

Assessment of Shelf-Life Stability and Bioactive Potency of HCA-Rich *Garcinia Atroviridis* Powder to Address Obesity and Metabolic Health

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Abstract *Garcinia atroviridis* (asam gelugur) is an underutilized plant commonly used in traditional medicine to reduce weight and excess body fat by inhibiting glycogen synthesis. The primary active compound in its fruit is (-)-hydroxy citric acid (HCA). This study aims to evaluate the stability of the physicochemical properties, microbiological quality, and hydroxycitric acid content in *Garcinia* powder during storage, ensuring its suitability as an ingredient in food products designed to support weight management and address metabolic health concerns. Two different bottle materials were chosen for the storage which were polyethylene terephthalate (PET) and high-density polyethylene (HDPE). The heavy metal content of the product, including arsenic, lead, cadmium, mercury, antimony, and tin, was below the detection limit (not detected, ND). The powder was a source of calcium (206.3 mg/100 g), iron (206 mg/100 g), and potassium (369.4 mg/100 g). Additionally, it was rich in fiber (30.9 g/100 g), copper (0.3 mg/100 g), and vitamins B2 (8.5 mg/100 g), B3 (45.4 mg/100 g), B5 (10.4 mg/100 g), B6 (2.5 mg/100 g), B9 (folic acid = 11.4 mg/100 g), and B12 (148.3 mcg/100 g). Both samples met the acceptable limits for microbial load in Dried or Powdered Botanicals, including total plate count, yeast and mould, and total coliforms. No significant differences were observed between the packaging materials (PET and HDPE) in terms of physicochemical properties, microbiological quality, or HCA content, making both materials suitable for product storage. The HCA content increased significantly by 31% after one and two months of storage compared to the initial value, but by the third month, a decrease was observed in both packaging types. Between months 3 and 4, the HCA content dropped by 32% in HDPE and 41% in PET. Overall, the HCA remained stable for up to three months, which is equivalent to 18 months of real-time storage.

Keywords: nutrient composition, physicochemical, microbiology, hydroxycitric acid (HCA), HPLC

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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]. Obesity increases the risk of chronic diseases such as type 2 diabetes, cardiovascular diseases, certain cancers, dyslipidemia, poor mental health, and osteoarthritis [3]. The Malaysian Non-Communicable Disease Surveillance highlighted the rapid rise of obesity [4] due to the low intake of fruits and vegetables. Additionally, 68.1% reported consuming high

sugar-sweetened beverages and calorie-dense local foods, depending on gender and ethnicity [5].

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

Potential new foods or compounds should be investigated to help prevent obesity and obesity-related health risks. *Garcinia atroviridis* is native and broadly scattered in Thailand, Myanmar, Peninsular Malaysia, and India [6]. *Garcinia* 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. The primary acid in this fruit, (-)-hydroxycitric acid (HCA), is marketed as a weight management supplement. It significantly regulates body weight and appetite by inhibiting the liver enzyme ATP citrate-lyase production [7]. This enzyme converts excess carbohydrates into fat. It also stimulates hepatic glycogen synthesis from glucose [8].

Parts of the *Garcinia* plant possess medicinal properties in addition to their use in food products [9]. Currently, extracts and bioactive compounds from *Garcinia atroviridis* exhibit various biological activities, such as antioxidant, antimicrobial, anticancer, anti-inflammatory, antihyperlipidemic, and anti-diabetic effects. These reported therapeutic properties have attracted the interest of numerous research groups over the past decade. Researchers believe that the plant's unique flavor, spiciness, low fat and calorie content, and medicinal benefits will contribute to its growing global popularity [10].

Even though the plants originated from the genus of *Garcinia* and become targets of HCA for weight management agents, the potential of other constituents should not be overlooked. Further exploration and investigation are anticipated to confirm the traditional medicinal value claimed of this plant and maximize the use of *G. atroviridis* for the betterment of health status.

On top of that, *Garcinia* is one of the priorities under the National Plan of Action For Nutrition of Malaysia (NPANM) III, 2016 - 2025, to promote the consumption of underutilized crops, healthy diets, and Food & Nutrition Security. Moreover, it falls under the Medical & Health Care section in 10-10 MySTIE. At present, there is a knowledge gap regarding the stability of *Garcinia* powder during storage. Therefore, this study aimed to evaluate the stability of *Garcinia* powder in terms of its physicochemical, microbiological properties, and hydroxycitric acid content over a 2-year storage period. Additionally, the nutrient composition was also examined.

