

## THE INFLUENCE OF THE DRYING PROCESS ON PLANT PIGMENT CONTENT IN SELECTED HERBS

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### Abstract

Plant pigments are important functional molecules in photosynthetic organisms and have been recognized as photoprotective compounds. This research aimed to determine the content of plant pigments in selected herbs and the influence of the drying process on the stability of chlorophylls and carotenoids. Coriander (*Coriandrum sativum*), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), and basil (*Ocimum basilicum*) were selected for analysis. Fresh herb material (0.5 g) was analyzed for plant pigment composition, including total chlorophyll, chlorophyll *a* and *b*, and total carotenoids. Following the initial assessment, samples were subjected to two dehydration treatments: air-drying and oven-drying at 45°C. Plant pigments were extracted from fresh and dried herbs using 80% (v/v) acetone. The concentrations of individual pigment fractions were determined spectrophotometrically by measuring absorbance at 470 nm, 648.6 nm, and 663.2 nm, following standard procedures for the quantification of photosynthetic pigments. Fresh parsley showed the highest total chlorophyll content ( $524.38 \pm 7.13$  mg/100g), which was followed by the highest chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) content, with a Chl *a*/Chl *b* ratio of 1:2. Oven-dried basil had the lowest total chlorophyll content ( $196.83 \pm 4.95$  mg/100 g dw). Fresh parsley showed the highest carotenoid content ( $114.99 \pm 2.04$  mg /100 g dw), and air-dried basil samples had the lowest carotenoid content ( $23.85 \pm 0.95$  mg/100 g dw). The drying process showed a significant impact on plant pigments, with a consistent trend toward their degradation during dehydration. Nevertheless, the specific drying technique employed exerted only a minimal effect on the overall pigment content.

**Keywords:** *plant pigments, chlorophyll, carotenoids, drying, herbs*

### INTRODUCTION

Herbs have been recognized as a valuable source of phytochemicals; hence, they have been utilized in traditional medicine for their therapeutic properties, thanks to the presence of essential oils (Muminović, 1998), and in culinary applications for enhancing

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the taste of foods. There is a wide range of phytochemicals in herbs, with a particular emphasis on plant pigments. Plant pigments represent a chemically diverse class of biomolecules that may be systematically classified according to their structural characteristics. This classification encompasses tetrapyrrolic compounds (chlorophylls), carotenoids (e.g.,  $\beta$ -carotene), phenolic pigments (anthocyanins), and N-heterocyclic pigments (betalains) (Schoefs, 2002). Chlorophylls play an essential role in photosynthesis, together with carotenoids, which fight against photooxidative destruction (Giri *et al.*, 2013). Plant pigments exhibit significant antioxidative activity against peroxide radicals as follows: chlorophyll a > lutein > pheophytin a > chlorophyll b >  $\beta$ -carotene > pheophytin b (Loranty *et al.*, 2010). Chlorophyll and carotenoids are, due to their chemical structure, liposoluble compounds: carotenoids with long carbon chains in the form of isoprenoid units and chlorophylls as tetrapyrrole compounds with an esterified phytol group (Schwartz *et al.*, 2008). Considering the fact that chlorophylls are found in green plants, their use in human nutrition is also significant. Anticancer and antimutagenic activity have been found in chlorophylls and their derivatives (Ferruzzi and Blakeslee, 2007). Chlorophyll stability, besides autumn plant aging, depends on heat and acidity, which can inhibit the degradation process (Schwartz *et al.*, 2008). Acting as antioxidants, carotenoids neutralize free radicals and reactive oxygen species while also reducing the detrimental effects of solar radiation (Howitt and Pogson, 2006; Matos, 2017). Compounds produced during carotenoid oxidation can act as signaling stress molecules (Havaux, 2013). The biological relevance of carotenoids is reflected in their antioxidant capacity and their function as provitamin A, demonstrating their importance in physiological processes (Hamed *et al.*, 2023). Beyond providing yellow, orange, and red pigmentation to plant tissues, carotenoids also serve as essential precursors in the biosynthetic pathways leading to various aromatic compounds. Meléndez-Martínez *et al.* (2022) emphasized the crucial role of carotenoids in reducing the risk of several chronic diseases (macular degeneration, cardiovascular diseases, and cancer).

