ANALYSIS OF FERULIC ACID CONTENT IN BAMBOO SHOOT AND PROCESSED PRODUCTS FROM BAMBOO SHOOT

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ABSTRACT

Ferulic acid is the one important phenolic acid since its good for health as antioxidant. Bamboo shoot used as the raw material to investigate ferulic acid .The aim of this work was to study feasibility on preparation of ferulic acid from each part of bamboo shoot such as the peel and flesh , then their processed product as boiling ,pickling were also tested by by reverse phase high performance liquid chromatography.The results presented that in raw Peel of bamboo shoot contained the maximum ferulic acid contents as 2197.08 ± 2.10 mg/100g and the pickled flesh of bamboo shoot showed the lowest ferulic acid content as 107.18 ± 2.95 mg/100g. This work showed that to feasibility on preparation of ferulic acid should be used the raw peel of bamboo shot as a raw material to get the valued antioxidant nutrient for supplement food.

Keywords: Bamboo shoot, Ferulic acid, processed products.

INTRODUCTION

Bamboo was the one type of popular plant that cultivated in Thailand like as neighbor countries such as Philipines,Indonesia and Malaysia. All part of bamboo was used for much purpose such as home construction, furniture, musical instrument, household utensils (Park, E. J and John, D. Y. 2010.), accessories products, soap from leave, and herbal treatment for health. However, the baby stem of bamboo which known as bamboo shoot was also the popular for food. There have many types of bamboo in the world but Rough Giant bamboo (*Dendrocalamus spp*). is the most popular for people for eat as food in Thailand, since young shoots are sweet , good taste which considerd as a delicious vegetable. Not only the good side of bamboo shoot as food but also found that bamboo shoot contains a cyanogenic glycoside 'amygdalin' composed of glucose, benzaldehyde and cyanide (Pillay VV ,2003). Thus it was the one of the cyanogenic plant which release the toxic substance as cyanide. The cyanogenic glycoside present in bamboo shoots is taxiphyllin an IUPAC name known as 2-(b-D-glucopyranosyloxy)-2-(4-hydroxy- phenyl) acetonitrile(Sang-A-Gad P et al.,2011). The Peel contains the highest cyanide (Pramod Kumar GN et al., 2011). From the toxic of bamboo shoot as above, before eating bamboo shoot, the peel of bamboo shoot must be take off and soaking the flesh bamboo shoot in water before boiling in water for a range of time (Liyi Ye .et al., 2015) The review literature about the ferulic acid showed the content of ferulic acid in many plants including in bamboo shoot (Zhaohui Zhao. Et al., 2008), but not in details too much. From the other side of bamboo shoot, it also contains many high dietary fiber and rich of various nutrients (Pandey, A. K.,et al,2012)(Nirmala, C.,et al,2007). The shoots contain many minerals, such as mainly potassium, calcium, manganese, zinc, chromium, copper, iron and also the minor amounts of phosphorus and selenium (Pandey, A. K.,et al,2012). In addition, fresh flesh bamboo shoot also contains many vitamins such as Vitamin A, vitamin B1, vitamin B3, vitamin B6 (Singhal,P,et al.,2013). The other compounds such as flavonoids, phenols and phenolic acids, which are bioactive compounds with putative health benefits Guta *et al*. (2010) also found in bamboo shoot. Bamboo shoot extracts may provide anti-inflammatory and anti-carcinogenic effects for human (Tamang, B.,& Tamang, J. P. 2009). Bamboo shoot have been reported as a source for

preparation of biochar(Liyi Ye.,et al.,2015), polyols (Liyi Ye.,et al.,2014),xylitol (Miura, M. et al.,2013) ,biomethane (Shen,S.C.,et al.,2013). Another representive product in bamboo especially bamboo leaf is flavonoid compound (Jiao et al., 2007; Xie et al., 2013; Zhang, Jiao, Liu, Wu, & Zhang, 2008), which will led to a added-value for bamboo utilization. Ferrulic acid is the one phenolic compound that claimed to present in bamboo shoot (Zhaohui Zhao. Et al.,2008), its IUPAC name is E -3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid) as showed structure in fig.1 (Cesare Mancuso and Rosaria Santangelo.2014).