2. Material and Methods

2.1. Chemical and Reagents

All of the reagents and HPLC standards were purchased from Sigma-Aldrich (Merk KGaA, Germany). Peptinase enzyme was purchased from the local distributor.

2.2. Materials

Nasuha Herba & Rempah Karya Sari Sdn.Bhd, Pagoh, Johor, supplied fresh *Garcinia* fruits. *Garcinia* at two maturity indexes were used with no presence of injury and were selected for the study according to the previous optimization study [11]. After arriving at the Food Science and Technology Research Centre, MARDI Serdang, the fruits were stored at room temperature before being processed.

2.3. *Garcinia* Powder Preparation

Upon arrival at the pilot plant processing hall, the fruits were cleaned and washed using a washer for 15 minutes. The edible portion of the fruits was cut into 6 pieces prior

to the pretreatment process. The sample was dried using a hot air circulating dryer (Model RXH-B-I, China) until the final moisture content reached below 10%. Subsequently, the sample was ground with 120 mesh size using an Industrial Pulverizer Machine (Model No. 30B, China). The sample was then processed into capsules for further analysis.

2.4. Nutrient Composition

Proximate and nutrient analyses were conducted using the Association of Official Analytical Chemists [12]. 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). Total dietary fiber was determined by MY/STP/378 based on US FDA 21 CFR101.9 Part 101 (2017).

The heavy metals content: arsenic, lead, cadmium, mercury, antimony, and tin were analyzed by MY/STP/375 (Method No. 999.10) analyzed by ICP-OES. Minerals content: calcium, phosphorus, magnesium, sodium, potassium, iron, zinc, and copper were determined by MY/STP/009 (Method No. 968.08). Vitamin A was determined by MY/STP/128 based on USP 30 -NF25; vitamins B1, B2, B3, B5, B6, B9, and K were determined by BP 2007 (Vitamin B and C injection) and LGC/GC/2007/019; vitamin B12 was determined by MY/STP/238 based on Direct Enzyme Immunoassay for Quantitative; vitamin C was determined by AOAC 967.21 & GB 5413.18 – 2010; and vitamin E was determined by MY/STP/404 based on ISO 9936:2016 (HPLC).

2.5. Accelerated Shelf-life Study

For the accelerated shelf-life study, two different bottle materials were chosen: polyethylene terephthalate (PET) and high-density polyethylene (HDPE). A 1-gram desiccant silica gel sachet was added to each bottle to absorb moisture. A total of 8 bottles of each material were stored in a climate chamber (40°C, 75% RH, Model KBF 240, Binder, Germany) for four months. Two bottles of each material were sampled at monthly intervals for physicochemical, microbiological, and hydroxycitric acid analysis.

2.6. Microbiological Analysis

The total plate count (TPC) was performed on plate count agar (PCA, Oxoid, United Kingdom). In contrast, yeast and mould (YM) were assessed on potato-dextrose agar (PDA, Oxoid, United Kingdom) media added with 10% tartaric acid for YM. The total coliform in the sample was evaluated using commercial Nissui plate agar. In contrast, Violet Red Bile Agar (VRBA) was used for total coliform. These analyses were conducted following the standard methods outlined in the United States Food and Drug Administration (US-FDA) Bacteriological Analytical

Manual (BAM), as described by Feng et al. [13].

Ten grams of the sample was aseptically weighed and homogenized with 90 mL of Ringer's solution (Oxoid, Hampshire, England) using a stomacher lab-blender (Seward Model 400, London, UK) for 60 seconds. Serial dilutions were prepared with the same diluent, and duplicate counting plates were prepared using appropriate dilutions. For pour plating, 1 mL of the appropriate dilutions were mixed with molten (45°C) media and poured into plates. The inoculum was evenly mixed by gently swirling the plates 6 times in each direction (6 clockwise, 6 anti-clockwise). Then, the agar with inoculum in the plate was allowed to solidify. Plating samples counted total mesophilic aerobic and anaerobic bacteria on PCA. Yeast and mould counts were cultivated on PDA. Microbial colonies were counted after 72 h incubation at $31 \pm 1^\circ\text{C}$ for both PCA and PDA. The coliform was incubated at 37°C for 24 and 48 hours. All microbial enumeration results were expressed in log colony-forming units, cfu/g.