Apiaceae is a family with more than 3000 species known for their aromatic characteristics, specific odor, and taste, which is the reason for their use as spices (Ašimović and Milić, 2017). Plants such as basil, mint, rosemary, oregano, and others from the Lamiaceae family are used in culinary and mostly in traditional medicine as a remedy for many conditions, thanks to bioactive compounds with antioxidative, anti-inflammatory, and antiviral properties (Kivilompolo and Hyötyläinen, 2007).

Herbs, chosen for this research, have a wide range of applications in human nutrition and are regular components of herb mixes produced in the industry. Although the selected herbs do not belong to the same family (parsley, dill, and coriander belong to Apiaceae, and basil to the Lamiaceae family) and were collected in different stages of maturity, their physiological properties and significance are very similar. These herbs are susceptible to spoilage after harvesting due to tissue damage, inadequate temperatures, and water loss during storage, which can lead to metabolic processes similar to spoilage. Biochemical changes, such as increased breathing rate, as well as decreased water content, favor microbial growth and development (Ouzounidou *et al.*,

2013). For this reason, drying is employed as a preservation method, enabling prolonged storage and maintaining the usability of these herbs over extended periods (Akpınar, 2006).

This study aimed to quantify the plant pigment content in selected herbs and to evaluate the impact of different drying methods on the stability of chlorophylls and carotenoids.

## MATERIALS AND METHODS

### Materials

Herbs used in the research were sourced fresh after harvest from local producers in the Kakanj area, Bosnia and Herzegovina. The herbs included parsley (*Petroselinum crispum* cv. *Rialto*), dill (*Anethum graveolens* cv. *Ella*), basil (*Ocimum basilicum* cv. *Italiano classico*), and coriander (*Coriandrum sativum*). Parsley and basil were greenhouse-grown, while dill and coriander were cultivated in open fields.

### Drying process

The herbs were subjected to two different drying methods to examine the effects on their properties:

- a) shade drying without sunlight for seven days at room temperature (average 25°C); and
- b) laboratory oven drying (MEMMERT, Germany) at 45±1°C for 4 hours, with periodic mixing.

Both drying processes were applied on fresh herb leaves with initial moisture as follows: parsley 83.32%, dill 84.12%, coriander 85.67%, and basil 89.21%.

### Methods

Photosynthetic pigments (Chl a, Chl b, and total carotenoids) were determined with the method suggested by Sumanta *et al.* (2014). The method is based on a pigment extraction with an 80% acetone solution from fresh and dried herbs. Pigment content was determined using a spectrophotometric method (measuring absorbance at 470 nm, 648.6 nm, and 663.2 nm, respectively). Briefly, 0.5 grams of the sample was mixed with 10 mL of 80% acetone, homogenized, and centrifuged at 10000 rpm for 15 minutes at 4°C. 0.5 mL of supernatant was then mixed with 4.5 mL of acetone, and the absorbance was read at three wavelengths. The content of photosynthetic pigments was calculated using the equations below:

$$\begin{aligned}
 Ch - a &= 12,25A_{663,2} - 2,79A_{646,8} \\
 Ch - b &= 21,5A_{646,8} - 5,1A_{663,2} \\
 Cx + c &= \frac{1000A_{470} - 1,82Ca - 85,02Cb}{198}
 \end{aligned}$$

The obtained results were expressed as micrograms of sample/mL, and calculated as mg of sample/100 g of dry weight (dw).

### Statistical analysis

Obtained data were expressed as mean  $\pm$  SD and statistically analyzed with a comparison of data by a one-way analysis of variance (ANOVA) with the level of significance at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The results for pigment content (Chl a, Chl b, total chlorophyll, Chl a/Chl b ratio, and carotenoids) are shown in Table 1. The results were expressed as mean  $\pm$  SD.

