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Quantitative Changes in Ascorbic Acid and Chlorophyll Contents of Parsley (Petroselinum crispum) and Dill (*Anethum graveolens*) Harvested in Three Consecutive Months of Spring

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ABSTRACT: Ascorbic acid has been used to treat many diseases and has recently been considered seriously in the Covid 19 event. Recent studies concerned with ascorbic acid status indicated high prevalence of vitamin C deficiency in many countries which plays an essential role in nutrition and lack of it in the diet causes disease. It is also an antioxidant in vegetables that prevent chronic diseases. Similar results have been observed for chlorophyll and its derivatives in treating acute diseases. There are several medicinal products derived from chlorophyll or its derivatives to treat diseases and variation of health benefits in chlorophyll. According to studies chlorophyll is a potential cancer-preventing agent and has been shown to have antioxidant and anti-mutagenic activities. The properties of chlorophyll are determined to reduce food wastage during processing and storage. Considering the importance of ascorbic acid and chlorophyll in food and especially the important presence of these two substances in vegetables, chlorophyll (spectrophotometric method for chlorophyll) and ascorbic acid (Ascorbic acid reduces oxidation-reduction indicator dye, 2,6-dichloroindophenol, to colorless solution) were measured in two popular vegetables: parsley and dill in three consecutive months of spring 2020 and the data were statistically analyzed using the SPSS 24. The results indicated that the last three months of plant growing just before harvesting, the ascorbic acid contents of both vegetables increased considerably. The results of chlorophyll contents in both substrates indicated increasing in all the stages. The average ascorbic acid reached its highest level in the third month of the spring (1.3000±0.120 $mg.kg^{-1}$ for parsley and 0.2233±0.120 $mg.kg^{-1}$ for dill); the difference in the amount of chlorophyll in the first and second harvest was nearly two times. The highest amount of chlorophyll in both vegetables belonged to the samples harvested in the third month of spring (0.343±0.006 $mg.kg^{-1}$ for parsley and 0.580±0.006 $mg.kg^{-1}$ for dill).The results of this study can help researchers to stablish a healthy and nutritious diet.

Keywords: Ascorbic Acid, Chlorophyll, Dill, Parsley, Spectrophotometer, Titration.

Introduction

The importance of nutrition in increasing immunity and reducing disease has been recognized for many years (Taghdir *et al*., 2020). For the curing of

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many diseases such as new viral diseases, Covid 19, paying attention to diet to strengthen the immune system is a priority (Hemila 2003, Nora *et al*., 2021). Many studies have shown that lack of certain nutrients reduces the immune system function and increases the risk of infections (Taghdir *et al*., 2020). Various ancient texts that deal with the benefits and harms of food and its relationship to health exist. Lack of essential nutrients causes diseases (Astley & Finglas, 2016). An active immune system is needed during the body's exposure to infection. Optimal nutrition supports the function of immune cells and effective responses to pathogens (Childs *et al*., 2019). Diet plays an important role in treating and managing diseases such as allergies and cancer, diabetes, and heart disease (Venter *et al*., 2020). Diets rich in fruits and vegetables are very important because they contain vitamins, minerals, dietary fiber and bioactive substances (Wallace *et al*., 2020). Vegetables are essential for human health due to their content of nutrients such as dietary fiber, vitamins and minerals and non-nutritious phytochemicals (Limantaraa *et al*., 2015). Vitamins are organic compounds needed in very small amounts for metabolism and vital functions of the body and growth and health. Their deficiency disrupts the role of different parts of the body and can cause or aggravate some diseases (Semba 1998). Vitamin C is a white to pale yellow crystalline powder, water-soluble and nontoxic vitamin that acts as a powerful antioxidant. In common colds and other infections rate of vitamin C is reduced in plasma, leukocytes, and urine. In many situations where oxidative stress increases in the body, the antioxidant effect of vitamin C can be significant and effective in preventing, shortening, and improving many infections (Li *et al*., 2006; Attar *et al*., 2015; Hemila, 2017). Vitamin C also acts as an antihistamine and prevents sneezing, itchy nose, runny nose, stuffy nose, swollen sinuses, or even pneumonia, reduce the risk of respiratory infections and sinus swelling (Atherton *et al*., 1978, Field *et al*., 2002; Hemila 1997; Rssetti *et al*., 2020). Humans (Mezeyova, 2016) and primates, bats, and guinea pigs (Gallie, 2016) have lost the ability to synthesize vitamin C; therefore, the only source of this vitamin is diet (Mezeyova, 2016). The richest sources of ascorbic acid are: Indian grapes, citrus fruits such as limes, oranges and lemons, tomatoes, papaya, green and red peppers, kiwi, strawberries and cantaloupe, green leafy vegetables such as broccoli (Devaki *et al*., 2017), enriched cereals (Devaki *et al*., 2017) parsley (Mezeyova, 2016) and Dill (Karklelienė *et al*., 2014). Chlorophylls play an important role as the primary photosynthetic pigment for absorbing light energy from the sun in living plants. Color determines the appearance of vegetables and influences consumer choice (Limantaraa *et al*., 2015). On the other hand, the acceptability of food largely depends on the color of food products. (Sarker, 2019). Chlorophyll-derived compounds are non-toxic, water-soluble, and a common dietary supplement (Clark *et al*., 2020). The quality and freshness of commercial vegetables can be controlled by measuring the amount of chlorophyll (Limantaraa *et al*., 2015). In the areas of safety, nutrition, and beauty, natural pigments such as carotenoids, β-xanthines, β-cyanines, anthocyanins, betalains, and chlorophylls have increased the interest of food consumers. A small number of families of Caryophyllales contain natural water-soluble pigments such as β-cyanines, β-xanthines, and betalains, respectively. Amaranth (red amaranth), which can detoxify potential free radicals, is a unique source of

