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Extraction and Quantitative Determination of Ascorbic Acid from Banana Peel Musa Acuminata 'Kepok'

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Abstract – *This paper discusses the extraction of antioxidant compound, which is ascorbic acid or Vitamin C, from banana peel using ultrasound-assisted extraction (UAE) method. The type of the banana used was a cooking banana, Musa Acuminata or also known as "Pisang Kepok" in Malaysia. The investigation includes the effect of solvent/solute ratio (4.5, 5g and 10 ml/g), sonication time (15, 30 and 45 mins) and temperature variation (30, 45 and 60 °C) on the extraction of ascorbic acid compounds from banana peel to determine the best or optimum condition of the operation. Out of all extract samples analyzed by redox titration method using iodine solution, it was found that the highest yield was 0.04939 ± 0.00080 mg that were resulted by an extraction at 30 °C for 15 mins with 5 ml/g solvent-to-solute ratio.*

Keywords: Musa Acuminata, Ultrasound-Assisted Extraction, UAE, Vitamin C, Redox Titration

1.0 INTRODUCTION

Banana is one of the main crops in the world that has significance to human for its nutrition and mineral contents. It must have been one of the first known tropical crops since there are records of it being in cultivation 4000 years ago [1]. According to [2], edible bananas originated from Melesia, a biogeographical region consisting of the primary centre including Malaysia, Indonesia, Philippines and New Guinea as well as the secondary centre which is India.

Every part of a banana plant has its own usage from a good source of nutrients to religious ceremonies. In Africa and Asia, banana fruit is a source of starch staple food and dessert bananas are also a main cash crop in many developed and developing countries [3]. Their contribution to human health is due to its great medicinal value. Banana fruit is claimed to prevent anemia, regulate blood pressure, prevent constipation, cure heartburns and prevent stroke [4]. Even the leaves are used as food wrappings and plates for its pleasant smell [5].

Due to the consumer demand for products from natural sources, the economic impact of plant-based extracts especially for food, nutraceuticals, cosmetics, flavors or fragrances, and pharmaceutical industry has developed in the last few years. Extraction technology like ultrasonic-assisted extraction and many other methods are usually used to fulfill the demands.

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Sound frequency more than 18 kHz is considered to be ultrasound and a lot of studies have been done on the application of both high and low power ultrasound [6]. The different intensity of powers has different usage in various fields. Low powers ultrasound are mainly applied in medical ultrasonography while high powers ultrasound have numerous uses such as ultrasonic welding, degassing of solutions, defect detection and sonochemistry [6].

According to Mason and Lorimer (1988), sonochemistry is a century old since it has been under ongoing investigation for over 50 years. Ultrasonic cleaning bath has been introduced into metallurgy and chemical laboratories in 1960's and considering how soiled glassware being cleaned and immiscible organic solvents dispersed in aqueous detergent, it was not surprising that it is being used by chemists to enhance chemical reactivity [7].

An important phenomenon associated with power ultrasound is called cavitation bubbles. When liquids are sonicated at high ultrasound intensities, the sound waves that propagate into the liquid media causes alternating high-pressure (compression) and low pressure (rarefaction) cycles, where rates are dependent on the frequency [8]. The bubbles are produced during rarefaction cycle of the wave when the liquid structure are broken resulting in the formation of tiny voids which collapse in the compression cycle [7].

According to Tabaraki and Nateghi (2011), antioxidants extracted from plants via ultrasonication promote sustainable green process as it replaces synthetic antioxidants. However, they found that at optimum operating condition, only 11 to 20.1 % yields were obtained by using rice bran which is considerably low. Therefore, this study explores the possibility of obtaining higher antioxidants yield by applying ultrasonication on other type of plant particularly banana.

2.0 METHODOLOGY

2.1 Materials

Banana from species *Musa Acuminata 'Kepok'* were obtained from Kota Samarahan Market in the state of Sarawak, Malaysia. The maturity of bananas was observed visually which can be described as follows; the bananas were fully ripe and have yellow skin. Besides, Methanol 99.8% (Sigma-Aldrich), Iodine 99.9% anhydrous (Sigma-Aldrich), Potassium Iodide anhydrous (ACS reagent) used were of analytical grade while Vitamin C was obtained from Abbot Laboratories, USA.

2.2 Sample preparation

Initially, all bananas were washed with municipal tap water to remove soil or dust. Then the cleaned bananas were peeled and the peels were collected for sampling. The peels were freeze-dried using FreeZone® 4.5 Liter Freeze Dry Systems (Labconco, USA) which was set to run automatically at pressure and temperature of 7 bar and -30 ºC respectively before sliced into smaller pieces using IKA A11 Basic Analytical mill (Sigma-Aldrich, USA) to a fine powder form.

