

Article

A Study on the Enhancement of Storage Stability in Formulated Gac Fruit Oil and Its Encapsulated Form

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Abstract: The fruit of *Momordica cochinchinensis* Spreng., commonly known as Gac fruit, contains arils rich in carotenoids and unsaturated fatty acids, making it suitable for use as a natural colorant, flavor enhancer, and dietary supplement. This study examined Gac oil extracted from locally cultivated Gac fruit in Taiwan and evaluated the impact of different encapsulation methods and press through packaging (PTP) packaging on its oxidative stability during storage. The Gac oil was found to contain exceptionally high levels of β -carotene (up to 6047.52 ± 16.15 ppm) and lycopene (3192.84 ± 20.21 ppm). Among the tested formulations, soft capsules demonstrated lower peroxide value (PV) and better retention of carotenoids, including lycopene β -carotene compared to hard capsules. Furthermore, capsules stored in PTP packaging exhibited enhanced protection against oxidation. Overall, soft capsules combined with PTP packaging provided the most effective approach for maintaining the nutritional quality and oxidative stability of Gac oil during storage.

Keywords: *Momordica cochinchinensis* Spreng oil; liquid capsule; storage protective; press through packaging (PTP); fat-soluble nutrient



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1. Introduction

Gac fruit (*Momordica cochinchinensis* Spreng.), a Cucurbitaceae perennial vine indigenous to Southeast Asia and northeastern Australia, has been known as “super fruit” or “fruit of paradise” due to its exceptional phytonutrient value and medicinal properties [1]. Among all the fractions of the fruit (e.g., seed, aril, pulp, and peel), the aril is exceptionally rich in lycopene, β -carotene, and unsaturated fatty acids. Gac aril can be consumed in both powdered and oil forms, and is often used as a nutritional supplement, colorant, and for medical use. Due to its high content of unsaturated fatty acids and the fat-soluble carotenoids, Gac aril oil offers superior health benefits, making it a more effective option for dietary supplementation and overall well-being [1].

Gac aril's high carotenoid and unsaturated fatty acid content make it a favorable choice for food coloring and flavor enhancer. It is also reported to pose several health benefits, including reducing the risk of cardiovascular disease, cancer, dry eye, and night blindness [2–4]. The aril is particularly rich in polyunsaturated fatty acids (PUFAs), mainly as linoleic acid, linolenic acid, and α -linolenic acid (ALA). ALA in the diet can desaturate and convert into docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), all of which are omega-3 fatty acids that can promote heart health and regulate inflammatory responses [5]. Additionally, the high concentrations of unsaturated fatty acids and carotenoids in the aril may help increase the bioavailability of carotenoids [6–8]. Carotenoid-rich edible oils, particularly those containing lycopene and β -carotene, provide enhanced health benefits, as these compounds have been shown to be beneficial in decreasing the risk of certain cancers and eye diseases, and play an important role in the prevention of chronic diseases [9]. Notable carotenoid-containing edible oils include Gac fruit oil, pumpkin seed oil, and argan oil. Among these, Gac fruit oil has significantly higher levels of lycopene and β -carotene [9] compared to pumpkin seed oil [10] and nut oil [11].

Despite their health benefits, these unsaturated fatty acids are susceptible to oxidation and rancidity, probably due to their increased numbers of double bonds [12]. The tocopherol (vitamin E) content may also affect the oxidative stability of oils, as it exerts a protective antioxidant effect [13]. Therefore, the processability and shelf life of oils largely depend on their oxidative stability. There are various methods available for measuring the oxidative stability of oil, with the storage test being the most common, yet time-consuming, method. Therefore, thermostated sped-up testing has been proposed as an alternative to traditional storage testing [12]. According to previous studies, storage temperatures higher than 40 °C can accelerate peroxide degradation through primary oxidation, leading to a decrease in peroxide value, an increase in acid value, and flavor deterioration. Primary oxidation involves the initial formation of hydroperoxides from polyunsaturated fatty acids (PUFAs) in the presence of oxygen. These hydroperoxides are unstable and may further degrade through secondary oxidation, producing aldehydes, ketones, alcohols, lactones, and other tertiary oxidation products. These secondary products are largely responsible for undesirable flavors, quality losses, and reduced shelf life. A storage temperature above 55 °C was reported to increase the accumulation of aldehydes in Gac oil and other lipid-based products derived from linoleic acid hydroperoxide degradation, particularly hexanal, which is the major cause of off-flavors [14–16].

