

## IDENTIFICATION OF CAROTENOID COMPONENTS IN GREEN VEGETABLES AS BASIC INGREDIENTS FOR ALTERNATIVE FOOD SUPPLEMENTS

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### ABSTRAK

Karotenoid yang dikenal sebagai prekursor vitamin A ( $\beta$ -karoten) saat ini sedang dikembangkan sebagai pelindung terhadap penyakit kanker dan jantung, mengurangi penyakit mata, anti oksidan, dan pengatur sistem imun tubuh. Penelitian ini bertujuan untuk mengetahui kandungan karotenoid pada beberapa sayuran hijau sebagai alternatif bahan dasar suplemen makanan. Penelitian ini dilakukan di Laboratorium Pendidikan Biologi Universitas Syiah Kuala dengan menggunakan metode observasi langsung yang mengukur nilai absorbansi karotenoid pada 5 jenis sayuran hijau, dengan menggunakan teknik purposive sampling. Sampel terlebih dahulu dipotong kecil-kecil dan ditimbang 0,03 mg kemudian diekstraksi dan dihitung nilai absorbansinya menggunakan spektrofotometer dengan panjang gelombang 480, 663, dan 664, diukur dengan 5 perlakuan dan 4 ulangan dengan pengenceran 20%, 40%, 60%, 80%, dan 100%. Sampel yang telah diencerkan diukur kandungan karotenoidnya. Hasil penelitian menunjukkan bahwa kandungan karotenoid tertinggi terdapat pada daun kacang panjang dan terendah pada daun pepaya.

**Kata Kunci:** Karotenoid, Sayuran Hijau, Suplemen Makanan.

### ABSTRACT

Carotenoid known as a precursor to vitamin A ( $\beta$ -carotene) is currently being developed as protection against cancer and heart disease, reducing eye disease, anti-oxidants, and regulators in the body's immune system. This study aimed to determine the carotenoid content in some green vegetables as an alternative to the basic ingredients of food supplements. This research was conducted in the biology education laboratory of Syiah Kuala University using direct observation methods that measure the absorbance value of carotenoids in 5 types of green vegetables, using a purposive sampling technique. The sample was first cut into small pieces and weighed 0.03 mg then extracted and the absorbance value was calculated using a spectrophotometer with a wavelength of 480, 663, and 664, measured by 5

treatments and 4 replications with a dilution of 20%, 40%, 60%, 80 %, and 100%. Samples that have been diluted are measured for the carotenoid content. The results showed that the highest content of carotenoid was found in long bean leaves and the lowest was in papaya leaves.

**Keyword:** Carotenoid, Green Vegetables, Food Supplement.

## INTRODUCTION

The Green vegetables for Indonesian people are vegetables that are often consumed. In green vegetables there are components that can be a source of micronutrients. One of the important componenets in green vegetables is carotenoid.

Carotene, known as a precursor of vitamin A ( $\beta$ -carotene), is currently being developed as protection against cancer, heart disease, reducing eye diseases, anti-oxidants, and regulators in the body's immune system [1]. The latest research revealed that lycopene was able to reduce the risk of prostate cancer, lung cancer, uterine cancer, and skin cancer [2]. The sources of carotene were dark green vegetables and yellow-orange vegetables and fruits, such as cassava leaves, peanut leaves, kale, spinach, long beans, beans, carrots, tomatoes, and papayas [3].

Currently, a supplement is being spread, namely liquid chlorophyll or chlorophyllin based on chlorophyll

extract of alfalfa leaves (*Medicago sativa* L.). It is widely traded as ready-to-eat supplements. Extensive studies have been conducted on alfalfa. All parts of this plant contain components that are functional for the body, including saponins, sterols, flavonoids, Kuramin, alkaloids, vitamins, amino acids, sugars, proteins, minerals, and other nutritional components [4].

Alfalfa plants are cultivated in Europe, Australia, the United States, South Africa, China, the Middle East, and Indonesia. In Indonesia, it is mostly cultivated in Central Java. The use of alfalfa leaf extract as a food supplement caused constraints related to its growing areas because the plant was a member of the Leguminosae from the subtropics so its cultivation in Indonesia required a cool climate [4]. Due to the constraints of using alpha leaves, other alternatives can be sought for chlorophyll supplements. The plants chosen to be used as chlorophyll supplements are

frequently-consumed green vegetables such as mustard greens, water spinach, long bean leaves, papaya leaves, and Gotu kola. Some experts state that the main key to maintaining health is the consumption of green foods, especially vegetables.

