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Carotenoid comparison among broccoli leaves, stems, and flowers dried with drying cabinet

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Abstract. Vegetables are essential for humanity due to their colour, taste, nutrition, and health. Carotene, a vitamin A precursor and a potent antioxidant, is one of the essential nutrients present in vegetables. The present work was initiated to study the carotenoid content in Broccoli plants dried by cabinet dryer with emphasis on the carotenoid distribution of β-carotene, α-carotene, phytofluene, phytoene and lutein in the leaves, stems and flowers of the plant. These compounds were quantified with the help of High-Performance Liquid Chromatography (HPLC). Based on the results, the highest amount of carotenoids histologically present was found in the broccoli leaves, particularly in β-carotene 131.51 μg/g, which was more than in the stems 77.52 μg/g and flowers 55.24 μg/g. One-way ANOVA analysis of variance was carried out. The results were significant in carotenoid content of leaves, stems and flowers $p < 0.05$, except for phytoene, where no significant difference was noted. It can be concluded that the broccoli leaves, which are usually discarded, are an excellent source of nutrients and therefore can be incorporated into food formulations, especially in the formulations meant to boost antioxidant and provitamin A levels. This study lays down the foundation for improving food processing methods for the better nutritional quality of dry vegetables and sheds light on using Broccoli leaves to achieve sustainable food production by reducing food waste.

1 Introduction

Consuming vegetables is vital for humans because of their unique characteristics, such as colours, flavours, nutritional value, and health benefits. In addition to making meals more creative and more enjoyable, they provide a source of essential nutrients. Vegetables, particularly green vegetables, have been popular as foods that contain high amounts of carotene, ascorbic acid (vitamin C), iron, zinc, folate and dietary fibre for the healthy nutrition of people [1]. Various studies have cited that vegetables are rich in nutrients, including their control of chronic illnesses such as cardiovascular diseases, diabetes, and some forms of cancer [2–5]. In particular, it has been well known that protein, carotene and ascorbic acid

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are abundant in green leafy vegetables. These nutrients can be further improved by using organic fertilizers. Depending on how they are managed, organic agriculture practices will enhance the nutritional value of such vegetables and create a rising demand among consumers for such vegetables that are grown without synthetic insecticides.

Broccoli (Brassica oleracea var italica) is considered a treasure house among all these vegetables packed with nutrients. They belong to the kingdom Plantae subfamily broccolisgae (crossplant eating family). Broccoli is a flowering plant possessing a thick central stalk with a green leaf and green flower buds, although some coloured varieties show purple for broccoli plants [6]. Phytochemicals and fibre nutrition of crucifer vegetables, like Broccoli, have been increasingly focused on because these nutrients have demonstrated potential health benefits, especially anti-inflammatory, antioxidant, and anticancer agents. For that reason, many people consider broccoli one of the most nutritious vegetables, so it is included in dietary recommendations in many countries.

Among the many benefits of eating Broccoli, the greatest health benefit comes from its high fibre content, which significantly helps promote digestive health. The indigestible carbohydrate constituents known as fibre are critical in sustaining a healthy gut microbiome, bowel regularity, and cholesterol below normal levels. Considering that a single cup of Broccoli consists of nearly 9% of the recommended fibre intake, it sufficiently improves the health of a person [7]. Besides aiding the digestive system, fibre helps control sugar levels in the blood. It assists with managing weight, making Broccoli the best diet option focusing on reducing weight [8].

Moreover, it is also known that, apart from being a source of dietary fibre, Broccoli contains carotenoids known for their antioxidant properties [9]. As one of the various carotenoids present, β-carotene can be singled out as one of the most significant. This compound is responsible for the orange colour in many fruits and vegetables; however, it is also available in green leafy vegetables like Broccoli, though the green chlorophyll primarily masks it. Most of all, β-carotene is essential because this substance is a precursor of vitamin A, which is necessary for vision and immune functions, as well as the health of the skin. The antioxidant effects of beta-carotene are remarkable as they capture and neutralize harmful oxygen radicals, thus eradicating the chances of most diseases that are common today, including cancer and heart failure.

