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Ultrasound assisted extraction of bitter gourd fruit (*Momordica charantia*) and vacuum evaporation to concentrate the extract

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Abstract

Bitter gourd (*Momordica charantia*) is a medicinal plant widely cultivated in Indonesia. The aim of this present study was to investigate application of ultrasound assisted extraction (UAE) and vacuum evaporation (VE) to process the fruit into a concentrate with focus on increasing extract's total phenolic content (TPC), antioxidant activity (AA) and storage stability. UAE was done under various level of time and temperature with the best combination found was 5 minutes and 25°C respectively. VE of the extract at 125 mmHg and water bath temperature set at 65°C showed that concentrate concentration factor (CF) was linearly correlated to its TPC and AA. Stability study of the concentrate under different storage temperature (4°C and 25°C) without preservative was done for 14 days. Higher CF enhanced shelf life and AA stability in both storage temperatures. This study concluded that UAE may reduce the required extraction temperature and time used for yielding extract with higher TPC and AA. Also, VE may concentrate the extract without damaging its TPC and AA. Lastly, VE may provide higher TPC, AA, and increasing storage stability while reducing extract volume compared to non-concentrated extract.

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Keywords: Antioxidant; bitter gourd; total phenolic content; ultrasound assisted extraction; vacuum evaporation.

1. Introduction

Bitter gourd fruit has several mechanisms of action that can be beneficial for human health. For instance, bitter gourd fruit extracts showed significant antioxidant properties' [1].The capability of antioxidants to inhibit oxidative reaction which are related to many health disorders makes fruit such as bitter gourd a valuable resources[2].The beneficial properties of bitter gourd fruit could be enhanced if it can be industrially processed to yield more phenolic compounds without damaging it severely as in the case of cooking, which is generally done to mask its bitter taste. This is due to the fact that phenolic compunds and flavonoids largely contribute to antioxidant capacity of a plant[3].

Several process must be studied in order to industrially process bitter gourd fruit into various forms of consumer product (e.g. food enrichment product, gelatin capsule, etc.). Extraction is crucial step for the isolation, identification and use of valuable phytochemicals from any plants. Extraction can be done by mixing the plant material with the

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extraction solvent and let the soluble pythochemicals diffuse out of the plant cell walls. This process is known as solid liquid extraction (SLE).

SLE of bitter gourd fruit can be assisted by heating or stirring as performed by Yunita (2010) [4]. Stirring is generally used to increased diffusion rate while heating is used to increase solubility and disrupt the cell structure. However, stirring will not help much in releasing desired component trapped inside hydrophobic cell matrix while high temperature may cause degradation of bioactive compounds responsible for extract antioxidant activity[5].

Advancement in extraction technology reveal the potential of ultrasonic waves to assist SLE and this process is known as ultrasound assisted extraction (UAE) [6]. UAE method may offer several advantages such as simplicity, inexpensive equipment, and remarkable reduction in solvent amount, temperature, and time of extraction [7].

Concentration is another important process to faciliate commercialization since there is a need to reduce costs associated with logistics (packaging, storage, and transporation). Concentrating bitter gourd fruit extract will also result in more health beneficial effect gained by consuming less extract quantity. Concentration can be done by vacuum evaporation (VE) which offer several advantages such as lower boiling temperature required to evaporate the solvent. There is no study providing information about influence of VE towards extract physichochemical charateristic and different concentration factor (CF) influence towards the concentrate stability during storage.

Bitter gourd fruit has a big potential to be developed into productsbenefial for many people. Therefore, this study aims to maximize this potential by studying UAE and VE performance in processing bitter gourd fruit with focus in increasing extract's total phenolic content (TPC), antioxidant activity (AA) and storage stability of the concentrate.