2.3 Extraction of antioxidant

The ultrasonic-assisted extraction of ascorbic acid from dried banana peel were executed in an Elmasonic S900H ultrasonic bath (Elma Schmidbauer GmbH, Germany) at a constant

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power of 2000 W and ultrasonic frequency at 37 kHz. The extraction was conducted with different solvent-to-solute ratio, temperature and sonication time. The solvent used was 60 % methanol. It must be noted that single electron transfer and hydrogen atom transfer are affected by the type and polarity of the solvent used to extract ascorbic acid from banana peel [9].

10 g of fine banana powders was used for this experiment. The experiment was conducted with a solvent-to-solid ratio of 4.5 ml/g, 5 ml/g and 10 ml/g. The samples in the beaker were then transferred to a 250 ml sample bottles and were placed in the ultrasonic bath to be extracted at different experimental conditions. Sonication time of extraction was varied at 15, 30 and 45 mins while the temperature at 30 ℃, 45 ℃ and 60 ℃. Extraction temperature was controlled by a built-in function of ultrasonic bath. After that, the crude extracts were filtered using Whatman filter papers before the extracts were heated until a significant amount of methanol solvent evaporated which was collected to estimate the oil yield. The extracts were then titrated with Iodine to determine the ascorbic acid or Vitamin C content.

2.4 Quantification of ascorbic acid

A 500 mg Vitamin C tablet was used for the construction of the standard curve. The single tablet was dissolved in 200 ml distilled water where 1 ml aliquot was taken using a pipette out of the sample solution and transferred into a 250 ml conical flask. 1 ml of starch indicator solution was added and the flask was filled to 200 ml with distilled water.

The sample was titrated with 0.005 mol/L iodine solution. The endpoint of the titration was identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex. It was repeated with further aliquots of sample solution until concordant results were obtained. Titration was repeated three times and the average value was taken.

1 ml of sample was diluted in a 250 ml conical flask with distilled water filling to 200 ml. Then, 50 ml aliquot of the solution was taken for titration and mixed with 1 ml starch solution and diluted again with distilled water to 200 ml. The sample was again titrated with 0.005 mol/L iodine solution. The end point of the titration was identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex. The titration was repeated three times with the same amount of aliquots of sample solution until concordant results were obtained and the average volume of iodine used was calculated.

3.0 RESULTS AND DISCUSSION

3.1 Effect of sonication time on Vitamin C yield

Fig. 1(a) shows the effect of sonication time; 15, 30 and 45 mins, solvent to solid ratio; 4.5ml/g, 5ml/g, 10ml/g, and extraction temperature: 30 °C on the extraction yield of Vitamin C from banana peel. The methanol concentration: used throughout this experiment is kept at a constant concentration which is 60%. At 30℃, the yield decreases as the time is extended from 15 to 30 mins. This may happen because longer sonication time can affect the extraction results since the extra sonication can degrade the quality of extracts [10]. The increase of yield occurred as the time increased to 45 mins. At 15 mins, 0.04939 ± 0.00080 mg of yield were obtained from extracts which is the highest yield throughout the experiment whereas at 30 mins and 45 mins, 0.03046 ± 0.00138 mg and 0.03416±0.00080 mg were obtained

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respectively. Both are lower than the yield at 15 mins by 61.68 % and 69.16 % correspondingly. Ultrasound usage in extraction makes the processing conditions for other non-ultrasonic extractions like temperature and pressure to decrease [11][12].

At 30 °C (Figure 1(a)) and solvent to solid ratio of 4.5 ml/g and 5ml/g both indicates that the yield levitates as the time is extended from 15 to 30 mins. However, the increase is immediately diminished when sonication time is extended to 45 mins. This is also true for the yield obtained at 45 °C (Figure 1(b)) and solvent to solid ratio of 10ml/g At 60 °C (Figure 1(c)), all three ratios shows the same pattern with the same temperature changes.. In the view of time usage, it is not recommended to exceed 30 mins.

Sonication time plays an important part in this extraction too. As discussed earlier, longer sonication time causes less ascorbic acid yield because the cavitation depletes as the sonication time increases. The optimum time found in this study indicates the lowest time experimented. Lesser time for extraction is feasible and practical for this extraction to be done at industrial scale.

