



Comparative Analyses of Drying and Extracting Efficacies and Carotenoid Components in Some *Momordica cochinchinensis* Samples Collected from the different areas in Vietnam

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Abstract *Momordica cochinchinensis* is a tropical vine that originates from South and Southeast Asia. It has traditionally been used in Asia for its ability to provide red coloring to cuisine and enhance human health. Gac fruit is now recognized as a valuable source of carotenoids, particularly β -carotene and lycopene. These carotenoids, along with other bioactive compounds such as astaxanthin and lutein, are associated with various beneficial activities including antioxidant, anticancer, and provitamin A properties. This study aimed to analyze and compare the drying and extracting efficiencies of different parts of gac fruit samples collected from various growing regions. The research also focused on detecting and comparing the compositions of astaxanthin, lutein, lycopene, and β -carotene in the peels, flesh (arils), and seeds of the fruit samples using HPLC analysis. The results revealed that the highest concentration of β -carotene was found in the flesh of Thai Binh samples (0.65%), followed by the flesh samples from Phu Xuyen (0.089%) and Ha Tinh (0.052%), while the lowest concentration was observed in the flesh of Hung Yen samples (0.025%). On the other hand, lycopene content was lower in the flesh of all samples, ranging from 0.013% to 0.089%. In terms of the peel samples, the Thai Binh sample exhibited the highest β -carotene concentration (~0.052%) followed by the Hung Yen sample (0.035%), the Phu Xuyen sample (0.024%), and the lowest composition of peels collected in Ha Tinh province (0.012%). It is worth noting that only the peels of Ha Tinh and Thai Binh samples contained the same concentration of lycopene composition (0.0031% and 0.0032%), while lycopene was not detected in the peel samples from Phu Xuyen and Hung Yen. The carotenoid compositions of the samples were found to vary depending on the variety and growing conditions. These findings provide valuable information for the development of effective isolation methods and the understanding of carotenoid compositions in gac samples from Vietnam.

Keywords astaxanthin, lycopene, β -carotene, lutein, Gac powder, carotenoids, HPLC

1. Introduction

Momordica cochinchinensis, commonly known as Gac or cochinchin gourd, belongs to the Cucurbitaceae family and is native to South-Eastern Asia. It is found in countries such as Thailand, Vietnam, China, and India. Gac is a dioecious plant, meaning it has separate male and female plants, and it can be grown from seeds,



branches, or roots. The plant typically blooms in the summer, and its fruits ripen around 9 to 10 weeks after pollination (Chuyen et al., 2014).

Carotenoid content is considered the main chemical component in gac fruit. It was first identified in 1941 by Guichard and Bui, and this finding was later confirmed by West and Poortvliet in 1993. Over the past two decades, numerous studies have been conducted on the chemical constituents and bioactive compounds in various gac species around the world due to their significant pharmacological activities. Within the fruit *M. Cochinchinesis*, there are beneficial carotenoid contents. These carotenoids are soluble pigments synthesized in plants, photosynthetic bacteria, and fungi through the mevalonic acid pathway using an isopentenyl pyrophosphate precursor (IPP) (Rodriguez-Amaya and Kimura 2004). The main carotenoids present are β -carotene and lycopene, responsible for the red and yellow-orange color of the fruit, respectively. Both β -carotene and lycopene have numerous benefits in biomedicine and nutraceuticals (Khan et al., 2021). They exhibit highly effective antioxidant activities due to their conjugated double bond system and free radical scavenging activity. Additionally, they have various biological activities that contribute to human health benefits (Khan et al., 2021; Bin-Jumah et al., 2022). Among the carotenoids studied, lycopene has the highest single oxygen-quenching rate (Perkins-Veazie et al. 2001). It also helps reduce the risk of cancer, macular dysfunction, and cardiovascular problems (Rao and Rao 2007).

