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Investigation the ratios of antioxidant supplementation into the mixture of GAC (*Momordica Cochinchinensis* spreng) and carrier to get the highest total carotenoid content during drying

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Abstract
Gac fruit, *Momordica cochinchinensis* Spreng, also known as baby jackfruit or sweet gourd, is one of the traditional fruits in Vietnam. Studies report that extraordinarily high levels of carotenoids, especially β-carotene and lycopene, are found in Gac fruit aril, the brightly coloured flesh covering the seeds. Gac fruit also contains significantly high levels of α-tocopherol (vitamin E) and of fatty acids. It is very important therefore, to preserve or enhance these constituents in processed Gac fruit products, particularly the high levels of carotenoids and the associated antioxidant activity. Dried Gac powder is usually the dried aril component having the high concentration of nutrients and colour. The aim of this study is to investigate the ratios of antioxidant supplementation such as vitamin C and vitamin E into the mixture of Gac and carrier. The result shows that vitamin C at content of 2000 ppm (according to weight of wet material) supplemented into the mixture of Gac and carrier gives the highest total carotene content.

Keywords: Gac fruit, antioxidant supplementation, vitamin C, vitamin E, carotene

1. Introduction

Gac fruit, *Momordica cochinchinensis* Spreng, is botanically classified as Family Cucurbitaceae, Genus *Momordica*, and Species *Cochinchinensis*. It is also known as baby jackfruit, sweet gourd or cochinchin gourd in English. The fruit is one of the traditional fruits in Southeast Asia in general, and in Vietnam in particular. In Vietnam, Gac fruit is most commonly prepared as “Xoi Gac” (the Gac aril cooked in glutinous rice) for Tet (Vietnamese New Year) and wedding celebrations.
The Gac plant can be cultivated from seeds or root tubers, and grows as devious vines that are separate male and female plants. It can be commonly seen in Vietnam growing wild or in domestic settings with the vines growing on lattices in rural homes or in gardens. Two months after planting root tubers the plant will start flowering; flowering usually begins in April and continues until August or September.

Momordica Cochinchinensis Spreng *(Gac)* is botanically classified as follows:
Family: Cucurbitaceae
Genus: *Momordica*
Species: *Cochinchinensis*

Several studies have reported that Gac fruit contains extraordinarily high levels of carotenoids, especially carotenes and lycopene, in comparison to other fruits and vegetables containing lycopene and β-carotene. According to Bauernfeind (1972) and Aoki *et al*. (2002), the lycopene concentration in Gac fruit is at least five times higher than in other fruits analysed (rosehip, pitanga (Brazil), tomato (USA), guava (pulp), water melon, papaya and grapefruit). Furthermore, when compared to a range of other fruits and vegetables the β-carotene level in Gac fruit is the highest. Vuong (2000) stated that Gac fruit has the highest β-carotene content of the edible plants of Northern Vietnam. For example it is eight times higher than the level in carrots, which are commonly recognised as being high in β-carotene [4].

1.1 Carotene

Since carotenoids are the major component in Gac fruit, it is important to review the carotenoid pigments in terms of their structure, classification and distribution. In general, carotenoids are isoprenoid compounds, containing eight isoprenoid units whose order is inverted at the molecule centre; these are widely distributed in nature as red, yellow andorange pigments. More than 600 different carotenoids have been identified from natural sources; however, approximately twenty-four carotenoids commonly occur in foods and fourteen carotenoids have been identified in human serum (Dutta *et al*., 2005; Xianquan *et al*., 2005). Carotenoids are chemically divided into two groups, carotenes and xanthophylls. The first group is the highly unsaturated hydrocarbons, known as carotenes, which contain no oxygen. Xanthophylls contain one or more oxygen functional group (most commonly hydroxyl, keto, epoxy, methoxy or carboxylic acid groups) at particular sites on the terminal rings. Additionally, carotenoids are also classified as primary and secondary. The primary carotenoid group includes compounds required for photosynthesis, such as β-carotene, violaxanthin, and neoxanthin. The secondary classification includes carotenoids that are localised in fruits and flowers; these are α-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin, capsanthin and capsorubin (Delgado-Vargas *et al*., 2000) [4].