2.7. Phytochemical Analysis

2.7.1. Color Analysis

The color of garcinia powder was evaluated using a Chroma meter (Model CR-400/410, Konica Minolta, Japan) by measuring the color parameters: the negative or positive a^* value indicates the sample's greenness or redness; the value of L^* , which ranges from 0 (darkness) to 100 (lightness) and the b^* value indicates the sample's color, which ranges from blue (negative b^* value) to yellow (positive b^* value), respectively [14]. The analysis was performed in triplicate, and the average weight was reported.

2.7.2. pH Analysis

The pH value of garcinia powder was determined using a pH meter (Metler Toledo, Switzerland). The analysis was performed in triplicates, and the average weight was reported.

2.7.3. Moisture Content Analysis

The moisture content of garcinia powder was determined using standard AOAC methods (AOAC,1990) and the following equation:

$$\text{Moisture content (\%)} = \frac{(mw - md)}{md} \times 100 \quad (1)$$

Where mw is the wet mass, and md is the dry mass of the sample. Analyses were performed in triplicates, and the average weight was reported.

2.7.4. Water Activity Analysis

The water activity of garcinia powder was determined using LabMaster-aw meter (LabMaster-aw Novasina, Switzerland). The equilibrium of the air humidity over a sample (water-vapour pressure), which is proportional to the Aw value, was measured. Values were taken after constant readings were obtained. The analysis was performed in triplicate, and the average weight was reported.

2.8. Hydroxycitric Acid Analysis

2.8.1. Hydroxycitric Acid Standard Solution Preparation

One milligram of the standard compound (-)-hydroxycitric acid lactone (HCAL) ($\geq 95\%$ purity, Sigma-Aldrich) was dissolved in 1 mL of dehydrated water. The resulting HCA stock solution (1 mg/mL) was then diluted to prepare a range of concentrations (50-700 $\mu\text{g/mL}$).

2.8.2. Sample Extraction

A 0.5 g sample of Garcinia was mixed with 20 mL of deionized water and sonicated for 30 minutes. The mixture was then shaken at 150 rpm for 1 hour, followed by centrifugation at 7500 rpm for 15 minutes. One millilitre of the supernatant was filtered through a 0.2 μm nylon syringe filter and diluted 1:9 with dehydrated water. Finally, 20 μL of the sample was injected into the HPLC system for the quantitative analysis of HCA.

2.8.3. Chromatography Analysis

The determination of HCA compounds using high-performance liquid chromatography (HPLC) was performed following the method of Jena et al. [15] with some modifications. The experiment utilized a Waters 2695 model HPLC system equipped with a photodiode array detector (Waters 2996) and an automatic injector system with adjustable volume. Separation of the sample was achieved using a C18 column (Gemini C18, 110 $\text{\AA}$, 3 μm particle size, 4.6 mm inner diameter $\times$ 250 mm length). The mobile phase consisted of 0.1% orthophosphoric acid and HPLC-grade methanol (100%). Both solvents were filtered through a 0.45 μm membrane filter and degassed using a sonicator to remove air bubbles.

The mobile phase flow was set in a gradient, starting with 0.1% orthophosphoric acid-methanol (90:10, v/v) up to 10.5 minutes, followed by 50:50 (v/v) until 13.5 minutes, and returning to 90:10 (v/v) by 20 minutes, with a flow rate of 0.6 mL/min. The separation column was maintained at 25°C , and the wavelength was set to 210 nm. The HCA content was quantitatively determined by measuring the area of the compound peak in the chromatogram and using a calibration curve generated from a series of HCA standard concentrations (Figure 1).