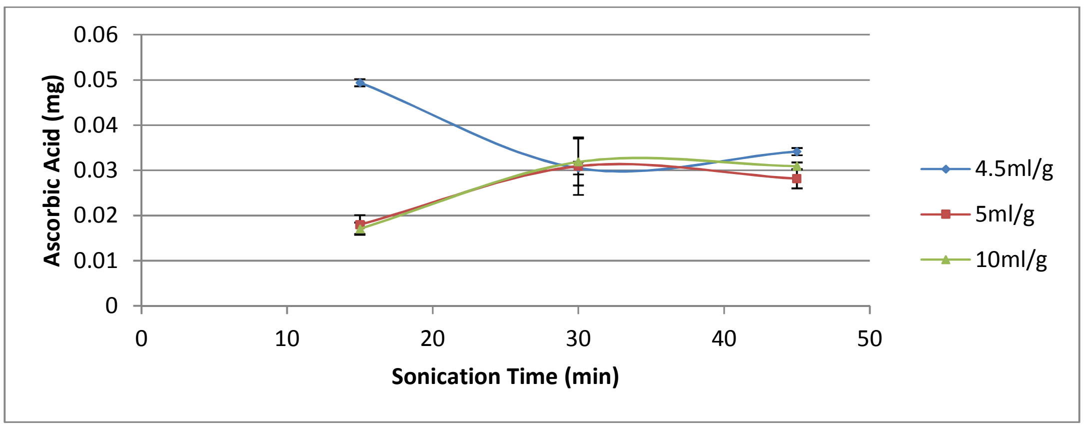

Figure 1(a): Effect of ultrasonication time; 15, 30 and 45 mins, solvent-to-solid ratio; 4.5ml/g, 5ml/g, 10ml/g, and extraction temperature: 30 °C on the extraction yield of Vitamin C from banana peel. Methanol concentration: 60%

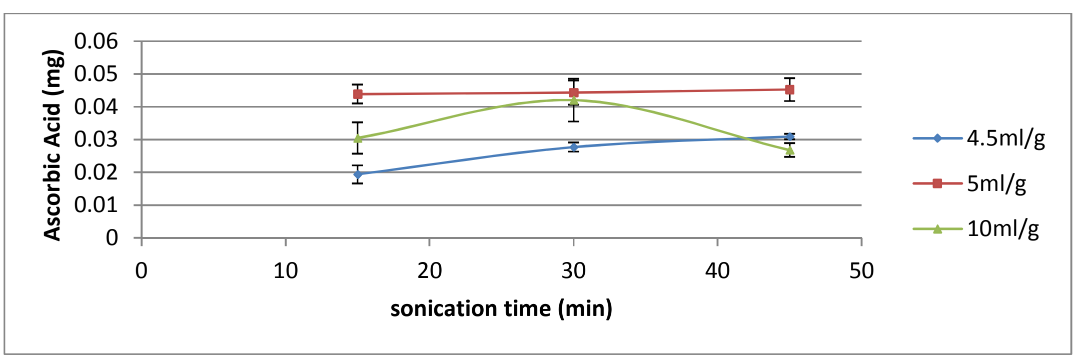

Figure 1(b): Effect of ultrasonication time; 15, 30 and 45 mins, solvent-to-solid ratio; 4.5ml/g, 5ml/g, 10ml/g, and extraction temperature: 45 °C on the extraction yield of Vitamin C from banana peel. Methanol concentration: 60%

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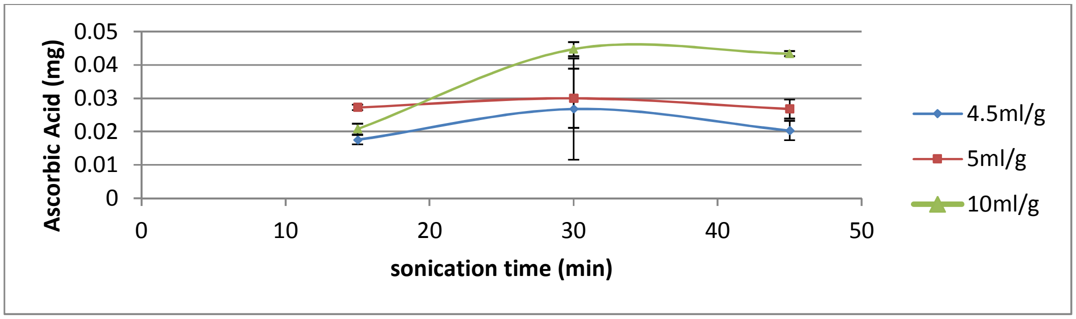

Figure 1(c): Effect of ultrasonication time; 15, 30 and 45 mins, solvent-to-solid ratio; 4.5ml/g, 5ml/g, 10ml/g, and extraction temperature: 60 °C on the extraction yield of Vitamin C from banana peel. Methanol concentration: 60%

3.2 Effect of Temperature on Vitamin C yield

Fig. 2 shows the effect of temperature on the extraction yield of Vitamin C from banana peel. Using solvent-to-solid ratio of 4.5 ml/g and sonication time of 15 mins, the extraction yield of Vitamin C decreases as temperature was increased from 30 °C to 60 °C. The temperature was manually changed by the built-in functions of the ultrasonic.

