JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Effect of Steam Explosion Treatment on Barley Bran Phenolic Compounds and Antioxidant Capacity

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ABSTRACT: A steam explosion pretreatment process followed by methanol extraction has been applied for releasing and extracting phenolic compounds, as well as other effective components, from barley bran. The steam explosion treatment was performed at different temperatures ranging from 210 to 250 °C, with a residence time of 30 s. The effect of residence time was also studied in the range 10 s to 120 s at 220 °C. The extracts were evaluated for their total soluble phenolic content (TSPC) including total free phenolic acids (TFPC) and total soluble conjugates (TSC), identified phenolic acids, total antioxidant capacity (TAC), water-soluble carbohydrates (WSC) and total methanol extracts (TME). High-performance liquid chromatography (HPLC) coupled with a photodiode array detector (PDA) was used in this study for the analysis of *p*-coumaric acid and ferulic acid in barley bran before and after steam explosion. Our results indicate that TSPC and TAC increased with residence time. They also increased dramatically with temperature up to 220 °C. After steam explosion at 220 °C for 120 s, the TSPC reached 1686.4 gallic acid equivalents mg/100 g dry weight, which was about 9-fold higher than that of the untreated sample. The TSPC and TAC obtained were highly positively correlated (r = 0.918−0.993), which meant that the increase of TAC for the steam explosion pretreated barley bran extracts was due, at least in part, to the increase of TSPC in the methanol soluble fraction. Also, under optimum conditions, the WSC in aqueous solution was 5 times as much as that of the untreated sample, which demonstrated that steam explosion also hydrolyzes carbohydrates into water-soluble sugars. It can be concluded that a proper and reasonable steam explosion pretreatment could be applied to release the bound phenolic compounds and enhance the antioxidant capacity of barley bran extracts.

KEYWORDS: *steam explosion, barley bran, phenolic compounds, total antioxidant capacity*

INTRODUCTION

In recent years, there has been growing interest in the phenolic compounds present in agricultural byproducts due to their positive effect against oxidative stress-induced diseases such as cancers, cardiovascular disease, diabetes, and Alzheimer's disease.[1,2] Cereal bran is produced worldwide in enormous quantities, as an important byproduct of the cereal industry. It is widely accepted that the bran constituent of cereal is rich in phenolic compounds, including *p*-coumaric acid and ferulic acid. Several investigations have been conducted to study profiles of the phenolic compounds present in cereals. These phenolic compounds can be divided into free, soluble esters or conjugates and insoluble bound forms. Insoluble bound phenolics are abundant in cell walls and are linked to celluloses and hemicelluloses. The soluble phenolics include free phenolics and esters or conjugates present within the plant cell vacuoles.[3−5] It has been reported that the proportion of bound phenolic acids was significantly higher (>93% of total) than that of free and soluble conjugated forms in cereals.[6]

The extraction of those important phenolic compounds with good antioxidant properties from agricultural byproducts is one of the key steps for developing value-added products from renewable byproducts. Due to the low solubility of bound phenolic compounds in organic solvents, conventional solvent-extraction processes give low extraction yields. Therefore, a number of special processing methods have been employed to release and extract phenolic compounds from cereal brans in order to obtain maximum yield of the phenolic compounds. These insoluble or bound phenolics are commonly released by alkaline, acidic, or enzymatic hydrolysis methods.[7−9] Alkaline hydrolysis is the method most often used for extracting esterified or bound phenolics at room temperature.[10,11] For example, the cell wall material is first isolated from the plant tissue and then sequentially extracted with 0.1 M NaOH (1 h, 25 °C), 0.1 M NaOH (24 h, 25 °C), 1 M NaOH (24 h, 25 °C), and 2 M NaOH (24 h, 25 °C). Each alkaline extract is acidified with HCl to pH < 2 and then extracted three times with ethyl acetate to recover the free phenolics.[12] These traditional hydrolysis methods are time-consuming and environmentally harmful. This has prompted increasing demand for an efficient alternative processing method.

Steam explosion is an economical and environmentally friendly processing method which has been extensively used for the pretreatment of structural components in plant biomass, e.g., cellulose, hemicelluloses, and lignin.[13,14] The principle of the steam explosion treatment is the use of steam hydrolysis at high temperature and pressure, followed by sudden reduction of the pressure for physical treatment of the product to produce low molecular weight substances.[15] Steam explosion is typically initiated at a temperature of 160−260 °C (with corresponding pressure 0.69−4.83 MPa) for a few seconds to a few minutes

Received: April 17, 2012
Revised: June 19, 2012
Accepted: June 19, 2012
Published: June 19, 2012

before the materials are exposed to atmospheric pressure. In the steam explosion process, the pressure is rapidly released to ambient, in 0.00875 s, simultaneously cooling the materials to 20 °C, a process which is different from normal thermal technology. The rapid pressure release leads to complex mechanical action. Meanwhile, the residence time of the materials with the high pressure steam causes various chemical reactions. Typical effects of this pretreatment are substantial breakdown of the lignocellulosic structure, hydrolysis of the hemicellulosic fraction, and depolymerization of the lignin components. Compared with other pretreatments, the advantages of steam explosion include a significantly lower environmental impact, lower capital investment, and the use of less hazardous process chemicals. Due to the disruption of the cell-wall matrix, the steam explosion treatment has been employed as an effective pretreatment process for extracting and separating bioactive phytochemicals from plant tissues. Kurosumi et al.[15] have utilized steam explosion before hot water and methanol extraction to separate antioxidant compounds from *Sasa palmata* tissues. Chen et al.[16] have reported that the yield of flavonoids from sumac fruits after steam explosion was about 8 times higher than that from raw samples. Steam explosion has also proved to be an effective method for releasing phenolic compounds bound to polysaccharides in the cell wall.

To the best of our knowledge, there is no comprehensive report on the extraction of phenolic compounds from cereal bran using steam explosion. Accordingly, the objective of this work was to investigate the possibility of extracting phenolic compounds with high antioxidant capacity using steam explosion. The steam explosion experiments were performed at different temperatures and with different residence times. The extracts were evaluated to determine the WSC yield, yield of phenolic compounds and TAC.