betaline, β-xanthine, and β-cyanine. These pigments which detoxify free radicals in the human body, act as powerful antioxidants and contribute to human health. These natural antioxidants prevent cancer, bloating, cardiovascular disease, atherosclerosis, diabetes, retinopathy or retinal disease, osteoporosis, neurodegenerative diseases, osteoarthritis, cataracts, inflammation and protect against aging (Sarker, 2019). Organic metal compounds derived from chlorophyll have antibacterial and antiviral properties and have been approved for human consumption. There is chlorophyll a-derived products that are medicinal and are used in intramuscular and intravenous injections. Chlorophyll-derived compounds are also used to treat cancer cells (Clark *et al*., 2020). Plants are one of the indicators of ecosystem health diagnosis (Xie K, 1998). Following legumes and fruits, vegetables also have important elements for balanced diet due to their vitamin content, dietary fiber content, and low carbohydrate content (Tuncay, 2011; Ziarati *et al*., 2018). Iran is one of the major producers of agricultural products globally, which ranked sixth in the world in the production of vegetables in 2013, and except for a few cases, its agricultural production has been increasing. This amount increased from 4,000 million tons in 1983 to nearly 16,000 million tons in 2013 (WFP, 2016). According to studies and reports, the average per capita consumption of vegetables in Iran is 286 grams per day (WFP, 2016). Parsley (Petroselinum crispum) is a leafy vegetable mainly used as an aromatic culinary plant to improve the sensory properties of food (Dobricevic *et al*., 2019). Parsley Petroselinum crispum, which is naturally found in the Mediterranean region, belongs to the Apiaceae family (Navazio, 2012). It is traditionally a vegetable that has been used in the world for a long time. This plant is a leafy vegetable that is rich in many biological compounds. Greece is the main origin of this plant. This plant can be considered as a plant with green leaves with a unique aroma (Navazio, 2012). Parsley may grow between 60 to120 cm, in sunny areas with suitable environmental conditions, in moist soil with a pH of 3.5 to 7.5. Parsley is sensitive to water stress, especially if planted in summer and late spring. To increase production and improve quality, a permanent source of water must be provided. Both growth and parsley type determine the sensitivity of plants to water stress (Daradkeh and Essa, 2019). The medicinal properties of parsley can be summarized as follows: Antioxidant properties (Taiz *et al*., 1998; Zhang *et al*., 2006; Nielsen *et al.*, 1999; Ozsoy Sacan *et al*., 2006; Kolarovic *et al*., 2010; Wong *et al*., 2006) and antimicrobial properties (Manderfeld *et al*., 1997; Wahba *et al*., 2010; Wong *et al*., 2006), diuretic or laxative properties (Heck *et al*., 2000; Jakovljvic *et al*., 2002), hypoglycemia (Luo *et al*., 1999; Kumar *et al*., 2011; Anand *et al*., 1981; Afkhami *et al*., 2003; Sener *et al*., 2003), liver protection (Ozsoy Scan *et al*., 2006), anticoagulant properties (Gadi *et al*., 2009; Chaves *et al*., 2011), contraception (Castleman, 2009), positive effect on the aorta (Sener *et al*., 2003), has Positive effect on bone health. Dill (Anethum graveolens) is an annual and sometimes biennial plant (Derakhshan *et al*., 2017) (Wright, 2009) of the family Apiaceae (Umbelliferae). In many countries, this plant has a long and ancient history as a medicinal and culinary plant (The Herb Society of America, 2009), and since ancient times, the leaves and seeds of this plant have been used as a spice to flavor cakes and sweets and in soups and salads