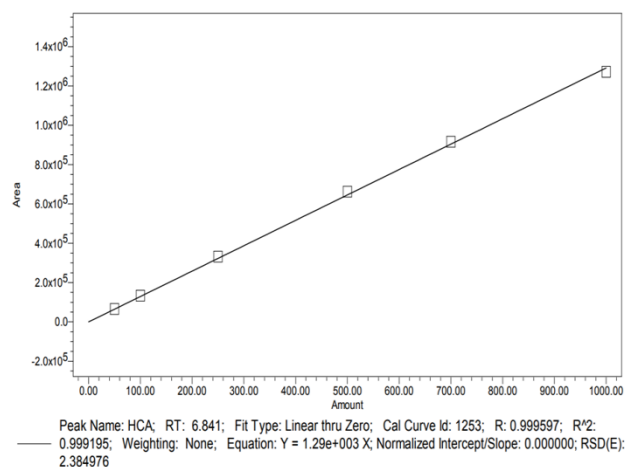


Figure 1. Calibration curve of (-)-hydroxycitric acid lactone (HCAL) standard

2.9. Statistical Analysis

Data were averaged and mean comparisons were performed using ANOVA and a Duncan Multiple Range Test at 95% confidence. P-values < 0.05 were regarded as statistically significant. All measurements were carried out in triplicate (n = 3). Statistical analyses were performed using IBM SPSS Statistics version 29.0.2.0 software (IBM Corp).

3. Result and Discussion

3.1. Nutrient Composition

Table 1 presents the nutrient composition. The findings indicated that the proximate values (energy, protein, fat, moisture, ash, and total carbohydrate) fell within the ranges documented by Kalsum and Mirfat [16]. Since the fruits were obtained from different locations, variations in their composition, such as sugar and carbohydrate content, may affect the hygroscopicity of the fruit powder. Additionally, differences in fruit maturity should also be taken into account [10].

Table 1. Nutrient composition of developed garcinia powder in 100 g dry weight (DW)

Nutrient (100g)	Value	According to the Guideline on Labelling Requirement Under Food Act 1983
Energy (Kcal)	359	
Protein (g)	ND < 0.1	
Fat (g)	ND < 0.1	
Moisture (% w/w)	8.1	
Ash content (% w/w)	2.2	
Total carbohydrate (g)	89.7	
Heavy metal (arsenic, lead, cadmium, mercury, antimony, tin) (mg/kg)	ND < 0.02	
Dietary fiber (g)	30.9	
Calcium (mg)	206.3	Source
Phosphorus (mg)	26.0	
Magnesium (mg)	2.8	
Sodium (mg)	107.9	
Potassium (mg)	369.4	Source
Iron (mg)	2.6	Source
Copper (mg)	0.3	High
Vitamin A (IU)	9.9	
Vitamin B1 (mg)	ND < 0.1	
Vitamin B2 (mg)	8.5	High
Vitamin B3 (mg)	45.4	High
Vitamin B5 (mg)	10.4	High
Vitamin B6 (mg)	2.5	High
Vitamin B9 (mg)	11.4	High
Vitamin B12 (mcg)	148.3	High
Vitamin C (mg)	2.0	

Moreover, the heavy metal content was analyzed, and the results have shown that the developed Garcinia powder was below the detection limit (not detected, ND) of arsenic, lead, cadmium, mercury, antimony, and tin. Rapid industrialization, air deposition, farmyard manure,

sewage sludge, and the widespread use of synthetic fertilizers all contribute to the presence of heavy metals in soils [17,18]. These metals can enter plant systems and contaminate the food chain, posing significant risks to both human health and food quality [19]. The fresh garcinia sample was sourced from an organic farm; hence, it is proven that the source is free from chemical fertilizers or the use of pesticides.

According to the Guideline on Labelling Requirement Under the Food Act 1983 and Regulations Thereunder, the Ministry of Health Malaysia [20], the developed garcinia powder was a source of calcium (206.3mg/100g), iron (206mg/100g) and, potassium (369.4mg/100g). On top of that, it is high in fibre (30.9g/100g), mineral cuprum (0.3mg/100g), vitamin B2 (8.5mg/100g), B3 (45.4mg/100g), B5 (10.4mg/100g), B6 (2.5mg/100g), B6 (2.5mg/100g), B9 (folic acid = 11.4mg/100g), B12 (148.3mcg/100g), copper, and vitamins B2, B3, B5, B6, B9, B12. High-fibre diets are linked to numerous health benefits, including enhanced nutrient absorption, the production of beneficial metabolites, strengthened immune responses, and the improvement of various conditions such as obesity, diabetes, and allergies [21]. The developed garcinia powder has the potential to be considered a superfood since it is supplemented with various natural nutrients.

