Effect of Hydrothermal Pre-Treatment on Ferulic Acid Content and Antioxidant Activities of Corn Hydrolysate

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This study investigated the effect of hydrothermal pre-treatment on ferulic acid content and antioxidant activities of corn hydrolysate. The low-grade corn was treated by hot water and autoclaving process at different temperatures and times. The highest phenolic content (471.7 ± 7.4 μg FA mL⁻¹, p < 0.05) was obtained by autoclaving at 121°C for 90 min. High performance liquid chromatography (HPLC) revealed 277.3 ± 3.2 μg mL⁻¹ of FA, which was 2.05 times higher than that of the control (unheated sample). The high yield of FA obtained here may be due to high-temperature autoclaving eliminating the FA-lignin and FA-polysaccharide bonds. Furthermore, the optimal hydrothermal pre-treatment at 121°C for 90 min improved the DPPH and ABTS radical scavenging activities by 1.3 and 1.5 fold, respectively, compared to the control. On the other hand, the dextrose equivalent (DE) values obtained from corn hydrolysate at the different heat treatments in this study were in the range of 23.2 ± 0.7 to 28.7 ± 0.9. These results illustrated that autoclaving method showed a promising pre-treatment process to add value of corn hydrolysate product.

Keywords: hydrothermal pre-treatment, ferulic acid, low-grade corn, corn hydrolysate, antioxidant activity

1. Introduction

Corn or maize (Zea mays L.) is one of the most important crops in the world because it is extensively used in several industries. As well it is the raw material in starch hydrolysate production, e.g. dextrin, maltodextrin, glucose and fructose syrup, which are often used in food and beverages [1, 2]. Many researchers have reported that corn contains high amounts of phenolic compounds, especially ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA) and p-coumaric acid (4-hydroxycinnamic acid, p-CA). For example, Boz [3] reported that refined corn bran contained approximately 26.1–33 g kg⁻¹ FA in dry matter; Mattila et al. [4] reported that corn flour contained 0.380 and 0.031 g kg⁻¹ of FA and p-CA, respectively. Also, Torre et al. [5] found that the values of FA and p-CA of corn hydrolysate solution were 1.171 g L⁻¹ and 2.156 g L⁻¹, respectively.

FA is usually found as an important structural component of plant cell walls [3, 6], located between the lignin and polysaccharide of the cell wall. It covalently attaches to lignin with ether bonds and to polysaccharides with ester bonds [7, 8]. This compound is used in a wide range of pharmaceutical, food, and cosmetic applications, because of its several physiological properties, including anti-microbial, anti-cancer, anti-inflammatory and free radical scavenging activities [9]. It has been reported that FA alleviated oxidative stress in diabetic rats and reduced glucose levels in their blood [10]. Kim et al. [11] found that FA increased HDL levels and decreased the total triglyceride and cholesterol levels in rats fed high-cholesterol diets.

Generally, acid-base hydrolysis [4, 12] and enzymatic hydrolysis [13, 14] are the treatment used to increase FA in corn. These treatment require many steps, hazardous chemicals and take a long time. Therefore, this study aimed to increase FA content using an efficient, simple, quick, cost-effective and green treatment. This was achieved by a simple hydrothermal pre-treatment of the corn sample during corn hydrolysate production. Corn hydrolysate are conventionally prepared from corn starch mixed with water and submitted to gelatinization by thermal heating. Then, the gelatinized starch is taken to the liquefaction by enzymatic hydrolysis [15, 16]. There are several products derived from corn hydrolysate, for example, maltodextrin, corn syrup and some alcoholic beverages [15].
Low-grade corn (corn that contains 90% broken or damaged kernels) is often used as a raw material in animal feed because it is high in protein and cheaper than other crops, such as soybeans or rice bran [17, 18]. Low-grade corn is also high in starch—up to 70% of dry matter. So, the low-grade corn was used as raw material for corn hydrolysate production in this work. This study is the first report to use heat to produce FA from low-grade corn during corn hydrolysate production. Moreover, this work investigated the antioxidant properties of the corn hydrolysates, including their DPPH and ABTS radical-scavenging activities, along with their dextrose equivalents.

2. Materials and Methods

2.1 Sample preparation

The low-grade corn (corn that contains 90% broken or damaged kernels) was kindly provided from Pattananikom Kaset Co., Ltd (Phrae, Thailand). The corn was ground by using the hammer mill machine to create a particle size less than 2 mm in diameter and dried at 80°C for 12 h, then stored in a desiccator until use.