Liquid oils are often encapsulated in both hard and soft capsules. These capsules not only provide excellent protection for physical and chemical stability but can also be easily administered orally or enterically. Soft vegetarian capsule shells are commonly made with methyl cellulose [17]. On the other hand, liquid-filled hard capsules were developed in the early 1980s as an alternative to soft capsules. They were produced using high melting point excipients, plasticizers, and preservatives, and were low in moisture content [18]. Previous studies have investigated the storage stability of soft-shell capsules containing different plant oils (e.g., soybean and flaxseeds) and their sensitivity to rupture and disintegration tests [19]. The effect of different storage conditions on the compatibility of hard gelatin capsules with lipid formulations was also explored [20]. However, there are limited studies that compare the compatibility of plant oil with soft and hard capsules. Therefore, this study aims to further compare the effects of different capsule shells on the changes in quality and nutrient content of encapsulated formulated Gac fruit oil during storage. Considering the commercial model as a key factor, the most widely adopted types of hard and soft capsules currently available in the market were chosen for subsequent experiments.

Liquid-filled capsules can be used to fill oily liquids or semi-solid formulations. The stability and leakage rate of these capsules are the major concerns during storage. Unpro-

tected capsules can absorb moisture from the air, resulting in capsule adhesion and leakage. Therefore, packaging is necessary to protect the capsules and their active ingredients, and to prevent damage caused by external factors such as light, temperature, atmospheric gases, microorganisms, and moisture [21]. Typically, press through packaging (PTP) and jars are used to protect the capsules and enhance their storage stability. The previous literature has shown that blister pack capsules can be stored for up to 20 years, with analysis of sensory properties, oil bacterial count, and anisidine value demonstrating compliance with the standards [21]. Hence, it becomes evident that capsule packaging can provide a significant protective effect. However, there is limited literature examining the storage stability of Gac oil capsules in PTP sheets versus unprotected capsules. Therefore, this study aims to compare the composition of Gac oil and evaluate the effectiveness of PTP packaging in extending the shelf life of encapsulated Gac oil. In collaboration with a biotechnology company, this study focuses on components that are commonly analyzed in routine industrial settings to establish practical quality control indicators. Based on insights from the previous literature, acid value, peroxide value, β -carotene content, and lycopene content were selected as the key analytical parameters.

2. Materials and Methods

2.1. Chemicals

Acetonitrile was obtained from Honeywell (Charlotte, NC, USA), methanol and dichloromethane were obtained from Avantor Performance Materials (Radnor, PA, USA), iso-octane and tetrahydrofuran were purchased from DUKSAN (Ulsan, Republic of Korea), ether was acquired from ECHO Chemical (Toufen, Taiwan), ethanol was obtained from CHENDING Biological (Taipei City, Taiwan), ammonium acetate, acetic acid, and potassium hydroxide were purchased from Nihon Shiyaku Reagent (Kyoto, Japan), sodium thiosulfate was purchased from Showa Chemical Industry (Tokyo, Japan), and potassium iodide was acquired from Uniregion Bio Tech (Nantou, Taiwan).

2.2. Sample Preparation

Organic Gac fruits were harvested from Jia Cheng Agricultural Technology Co., Ltd. in Wanluan Township, Pingtung County, Taiwan. The Gac fruit used in this study was a native Taiwanese variety, cultivated in Wanluan Township, Pingtung County. It was organically farmed to ensure it was free from contamination by pesticides and other chemicals. The processing and extraction of Gac fruit oil followed the principles of organic food processing, with no additional chemical additives used. The ripening period of the fruit varies depending on the cultivar and the season, but in general, Gac fruit is harvested between July and December. It is picked after the skin has fully changed color from green to a bright reddish-orange [22]. The Gac arils were removed and subjected to hot air drying at 60 °C for 48 h until the moisture content reached approximately 6–7%. The samples were then kept frozen in a −20 °C freezer until ready for oil extraction.

2.3. Gac Aril Oil Extraction

The extraction of Gac aril oil was modified based on previous research [23]. Gac aril oil was extracted using a screw press (Health Valley, YFM160, Taipei City, Taiwan). The press was preheated for 30 min, and the dried Gac fruit samples (finely chopped into 1 cm pieces) were fed into the press. Crude oil was collected and centrifuged at 11,000 rpm for 15 min to remove impurities and then stored at room temperature before filling.

The carotenoid content of the Gac fruit oil extracted in this study is shown in Table 1. As indicated in the table, the Gac fruit oil contains up to 6047.52 ± 16.15 ppm of β -carotene and 3192.84 ± 20.21 ppm of lycopene. These levels are significantly higher than the 67 ppm

typically found in nut oils [18], 1.6 ppm found in pine nut oil [19], and 111 ppm found in pumpkin seed oil [10]. Gac oil was found to be rich in carotenoids, including lycopene and β -carotene [20]. Lycopene imparted a red color to the oil, while β -carotene contributed to a yellow color [21]. Therefore, Gac oil appeared to be orange-red. Furthermore, the color varied with the lycopene content, as previous research demonstrated that the degradation of lycopene resulted in a linear reduction in red hues [23].