3.2. Microbiological Analysis

Table 2 summarizes the microbial counts, including TPC, YM, and total coliform, for the two bottle materials over four months of storage in the climatic chamber (equivalent to 2 years at normal temperature). The initial count of TPC and total coliform at 0 days of storage showed that the garcinia samples of both bottles contained 2.20×10^2 and 5.30×10 cfu/g, respectively. In addition, no growth of YM was observed for either bottle. Elevated levels of YM can negatively impact human health, depending on the specific type of Y&M present. Certain strains may produce toxins, while others can trigger allergic reactions [22].

Table 2. Microbiological data of developed garcinia powder over 4 months of storage in the climatic chamber

Month	PET (cfu/g)			HDPE (cfu/g)		
	Total plate count	Yeast and mould	Total coliform	Total plate count	Yeast and mould	Total coliform
0	2.20×10^2	<1.00 x 10	5.30×10	2.20×10^2	<1.00 x 10	5.30×10
1	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10
2	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10
3	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10
4	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10	<1.00 x 10

Note: <1.00 x 10 indicates the microorganisms tested were not detected in the analyzed samples

The result shows that both samples were within the acceptable limits for Dried or Powdered Botanicals according to the Recommended Microbial Limits for Botanical Ingredients, – United States Pharmacopeial

Convention, USP-NF 35-30 (2012) [24], indicating that the maximum limit for TPC should not exceed 1.0×10^5 cfu/g. In addition, a total coliform must be lower than 1.0×10^3 cfu/g. The total plate count (TPC), also referred to as aerobic colony count (ACC), total viable count (TVC), or standard plate count (SPC), serves as a hygienic indicator rather than a safety measure. Elevated TPC levels may indicate unhygienic handling, insufficient processing, or inadequate temperature control of the product [23]. The results showed that the processing was conducted hygienically and in accordance with standard operating procedures (SOP). A study reported that a garcinia product has an inhibitory and killing effect; hence, it showed an ability to exert antimicrobial properties [24]. Similarly, research on different garcinia species reported that garcinia demonstrated valuable antimicrobial properties [25]. The aforementioned findings could be speculated in relation to our developed garcinia powder, which may have an antimicrobial effect, as no growth was detected throughout 4 months of storage.

3.3. Physicochemical Characteristics

3.3.1. Color

Figure 2 presents the result of the colormeter analysis of developed garcinia powder upon four months of storage in the climatic chamber. According to the color analysis, the L, a, and b values decreased significantly during the first two months of storage in the chamber, remaining stable from the 3rd to the 4th month. No significant differences were observed between the two bottle materials across all values (L, a, b). The color darkened considerably (with the L value decreasing by 50% from 0 days to 4 months of storage) in both bottles, in proportion to the storage duration.

Although color changes do not impact consumer acceptance, as the powder is encapsulated and color is not a critical characteristic of the product, it is still essential to examine the relationship between color changes and other factors, such as pH and HCA content.

3.3.2. pH Analysis

Figure 3 indicates the pH values of developed garcinia powder. The pH values for both bottles ranged from 1.5 to 1.6, with no significant differences observed. The pH remained stable during the first 3 months (1.6) of storage and decreased at 4 months (1.5). Overall, the pH value declined from day 0 to 4 months of storage, indicating that the garcinia samples turned more acidic over time. To the best of our knowledge, no data has been previously reported on the pH changes in garcinia products during storage. Nurminah et al. reported that the pH of garcinia ranged from 1.2 to 1.7 depending on the variety and maturity index [26]. Our results are in accordance with the aforementioned findings.

3.3.3. Moisture Content Analysis

Figure 4 reports the moisture content of developed garcinia powder upon storage. In a PET bottle, the moisture content increased during the first 2 months of storage from 7.21% and remained stable from the 2nd to the 3rd month and reached 13.5% at 4th month. In an

HDPE bottle, the moisture content remained stable from 0 to 2 months, at 7.21%, and increased in the 3rd month and stabilized up to the 4th month (9.43%). Overall, the moisture content rose more in PET bottle storage compared to HDPE bottles.

The stability of all medical products is linked to the retention of the active ingredient throughout the entire shelf life without any degradation reactions occurring [27]. Moisture or gas permeability, particularly oxygen, can significantly impact product quality and shelf life, potentially compromising both the safety of the product and the effectiveness of the active pharmaceutical ingredient [28]. A study by Jaime et al. showed that PET represented a better moisture barrier compared to HDPE materials [29], which is consistent with our findings.