MATERIALS AND METHODS

Chemicals. The three phenolic acid standards, gallic acid, *p*-coumaric acid, and ferulic acid, together with glucose, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich. The Folin−Ciocalteu phenol reagent was purchased from Huadong Pharmaceutical Co., Ltd. All other chemicals and solvents were of analytical grade.

Materials. Samples of Tibetan hull-less barley bran (*Hordeum vulgare L.*) (THBB) for this study were kindly offered by Hongfa Enterprise Co., Ltd. (Tibet, China). The bran was sieved with a 32 mesh sieve before the steam explosion pretreatment.

Steam Explosion. Steam explosion was performed using a QBS-80 batch steam explosion apparatus (Hebi Gentle Bioenergy Ltd., China). The apparatus consists of a high pressure vessel, a steam generator, a material tank, a receiver, and a rapid-opening ball valve. The capacity of the vessel was 400 mL, and the highest pressure was 4.0 MPa (265 °C). The materials were steamed for different times and temperatures, prior to rapid decompression (explosion) brought about by opening the ball valve. The exploded materials, together with a little water, were recovered in the receiver. About 100 g of dry THBB was placed inside the vessel and exposed to the saturated steam in each batch. Steam explosion was performed at 210, 220, 230, and 250 °C for 30 s and at 220 °C for 10, 30, 60, and 120 s. After steam explosion, the THBB was allowed to cool to ambient temperature before extraction.

Yield of Water-Soluble Carbohydrates. The samples of steam exploded THBB were extracted with distilled water to fractionate the degraded hemicellulose and cellulose derivatives, e.g., monosaccharides, oligosaccharides, furfural, and 5-HMF. Extraction was carried out following Hodge et al.,[17] with some modifications. Briefly, treated or untreated THBB powder (2 g) was extracted three times with 20 mL of water at 60 °C for 1 h. After centrifugation at 5000 rpm for 10 min, the supernatants were combined, and water was added to make a final volume of 100 mL. This was then analyzed for water-soluble carbohydrates (WSC).

The WSC was further analyzed by a photometric method.[18] 2.0 mL of extraction or standard was mixed with 1.0 mL of aqueous phenol solution (5%, w/v), and the mixture was vortexed for 20 s. Then, 10.0 mL of sulfuric acid (98%) was added. After being vortexed for 20 s, the mixture was kept at 100 °C for 10 min to complete the reaction. Finally, this mixture was again vortexed, and the absorbance at 485 nm was recorded. Glucose was used as the standard, and the total soluble sugar concentration was expressed as glucose equivalents g/g DW.

Extraction of Phenolic Compounds from THBB. Methanol extraction was used to separate the phenolic compounds as well as their derivatives from lignin. Treated or untreated THBB powder (2 g) was extracted three times with 20 mL of 80% methanol. The samples were shaken at 300 rpm for 30 min at room temperature. After centrifugation at 5000 rpm for 10 min, the supernatants were combined, and 80% methanol was added to make a final volume of 250 mL. This was then used for analysis of the total soluble phenolic content (TSPC) and total antioxidant capacity (TAC). Another extraction was performed in the same way, but the supernatant was evaporated at 40 °C to dryness. This was then used to determine the yield of total methanol extracts (TME).

The phenolic compounds were isolated from the extracts following previously described methods, with certain modifications.[19−21]THBB powder (2 g) was extracted three times with 20 mL of 80% methanol and shaken at 300 rpm for 30 min at room temperature. After centrifugation at 5000 rpm for 10 min, the combined supernatant was evaporated under vacuum at 40 °C to about 20 mL. This was used for analysis of total free phenolic acids (TFPA) and identification of the phenolic acids. The aqueous suspension was adjusted to pH 2 (6 M HCl) and centrifuged. The supernatant was extracted five times with diethyl ether at 1:1 (v/v) solvent to supernatant ratio. The combined extract was evaporated to dryness in vacuum at 40 °C to obtain a free phenolic acid fraction, which was subsequently dissolved in methanol. The aqueous phase containing conjugates was subsequently treated by alkaline hydrolysis (4 M NaOH) under N_2 for 4 h at room temperature. The hydrolysate was acidified to pH 2 using 6 M HCl followed by extraction with diethyl ether. The phenolic acids released from the soluble conjugates were extracted as described above and used for analysis of total soluble conjugates (TSC).

HPLC Analysis. The analysis of phenolic compounds was conducted using the method of Kim et al.,[22] with certain modifications. A sample of 20 μL was analyzed using an Alliance 2695 separation module (Waters) equipped with a PDA 2996 on a Luna C18 ODS column (250 mm × 4.6 mm i.d., 5 μm). Empower software was used to control the HPLC system and data processing. Binary gradient mixtures, consisting of purified water with 1% acetic acid (solvent A) and acetonitrile (solvent B), were used as the mobile phase at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: 0−4 min, 21−23% solvent B; 4−12 min, 23−40% solvent B; 12−22 min 40−63% solvent B; 22−25 min, 63−100% solvent B; re-equilibrium for 5 min. The column temperature was set at 35 °C. The PDA detector was set to a scanning range from 210 to 400 nm with a resolution of 1.2 nm. The phenolic acids were identified by the retention time and by comparison with UV−vis spectra of standards. Quantification of the phenolic acids was carried out by an external standard method using calibration curves, and is expressed as micrograms per gram of dry weight (DW).

Total Soluble Phenolic Content (TSPC). The total soluble free phenolic acids and conjugated phenolics were determined according to the Folin−Ciocalteau colorimetric method,[23] with modifications. 0.1 mL of the methanol extract was mixed with 5.9 mL of distilled water and 0.5 mL of Folin−Ciocalteau reagent (Huadong Medicine Co., China). After 2 min, 1.5 mL of 20% sodium carbonate solution was added and mixed thoroughly. After 30 min reaction at 40 °C, the absorbance was read at 765 nm. The amount of total phenolics was expressed as milligrams of gallic acid equivalents (GAE) per gram of

the sample dry weight (DW). The total soluble phenolic compounds content (TSPC) is the sum of TFPA and TSC.