Beta-carotene is generally good for health in many ways, but it has to be understood that not all sources of beta-carotene are beneficial [10]. It has also been demonstrated that beta-carotene from whole foods, such as broccoli, can help prevent the incidence of chronic diseases, cancer, and age-related degeneration of several forms. Beta-carotene supplements, on the contrary, especially in high doses, can be harmful, for example, in some specific instances to smokers where studies have shown increased lung cancer risk. Thus, this explains why eating foods rich in beta-carotene instead of supplements is appropriate.

Beta-carotene contains lipophilic vitamins, so ingesting dietary fat enhances its absorption [11]. This trait is especially beneficial in natural plant food sources where fats in the food or vegetables help absorb the beta-carotene. Broccoli, known to contain a high level of beta-carotene, is a very healthy food that can be used by persons seeking ways to enhance their beta-carotene intake without the risk of synthetic beta-carotene supplementation.

Due to the nutritional importance of Broccoli, the present study will focus on the effects of different drying methods on the carotenoid content of Broccoli, mainly beta-carotene. The primary purpose of drying cut vegetables is to make them edible for a long time compared to unprocessed forms. However, the drying type employed can significantly influence the stability of heat-sensitive compounds such as carotenoids. An investigation of how this particular drying cabinet— a cabinet dryer— affects the amount of beta carotene in Broccoli will be done in this study. Since carotenoid levels are focused on foods, in this case, vegetables devoid of moisture content, this research aims to improve food processing to ensure dried vegetables retain their nutritive value.

2 Material and Methods

This study focused on conducting validation experiments to consider the effectiveness of different methods of drying broccoli, such as sun drying and cabinet drying. This experiment used a Completely Randomized Design (CRD) to avoid bias and reduce experimental error. In this manner, the spreads of experimental treatments using test units were randomised, enabling a smoother analysis of the impacts of the drying methods on the set of organisms tested.

Some preliminary research was done to understand the impacts of different drying methods on the different varieties of Broccoli and their leaves. This first stage matters in sieving the specific varieties of Broccoli to be tested to yield enough relevant data. Blanching was one of the essential pre-treatments, and it was necessary to inhibit the active enzymes inside the Broccoli. Blanching is essential and helpful in starving enzyme activity, which may change the food's nutrients and other chemical properties during drying and storage.

Blanched Broccoli and broccoli leaves were processed by drying, after which they were either dried as large pieces or ground to powder for incorporation into food products. The impact of this procedure is especially significant on the carotenoid reserves, which is why this study focused on them.

High-Performance Liquid Chromatography (HPLC) analysis was conducted to accurately determine the carotenoid content in the samples. This methodology is characterized by extreme selectivity, allowing compounds to be separated and measured accurately, especially carotenoids that are known to possess a high degree of geometric resemblance. Once again, the HPLC method made distinguishing beta-carotene and other similar substances possible.

Beta-carotene is a non-polar compound that is predicably exceptional in that it can dissolve in polar mediums. When Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) is employed, the beta-carotene binds to the non-polar stationary phase (C18), and the polar mobile phase (solvents like acetonitrile or isopropanol) at the same time. Such interaction enhances the separation of beta carotenes from other constituents in the sample.

Once the separation had been done and the search for analysis proceeded, beta-carotene was found and measured. The significant outputs provided by the HPLC detector were peak endpoints and retention time. The retention time was used to locate beta carotene by measuring the time taken to pass through the column compared to other compounds. In contrast, the peak area was used for quantitative analysis to measure the amount of beta-carotene within the sample.

Also, to improve the accuracy of detection and quantization, Ultraviolet-Visible Spectroscopy (UV-Vis) and Multiple Wavelength Detectors (MW) could be employed. These detection methods assess how much of a particular compound is absorbed at a specific wavelength. An example of the UV-Vis detector can analyse bacteria containing pigments, such as carotenoids, that absorb light optimally in the visible region. Without the MW detector, the analysis would be significantly sieved as one would be limited to single wavelength acquisitions from the source under venting the detection analysis cap.