2. Research method

2.1 Materials and Equipment

Bitter gourds were collected from "Pasar Moderen", Tangerang, Banten, Indonesia. Raw bitter gourds were selected visually based on the color (emerald green with no yellow color), hardness and overall condition of the fruit (no bruise). Transparent polypropylene (PP) plastic bags were used to carry the material from the market to the designated research location. The chemical materials and reagent used in this research consist of: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany), Ethanol 99% (PT. Smart Lab, Indonesia), Folin-Ciocalteau phenol reagent (Sigma-Aldrich, Germany), Gallic acid (Sigma-Aldrich, Germany), and Sodium carbonate (AnalaR BDH, England). The equipment used in this research were: Analytical Balance TP 214 (Denver, United States of America), Blender HR2116 (Philips, Indonesia), Digital Balance ED 6202S-CW (Sartorius, Germany), Hot Plate Cimarex (Barnsteadthermolyne, USA), Mesh 35 (Retsch, Germany), Tray Oven (Daihan, South Korea), Sonicator Bath Sonorex Super 10P (Bandelin, Germany), Genesys 10 UV-Vis spectrophotometer (Thermo Electron Corporation, USA), Whatmann filter paper 1001 125 (GE, UK), Centrifuge Rotina 35 R (Hettich, Germany)

2.2 Analytical Procedure and Statistical Analysis

Total phenolic content (TPC) analyses were done using Folin-Ciocalteu method. Folin reagent solution with 1:10 ratio was prepared and Gallic acid standard solutions were prepared into 5 different concentrations: 25 ppm, 50 ppm, 100 ppm, 200 ppm, and 250 ppm. About 1 ml of Folin-Ciocalteau solution prepared was added to 0.2 mL sample, followed by the addition of 0.8 mL of aqueous Na_2CO_3 (7.5%, w/v). The mixture was kept for 1 hour under dark condition. The absorbance was measured with UV-vis spectrophotometer at wavelength 765 nm against blank (distilled water). TPC yield in mg Gallic acid equivalent (GAE) per 100 g dry mass (DM) was calculated accordingly from Gallic acid standard curve.

Scavenging activity of the samples towards 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was measured based on the modified method of Molyneux (2004) [8] and was used as indicator of antioxidant activity (AA). Stock solution of DPPH (250 μM) was prepared by diluting 9.9 g of DPPH powder (Mr = 394.33) with 100 ml ethanol (96% V/V). To test antioxidant activity of the sample, 1 ml of extracts with various concentrations were added with 1 ml of DPPH stock solution (ratio 1:1). Control solution was made by replacing the sample with distilled water. Blank solution containing 1 ml of water and 1 ml of ethanol was prepared prior to incubation. Sample, control, and blank solution were homogenized using vortex and incubated for 30 minutes at 25ºC in a dark chamber. After incubation, the absorbance was read at 517 nm using UV-vis spectrophotometer. The scavenging of DPPH radical (%) was measured by subtracting control absorbance with those of samples and then dividing it with control value.

Unless otherwise stated, statistical test in this research were using 0.05 significance level. Tukey HSD test was done for post hoc test of two ways ANOVA. RStudio (R version 3.20) were used to do the statistical comparison and Minitab 17 trial version were used to assist stability study data analysis.

2.3 Experiment procedure

The experiments were done in series. Firstly, normal solid liquid extraction (SLE) and ultrasound assisted extraction (UAE) were run with varying extraction temperature (25, 35, 45, 55, and 65°C) and extraction time was kept constant (5 minutes). Afterwards, temperature was kept constant at 25°C while extraction time being varied (5, 10, 15, 25, 35, 45, 55, 65, 85, 125 minutes). Solid to solvent ratio used was 1:40 (g/ml) with water as solvent. The results were analyzed using Two Way ANOVA to investigate significance of ultrasound treatment and its interaction with other factors studied in influencing extract physicochemical characteristic.

Different levels of concentration duration which correlate to concentration factor were obtained by VE of extract (@75 ml). The levels were 0, 35, 55, and 75 minutes. VE was run at 125 mmHg, water bath set at 65°C, and condenser set below 20°C. Concentration factor was calculated using equation 1.

$$CF = \frac{B_t}{B_0} \tag{1}$$

Where B_0 and B_t stands for %Brix at initial and at time (t). Linear correlation between concentration factors with physicochemical characteristic was assessed using Pearson's product moment correlation test.

Stability study of the sample was done to estimate its shelf life during storage at different temperature (4°C and 25°C). Extract with different levels of CF obtained by VE were used as sample for stability test. The test was replicated twice. TPC stability was chosen as the key response studied and analyzed every 48 hours for 14 days. AA was measured at Day 0 and Day 14. Batch code and its respective description can be seen in Table 1.

