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Optimisation of Pressurised Liquid Extraction of Bioactive Compounds from *Ananas comosus* (Pineapple) Fruit

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ABSTRACT

In this study, pressurized liquid extraction (PLE) was used with methanol as extraction solvent to extract bioactive compounds from *Ananas comosus* (pineapple) flesh. Response surface methodology (RSM) was used to evaluate the correlative effects of temperature (60 – 150°C) and extraction time (10 – 30 min) on the yield of selected bioactive compounds. In this model, the R^2 obtained was 0.8788 for selected bioactive compounds for *Ananas comosus* suggesting a satisfactory agreement between the predicted and experimental values. Two-dimensional high-performance liquid chromatography (2D-HPLC) with a diode array detector (DAD) was used for the separation and detection of the bioactive compounds. Extraction temperature was found to significantly increase the yield of three selected bioactive compounds following which the optimum operating extraction conditions for PLE for *Ananas comosus* was determined to be 105°C and a static time of 20 min.

Keywords: Ananas comosus, bioactive compounds, extraction optimization, HPLC, pineapple, pressurized liquid extraction, RSM

INTRODUCTION

Tropical fruits have an important role in promoting health particularly its antioxidant properties. *Ananas comosus* (pineapple) is rich in bioactive compounds such as anthocyanins, polyphenols and health inducing enzyme bromelain. *Ananas comosus* belongs to Bromeliaceae family that produced edible fruits (Kudom & Kwapong, 2010), and ranked as the third most important tropical fruit produced in the world (Bartholomew et al., 2003). In Malaysia, out of the eleven reported varieties of pineapple available in the market, only Josephine, Morris, MD2 and Sarawak are popularly cultivated (Yuris & Siow, 2014).

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While popular with its nicknames, 'healthy fruit' that rich with antioxidant, pineapple reported to consist chlorogenic acid, catechin, kaempferol, myricetin, gallic acid, gentisic acid, vanillin, ferulic acid, sinapic acid, isoferulic acid, o-coumaric acid, protocatechuic acid, tyrosine, p-coumaroylquinic acid and arbutin, tons of bioactive compounds as claimed (Yapo et al., 2011; Wen, 2001; Taussig & Batkin, 1988).

Fast extraction and low solvent consumption are the main advantages of automated pressurized liquid extraction (PLE). PLE has been successfully applied for the extraction of thermal-sensitive bioactive compounds from plants and fruits (Petersson et al., 2010). It involves extraction using solvents in liquid state at elevated temperature and pressure to enhance the extraction process. The properties possess by solvent to remain liquefied above their boiling point helps high-temperature extraction activity that happened due to enhanced solubility and mass transfer properties (Richter et al., 1997). Solvents such as ethanol, methanol and water which are reported to be inefficient for extracting anthocyanins and other bioactive compounds at low temperatures, may be much more efficient at the elevated temperatures used in PLE (Ju & Howard, 2003) and thereby obtain an anthocyanin rich extract (Gizir et al., 2008). The present study was undertaken to evaluate the extraction temperature and extraction time on the recovery of bioactive compounds from *Ananas comosus* and to determine the optimal conditions for obtaining maximum yield of bioactive compounds using response surface methodology (RSM). The bioactive compounds selected in this study are catechin, myricetin and bromelain.

METHOD

Catechin, bromelain and myricetin reference standards was purchased from Sigma. Methanol (MeOH) and acetonitrile (ACN) of HPLC grade were purchased from Merck (Germany). Fruits of *Ananas comosus* with different varieties (Morris, MD2 and Josaphine) were obtained locally in Malaysia.

Preparation of Extract

Fresh ripened fruits of *Ananas comosus* were purchased from the market in Selangor, Malaysia. Following which the fruits were peeled, cut into thin pieces and dried for 48 hours at 45°C in an oven (Memmert UN110). The dried flesh was stored in dark-covered container prior to extraction.