Table 1. The carotenoid content of the Gac fruit oil.

Carotenoid	Content (ppm)
β -carotene	6047.52 ± 16.15
Lycopene	3192.82 ± 20.21

2.4. Hard Capsule, Soft Capsule, and PTP Packaging Manufacturing

Hard capsule shells were made of hydroxypropyl methylcellulose (HPMC), potassium chloride, and distilled water. The hard capsules were filled with Gac oil and banded with gelatin to prevent moisture and leakage, then packed in PTP packaging (performed by Yu Luen Biotech Scientific Co., Ltd., Tainan, Taiwan). The thickness of the capsule is controlled at 1–3 mm.

Seamless soft capsule shells were made with plant-based materials such as HPMC and carrageenan, then filled with Gac oil (performed by B. M. Bio. International Company, Taichung, Taiwan). The thickness of the capsule is controlled at 1–3 mm.

The storage test was determined by the method described by Li et al. [16]. The accelerated test was conducted under conditions of light and temperature avoidance at 55 °C for 0, 6, 12, 18, and 24 days. Quality indicators and changes in carotenoid content were monitored.

PTP-packet capsules refer to blister pack capsules sealed in aluminum foil, which serve to protect the capsules and enhance their storage stability. Capsules not packaged with PTP are referred to as unprotected capsules. The sampling method involves triplicate sampling of three capsules from three different types of PTP packaging.

To evaluate the storage stability of the Gac fruit oil produced in this study after encapsulation and to promote local production, hard and soft capsules manufactured by local Taiwanese factories were selected. The companies responsible for capsule production and the primary components of the capsules are listed in Table 2.

Table 2. Comparison of ingredients between hard capsules and soft capsules.

Capsules	Hard Capsules	Soft Capsules
Element	HPMC, potassium chloride, and distilled water	HPMC and carrageenan
Manufacturer	Yu Luen Biotech Scientific Co., Ltd., Taiwan	B. M. Bio. International Company, Taiwan

The primary component of both types of capsules is hydroxypropyl methylcellulose (HPMC). The major difference lies in the production of hard capsules, where potassium chloride is used as a coagulant, along with the addition of distilled water. In contrast, soft capsules use carrageenan for the capsule shell, employing a gelation process to fully encapsulate the oil within the soft capsule [24]. As to capsule compatibility, soft capsules, which take on a spherical or oval shape, are suitable for liquid and semi-solid formulations. On the other hand, the hard capsule is composed of a cylinder body and cap, but gaps can occur between the capsule shell and its contents during the filling and molding process [18].

2.5. Acid Value (AV)

The acid value was determined by the method described by Chen et al. [24]. An oil sample of 5 g was dissolved in 100 mL of ethanol and ether (1:1) mixture. The titration was performed using a 0.01 N potassium hydroxide in ethanol solution with an automatic titrator (KEM, AT-710S, Kyoto, Japan).

2.6. Peroxide Value (PV)

The peroxide value was determined according to the method modified from AOCS [25] and Karami et al. [26]. An oil sample of 5 g was weighed and dissolved in acetic acid-isooctane (3:2) solution, mixed with 0.5 mL of saturated potassium iodide solution and 80 mL of distilled water, and then titrated using a 0.1 N sodium thiosulfate solution with an automatic titrator.

2.7. High-Performance Liquid Chromatography (HPLC) Analysis of Lycopene and β -Carotene

The method described by Kubola and Siriamornpun [22] was adopted for the extraction of β -carotene from the samples. The samples were diluted with the mobile phase and filtered through a 0.45 μ m membrane filter. Then, 20 μ L of the filtered sample was injected for HPLC analysis. The analysis was performed using a Hitachi CM 5430 Diode Array Detector, Hitachi CM 5110 pump, Hitachi CM 5310 Column Oven (Hitachi High-Technologies Corporation, Tokyo, Japan), and Kanto Mightysil RP-18 GP (4.6 $\times$ 250 mm, 5 μ m, Kanto Chemical, Tokyo, Japan) column. The mobile phase consisted of acetonitrile, dichloromethane, and methanol (6:2:2) + 0.1% ammonium acetate, with a flow rate of 1.0 mL/min. The absorbance was measured at 450 nm.

Lycopene extraction was performed using the method described by Kubola and Siriamornpun [22]. The samples were diluted with tetrahydrofuran and filtered through a 0.45 μ m membrane filter. Then, 20 μ L of the filtered sample was injected for HPLC analysis. The mobile phase consisted of acetonitrile, tetrahydrofuran, and methanol (7:2:1), with a flow rate of 1.5 mL/min. The absorbance was measured at 450 nm.