(Derakhshan *et al*., 2017). The addition of this plant to food is known to be beneficial for health (Crage, 2021). In addition to vitamin C and fiber, which play an important role in regulating lipids and absorbing saccharides, Dill contains large amounts of vitamin A as carotene (Hammod *et al*., 2019). It is believed that the origin of this plant was the Mediterranean region. Dill grows in Southeast Europe and South Central Asia (Stanojević *et al*., 2016). Dill grows throughout the Indian subcontinent, the archipelago of Malaysia and Japan (Jana and Shekhawat, 2010). Historical records show that this plant was used as a sedative in Egypt 5000 years ago. (The Herb Society of America, 2009). This plant is usually planted and grows as a seed. In ideal conditions for dill growth, the height varies between 30.5 to122 cm. This plant has hollow branch stems without leaves. Dill grows well in light, medium-textured soil with adequate moisture and drainage. This plant has an excellent response to organic soils. The recommended soil pH is in the range of 5 to 8.2. During long periods of drought, Dill needs extra irrigation, and the soil should be kept moist (not flooded). The use of some irrigation methods may affect the quality of Anethum graveolens and its cultivars and cause stem breakage, premature seed crushing and disease susceptibility. Dill grows best in full sun climates. However, heat can be a determining factor in the early flowering of the plant, which stops leaf production, and the plant grows at different temperatures from 6 to 26 degrees Celsius, and strong winds can cause significant damage to the dill crop because Hollow stems are easily broken and bent. (Wright, 2009) The medicinal properties of Dill can be summarized as follows: Treatment of pulmonary bronchial disorders such as cough and gastrointestinal disorders. Also healing, clear, anticonvulsant, appetite stimulant and analgesic, biofilm formation to reduce bacterial pathogenicity. (Derakhshan *et al*., 2017) Anti-diabetic properties (Goodarzi *et al*., 2016) antispasmodics, appetite suppressant, colds, menstrual cramps, liver problems, anti-cancer, oral care, strengthening the immune system, protection against bone destruction, anti-inflammatory, urinary tract disorders, and Urinary tract (Meena *et al*., 2019) Antimicrobial activity (Jana and Shekhawat, 2010), anticancer properties (Craig, 2021). As an intestinal activator, Dill reduces cholesterol absorption in gallbladder salts (Hammod *et al*., 2019). Dill and parsley plant in the world that are popular for their valuable aromatic and biochemical compounds. Their diversity has continued to increase regularly for high production and compounds with valuable biochemical contents. (Karklelienė *et al*., 2014). It should also be noted that the use of natural products in the pharmaceutical and food industries is significant. Manufacturers are looking for natural ingredients that preserve food and enhance the perception of flavors and aromas (Biesiada *et al*., 2019). Since parsley and Dill are two popular plants and contain useful biochemical compounds and natural nutrients, including minerals, ascorbic acid, and other biologically active compounds, this study investigates the changes in ascorbic acid and chlorophyll the two vital and important micronutrients during three consecutive months of April, May, and June.

Materials and Methods

- ***Parsley and Dill (planting and harvesting)***

Seeds of Parsley (*Petroselinum crispum*) and Dill (*Anethum graveolens*) were taken from the Ministry of

Agriculture, Tehran (Bean, vegetable and seed production section) and planted in pots with suitable soil for cultivation of these plants. Both plants for suitable growing conditions, from the beginning of planting to the end of harvest, were monitored carefully. Since vegetables are plants sensitive to water stress, watering was carried out at regular intervals . Total treatment were 18 treatments (9 treatments for parsley and 9 treatments for dill). In April, May, and June when the plants reached the harvesting, the samples were collected and assessment for ascorbic acid and chlorophyll were carried out.

The traits were measured as follows:

Measurement of ascorbic acid in the aerial part of sample was performed by AOAC 967.21. This method is based on the extraction of ascorbic acid from the sample, using a solution of metaphosphoric acid and acetic acid and finally titration with 2, 6-dichlorophenol indophenol.