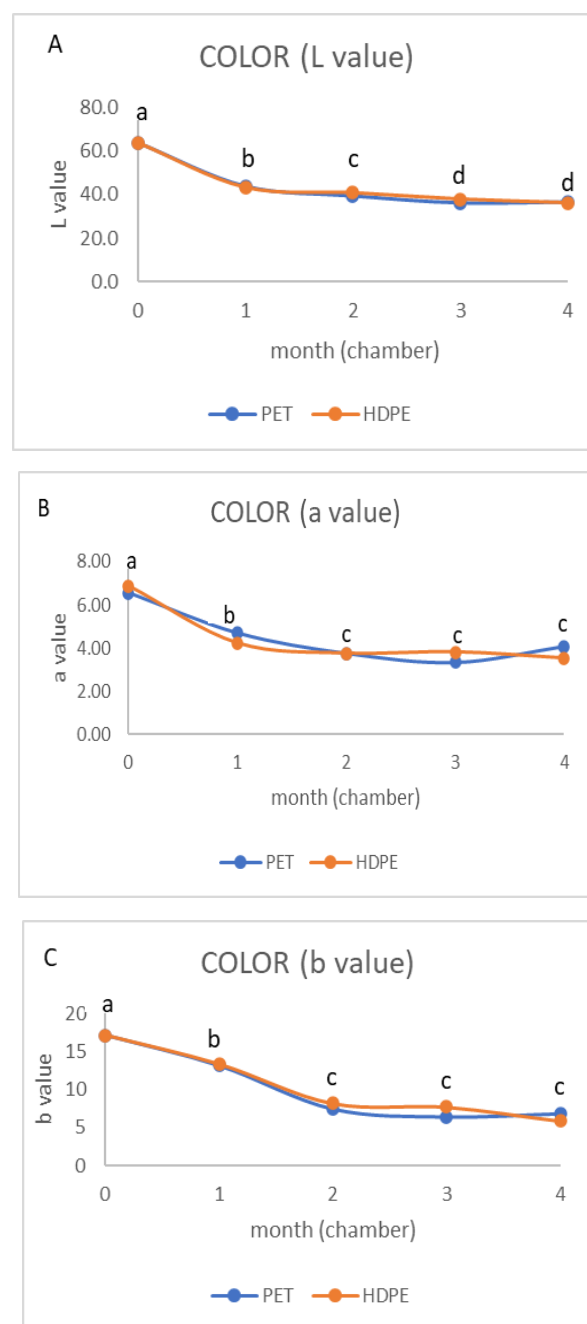


Figure 2. Color result of A) L value, B) a value, and C) b value of developed garcinia powder (mean $\pm$ SD, $n=3$). Different lowercase letters indicate a significant difference within a month. PET= polyethylene terephthalate, HDPE= high density polyethylene

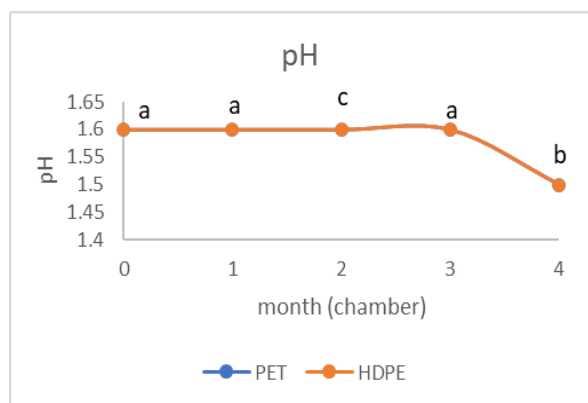


Figure 3. pH result of developed garcinia powder (mean $\pm$ SD, $n=3$). Different lowercase letters indicate a significant difference within a month. PET= polyethylene terephthalate, HDPE= high density polyethylene

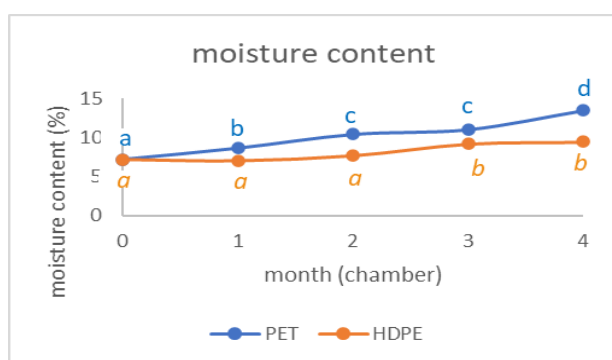


Figure 4. Moisture content of developed garcinia powder (mean $\pm$ SD, $n=3$). Different lowercase letters indicate a significant difference within a month. PET= polyethylene terephthalate, HDPE= high density polyethylene