2.8. Statistical Analysis

Data are expressed as mean $\pm$ SD ($n = 3$). The experiments were repeated in triplicates ($p < 0.05$), and the results were analyzed using one-way analysis of variance (ANOVA) with IBM SPSS Statistics 25 software.

3. Results

3.1. Effects of Capsule Types on the Acid Value and Peroxide Value

Acid value (AV) measures the free fatty acids (FFA) generated from the hydrolysis of triglycerides [27]. These FFAs are more susceptible to oxidation and rancidity, probably due to their higher degree of unsaturation and increased numbers of double bonds [12]. As shown in Figure 1, there is no significant increase in AV for both the unprotected hard capsule (HCN) and unprotected soft capsule (SCN) groups, indicating that encapsulation in both types of capsules slows down FFA formation. Recent research conducted by Wang et al. [28] has revealed the effect of capsule thickness and composition on the oxidative stability of fish oil capsules. The findings suggested that these factors may play a role in explaining the observed similarities in the protective effects offered by both soft and hard capsules. However, when considering the AV of Gac oil, it appears that the composition difference between hard capsules (made from HPMC) and soft capsules (made from starch) does not have a significant impact. The mechanism underlying the protective effects of hard and soft capsules was studied. HPMC capsules exhibit protective properties against reactions, stress, leakage, disintegration, and dissolution under various conditions [29]. On

the other hand, soft capsules made of HPMC have better sealing effects [30], which can effectively inhibit the formation of FFA in the oil. Furthermore, the synergistic effect of β -carotene and tocopherol present in the Gac fruit contributes to oxidative stability [31,32], preventing FFA formation.

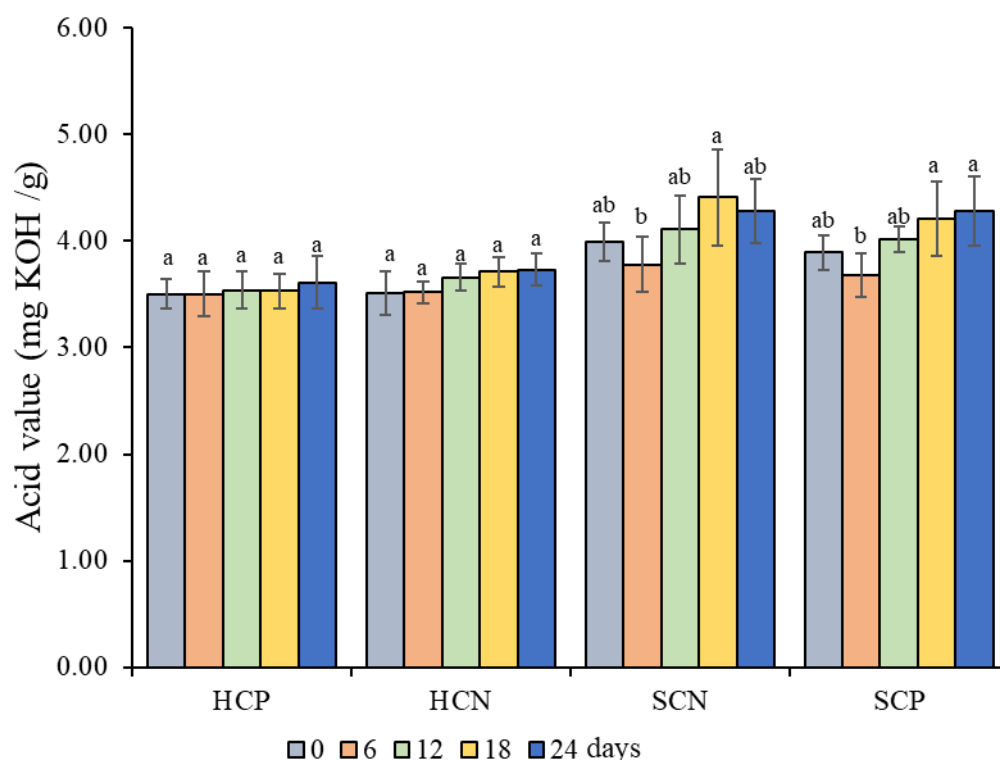


Figure 1. Effects of capsule type on the acid value (AV) of encapsulated Gac oil at different storage times. HCN: unprotected hard capsules; SCN: unprotected soft capsules; HCP: PTP-packed hard capsules; SCP: PTP-packed soft capsules. Data are expressed as mean $\pm$ SD ($n = 3$). Values with different letters are significantly different ($p < 0.05$).