2.2 Analysis of the components of the corn

The moisture and ash contents were determined according to the methods 930.15 and 942.05 of the Association of Official Analytical Chemists (AOAC) [19], respectively. Protein content was determined by the Kjeldhal method, as described by the method 991.20 of AOAC [19]. Fat content of the sample was determined according to AOAC, using the soxhlet extraction method (method 954.02) [19]. The starch content was determined according to the method 920.44 of AOAC [20].

2.3 Hydrothermal pre-treatment of corn sample

Corn kernel powder (25 g) was mixed with 100 mL distilled water in a close 250 mL laboratory bottle (Duran) and the mixture were hydrothermally pre-treated using 2 methods, hot water and autoclaving. In hot water pre-treatment, the samples were put into the water bath (GFL1083, Germany) and heated at 80 and 100°C, with each held for 15, 30, 60 and 90 min. In autoclaving pre-treatment, the samples were put into an autoclave (Purister60, Korea) and heated at 115 and 121°C with each held for 15, 30, 60 and 90 min. Unheated sample served as a control.

2.4 Corn hydrolysate production

For corn hydrolysate production, α-amylase from Bacillus licheniformis with activity of 150,000 U.mL⁻¹ (purchased from Union Science Co. Ltd, Thailand) was used. After gelatinization at 80–121°C, the samples were cooled down to the temperature in range of 70–75°C. Then the heated sample and control were mixed with 1 mL of 10 mM CaCl₂ and α-amylase enzyme, 72 U.g⁻¹ of corn. The mixtures were incubated with the shaker water bath at 75°C, 150 rpm for 5 h. Then, each sample was centrifuged at 3500 rpm for 10 min and the supernatant was collected as a sample solution to determine the dextrose equivalent (DE), total phenolic content (TPC), antioxidant activity and ferulic acid content.

2.5 Determination of TPC

TPC was determined by the Folin–Ciocalteu method according to a procedure adapted from Jadhav et al. [21]. Two mL of corn hydrolysate samples were mixed with 0.5 mL of methanol. Aliquots of 1 mL of the mixed solution were transferred to 20–mL test tubes. Then, 0.5 mL of 50% Folin–Ciocalteu’s phenol reagent and 2 mL of 15% Na₂CO₃ were added to the test tubes. The absorbance was measured at 718 nm against a blank solution. A standard curve of 0–80 μg.mL⁻¹ ferulic acid was used to quantify the TPC. The total phenolic content was expressed as μg FA.mL⁻¹ (microgram ferulic acid equivalents per milliliter of corn hydrolysate).

2.6 HPLC analysis of ferulic acid

The concentration of ferulic acid in the corn–hydrolyzed solutions was measured using HPLC according to the method of Fusawat [22] with slight modification. The HPLC conditions consisted of Ultra C18, 4.6×150 mm, 5 μm, column (RESTEK, USA) and Eluent: 0.5% acetic acid in water (solvent A) and acetonitrile (solvent B) with a constant flow rate of 0.6 mL.min⁻¹. The gradient started at 25°C over 15 min from 5–20% and over 15–40 min from 20–40% of solvent B. The injection volume of all samples was 20 μL. The ferulic concentrations of the corn hydrolysate samples were calculated by interpolation on the calibration curve.

2.7 DPPH radical scavenging activity assay

The DPPH radical scavenging activity of the corn–hydrolyzed solutions was measured using the modified method of Duan et al. [23]. Each sample solution (100 μL) was mixed with 100 μL of 250 μM DPPH in 100% methanol. The mixed solution was placed in the dark at room
temperature for 20 min. The absorbance was read at 517 nm. The blank was measured without a sample solution and 100% methanol was used as a control. The ferulic acid standard was used for calibration and the percent of inhibition was calculated from the following equation:

% Inhibition = \((A_{517\text{ control}} - A_{517\text{ test sample}})/A_{517\text{ control}}\) \times 100

2.8 ABTS radical scavenging activity assay

The ABTS radical scavenging activity of corn hydrolysate was measured using the modified method of Re et al. [24]. The sample solution (10 μL) was mixed with 1000 μL of ABTS solution. The absorbance at 734 nm was recorded at 6 min after mixing. The blank was measured without a sample solution and 100% methanol was used as a control. The ferulic acid standard was used for calibration and the percent of inhibition was calculated from the following equation:

% Inhibition = \((A_{734\text{ control}} - A_{734\text{ test sample}})/A_{734\text{ control}}\) \times 100

2.9 Determination of DE

The reducing sugar content in the corn-hydrolyzed solutions was measured using the dinitrosalysilic acid (DNS) method [25]. The sample solution (1 mL) was mixed with 1 mL of DNS reagent. The mixtures were heated in a boiling water bath for 15 min and then placed on an ice bath until cool. Distilled water (10 mL) was added to the mixtures. The absorbance was recorded at 540 nm. The glucose standard was used for calibration and DE value was estimated following the formula [26]:

DE = reducing sugar content (glucose)/total starch content \times 100%

2.10 Statistical Analysis

Three replications were performed for each experiment. All results were analyzed by one-way ANOVA using SPSS version 17.0 for Windows. A value of \(p<0.05\) was considered statistically significant.