Table 1. Plant pigment content (leaves) as mg/100 g dw

| Herb      | Sample type<br>(leaf) | Chlorophyll a                   | Chlorophyll b                  | Total<br>chlorophyll            | Total<br>carotenoids           |
|-----------|-----------------------|---------------------------------|--------------------------------|---------------------------------|--------------------------------|
| Parsley   | Fresh                 | 332.92 $\pm$ 7.58 <sup>a</sup>  | 192.56 $\pm$ 0.45 <sup>a</sup> | 524.38 $\pm$ 7.13 <sup>a</sup>  | 114.99 $\pm$ 2.04 <sup>a</sup> |
|           | Air-dried             | 215.71 $\pm$ 7.54 <sup>b</sup>  | 104.80 $\pm$ 3.95 <sup>b</sup> | 319.58 $\pm$ 22.45 <sup>b</sup> | 34.40 $\pm$ 2.10 <sup>c</sup>  |
|           | Oven-dried            | 208.37 $\pm$ 1.21 <sup>b</sup>  | 116.62 $\pm$ 0.75 <sup>b</sup> | 323.54 $\pm$ 21.64 <sup>b</sup> | 41.15 $\pm$ 1.28 <sup>b</sup>  |
| Dill      | Fresh                 | 269.14 $\pm$ 10.98 <sup>a</sup> | 183.63 $\pm$ 2.16 <sup>a</sup> | 460.08 $\pm$ 7.95 <sup>a</sup>  | 80.72 $\pm$ 3.86 <sup>a</sup>  |
|           | Air-dried             | 237.89 $\pm$ 4.23 <sup>a</sup>  | 121.94 $\pm$ 3.45 <sup>b</sup> | 360.89 $\pm$ 7.32 <sup>b</sup>  | 35.43 $\pm$ 1.18 <sup>c</sup>  |
|           | Oven-dried            | 214.63 $\pm$ 4.71 <sup>b</sup>  | 126.76 $\pm$ 3.45 <sup>b</sup> | 346.11 $\pm$ 2.31 <sup>b</sup>  | 48.57 $\pm$ 1.46 <sup>b</sup>  |
| Coriander | Fresh                 | 214.12 $\pm$ 3.88 <sup>a</sup>  | 156.21 $\pm$ 7.02 <sup>a</sup> | 363.15 $\pm$ 1.85 <sup>a</sup>  | 47.60 $\pm$ 3.19 <sup>a</sup>  |
|           | Air-dried             | 208.37 $\pm$ 2.39 <sup>a</sup>  | 106.28 $\pm$ 0.98 <sup>b</sup> | 327.46 $\pm$ 1.44 <sup>a</sup>  | 37.87 $\pm$ 1.61 <sup>b</sup>  |
|           | Oven-dried            | 191.65 $\pm$ 1.21 <sup>b</sup>  | 101.92 $\pm$ 0.95 <sup>b</sup> | 297.07 $\pm$ 8.27 <sup>b</sup>  | 41.82 $\pm$ 0.98 <sup>c</sup>  |
| Basil     | Fresh                 | 252.38 $\pm$ 17.71 <sup>a</sup> | 138.41 $\pm$ 6.57 <sup>a</sup> | 387.70 $\pm$ 24.28 <sup>a</sup> | 64.07 $\pm$ 5.81 <sup>a</sup>  |
|           | Air-dried             | 233.06 $\pm$ 3.38 <sup>a</sup>  | 135.32 $\pm$ 1.61 <sup>a</sup> | 371.46 $\pm$ 2.95 <sup>a</sup>  | 23.85 $\pm$ 0.95 <sup>c</sup>  |
|           | Oven-dried            | 134.18 $\pm$ 6.30 <sup>b</sup>  | 62.65 $\pm$ 3.36 <sup>b</sup>  | 196.83 $\pm$ 4.95 <sup>b</sup>  | 40.01 $\pm$ 1.37 <sup>b</sup>  |

Values marked with different letters are significantly different ( $p < 0.05$ )