Lycopene serves as a precursor for several cyclic carotenoids, such as β -carotene, which it converts into vitamin A. Vitamin A is crucial for cell membrane development, immune function, and skin health (Renzi et al., 2013; Rodriguez-Amaya and Kimura 2004). *M. Cochinchinesis* is an established food source with a high concentration of carotenoids, making it potentially valuable for the nutraceutical and functional food industries. In fact, *M. Cochinchinesis* contains significantly higher amounts of β -carotene and lycopene compared to other vegetables and fruits, including tomatoes (8.8–42.0 $\mu\text{g/g}$ FW lycopene) (Rao and Rao, 2007) and carrots (47–11,210 $\mu\text{g/g}$ FW β -carotene) (Gul et al., 2015). Despite its exceptional carotenoid content and phytochemical potential (Chuyen et al., 2015), *M. Cochinchinesis* exhibits genetic diversity and limited geographical distribution (Bootprom et al., 2012). It is primarily found in Southeast Asia, and there is limited information on varietal differences and the effects of carotenoids, which hampers breeding programs.

Other fruits, such as apricots, carrots, and tomatoes, also contribute significantly to the industrial economy due to their carotenoid content (Dragovic-Uzelac et al., 2007; Leja et al., 2013). The levels of carotenoids in these fruits are influenced by various environmental factors, including elevation (Rodriguez-Amaya and Kimura, 2004), rainfall (Brandt et al., 2003), and temperature (Leoni, 1992; Dumas et al., 2003). *M. Cochinchinesis* is distributed across Southeast Asia, encompassing tropical to temperate regions with diverse eco-geographical systems (Winalasiri et al., 2017). Various methods have been employed for the effective extraction, storage, and isolation of bioactive compounds in Gac fruit, such as oven air drying, vacuum drying, heat pump drying, spray drying, and freeze drying (Mai et al., 2014). Additionally, different oil extraction techniques, including mechanical and organic solvent extractions, supercritical fluid extraction, and enzymatic aqueous extraction, have been extensively utilized. However, there is a need for further development of advanced isolation and processes that are simple and cost-effective.

Gac is a widely grown fruit in Vietnam, found in various regions from north to south, including mountainous, coastal, and delta areas. In rural areas, Gac plants grow on dioecious vines and are typically harvested from climbing hedges or wild plants. There are different varieties of Gac, referred to as gac Nep (sticky) and gac Te (non-sticky). The gac Nep fruit is known for its larger size, thicker, and darker red arils compared to gac Te. Because of these characteristics, gac Nep is primarily cultivated in Vietnam for its size, color, and nutritional composition. Currently, gac Nep is being cultivated on a large scale in Vietnam to extract oil from its arils, which is used for its colorant and health benefits (Mai and Debaste, 2014).

The objectives of this study were to analyze and compare the drying and extracting efficiencies of different parts of gac fruit samples collected from various growing regions. Additionally, the research aimed to detect and compare the compositions of astaxanthin, lutein, lycopene, and beta-carotene in the peels, flesh (arils), and seeds of the fruit samples using HPLC analyses.

2. Materials and Methods



Material collection



Figure 1: The morphology characteristics of *M. Cochinchinesis* fruits collected from different provinces. A: Ha Tinh; B: Thai Binh; C: Phu Xuyen; D: Hung Yen.

Fruits of *M. Cochinchinesis* were collected from four different regions in Vietnam: Ha Tinh, Hung Yen, Thai Binh, and Phu Xuyen. The fruits were purchased and collected from gardens and immediately separated into three parts: peels, seeds, and flesh. The peels consisted of the exocarp and mesocarp, while the flesh contained the sarcotesta aril; the arils were removed from the seeds. The flesh of the Gac fruit is edible. All three parts were dried at 60°C for three days until completely dehydrated and then blended into a fine powder. However, half of the peels and flesh from Ha Tinh were kept undehydrated for steam distillation to determine the essence oils.