The careful application of the above approach – that is, by fine-tuning the drying conditions, isolating the necessary enzymatic treatment, and applying sophisticated instruments, including HPLC and UV-Vis spectrophotometry – constitutes a reliable tool for determining the nutritional value and retention of carotenoids in ready-to-eat frozen Broccoli and broccoli leaves. Such results would pave the way for the creation of other food processes that could cook food in the most efficient manner and, at the same time, preserve the essential substances of the food, which undergo drying and other forms of preservation.

3 Results and Discussion

The findings of the carotenoid content in the dried broccoli powder in terms of the individual parts of the plant (leaves, stems, and flowers) are shown in Table 1. It is evident from these findings that there is a notable difference in the concentration of carotenoids among the different parts of the Broccoli. The data shows that the leaves are the most abundant in carotenoid concentration, mainly beta-carotene, of the three- stems, leaves, and flowers.

Table 1. Carotenoid content of dried broccoli powder.

Sample (μg/g)	Leaves	Stem	Flower
β-Carotene	131.51	77.52	55.24
α-Carotene	24.39	14.78	10.50
Phytofluene	14.92	9.15	6.53
Phytoene	4.35	2.90	1.34
Lutein	11.53	7.25	5.39

Results in Table 1 showed that dried broccoli leaves have the highest carotenoid content compared to Broccoli stems and flowers. β-Carotene in broccoli leaves has the highest content among other carotenoid compounds. One Way ANOVA test results $p<0.05$ showed significant differences in each part of dried Broccoli in terms of the content of β-Carotene, α-Carotene, Phytofluene, and Lutein. Meanwhile, the Phytoene content in the leaves, stems, and flowers was not significantly different.

3.1 β-Carotene Content on Broccoli Powder

β-carotene is one of around 600 carotenoid compounds found in plants. Carotenoids are a class of C40 isoprenoid and tetraterpene chemicals found in the plastids of plants, both those that photosynthesis and those that do not. Consuming various fruits and vegetables high in beta-carotene reduces the chance of developing several types of cancer and cardiovascular disease [12]. Beta-carotene is a kind of carotene found in dark green or dark yellow foods such as carrots and Broccoli [13].

Beta-carotene is a naturally occurring carotenoid. Apart from providing colours to various fruits and vegetables, beta-carotene also works as a cofactor in the food generation process known as photosynthesis in plants. Beta-carotene is critical not just for plants but also for human health. When beta-carotene is taken in the form of fruits and vegetables, it is transformed into vitamin A, a potent antioxidant [14].

β-Carotene has many benefits, as an antioxidant and increases the immune system. A study showed that patients with Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) treatment given micronutrient supplementation and carrot-ginger blend (β-Carotene-748.44 g) for 90 days can improve the immune system and strengthen the nutritional status [15].

The ANOVA test analysis revealed a significant difference in the β-carotene concentration of broccoli powder in stem, leaf, and flower samples at the 5% significance level. The Duncan's Multiple Range Test (DMRT) test analysis indicated that broccoli powder samples taken from leaves had a more significant rise in beta-carotene concentration than broccoli powder samples from stems and flowers. The increase in -carotene content in broccoli powder leaf samples averaged 131.51 μg/g, the increase in β-carotene content in broccoli powder stem samples averaged 77.52 μg/g, and the increase in -carotene content in broccoli powder flower samples averaged 55.24 μg/g.

Broccoli is a vegetable that has a moderate amount of beta-carotene, 623 IU/100 grams (2). Broccoli also has 361g of beta carotene per 100 grams, according to the Indonesian Food Composition Database and the National Database for Standard Reference [16]. The beta-carotene content of broccoli leaf powder was determined to be 14.42 0.18 mg GAE/g. When converted to mg/g, the beta-carotene concentration of broccoli leaf powder is 131.511815 μg/g [17]. The beta-carotene content of broccoli leaf powder is lower than the value reported in research by Liu et al. [18]. The decrease in beta-carotene output can be attributed to various factors, including washing, cutting, and processing operations. As a result, the beta-carotene concentration of broccoli powder reduces as well.

