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Feasibility Study on Vanillin Production from *Jatropha curcas* Stem Using Steam Explosion as a Pretreatment

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Abstract*—Jatropha curcas* stem was analyzed for chemical compositions: 19.11% pentosan, 42.99% alphacellulose and 24.11% lignin based on dry weight of 100-g raw material. The condition to fractionate cellulose, hemicellulose and lignin in *J. curcas* stem using steam explosion was optimized. The procedure started from cutting *J. curcas* stem into small pieces and soaked in water for overnight. After that, they were steam exploded at 214 °C and 21 kg/cm^2 for 5 min. The obtained hydrolysate contained 1.55 g/L ferulic acid which after that was used as substrate for vanillin production by *Aspergillus niger* and *Pycnoporus cinnabarinus* in one-step process. The maximum 0.65 g/L of vanillin were obtained with the conversion rate of 45.2% based on the initial ferulic acid.

***Keywords*—**Vanillin, production, *Jatropha curcas* stem, steam explosion.

I. Introduction

VANILLIN or 4-hydroxy-3-methoxybenzaldehyde is the most often used flavor for the industries of food, perfume, drink and pharmaceuticals [1]. There are 2 types of commercially available vanillin. The first one is a natural vanillin extracted from the vanillin pods, with 50 tons/year consumption [2]. The second type is a pure vanillin chemically synthesized from various chemical substrates, with 12,000 tons/year consumption [3]. Natural vanillin is 250 times more expensive than the chemical vanillin. However, the United Kingdom and European laws begin to against the chemical vanillin [4], therefore, the need for a natural vanillin is increased [5].

Vanillin can be produced from phenolic compounds such as phenolic stibenes, lignin, isoeugenol, eugenol, ferulic acid, vanillic acid, aromatic amino acid, sugar beet pulp, wheat straw and biomass substances [6]. Falconnier *et al*. (1994) produced vanillin from ferulic acid by the fungi *Pycnoporus cinnabarinus* I-937 [7]. It was found that within 6 days, the vanillin yield reached 64 mg/L or 27.5%. Lesage-Meessen *et al*. (1996) experimented the utilization of ferulic acid extracted from lignocellulosic biomass such as oil palm as a substrate for vanillin production [8]. It showed that the fungi *Aspergillus niger* I-1472 changed ferulic acid to 88% vanillic acid and after that *P. cinnabarinus* MUCL39532 changed vanillic acid to 22% vanillin. Bonnin *et al*. (2000) also used *P. cinnabarinus* MUCL39533 for vanillin production from vanillic acid using sugar beet pulp as substrate [9]. The results demonstrated that the addition of cellobiose as a carbon source was essential as it increased vanillin yield for 3.3 times when compared with the reaction without cellobiose. Bonnin *et al*. (2001) used ferulic acid in sugar beet pulp and maize bran as substrates for vanillin production using the combination of *A. niger* I-1472 and *P. cinnabarinus* MUCL39533 [10]. The results indicated that *A. niger* I-1472 preferred maize bran as a carbon source as observed by the increase in feruloyl esterases enzyme. Klinke *et al*. (2002) fractionated wheat straw by the wet oxidation technique and found that approximately 0.04-0.12 g/100 g straw of vanillic acid, ferulic acid and vanillin were obtained [11]. Topakas *et al*. (2003) used *Sporotrichum thermophile* ATCC34628 for the conversion of ferulic acid to vanillic acid [12]. Within 20 h, the fungi produced as high as 4,798 mg/L vanillic acid or equivalent to 35% yield. Brunati *et al*. (2004) screened *Streptomyces halstedii* GE107678 from soil [13]. This strain converted ferulic acid to vanillic acid for 80% based on 1 g/L substrate. Zheng *et al*. (2007) applied wastes from rice bran oil production process for ferulic acid and vanillin productions [14]. *A. niger* CGMCC0774 and *P. cinnabarinus* CGMCC1115 were used in a two-step process. The first step produced 2.2 g/L vanillic acid whereas the second step gave 2.8 g/L vanillin.

Steam explosion is a technique for fractionation of wood components under high temperature and pressure. This technique could be called a thermomechanochemical process because it relates to steam heat, mechanical force for fiber separation and chemical reaction for glycosidic bond degradation [15]. Usually, this technique is operated under temperature 180-210 °C for a period of time not longer than 10 min. The principle is that the steam degrades hemicellulose from the fiber and therefore cellulose and lignin remain in the pulp. The lignin can be separated from the pulp by boiling in diluted base solution. After that, the lignin is precipitated by chemical reagents.