ABTS Free Radical Scavenging Assay. The ABTS assay was determined based on the procedure described by Re et al.[24] The ABTS solution was formed by reacting ABTS stock solution (7 mM) with 140 mM potassium persulfate at a ratio of 500:88 (v:v) in the dark at room temperature for 12−16 h before use. The ABTS solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. 0.1 mL of extract was mixed with 3.9 mL of diluted ABTS solution, the mixture was vortexed for 20 s, and the absorbance was read after 6 min at 734 nm. The blank was made from 0.1 mL of methanol and 3.9 mL of diluted ABTS solution. The ABTS radical scavenging activity of the extracts was expressed as the Trolox equivalent antioxidant capacity (TEAC) in milligrams per gram of DW. The radical stock solution was freshly prepared daily.

Ferric Reducing Antioxidant Power (FRAP) Assay. For the FRAP assay a modified method using a total volume of 4.3 mL was used.[25,26] The working solution included 10 volumes of 300 mM acetate buffer (pH = 3.6), one volume of 10 mM TPTZ in 40 mM hydrochloric acid, and one volume of 20 mM $FeCl_3$ solution. Extracts (0.1 mL) and deionized water (0.3 mL) were allowed to react with 3.9 mL of the FRAP working solution at 37 °C for 4 min. The absorbance at 593 nm of the mixture was recorded and used to calculate FRAP using standard curves prepared with known concentrations of $FeSO_4$. The results were expressed as mmol of $FeSO_4 \cdot L^{-1} \cdot g^{-1}$.

Statistical Analysis. All experiments were carried out in triplicate. The data were expressed as means ± standard deviations (SD). The differences between groups were tested by the ANOVA and Duncan's multiple range tests (SPSS version 16.0 for Windows, SPSS Inc., Chicago, IL). A probability value of $P < 0.05$ was adopted as the criterion for significant differences.

RESULTS AND DISCUSSION

Decomposition of Carbohydrate Part of the THBB. Figure 1 shows the influence of steam explosion on the yield of water-soluble carbohydrates in the aqueous solution and total soluble methanol extracts. It demonstrates that the WSC yield increased with increasing temperature to reach a peak at 220 °C, and then decreased sharply to the level of untreated THBB. The effect of treatment time on the yield of WSC at 220 °C is also shown in Figure 1. The yield profile shows a peak at 60 s, after which it decreased. It is widely accepted that the steam explosion treatment can hydrolyze hemicelluloses and celluloses to oligosaccharides, monosaccharides, and sugar degradation products such as furfural, hydroxymethylfurfural, furan, and levulinic acid.[27,28] The phenol sulfuric acid method relies on the degradation of sugars to furan derivatives for the color reaction. Thus, the absorbance in the steam exploded samples can give an estimate of the relative degree of hydrolysis of the lignocelluloses in THBB. The decreasing yield under the more drastic conditions of higher temperature or longer residence time may arise because the soluble carbohydrates undergo a series of secondary reactions to form carboxylic acids and soluble polymers.[29,30] On the other hand, the color of the aqueous solution after steam explosion became darker by increasing the temperature and residence time (not shown). This effect is also attributed to the formation of undesired materials formed via the Maillard browning reaction.

As is also shown in Figure 1, the TME yield gradually increased with temperature increasing and treatment time expending. This result may be explained by the production of low molecular weight hydrolysis products from lignins. During pretreatment, lignin is primarily degraded to produce a series of monomeric compounds and condensation byproducts, e.g., coumaryl alcohol, vinyl phenol, and di- and trihydroxybenzenes.[31]

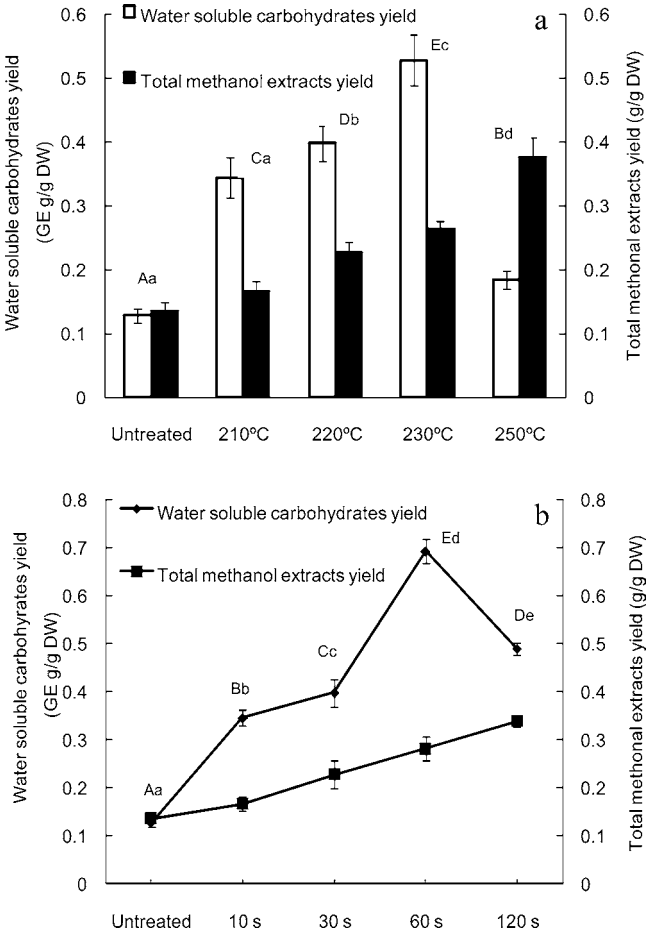

Figure 1. Effect of steam pretreatment on yield of total soluble sugars and total soluble methanol substance (a, steam pretreated at 210, 220, 230, and 250 °C for 30 s; b, steam pretreated at 220 °C for 10, 30, 60, and 120 s). The data are expressed as the mean ± SD ($n = 3$). The significance is expressed in uppercase letters (yield of water-soluble carbohydrates) and in lowercase letters (yield of total methanol extracts). The same letter is not significantly different ($p > 0.05$).