Isolation methods

The isolation methods are presented in Figure 2. In summary, each fruit sample was divided into arils (flesh), peels, and seeds. All parts were then cut into small pieces and dried until they reached less than 15% humidity. Next, they were finely ground using an experimental grinder. The weight of each sample was recorded to determine drying efficiency (%). To evaluate extracting efficiency, the powdered parts of *M. Cochinchinesis* were separated into labeled regions and labeled fruit parts in order to isolate the aromas. Approximately 2 grams of each part from all samples were weighed out and thoroughly mixed with CH₂Cl₂, which serves as a crucial solvent. The ratio of *M. Cochinchinesis* weight to solvent is 1:20 (g/mL); for this analysis, 40mL of CH₂Cl₂ was used and combined with 2g of *M. Cochinchinesis*.

The solution was placed in an Ultrasound-Assisted Extraction (UAE) machine for a total of 3 rounds, with each round lasting 60 minutes (Ashokkumar et al., 2008). The temperature for the UAE process was set to 30-35°C, as the solvent, CH₂Cl₂, evaporates at its boiling point of 60°C. Once all the solutions had been processed through UAE, they were combined with their respective fruit parts and subjected to a rotary evaporator to remove any excess solvents. A standardized method for HPLC 1260 and HPLC 1600 (Figure 2) was then used to inject and analyze 5mL of each fruit part solution.



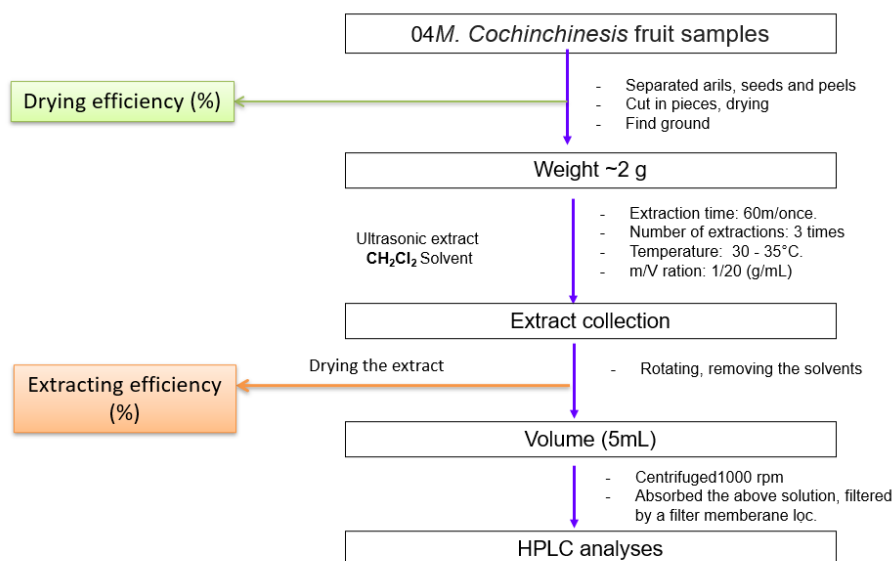


Figure 2: Isolating method to identify the carotenoid composition in the fruit sample

HPLC analysis

The volatile compounds in the samples were analyzed using Agilent HPLC 1200 and Agilent HPLC 1260 instruments, following the standardized methods described by Bohoyo-Gil et al. (2012) and Park et al. (2019). The carotenoids xanthosine and lutein were analyzed using the HPLC 1200 Agilent machine. The mobile phase consisted of MeOH-H₂O (95-5) and the column used was XDB-C18 (4.6 x 150 mm; 5 μm). The flow rate was set at 1.0 mL/min, and the pressure was maintained at 103 bar. The temperature was kept at a cool 25°C. The injection volume was 5 μL, and the UV/Vis detectors were set at 474 nm. The analysis was conducted for a duration of 20 minutes.

For the analysis of beta-carotene and lycopene, the HPLC 1260 Agilent instrument was used, following a standardized method. The mobile phase consisted of ACN-CH₂Cl₂ (90-10), and the column used was Eclipse XDB-C18 (4.6 x 150 mm; 5 μm). The pressure was set at 60 bar, and the temperature was maintained at 20°C. The injection volume was 10 μL, and the UV/Vis detectors were set at 450, 470, and 504 nm. The analysis was conducted for a duration of 30 minutes. The concentration of the tested solutions was 2000 ppm. All data were statistically analyzed using Excel version 2016.

