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Oral Presentation

## CAROTENOIDS CONTENT OF COMMERCIAL SEAWEED IN BALI

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### ABSTRACT

In Bali there are several types of seaweed that has long been used as a source of food by people. These seaweed local name are *Bulung Boni* (*Caulerpa* spp.) and *Bulung Sangu* (*Gracilaria* spp.). However the study of total carotenoids content and types of carotenoids of these seaweed are very limited, therefore need to be further study. The types of carotenoids in this study identified based on the retention of value (Rf) on thin layer chromatography. This research concluded that total carotenoids of *Bulung Boni* (*Caulerpa* spp.) higher than *Bulung Sangu* (*Gracilaria* spp.). Total carotenoids were 57.734 mg / 100 g in *Bulung Boni*, and 1.776 mg /100 g in *Bulung Sangu*. The separation of carotenoids *Bulung Boni* and *Bulung Sangu* on thin layer chromatography obtained several types of carotenoids. Based on the calculation Rf values on *Bulung Boni* found as many as nine types of carotenoids such as neoxanthin, astaxanthin free, antheraxanthin, canthaxanthin, astaxanthin monoester, fucoxanthin, chlorophyll b, astaxanthin diester, and beta carotene. In *Bulung Sangu* found eight types of carotenoids, such as neoxanthin, violaxanthin, astaxanthin free, antheraxanthin, lutein, chlorophyll b, chlorophyll a, and beta carotene.

*Keywords* : *Caulerpa* spp., *Gracilaria* spp., and carotenoids

### INTRODUCTION

Seaweeds are nutritious because of their high protein content and high concentration of minerals, trace elements, vitamin, and carotenoids. As precursors of vitamin A, carotenoids are fundamental components in our diet, and play additional important roles in human health. Industrial uses of carotenoids include pharmaceuticals, food supplements, animal feed additives, and colorants in cosmetics (Mann *et al.*, 2000; Thomas, 2005). Carotenoids are synthesized in all photosynthetic organisms, some bacteria, and fungi (Stahl *et al.*, 2002). Animals are unable to synthesize them *de novo*, they must obtain them by dietary means (Deming *et al.*, 2003; Liu, 2004). Carotenoids content and composition in plants can improve their nutritional value, and provide a new source for valuable materials for industry. Some carotenoids have also been shown as potent antioxidant, inhibitor of lipid peroxidation, anti stress, anti inflammatory, and anti aging (Siems *et al.*, 2002; Ronen *et al.*, 2005). Antioxidants are molecules which can safely interact with free



radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. The body cannot manufacture these micronutrients so they must be supplied in the diet (Salganik, 2001).

In Bali, there are two types of seaweeds that have been consumed as vegetables. These seaweeds are *Bulung Boni* (*Caulerpa* spp.), and *Bulung Sangu* (*Gracilaria* spp.). People in Bali has long been used these seaweed as a source of food , but untill this time the study or publication about the total carotenoids content and types of carotenoids *Bulung Boni* and *Bulung Sangu* are very limited, therefore needs to be further study.

## MATERIALS AND METHOD

### 1. Collection of seaweeds

Seaweeds were collected from Serangan Beach in Bali, and dried without sunlight.

### 2. Analysis of total carotenoid

Dried seaweed grinded become powder. Aproximately 0,5 g samples in powder put into centrifuge tube, added 5 ml acetone and petroleum ether, centrifuge in 3000 rpm for five minute. Take supernatant, and put into new reaction tube. The lisat added 5 ml acetone and 5 ml petroleum ether, do it for twice. Supernatant put into separate tube, and wash with distilled water. Take supernatant, and put into microtube, added 1 g Na<sub>2</sub>SO<sub>4</sub>, and then vortex. After this step take supernatant, added petroleum ether until volume 10 ml. Read absorbance in λ 450 nm with PE as blanco.

$$\text{Total carotenoid} = \frac{\text{total volume} \times \text{absorbance} \times 100 (\mu\text{g}/100 \text{ g})}{0,2 \times \text{sample weight}}$$