Clearly, steam explosion leads to the hydrolysis of glycosidic bonds in the hemicelluloses and celluloses. It also results in the depolymerization of lignin through the cleavage of β-*O*-4 ether and other acid-labile linkages. In general, the hydrolysis of lignocelluloses depends on the treatment conditions as well as on the type and physical accessibility of the raw material used. In the present work, the results proved that steam explosion allowed use of a lower temperature (220 °C) and shorter time (60 s) to effectively increase water solubilization of hemicelluloses and increase solubility of lignin in methanol. On the other hand, as demonstrated by Chen and co-workers,[16] steam explosion caused breakage and destruction of cell walls, leading to the formation of large cavities and intercellular spaces, which aided extraction of the soluble substances by increasing the specific surface area of the material.

Effect of Steam Explosion on the Phenolic Compounds of THBB. The phenolic compounds in THBB were divided into three fractions, the free phenolics, conjugates, and the bound form. In order to establish the applicability of steam explosion for the release of phenolic compounds from THBB, a series of experiments were performed at different temperatures and times. In this study, the total soluble phenolic compounds, including free and conjugates, was evaluated. Figure 3 shows

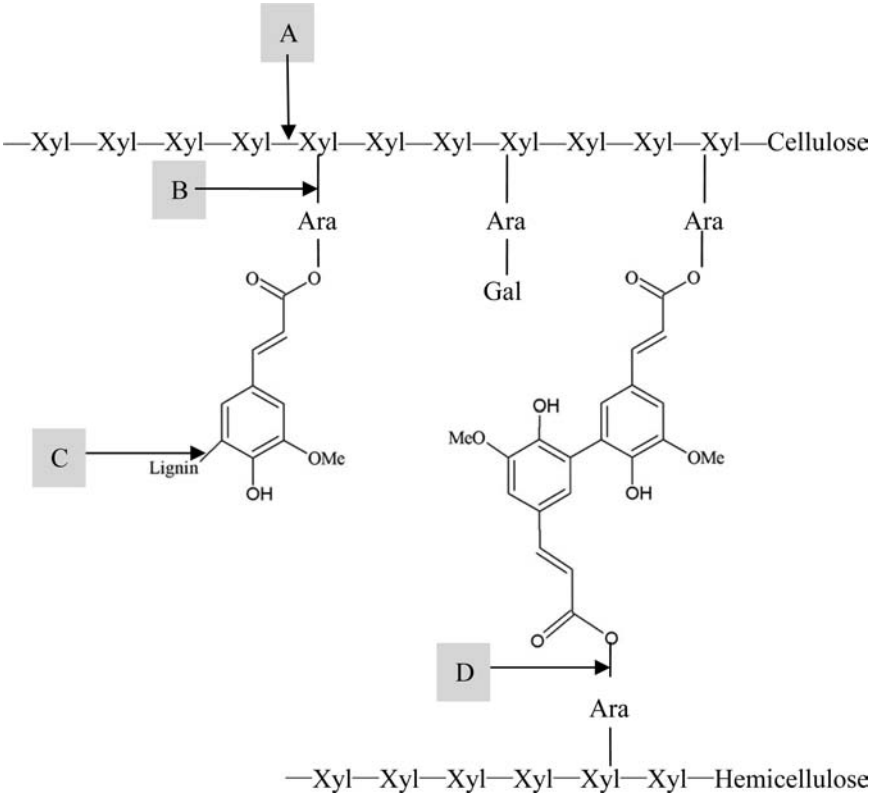

Figure 2. Proposed decomposition of lignin−phenolics−cellulose/hemicellulose complex under steam pretreatment conditions (A, B, glycosidic linkage; C, ether linkage; D, ester linkage).

the effect of temperature and residence time on the yield of TSPC, which is the sum of the quantities of free phenolic acid and soluble conjugates. Generally speaking, TSPC of THBB significantly increased after steam explosion. The TSPC of untreated hull-less barley is 172 GAE mg/100 g DW, which is in accordance with the results of Lee et al.[32]

Figure 3 shows the influence of explosion temperature in the range 210−250 °C on the yield of TSPC. The forms of the TFPA and TSC curves are similar. Both TFPA and TSC showed a maximum yield at around 220 °C, increasing from 98.9 to 664.1 and 73.2 to 208.2 GAE mg/g DW, respectively. Similar increased phenolic yields after steam explosion were reported by Conde[33] and Kurosumi.[15] The phenolic extraction yield achieved at 240 °C was about 2 times higher than the value obtained at 200 °C from olive tree prunings. Similarly, it has been reported that the amount of phenolic compounds in *Sasa palmata* leaf increased gradually with an increase of steaming temperature from 180 to 250 °C, but at a temperature of 260 °C the amount of phenolic compounds decreased.[15] The amount of phenolic compounds in methanol extracts of steam exploded *Sasa palmata* leaf increased by about 52 times compared with that of untreated leaf. Figure 3 also shows that the TFPA and TSC yield decreased sharply with increasing temperature.

On the other hand, TFPA and TSC sharply increased by increasing the treatment time from 10 to 120 s (Figure 3). The maximum yields of TFPA and TSC were 1022.0 and 663.9 GAE mg/g DW, respectively. The TSPC extracted from THBB after steam explosion was nearly 9 times higher than that of untreated THBB. Steam explosion has been carried out using a wide variety of conditions for diverse types of plant biomass. However, the optimal conditions will depend on the pretreatment strategy as well as on the type and physical accessibility of the raw material used. Kurosumi[15] found that the amount of phenolic compounds in *Sasa palmata* leaves decreased at a steam temperature of 250 °C for 5−20 min. These results showed that a steaming time of 1 min was optimal for hydrolysis of lignins or phenolic carbohydrates in *Sasa palmata* leaves.

Results have also indicated that the steam explosion technique can successfully release insoluble bound phenolic compounds from THBB. It has been reported that insoluble bound phenolics are abundant in cell walls and linked by hydrogen bonding (between the hydroxyl group of the phenolic compounds and oxygen atoms of the glycosidic linkages of polysaccharides), hydrophobic interactions, and covalent bonds such as ester bonds between phenolic acids and polysaccharides (Figure 2).[34]

For example, in the cell walls of cereal straws, ferulic acid is ester-linked to arabinoxylans, and ether links with lignin to allow its intricate incorporation with the polysaccharides that make up hemicelluloses and cellulose.[35,36] The steam explosion can hydrolyze the glycosidic linkages in hemicelluloses or celluloses and the β-*O*-4 ether bonds in lignin. Steam explosion can also effectively hydrolyze the ester and/or ether bonds between phenolic compounds, lignin, and carbohydrate.[15] Therefore, extraction yields of free phenolic acids and soluble conjugates increased sharply after steam explosion.

