



BIOCHEMICAL STATUS OF PLANTS *BRASSICA OLERACEA* VAR. *SABELLICA*, *OCIMUM BASILICUM* AND *PETROSELINUM CRISPUM* INDUCED BY A DIFFERENT SPECTRUM OF LIGHT

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Abstract

Plants, in addition to primary metabolites, such as carbohydrates and amino acids, contain a wide variety of chemical compounds other than intermediate products and products of primary metabolism, which are called secondary metabolites. A person uses secondary plant metabolites as a medicinal raw material.

In the framework of this study, the biochemical status of the plants *Brassica oleracea* Var was determined. *Sabellica*, *Ocimum basilicum*, *Petroselinum crispum* when grown in the light of different spectral composition in closed ground conditions. For this purpose, these plants were analyzed for the content of quercetin, provitamin A and protein in them. As a result of the study, it was found that the blue part of the spectrum induces the accumulation of protein, quercetin and provitamin A in all studied species. The greatest accumulation of these substances was observed in the cabbage of feces *Brassica oleracea* VAR. *Sabellica* influenced by the blue rays of the spectrum. The content of quercetin in Kale cabbage when growing on the blue part of the spectrum is an order of magnitude higher compared to other plant species. Thus, when growing plants in closed ground, blue light is needed to accumulate secondary metabolites in the lighting spectrum.

Key words : Kale cabbage, curly parsley, aromatic basil, spectral composition of light, provitamin A, quercetin, protein.

Introduction

Growing plants with a large number of nutrients will allow people to take care of their health independently and without any negative consequences for the body. It is known that plants, in addition to primary metabolites, such as carbohydrates and amino acids, contain a wide variety of chemical compounds other than intermediate products and products of primary metabolism, which are called secondary metabolites. At present, little is known about the physiological relationship between photosynthesis and secondary metabolism of plants grown in greenhouses both under LED lamps and under natural lighting in greenhouses (Li & Kubota, 2009; Ouzounis *et al.*, 2015).

Many secondary metabolites are key components for protection against herbivores, microbes, and viruses, and are also the main sources of specific plant odors and tastes (Bennett & Wallsgrove, 1994). Phenolic acids and

flavonoids are an example of metabolic plasticity that allows plants to adapt to biotic and abiotic environmental changes (Wink, 2010). Phenolic compounds are found in the cuticle, epidermis and / or mesophyll (Solovchenko & Merzlyak, 2008). The concentration of these compounds depends on the season and varies at different stages of growth and development (Lynn & Chang, 1990). A person uses secondary plant metabolites as a medicinal raw material. Flavonoid, campferol glucoside exhibits both antimicrobial and antioxidant activity and is an effective absorber and inhibitor of xanthine oxidase (an enzyme that generates reactive oxygen species) and has the properties of chelation of metal ions (Seigler, 2012).

Rutin and quercetin also exhibit antioxidant, antimicrobial and radical activity. Anthocyanins are involved in the coloring of flowers and fruits and can serve as an attractant of insects; however, at the same time, they exhibit antimicrobial activity and protect cells from strong light damage by absorbing blue or ultraviolet light (Seigler, 2012).

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The problem of increasing the concentration of necessary and useful substances for humans synthesized by plants is more acute than ever. Growing plants in closed ground using various cultivation technologies has allowed plant physiologists to influence plant metabolism and regulate it to obtain the necessary useful chemicals in the winter. Today, the technology with the use of various growth and fertilizer regulators is popular, but perhaps the most “environmentally friendly”, and not fully understood, is the regulation of plant metabolism using different spectral composition of light (Rybakova *et al.*, 2002). Currently, plant physiologists pay special attention to the study of curly cabbage or Kale cabbage (lat. *Brassica oleracea* var. *Sabellica*), which is a specific variety of *Brassica oleracea* cabbage. It is known that it contains a large amount of vitamins and minerals. In particular, vitamins: A, C, K, PP, as well as B vitamins. This type contains minerals: potassium, calcium, sodium, magnesium, phosphorus, as well as beta-carotene (Kaulmann *et al.*, 2014).