In general, people only know that green vegetables only contain nutrients such as iron, vitamins, and fiber but they do not know the role of chlorophyll and carotenoids, so it is necessary to inform the public about the role of chlorophyll and carotenoids. The higher the absorbance value of the vegetables to be studied, the content higher is the chlorophyll and carotenoid.

Based on the background, the researchers aimed to test the carotenoid content as conducted in previous research on the chlorophyll content test to the same vegetables. Chlorophyll and carotenoids are the two main substances in green vegetables, so it is necessary to do content testing to identify high chlorophyll and carotenoid content plants and alternative or worthy plants to be used as references in the manufacture of food supplements.

## **RESEARCH METHOD**

The approach in this research was quantitative with exploratory survey. The population and sample in this study were green vegetables consumed by the community. The samples were mustard greens, water spinach, Gotu kola, long bean leaves, and papaya leaves. The research tools are cuvette, spectrometer, filter paper, label paper, mortar, beaker, measuring cup, funnel, scissors, and cutter. The materials were mustard greens, water spinach leaves, Gotu kola, long bean leaves, papaya leaves with 80% alcohol solution, and Aquadest (distilled water/pure water).

Data were collected by observing the samples (green vegetables), taking the extracts of them and measuring their absorbance. Furthermore, the chlorophyll and carotenoid contents of each vegetable were calculated using a spectrophotometer at wavelengths of 646 and 663 nm for chlorophyll and wavelengths 645,663 and 480 nm for carotenoids.

Before the procedure was conducted in the laboratory, five types of green vegetables had been studied to

find the eligible plants (not too young and not too old), so as to avoid differences in carotenoid content from the age of the plant. After that, the 5 types of vegetables were brought to the laboratory to measure their absorbance using a spectrophotometer with wavelengths of 645,663 and 480 nm. The procedure can be seen in Figure 1.