3.2 α-Carotene Content on Broccoli Powder

Caballero (2005) notes that α-carotene, another carotenoid in various foods, also produces provitamin activity. Following central cleavage, it is transformed into a single physiologically active retinol molecule, and twice the molar amount is comparable to -carotene. As with other carotenoids, it possesses antioxidant and possibly anti-carcinogenic qualities and the ability to boost immunological function [19]. Carotenoids, including α-carotene, are precursors to vitamin A. These pigments give fruits and vegetables yellow, orange, red, and dark green hues. They act as antioxidants, preventing oxidative damage, which contributes to cardiovascular disease and cancer development. While alpha-carotene is chemically similar to beta-carotene, certain studies indicate that it is significantly more beneficial in reducing mortality from certain types of cancer (lung, prostate, liver, etc.) and lowering the risk of cancer and cardiovascular death.

The ANOVA test revealed that the α-carotene concentration of broccoli powder in stem, leaf, and flower samples varied considerably at a 5% significance level. The DMRT test analysis revealed that broccoli powder in leaf samples had more α-carotene than broccoli stem and flower samples. The average content of -carotene in broccoli powder leaf samples was 24.39 μg/g, the average content of α-carotene in broccoli powder stem samples was 14.78 μg/g, and the average content of α-carotene in broccoli powder flower samples was the lowest. That is, 10.50 μg/g. α-carotene was identified at concentrations ranging from 0.0 to slightly less than 0.1 mg/100 g. The amount of beta-carotene in broccoli powder may decrease due to various causes. One of them is due to the broccoli washing process, which is capable of reducing the α-carotene content of the Broccoli by cutting it smaller in size; additionally, it might be owing to the drying temperature procedure and the prolonged drying time applied to the broccoli powder [20].

3.3 Phytofluene Content on Broccoli Powder

Phytofluene is a colourless carotenoid found in natural foods such as tomatoes and vegetables. It is the second carotenoid biosynthetic product. It is synthesized from phytoene through a desaturation reaction that produces five conjugated double bonds—adding a carbon-carbon conjugated double bond in the following step results in the production of z-carotene and the development of visible colour. Phytofluene has a UVA absorption spectrum, with a maximum absorption at 348 nm and a 1% absorption at 1557 [21].

After the Anova test revealed a significant difference in the phytofluene concentration of broccoli powder in stem, leaf, and flower samples at the 5% significance level, the DMRT test was performed. The DMRT test analysis revealed that the broccoli powder in the leaf samples had more phytofluene than the broccoli stem and flower samples. The phytofluene content of broccoli powder in the leaf sample averaged 14.92 μg/g, the phytofluene content of broccoli powder in the stem sample averaged 9.15 μg/g, and the phytofluene content of broccoli powder in the flower sample was 6.53 μg/g.

According to Khachik et al. (1991), phytofluene was detected in most fruits and vegetables during an analysis. Unlike all other carotenoids, phytofluene, the initial carotenoid precursor in the biosynthesis of other carotenoids, absorbs ultraviolet light [22]. This buildup of carotenoids protects the skin in various ways: UV absorbers, antioxidants, and anti-inflammatory agents [23,24]. Broccoli leaves produce more phytofluene than other leaves because they can photosynthesize [25]. The leaf is the primary organ of plants where photosynthesis occurs. Plants need a pigment called chlorophyll to absorb light, giving them a green colour. Chlorophyll is located in organelles called chloroplasts, which are precisely where photosynthesis occurs. Although chloroplasts are found in all green regions of the plant, the majority of energy is produced in the leaves [26].

3.4 Phytoene Content on Broccoli Powder

Phytoene synthase (PSY) is the primary enzyme that regulates the onset of the carotenoid biosynthetic pathway, significantly affecting the amount of carotenoids produced. It is encoded by three genes, two of which (PSY1 and PSY2) exhibit widespread expression, while PSY3 expression is deficient and restricted to roots [25,27]. Carotenoid biosynthesis begins by condensing two GGPP (C20) molecules into phytoene via phytoene synthase (PSY). The enzyme phytoene desaturase (PDS) then catalyzes the desaturation of phytoene, resulting in the synthesis of 9,15,9'- tri-cis-ζ carotene.