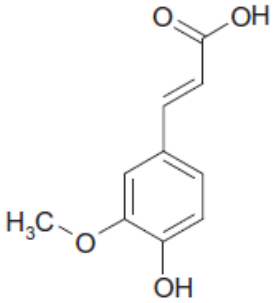

Figure1. Structural formula of ferulic acid

This acid is a derivative of caffeic acid that found in vegetables, fruit and beverage (D'Archivio, M.et al., 2007)(Rechner, A.R., et al,2001). Ferulic acid is also inhibit the secondary free radicals in mice (Srinivasan, M .et al.,2005), prevent liver damage,(Zheng, R.L. and Zhang, H., 1997), prevent induction and apoptosis in cerebral artery(Koh, P.O., 2012)(Cheng, C.Y., et al.,2010) , decrease Alzheimer's discease symptom (Mori, T ,et al.,2013), anti cancer(Hemaiswarya, S.& Doble, M., 2013)(Mancuso, C., et al.,2012), cardiovascular discease (Pagidipati, N.J.& Gaziano, T.A., 2013) and diabetes(Prabhakar, P.K.et al.,2013). Since, the bamboo shoot contains ferulic acid as refered in many researcher, thus the aim of this work was to analyse ferulic acid content in bamboo shoot by survey its content in the peel of bamboo shoot and flesh body of bamboo shoot as the raw status and processed as cooking by boiling and pickling. This research will evaluate the possible source to prepare ferulic acid from bamboo shoot. There has no report about the preparation of ferulic acid from bamboo shoot,so this is the first time to get an estimation idea in preparation of ferulic acid from natural source.

MATERIALS AND METHOD

Chemicals and Apparatus

Standard ferulic acid (HPLC grade) was purchased from Sigma-Aldrich. Acetic acid, AR grade brought from Merck . Sodium chloride , sodium hydroxide , potassium carbonate and potassium hydroxide (all AR grade) purchased from RCI Lab Scan. Dichloromethane, trichloromethane , acetone and ethanol (all AR grade) were purchased from Carlo Erba Reagents. Acetonitrile, and methanol (HPLC grade) were purchased from Carlo Erba Reagents. Ultrapure water system was generated from Siemens (Alpharetta, USA).

The high performance liquid chromatography coupled with diode array detector was purchased from Hewlett-Packard model HP 1100.The C18 was Sphere Clone packed with 5 µm ODS, 250 x 4.60 mm from Phenomenex. Bamboo shoot sample is Rough Giant bamboo (Dendrocalamus spp.) , from the local market Prachinburi province, Thailand.

EXPERIMENTAL

Optimization Method For HPLC Analysis (modified from Ndolo,V.U., et al.,2013)
The standard ferulic acid was prepared as a stocking solution and prepared as a working calibration solution as 0.5, 2.0, 10.0, 25.0, 50.0 and 100.00 µg/ml. All standard solution was injected to the C18 column and operated the HPLC as the following condition as using 1% of acetic acid : methanol (system1) and 1% of acetic acid : acetonitrile (system 2) as mobile phase solvent by variation ratio between 50:50, 55:45, 60:40,65:35 and 70:30 with control flow rate at 1.0 ml/min by isocratic technique. The area of chromatogram and R_T of standard ferulic acid peak were recorded. After, the method was accepted , the LOD and LOQ were evaluated.

Preparation Bamboo Samples
The raw bamboo shoots were separated into peels and flesh body. Each part of bamboo shoot sample was cooked by boiling in hot water for 20, 30, 40, 50 and 60 mins. Then each part was placed in 2% of sodium chloride solution for 1days and the flesh body was separated the salted solution and placed agained into solid sodium chloride 2kg. for bamboo shoot 10 kg. And placed into the bottle and covered with lid, left it at room temperature for 10 days.

