeyer flask and extracted using a water bath with magnetic stirring (at 160 rpm) under inverse flow. After extraction, the mixture was cooled in a water bath and then vacuum filtered. The viscosity of the filtrate was determined using an Ubbelohde viscometer (Sunlex, Shanghai, China). Ferulic acid content was determined using HPLC.

A magnified test was carried out to verify the optimal extraction technology obtained from the orthogonal experiment design and to prepare extracts for membrane separation. Triplicates of 1500 g corn bran were suspended in 15 L of solution containing 150 g NaOH, 7.5 L ethanol and 7.5 L distilled water. The mixtures were extracted in a 30 L multifunction reactor (SENCO Technology Co. Ltd., Shanghai, China) at 160 rpm for 2 h at 75 °C. After the extraction, the mixtures were cooled and centrifuged using a basket centrifuge (Tongyong, Centrifuge, Xiangtan, China). The residue was washed twice with 3 L of water. The filtrates were combined, and ethanol was vacuum removed at 45 °C. Ferulic acid in ethanol-removed extracts was determined by HPLC and then kept at 4 °C for membrane separation.

### 2.3. Membrane separation of ferulic acid

Ethanol-removed extracts were ultrafiltered using an NUF model hollow fiber ultrafiltrator (Wuxi Ultrafiltrating Equipment Company, Jiangsu Province, China) with a 5000 Da molecular cut-off membrane (UPVC De63 hollow fiber ultrafiltration membrane from Wuxi Ultrafiltrating Equipment Company, Jiangsu Province, China; 0.6 m<sup>2</sup>). The effect of different pressures (0.2–1.5 bar) on ultrafiltration was investigated. Retentate was diluted and re-ultrafiltered twice by adding 2.0 L water in each. The permeates were further nanofiltrated using a NF2295 model nanofiltrator (Wuxi Ultrafiltration Equipment Factory, China) with 150 Da molecular cut-off (NFT50 model from Alfa laval; 0.25 m<sup>2</sup>). The effects of different pressures (1.5–4.5 bar) and temperatures (15–45 °C) on the concentration of ferulic acid by nanofiltration were investigated.

Ultrafiltration and nanofiltration parameters were calculated as follows:

Concentration factor ( $F_c$ ):

$$F_c = \frac{V_o}{V_o - V_t} \quad (1)$$

where  $V_o$  is feed volume and  $V_t$  is permeate volume

Permeate flux ( $J$ ):

$$J = \frac{V_t}{t \cdot A} \quad (2)$$

where  $V_t$  is permeate volume and  $A$  is the membrane available area.

Transmissivity ( $Y$ ) and recovery rate ( $R$ ):

$$Y(\%) = \frac{C_p}{C_f} \times 100 \quad (3)$$

$$R(\%) = \frac{C_r}{C_f} \times 100 \quad (4)$$

where  $C_p$ ,  $C_r$  and  $C_f$  are the contents of ferulic acid in the permeate, retentate and feed, respectively.

### 2.4. Crystallisation

After nanofiltration, the retentate was adjusted to pH 2.0 with 6.0 mol/L HCl and then kept at room temperature for 24 h for crystallisation. The crystals formed were collected by filtration, carefully washed with 100 mL of 0.1% HCl solution and then lyophilised at –20 °C and 10 Pa to 100 Pa for 20 h (FD-1 model lyophiliser, Beijing Boyikang Instrument CO. Ltd., Beijing, China). The crystal was weighed, and ferulic acid content was determined by HPLC.

### 2.5. Digestibility of the residue by xylanase after extraction of ferulic acid

After hydrolysis, the residue was neutralised using 0.5 M HCl (the amount of HCL needed was recorded by titration), vacuum filtered, and oven dried. The dried corn bran and the residue were then ground to pass a 45-mesh sieve. The samples (1.0 g) were suspended in 10 mL of 1% xylanase (2500FXU-w/g, Novozymes) in 0.2 M phosphate buffer (pH 5.0). The mixture was reacted in a 100 mL Erlenmeyer flask at 55 °C for 1, 2, 4, 6 and 8 h at 120 rpm. After the reaction, the enzyme was deactivated by putting the flask in a boiling water bath for

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