However, steam explosion caused degradation or polymerization of phenolic compounds using more severe pretreatment, such as high temperatures and long residence times. There is much evidence to indicate that heat treatment causes the phenolic compounds to undergo degradation to different degrees, depending on the type and orientation of aryl ring

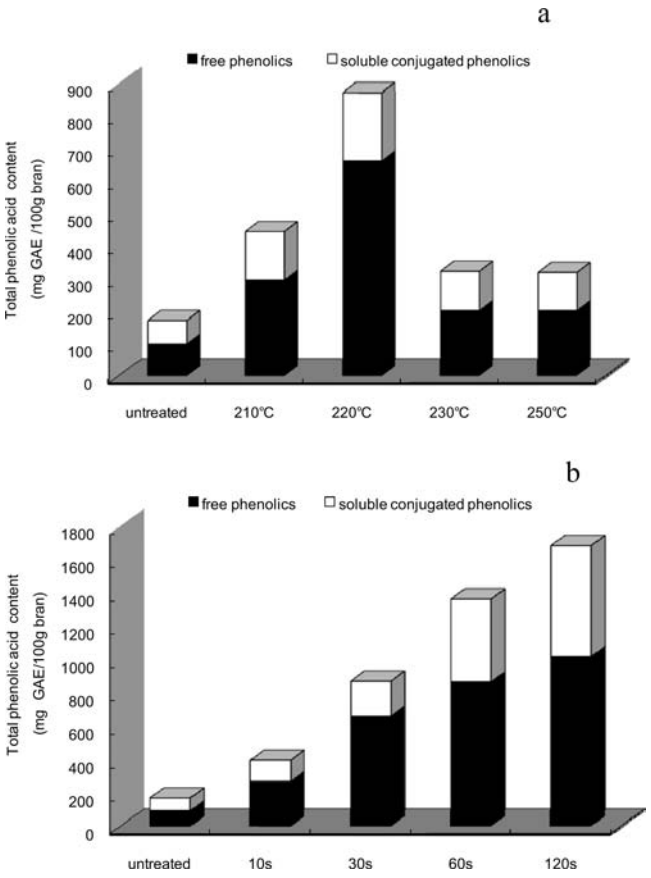

Figure 3. Effect of steam pretreatment on contents of total phenolic acids (a, steam pretreated at 210, 220, 230, and 250 °C for 30 s; b, steam pretreated at 220 °C for 10, 30, 60, and 120 s).

substituents.[37,38] On the other hand, polymerization reactions of phenolic compounds also cause discoloration of the product. It is well-known that organic acids (e.g., acetic acid, formic acid, and levulinic acid) are formed from the biomass itself during the steam explosion process.[39] Under acidic conditions, the polymerization of phenolic compounds is caused by the formation of carbonium ions.[40] This may be the cause of the discoloration of the samples at higher temperatures (>230 °C).

Identified Phenolic Compounds in THBB Extracts. Ferulic and *p*-coumaric acids are the major bound phenolic acids detected in barley.[41] Ferulic acid is the predominant free phenolic acid in barley seeds and barley brans.[42] Consequently, ferulic and *p*-coumaric acid were detected and quantified after steam explosion.

The effect of temperature on ferulic and *p*-coumaric acid with a residence time of 30 s is shown in Figure 4. As with TFPA and TSC, the maximum yields of ferulic and *p*-coumaric acid occurred at a temperature of 220 °C. More ferulic acid was formed, nearly 1.7 and 3.34 times the quantities of *p*-coumaric acid in its free and conjugated forms, respectively. By comparing the gradient of profiles, it is concluded that ferulic acid is more sensitive to steam explosion. Due to the decomposition reactions, its yield sharply decreased at high temperatures.

The treatment time dependence of the yield of ferulic and *p*-coumaric acid at 220 °C is shown in Figure 5. The yields of ferulic and *p*-coumaric sharply increased when the residence time was increased from 10 to 60 s. The yields of ferulic acid in its free and conjugate forms increase by about 59.0 and 8.45

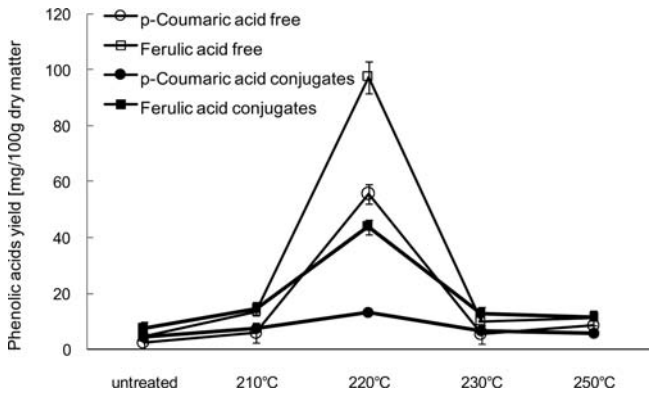

Figure 4. Effect of steam pretreatment temperature on the identified phenolic compounds at treatment time of 30 s.