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The aim of this work is to evaluate the feasibility of vanillin production starting from one of the most potential used lignocellulosic biomass, *J. curcas* stem. The work was carried out at the laboratory bench scale, and discusses the chemical composition and recovery potential of vanillin product.

II. Materials and Methods

A. Preparation of Raw Material

The *J. curcas* stem used in this study was obtained from Kasetsart University, Kumpangsaen campus, Thailand. It was chopped into 1 x 1 inch pieces, dried and stored in sealed plastic bag at -20 °C until use. *A. niger* and *P. cinnabarinus* were used for the biological conversion to vanillin.

B. Analytical Method

The content of lignin, pentosan and alpha-cellulose were determined using the standard method [16]. Ferulic acid, vanillic acid and vanillin were analyzed by an LC10A High Performance Liquid Chromatography (Shimadzu Co. Ltd., Kyoto, Japan). The ODS Hypersil (25 x 0.46 cm ID, 5μm particle size, 12.0 nm pore size) was used at 30 ^{o}C with the mixture of 0.6% acetonitrile:methanol:acetic acid (10:10:80 v/v) as an eluent with connection to UV-vis detector at 280 nm. The flow rate was set at 1.0 ml/min with a sample injection volume of 20 μl.

C. Preparation of Lignin Substrate from Jatropha Stem

The scheme of vanillin production from *J. curcas* stem was shown in Fig. 1. The *J. curcas* stem sample (100 g oven dry weight) was soaked in water for overnight, and placed in a stainless steel batch reactor of 2.5 L capacity (Nitto Kouatsu Co. Ltd., Tokyo, Japan). Heating was accomplished by direct steam injection into the reactor. The sample was treated for 5 min at the desired temperature 214 °C (21 kg/cm^2). Explosive discharge of the digester content into a collecting tank was actuated by rapidly opening a pressure release valve. The pulp slurry was collected and extracted with hot water (80°C) with a ratio of fibre to water of 1:10 (w/v) for 15 min. The steam exploded mixture was adjusted to 1 L final volume. Then, it was boiled at 80 °C for 30 min and filtered through the cheese cloth to separate pulp and hydrolysate. Lignin was extracted from the pulp (cellulose+lignin) by boiling in 25% NaOH solution. After that, lignin was precipitated from the NaOH solution by the addition of sulfuric acid to pH 2. The lignin precipitate was dried and used as substrate in the next step.

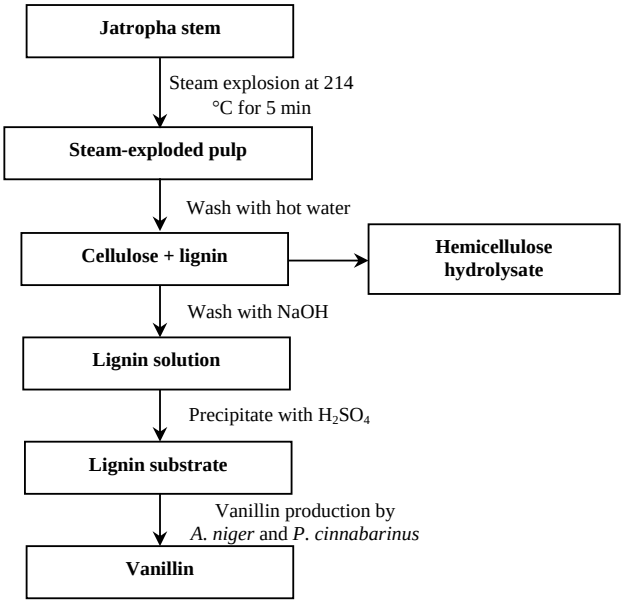
D. Conversion of Pure Ferulic Acid to Vanillin by a Single-Step Process

The stocks of *A. niger* and *P. cinnabarinus* were inoculated on a nutrient agar slant and incubated at room temperature for 48 h. After that, it was transferred into 100-ml basal medium broth (2% maltose, 0.18% $(NH_4)_2O_6C_4H_4$, 0.05% yeast extract, 0.05% $MgSO_4$, 0.02% K_2HPO_4, 0.0013% $CaCl_2$, 0.0025% vitamin B2) and incubated at 30°C for 48 h with 150 rpm shaking.