3. Results and Discussion

3.1 Corn components

Figure 1 shows the low-grade corn before (a) and after (b) grinding. The components of corn sample are shown in Table 1. The moisture content of the corn sample in the current study was 8.43%. The low moisture content is important, as it enables long-term storage by minimizing fungal contamination and spoilage [27,28]. The 1.83% of ash content found in the low-grade corn in this study. This agreement with Enyisi et al. [28] who reported ash content of corn in the range of 1.1–2.95%. The fat content obtained from the corn sample in this study was similar to Ijabadeniyi and Adebolu [29] and differed slightly from Richardson et al. [18], Böhme et al. [30] and Bourg [31]. The starch and protein contents of the low-grade corn sample (>90% broken kernels) in this study were 68.96% and 8.68%, respectively. These results were similar to Richardson et al. [18], who found 70.27% starch and 8.54% protein in low-grade corn (55% broken kernels). The starch content of the low-grade corn sample in this study was similar to normal-grade corn kernels that have been reported to contain starch in the range of 70.06–73.04% [18,29–31]. This indicated that the low-grade corn sample in this study was suitable to use as raw material for corn hydrolysate production. Corn hydrolysate can be extensively used in several industries, for instance, pharmaceutical, food, beverage and cosmetic. Furthermore, using low-grade corn can be significantly reduced the cost of corn hydrolysate production which is highly beneficial to the industrial sector.

3.2 Effect of hydrothermal pre-treatment on TPC and FA contents of corn hydrolysate

Phenols or phenolic compounds are found widely in plants and produced from their secondary metabolism. Phenolic acids are the main phenolic compounds in corn grain, the most abundant of which are FA and p-CA [32]. In this study, the total phenolic content was determined using the Folin–Ciocalteu method, expressed as μg feru-
lished content per mL of corn hydrolysate. Table 2 shows the TPC of corn hydrolysate under hot water and autoclaving treatments at different temperatures and times. Heat treatment significantly increased the TPC in the corn hydrolysate compared with the control. The TPC of the control (unheated sample) was 324.2 ± 14.6 μg FA mL⁻¹. The TPC in the corn hydrolysate increased from 324.2 ± 14.6 μg FA mL⁻¹ in the control to 404.7 ± 19.6 μg FA mL⁻¹ in the sample at 115°C for 90 min (p < 0.05). The TPC in the sample heated at 121°C or 90 min was even higher 471.7 ± 7.4 μg FA mL⁻¹ (p < 0.05), while the TPC in the sample heated at 80°C did not change significantly compared to the control (p > 0.05). These results indicated that heating released the TPC in corn hydrolysate. In addition, autoclaving yielded more TPC than hot water treatment. These results are in agreement with Dewanto et al. [33], who found that the total free phenolic content of yellow sweet corn increased after heating at 121°C for 25 min. Our results also agree with Bruijin et al. [34], who reported that polyphenols in Grifola gargar increased after autoclaving at 121°C for 45 min. Choi et al. [35] found that the free phenolic compounds in Shiitake mushrooms (Lentinus edodes) significantly increased after autoclaving. These researchers suggested that the liberation of phenolic contents was enhanced by autoclaving.

Next, the FA content of corn hydrolysate was analyzed using HPLC. The results are shown in Fig. 2. The FA content tended to increase with increasing heating temperature and time. Moreover, the ratio of FA content to TPC in all samples was in the range of 37.9–58.79%. The contents of TPC and FA were not consistent may be due to the different determinations. FA was specifically analyzed by HPLC while TPC was measured by spectrophotometry which is a rough analysis method. The amount of FA in the control was 135.5 ± 0.1 μg mL⁻¹. Autoclaving at 115°C for 60 and 90 min increased the FA content in the corn hydrolysate by 30.4% and 43.3%, respectively (p < 0.05). Autoclaving at 121°C for 90 min yielded the biggest increase (277.3 ± 3.2 μg mL⁻¹, or 51.1%). The obtained high FA yield may be due to the sufficient elimination of FA-lignin and FA-polysaccharide bonds at high temperature. Santos et al. [26] has shown that FA is thermally stable below 147°C. In this study, the result agreed with Xu et al. [36], who reported that heat treatment at 121°C for 90 min enhanced the extractability of FA in Huyou (Citrus paradisi Changshanhuyou) peel about 13 fold. Furthermore, Juániz et al. [37] noted that heat treatments tended to increase the concentration of phenolic compounds in vegetables, due to thermal destruction of cell walls and sub-cellular compartments during cooking that favors the release of these compounds.