There is no significant increase in AV for both the PTP-packed hard capsule (HCP) and PTP-packed soft capsule (SCP) groups, indicating that PTP-packed hard capsules and soft capsules both provide barriers that slow down the increase in FFA. SCP demonstrated higher AV values than HCP throughout the 24-day storage period, suggesting a higher initial AV content, but the difference is not statistically significant. Kwon et al. [33] found out that when omega-3 dietary supplements were PTP packed or placed in polyethylene or polypropylene containers, no significant difference was observed in the final AV content, indicating that these types of outer packaging provide certain protection against the formation of FFA [33]. Additionally, moisture content is a facilitating factor of rancidity, leading to an increase in AV [34]. It can be inferred that both soft capsules and hard capsules, in the presence or absence of PTP packaging, have protective effects against moisture permeation and therefore prevent the increase in AV. Furthermore, the synergistic effect of β -carotene and tocopherol present in the Gac fruit contributes to oxidative stability [31,32], preventing FFA formation.

Autoxidation is the process that leads to the oxidative deterioration of fats and oils. Primary oxidation products, such as hydroperoxides, are relatively stable at room temperature but can decompose into alcohols, aldehydes, ketones, and other secondary oxidation products, which can generate off-flavors and cause illness [35]. PV is the most commonly used parameter to assess the degree of primary oxidation in oils, measuring the formation of hydroperoxides during the oxidation process [36]. Gac aril contains approximately 70% unsaturated fatty acids in the total fatty acid composition, with 50% being polyunsaturated

fatty acids and a high concentration of oleic acid and omega-3 fatty acids [37]. Its highly unsaturated nature may contribute to oxidation and rancidity issues [38].

Figure 2 shows that the PV of the HCN group significantly increased from day 0 to day 18, indicating that unprotected hard capsules promote the primary oxidation and formation of peroxides in Gac oil. It was speculated that there are gaps between the hard capsule shells and the encapsulated oil, allowing air and moisture contact. The studies reported that various oxidative conditions, such as oxygen permeability, high temperature, light exposure, and the presence of iron and copper ions during processing, can compromise the stability of fish oil capsules, and the instability of fish oil due to its high content of omega-3 long-chain polyunsaturated fatty acids [39]. Therefore, it can be inferred that the high content of unsaturated fatty acids in Gac oil may contribute to oxidation during storage, and the capsules may have limited protective effects on the oxidative stability of the oil [39,40]. The permeability of the hard capsules and the gaps between the capsule shell and encapsulated component that allow oxygen contact may compromise the stability of the Gac oil capsules, leading to a rapid increase in PV. In contrast, the PV of the SCN group gradually decreased over the storage period, indicating the decomposition of primary oxidation products into secondary oxidation products. Primary oxidation products are prone to decomposing into alkoxy radicals at high temperatures, which then form aldehydes, ketones, acids, esters, alcohols, etc. SCN appeared to have lower primary oxidation products than the HSN, leading to a decrease in PV.