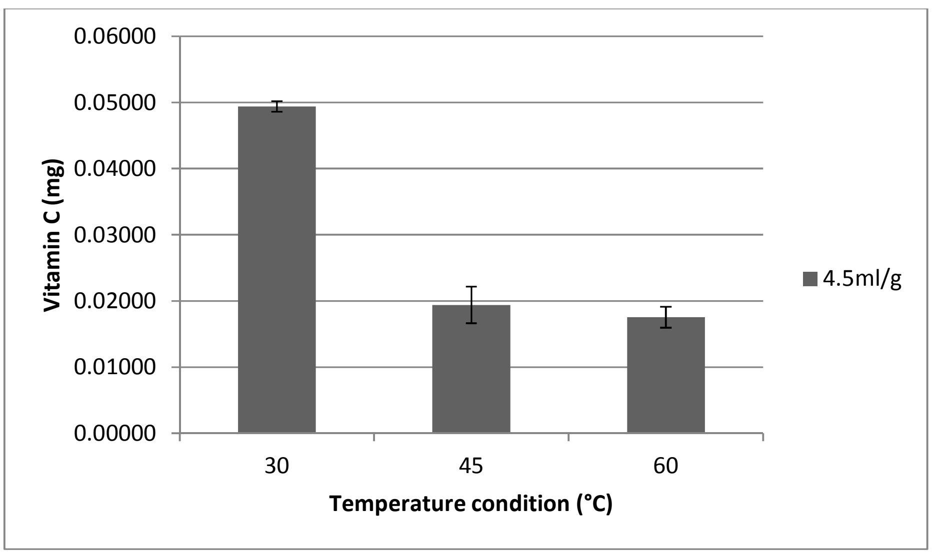

Figure 2: Effect of Temperature Changes at 15 mins and 4.5 ml/g Solvent to Solid Ratio

The highest yield was obtained at 30 °C with the value of 0.04939 ± 0.00080 mg which was 60.7% higher than yield at 45°C. At 30 °C, the mass transfer has improved due to the increasing solubility of Vitamin C and the decreasing viscosity of the solvent.

The yield at 45 °C is 60.75 % less than the yield at 30 °C while at 60°C, it is lower by 64.49 %. This may be caused by the degradation of Vitamin C at elevated temperature where the ultrasonic probe causes an increase in the bulk temperature [13].

When the amplitude is higher, the temperature increases faster that it can go beyond the desired temperature in a short period of time. The most apparent effects that can occur are the degradation of compounds tested, and the volatilization of low volatile analytes. In addition,

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the increase of temperature changes the physical characteristics of the liquid media in such a way that it affects the ultrasonic transmission with no cavitation achieved, which is a phenomenon known as decoupling [14][15].

As temperature is a very important parameter, it must be controlled but it is not easy to achieve the desired value. This has also been admitted by Salisova et al, (1997) where they stated that it is crucial to accurately control the extraction temperature when ultrasound is used for it constantly generates heat. On the other hand, lower heat supply in addition to the heat generated by ultrasound calls for a lesser energy use in the industry which could attracts more interest on its investment [16].

3.3 Effect of solvent-to-solid ratio on Vitamin C yield

Figure 3 displays the effect of solvent-to-solid ratio on the extraction yield of Vitamin C from banana peel. The experiment was carried out with constant methanol concentration of 60 %, extraction temperature of 30 °C and sonication time of 30 mins. The yield shows an increasing trend as the solvent-to-solid ratio increases. At 4.5 ml/g solvent-to-solid ratio, the extraction yielded 0.03046 ± 0.00138 mg of Vitamin C and a slight increase to 0.03093 ± 0.00524 mg can be seen as the ratio was increased to 5 ml/g. It increased further to 0.03185 ± 0.00635 mg at 10 ml/g solvent-to-solid ratio.

A larger solvent volume can dissolve targeted components more efficiently and therefore resulted in an enhancement of the extraction yield. The observation therefore obeys the mass transfer principle, where the driving force during the mass transfer was the concentration gradient between the solid and bulk liquid in which was escalated with a higher solvent-to-solid ratio usage [17][18].