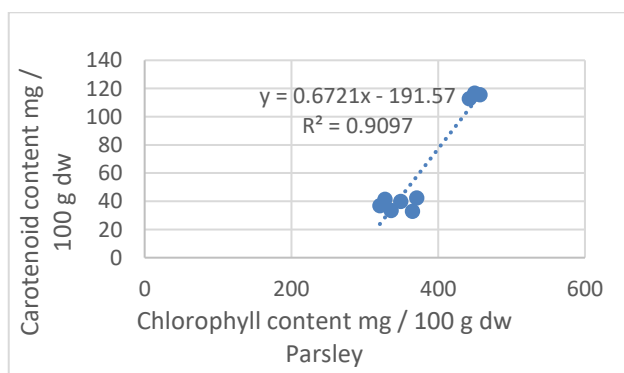
It can be seen that the extract from fresh parsley showed the highest total chlorophyll content (524.38  $\pm$  7.13 mg/100 g dw), and oven-dried basil showed the lowest content (196.83  $\pm$  4.95 mg/100 g dw). Analyzed samples had a Chl a to Chl b ratio of 1:2, which is in accordance with previous research and literature (Śledź and Witrowa-Rajchert, 2012). Kathirvel *et al.* (2006) reported 130.7, 159.4, and 304.9 mg/100 g of total chlorophyll content in coriander, dill, and parsley, respectively. According to Hawrylak-Novak (2009), basil had 118 and 28 mg/100 g of chlorophyll a and chlorophyll b, whilst Jangra *et al.* (2018) reported 141-172 mg/100 g for chlorophyll a, and 70-93 mg/100 g for chlorophyll b for different coriander varieties. In this same research (Jangra *et al.*,

2018), it was reported that the type of extraction solvent had a high influence on chlorophyll content. Dobričević *et al.* (2019) found pigment content lower than in our research. It can be explained by the fact that pigment content is dependent on plant genotype and environmental factors. The chlorophyll content is influenced by various factors, such as the place where the plant is grown, leaf maturity, and position (Schwartz *et al.*, 2008). Total chlorophyll content was significantly lower during the drying process, whereas there was no significant difference in total chlorophyll content between oven-dried and air-dried samples. The influence of temperature on chlorophyll degradation during the drying process has been widely studied. Various authors showed that degradation kinetics followed pseudo-first order with a high impact of temperature. Kathirvel *et al.* (2006) reported a decrease of total chlorophyll in oven-dried (45°C) samples of coriander, parsley, and dill for 1.66, 1.86, and 2.41 times, respectively. Similar trends were observed in the present study. The chlorophyll content in oven-dried parsley samples decreased 1.6 times, in oven-dried dill samples 1.33 times, and in coriander samples the chlorophyll content was 1.22 times lower than in fresh samples. Oven-dried basil samples showed a chlorophyll content approximately 50% lower than that of fresh samples. These results, which correlate with previous studies (Mafakheri *et al.*, 2010; Ghorbanli *et al.*, 2013; Giannakoula and Ilias, 2013; Al Hassan *et al.*, 2015), could be an indicator for the rapid decrease of photosynthetic pigment synthesis in the stress conditions. During the thermal processing of herbs, chlorophylls have been converted to pheophytins with a change of color from light green to olive green, which has been recognized as unpleasant by consumers. Hence, preventive procedures, like blanching or microwave drying, which can minimize degradation, have been developed (Alibas, 2006; Shaw *et al.*, 2007).

Levels of total carotenoids found in selected herbs are given in Table 1. The results ranged from 47.60 to 114.99 mg/100 g in fresh samples, and 23.85 to 48.57 mg/100 g in dried samples. The highest total carotenoid content was found in fresh parsley leaves ( $114.99 \pm 2.04$  mg/100 g dw), whilst the lowest total carotenoid content was detected in air-dried basil leaves ( $23.85 \pm 0.95$  mg/100 g dw). Carotenoids are ubiquitous in all plants, mostly in reproductive organs in high concentrations. Beta-carotene, the most abundant, is known for its role as a radical scavenger in photosynthesis (Speek *et al.*, 1988). Recent studies have highlighted the bioactivity and bioavailability of carotenoids, including  $\beta$ -carotene, emphasizing technological and innovative approaches for their application in human health (Bas, 2024). In addition, dietary intake of provitamin A carotenoids, such as  $\beta$ -carotene, has been associated with cognitive function in adults, suggesting potential neurobiological benefits (Hailili *et al.*, 2025). Furthermore, microalgae have been explored as an alternative source of carotenoids, including  $\beta$ -carotene, with study focusing on challenges and strategies associated with obtaining stable compounds from these emerging sources (Mostafa and Hashem, 2025). Statistical analysis revealed significant differences between fresh and dried samples. Carotenoid content is influenced by ecological factors, geographical and geological conditions, and the level of plant maturity at the moment of analysis, as well as treatments after harvesting (Aizawa and Inakuma, 2007). Analysis of carotenoid content