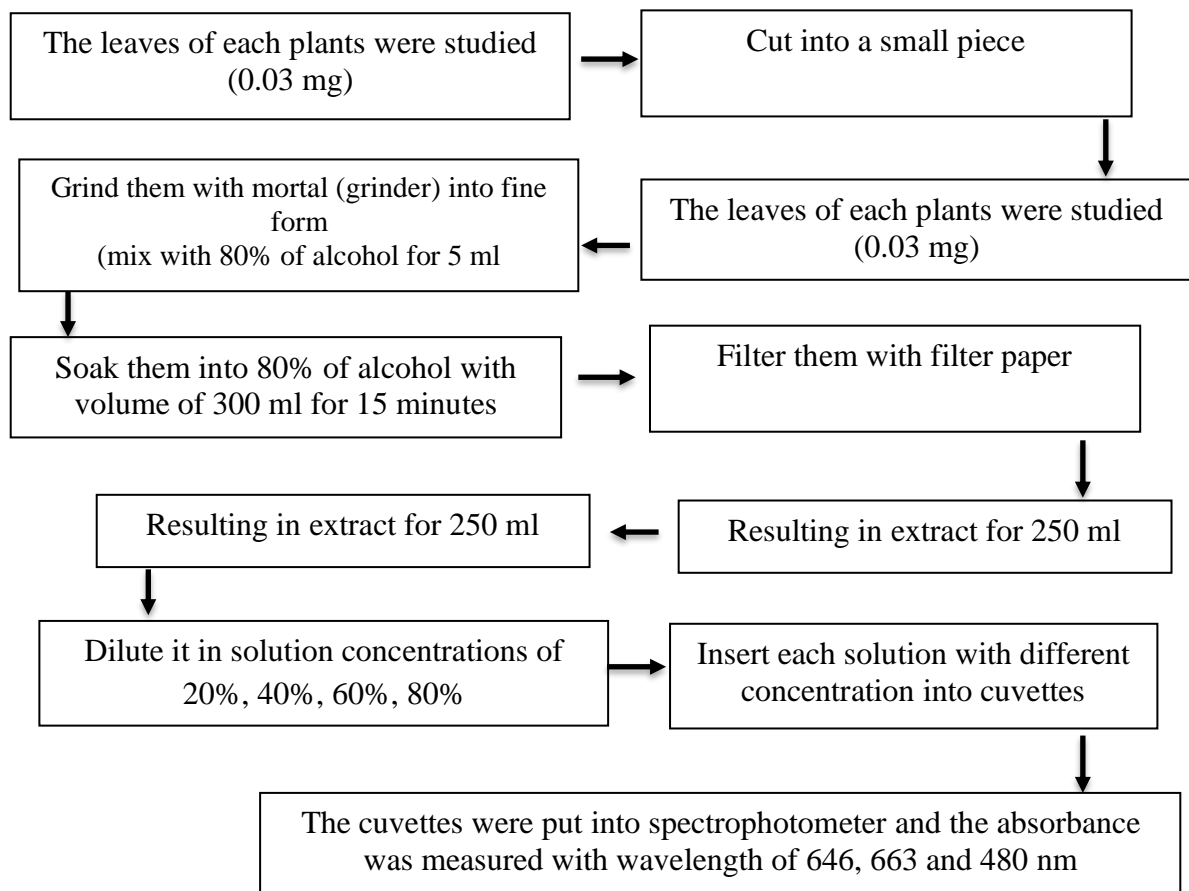


Figure 1. Scheme of Working Procedure

Data were analyzed by calculating the value of absorbance of each sample with the formula below:

A663=absorbance wavelength 663 nm  
 V=Extract volume (mL)  
 W=sample weight (g).

$$\text{Carotenoid } (\mu\text{mol/L}) = \frac{(A_{480} + (0.114 \times A_{663})) - (0.638 \times A_{645} \times V \cdot 10^3)}{112.5 \times W}$$

Description:

A480=absorbance wavelength 480 nm

A645=absorbance wavelength 645 nm

## RESULTS AND DISCUSSION

The result of test toward the samples (some green vegetables) to find carotenoid content conducted at The

Biology Laboratory in Syiah Kuala University is showed in table 1, as follows.

Table 1. Carotenoid Content of Green Vegetables

No	Vegetables	Dilution ( $\mu\text{mol/L}$ )				
		20%	40 %	60 %	80 %	100 %
1	Papaya	0.46	1.4	2.59	4.3	7.7
2	Water Spinach	0.58	2	4.38	7.11	1.49
3	Mustard Greens	0.74	0.43	4.3	7.17	13.8
4	Gotu Kola	0.47	1.5	2.7	4.5	11.7
5	Long Beans	1.16	2.89	5.25	7.5	18.61

Based on Table 1, the lowest carotenoid content with 20% dilution was found in papaya (0.46  $\mu\text{mol/L}$ ) and the highest was found in long beans (1.16  $\mu\text{mol/L}$ ). The lowest carotenoid content with the 40% dilution was found in mustard greens (0.43  $\mu\text{mol/L}$ ) and the highest in long beans (2.89  $\mu\text{mol/L}$ ). The lowest carotenoid content with 60% dilution was found in papaya (2.59  $\mu\text{mol/L}$ ) and the highest in long beans

(5.25  $\mu\text{mol/L}$ ). The lowest carotenoid content with 80% dilution was found in papaya (4.3  $\mu\text{mol/L}$ ) and the highest was found in long beans (7.5  $\mu\text{mol/L}$ ). The lowest Carotenoid content with 100% dilution was found in papaya (7.7  $\mu\text{mol/L}$ ) and the highest was found in long beans (18.61  $\mu\text{mol/L}$ ).

Papaya is a fruit containing a considerable amount of carotenoids, potassium, fiber, and ascorbic acid

content. The total carotene in papaya was 2.740  $\mu\text{m}$  [5]. The value of  $\beta$ -carotene in papaya was 0.37 mg/100 g [6]. Papaya contains ascorbic acid and is also a good source of serotonin (0.99 mg/100 mg) which is directly related to the digestive system and able to reduce the risk of thrombosis [7].

Long beans are plants whose leaves are commonly consumed by Indonesian and Aceh people, especially in making *Pecal* (kind of food) or boiled. Long bean is in the same family as alfalfa so the carotenoid content is as high chlorophyll content as in alfalfa leaves. The chlorophyll content in water spinach is low, it could be because the pigment is scattered almost throughout the plant, both in the leaves and stems, while carotenoids in water spinach as we tested were only in the leaves. In addition, long beans contain a source of protein, amino acids (lysine and tryptophan), and minerals.