Pressurized Liquid Extraction (PLE)

Pressurized Liquid Extraction (PLE) was performed on a Dionex ASE 350 accelerated solvent extractor (Thermo Scientific Ltd. Surrey, UK). Dried *Ananas comosus* was weighed 30.0 grams and were thoroughly put in 100 mL stainless steel extraction cell (PLE) with an adequate amount of diatomaceous earth. The cell containing the sample was heated before filled with methanol and undergone pressurization process. Methanol was selected as extraction solvent based on the polarity of targeted bioactive compounds. To ensure the sample of *Ananas comosus* reaches the desired temperature (60 - 150°C), the cell was placed in heating system for 5 minutes. The sample with pressurized solvent at 1500 psi for 10-30 minutes. All extracts

were covered in aluminium foil to prevent exposure to light and stored at -4°C. A total of 14 experiments were performed to determine the effects of temperature and extraction time on extraction yield. See Table 1.

Experimental Design and Data Analysis

The design of experiments, analysis of the results and prediction of the response were carried out using Design-Expert software (Version 6.0.4). Total of 14 experiments were carried out in randomised run order and central composite design (CCD) was used consisting 5 coded level; -α, -1, 0, 1, α. The response variable (catechin, bromelain and myricetin) was evaluated using response surface methodology (RSM) and comparison of means was performed by one-way ANOVA (analysis of variance).

Model Verification

The optimum extraction conditions were based on the maximum yield of bioactive compounds in sample and analysed based on the regression analysis and 3D surface plots of the independent variables. The response was determined under the recommended conditions of extraction and the predicted value was compared with the experimental value to prove the validity of the model.

Analysis using Two-Dimensional Liquid Chromatography (2D-LC)

2D-LC analysis was performed on a Dionex Ultimate 3000 Liquid Chromatography system equipped with diode array detector. 2D-LC chromatographic separation was done on C_{18} column (5 µm, 4.6 x 250 mm). The mobile phase comprised of 0.01% acidified pure water (A), methanol (B) and acetonitrile (C) using gradient elution (0-2.5 minutes; 50:20:30, 2.5-10.00 minutes; 40:30:30 and 10.00-25.00min ;20:50:30) with flow rate kept at 1 mL/min.in HPLC system, column temperature was maintained at 37 °C and injection volume was 100 µL. Data acquisition was performed by Chromeleon software version 6.8 Dionex, U.S.A. Eluted compounds were monitored over a wavelength range of 200 – 400 nm.

RESULTS AND DISCUSSION

Optimization of PLE Parameters

For the optimization of extraction of *Ananas comosus* using pressurized liquid extraction static time and temperature ranging from 10 min to 30 minutes and 60°C to 150°C respectively was assigned. Multilinear regression was applied to the results of the central composite design. The results were based on the amount of bioactive compounds obtained from the extract as shown in Table 1. Repetition of same combination of temperature and extraction time which can be seen in Table 1 was designated experiment that determined by central composite design (CCD) method. The effect of independent variables; (a) extraction temperature (T); and (b) extraction time (t), on the amount of bioactive compounds was evaluated by second order (quadratic) model.

Fitting the Model

The adequacy of the model was determined using model analysis, lack of fit test and coefficient of determination (R^2), and its fitness assessed with lack of fit test ($p>0.05$). ANOVA result showed that the model was significant, $p<0.05$ ($p=0.042$) with not significant lack of fit, $p > 0.05$ ($p = 0.1793$). The significance of the equation parameters for each response variable was also assessed by F-ratio at a probability (p) of 0.05. The closer the value of R^2 to unity, the better the empirical model fits the actual data. The quality criteria for a good fit of a model, the coefficient of determination (R^2) should be at least 0.80. In this model, the R^2 was 0.8788 for selected bioactive compounds for *Ananas comosus*. The high value of R^2 (>0.80) indicates the adequacy of the applied quadratic model. In Table 2, the summary for optimization using Central Composite Design were tabulated and be concluded.

