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Enhancing Hydroxycitric Acid Yield in *Garcinia atroviridis*: A Strategic Optimization Approach

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Abstract *Garcinia atroviridis* (asam gelugur) is one of the underutilized plants used frequently in medicine as fruit for reducing weight and excess body fat by halting the glycogen process. The principal acid in this fruit is (-)-hydroxycitric acid (HCA). The objective of this research was to enhance the concentration of HCA in garcinia powder, intended for utilization as an ingredient in a range of health food items designed to aid in weight management. A pre-treatment method was used on the fruit to achieve the optimal level of HCA content. Three maturity indexes were employed to evaluate and determine the optimum level of HCA. Ten different pre-treatments were analyzed (five pre-treatments added sodium meta-bisulfate) and (five pre-treatments without sodium meta-bisulfate). The existing method for the determination of HCA uses an acid-base titration which gives the total acidity of fruit extracts. The analysis showed that all treatments that added sodium meta-bisulfate gave lower acidity content significantly compared to pre-treatment without sodium meta-bisulfate. Five pre-treatments of the sample showed that the blanching process and enzyme added gave the optimum acidity content compared to the other three treatments. There was no notable distinction in acidity content between the blanching and enzyme-added methods, registering at 45.6% and 46.84%, respectively. These two samples were chosen for the determination of HCA using HPLC. HCA content for blanching and enzyme added were 1.23 and 1.87 gm/freeze-dry samples, respectively. The findings indicate that there were no statistically significant variations in acidity levels between samples at 3 months and 4 months of maturity. Similarly, the analysis of HCA content revealed only marginal differences between the two maturity stages.

Keywords: *weight management, underutilized plants, pre-treatment, maturity index*

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1. Introduction

Obesity has become a global epidemic and a public health crisis, particularly in recent decades, and the prevalence of obesity is still increasing at an alarming rate [1]. The worldwide growth in being overweight or obese is largely due to dietary and lifestyle changes. In Malaysia, approximately 15.7% of adults are obese [2]. According to World Health Organization (WHO) statistics from 2016, more than 42% of Malaysians are overweight. Furthermore, Malaysia has the second-highest obesity rate among children and adolescents in Asia, at around 12.7% [3]. Globalization affects Malaysia, causing a change from traditional diets and lifestyles to more Westernized versions. Obesity increases the risk of chronic diseases such as type 2 diabetes, cardiovascular diseases, certain cancers, dyslipidemia, poor mental health, and osteoarthritis [4].

It's not surprising given the obesity and obesity-related health risk statistics that the market for weight loss products continues to grow locally and globally. Due to this lucrative industry and advancement in technology, the competition and marketing efforts of every weight loss product are intensified. Some of these products are formulated with ingredients that have been used traditionally for weight loss.

Potential new foods or compounds should be investigated to help prevent obesity. *Garcinia atroviridis*, known as Asam Gelugur, asam gelugo, or asam keping in Malay is a medium-sized fruit and a large rainforest tree native to peninsular Malaysia. Asam gelugor is frequently used to provide a sour flavor to foods and is typically processed as dried slices. Additionally, it is employed as a conventional treatment for post-partum discomfort. The leaves are also used to treat obesity and high blood pressure. The possibility for garcinia products to be commercialized is increased with the development of such a plethora of products as well as planting material will increase the value of the species within the community and thus contribute to the conservation of the species.

The principal acid in this fruit is (-)-hydroxycitric acid (HCA) [5] is commercialized as a weight management product. It plays crucial roles in body weight and

appetite; it prevents the hepatic enzyme production of ATP citrate-lyase. This enzyme converts excess carbohydrates into fat. It also stimulates hepatic glycogen synthesis from glucose [6].

There was limited data on garcinia's clinical uses in treating any kind of health condition. However, an interesting investigation conducted in Thailand using obese women as subjects resulted in promising HCA as an effective weight management agent [7]. Obese women who received HCA as garcinia lost more weight than the control group. It was also proved by a significant reduction in triceps skin fold thickness. A further clinical investigation may be proposed since such a finding provides promising insight into HCA's benefits in managing weight. Moreover, garcinia contains many active constituents that could provide a promising source for treating various diseases. Thus, the current work aimed to enhance the concentration of HCA in garcinia powder using various pre-treatments. In addition, the maturity index has been investigated, given its high content to HCA. For this purpose, the acidity content was determined for the pre-treatment process, and the HCA content was analyzed with HPLC.

2. Material and Methods

2.1. Chemical and Reagents

Sodium metabisulfite, sodium hydroxide, phenolphthalein, acetonitrile, methanol, and enzyme pectinase from *Aspergillus niger* were purchased from Sigma-Aldrich (Merk KGaA, Germany).