3.3.4. Water Activity Analysis

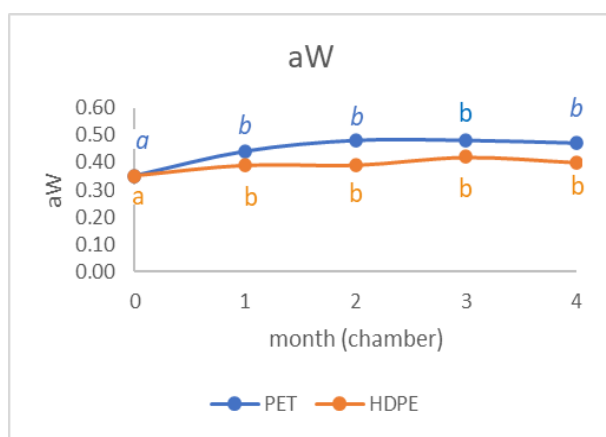


Figure 5. Water activity value of developed garcinia powder (mean $\pm$ SD, $n=3$). Different lowercase letters indicate a significant difference within a month. PET= polyethylene terephthalate, HDPE= high density polyethylene

Figure 5 shows the water activity (A_w) values of the developed Garcinia powder, ranging from 0.35 to 0.47 over the four months of storage. The values remained relatively stable for both types of bottles. The low A_w values, as indicated in Table 2, are associated with reduced microbial growth. The water activity value of the developed garcinia powder shows that they are not likely to support microbial growth under intended product

storage. A_w is a crucial factor in preventing or limiting microbial growth. In many instances, A_w is the main factor influencing food stability, affecting microbial growth, and determining the types of microorganisms present in the food [30].

3.4. Hydroxycitric Acid Content

Figure 6 presents the hydroxycitric acid (HCA) content upon four months of storage. The HCA content showed a significant increase of about 31% after one and two months of storage compared to the initial value at 0 months. However, by the third month, it decreased for both types of packaging materials. Between months 3 and 4, the HCA content dropped by 32% in HDPE and by 41% in PET. Overall, the HCA remained stable for up to 3 months, which is equivalent to 18 months of real-time storage.

To the best of our knowledge, no data are available on the stability of HCA during storage. Nevertheless, several studies found that capsulated powder could optimize the chemical stability of the bioactive compounds [31]. Encapsulation is a promising approach for enhancing the therapeutic potential of these compounds [32]. The encapsulation process results in the formation of capsules, particles, or spheres [33]. It improves their stability, extends shelf life, shields them from degradation during digestion, and allows for better release control by increasing their bioaccessibility and bioavailability [32].

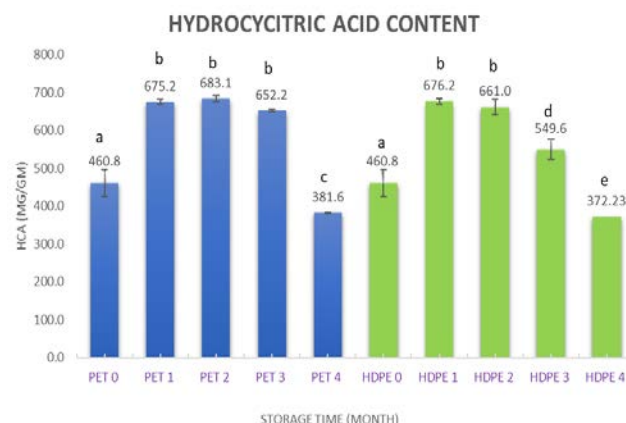


Figure 6. Hydroxycitric acid (HCA) content of developed garcinia powder (mean $\pm$ SD, $n=3$). Different lowercase letters indicate a significant difference within a month. PET= polyethylene terephthalate, HDPE= high density polyethylene

All in all, these findings demonstrate that encapsulation of garcinia powder could be considered a suitable approach to retain the HCA content.

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been transferred to the company for commercialization.

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