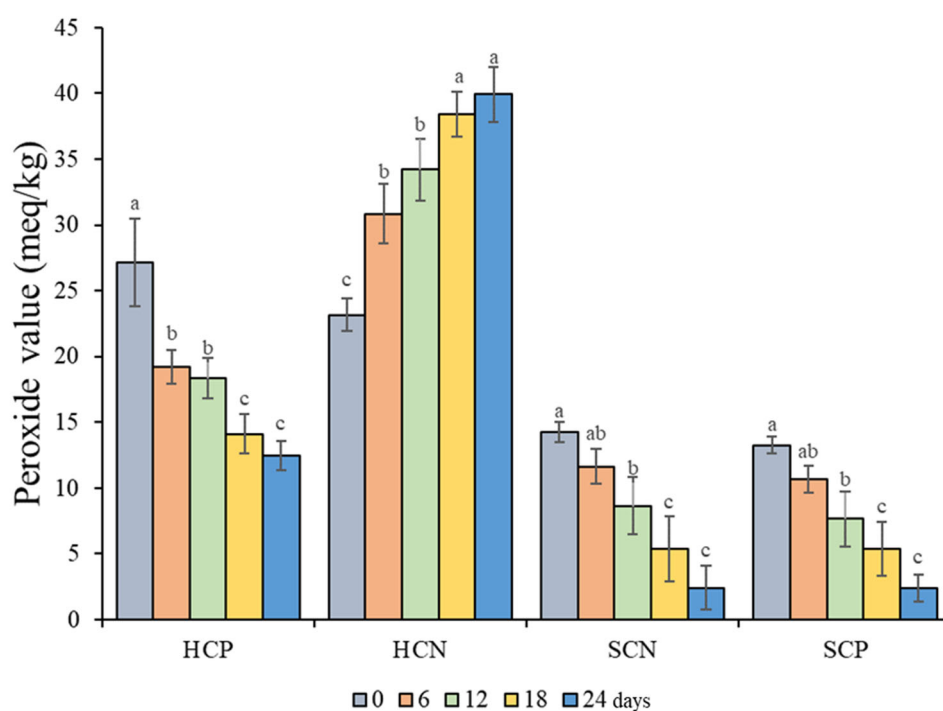


Figure 2. Effects of capsule type on the peroxide value (PV) in encapsulated Gac oil at different storage times. HCN: unprotected hard capsules; SCN: unprotected soft capsules; HCP: PTP-packed hard capsules; SCP: PTP-packed soft capsules. Data are expressed as mean $\pm$ SD ($n = 3$). Values with different letters are significantly different ($p < 0.05$).

The literature suggests that the formation of secondary oxidation products in the same type of oil generally occurs at similar times [41]. Therefore, it could be inferred that the absence of gaps inside the soft capsules and low oxygen concentration in the capsule contribute to lower oxidation [42] and a decrease in PV in the SCN [43].

According to Figure 2, the PV of the HCP and SCP groups significantly decreased from day 0 to day 24, indicating that storing them at 55 °C promotes the formation of

secondary oxidation products in the oil. Due to their different initial PVs, it can be inferred from the trend that PTP-packed soft capsules and hard capsules have the ability to reduce the formation of primary oxidation products and simultaneously initiate the formation of secondary oxidation products. Within the 24-day storage period, all PTP-packed hard capsules demonstrated the ability to reduce the formation of primary oxidation products in the oil, without exceeding the limit of PV (<15 meq/kg oil) set by the Codex Alimentarius Commission [44]. The literature suggests that PTP packaging provides protective properties for the oil within the capsules, enabling the PV to remain within the limit [33]. It is known that both PTP packaging and bottles made of polyethylene and polypropylene have protective effects. Since PTP packaging is individually sealed, it reduces the possibility of capsules being exposed to air during handling and interactions between capsules. Therefore, we used PTP in our study for its simplicity analysis. Gac oil contains abundant carotenoids and vitamin E, which can act as antioxidants in the oil, reducing the rate of formation of oxidation products. The literature indicates that the addition of antioxidants effectively slows down the increase in PV of plant oil and increases the stability of the oil [45]. Previous studies have demonstrated that storing olive oil in transparent polyethylene terephthalate (PET) containers for a period of six months, with a PV below 35 meq/kg oil, effectively reduces contact between the oil and oxygen, thereby slowing down the increase in PV [46]. Furthermore, the addition of antioxidants to refined olive oil stored at 60 °C for up to 48 days resulted in a lower PV [47]. Consequently, it can be speculated that the high vitamin E and phenolic compound content present in Gac oil [20] act as effective antioxidants, thereby slowing down the formation of oil peroxides.

3.2. Effects of Capsule Types on Lycopene and β -Carotene Content

Gac oil was found to be rich in carotenoids, including lycopene and β -carotene [20]. Therefore, Gac oil is often used as a nutritional supplement, colorant, and for medical use [1]. At the end of the storage test, the SCN group exhibited significantly higher lycopene content compared to the HCN group (Figure 3). Moreover, the SCN group maintained a stable lycopene level after day 6, while the lycopene content in the HCN group approached zero from day 12 onwards, indicating that unprotected hard capsules failed to protect lycopene from degradation, whereas soft capsules significantly slowed down the degradation of lycopene in the oil.

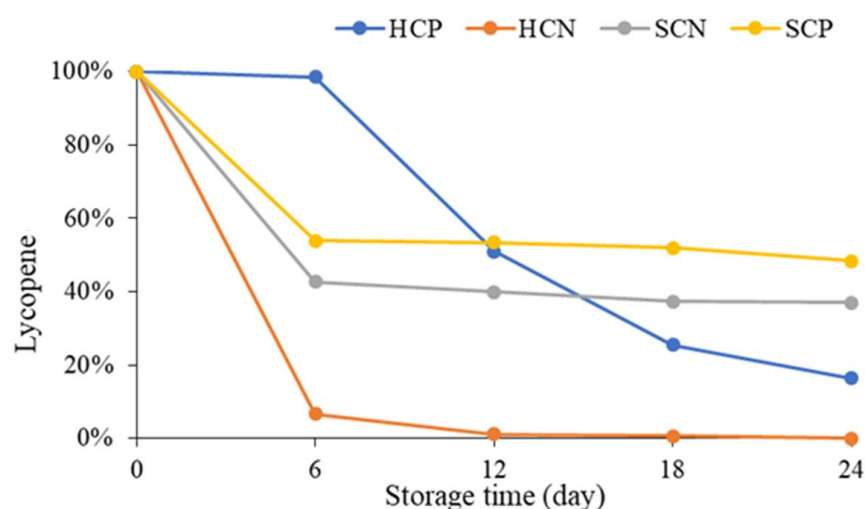


Figure 3. Effects of PTP packaging on the lycopene content loss rate of encapsulated Gac oil at different storage times. HCN: unprotected hard capsules; SCN: unprotected soft capsules; HCP: PTP-packed hard capsules; SCP: PTP-packed soft capsules. Data are expressed as mean $\pm$ SD ($n = 3$).