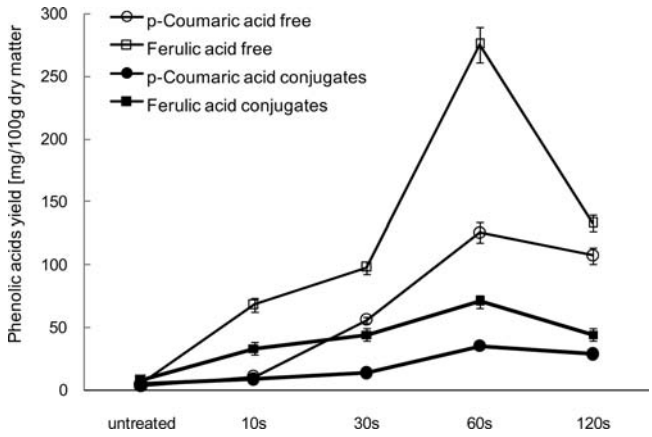

Figure 5. Effect of steam pretreatment time on the identified phenolic compounds at a temperature of 220 °C.

times, respectively. Meanwhile, the corresponding increases of *p*-coumaric acid were 47.6 and 7.25 times. These levels were similar to other data in the literature, which found that the quantities of phenolic compounds increased by about 52 and 27 times.[15] Meanwhile, our results showed that the maximum amount of ferulic acids released by steam explosion was nearly 340 mg/100 g DW, meaning that the steam explosion must release some ferulic acids bound in the cell walls. Renger and Steinhart[43] had isolated and identified ester- and ether-bound FA from barley bran by alkaline hydrolysis with 1 M NaOH under pressure for 24 h. The total amount of FA was about 600−700 mg/100 g DW. This result reflects the very high effectiveness of steam explosion for the release of bound phenolic acids in THBB. Saulnier et al.[44] had applied flash explosion and autoclaving to treat maize bran. In their studies, 70% of the ferulic acid was significantly solubilized at 210 °C maintained for 1 min, while ferulic acid remained esterified to neutral sugars. Unlike steam explosion, the maize brans were suspended in water during the flash explosion or autoclaving. The temperature was raised by heating the water for 15−25 min. At the point of pressure release, the intra- and extracellular water was in equilibrium, which will therefore not give rise to a loss of water from the cells. However, in the steam explosion, saturated steam at high pressure is used to rapidly heat the materials in a vessel. The material is maintained at the desired temperature for a short time, and during this period the material undergoes autohydrolysis and other chemical processes. At the end of this period, the pressure rapidly

decreases to atmosphere (in 0.00875 s) to stop the chemical reaction. The explosive decompression gives rise to a loss of water from the cells and the breakdown of the cellular and even molecular structures.

But when temperature rose higher than 220 °C or residence time increased to 120 s, the yield of the two phenolic acids decreased significantly. In cereals, phenolic acids mainly occur in pectic or hemicellulosic polysaccharides through its dimers, and/or esterified with arabinose and galactose residues. During steam explosion, an almost simultaneous formation and decomposition take place with phenolic acids simply due to the physical−chemical processes. Bound phenolic acids are released by mechanical and chemical action resulting from the high pressure and explosion. On the other hand, the yield of phenolic compounds may also be increased by depolymerization of lignins. However, in very severe conditions, ferulic acid may decompose to other chemicals, e.g., 4-methyl-, 4-ethyl-, and 4-vinylguaiacol and vanillin.[45] Moreover, ferulic and *p*-coumaric acids undergo decarboxylation, yielding dimeric products formed through their corresponding radical intermediates under thermal conditions.[46]

The mass balance difference between the TSPC yield and the sum of identified phenolic compounds in the treated and untreated THBB confirmed the presence of other unknown phenolic compounds. Our future work aims to identify these additional phenolic compounds and study their chemical changes under steam explosion.

Effect of Steam Explosion on the Antioxidant Capacity of THBB Extract. The antioxidant capacity of the 80% methanol extract of THBB was estimated by using the ABTS and FRAP assays. The results are shown in Figure 6. Clearly, the antioxidant capacity of the THBB extract increased after steam explosion. According to the results, the maximum TAC of the THBB extract was obtained after being treated at 220 °C for 120 s. The TAC increased from 326 to 2983 TEAC mg/100 g of DW by the ABTS assay, and from 4.78 to 13.45 mmol $FeSO_4 \cdot L^{-1} \cdot g^{-1}$ using the FRAP assay.

The effect of temperature on the TAC for 30 s residence time is shown in Figure 6a. The profile shows a peak at 220 °C, and then a sharp decrease at higher temperature; it remained constant at temperatures of 230 and 250 °C.

The effect of treatment time on the TAC at 220 °C is shown in Figure 6b. The TAC of THBB after steam explosion increased with treatment time in the range 10−120 s in this study.

The shape of the profile in Figure 6 is quite similar to the TSPC yield profile. Generally, besides phenolic compounds, other nonphenolic compounds with antioxidant activity were also produced and/or extracted from THBB after steam explosion. Therefore, the correlation coefficients for the TSPC, ABTS assay, and FRAP assay in the THBB extracts have been investigated, with the results shown in Figure 7. As can be seen, correlation coefficients in each case were high (r = 0.993 and 0.918 for ABTS and FRAP, respectively), which means that the increase of TAC of the treated THBB extract was due at least in part to the increase of TSPC.

In conclusion, decomposition and conversion of THBB into valuable chemical compounds has been successfully carried out using steam explosion followed by methanol extraction. The yield of total soluble phenolic compounds increased by 8.83 times after steam explosion treated for 120 s at 220 °C. During the steam explosion, the hydrolysis of esters or ethers of the phenolic carbohydrates takes place in the presence only of steam, and without additional chemicals. Moreover, at high temperature the hemiculluloses are partially hydrolyzed and lignin is depolymerized, giving rise to sugars and phenolic compounds that are soluble in water and organic solvents. Therefore, it is feasible to extract those nutritionally, functionally, and medically effective ingredients from THBB using steam pretreatment and apply them in the food and pharmaceutical industries.