Preparation of Sample For Analysis (modified from Eun-Jin Park, Deok-Young Jhon .2010)
All samples were approximately weighed as 1.xxxx g (each), ultrasonicated with methanol for 20 minutes. Then, the mixture was centrifugated at 4500 rpm for 5 minutes, and adjusted with methanol to a final volume of 5 ml, and filtered through a 0.45 µm filter. The extracts were stored at -1 °C until the analysis. The analysis was performed as in part 2.2.1.

Statistical Analysis
All determinations were carried out at least in five replication and values were averaged. The results are expressed as mean ± S.D. values; statistical differences between means were determined by one way ANOVA. Microsoft Excel were used for calculate and graph presentation in this work.

RESULTS AND DISCUSSION

The Optimization Method

The result from optimization study in part 2.2.1 by using 1% of acetic acid: methanol and 1% of acetic acid : acetonitrile as mobile phase solvent to find out the best solvent between methanol and acetonitrile as showed in fig.2.

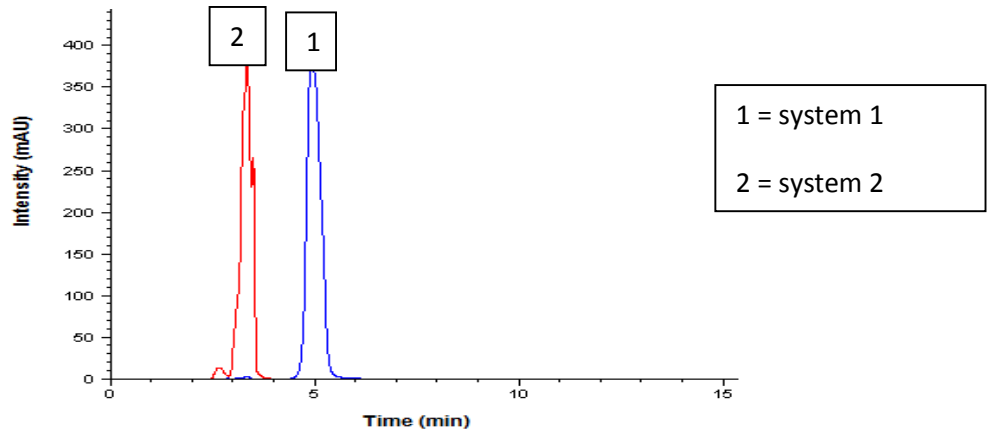

Figure 2 Chromatogram of standard ferulic acid by elution of system1 and 2

The system 1 (1% acetic acid: methanol) = 1:1 or 50:50 gave the symmetrical peak which good characteristics than using system 2. The optimized ratio also good at equal ratio of each solvent in system. However, the retention time of ferulic acid also showed at approximately 5 mins , at this time is quite good for analysis in true samples. However, the flow rate of mobile phase solvent was studied the flow rate in the range of 0.7, 0.8, 0.9 and 1.0 ml/min., the result presented that the 1.0 ml/min flow rate was the best as in fig.3

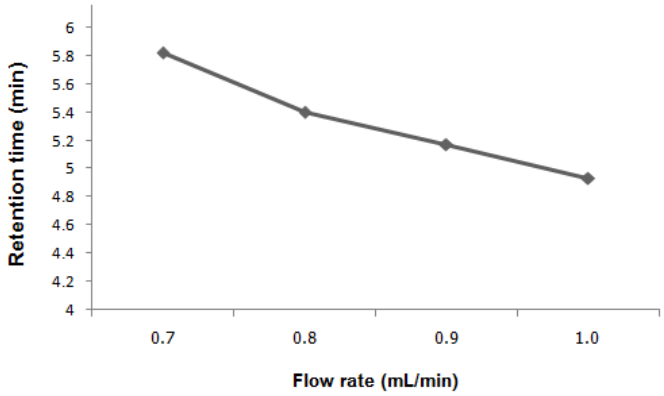

Figure 3. The effect of variation flow rate of mobile phase system

The standard calibration curve was obtained by drawing the graph between peak area versus the concentrations of standard ferulic acid. Linearity obtained from the calibration curve was in the range 0.2 to 10.0 μgmL^{-1} with the regression coefficient (r^2) of 0.999. as presented in fig.4.The limit of detection (LOD) and limit of quantitation (LOQ) were evaluated based on a signal to noise ratio of 3 and 10, respectively. The LOD of 0.02 μgmL^{-1} and LOQ of 0.06 μgmL^{-1} were obtained.