The ANOVA test revealed a significant difference in the phytoene concentration of broccoli powder in stem, leaf, and flower samples at the 5% significance level. The DMRT analysis revealed that the broccoli powder in the leaf samples contained more phytoene than the stem and flower samples. The phytoene content of broccoli powder in the leaf sample averaged 4.35 μg/g, the phytoene content of broccoli powder in the stem sample averaged 2.90 μg/g, and the phytoene content of broccoli powder in the flower sample was 1.34 μg/g. According to Steinbrenner and Linden (2001), an increase in the enzymes carotenoid hydroxylase (CH) and phytoene synthase (PSY) increased the amount of phytoene. Because phytoene is a constituent of β-carotene, an increase in phytoene results in an increase in β-carotene synthesis. As a result, the broccoli powder in the leaf sample had more phytoene than the other samples, owing to the leaf's higher β-carotene content.

3.5 Lutein Content on Broccoli Powder

Lutein is a kind of xanthophyll, an oxidized carotenoid with anti-inflammatory properties. Lutein is a carotenoid that exhibits significant antioxidant action [28]. People can only obtain lutein through food because lutein cannot synthesise carotenoids in the body. Broccoli contains phytochemicals that can reduce cancer risk, including glucosinolates and lutein, which are carotenoid molecules [29]. Broccoli's lutein content also has anticancer properties and may help prevent macular degeneration [30]. Research indicates that lutein may help reduce cancer risk and enhance cardiovascular health [31].

The ANOVA test revealed a significant difference in the lutein content of broccoli powder in stem, leaf, and flower samples at the 5% significance level; hence, the DMRT test was performed. The DMRT test analysis revealed that broccoli powder in leaf samples increased the lutein concentration more than in stem and blossom samples. The average increase in lutein content in broccoli powder in leaf samples is 11.53 μg/g, the average increase in lutein content in broccoli powder in stem samples is 7.25 μg/g, and the average increase in lutein content in broccoli powder in flower samples is 5.39 μg/g.

According to Tarwotjo (1998), raw Broccoli contains around 0.0283 mg/g lutein. According to the USDA (2016), 100 g of Broccoli contains 1403.00 g of lutein [31]. Meanwhile, the USDA (2018) reports that 100 g of dried Broccoli has 15 g of lutein + zeaxanthin. These antioxidant chemicals are most effective when the Broccoli is not overripe, as excessive heat degrades the compounds. Due to its antimutagenic and antitumor qualities, lutein works well in conjunction with -carotene to reduce the risk of cancer [32]. According to Calvo (2007), green vegetables (lettuce, Broccoli, watercress, parsley, beans, and spinach) contain a higher quantity of lutein than yellow-white foods [33]. The lutein content of broccoli blossoms was 157.53 mg/ml. This is because broccoli flower extract (*B. oleracea var. italica*) includes lutein, a non-polar compound soluble in organic solvents and only weakly soluble in lipids or fats [34]. Zhou, Z. et al. (2021) reported a lutein concentration of 641.45 g/g dry weight of Broccoli in broccoli flowers [35]. According to prior research by Liu et al. (2018), broccoli leaves contained 484 μg/g lutein [18].

4 Conclusion

This paper emphasizes the superiority in terms of nutrition of the Broccoli leaves over other parts of the plant, including stems and flowers. The results demonstrated that broccoli leaves are the most abundant source of carotenoids, especially β-carotene, which is crucial in fortifying food products. The eminent relative levels of carotenoids such as α-carotene, phytofluene, phytoene, and lutein can also illustrate the superiority of the tissues above the ground level.

The one-way ANOVA test showed that the three separate anatomical structures, i.e., leaves, stems, and flowers, will vary in their contributions due to the presence of tensions in carotenoids. Instead of being wasted, the green leaves of Broccoli, which are now usually thrown away, can be transformed into stimulators and or additives rich in nutrients, especially in dried powder, for the nutritional enhancement of foods with a particular emphasis on the enhancement of β-carotene content.

The implications of these findings are robust for the food and nutrition industries, particularly in the formulation of functional foods fortified with antioxidants and provitamin A. In future research endeavours, efforts should be made to investigate further the most suitable processing methods, such as drying, that maximize retention of nutrients, especially heat-labile inclusions, such as carotenoids. This research has implications for more sustainable food systems by promoting the use of currently unutilized parts of vegetables, such as broccoli leaves, which will enhance health by reducing food waste.