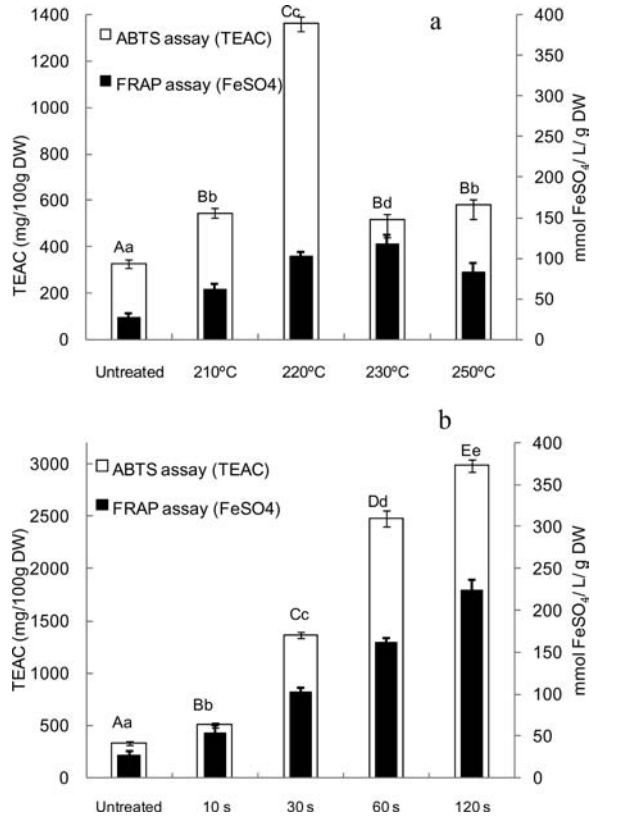

Figure 6. ABTS and FRAP assays of methanol extract of untreated and treated THBB (a, steam pretreated at 210, 220, 230, and 250 °C for 30 s; b, steam pretreated at 220 °C for 10, 30, 60, and 120 s). The significance is expressed in uppercase letters (ABTS assay) and in lowercase letters (FRAP assay). The same letter is not significantly different ($p > 0.05$).

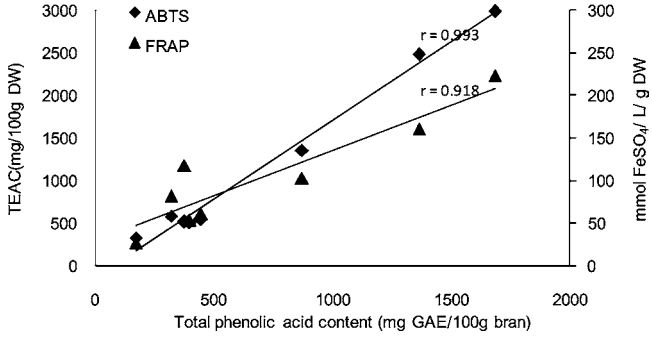

Figure 7. The correlation coefficients among TPC, ABTS assay, and FRAP assay.

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Funding

This work was supported by National Natural Science Foundation of China [Grant No. 31171746].

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Hongfa Enterprise Limited Company for providing Tibetan hull-less barley bran and to Hebi Gentle Bioenergy Limited Company for steam explosion technical support.

■ ABBREVIATIONS USED

TSPC, total soluble phenolic content; TFPC, total free phenolic acids; TSC, total soluble conjugates; TAC, total antioxidant capacity; WSC, water-soluble carbohydrates; TME, total methanol extracts; HPLC, high-performance liquid chromatography; PDA, photodiode array detector; THBB, Tibetan hull-less barley bran

■ REFERENCES

(1) Liu, R. H. Whole grain phytochemicals and health. *J. Cereal Sci.* **2007**, *46*, 207−219.

(2) Mateo, A. N.; van denBerg, R.; Havenaar, R.; Bast, A.; Haenen, G. R. M. M. Bioavailability of ferulic acid is determined by its bioaccessibility. *J. Cereal Sci.* **2009**, *494*, 296−300.

(3) Bonoli, M.; Verardo, V.; Marconi, E.; Caboni, M. F. Antioxidant phenols in barley (Hordeum vulgare L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. *J. Agric. Food Chem.* **2004**, *52*, 5195−5200.

(4) Robbins, R. J. Phenolic acids in foods: an overiew of analytical methodology. *J. Agric. Food Chem.* **2003**, *51*, 2866−2887.

(5) Naczk, M.; Shahidi, F. Extraction and analysis of phenolics in food: a review. *J. Chromatogr., A* **2004**, *1054*, 95−111.

(6) Adom, K. K.; Liu, R. H. Antioxidant activity of grains. *J. Agric. Food Chem.* **2002**, *50*, 6182−6187.

(7) Kim, K. H.; Tsao, R.; Yang, R.; Cui, S. W. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem.* **2006**, *95*, 466−473.

(8) Verma, B.; Hucl, P.; Chibbar, R. N. Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chem.* **2009**, *116*, 947−954.

(9) Sancho, A. I.; Bartolomé, B.; Gómez-Cordovés, C.; Williamson, G.; Faulds, C. B. Release of ferulic acid from cereal residues by barley enzymatic extracts. *J. Cereal Sci.* **2001**, *34*, 173−179.

(10) Buranov, A. U.; Mazza, G. Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurized solvents. *Food Chem.* **2009**, *115*, 1542−1548.

(11) Sancho, A. I.; Bartolomé, B.; Gómez-Cordovés, C.; Williamson, G.; Faulds, C. B. Release of ferulic acid from cereal residues by barley enzymatic extracts. *J. Cereal Sci.* **2001**, *34*, 173−179.

(12) Parker, M. L.; Waldron, K. Texture of Chinese water chestnut: involvement of cell wall phenolics. *J. Sci. Food Agric.* **1995**, *68*, 337−346.

(13) Li, J.; Gellerstedt, G.; Toven, K. Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals. *Bioresour. Technol.* **2009**, *100*, 2556−2561.

(14) Han, G.; Deng, J.; Zhang, S.; Bicho, P.; Wu, Q. Effect of steam explosion treatment on characteristics of wheat straw. *Ind. Crop Prod.* **2010**, *31*, 28−33.

(15) Kurosumi, A.; Sasaki, C.; Kumada, K.; Kobayashi, F.; Mtui, G.; Nakamura, Y. Novel extraction method of antioxidant compounds from Sasa palmata (Bean) Nakai using steam explosion. *Process Biochem.* **2007**, *42*, 1449−1453.

(16) Chen, G.; Chen, H. Extraction and deglycosylation of flavonoids from sumac fruits using steam explosion. *Food Chem.* **2011**, *126*, 1934−1938.

(17) Hodge, J. E.; Hofreiter, B. T. Determination of reducing sugars and carbohydrates. *Methods Carbohydr. Chem.* **1962**, *1*, 380−394.

(18) Dubios, M. G. Colorimetric method for determination of Sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−357.