Table 1
Results of the CCD for the analysis of Ananas comosus

No.	Factor		Compounds			
	Temperature T (°C)	Extraction Time t(min)	Catechin (mAu*min)	Myricetin (mAu*min)	Bromalein (mAu*min)	Total Area (mAu*min)
1	169	34	295.7	13.57	40.16	322.5
2	41	06	88.23	0.360	10.54	397.9
3	169	06	213.6	73.58	35.49	99.13
4	105	20	271.1	12.32	39.17	104.2
5	105	20	333.7	20.02	44.88	349.4
6	41	34	92.26	0.140	11.73	322.6
7	105	20	348.1	39.43	42.27	429.8
8	169	20	308.9	74.09	38.07	421.0
9	105	34	325.1	41.49	44.15	103.5
10	105	20	344.4	27.57	42.87	206.2
11	41	20	92.29	0.250	11.01	450.6
12	105	06	163.7	12.24	30.24	410.7
13	105	20	375.1	27.17	48.39	414.8
14	105	20	311.6	26.81	42.57	381.0

Table 2
Summary of Central Composite Design for selected bioactive compound in Ananas comosus

Response	Transform	Model	Lack of Fit	R^2	Equation
Total Area	Square root	Quadratic Significant	Not Significant	0.8788	Sqrt (Total Area) = +394.03+131.05* A+39.38*B-114.98*A^2-68.78*B^2+5.44*A *B

Effect of Extraction Conditions on Total Bioactive Compounds Content

Parameters such as time and temperature used in extraction plays a vital role in determining the quantity of extractable bioactive compounds from a sample. Temperature is a critical factor that affects extraction processes, as it increases solubility of the substances and recovery of bioactive compounds (Karacabey & Mazza, 2010). Variation in extraction temperature (T) for selected bioactive compounds revealed that increases in temperature also increases the amount of each compounds extracted. When the temperature is increased from 41°C to 73°C the yield of total selected bioactive compounds increased simultaneously, to reach stability when it reaches 105°C before declining. This may be due to thermal degradation occurring in bioactive compounds in extreme temperature thus affecting yields of extraction. It was observed that temperature significantly affects the yield of selected major bioactive compounds in *Ananas comosus* during the extraction process. In general, more components are extracted, when the temperature is elevated through the increased of solubility and mass transfer properties which improved the penetration of solvent into sample matrix.

Long extraction time resulted in high yield of compounds extracted as shown in Figure 1 with maximum yield at 20 minutes. However, prolonged extraction time may increase degradation of bioactive compounds which will affect the yield of response as noted in this study.

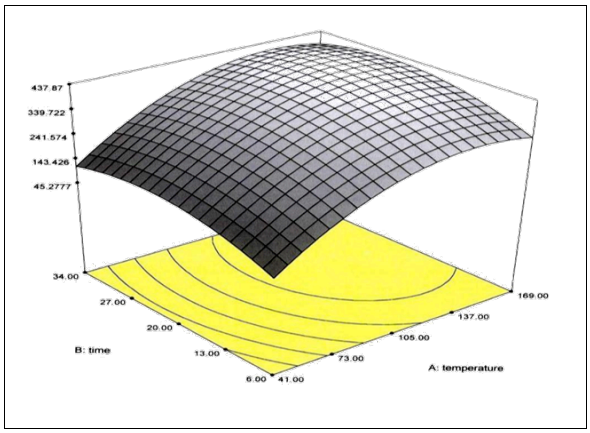
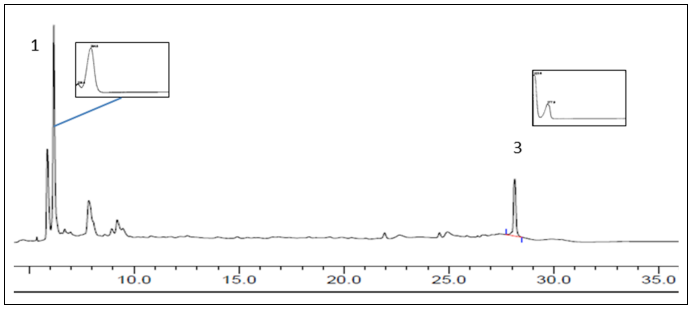


Figure 1. Contour and 3D-response surface plot of static time against temperature for selected major compounds in *Ananas comosus* obtained using Central Composite Design