Figure 3 shows that from day 0 to day 24, in a comparison between the HCP and SCP groups, the SCP group exhibits a significantly higher lycopene content than the HCP group, which maintains over 40% content of lycopene. Furthermore, the SCP group maintains a consistent level after day 6, indicating that PTP packaging provides less protection for lycopene in hard capsules compared to soft capsules.

Most of the lycopene naturally occurs in the all-trans form. Lycopene has seven double bonds and can isomerize to cis or all-trans isomers. Among the isomeric configurations, 5-cis-lycopene is the most stable, followed by all-trans and 9-cis-lycopene. Furthermore, 5-cis-lycopene exhibits the lowest isomerization rate among other cis-lycopene isomers due to the significant rotational barrier, limiting its conversion to the all-trans form [48]. Previous studies have reported that the content of all-trans-lycopene in the peel of Gac fruit is 1342.1 $\mu\text{g/g}$ FW, while the cis-lycopene content is 82.1 $\mu\text{g/g}$ FW [49]. During storage at 50 °C, it was observed that the all-trans-lycopene did not significantly change within the first 12 h but started to decline afterward, whereas the cis-lycopene content decreased with increasing storage time, suggesting that the degradation rate of cis-lycopene might be higher than its formation rate [50]. Moreover, cis-lycopene is an unstable compound, and its unsaturated hydrocarbon chain consisting of multiple conjugated double bonds makes it susceptible to oxidation when exposed to oxygen, light, and heat [51,52]. The rapid decline in lycopene content in the HCN group may be attributed to the presence of gaps between the capsule shell and the oil, allowing rapid oxygen penetration. Previous research has also suggested that although oil can be a protective component against lycopene degradation, its efficacy is not significant [53], consistent with the findings of this study. Furthermore, factors such as temperature and light exposure can contribute to the reduction in carotenoid content [54].

PTP-packed soft capsules maintained a lycopene content of approximately 50% from day 6 to day 24, without significant changes, indicating that the soft capsule shell effectively protected lycopene from degradation. It has been suggested that the synergistic effect of carotenoids and phenolic compounds can enhance the stability of oil during storage [34]. Due to the protective nature of the soft capsule and the synergy between its components, lycopene remained stable without significant changes in content from day 6 to day 24. Studies have indicated that soft capsules formulated with lipophilic fillings exhibit high stability during storage and higher mechanical stability compared to soft gelatin capsules when exposed to increased humidity and temperature [55]. Therefore, the stability between the raw material of soft capsules and Gac fruit oil helps maintain a constant level of lycopene.

Figure 4 demonstrates a significantly higher β -carotene content in the SCN group compared to the HCN group, indicating that superior protection of β -carotene by soft capsules could maintain over 80% β -carotene content after being stored for 24 days. The β -carotene content in the HCN group shows a significant decline during storage, falling below the detection limit by the 24th day, suggesting inadequate protection against β -carotene degradation by the hard capsule shell. Figure 4 reveals a gradual decrease in β -carotene content in the HCP group (60%) during storage, while the SCP group (100%) exhibits no significant difference, indicating effective preservation of β -carotene in soft capsules with PTP packaging, but poorer protection for hard capsules.

Carotenoids, being lipophilic, are highly sensitive to environmental stressors such as high temperatures, oxygen exposure, and light during processing [56]. Variations in capsule filling conditions and the presence of gaps inside capsules increase oxygen exposure, impeding the effective prevention of β -carotene oxidation in HCN. Studies indicate that β -carotene in extra virgin olive oil shows a minimal decrease after 18 and 36 months of

storage, as it acts as an antioxidant [57,58]. Hence, factors beyond light are presumed to contribute to the rapid β -carotene decline in HCN, possibly linked to oxidation.