(19) Verma, B.; Hucl, P.; Chibbar, R. N. Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chem.* **2009**, *116*, 947−954.

(20) Pham, V. H.; Tomoko, M.; Kazutaka, M.; Naofumi, M. Total phenolic compounds and antioxidant capacity of wheat graded flours by polishing method. *Food Res. Int.* **2009**, *42*, 185−190.

(21) Ann, K. H.; Claudia, K.; Svein, , H.K. Flavanol and Bound Phenolic Acid Contents in Different Barley Varieties. *J. Agric. Food Chem.* **2006**, *54*, 2253−2260.

(22) Kim, M.-J.; Hyun, J.-N.; Kim, J.-A.; Park, J.-C.; Kim, M.-Y.; Kim, J.-G.; Lee, S.-J.; Chun, S.-C.; Chung, I.-M. Relationship between phenolic compounds, anthocyanins conten and antioxidant activity in colored barley germplasm. *J. Agric. Food. Chem.* **2007**, *55*, 4802−4809.

(23) Singletion, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods Enzymol.* **1999**, *299*, 152−178.

(24) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying and improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.

(25) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(26) Politeo, O.; Jukic, M.; Milos, M. Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum L.*) compared with its essential oil. *Food Chem.* **2007**, *101*, 379−3857.

(27) Shevchenkl, S. M.; Chang, K.; Robinson, J.; Saddler, J. N. Optimization of monosaccharide recovery by post-hydrolysis of the water-soluble hemicelluloses component after steam explosion of softwood chips. *Bioresour. Technol.* **2000**, *72*, 207−211.

(28) Persson, T.; Ren, J. L.; Joelsson, E.; Jönsso, A.-S. Fractionation of wheat and barley straw to access high-molecular-mass hemicelluloses prior to ethanol production. *Bioresour. Technol.* **2009**, *100*, 3906−3913.

(29) Ramos, L. P. The chemistry involved in the steam treatment of lignocellulosic materials. *Quim. Nova* **2003**, *26*, 863−871.

(30) Sun, X. F.; Xu, F.; Sun, R. C.; Geng, Z. C.; Fowler, P.; Baird, M. S. Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. *Carbohydr. Polym.* **2005**, *60*, 15−26.

(31) Amen-Chen, C.; Pakdel, H.; Roy, C. Production of monomeric phenols by thermochemical conversion of biomass: a review. *Bioresour. Technol.* **2001**, *79*, 277−299.

(32) Lee, N. Y.; Kim, Y. K.; Choi, I.; Cho, S. K.; Hyun, J. N.; Choi, J. S.; Park, K. H.; Kim, K. J.; Lee, M. J. Biological activity of barley (Hordeum vulgare L.) and barley by-product extracts. *Food Sci. Biotechnol.* **2010**, *19*, 785−791.

(33) Conde, E.; Cara, C.; Moure, A.; Ruiz, E.; Castro, E.; Domínguez, H. Antioxidant activity of the phenolic compounds released by hydrothermal treatments of olive tree pruning. *Food Chem.* **2009**, *114*, 806−812.

(34) Saura-Calixto, F. Dietary fiber as a carrier of dietary antioxidants: an essential physiological function. *J. Agric. Food Chem.* **2011**, *59*, 43−49.

(35) Renger, A.; Steinhart, H. Ferulic acid dehydrodimers as structural elements in cereal dietary fibre. *Eur. Food Res. Technol.* **2000**, *211*, 422−428.

(36) Sun, R. C.; Sun, X. F.; Wang, S. Q.; Wang, S. Q.; Zhu, W.; Wang, X. Y. Ester and ether linkages between hydroxycinnamic acids and lignins from wheat, rice, rye, and barley straws, maize stems, and fast-growing poplar wood. *Ind. Crop Prod.* **2002**, *15*, 179−188.

(37) Rizzi, G. P.; Boekley, L. J. Observation of ether-linked phenolic products during thermal degradation of ferulic acid in the presence of alcohols. *J. Agric. Food Chem.* **1992**, *40*, 1666−1670.
(38) Stadler, R. H.; Welti, D. H.; Stämpfli, A. A.; Fay, L. B. Thermal decomposition of caffeic acid in model systems: indentification of novel tetraoxygenated phenylindan isomers and their stability in aqueous solution. *J. Agric. Food Chem.* **1996**, *44*, 898−905.
(39) Li, J.; Henriksson, G.; Gellerstedt, G. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresour. Technol.* **2009**, *98*, 3061−3068.
(40) Robert, D.; Bardet, M.; Lapierre, C.; Gellerstedt, G. Structural changes in aspen lignin during steam explosion treatment. *Cell. Chem. Technol.* **1988**, *22*, 221−230.
(41) Yu, J.; Vasanthan, T.; Temelli, F. Analysis of phenolic acids in barley by high-performance liquid chromatography. *J. Agric. Food Chem.* **2001**, *49*, 4352−4358.
(42) Madhujith, T.; Izydorczyk, M.; Shahidi, F. Antioxidant properties of pearled barley fractions. *J. Agric. Food Chem.* **2006**, *54*, 3283−3289.
(43) Renger, A.; Steinhart, H. Ferulic acid dehydrodimers as structural elements in cereal dietary fibre. *Eur. Food Res. Technol.* **2000**, *211*, 422−428.
(44) Saulnier, L.; Marot, C.; Elgorriaga, M.; Bonnin, E.; Thibault, J. F. Thermal and enzymatic treatments for the release of free ferulic acid form maize bran. *Carbohydr. Polym.* **2001**, *45*, 269−275.
(45) Fiddler, W.; Parker, W. E.; Wasserman, A. E.; Doerr, R. C. Thermal decomposition of ferulic acid. *J. Agric. Food Chem.* **1967**, *15*, 757−761.
(46) Arrieta-Baez, D.; Dorantes-Álvarez, D.; Martinez-Torres, R.; Zepeda-Vallejo, G.; Jaramillo-Flores, M. E.; Ortiz-Moreno, A.; Aparicio-Ozores, G. Effect of thermal sterilization on ferulic, coumaric and cinnamic acids: dimerization and antioxidant activity. *J. Sci. Food Agric.* **2012**, DOI: 10.1002/jsfa.5695.