2.2. Materials

Fresh garcinia fruits were collected from Kampung Gajah, Perak. Garcinia at three different maturity unripe (1 month), partially ripe (3 months), and fully ripe (4 months) with no presence of injury was selected for the study. The fruits were stored at room temperature after arriving at the Food Science and Technology Research Centre, MARDI Serdang before being processed.

2.3. Sample Preparation

Upon arrival at the laboratory, the fruits were cleaned and washed using a washer for 15 minutes. The edible portion of the fruits was cut and ground prior to the pretreatment process. The maturity stage of four months was chosen for conducting acidity analysis on 16 pre-treatments (8 pre-treatment added sodium meta-bisulfate) and (8 pre-treatment without sodium meta-bisulfate).

2.4. Proximate Compositions

Proximate analysis was conducted using the Association of Official Analytical Chemists [8]. The moisture content was determined by drying samples (10 g) in an air oven at 105°C overnight until a constant weight was achieved. The ash content was determined by incineration at 600±15°C (Method No. 930.05). Protein content was determined based on the Kjeldahl method (Method No. 978.04). A conversion factor of 6.25 was used to convert the measured nitrogen content to protein content. Fat content was determined by using a semi-continuous solvent extraction method (Method No. 930.09). Carbohydrate was calculated by the difference: carbohydrate = 100 - (g moisture + g protein + g fat + g ash).

2.5. Determination of Color

The color of the samples was measured using a reflectance colorimeter (Minolta, CM 3500d, Minolta, Osaka, Japan). The instrument was calibrated with standard black and white tiles before analysis and the mean values of three replicates were reported. The surface color of fruit pulp was measured using the L*, a*, b* scales for the top (central and outer parts) which indicate the lightness, red to green and yellow to blue, respectively.

2.6. Acidity Analysis

10gm of samples were weighed and transferred to the 100ml volumetric flask and made up with distilled water to 100ml. The samples were mixed well and 10ml of the sample mixture was discharged into a 250ml beaker. Then, 3 drops of phenolphthalein were added sample as an indicator. Next, 0.1M of NaOH was titrated into the samples till the pink color persisted for at least 15 seconds. Results were expressed as percentage acid:

$$\text{Percentage acid} = \frac{\text{Titrate X acid factor X 100}}{100\ (\text{ml juice})}$$

2.7. Determination of Hydroxycitric Acid (HCA)

HPLC was utilized to determine the hydroxycitric acid (HCA) content in extracts from fruits at three stages of maturity: unripe (1 month), partially ripe (3 months), and fully ripe (4 months). The samples underwent a process of being cut into small pieces and subsequently freeze-dried. Then, 2 grams of the dried samples were extracted using 50 mL of methanol. The analysis was done using IC-PAK Anion HR 4.6x75 column and consisted of acetonitrile and water as a mobile phase. Data were collected using a PDA detector with quantification performed at 210nm. The retention time was 4 minutes (Figure 1).

2.8. Statistical Analysis

Experimental data obtained were subjected to statistical analyses using the commercial SPSS computer program (SPSS Version 21.0 SPSS Inc., Chicago, IL, USA). Data were averaged and mean comparisons were performed using ANOVA and a Duncan Multiple Range Test at 95% confidence. All measurements were carried out in triplicate (n = 3).

3. Result and Discussion

3.1. Proximate Compositions and Color Value

The proximate composition and color value are shown in Table 1. The findings indicated that there were no notable variances in proximate composition among the three maturity samples. Similarly, the proximate values fell within the ranges documented by Kalsum and Mirfat [9]. Since the fruits were sourced from various locations, variations in their composition, including sugar and carbohydrate content, could influence the hygroscopicity of the fruit powder. Furthermore, differences in fruit maturity should also be considered [10]. In addition, the L reading revealed a progression in color intensity from unripe (1 month) to fully ripe (4 months). This suggests that the samples become lighter as they ripen.