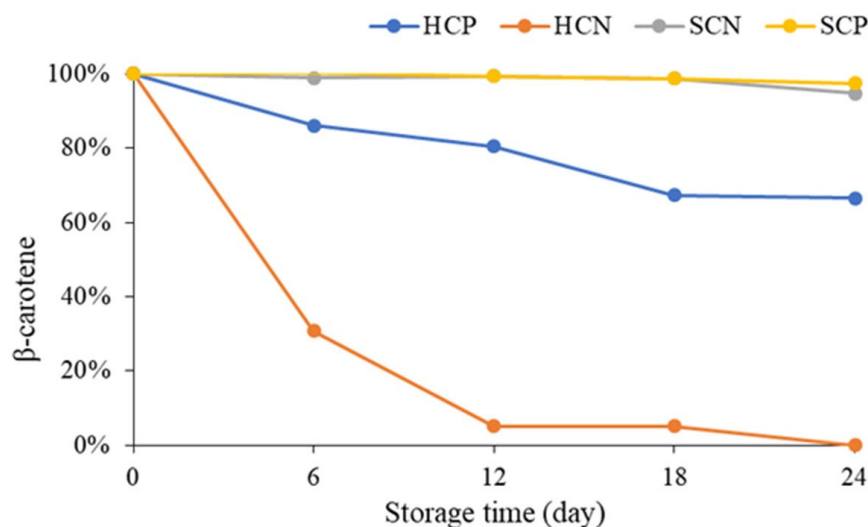
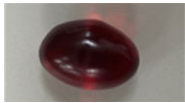


Figure 4. Effects of PTP packaging on the β -carotene content loss rate in encapsulated Gac oil at different storage times. HCN: unprotected hard capsules; SCN: unprotected soft capsules; HCP: PTP-packed hard capsules; SCP: PTP-packed soft capsules. Data are expressed as mean $\pm$ SD ($n = 3$).

SCP exhibited a minimal decline in β -carotene content from day 0 to day 24 of storage, reflecting effective protection by the soft capsule shell. Conversely, HCP showed a significant decrease, indicating insufficient preservation of β -carotene content even with PTP packaging. This could be attributed to the presence of gaps between the oil and the capsule, allowing oxygen penetration and consequent β -carotene degradation. In addition, the capsule exterior was free of cracks and pores, which are important to effectively prevent the oil and carotenoids from oxidation to the particle surface [59]. In a light-protected environment, β -carotene functions as a photo-oxidation antioxidant, slowing down oil oxidation [60,61]. However, factors such as temperature, light exposure, oxygen free radicals, lipid peroxidation-derived free radicals, and transition metal cations accelerate β -carotene degradation [62]. Higher PV in HCP may be due to the formation of oxygen free radicals and lipid peroxidation-derived free radicals, leading to β -carotene degradation. Another study also demonstrated the ability of soft capsules to maintain carotenoid content in concentrated pine needle extracts during storage [63].

Table 3 shows the appearance of hard capsules at the beginning of the storage test. The orange-red color is indicative of high carotenoid content, while the soft capsule shows a darker red color, probably due to the thicker shell of the soft capsules resulting in color differences [18]. Meanwhile, the hard capsules stored for 24 days at 55 °C, resulting in a dark yellow color. According to Figures 3 and 4, lycopene content in Gac oil decreased from 1916.7 ppm to 314.4 ppm after 24 days of storage, while β -carotene decreased from 6047.5 ppm to 4022.6 ppm after 24 days of storage. This indicates a significant degradation of lycopene, leading to a diminished red color, and the yellow color from β -carotene became more prominent. This represents the soft capsules stored for 24 days at 55 °C, exhibiting a deep red color. As shown in Figures 3 and 4, lycopene decreased from 3192.8 ppm to 1544.3 ppm after 24 days of storage, while β -carotene showed no significant decrease. This suggests that lycopene degradation is the major factor causing the reduction in red color in Gac oil, which is consistent with the observation of Galdeano et al. [64].

Table 3. Appearance of capsules stored at different storage times.

Packaging	Capsule	Day 0	Day 24
Press through packaging	Hard capsule		
	Soft capsule		
Naked particles	Hard capsule		
	Soft capsule		

4. Conclusions

Gac oil is rich in carotenoids and unsaturated fatty acids, making it a promising edible oil for direct consumption, with potential health benefits exceeding those of many conventional oils. With its high content of bioactive compounds, Gac oil has been increasingly used in nutritional supplements and functional food products. Its availability from locally cultivated varieties, such as the native Taiwanese Gac fruit used in this study, supports its commercial viability and regional sourcing potential. This study, which utilized a native Taiwanese variety of Gac fruit from Pingtung County, compared the stability of Gac oil in hard and soft capsules, with and without PTP packaging, under different storage conditions. Peroxide value (PV), acid value (AV), β -carotene, and lycopene content were used as indicators of oxidative degradation. The results showed that hard capsules with PTP packaging provided better protection against oxidation, particularly at elevated temperatures. These findings provide valuable insights into the formulation and packaging strategies for improving the shelf life of Gac oil capsules.

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