### 3. Types of carotenoids *Bulung Boni* (*Caulerpa* spp.) and *Bulung Sangu* (*Gracilaria* spp.)

Dried seaweed grinded until smooth and become powder then soaked in a solution of dietilether for 24 hours. Furthermore carotene solution was evaporated by poured into Eppendorf tubes. Carotene solution is the result of carotenoids extracted from seaweed that will be placed on a thin layer chromatography. The analysis was performed with silica plates using solution. The movement of

carotenoids in the thin layer of silica was observed and calculated by the formula Rf by Crowe (2005) as follows:

$$R_f = \frac{D_1}{D_2}$$

Description: Rf = Relative to the front value

D1 = distance components

D2 = distance solvent

## RESULTS AND DISCUSSION

### 1. Analysis of total carotenoids

Analysis of total carotenoids in *Bulung Boni* (*Caulerpa* spp.) higher than *Bulung Sangu* (*Gracilaria* spp.). Total carotenoids contents in *Bulung Boni* is 57.734 mg / 100 g and in *Bulung Sangu* 1.776 mg /100 g.

### 2. Types of Carotenoids *Bulung Boni* (*Caulerpa* spp.) and *Bulung Sangu* (*Gracilaria* spp.)

The result of the separation of carotenoids *Bulung Boni* (*Caulerpa* spp.) and *Bulung Sangu* (*Gracilaria* spp.) on thin layer chromatography obtained several types of carotenoids. Based on the calculation Rf values on *Bulung Boni* found as many as nine types of carotenoids such as neoxanthin, astaxanthin free, antheraxanthin, canthaxanthin, astaxanthin monoester, fucoxanthin, chlorophyll b, astaxanthin diester, and beta carotene. In *Bulung Sangu* found eight types of carotenoids, such as neoxanthin, violaxanthin, astaxanthin free, antheraxanthin, lutein, chlorophyll b, chlorophyll a, and beta carotene (Table 1).

*Bulung Boni* (*Caulerpa* spp.) is a group of divisio Chlorophyta; Class Chlorophyceae; Ordo Caulerpales; Family Caulerpaceae; Genus *Caulerpa*; Species *Caulerpa* spp. *Bulung Sangu* is a group of Divisio Rhodophyta; Class Rhodophyceae; Ordo Gigartinales; Family Gracillariaceae; Genus *Gracillaria*; Species *Gracillaria* spp. Burtin (2002), reported that the main carotenoids in the red algae are  $\beta$ -carotene and  $\alpha$ -carotene, and their dihydroxylated derivatives zeaxanthin and lutein. The carotenoid composition of the green algae is similar to that of higher plants. The main carotenoids present are  $\beta$ -carotene, lutein, violaxanthin, antheraxanthin, zeaxanthin, and neoxanthin.

Table 1. Rf value of carotenoids from *Bulung Boni* (*Caulerpa* spp.) and *Bulung Sangu* (*Gracilaria* spp.)

| Types of seaweed       | Rf value      | Types of carotenoids  |
|------------------------|---------------|-----------------------|
| <i>Caulerpa</i> spp.   | 0,09          | Neoxanthin            |
|                        | 0,30          | Astaxanthin free      |
|                        | 0,36          | Antheraxanthin        |
|                        | 0,41          | Canthaxanthin         |
|                        | 0,48          | Astaxanthin monoester |
|                        | 0,50          | Fucoxanthin           |
|                        | 0,59          | Chlorophyll b         |
|                        | 0,70          | Astaxanthin diester   |
|                        | 0,98          | Beta carotene         |
| <i>Gracilaria</i> spp. | 0,09          | Neoxanthin            |
|                        | 0,21          | Violaxanthin          |
|                        | 0,30          | Astaxanthin free      |
|                        | 0,36          | Antheraxanthin        |
|                        | 0,42          | Lutein                |
|                        | 0,55          | Chlorophyll b         |
|                        | 0,69          | Chlorophyll a         |
| 0,98                   | Beta carotene |                       |

There are two main classes of naturally occurring carotenoids: carotenes, which are hydrocarbons that are either linear or cyclized at one or both ends of the molecule (such as  $\beta$ -carotene,  $\alpha$ -carotene), and xanthophylls, which are oxygenated derivatives of carotenes. All xanthophylls produced by higher plants, for examples violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and lutein, are also synthesized by green algae. However specific green algae possess additional xanthophylls such as astaxanthin and canthaxanthin. In addition, diatoxanthin, diadinoxanthin, and fucoxanthin, are produced in brown algae or diatoms (Eonseon et al., 2003).