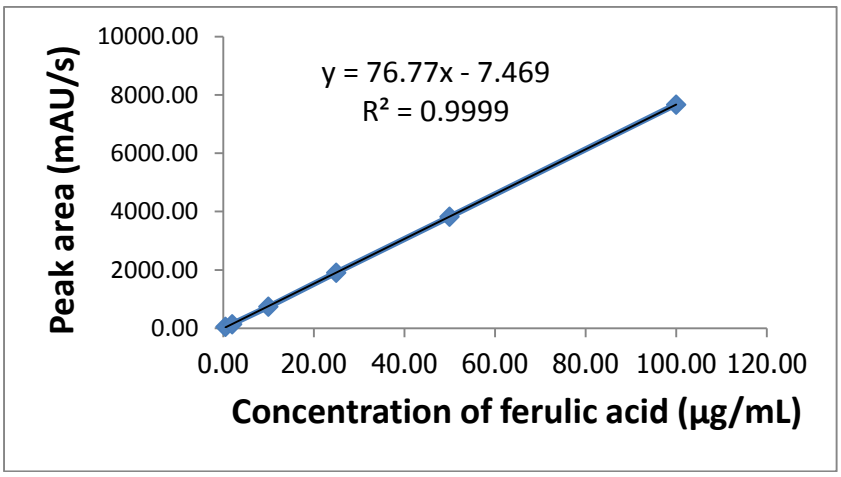

Figure 4 Calibration curve of standard ferulic acid

Analysis Results In Bamboo Shoot Sample

The boiling temperature was studied from 100 to 120 ^{o}C and the boiling time was varied from 20 to 40 min. It was found that boiling temperature and time did not affect significantly on the peak area of the extracted ferulic acid in all samples. Therefore, boiling at 100 ^{o}C for 20 min was chosen for preliminary sample preparation. The extraction of ferulic acid from samples was performed under ultrasonication and methanol was used as the extraction solvent. The extraction time was studied in term of sonication time in the period of 10 to 25 mins. The peak

area of the extracted ferulic increased with the increasing time from 10 to 20 min. Further increasing the extraction time to 25 mins did not increase the peak area. Therefore, sonication time of 20 mins was chosen.After the samples were analysed ferulic acid contents as showed the chromatogram of ferulic acid in bamboo shoot sample as in fig.5

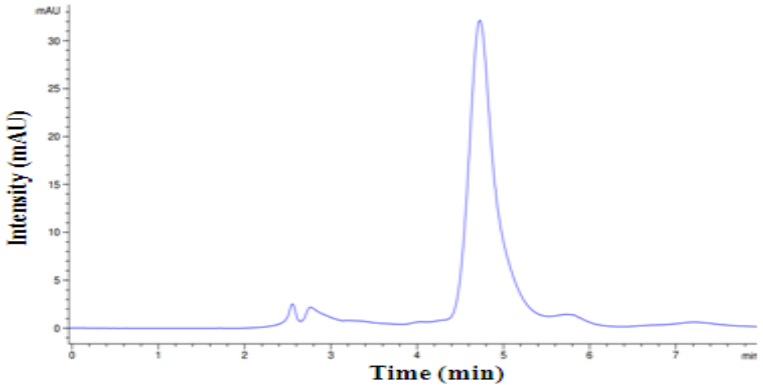

Figure 5. Chromatogram of ferulic acid in the peel of bamboo shoot sample

The quantity of ferulic acid in all samples showed in fig.6.

