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EFFECT OF ULTRASONIC-ASSISTED EXTRACTION ON PHENOLIC CONTENT OF AVOCADO

(Kesan Pengekstrakan Bantuan Ultrasonik ke Atas Kandungan Komponen Phenolik Dalam Buah Avokado)

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Abstract

This study evaluate the effect of ultrasonic application in the extraction process on total phenolic content (TPC) of Hass avocado (*Persea americana* Mill) pulp. In this study, the solid/solvent ratio of 1/30 (wt/vol) and extraction temperature of 40°C gave higher TPC value. This ratio and temperature was applied in the ultrasonic-assisted extraction (UAE) of avocado pulp. This study then compared the TPC obtained from the avocado pulp extract without involving ultrasonic and the TPC obtained from the UAE. Results showed that the TPC value of avocado pulp was significantly higher in the UAE (235.77 mg GAE/100g dried sample) compared to the TPC in the non-UAE (166.32 mg GAE/100g dried sample). The increase in the TPC was between ~31% and ~41% when 5 to 20 min of ultrasonication applied in the extraction. Ultrasonication duration of 15 min gave the highest TPC where the value was significantly higher compared to the other duration.

Keywords: avocado pulp, ultrasonic-assisted extraction, total phenolic content

Abstrak

Kajian ini memperlihatkan kesan aplikasi ultrasonik dalam proses pengekstrakan ke atas jumlah kandungan komponen phenolik (TPC) dalam isi buah avokado (*Persea americana* Mill). Dalam kajian ini, nisbah pepejal/pelarut 1/30 (berat/isipadu) dan suhu pengekstrakan 40°C telah memberikan nilai TPC yang lebih tinggi. Nisbah dan suhu ini kemudiannya diaplikasikan dalam pengekstrakan bantuan ultrasonic (UAE) bagi isi buah avokado. Kajian ini kemudiannya membandingkan di antara nilai TPC dalam isi avokado yang telah diekstrak tanpa UAE dengan nilai TPC dalam ekstrak avokado yang telah melalui UAE. Keputusan telah menunjukkan bahawa nilai TPC adalah lebih tinggi (perbezaan bererti) dalam ekstrak isi avokado yang telah melalui UAE (235.77 mg GAE/100g sampel kering) berbanding ekstrak isi avokado tanpa UAE (166.32 mg GAE/100g sampel kering). Peningkatan dalam nilai TPC adalah di antara ~31% dan ~41% apabila 5 hingga 20 minit ultrasonik diaplikasikan. Tempoh ultrasonik 15 minit adalah tempoh yang memberikan nilai TPC yang paling tinggi (perbezaan bererti) berbanding tempoh lain yang digunakan.

Kata kunci: isi avokado; pengekstrakan bantuan ultrasonik; kandungan komponen phenolik

Introduction

The avocado (*Persea Americana* Mill), belonging to the Lauraceae family is a tropical-subtropical fruit native to Mexico and Central America. They ripen after being harvested where the fruit depicts a green-skinned, fleshy body that resembles the shape of a pear [1]. Many studies have reported on the bioactive phytochemicals of this fruit. They include carotenoids, phenolic acids and flavonoids [2–4]. Antioxidant activities have also been reported in avocado [5]. It has been reported that avocado contains many different polyphenols including catechins,

hydroxybenzoic acids, hydroxycinnamic acids, procyanidins, flavanol, gentisic, chlorogenic, caffeic acids, laricitrin, aringenin, chrysin, kaempferide, protocatechuic, p-coumaric, ferulic, sinapic, and benzoic acids [4–6].

Avocado extract is usually obtained by solid-liquid extraction with different solvents. In research involving plants, various extraction methods have been developed for the extraction of phytochemicals in order to increase the extraction yield, shorten the extraction time as well as enhance the quality of extracts. Ultrasound-assisted extraction is among the methods used to enhance the process of extraction. It is inexpensive, simple and efficient alternative to conventional extraction techniques. Like soxhlet extraction, it can be used with any solvent for extracting a wide variety of natural compounds. In solid-liquid extraction, it offers advantages which include increased yield and faster kinetics. The apparatus is cheaper and easier to operate compared to other novel extraction techniques such as microwave-assisted extraction and supercritical fluid extraction [7].

Ultrasound-assisted extraction is widely used in the extraction of plant compounds. However, no information is available on the effect of ultrasound-assisted extraction on polyphenol content in avocado. This study is carried out to evaluate the usage of ultrasound in overcoming the limitation of conventional extraction of avocado fruit as well as its effect on the phenolic content of the fruit.

Materials and Methods

Folin-Ciocalteu's phenol reagent and gallic acid ($C_7H_6O_5$) were purchased from Sigma-Aldrich, Germany. Sodium carbonate (Na_2CO_3) was purchased from Merck, Germany. All chemicals were of analytical grade.

Sample preparation

Fresh avocado fruits (Hass avocado) were purchased from the local grocery store in Kitakyushu, Japan. Once ripened, all the fruits were cleaned, cut and peeled. The pulp of the fruit was dried using a freeze dryer (EYELA FDU-1200, Tokyo Rikakikai Co. Ltd Japan) and used in this study. The dried pulp was ground to fine powder using Waring commercial blender 8011S (Connecticut, USA) and kept at -40°C until further use.