A 10 ml of each suspension of *A. niger* and *P. cinnabarinus* were then transferred into 250-ml flask containing 120 ml basal medium broth (supplemented with 0.3% yeast extract). The initial cell density at 660 nm was adjusted to 1.0. The mixture was incubated at 30 °C for 48 h with 150 rpm shaking. After that, the concentrations (2, 4, 6, 8 and 10 g/L) of pure ferulic acid were added for the conversion of ferulic acid to vanillic acid by *A. niger* and of vanillic acid to vanillin by *P. cinnabarinus*. The culture samples were daily taken for 5 days for the determination of produced vanillin and remained ferulic acid by HPLC.

E. Conversion of J. curcas ferulic acid to Vanillin by a Single-Step Process

The previously mentioned protocol was followed, except ferulic acid from *J. curcas* stem was used as substrate instead of pure ferulic acid. The culture samples were also daily taken for 5 days for the determination of produced vanillin and remained ferulic acid by HPLC.



Experimental scheme of vanillin production from *J. curcas* stem

III. Results and Discussion

The chemical composition of *J. curcas* stem was shown in Table I. It demonstrated that *J. curcas* stem contained high alpha-cellulose as in oil palm trunk (37.14%; [17]). This was high enough to be used in the ethanol production process. Pentosan was the hemicellulose in form of xylose polymer which could be applied for furfural or xylose syrup production. However, this amount was far less than that detected in oil palm (30.59%; [17]). The lignin amount was very similar to that in oil palm trunk (22.32%; [17]). This information was useful for further adjustment of steam explosion condition in chemical composition fractionation of lignocelluloses for many purposes.

TABLE I
CHEMICAL COMPOSITION OF *J. CURCAS* STEM

composition	% based on dry weight of raw material
Lignin	24.11
Pentosan	19.11
Alpha-cellulose	42.99

The steam explosion technique was used for fractionation of *J. curcas* stem. After the explosion, the hydrolysate or hemicellulose solution contained both monomer and oligomers of xylose whereas the pulp contained mainly cellulose. The steam-exploded *J. curcas* stem was then washed with NaOH solution and quantitatively determined for phenolic compounds (ferulic acid, vanillic acid and vanillin) by HPLC. The retention times (Table II) and area peaks were used for calculation of phenolic compounds concentrations. The results demonstrated that the steam-exploded *J. curcas* stem contained 0.0008 g/100 g *J. curcas* stem of ferulic acid at retention time 17.935 min (close to standard ferulic acid) and 0.15 g/100 g *J. curcas* stem of vanillic acid at retention time 8.737 min (close to standard vanillic acid). However, no vanillin was detected. The concentration of ferulic acid was relatively lower when compared with the experiment by [18]. They prepared 0.5-1.0 g ferulic acid/ 100 g raw material from wheat straw and sugar beet pulp using selected fungi. Furthermore, Lesage-Meessen *et al*. (1996) produced 1.0-1.2 g ferulic acid/ 100 g raw material from oil palm, wheat straw and wheat bran using the enzymatic technique. However, more ferulic acid could be detected in other lignocelluloses. For example, Zheng *et al*. (2007) extracted 13.6 g ferulic acid/100 g of waste from the production process of rice bran oil. The differences of extracted ferulic acid among various lignocellulose sources could be due to the extraction condition from lignocellulose raw materials. The steam explosion condition used in this experiment might be too harsh and then resulted in the conversion of most ferulic acid into vanillic acid as observed by the relatively lower concentration of ferulic acid when compared with other mentioned examples in which the enzymatic technique was applied instead.

TABLE II
RETENTION TIMES OF SUBSTANCES FROM HPLC ANALYSIS

substances	Retention time (min)	
Ferulic acid	17.84	y = 56564x
Vanillic acid	8.62	y = 35400x
Vanillin	12.54	y = 83333x

A concentration series of pure ferulic acid were applied as substrate for vanillin production. The results showed the ability of *A. niger* and *P. cinnabarinus* in converting pure ferulic acid into vanillin (Table III). The production of vanillin clearly depended on the reaction time and ferulic acid initial concentration. As the reaction time increased, vanillin yield also increased (Fig. 1). Table III indicated that the maximum vanillin (5.15 g/L) was obtained from 10 g/L ferulic acid or equivalent to 51.5% conversion. However, the maximum conversion percentage at 69.5% (2.78 g/L vanillin yield) was obtained when 4 g/L ferulic acid was used as starter. Thus, it could be concluded that the most appropriate ferulic acid initial concentration for vanillin production was 4 g/L. This result was also consistent with that by Zheng *et al*. (2007) that the ferulic acid concentration influenced on the production of vanillic acid and vanillin. However, vanillin yield did not vary on the concentration of ferulic acid and the maximum conversion at 61.9% was obtained when 4.5 g/L ferulic acid was applied as substrate to produce 2.8 g/L vanillin yield.