in basil grown in an open field and in a greenhouse showed different results: 1.22 mg/100 g for field samples and 0.92 mg/100 g for greenhouse samples (Kopsel *et al.*, 2005). Daly *et al.* (2010) reported carotenoid content in basil, dill, coriander, and parsley as follows: 25.8 mg/100 g, 8.7 mg/100 g, 14.4 mg/100 g, and 6.6 mg/100 g dw, respectively. Divya *et al.* (2012) found statistically significant differences in carotenoid content among different varieties of coriander, from 152.79 to 159.15 mg/100g dw in fresh leaves of a young plant and from 203.55 to 217.50 mg/100 g of a mature plant. Aizawa and Inakuma (2007) reported carotenoid content in fresh basil leaves (15.56 mg/100 g) and in parsley (18.33 mg/100 g). The total carotenoid content among the analyzed fresh samples varied, with parsley exhibiting the highest levels, followed by coriander, dill, and basil, which showed the lowest content. Herbs with darker green leaves also have higher anthocyanin content, which can reduce damage to leaves, acting both as light filters and as free-radical scavengers, thereby reducing photo-oxidative damage (Gould *et al.*, 2018; Oliveira *et al.*, 2020). Heat treatments used for herb processing can cause a loss of bioactive compounds. The drying process of parsley, dill, coriander, and basil samples resulted in a decrease of carotenoid content. However, the total carotenoid content in oven-dried samples was higher than in air-dried samples. Oven-dried coriander leaves resulted in a loss of total carotenoids of 35% and chlorophylls of 65% (Divya *et al.*, 2012). Our research showed a loss of total carotenoid content in oven-dried samples of 64.2% for parsley, dill, and basil (39.8% and 37.55%), and coriander of 12%. Air-dried samples showed a total carotenoid content loss from 14% for coriander to 70.08% for parsley. Mazzeo *et al.* (2011) studied the influence of heat treatment on carotenoid content and determined the loss of total carotenoid content from 8-10 %. El Abassy *et al.* (2010) showed that a fast and conventional drying process at high temperatures resulted in carotenoid degradation, but that was not the case with the microwave drying process.

The correlation between chlorophyll and total carotenoid content in dependence on the drying process has been calculated for all samples. A positive correlation between total chlorophyll and carotenoid content in fresh and dried samples among selected herbs has been found ( $r^2$  ranged from 0.849 to 0.954) (Figure 1).