Table 1. Proximate and color value of garcinia

sample	unripe (1 month)	partially ripe (3 month)	fully ripe (4 month)
Moisture	14.61 ± 0.22^{a}	14.55 ± 0.34^{a}	13.95 ± 0.42^{a}
Ash	0.21 ± 0.01^{a}	0.16 ± 0.00^{a}	0.17 ± 0.03^{a}
Protein	0.47 ± 0.02^{b}	0.50 ± 0.00^{ab}	0.52 ± 0.01^{a}
Fat	0.06 ± 0.00^{a}	0.07 ± 0.00^{a}	0.06 ± 0.00^{a}
Carbohydrate	84.65 ± 0.22^{a}	84.72 ± 0.33^{a}	85.30 ± 0.37^{a}
energy	341.05 ± 0.81^{a}	341.47 ± 1.37^{a}	343.86 ± 1.53^{a}
Color (L)	43.71 ± 0.25^{a}	49.00 ± 0.20^{b}	56.66 ± 0.04^{c}
a*	-11.26 ± 0.23^{b}	-10.43 ± 1.03^{b}	-7.84 ± 1.38^{a}
b*	19.21 ± 0.13^{b}	21.89 ± 0.08^{ab}	25.03 ± 1.65^{a}

Values are presented as mean ± SD. Means with the same letter in the same column are not significantly different (p <0.05) (n=3)

3.2. Acidity Analysis

In the present research, the acidity content was assessed using titratable acidity analysis. The fully ripe (4 months) was used for the acidity analysis. The pre-treatment samples with no added sodium meta-bisulfate gave the highest acidity content compared with added sodium meta-bisulfate (Table 2). It can be concluded that the sodium meta-bisulfate does not influence the yield of acidity content in samples.

Table 2. Acidity content of the pre-treatment process of garcinia

Pre-treatment	Acidity content (%)	
	Without sodium meta-bisulfate	Added sodium meta-bisulfate
Blanched 5 minutes	42.66 ± 1.06^{b}	35.77 ± 1.79^{c}
Blanched 30 minutes	42.52 ± 1.00^{b}	35.19 ± 0.91^{f}
Soaked with water (room temperature)	39.63 + 0.37^{c}	34.13 + 0.61^{a}
Soaked with hot water	34.73 ± 0.97^{d}	23.12 ± 0.84^{a}
Steamed 5 minutes	26.14 ± 0.72^{e}	16.73 ± 0.47^{c}
Steamed 30 minutes	25.22 ± 1.17^{e}	22.22 ± 1.15^{b}
Enzyme 0.1%	46.64 ± 0.42^{a}	36.51 ± 0.79^{a}
Enzyme 0.5%	45.26 ± 0.34^{a}	35.81 ± 0.58^{a}

Values are presented as mean ± SD. Means with the same letter in the same column are not significantly different (p <0.05) (n=3)

Among all the analyses, pre-treatment samples with added enzyme showed the highest acidity content, followed by blanched treatment, 46.64 ± 0.42%, and 42.66 ± 1.06%, respectively. Research revealed that enzymatic treatment led to an increase in both yield and filterability in fruit juice [11]. In addition, another study demonstrated that pre-treatments involving blanching and pectinase enzyme pretreatments have an impact on the extraction of papaya fruit juice [12]. Enzymes, specialized proteins, serve as catalysts, aiding chemical reactions by either constructing or breaking down molecules within all living organisms [13], hence increasing some active compounds.

The lowest acidity content was detected in the steam samples. Similarly, a study reported that conventional steaming reduced the volatile compound in salmon [14]. Based on the aforementioned findings, it is suggested that the steam process has the potential to reduce the presence of the compound in the samples.

3.3. Determination and Optimization of Hydroxycitric Acid (HCA)

Further analysis of HCA was conducted for both blanching and enzyme treatment. From the analysis, HCA content for blanching and enzyme added were 1.23 and 1.87 gm/freeze-dry samples, respectively (Table 3). No statistically significant difference was observed between both pre-treatments. As a result, both blanching and enzyme addition were utilized for powder development.

Table 3. Acidity and HCA content in 3 maturity stages of garcinia

sample	HCA (gram/dry weight, DW)
Blanching 30 minutes	1.22 ± 0.36^{a}
Enzyme (1%)	1.87 ± 0.31^{a}

Values are presented as mean ± SD. Means with the same letter in the same column are not significantly different (p <0.05) (n=3)

Next, the HCA content in 3 maturity stages was further investigated. The findings revealed no notable disparities in acidity levels between samples harvested at 3 and 4 months of maturity (35.09 ± 0.90 % and 34.73 ± 0.96 %), respectively (Table 4). Similarly, no significant distinctions were observed in hydroxycitric acid content between both maturity stages, 1.17 ± 0.20 g/DW and 1.30 ± 0.16 g/DW, respectively. Considering these findings, it is justifiable to choose samples from both the 3 and 4-month maturity stages for further applications.