Biosynthetic pathway for carotenoids are Farnesyl pyrophosphate (FPP) combining with C5- isoprenoid units is extended to C20 molecules, geranylgeranyl pyrophosphate (GGPP) by geranylgeranyl pyrophosphate synthase (crtE). The common C40 carbon results from the condensation of two C20 molecules by phytoene synthase (crtB). The sequential desaturation steps and cyclization of the ends of the molecule to generate carotenes are catalyzed by phytoene desaturase (crtP/crtI),  $\zeta$ - carotene desaturase (crtQ) and lycopene cyclase (Figure 1) (Liang, 2006). Figure 1. Show The names of enzymes are according to the crtE, geranylgeranyl pyrophosphate synthase; crtB, phytoene synthase; crtP, phytoene desaturase; crtQ, zeta-carotene desaturase; crtL, lycopene beta cyclase, ; lycopene

epsilon cyclase; *cruA*, the most like candidate for lycopene cyclase by comparison to CT0456 in the species lacking *crtL*. *crtO/crtW*, beta-carotene ketolase; *crtR*, betacarotene hydroxylase.

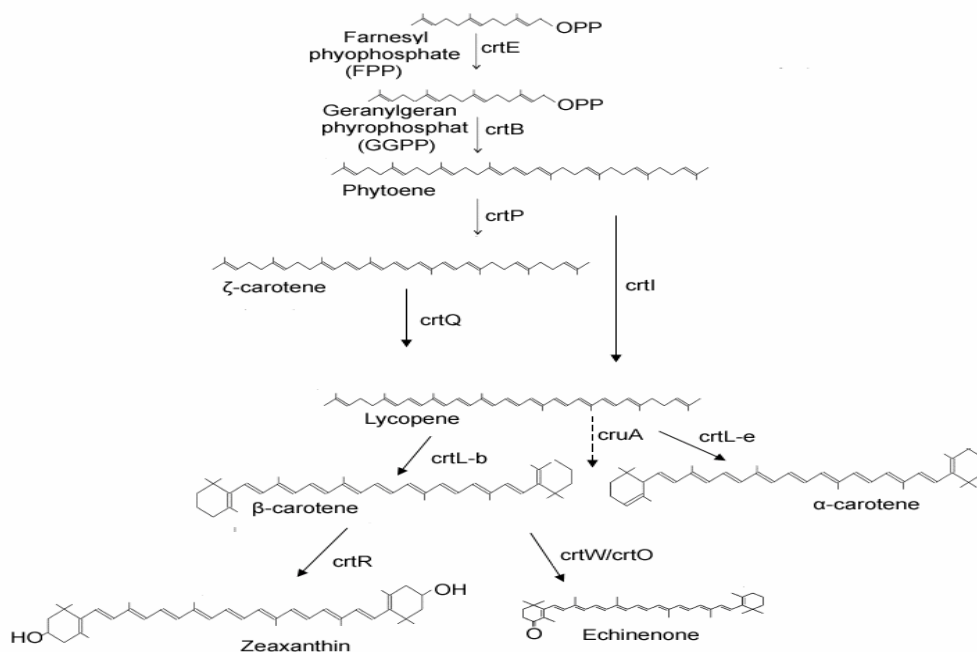


Figure. 1. Biosynthetic pathway of carotenoids.

## CONCLUSION

Total carotenoids of *Bulung Boni* (*Caulerpa* spp.) higher than *Bulung Sangu* (*Gracilaria* spp.). Total carotenoid are 37,249.00  $\mu\text{g}/100\text{ g}$  in *Bulung Boni*, and 1,777.63  $\mu\text{g}/100\text{ g}$  in *Bulung Sangu*. In *Bulung Boni* found as many as nine types of carotenoids such as neoxanthin, astaxanthin free, antheraxanthin, canthaxanthin, astaxanthin monoester, fucoxanthin, chlorophyll b, astaxanthin diester, and beta carotene. In *Bulung Sangu* found eight types of carotenoids, such as neoxanthin, violaxanthin, astaxanthin free, antheraxanthin, lutein, chlorophyll b, chlorophyll a, and beta carotene.

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