Table 1. Batch code used for stability study

Bath Code	CF Level	Storage Temperature (°C)
1	1	25
2	1	4
3	2	25
4	2	4
5	3	25
6	3	4
7	4	25
8	4	4

3. Results and ddiscussionss

The findings from extraction stage were presented in Figure 1 and Figure 2. Statistical analysis revealed that ultrasound is a significant factor in influencing the extraction process and has interaction with extraction temperature as well as time. Ultrasound assisted extraction (UAE) run at 25°C for 5 minutes was not significantly different than normal solid liquid extraction (SLE) at 65°C for 5 minute or SLE at 25°C for 125 minutes in term of extract's TPC yield and AA. When varying extraction temperature for both UAE and SLE, extract's TPC and AA value was the lowest at 45°C. The value recovered again when extraction temperature was raised by 10°C. These fluctuation may be caused by the action of oxidative enzyme capable of oxidizing polyphenol which is present in almost all plants and has optimum temperature of 45°C [(9)].

TPC and AA profile from various level of extraction time were different between SLE and UAE. Rapid increase followed by slow increase of physicochemical characteristics which were generally observed trend in SLE was also observed in SLE of bitter gourd. In UAE however, TPC and AA were already very high at early phase of extraction (5 minute).Prolonging UAE from minute 5 until minute 45 resulted in decrease of TPC and AA value. In contrast, from minute 45 to minute 125, recoveries of these values were observed.

4.

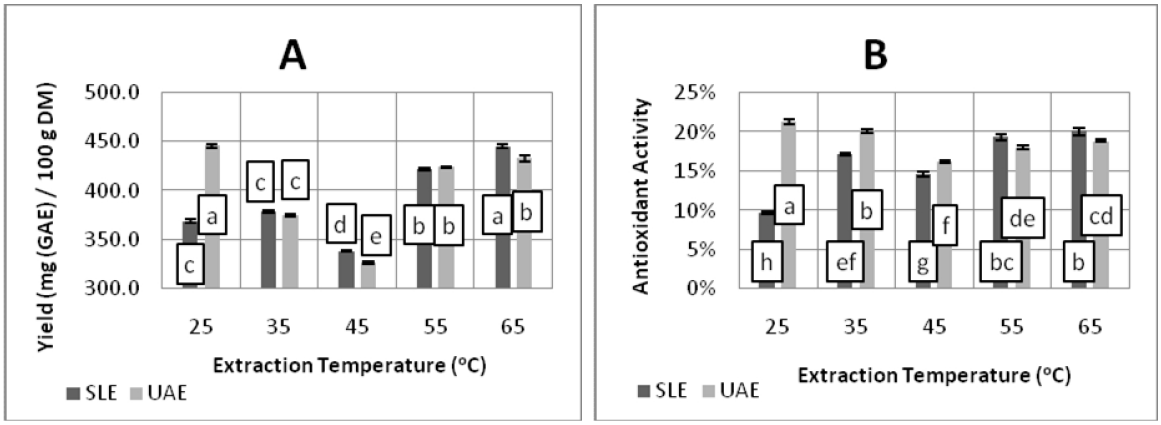

Figure 1. Bitter gourd fruit extract total phenolic content yield (A) and antioxidant activity at 2500 ppm (B) under various extraction temperatures in ultrasound assisted extraction (UAE) and normal solid liquid extraction (SLE). Note: Mean value with the same label is not significantly different.