TABLE III
THE EFFECT OF FERULIC ACID CONCENTRATION ON VANILLIN PRODUCTION

Initial ferulic acid (g/L)	Produced vanillin (g/L)	Remained ferulic acid (g/L)	conversion (%)
1	0.56	0.00	56.0
2	1.25	0.21	62.5
4	2.78	1.02	69.5
6	3.98	1.09	66.3
8	5.10	1.13	63.8
10	5.15	0.94	51.5

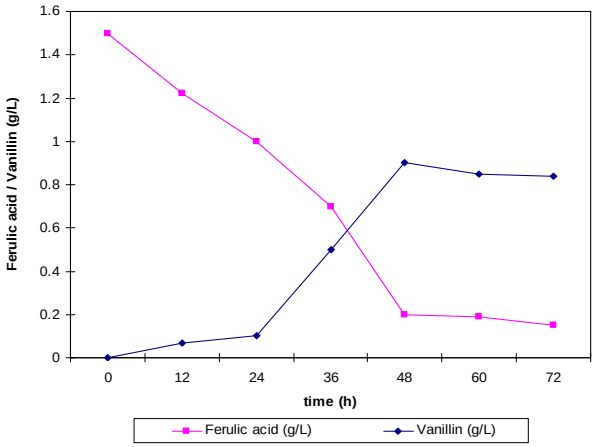


Fig. 1 Conversion of pure ferulic acid to vanillin

Ferulic acid from *J. curcas* was also used as substrate for vanillin production. The initial concentration of ferulic acid at 4 g/L was prepared according to the previous result. The results also showed the ability of *A. niger* and *P. cinnabarinus* in converting *J. curcas* ferulic acid into vanillin. The reaction time also influenced the production of vanillin. As the time increased, the yield of vanillin also increased (Figure 2). The maximum vanillin at 0.70 g/L (45.2% conversion based on initial ferulic acid) was achieved within 48 h with 0.18 g/L remained ferulic acid. The lower conversion percentage from this experiment (45.2%) compared with the previous experiment (69.5%) could be due to some derived compounds such as furfural or acetic acid from the steam explosion. These substances were shown to inhibit the growth of

microorganisms. Thibault *et al*. (1998) demonstrated that the application of adsorption technique by resin or activated carbon could remove these toxic substances and improve up to 50% of the purity of ferulic acid from sugar beet pulp.

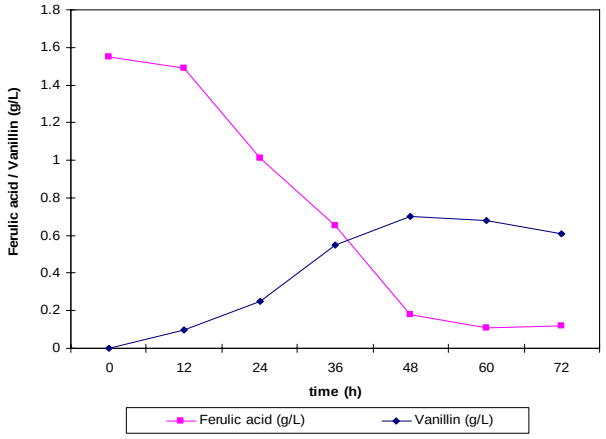


Fig. 2 Conversion of *J. curcas* ferulic acid to vanillin

IV. Conclusion

This research was the first document to reveal the possibility of *J. curcas* stem value-adding as a raw material for vanillin flavor production. The *J. curcas* stem contained 19.11% pentosan, 42.99% alphacellulose and 24.11% lignin based on dry weight of 100-g raw material. The condition to fractionate cellulose, hemicellulose and lignin in *J. curcas* stem using steam explosion pretreatment was optimized. The one-step process of fermentation by *A. niger* and *P. cinnabarinus* was applied for vanillin production starting from ferulic acid extracted from *J. curcas* stem. The maximum 0.65 g/L of vanillin was obtained within 48 h with the conversion rate of 45.2% based on the initial ferulic acid. The above results indicated the possibility of lignocellulosic biomass utilization for vanillin flavor production by microorganism. However, the optimization of the process and the evaluation of cost investment are extremely needed.

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