Carotenoid are beneficial for the human body because containing a protective effect against cancer cells, heart disease, eye disease, antioxidants, and becoming immune system regulators in the body known as precursors of vitamin A (beta carotene)

[8]. In addition,  $\beta$ -Carotene can protect the skin from the harmful effects of UV rays by preventing the formation of reactive oxygen species and has anti-inflammatory properties [9]. Consuming foods that contain  $\beta$ -carotene is very good for daily consumption patterns. The daily intake of  $\beta$ -carotene was 4,117.0 /g per capita [10].

$\beta$ -Carotene is the main pro-vitamin A component of most carotenoid-containing foods, fruits, and vegetables. It is also a nutrient known to exhibit pro-vitamin A activity [11]. This compound is a precursor of vitamin A which is known to prevent serious eye diseases, such as night blindness [12]. In addition, to function physiologically as vitamin A, carotenoid containing foods must be properly digested to release carotenoids from the food matrix [13]. Recent studies have demonstrated the preventive and protective effects of  $\beta$ -Carotene on hepatic steatosis, fibrosis, oxidative stress, inflammation, and apoptosis [14]. In addition, this antioxidant functions as a pre-hormone, because through metabolism it is converted into retinoic acid, functioning as a ligand, regulating the expression of

genes involved in metabolic processes [15].

Measured the carotenoid content in the aquatic plant *Nymphaea* sp and suggested that carotenoids acted as additional pigments in the photosynthesis process because carotenoids helped chlorophyll in the absorption of light [8]. Carotenoids act as additional pigments that help chlorophyll absorb light. In chlorophyll and carotenoids were important pigments in photosynthesis that had complementary functions in absorbing light [16].

The biosynthesis of chlorophyll pigments, carotenoids, and anthocyanins was controlled by the activity of several enzymes [17][18]. The activity of these enzymes was influenced by environmental factors such as temperature, soil pH, light, and nutrients. The effect of light could increase the work of enzymes that play a role in the synthesis of chlorophyll. Chlorophyll is an enzyme that plays a role in catalyzing protochlorophyll-A. The conversion of protochlorophyllide into protochlorophyll-A in plants absolutely requires light.

An important factor in the biosynthesis of carotenoids. Enzymes that play a role in the biosynthesis of carotenoids are phytoene synthase (PSY) and carotenoid hydroxylase (CH). The intensity of light can increase the level of carotenoid hydroxylase (CH) and phytoene synthase (PSY) mRNA. As the mRNA level of these enzymes increases, the phytoene components of carotenoids also increase. Increasing phytoene can increase the content of carotenoids in plants [19].

The benefits of carotenoid and chlorophyll have not been widely known by the community so this kind of research needs to be published to make many people know the benefits of consuming green vegetables because of their carotenoid content. The benefits of carotenoids play a very important role in supporting human health and survival [20]. Carotenoids exhibit biological activity as antioxidants, influence the regulation of cell growth, and modulate gene expression and immune responses. Carotenoids can protect cells and organisms from oxidative damage caused by free radicals produced by the body during metabolism, sunlight,

radiation, and polluted materials. This protection occurs because carotenoids can eliminate the activity of free radical species. Free radical inhibition by

carotenoids is mainly carried out by  $\beta$ -carotene [21].

## **CONCLUSION**

Carotenoid in papaya with dilution of 20% was 0.46 mmol/L, 40% was 1.4 mmol/L, 60% was 2.59 mmol/L, 80% was 4.3 mmol/L, and 100% was 7.7 mmol/L. Carotenoid in water spinach with a dilution of 20% was 0.58 mmol/L, 40% was 2 mmol/L, 60% was 4.38 mmol/L, 80% was 7.11 mmol/L, and 100% was 1.49 mmol/L. Carotenoid in mustard greens with dilution of 20% was 0.74 mmol/L, 40% was 0.43 mmol/L, 60% was 4.3 mmol/L, 80% was 7.17 mmol/L, and

100% was 13.8 mmol/L. Carotenoid in Gotu kola with dilution of 20% was 0.47 mmol/L, 40% was 1.5 mmol/L, 60% was 2.7 mmol/L, 80% was 4.5 mmol/L, and 100% was 11.7 mmol/L. Carotenoids in long beans with a dilution of 20% were 1.16 mmol/L, 40% was 2.89 mmol/L, 60% was 5.25 mmol/L, 80% was 7.5 mmol/L, and 100% was 18.61 mmol/L. The highest carotenoid content was in long beans (18.61 mmol/L), and the lowest was in mustard greens (1.49 mmol/L).



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