1.2 Many studies have reported about Gac

- Hiromitsu Aoki *et al*. (2002) determined carotene in Gac and concluded lycopene in Gac seed membrane with carotenoid concentrations to 380µg/g, 10 fold higher than those in any of the plant sources [4].
- L.T.Vuong *et al*. (2005) determined the acceptance of Gac supllementation to Vietnamese children. Results showed that vitamin A in Vietnamese children body was higher in Gac consumption than using β-carotene synthetic. They Vuong also reevaluated β-carotene content in fresh Gac fruit 408µg/g [3].
- Tran Hoang Thao *et al*. (2007) produced Gac powder by different drying methods. They proved that freeze drying method retained the highest β-carotene content. They also researched pretreatment methods to detach Gac seed membrane more easily, including thermal and enzyme. Loss of carotene by these pretreatment methods was 35%. If these products kept in vacuum below 25 ^{0}C would maintain red color and carotene to 70% in 4 month [5].
- Nguyen Minh Thuy *et al*. (2009) manufactured variety of Gac products such as: dried Gac seed membrane, jelly, gum, paste, oil and juice. They also proved the change of carotene in Gac seed membrane after 6 days harvested [1].
- Dang Thi Tuyet Nhung *et al*. (2009) evaluated the change of lycopene and β-carotene in Gac seed membrane and Gac oil during preservation. Gac seed membrane primarily contained lycopene 2.378–3.728 mg/g (raw material), β-carotene 0,257–0,379 mg/g (raw material), carotene stabilized within the first one week by strongly decomposed in the second week of preservation. Gac oil extracted from seed membrane with addition of 0.02% BHT, it could be preserved 15 to 19 weeks at 5 ^{0}C, 40 ^{0}C, 60 ^{0}C; lycopene and β-carotene also reduced dramatically [2].
- Tuyen Chan Kha *et al*. (2010) produced Gac powder by using spray drying method with maltodextrin supplementation. They concluded that the appropriate drying process to keep red color was in temperature 120 ^{0}C, 10% maltodextrin as carrier material (w/v) [6].

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2. Material and Methods

Raw Gac fruit source

Gac fruits *(Momordica cochinchinensis* Spreng*)* are originally collected from Trang Bang, Tay Ninh province, Vietnam when they are in half ripen stage. They are kept 6 days and then experimented.

2.1 Raw material preparation:

Gac fruits are chopped into two parts, collect seed membrane, discard seed. In our experiments, we only use seed membranes without seed, pulp and skin.

2.2 Antioxidant:

L (+)-Ascorbic acid in white crystal is provided from Shanghai Yuking Chemtech Co., Ltd.

D-alpha - tocopherol 96% is purchased from Jiangsu Xixin Vitamin Co., Ltd.

Fig 1: Gac cultivation farm

Fig 2: Overal ripen Gac after 6 days

3.1 Effect of vitamin C addition to carotene content in Gac powder

a. Experimental parameter:

- Concentration of vitamin C supplementation into carrier gelatin: maltodextrin (0.5:0.5) is 1000 ppm, 2000 ppm, 3000 ppm, 4000ppm (wet material).
- Control sample: Gac seed membrane being steamed in 6 minutes.

b. Fixed parameter:

- Gac seed membrane after being pretreated in preserved in refrigerator 5 ^{0}C, 15 minutes.
- Sample weigth: 35 g raw Gac seed membrane.
- Scatter sample in drying: 0.2g/cm^2.
- Temperature of drying: 60 ^{o}C.
- Moisture content of sample after being dryed: 6 ± 1%.

c. Target parameter:

- Total carotenoid µg/g Gac seed membrane (dry matter).

3.2 Effect of vitamin E addition

a. Experimental parameter:

- Concentration of vitamin E supplementation into carrier gelatin: maltodextrin (0.5:0.5) is 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm (wet material).
- Control sample: Gac seed membrane being steamed in 6 minutes.

b. Fixed parameter:

- Gac seed membrane after being pretreated in preserved in refrigerator 5 ^{0}C, 15 minutes.
- Sample weigth: 35 g raw Gac seed membrane.
- Scatter sample in drying: 0.2 g/cm^2.
- Temperature of drying: 60 ^{o}C.
- Moisture content of sample after being dryed: 6 ± 1%.

c. Target parameter:

- Total carotenoid µg/g Gac seed membrane (dry matter).