3. Results & Discussion

Evaluation of drying and extraction efficacies in different parts of the fruit gac samples

In this study, four samples of gac fruits were collected from different growing regions. Ha Tinh Province is located in the North Central Coast region, while Phu Xuyen is a district of Hanoi, and Hung Yen and Thai Binh Provinces belong to the Red River Delta. The growing areas of Gac in those provinces have significantly expanded in recent years. All fruit samples were separated into peels, flesh, and seeds. Specifically, the peels included the exocarp and mesocarp, while the flesh consisted of sarcotesta aril. The black seeds were removed from the aril. The flesh of the Gac fruit is edible (Figure 2).

The collected gac fruits had oblong morphological traits. The Phu Xuyen fruit sample was slightly round. The sizes of the fruits were 10 to 13 cm in length and 9 cm in diameter. When ripe, the colors of the fruits were dark orange and red (Figure 1). Figure 2 displays the morphology of the collected gac fruits, including the exocarp, mesocarp (peels), flesh (arils), and seeds. The exocarp of gac fruits is typically thorny. The mesocarp, which constitutes approximately 50% of the fruit's weight, is spongy, orange, and 4 cm thick. Inside the mesocarp, there is an aril (flesh) that covers a black or brown seed, as well as yellow connective tissue in the middle. This description aligns with the findings of Vuong (2000).





Figure 3: The morphology of freshly cut *M. Cochinchinensis* fruits collected from different provinces. A: Hung Yen; B: Thai Binh; C: Ha Tinh; D: Phu Xuyen. Scale bar: 5 cm

After performing steam distillation on the fruit sample from Ha Tinh, which served as a representative sample, it was determined that no essential oils were present in either the flesh or the peel. Based on this result, it was assumed that other *M. cochinchinensis* samples would also not contain any essential oils. The peels and flesh of all the samples were manually separated and assessed for their drying efficiency. The drying efficiency ranged from 8.0% to 9.85%, with the Thai Binh sample having the highest (9.85%), followed by the Ha Tinh (8.0%) and Hung Yen (8.13%) samples. The Phu Xuyen sample observed the lowest drying efficiency (8.81%).

Table 1: Drying Efficiency (%) of the peels and flesh of the collected samples

No.	Part of fruits	Sample's names	Drying efficiency (%)
1		Ha Tinh	8.13
2	Peel	Hung Yen	8.00
3		Thai Binh	9.85
4		Phu Xuyen	7.81
5		Ha Tinh	43.90
6	Flesh	Hung Yen	42.30
7		Thai Binh	41.28
8		Phu Xuyen	47.39



According to the extraction methods, as shown in Figure 2, the peels, flesh, and black seeds were evaluated for their extraction efficiency. Among these parts, the peels collected from the Ha Tinh sample showed the highest efficiency (3.43%), followed by the Phu Xuyen sample (2.90%), the Thai Binh sample (1.01%), and the lowest was the Hung Yen sample (0.96%), as presented in Table 2. In terms of the flesh, the Phu Xuyen sample had the highest extraction efficiency (25.47%), followed by the flesh from the Hung Yen sample (20.79%), the Ha Tinh fresh sample (19.29%), and the lowest was found in the Thai Binh flesh sample (18.32%). As for the black seeds, both the Thai Binh and Phu Xuyen seeds had a percentage of over 21.20%, while the black seeds from Ha Tinh and Hung Yen were 12.94% and 14.49%, respectively (Table 2).

Table 2: Extraction efficiency (%) of the different parts of the fruit samples

No.	Part of fruits	Collection areas	Extraction efficiency (%)
1	Peel	Ha Tinh	3.43
2		Hung Yen	0.96
3		Thai Binh	1.01
4		Phu Xuyên	2.90
5	Flesh	Ha Tinh	19.29
6		Hung Yen	20.79
7		Thai Binh	18.32
8		Phu Xuyên	25.47
9	Black seed	Ha Tinh	14.49
10		Hung Yen	12.94
11		Thai Binh	21.29
12		Phu Xuyên	21.84

As shown in Figure 3, there was a significant difference in the total average extraction efficacy among the three parts of fruits in all collected fruit areas. The flesh had the highest extraction efficacy (20.9%), followed by the seeds (17.64%), and the peels had the lowest efficacy (2.07%) (Table 3).