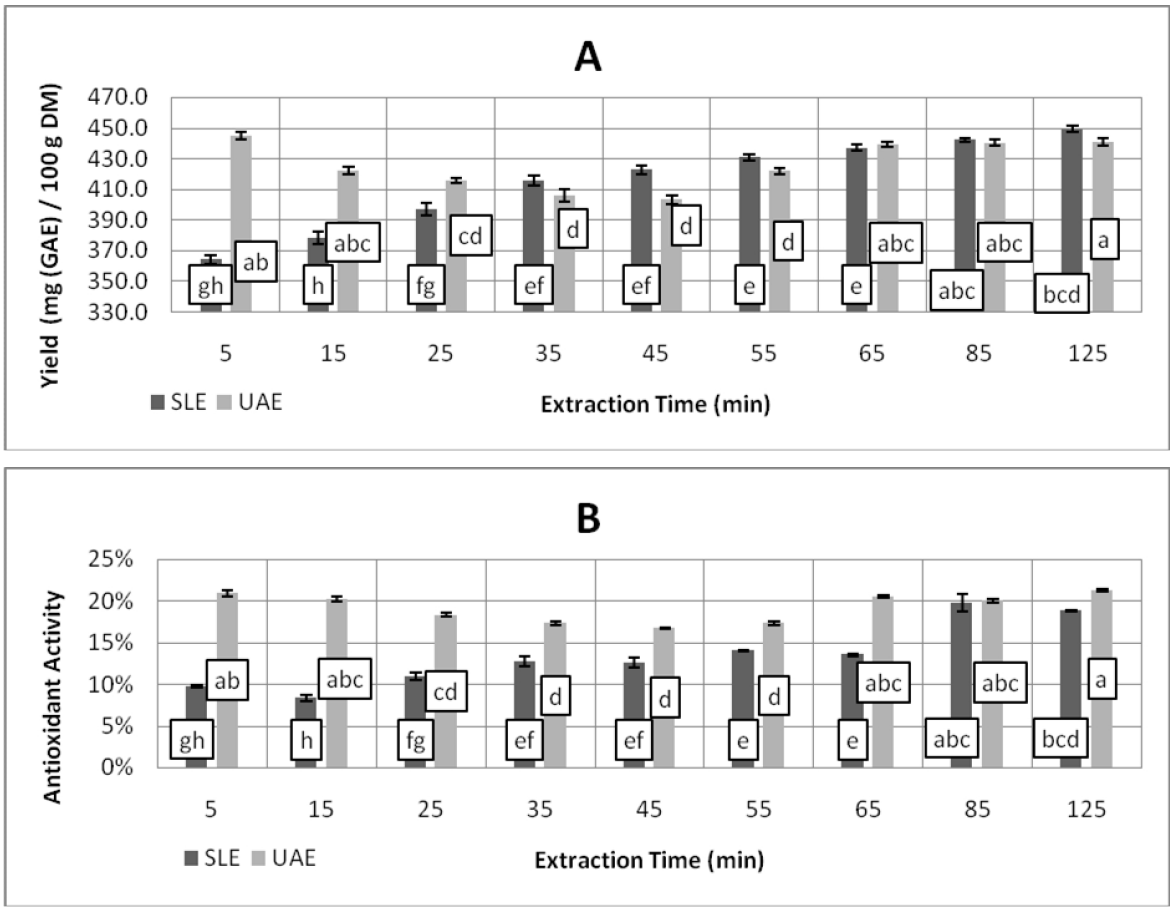

Figure 2. Bitter gourd fruit extract total phenolic content yield (A) and antioxidant activity at 2500 ppm (B) under various extraction times in ultrasound assisted extraction (UAE) and normal solid liquid extraction (SLE). Note: Mean value with the same label is not significantly different.

High value of TPC and AA at initial stage of UAE could happened due to increased mass transfer of soluble phytochemicals from the effect of ultrasound treatment which improve swelling and hydration. Improved swelling and hydration caused enlargement of cell wall pores and in some cases leads to cell rupture [10]. Radical attack may cause phenolic compound to enter state of phenoxy radical which could lead to hydroxylation [11]. This transformation may not happen spontaneously and the decreasing value observed until minute 45 may indicate more phenoxy radical intermediate were present in the extract rather than its various possible end product. After minute 45 of UAE, more products were formed and thus the recovery of TPC and AA value were observed.

VE run with water bath set not to exceed 65°C revealed no significant damage towards the extract physicochemical characteristic. Increasing total soluble solid concentration due to VE process was linearly correlated with the rise of concentrate's TPC and AA as seen in Figure 3.