3.3 Comparison the mixing methods among carrier (gelatin: maltodextrin) and antioxidants:

a. Experimental parameter:

- Compare the results of carotene content in Gac seed membrane in three groups: (1) with carrier gelatin: maltodextrin 0.5:0.5; (2) vitamin C supplementation optimized among 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm; (3) vitamin E supplementation optimized among 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm.
- Gac seed membrane being steamed in 6 minutes.

b. Fixed parameter:

- Gac seed membrane after being pretreated in preserved in refrigerator 5 ^{0}C, 15 minutes.
- Sample weight: 35 g raw Gac seed membrane.
- Scatter sample in drying: 0.2 g/cm^2.
- Temperature of drying: 60 ^{o}C.
- Moisture content of sample after being dryed: 6 ± 1%.

c. Target parameter:

- Total carotenoid µg/g Gac seed membrane (dry matter).
- β- carotene µg/g Gac seed membrane (dry matter).

4. Results and Discussion

4.1 Effect of vitamin C addition to total carotene content in Gac powder:

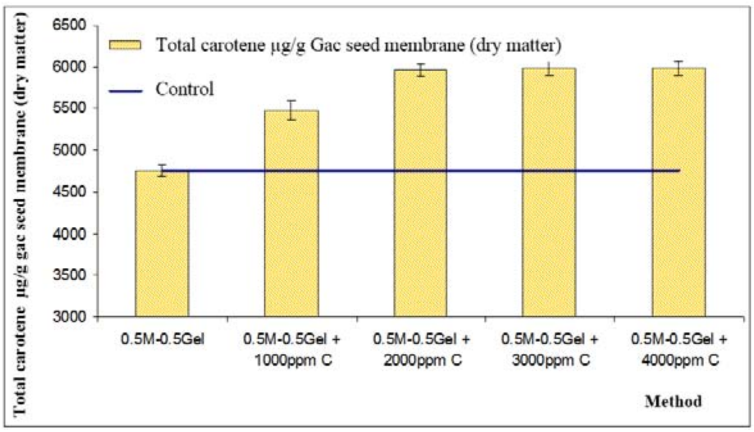

Fig 3: Effect of vitamin C concentration supplemented into Gac seed membrane to carotene content in gac powder (µg carotene/g gac seed membrane) (dry matter)

Table 1: Effect of vitamin C concentration supplemented into Gac seed membrane to carotene content in Gac powder

Method	Replication	Average of carotene (µg/g seed membrane) (dry matter)	Difference to control (%)
0.5M-0.5Gel (Control)	3	4756.43[a]	0.00
0.5M-0.5Gel + 1000ppm C	3	5476.42[b]	15.14
0.5M-0.5Gel + 2000ppm C	3	5962.69[c]	25.36
0.5M-0.5Gel + 3000ppm C	3	5984.52[c]	25.82
0.5M-0.5Gel + 4000ppm C	3	5987.44[c]	25.88

In this experiment, we gradually increase vitamin C concentration (0 ppm, 1000 ppm, 2000 ppm) into raw material before drying; we can obviously see total carotene loss decreased respectively. If we continue increasing vitamin C concentration (3000 ppm, 4000 ppm), we get no significant total carotene increment. Through ANOVA analysis, total carotene isn't significantly different at 2000 ppm, 3000 ppm, 4000 ppm ($\alpha = 0.05$). Vitamin C plays role as an anti-oxidant. While contacting to oxygen, it will be oxidized first so it can protect carotene in drying step. Because the amount of oxidable carotene is in limit so we don't need to use too much vitamin C. We choose vitamin C concentration 2000 ppm supplemented into raw material before drying (wet material).

Effect of vitamin E addition to total carotene content in Gac powder

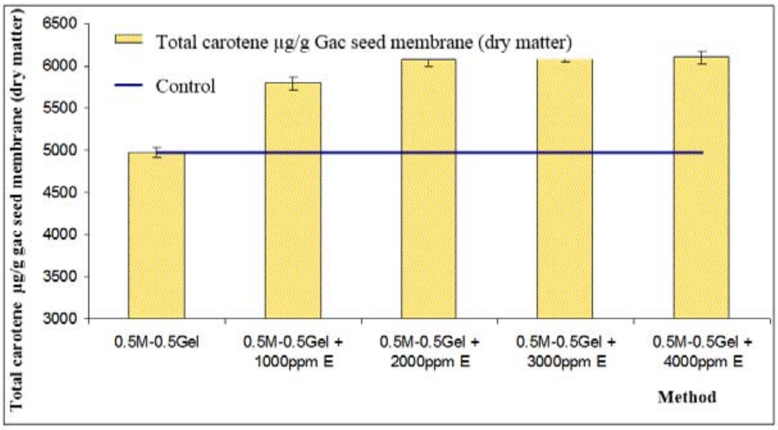

Fig 4: Effect of vitamin E concentration supplemented into Gac seed membrane to carotene content in gac powder (µg carotene/g gac seed membrane) (dry matter)