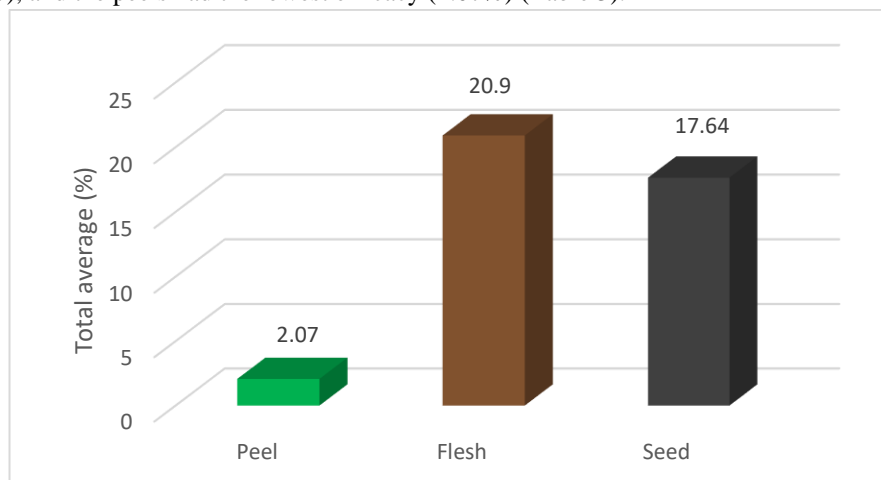


Figure 4: Total average of extraction efficacy (%) of different parts of the samples



Detection of some major Carotenoid compositions in the different parts of the gac fruits

In this study, we utilized the HPLC analysis system with CH₂CL₂ as the solvent. This system allowed us to detect various carotenoids in the flesh, peel, and seed of the fruits. However, not all carotenoids could be identified due to their low or non-existent concentration in these parts.

Table 3: Detection of carotenoid compositions in the different parts of fruits of 4 provinces.

No	Part of fruits	Samples collection	Detection			
			Astaxanthin	Lutein	Lycopene	β-carotene
1	Flesh	Ha Tinh	-	-	+	+
2		Phu Xuyen	-	-	+	+
3		Thai Binh	-	-	+	+
4		Hung Yen	-	-	+	+
1	Peel	Ha Tinh	-	-	+	+
2		Phu Xuyen	-	-	-	+
3		Thai Binh	-	-	+	+
4		Hung Yen	-	-	-	+
1	Black seed	Ha Tinh	-	-	-	-
2		Phu Xuyen	-	-	-	-
3		Thai Binh	-	-	-	-
4		Hung Yen	-	-	-	-

Specifically, both astaxanthin and lutein have not been detected in all parts of the fruit samples. Only lycopene and beta-carotene have been detected in all fruit samples' aril (fresh) and peels. However, the peels of samples collected in Phu Xuyen and Hung Yen provinces were not detected (Table 3). As a result, it can be explained that the composition of carotenoids depends significantly on fruit varieties and growing conditions.

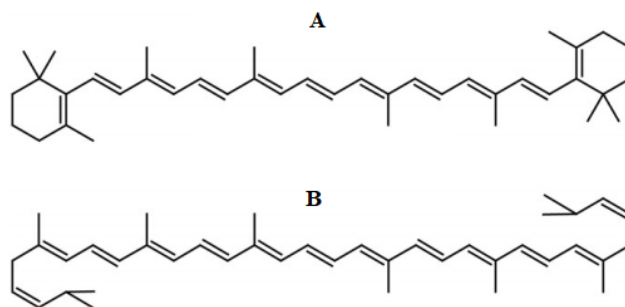


Figure 5: Chemical structures of beta-carotene (A) and lycopene (B) detected in peels and flesh of gac samples