The maximum FA found in this study was 69.9 mg/100g of corn from the sample heated by autoclaving at 121°C for 90 min. This was similar to Topakas et al. [13], who used enzymatic hydrolysis to release FA from corn cobs, but lower than that of the alkaline hydrolysis method [12]. However, heat treatment by autoclaving is a non-vigorous pretreatment method that does not require any catalysts (e.g., enzyme, acid, or base) and does not lead to corrosion problems. Autoclaving is also quick and inexpensive compared with enzymatic hydrolysis.

Table 2  Total phenolic content (TPC) of corn hydrolysate under different hydrothermal pre-treatment process.

| Pre-treatment process | Temperature (°C) | 15 min | 30 min | 60 min | 90 min |
|-----------------------|-----------------|--------|--------|--------|--------|
| Hot water             | 80              | 339.1 ± 19.5 ay | 338.3 ± 5.8 az | 357.6 ± 6.3 az | 333.3 ± 14.6 az |
|                       | 100             | 336.6 ± 18.8 aby | 338.5 ± 6.0 abz | 360.7 ± 8.1 az | 347.8 ± 3.9 az |
| Autoclaving           | 115             | 366.7 ± 2.9 cy | 376.0 ± 2.6 bcz | 410.4 ± 3.6 ay | 404.7 ± 19.6 aby |
|                       | 121             | 406.6 ± 4.4 bx | 414.8 ± 0.6 bx | 450.0 ± 8.9 ax | 471.7 ± 7.4 ax |

Different letters (a–c) within a row are significantly different (p<0.05)
Different letters (x–z) within a column are significantly different (p<0.05)
3.3 Effect of hydrothermal pre-treatment on antioxidant activity of corn hydrolysate

The antioxidant activities of the unheated and heat-treated corn samples, as determined by scavenging DPPH radical, are shown in Table 3. Compared with the control, the DPPH scavenging radical (%) of corn hydrolysate significantly increased when heated by autoclaving. The DPPH scavenging activities of the control was 57.1 ± 2.3%. After heating at 115°C for 90 min, the DPPH scavenging activities increased to 69.1 ± 4.7 % (p < 0.05). After heating at 121°C for 90 min, the DPPH scavenging activities increased to 73.1 ± 4.9 % (p < 0.05).

The ABTS scavenging radical activities (%) of the corn hydrolysate are presented in Table 4. Autoclaving significantly increased the ABTS activity, compared to the control; hot water treatment showed no significant change. The ABTS radical scavenging activity of the corn samples heated at 115°C for 90 min and 121°C for 90 min increased from 50.3 ± 4.3% in the control to 69.0 ± 1.1% and 76.5 ± 1.8%, respectively (p < 0.05).

This study has shown that hydrothermal pre-treatment by autoclaving method positively affected the antioxidant activities of corn hydrolysate. This is because many antioxidants, e.g., ferulic acid and p-coumaric acid, are mainly present as esters covalently bound to the cell-wall polysaccharides of corn [4, 6]. Heat treatment may disrupt the cell wall and release these antioxidants. However, it was observed that the contents of FA and TPC was not related to the antioxidant activities (DPPH and ABTS). This may be attributed to the present of other antioxidants in corn hydrolysates such as p-coumaric acid, vanillic acid, p-hydroxybenzoic acid and syringic acid [4] that were not measured in this study. In addition, our results showed that a prolonged heating time (60–90 min) and higher temperature (121°C) significantly enhanced the overall antioxidant activities of corn hydrolysate. These results concurred with Indrawati et al. [38], who observed that the water soluble antioxidant capacities of orange and carrot juice increased after thermal treatment at 120°C for 60–90 min. Dewanto et al. [33] reported that the total antioxidant activity of sweet corn increased by 44–54%, after heating at 100–121°C for 10–50 minutes. Also, the antioxidant activities of Citrus unshiu peels increased after heating at a high temperature (150°C for 60 min) [39].