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References

1. F. A. Tomas-Barberan and M. I. Gil, Improving the health-promoting properties of fruit and vegetable products, *Improving the Health-Promoting Properties of Fruit and Vegetable Products* (Elsevier, 2008)
2. U. Sarma and B. TR, Dietary phytonutrients in common green leafy vegetables and the significant role of processing techniques on spinach: a review, Food Prod. Process. Nutr. **6**, 10 (2024)
3. F. Cristofano, C. El-Nakhel, and Y. Rouphael, Biostimulant Substances for Sustainable Agriculture: Origin, Operating Mechanisms and Effects on Cucurbits, Leafy Greens, and

Nightshade Vegetables Species, Biomolecules **11**, 1103 (2021)
4. T. Sarkar, M. Salauddin, S. Roy, R. Chakraborty, M. Rebezov, M. A. Shariati, M. Thiruvengadam, and K. R. R. Rengasamy, Underutilized green leafy vegetables: frontier in fortified food development and nutrition, Crit. Rev. Food Sci. Nutr. **63**, 11679 (2023)
5. M. Haghighi, A. Barzegar Sadeghabad, and R. Abolghasemi, Effect of exogenous amino acids application on the biochemical, antioxidant, and nutritional value of some leafy cabbage cultivars, Sci. Rep. **12**, 17720 (2022)
6. D. Ramirez, A. Abellán-Victorio, V. Beretta, A. Camargo, and D. A. Moreno, Functional Ingredients From Brassicaceae Species: Overview and Perspectives, Int. J. Mol. Sci. **21**, 1998 (2020)
7. O. Dorcas Adegbaju, G. Aderonke Otunola, and A. Jide Afolayan, Suitability of Fruits and Vegetables for Provision of Daily Requirement of Dietary Fiber Targets, in *Diet. Fibers* (IntechOpen, 2022)
8. Y. He, B. Wang, L. Wen, F. Wang, H. Yu, D. Chen, X. Su, and C. Zhang, Effects of dietary fiber on human health, Food Sci. Hum. Wellness **11**, 1 (2022)
9. N. Tomlekova, V. Spasova-Apostolova, I. Pantchev, and F. Sarsu, Mutation Associated with Orange Fruit Color Increases Concentrations of β-Carotene in a Sweet Pepper Variety (Capsicum annuum L.), Foods **10**, 1225 (2021)
10. Q.-H. Chen, B.-K. Wu, D. Pan, L.-X. Sang, and B. Chang, Beta-carotene and its protective effect on gastric cancer, World J. Clin. Cases **9**, 6591 (2021)
11. S. L. Stevens, Fat-Soluble Vitamins, Nurs. Clin. North Am. **56**, 33 (2021)
12. M. Ebadi, M. Mohammadi, A. Pezeshki, and S. M. Jafari, Health Benefits of Beta-Carotene, in *Handb. Food Bioact. Ingredients* (Springer International Publishing, Cham, 2023), pp. 1–26
13. S. Akram, M. Mushtaq, and A. Waheed, β-Carotene: Beyond provitamin A, in *A Centum Valuab. Plant Bioact.* (Elsevier, 2021), pp. 1–31
14. R. Anand, L. Mohan, and N. Bharadvaja, Disease Prevention and Treatment Using β-Carotene: the Ultimate Provitamin A, Rev. Bras. Farmacogn. **32**, 491 (2022)
15. Z. P. Joshua, M. M. Abarshi, I. Sani, O. A. Owolabi, M. A. David, S. B. Mada, M. A. Saliu, and A. Muhammad, Impact of carrot-ginger blend on micronutrient status and CD4+ cell-counts of HIV-positive-patients on antiretroviral therapy in Kaduna, Nigeria, Hum. Nutr. Metab. **26**, 200133 (2021)
16. E. Landais, Fruit and vegetable consumption and its determinants amongst Moroccan women, in the context of nutrition transition, (2012)
17. U. Krupa-Kozak, N. Drabińska, N. Bączek, K. Šimková, M. Starowicz, and T. Jeliński, Application of Broccoli Leaf Powder in Gluten-Free Bread: An Innovative Approach to Improve Its Bioactive Potential and Technological Quality, Foods **10**, 819 (2021)
18. M. Liu, L. Zhang, S. Ser, J. Cumming, and K.-M. Ku, Comparative Phytonutrient Analysis of Broccoli By-Products: The Potentials for Broccoli By-Product Utilization, Molecules **23**, 900 (2018)
19. B. Caballero, Encyclopedia of human nutrition, *Encyclopedia of Human Nutrition* (Elsevier, 2005)
20. M. W. Farnham and D. A. Kopsell, Importance of Genotype on Carotenoid and Chlorophyll Levels in Broccoli Heads, HortScience **44**, 1248 (2009)
21. G. R. Greenstein, The Merck index: An encyclopedia of chemicals, drugs, and biologicals, Ref. Rev. **21**, 40 (2007)
22. F. Khachik, G. R. Beecher, M. B. Goli, and W. R. Lusby, Separation, identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography, Pure Appl. Chem. **63**, 71 (1991)
23. O. Aust, W. Stahl, H. Sies, H. Tronnier, and U. Heinrich, Supplementation with Tomato-Based Products Increases Lycopene, Phytofluene, and Phytoene Levels in Human Serum