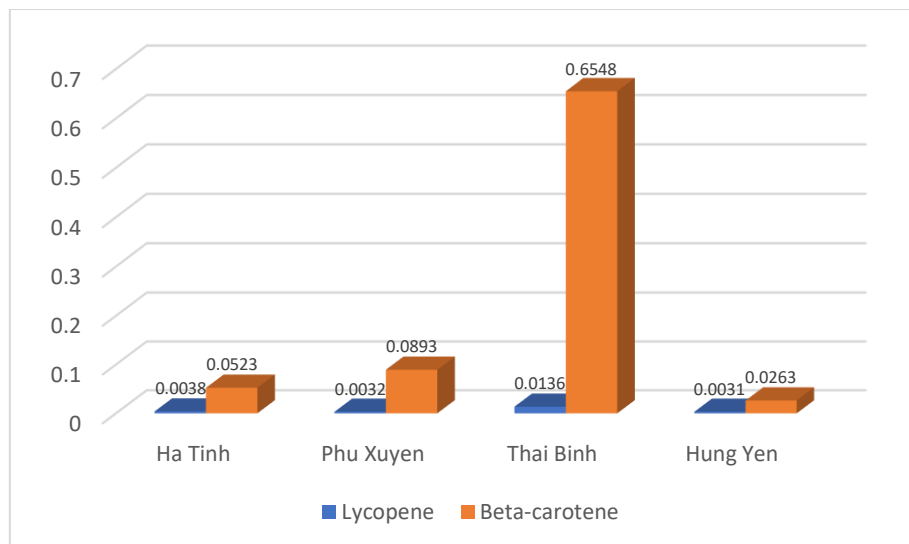


Figure 6: The concentration (%) of lycopene and beta-carotene in the flesh of the fruit samples

As shown in Figure 5, the Thai Binh flesh samples had the highest beta-carotene concentration (0.65%), followed by the Phu Xuyen flesh sample (0.089%), the Ha Tinh flesh sample (0.052%), and the lowest concentration was found in the Hung Yen flesh sample (0.025%). In contrast, the concentration of lycopene in the flesh of all samples was low, ranging from 0.013 to 0.089 (Figure 5). The chemical structures of lycopene and beta-carotene are illustrated in Figure 4.

As presented in Figure 6, the beta-carotene concentration in the peel parts of all samples was the highest in the Thai Binh sample (~0.052%), followed by the Hung Yen sample (0.035%), the Phu Xuyen sample (0.024%), and the lowest concentration was found in the peels collected in Ha Tinh province (0.012%). It is worth noting that only the peels of Ha Tinh and Thai Binh samples had the same concentrations of lycopene (0.0031% and 0.0032%), while lycopene was not detected in the Phu Xuyen and Hung Yen peel samples (Figure 6).

Our study aimed to analyze carotenoid contents in different cultivating regions of Vietnam: Ha Tinh, Phu Xuyen, Thai Binh, and Hung Yen provinces. *M. Cochinchinesis* is commonly quantified using HPLC (High-Performance Liquid Chromatography) (Kubola and Siriamornpun 2011; Vuong et al. 2006) and steam distillation using dried extracts of the fruits. These methods allow for accurate quantification of independent carotenoids and separation of isomers. However, they are laborious and the standardized procedures may not be suitable for developing nations. On the other hand, steam distillation methods (Machado et al. 2022) could be used to determine the richness of carotenoids in fruits. This method is simpler, cost-effective, and routinely used for extracting and identifying essential oils in fruits and vegetables as well as *M. Cochinchinesis*. In this study, we applied modified isolating methods to detect the carotenoid compositions in the fruit samples. These methods are simple, fast, and easily applicable to all parts of gac fruits.