Table 2: Effect of vitamin E concentration supplemented into Gac seed membrane to carotene content in gac powder

Method	Replication	Average of carotene (µg/g seed membrane) (dry matter)	Difference to control (%)
0.5M-0.5Gel (Control)	3	4971.43[a]	0.00
0.5M-0.5Gel + 1000ppm E	3	5793.52[b]	16.54
0.5M-0.5Gel + 2000ppm E	3	6073.85[c]	22.18
0.5M-0.5Gel + 3000ppm E	3	6105.78[c]	22.82
0.5M-0.5Gel + 4000ppm E	3	6104.39[c]	22.79

In this experiment, we gradually increase vitamin E concentration (0 ppm, 1000 ppm, 2000 ppm) into raw material before drying; we can obviously see total carotene loss decreased respectively. If we continue increasing vitamin E concentration (3000 ppm, 4000 ppm), we get no significant total carotene increment. Through ANOVA analysis, total carotene isn't significantly different at 2000 ppm, 3000 ppm, 4000 ppm ($\alpha = 0.05$). Vitamin E plays role as an anti-oxidant. While contacting to oxygen, it will be oxidized first so it can protect carotene in drying step. Because the amount of oxidable carotene is in limit so we don't need to use too much vitamin E. We choose vitamin E concentration 2000 ppm supplemented into raw material before drying (wet material).

4.2 Comparison the mixing methods among carrier (gelatin: maltodextrin) and antioxidants:

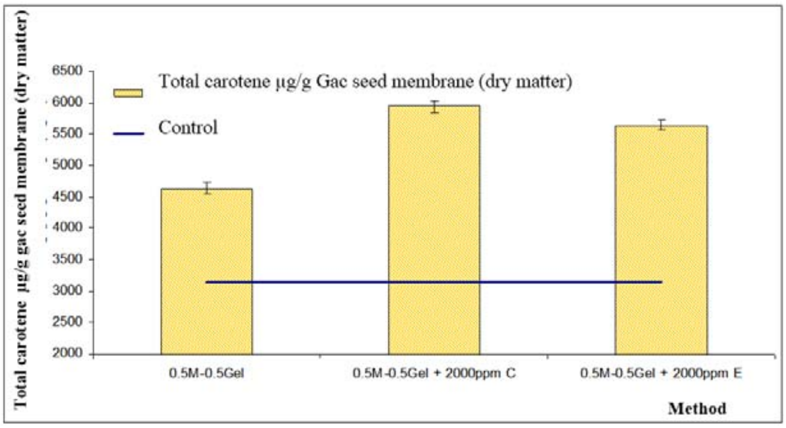

Fig 5: Effect of different anti-oxidants to total carotene (µg/g Gac seed membrane) (dry matter)

Table 3: Effect of different anti-oxidants to total carotene

Method	Replication	Average of carotene (µg/g seed membrane) (dry matter)	Difference to control (%)
Control	3	3138.17[a]	0.00
0.5M-0.5Gel	3	4636.64[b]	44.85
0.5M-0.5Gel + 2000ppm C	3	5939.32[d]	89.26
0.5M-0.5Gel + 2000ppm E	3	5643.37[c]	79.83

Basing on ANOVA analysis at significant level α =0.05, we can completely see the significant differences while using carrier maltodextrin: gelatin at ratio 0.5: 0.5 (w/w), 2000ppm vitamin C or 2000ppm vitamin E supplementation in respect of total carotene (µg/g gac seed membrane) and higher than control sample (steaming 6 minutes, without carrier). Moreover, we can conclude some important points as follow:

- Maltodextrin: gelatin at ratio 0.5: 0.5 (w/w) with 2000ppm vitamin C show the best result, 89% higher than control sample.
- Maltodextrin: gelatin at ratio 0.5: 0.5 (w/w) with 2000ppm vitamin E show the best result, 80% higher than control sample.
- Maltodextrin: gelatin at ratio 0.5: 0.5 (w/w) without antioxidant show the best result, 45% higher than control sample.

From above experiments, we comprehensively defined the role of each supplement added into gac seed membrane pretreated. Maltodextrin – gelatin protects raw Gac material out of oxygen in drying step. Vitamin C and vitamin E which are supplemented to seed membrane-carrier will be anti-oxidant in drying step because they will be primarily oxidized.