and Protects Against UV-light-induced Erythema, Int. J. Vitam. Nutr. Res. **75**, 54 (2005)
24. B. Fuller, D. Smith, A. Howerton, and D. Kern, Anti-inflammatory effects of CoQ10 and colorless carotenoids, J. Cosmet. Dermatol. **5**, 30 (2006)
25. A. Sugiyama, Y. Ikoma, H. Fujii, T. Endo, H. Nesumi, T. Shimada, and M. Omura, Allelic diversity of phytoene synthase gene influences the transcription level in citrus fruit among a citrus F1 hybrid population, Breed. Sci. **67**, 382 (2017)
26. J. Dobrogojski, M. Adamiec, and R. Luciński, The chloroplast genome: a review, Acta Physiol. Plant. **42**, 98 (2020)
27. G. Giuliano, Plant carotenoids: genomics meets multi-gene engineering, Curr. Opin. Plant Biol. **19**, 111 (2014)
28. P. Bernstein and R. Hobbs, Nutrient supplementation for age-related macular degeneration, cataract, and dry eye, J. Ophthalmic Vis. Res. **9**, 487 (2014)
29. D. Ağagündüz, T. Ö. Şahin, B. Yılmaz, K. D. Ekenci, Ş. Duyar Özer, and R. Capasso, Cruciferous Vegetables and Their Bioactive Metabolites: from Prevention to Novel Therapies of Colorectal Cancer, Evidence-Based Complement. Altern. Med. **2022**, 1 (2022)
30. B. Agin and S. Jegtvig, Superfoods for dummies, *Superfoods for Dummies* (John Wiley & Sons, 2009)
31. G. Bjørklund and S. Chirumbolo, Role of oxidative stress and antioxidants in daily nutrition and human health, Nutrition **33**, 311 (2017)
32. M. Ayaz, A. Nawaz, S. Ahmad, O. F. Mosa, A. A. Eisa Hamdoon, M. A. Khalifa, A. Sadiq, F. Ullah, A. Wadood, A. Kabra, and H. C. Ananda Murthy, Underlying Anticancer Mechanisms and Synergistic Combinations of Phytochemicals with Cancer Chemotherapeutics: Potential Benefits and Risks, J. Food Qual. **2022**, 1 (2022)
33. M. M. Calvo, Lutein: A Valuable Ingredient of Fruit and Vegetables, Crit. Rev. Food Sci. Nutr. **45**, 671 (2005)
34. . K. and N. W. Sri Agustini, Potential Lutein Extract of Broccoli (Brassica oleracea L. var. italica) as Antiradical Abts (2,2 - Azinobis Acid, 3-Ethyl Benzothiazoline-6-Sulfonic Acid), KnE Life Sci. **3**, 125 (2017)
35. Z. Zhou, W. He, D. Li, Q. Fu, Y. Xiao, Y. Bao, Z. Zhang, J. Song, and C. Liu, Accumulation of lutein in broccoli sprouts based on the cultivation conditions of GABA combined with NaCl optimized by response surface methodology, J. Food Process. Preserv. **45**, (2021)