Sample extraction

One gram of powdered sample was extracted in 80% ethanol where ratio of solid/liquid were at 1/30 and 1/40 (wt/vol). Extraction was carried out at 400 rpm at the temperature of 30°C and 40°C for 2 hours using Heidolph Instrument Unimax 1010DT orbital shaker, Germany. The extracts were then filtered and analysed for total phenolic content. The solid/liquid ratio and temperature which gave better result was selected and applied in ultrasonic-assisted extraction.

Ultrasonic-assisted extraction (UAE)

One gram of powdered sample in 80% ethanol was ultrasonicated for the duration of 5, 10, 15 and 20 min using a 37kHz ultrasonic generator (UT-106, SHARP, Japan). The mixture was then centrifuged at 400 rpm for 30 min using Heidolph Instrument Unimax 1010DT orbital shaker, Germany. The extracts were filtered and used for further analysis.

Total Phenolic Content

Total phenolics were determined using a modified Folin-Ciocalteau colorimetric method [8,9]. A volume of 0.25ml of ethanolic extract was mixed with 1ml distilled water in a test tube. 0.25ml Folin-Ciocalteau reagent was added to the solution and allowed to react for 6 min. Then, 2.5ml of 7% sodium carbonate solution was added into the test tubes, and the mixture was diluted to 6ml with deionized water. Each sample was allowed to stand for 90 minutes, and the absorbance was measured at 760 nm using UV-Vis spectrophotometer UV1601 (Shimadzu Corporation, Australia). The measurement was compared to a standard curve of gallic acid concentrations and expressed as milligrams of gallic acid equivalents (GAE) per 100g dried sample.

Statistical Analysis

Experiments were performed in triplicates. The results were expressed as mean ± standard deviation (S.D). The experimental data were analysed using analysis of variance (ANOVA). The mean values were considered at the 95% confidence level ($p = 0.05$). Statistical analysis was done using IBM SPSS Statistics (version 19).

Results and Discussion

Total phenolic content

In avocado, the pulp is consumed while the peel and seed are considered as wastes. It has been reported that the moisture content of avocado pulp was at ~74% [10,11]. In determining total phenolic content of avocado pulp, freeze dried material was prepared in this study. The total phenolic content of the pulp were estimated using the Folin-Ciocalteu method, which relies on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium [12].

As recorded in Table 1, solid/liquid (wt/vol) ratio that gave a higher total phenolic content (TPC) reading was 1/30 at both temperatures used. Among the two temperatures, TPC was higher at the temperature of 40°C for both solid/liquid ratios. Thus, it was concluded that TPC was highest at solid/liquid ratio of 1/30 and at the temperature of 40°C. This condition gave the best result and it was taken as the best condition to perform further extraction.

Table 1. Total phenolic content of avocado pulp without UAE.

	Temperature			
	30°C		**40°C**	
Wt/Vol (g/ml)	mg GAE	±S.D.	mg GAE	±S.D.
1/30	163.27	1.43	166.32	0.98
1/40	130.21	2.26	155.95	2.26

GAE, gallic acid equivalents per 100g dried sample. Mean ±S.D. n=3.

Effect of UAE on total phenolic content

As indicated in Table 2, when extraction was repeated using UAE method, the TPC readings were higher compared to the extraction without UAE (166.32 mg GAE/100g dried sample extracted using 1/30 ratio at 40°C). The increase in TPC was at ~31% – 41% when 5 to 20 min of ultrasonication was applied. Among the four different durations used, the duration of 15 min gave the highest TPC reading at 235.77 mg GAE/100g dried sample. This TPC value was significantly higher when compared to the other duration.

Table 2. Total phenolic content for UAE of avocado pulp with different ultrasonication duration

	mg GAE	**±S.D.**	**% Increase**
5 min	226.69	0.56	36.30
10 min	219.19	0.28	31.79
15 min	*235.77	0.80	*41.75
20 min	222.44	0.85	33.74

GAE, gallic acid equivalents per 100g dried sample. Mean ±S.D. n=3. *$p < 0.05$.

Ultrasound-assisted extraction is an attractive alternative to conventional extraction techniques because it is easy, inexpensive and efficient. The main benefit of including ultrasound in an extraction procedure is that it increases yield and the extraction process can be done at a faster rate [7]. In this study, the usage of ultrasound has successfully enhanced the extraction process as indicated by the significantly higher TPC values and furthermore, has reduced the centrifuge time from 2 hours to 30 minutes (75% reduction).

This is made possible due to the propagation of ultrasound pressure waves through the solvent resulting in cavitation phenomena. The controlling mechanism of ultrasound-assisted extraction is generally attributed to mechanical, cavitation, and thermal effects which can result in disruption of cell walls, particle size reduction, and enhanced mass transfer across cell membranes, which lead to target compounds dissolving in the solvent, hence increasing yield with shorter time [13]. Research have found that ultrasonication is a critical pretreatment to obtain high yields of oils from almond, apricot and rice bran [14]. For extraction of saponin from ginseng assisted by ultrasound, the total yield and saponin yield increased by 15 and 30%, respectively [15]. The yield of oil extracted from soybeans also increased significantly when ultrasound was applied [16].

Conclusion

The TPC of avocado pulp extracted with and without the application of ultrasonic were compared in this study. The results clearly indicated that the values of TPC were significantly higher when the extraction procedure included ultrasonic as assistance in the method. This study showed that avocado extraction can benefit from UAE especially by reducing the extraction time.

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