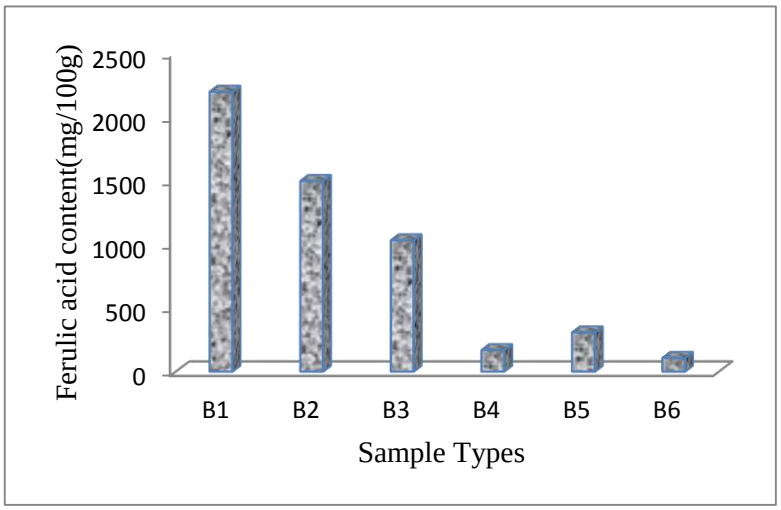

Figure 6 Ferulic acid in bamboo shoot sample and processed samples

Note: Each experiment trial for 5 replications.
B1 = Raw peel of bamboo shoot
B2 = Boiled peel of bamboo shoot
B3 = Raw flesh of bamboo shoot
B4 = Boiled flesh of bamboo shoot
B5 = Pickle peel of bamboo shoot
B6 = Pickle flesh of bamboo shoot

From the fig.6 , it revealed that the raw peel of bamboo shoot showed the highest quantity of ferulic acid as 2197.08 ±2.10 mg/100 g bamboo shoot approximately as 2.2% but after the peel was boiled in water the ferulic acid reduced to be as 1497.73 ±1.86 mg/100 g bamboo

shoot approximately as 1.5%. For the aim of this work was to evaluate ferulic acid content in bamboo shot, the peel of bamboo shoot showed the good feasibility to be a raw material for preparation of ferulic acid source.This is the good application from raw material as the peel of bamboo shoot that was left as a waste from agricultural industry, since all people just only eat flesh of bamboo shoot. The reason for the peel contained the high ferulic acid content may concerning about the structure of peel is hard with highest fiber as state in Sang-A-Gad P.,et al.(2011) and this part also showed the highest cyanide content, ferulic acid which was derived from caffeic acid could bound with cyanide(Pramod Kumar GN .,et al.,2011) and this system is normally found as natural protection of plant.

The trend of using agricultural waste is now blooming for mankind to choose in changing waste to new products. As the result, there was no report that report the utilization of bamboo peel to prepare the useful supplement product.The recommendation from this work to prepare ferulic acid should be use the raw peel of bamboo shoot, but from this work the flesh of bamboo shot also could be a raw material.For the food concept, the people who ate the boiled flesh of bamboo shoot also got the ferulic acid in the quantity of 169.16 ±2.10 mg/100 g bamboo shoot.The peel must be took out from bamboo shoot before processing as food so this is good for health as remove of cyanide compound. The pickle bamboo shoot is the popular products from bamboo shoot showed the lowest ferulic acid content since the flesh of bamboo shoot was cut into small pieces and washed many times with sodium chloride salt , the proceesed to prepare pickle bamboo shoot products reduced the toxic substance as cyanide and also reduced ferulic acid too.

CONCLUSIONS

In the optimization method of analysis found that the optimum condition was using reverse phase column C18 as the stationary phase and mobile phase system was 1% acetic acid: methanol as 1:1, the solvent eluted as isocratic gradient system with 1.0 ml /min flow rate that detected signal by Diode array detector at 320 nm. The retention time of ferulic acid showed at 5.0 min. The LOD and LOQ of method were 0.02 μgmL^{-1} and 0.06 μgmL^{-1}, respectively. The result from the analysis ferulic acid in bamboo shoot showed that the raw peel of bamboo shoot contained the ferulic acid as 2197.08±2.10 mg/100 g bamboo shoot. This conclude that the raw peel of bamboo shoot was the best source of ferulic acid and could be the raw material to prepare the antioxidant substance to be a supplement food for human.

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