The aim of this study was to study the biochemical parameters of Kale cabbage, when grown at different light quality in comparison with the traditionally grown in winter species of fragrant basil (*Ocimum basilicum* L.) and curly parsley (*Petroselinum crispum*).

Materials and Methods

Objects of study

The following plants were selected as objects of our study:

Curly cabbage or Kale cabbage (lat. *Brassica oleracea* Var. *Sabellica*) is a specific type of *Brassica oleracea* cabbage. The plant has green or purple leaves, and the central leaves do not form a head (as with cabbage). Kale is considered closer to wild cabbage than most domesticated forms of *Brassica oleracea*. Raw cabbage consists of 84% water, 9% carbohydrates, 4% protein and 1% fat. It contains a large amount of vitamin K: several times higher than the daily norm (DC). It is a rich source (20% or more of DC) of vitamin A, vitamin C, vitamin B6, folic acid, and manganese.

Kale is a good source (10-19% DC) of thiamine, riboflavin, pantothenic acid, vitamin E, and several dietary minerals, including iron, calcium, potassium, and phosphorus. Kale is a source of carotenoids, lutein and zeaxanthin (Walsh *et al.*, 2015). As with broccoli and other cruciferous vegetables, cabbage contains glucosinolate compounds, such as glucoraphanin, which promotes the formation of sulforaphane, a compound that allows cancer prevention (Houghton *et al.*, 2013).

Cabbage contains high levels of polyphenols, such as ferulic acid (Korus & Lisiewska, 2011). Sacred Basil, or Common Basil (lat. *Ocimum basilicum*). The composition of various varieties of basil includes various essential oils in different proportions. Essential oil from European Basil contains high concentrations of linalool and methylchavicol (estragol) in a ratio of about 3: 1. Other ingredients include: 1,8-cineole, eugenol and myrcene. The clove smell of sweet basil is due to the presence of eugenol in it. The aromatic profile of basil includes 1,8-cineole methyleugenol (Miele *et al.*, 2001). Curly parsley (lat. *Petroselinum crispum*) - a two-year plant in a temperate climate or an annual grass in subtropical and tropical areas. Parsley is a source of flavonoids and antioxidants, it contains luteolin, apigenin (Meyer *et al.*, 2006), folic acid, vitamin K, vitamin C and vitamin A. One gram of dried parsley contains about 6.0 µg of lycopene and 10.7 µg of alpha-carotene, and also 82.9 µg of lutein + zeaxanthin and 80.7 µg of beta-carotene. To grow indoor plants, a phytotron with various light sources (fluorescent lamps) was used: daylight white light (control), red and blue.

Quantitative determination of secondary metabolites in terms of quercetin

One gram of the investigated raw material is placed in a mortar and crushed to a state of gruel. Then place a flask with a thin section with a capacity of 150 ml, add 30 ml of 90% alcohol containing 1% concentrated hydrochloric acid. The extracts are filtered through the same filter into the same volumetric flask, washed with 90% alcohol and the filtrate volume adjusted with 90% alcohol to the mark (solution A). In a volumetric flask with a capacity of 25 ml, add 2 ml of solution A, add 1 ml of a 1% solution of aluminum chloride in 95% alcohol and adjust the volume of the solution with 95% alcohol to the mark.

After 20 minutes, the optical density of the solution was measured on a spectrophotometer at a wavelength of 430 nm in a cell with a layer thickness of 10 mm.

As a comparison solution, use a solution consisting of 2 ml of solution A, adjusted to 95% alcohol with a mark in a 25 ml volumetric flask. The content of the sum of flavonoids in terms of quercetin and absolutely dry raw materials in percent (X) is calculated by the formula:

$$x = \frac{D \times 25 \times 100 \times 100 \times 100}{764.6 \times m \times 2 \times (100 - W)}$$

where:

D - is the optical density of the test solution;

764.6 - specific absorption rate of the complex of quercetin with aluminum

chloride at 430 nm;

m - is the mass of raw materials in grams;

W - is the loss in mass upon drying of the raw material as a percentage.