Table 4. Acidity and HCA content in 3 maturity stages of garcinia

sample	Acidity content (%)	HCA (gram/dry weight, DW)
unripe (1 month)	19.97 ± 1.66^{a}	0.17 ± 0.16^{a}
partially ripe (3 month)	35.09 ± 0.90^{b}	1.17 ± 0.20^{b}
fully ripe (4 month)	34.73 ± 0.96^{b}	1.30 ± 0.16^{b}

Values are presented as mean ± SD. Means with the same letter in the same column are not significantly different (p <0.05) (n=3)

The acid-base titration method yields elevated values for HCA likely because of the presence of other acids. In contrast, the HPLC method specifically quantifies HCA by measuring its area in the chromatogram. To verify the identity of HCA, its relative retention time was determined by spiking with a standard HCA, which was found to be 4.725 minutes (Figure 1). Our study is in agreement with Asish's findings, indicating that the titration method tends to yield slightly higher percentage values (ranging from 0.09 to 0.879 in leaves and 0.14 to 3.53 in fruit rinds) compared to the HPLC method. This discrepancy is attributed to the titration method's estimation of total organic acids [15].

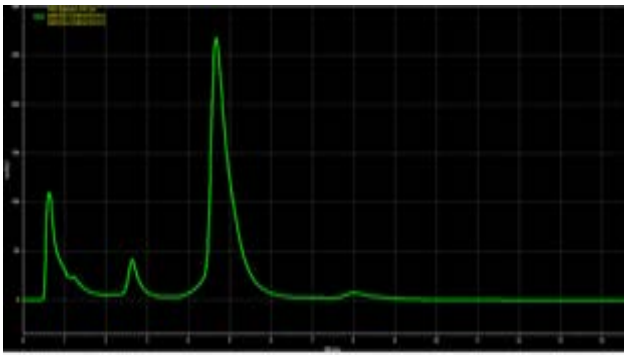

Figure 1. Retention time of standard chromatogram

Processing parameters for garcinia powder production were established. A combination of enzyme pretreatment and blanching was applied to a mixture of 3 and 4-month garcinia fruits. The determination of enzyme concentration and blanching duration percentages was conducted using Response Surface Methodology with a Composite Design Center, resulting in a total of 13 runs (Table 5). Statistical analysis revealed that enzyme concentration at 0.30% significantly influenced ($p<0.05$) the hydroxycitric acid (HCA) content, as did the blanching duration for 13 minutes (Figure 2). In conclusion, both pretreatment methods positively impacted the increase in HCA content in garcinia powder. In conclusion, the optimal parameters for minimizing HCA content in the developed powder were found to be 0.30% enzyme concentration and a blanching duration of 13 minutes.

A study by Bainto et al. reported a negligible change in acid content was observed when heating continued for 20 minutes. HCA present in the inner sections of the fruit proved more resistant to removal through heat treatments, which explains the observed HCA value at 20 minutes [16].

Enzymes play a significant role in the food industry by enhancing yield and production. They facilitate the breakdown and proliferation of cells found in plant tissues into simpler molecules [17,18]. Similarly, another research reported that the enzymatic breakdown of cell walls enhances extraction yield while reducing sugars, soluble dry matter content, galacturonic acid content, and titratable acidity of the resulting products [19]. Our findings align with the aforementioned studies, indicating that blanching and enzyme treatment can elevate the HCA content in the final product.

Table 5. Regression coefficients (R^2) and *p* (probability values) for HCA of garcinia

	HCA
Suggested model:	quadratic
Regression coefficient	
Constant,	2.96
* Percentage of enzyme, b_1	-3.193E-003
* Blanching duration, b_2	-0.13
* Percentage of enzyme, b^2_1	-0.35*
* Blanching duration, b^2_2	-0.39*
* Percentage of enzyme * blanching duration, b_{12}	-0.11
R^2	0.7603
P or probability	0.1133

Subscripts: 1 = ultrasound amplitude, 2 = sonication time
*Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level

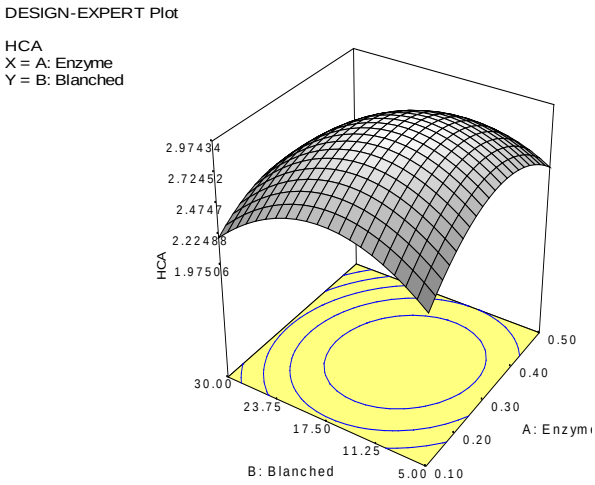

Figure 2. Respond surface curve plot of pre-treatment of garcinia

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