According to our results, vitamin C addition at 2000 ppm will be superior to vitamin E 2000 ppm regarding total carotene. So we finally select the treatment method using: carrier: Gac seed membrane 1:1; maltodextrin: gelatin 0.5: 0.5 (dry matter); 2000 ppm vitamin C addition.

Determine β-carotene by High Performance Liquid Chromatography (HPLC)

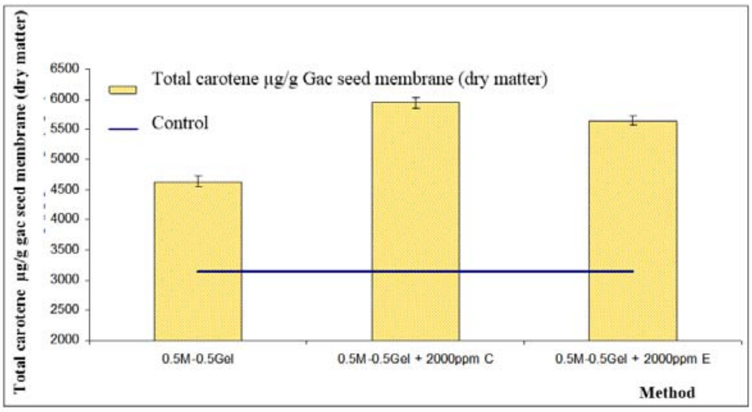

Fig 6: Effect of different anti-oxidants to β-carotene (µg/g Gac seed membrane) (dry matter)

Table 4: Effect of different anti-oxidants to β-carotene

Method	Replication	Average of carotene (µg/g seed membrane) (dry matter)	Difference to control (%)
Control	1	261.14	0.00
0.5M-0.5Gel	1	378.82	45.07
0.5M-0.5Gel + 2000ppm C	1	499.44	91.26
0.5M-0.5Gel + 2000ppm E	1	475.00	81.89

Analysis results of the β-carotene determined by HPLC and photography method are equivalent. We can conclude some important points as follow:

- Method using carrier: Gac seed membrane 1:1, maltodextrin: gelatin at ratio 0.5: 0.5 (w/w) with 2000ppm vitamin C shows the best result, higher 97% than control sample.
- Meanwhile, using carrier: Gac seed membrane 1:1, maltodextrin: gelatin at ratio 0.5: 0.5 (w/w) with 2000 ppm vitamin E shows the second result, higher 82% than control sample.
- Maltodextrin: gelatin at ratio 0.5: 0.5 (w/w) without antioxidant shows the third result, higher 45% than control sample.

All three above groups, they are all superior to control sample regarding β-carotene (steaming 6 minutes without carrier). We also use HPLC testing method to check stability and nutritional value of Gac powder; results are as follow: protein (24.50%), total sugar (38.75%), lipid (11.24%).

Fig 7: Gac powder without carrier

Fig 8: Gac powder with carrier: Gac seed membrane (1:1), maltodextrin: gelatin (0.5: 0.5)

Fig 9: Gac powder with carrier: Gac seed membrane (1:1), maltodextrin: gelatin (0.5: 0.5, 2000 ppm vitamin E

Fig 10: Gac powder with carrier: Gac seed membrane (1:1), maltodextrin: gelatin (0.5: 0.5), 2000ppm vitamin C

From 1 kg raw Gac fruit, we get 200g seed membrane (20%), remove 80g seed (8%). Moisture of seed membrane is about 80% so 200g seed membrane is equal to 40g dry matter. In general, raw Gac fruit contains about 4% dry matter of seed membrane. On our calculation for above experiments, we decide the pretreatment method by steaming in 6 minutes, carrier ratio 1:1, ratio of maltodextrin: gelatin 0.5: 0.5 (dry matter); addition of 2000 ppm vitamin C will keep total carotene higher 2-3 times to control. Total carotene in gac powder (dry matter) is about 6000 µg/g seed membrane; β-carotene is about 500 µg/g seed membrane.

5. Conclusion

The effects of different treatments to total carotene and β-carotene of Gac fruit powder were investigated. Addition of some anti-oxidants such as vitamin C and vitamin E will be the appropriate approach to limit the carotene loss in drying step. Concentration of these vitamins should be 2000 ppm based on wet material weight. Mixing carrier (maltodextrin: gelatin, 0.5:0.5) together with anti-oxidant (2000 ppm vitamin C) will successfully preserve total carotene 2.3 times.

6. References

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