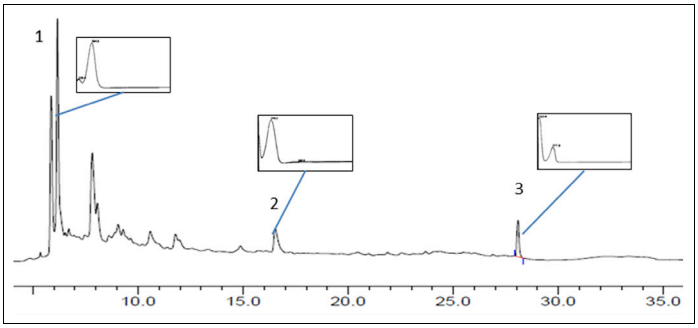
Experimental Validation of the Predictive Model

Establishing the optimal extraction parameters was done using Design-Expert statistical software. The goal was to obtain the highest yield of bioactive compounds extraction from the fruit flesh. The optimal PLE conditions for the extraction of catechin, myricetin and bromelain were pressure of 1500 Psi, and optimal temperature of 105°C overall extraction time of 20 min. Under these optimal conditions and parameters a large amount of extract with a high content of bioactive compounds from *Ananas comosus* flesh was obtained. The adequacy of

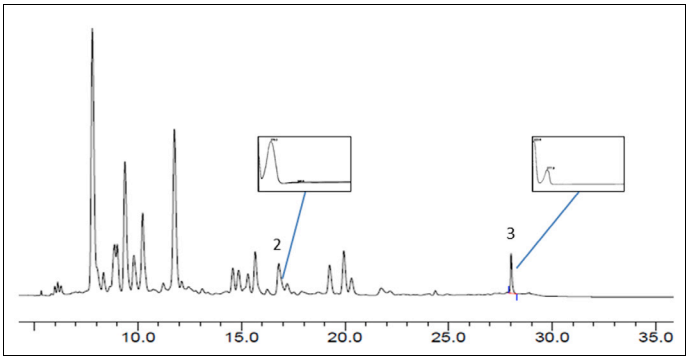
the model was verified as the experimental values were in close agreement with the predicted value, and the deviation too was found to be insignificant. By minimizing the possible strong effect such as origin, time of production and pre-processing storage, the performance of extraction technique can fairly be compared and optimized (Al-Farsi et al., 2005; Dourtoglou et al., 2006; Gizir et al., 2008).



(a)



(b)



(c)

Figure 2. Chromatogram of extract from three varieties of *Ananas comosus*; (a) MD2; (b) Morris; and (c) Josaphine using PLE optimum condition with static time of 20 minutes and temperature of 105°C with their respective UV spectra

HPLC Analysis of *Ananas comosus* Extract

Chromatographic analysis of bioactive compounds in the extract at optimized conditions was conducted using high performance liquid chromatography (HPLC). Several samples of *Ananas comosus* from Morris, MD2 and Josaphine varieties were extracted using the PLE optimum parameters at 105°C, pressure of 1500 psi and a static time of 20 min. Figure 2 shows the chromatograms of *Ananas comosus* extract from respective varieties of pineapple revealed the bioactive compounds mainly present are catechin, myricetin and bromelain. The concentration of the compounds in particular varieties can help to distinguish them apart. Such as in Morris, each of these bioactive compounds were reported present compared to the absence of myricetin in MD2 varieties. In Josaphine, the absence of catechin that appeared in other types of pineapple acts as a distinguishing marker. The identities of the compounds were confirmed by standard reference of the compounds and UV comparison that detected at selected wavelength, 280 nm.

Peak 1 representing catechin with retention time of 6.32 min followed by peak 2, myricetin with retention time of 11.82 min and peak 3; bromelain retention with time 28.08 min.

CONCLUSION

Optimization of extraction procedure for selected bioactive compounds of *Ananas comosus* fruit extract was examined using response surface methodology. This is the first report on optimization of bioactive compound from *Ananas comosus* using this particular extraction technique i.e. pressurized liquid extraction (PLE). The optimized condition was validated and found to be in close agreement with experimental values. The results showed that extraction temperature and extraction time play significant roles in measured responses. Under the optimal conditions of 105°C extraction temperature and 20 min of extraction time, pressurized liquid extraction yield maximum responses of selected bioactive compounds. These conditions can be used to produce extraction yields with higher concentration. The PLE method can contribute to the further isolation of bioactive compound from varieties of *Ananas comosus*.

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