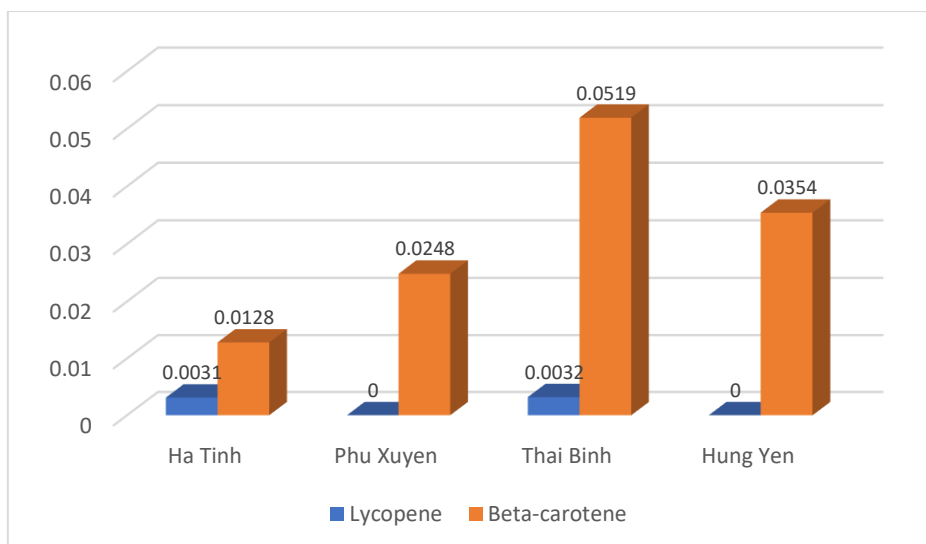


Figure 7: The concentration (%) of lycopene and beta-carotene in the peels of the fruit samples

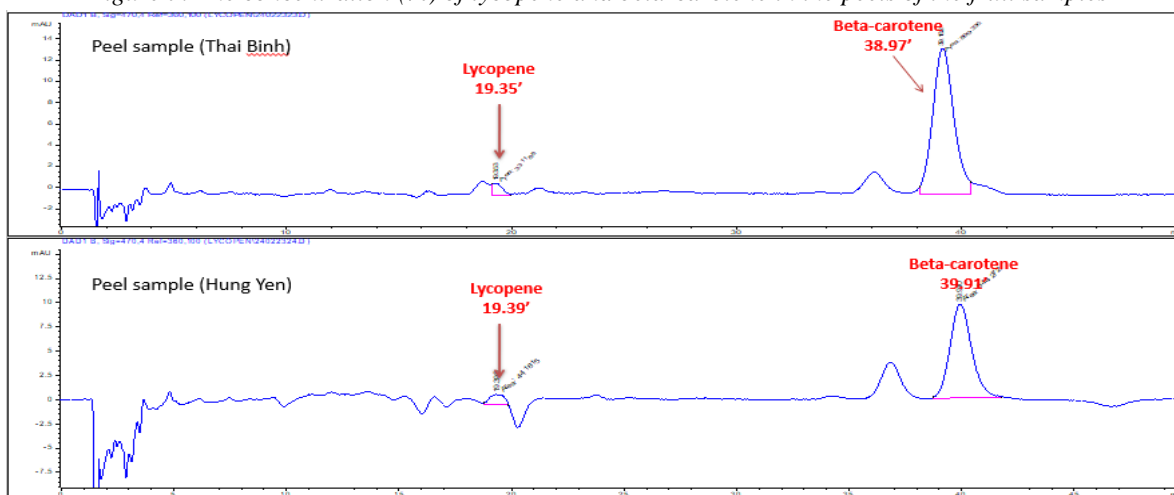


Figure 8: HPLC chromatogram of lycopene and beta-carotene detected from gac peel samples collected from Thai Binh and Hung Yen provinces

Conclusion

In conclusion, this study analysed and compared the drying and extracting efficiencies of the different parts of some gac fruit samples collected from different growing regions. We detected the carotenoid compositions and compared the astaxanthin, lutein, lycopene and beta-carotene compositions in the peels, flesh (arils) and seeds of the fruit samples using HPLC analyses. Our finding may provide useful information for effective isolation methods and carotenoid compositions in some gac samples in Vietnam.

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