3.4 Estimation of DE in corn hydrolysate

DE value is typically used to characterize the molecular weight of starch hydrolysis products, such as corn syrups and maltodextrins. DE refers to the percentage of reducing sugars present in a hydrolysate product calculated as dextrose (D-glucose). It also indicates the degree of polymerization during starch hydrolysis with an enzyme [15, 40, 41]. In corn starch hydrolysis, the

| Table 3  | DPPH radical scavenging activity of corn hydrolysate under different hydrothermal pre-treatment process. |
|---------|----------------------------------------------------------------------------------------------------------|
| Pre-treatment process | Temperature (°C) | 15 min | DPPH radical scavenging activity (% inhibition) | 30 min | 60 min | 90 min |
| Hot water | 80 | 63.0±1.9 ax | 63.4±0.5 ax | 64.5±4.1 ax | 64.9±4.2 axy |
| | 100 | 59.5±5.8 ax | 61.7±6.7 ax | 60.5±6.9 ax | 59.4±2.4 ay |
| Autoclaving | 115 | 66.8±3.1 ax | 68.2±3.9 ax | 69.1±4.7 axy | 66.8±3.1 ax |
| | 121 | 68.8±1.4 ax | 70.5±4.0 ax | 73.1±4.9 ax | 68.8±1.4 ax |

Different letters (a and b) within a row are significantly different (p < 0.05)
Different letters (x and y) within a column are significantly different (p < 0.05)

| Table 4  | ABTS radical scavenging activity of corn hydrolysate under different hydrothermal pre-treatment process. |
|---------|-----------------------------------------------------------------------------------------------------------|
| Pre-treatment process | Temperature (°C) | 15 min | ABTS radical scavenging activity (% inhibition) | 30 min | 60 min | 90 min |
| Hot water | 80 | 53.4±9.8 axy | 53.8±9.7 ax | 54.9±6.4 ayx | 53.7±11.7 ay |
| | 100 | 43.9±2.3 ay | 50.7±0.7 ax | 47.7±1.6 az | 52.4±4.0 ay |
| Autoclaving | 115 | 56.6±2.1 bcxy | 57.0±1.1 bcx | 58.9±2.6 by | 69.0±1.1 axy |
| | 121 | 61.7±0.9 bx | 62.1±2.4 bx | 69.9±1.0 ax | 76.5±1.8 ax |

Different letters (a-c) within a row are significantly different (p < 0.05)
Different letters (x-z) within a column are significantly different (p < 0.05)
starch is gelatinized primarily at 65–78°C [16], before hydrolysis with the enzyme. However, this study used higher gelatinization temperatures of 80–121°C. The DE values of the control was 23.1 ± 0.6. DE values obtained from corn hydrolysate under hot water and autoclaving pre-treatments at different temperatures and times are shown in Table 5. The DE values of heated samples in our study ranged from 23.2 ± 0.7 to 28.7 ± 0.9 – similar to low-DE maltodextrins (15–30) that are used in clinical feed formulations and as raw materials for enzymatic saccharification, thickeners, stabilizers, etc. [42].

As corn mixture solution was heated, starch granule becomes swollen. During heating, the intermolecular bonds between amylose and amylopectin of starch molecules are broken down and starch granule transforms into the viscous paste, known as gelatinization [43]. As the temperature increased, the viscous paste transforms into the solution due to the separation of the amylose and amylopectin chains. However, the individual amylose and amylopectin molecules are capable to be recrystallized when the sample was cooled down to a lower temperature. This process is called retrogradation [44]. In this work, it was found that the DE values significantly increased at 121°C using autoclave hydrothermal pre-treatment. However, the other pre-treatment conditions did not affect the DE value. This may be due to the difference of the morphology of the recrystallized amylose and amylopectin molecules in the cooled down step resulting in different in amylase activity, leading to different DE value. The parameters affected DE value will be investigated in the future study.

### 4. Conclusions

This study obtained a significant amount of FA from low-grade corn by a simple and green pre-treatment method during corn hydrolysate production. Autoclaving at 121°C for 90 min yielded the highest FA. This optimal pre-treatment condition efficiently broke down the FA-lignin and FA-polysaccharide bonds. Furthermore, the resultant corn hydrolysate product exhibited good antioxidant activities, including DPPH and ABTS radical scavenging activities. On the other hand, the DE values of the unheated and heated low-grade corn samples in this study were similar to low-DE maltodextrins. This research has highlighted a potentially value-added treatment for processing low-grade corn.

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