Titration with dye 2,6-dichlorophenol indophenol continues until a light pink color is obtained.

In a 50 ml volumetric flask, 50 mg of ascorbic acid was poured and brought to volume. According to the concentration of the sample, a certain amount of sample was carefully weighed. Five milliliters of the standard ascorbic acid solution were diluted with 5 milliliters of the extraction solution. It was titrated immediately with the color solution until a bright pink color appeared. The final calculation to determine the amount of ascorbic acid was based on the initial weight of the sample.

Determination of chlorophyll a content was based on the amount of absolute adsorption at 660 nm. This measurement requires a high-performance spectrophotometer. The determination was carried out according to the AOAC 940-03 method.

Liquid nitrogen was added to a certain amount of chopped plant. A certain amount of 80% acetone was added to the sample. The sample was placed in a centrifuge. The upper extract was separated, and the resulting solution (that was centrifuged) was transferred to a flask. Samples were poured into the cuvette of the spectrophotometer, and then the absorbance value was read by the spectrophotometer at a certain wavelength for chlorophyll a.

All measurements were carried out in triplicate order.

- ***Statistical Analysis***

All experiments and measurements were carried out in triplicate order. Data were expressed as the mean ±SE (standard error). The data were statistically analyzed using the SPSS 24 on replicated test data. Analyses of variance were performed by SS Type 3. Significant differences between the means were determined using the Duncan multiple range test. P-value of 0.05 or less was considered statistically significant.

Results and Discussion

- ***Ascorbic acid***

The results obtained from the test showed that the accuracy of measurements for both leafy vegetables, dill, and parsley was very high, and there was a statistically significant difference between the measured items ($P \leq 0.05$). The following results can be an excellent report to know the extent of ascorbic acid changes for researchers and those looking for a source of vitamin C through natural substances and choosing an appropriate nutritional pattern, effective and helpful (Table 1).

Table 1. Mean concentration of ascorbic acid measured in different treatments in parsley and dill

Treatment		Mean±SE	95% Confidence Interval	
			Lower Bound	Upper Bound
April (A)	Dill	0.1933±0.120	-0.069	0.456
	Parsley	0.6900±0.120	0.427	0.953
May (B)	Dill	0.2133±0.120	-0.049	0.476
	Parsley	1.0933±0.120	0.831	1.356
June (C)	Dill	0.2233±0.120	-0.039	0.486
	Parsley	1.3000±0.120	1.037	1.563

In parsley the concentrations of ascorbic acid increased considerably in May as well as June harvested while in dill increased slightly. In April treatments, represent the sample harvested in April, the measured mean amount of ascorbic acid in parsley samples shows 0.69 ±0.51 $mg.kg^{-1}$ DW. The ascorbic acid in the dill in the first harvest shows the amount of 0.193 00± 0.005 $mg.kg^{-1}$, which was much less than measured in the first harvest of parsley. In a study by Kabasakails *et al.* in 2000 (using the HPLC method), the amounts of ascorbic acid in 100 ml of orange juice were measured using the dichlorophenol-indophenol method. The reported dose was 52.3 $mg.kg^{-1}$. In 1995, Vinci *et al* measured ascorbic acid levels in oranges, grapefruits, and lemons by the same method as Kabasakails *et al*. 2000.

Jain *et al*. (1995) also measured the amount of ascorbic acid in 100 ml of orange juice and lemon juice in India using iodine titration and reported 67.1 and 52.8 $mg.kg^{-1}$. In a similar study conducted in 2019 by Elhefian *et al*., The content of ascorbic acid in some fruits such as guava, kiwi, oranges, lemons, and vegetables; Green pepper, red pepper, green pepper, tomato, and parsley were measured by titration method. This study found that the most ascorbic acid content of fruits was in guava (217.32 mg per 100 g DW), and the lowest was in lemons (58.12 mg per 100 g). In vegetables, the highest amount was in green pepper (123.94 mg per 100 g) and the lowest in green pepper grown in a greenhouse (24.12 mg per 100 g).

- ***Chlorophyll***

Chlorophyll content in parsley and dill were determined using a spectrophotometer apparatus. Parsley and dill were harvested in April, May, and June (to study chlorophyll changes in three consecutive months of spring). They were transferred to the laboratory to determine chlorophyll change levels according to the standard methods. The results obtained from the chlorophyll analyses in both parsley and dill showed a very high accuracy of chlorophyll assay. There was a statistically significant difference between the measurements ($P \leq 0.05$). The Table 2 shows the changes in chlorophyll content in parsley and dill in three consecutive months of spring.