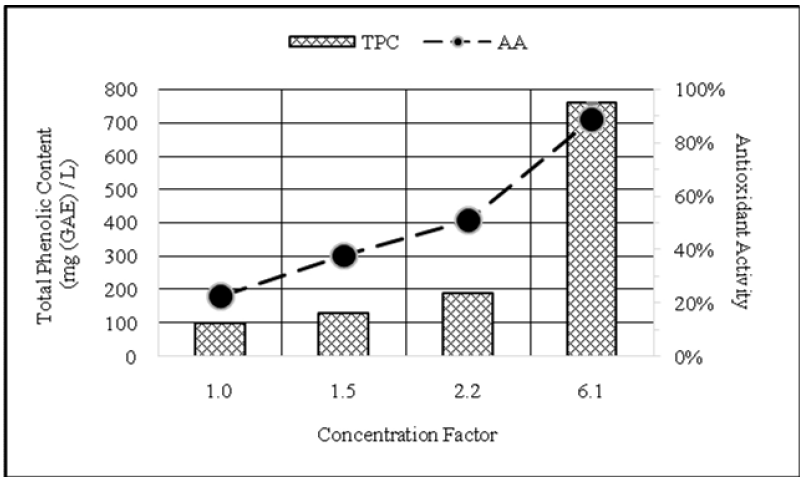

Figure 3. Total phenolic content (TPC) and antioxidant activity (AA) of concentrate with different concentration factor

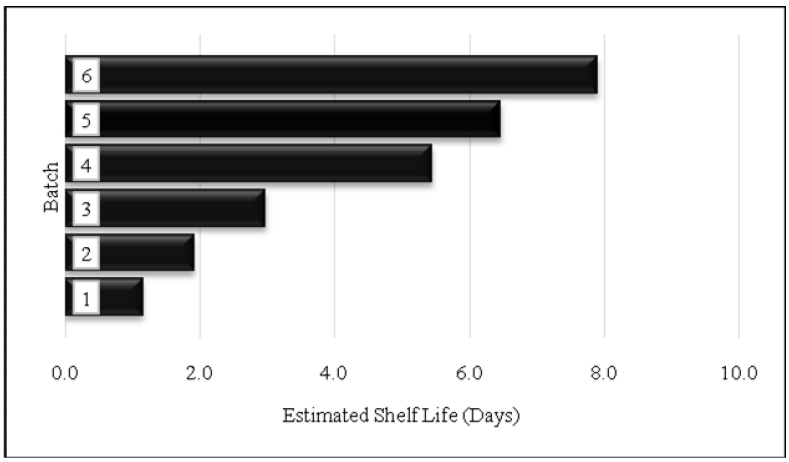

Figure 4. Estimated shelf life of bitter gourd fruit concentrates from different batches.
Note: Refer to table 1 for batch codes description.

Stability study of bitter gourd fruit concentrate for 14 days of the concentrate revealed that higher concentration factor (CF) could improve extract's shelf life without addition of preservative. Lower temperature was also significant in improving the concentrate shelf life. Batch 7 and 8 TPC and AA showed no significant degradation in

quality (does not exceed the fluctuation limit set as ± 5% from initial value) thus its shelf life estimate could not be included in Figure 4. The same could also be observed from AA analysis as seen in Table 2.

Table 2. Stability of bitter gourd fruit concentrates from different batches

Batch	Statistical Grouping	DPPH Radical Scavenging Activity Stability	
		Day 14	% Change
1	g	6.00%	17.00%
2	f	12.00%	11.00%
3	e	23.20%	14.80%
4	d	29.60%	8.40%
5	c	38.30%	12.70%
6	b	43.50%	7.50%
7	a	86.40%	2.60%
8	a	86.00%	3.00%

Low storage stability of sample with low concentration factor may occur since high water content may improve diffusion of oxygen in the sample [12]. This process may leads to degradation of phenolic compounds in the sample by microbial activity or enzyme catalyzed oxidation. Lower storage temperature may lead to better storage stability of the sample since kinetic energy of molecules was reduced at low temperature. This phenomenon may reduce the reaction rate of various chemical reactions which may degrade samples' phenolic compounds.

5. Conclusion

From statistical analysis of the result, ultrasound treatment was significant factor in influencing TPC and AA of the bitter gourd fruit extract. In addition, there was interaction observed between ultrasound treatment with temperature and extraction time. UAE of bitter gourd fruit run at 25^{o}C for 5 minute produced extract with highest value of TPC and AA, and therefore concluded to be the optimum process condition in this research. UAE could be used as an alternative method of extraction by industry to process bitter gourd fruit since it significantly reduce extraction time and temperature required to yield higher physicochemical characteristics desired. Vacuum evaporation between 50^{o}C-65^{o}C to concentrate the extract may improve logistic consideration (lower volume, better storage stability) without significantly damaging bitter gourd extract's TPC and AA.

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