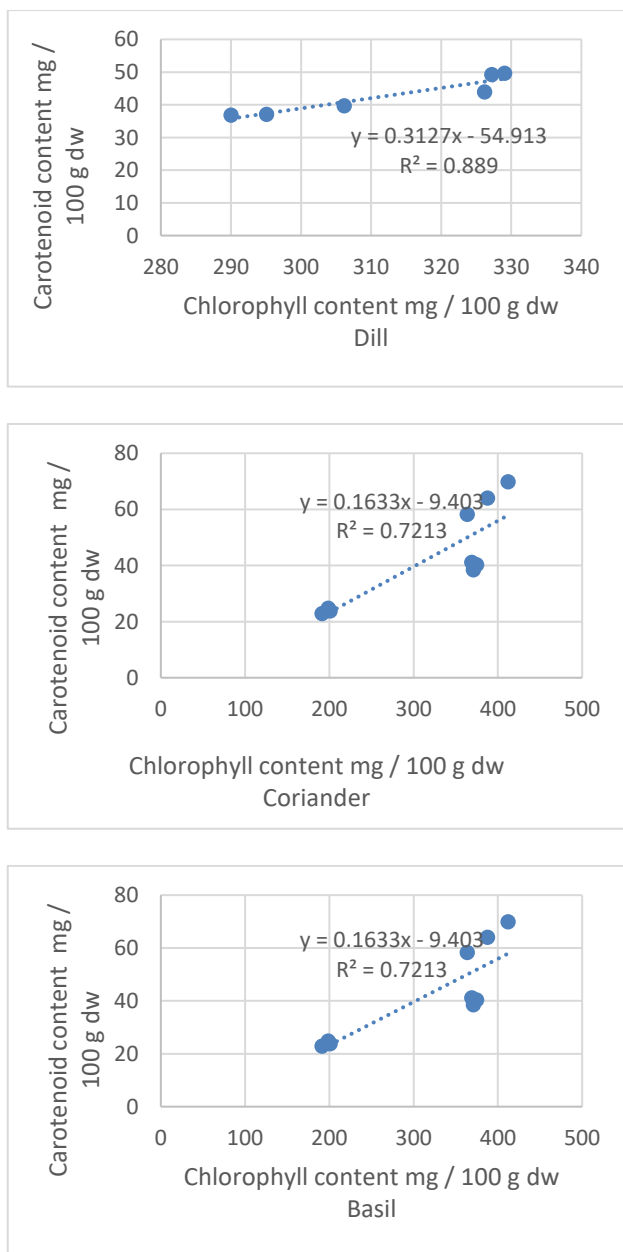


Figure 1. Linear regression between total chlorophyll and carotenoid content among selected herbs (parsley, dill, coriander, and basil)

Previous studies have demonstrated a positive correlation between chlorophyll and carotenoid content in various green plants, such as kale (Kopsell *et al.*, 2004) and basil (Kopsell *et al.*, 2005). Falcioni *et al.* (2023) proposed a sensor-based method for the estimation of photosynthetic pigments, which can be applied in plant research and for pigment analysis. Sumi *et al.* (2024) reported in their research that most of the investigated species exhibited a positive correlation between pigments, including chlorophylls and carotenoids, indicating that higher chlorophyll content is often associated with higher carotenoid levels. Such strong correlations may serve as a useful indicator for estimating carotenoid levels based on chlorophyll content.

## CONCLUSIONS

This research showed that selected herbs, including parsley, dill, coriander, and basil, which are rich in chlorophyll and carotenoids, represent valuable sources of plant pigments and may contribute to improved nutritional quality. The drying process had a significant impact on plant pigment content, with a general trend toward degradation, whereas the specific drying method exerted only a minor effect on the overall pigment levels.

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## UTICAJ PROCESA SUŠENJA NA SADRŽAJ BILJNIH PIGMENATA U ODABRANIM ZAČINSKIM BILJKAMA

### Sažetak

Biljni pigmenti važne su funkcionalne molekule u fotosintetskim organizmima i prepoznati su kao fotoprotektivni spojevi. Ovaj rad je imao za cilj kvantifikaciju biljnih pigmenata u odabranim začinskim biljkama te procijeniti utjecaj procesa sušenja na stabilnost hlorofila i karotenoida. Za analizu su odabrani korijander (*Coriandrum sativum*), peršin (*Petroselinum crispum*), kopar (*Anethum graveolens*) i bosiljak (*Ocimum basilicum*). Svježe biljke (0,5 g) analizirane su na sadržaj biljnih pigmenata (ukupni hlorofil, hlorofil a, b i ukupni karotenoidi) nakon čega su uzorci sušeni na zraku i u sušnici (45°C). Biljni pigmenti ekstrahirani su 80% acetonom iz svježeg i osušenog bilja i određeni spektrofotometrijskom metodom (mjerjenje apsorbancije na 470 nm, 648,6 nm, odnosno 663,2 nm). Svježi peršin pokazao je najveći ukupni sadržaj hlorofila (524,38±7,13mg/100 g), a zatim najveći sadržaj hlorofila a (Chl a) i hlorofila b (Chl b), s odnosom Chl a/Chl b 1:2. Najniži ukupni sadržaj hlorofila zabilježen je kod bosiljka sušenog u sušnici (196,83±4,95 mg/100g suhe tvari). Svježi peršin pokazao je najveći sadržaj karotenoida (114,99±2,04 mg/100 g), a uzorci bosiljka osušeni na zraku imali su najmanji sadržaj karotenoida (23,85±0,95mg/100 g t.v.). Proces sušenja pokazao je značajan utjecaj na biljne pigmente s tendencijom njihove razgradnje tokom sušenja, iako je način sušenja pokazao minoran utjecaj na sadržaj biljnih pigmenata.

**Ključne riječi:** biljni pigmenti, hlorofil, karotenoidi, sušenje, začinske biljke