In the parsley harvested in April, the measured mean of chlorophyll in three replications of the samples was 0.173± 0.005 $mg.kg^{-1}$ DW (Table 2). The mean measured chlorophyll in dill (in April) was similar to the amount of chlorophyll in parsley. The accuracy of measurement in the results of parsley harvest in May was very high. Chlorophyll levels in the second month of spring showed an increase in

both dill and parsley samples than in April's first analyses. Chlorophyll levels of parsley in May increased rapidly, almost two times as much as the second harvest in April. With more increase than parsley, chlorophyll levels of dill were climbs rapidly almost three times as much as the second harvest in April. In June, laboratory and statistical studies reveal that chlorophyll in both parsley and dill increased gradually to 0.343±0.005 $mg.kg^{-1}$ in parsley and to 0.58 $mg.kg^{-1}$ in dill (Table 2). The results describe high accuracy in all stages of chlorophyll assay in dill. Studies show that chlorophyll changes in both parsley and dill from the first to the second harvest were much higher than the second to the third harvest. Changes in parsley in the second harvest (May) indicate a significant increase nearly two times; nevertheless, the data shows tripled increase in dill. There is a slight increase in the levels of chlorophyll in both parsley and dill from the second to third harvest. The difference in chlorophyll content in analyzed samples of parsley and dill (April, May, and June) was significant. The research of Olsson *et al*. in 2005 showed that climatic factors affect the production of plant pigments. They also showed that the production of some pigments in different fruits with different climatic factors such as light, Humidity, temperature, and altitude from sea level are related, and these factors are affected by each other. Another study was conducted by Seljasen *et al*. in 2013 and showed that several factors, such as genetic factors and climatic conditions such as light, temperature, and precipitation, affect quality during the growth to harvest stage. Also the content of chemical compounds present in carrots is effective. Carrying out Duncan test, statistical studies showed that analyzed samples of April, May, and June in both parsley and dill were not similar and had a statistically significant difference ($P \leq 0.05$).

Conclusion

Measurement of ascorbic acid and chlorophyll can provide a good preference for vegetables that are naturally high in nutrients. The ascorbic acid and chlorophyll content varies between different plant species consumed around the world. Ascorbic acid and chlorophyll play an essential role in the human diet, therefore it is crucial to determine the concentrations in vegetables during the growing season. In this study, in addition to examine the levels of ascorbic acid and chlorophyll in two different vegetables, the changes in their concentrations in three consecutive months of spring were determined and measured. The results showed that the amount of ascorbic acid in

Table 2. Mean chlorophyll a concentration levels in parsley and dill

Treatment		Mean±SE	95% Confidence Interval	
			Lower Bound	Upper Bound
April (A)	Dill	0.173±0.006	0.160	0.186
	Parsley	0.173±0.006	0.160	0.186
May (B)	Dill	0.543±0.006	0.530	0.556
	Parsley	0.340±0.006	0.327	0.353
June (C)	Dill	0.580±0.006	0.567	0.593
	Parsley	0.343±0.006	0.330	0.356

parsley varies from the beginning to the end of spring. However, these changes in dill were not observed. The amount of ascorbic acid measured in dill from the beginning to the end of the harvest season showed little changes. This study indicated, that the amount of ascorbic acid of parsley increased rapidly during harvesting stages. In addition, in the case of dill, a similar trend in all the stages of the study were observed. There are statistically significant differences in both parsley and dill in the studied treatments (April, May, and June) ($P \leq 0.05$). Also, the research results in this study showed the extent to which these values can be in different conditions of a different months. According to observations, this difference can be about twice from the beginning to the end of a season. This study also showed that vegetables should be considered a rich source of vitamin C, and their role and presence in the diet should not be overlooked. In the other hand this study showed that the amount of chlorophyll changes in both vegetables was different from the beginning to the end of the study, and the amount of chlorophyll changes in parsley was more than dill. Focus on this issue is also a pattern to know how and from what source to use these substances containing such beneficial compounds to enjoy their significant benefits. The experiments in this study showed how these values could be different in different months of a season. According to laboratory observations, this difference was more than 50% in the different months. The study results of ascorbic acid and chlorophyll content showed that ascorbic acid in parsley and dill has increased with approaching the summer. Further study on minerals and vitamins of these plants and other plants, also in the year's cold season, is recommended.

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