At 60 °C, the Vitamin C yields also exhibited increasing value when the solvent-to-solid ratio was increased. From Fig. 3, it can be seen that extracts obtained by using 4.5 ml/g contained 0.02677 ±0.00212 mg Vitamin C and had10.76% more that was 0.03000 ±0.00890 mg at 5 ml/g ratio. The highest yield obtained was when 10 ml/g solvent was used that resulted with 0.04477 ±0.01525 mg Vitamin C. This shows that the extraction yields more compounds with more solvent used.

Ultrasound-extraction at 30°C yielded higher ascorbic acid at any solvent-to-solid ratio in comparison to the extraction at 60 °C. For instance at 4.5 ml/g solvent, the difference between the yield values of ascorbic acid at 30°C and 60°C were 12.12 % and 2.99 % at 5 ml/g.

This substantiated the findings discussed in section 3.2 that the decrease in Vitamin C was caused by thermal effect as it is a sensitive molecule that is altered at temperature above 60°C. The presence of ultrasound had caused the temperature to increase beyond the targeted temperature, so lower heating supply is needed for a particular extraction temperature. The phenomenon where decreasing acoustic cavitation bubbles created by the ultrasound had also caused the yield to decrease at 60°C. Therefore, even though the yield increased with increasing solvent-to-solid ratio, the yield had a slight decrease when the temperature was increased.

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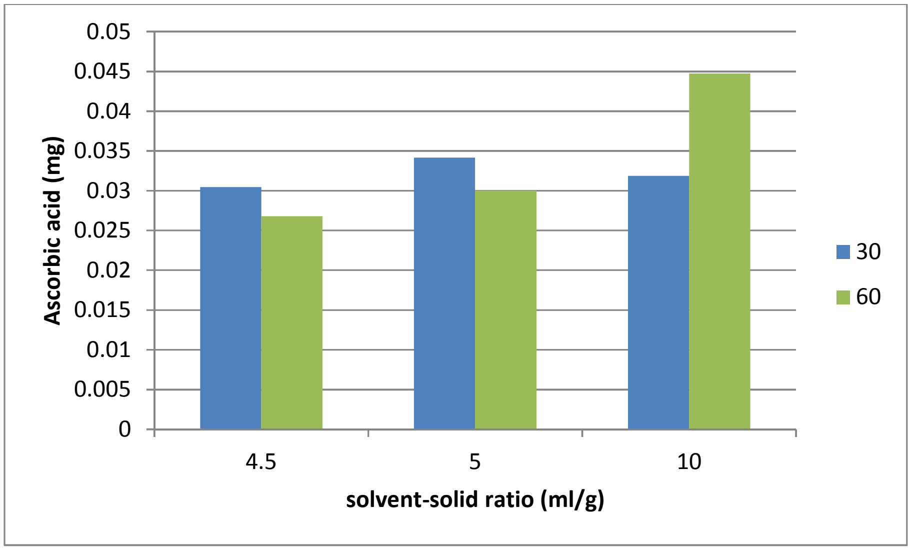

Figure 3 Comparison between yields at 30°C and 60 °C, solvent to solid ratio of 4.5, 5 and 10 ml/g and sonication time of 30 mins.

4.0 CONCLUSSION

Ultrasonic extraction provides cavitation towards the banana peel samples. Such phenomenon gives the advantage to extraction process through the mechanical disruption of the cell structure causing it to release its contents. Another is the heat it provides to the liquid which causes an increase to the diffusion. The high efficiency of mass transfer across the solid-liquid boundary is greatly affected by the kinetic energy applied by the cavitation phenomenon. From the results obtained in this study, ultrasonic-assisted extraction method is a feasible method to extract desired compounds from the banana peel.

The investigation of the effect of solvent-to-solute ratio, sonication time and operating temperature on the extraction of ascorbic acid compounds from banana peel was conducted to determine the best or optimum condition for this extraction. From all extract samples analyzed by redox titration method using iodine solution, it was found that the highest yield was 0.04939 ± 0.00080 mg obtained at the temperature 30 ℃, sonication time of 15 mins and solvent-to-solid ratio of 5 ml/g amongst other conditions.

From the discussion, extraction yield increases with the increase of solvent used. Nevertheless, the type of solvent used is also a significant factor, because the use of methanol for example, is a very good solvent but very toxic and harmful for human consumption.

Even though a conclusion may review the main results or contributions of the paper, do not duplicate the abstract or the introduction. For a conclusion, you might elaborate on the importance of the work or suggest the potential applications and extensions.

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