Quantification of protein according to the Lowry method

Determination of protein content was determined spectrophotometrically according to Lowry (Lowry *et al.*, 1951). The method is based on the formation of a biuret complex, which in the presence of phenol gives a characteristic color in proportion to the amount of protein.

Determination of carotenoid content on a spectrophotometer

The pigments were extracted with 85% acetone and the absorbance of the extracts was measured on a Unico 2800 UV / VIS spectrophotometer.

The concentration of pigments is calculated according to the equations:

for 85% of acetone (Rebbelen):

$$C_{chl.a} + chl.b = 6.4 D_{663} + 18.8 D_{664};$$

$$C_{car} = 4.75 D_{452.5} - 0.226 C_{chl.a} + chl.b$$

Having determined the concentration of the pigment, we find its content in the experimental material by the formula:

$$x = \frac{100 \cdot B}{A},$$

where:

B - is the amount of chlorophyll in the extract, mg;

A - is the mass of raw leaves taken for analysis, mg;
100 - coefficient for expression in percent.

Statistical data processing

Repeatability within one experiment is threefold. The experiments were carried out for all three types of plants grown on three different light spectra. Arithmetic mean values and their standard errors obtained during the research period are presented. The experimental results were processed in the Exsel program.

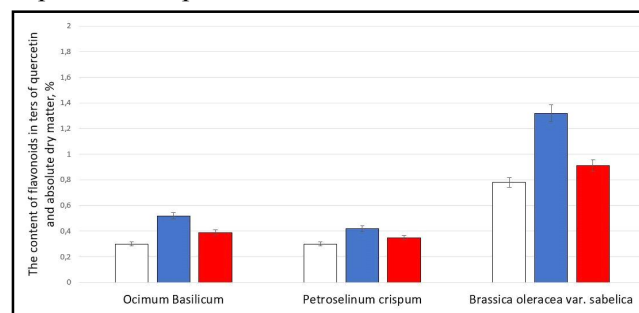
Results and Discussion

Dependence of quercetin concentration on exposure to various light spectra

Quercetin is a vegetable flavonoid found in many fruits and vegetables. The main advantage of quercetin

is that it is a powerful antioxidant that helps fight free radicals that damage cell membranes and DNA, causing cell death. Quercetin is one of the most common antioxidants in human nutrition, which has a significant effect on human health, including the aging process. The main properties of quercetin are: antiviral, antimicrobial, anti-inflammatory antitumor, antiallergic (Endale *et al.*, 2013; Chirumbolo, 2010). Quercetin has a strong effect on inflammation caused by high white blood cell counts. Plants that contain this substance can have an anti-inflammatory effect on the human body, including heart disease, blood vessels, allergies, chronic fatigue, the development of infectious and autoimmune diseases, such as arthritis. Being one of the powerful bioflavonoids, it helps to slow down the aging process, as it reduces oxidative reactions in the body, which are directly related to poor nutrition, stress level, lack of sleep, and the effect of toxins on the human body (Mamani-Matsuda *et al.*, 2006).

When studying the content of quercetin in all three plant species, we observe that the blue spectrum of light has the most significant effect on the accumulation of quercetin. At the same time, the content of quercetin in Kale cabbage significantly exceeds this indicator in other experimental species.



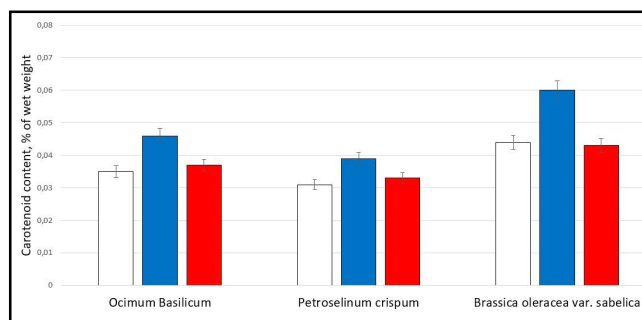
The content of carotenoids in plants grown on a different spectrum of light

In plants, carotenoids are involved in photosynthesis. However, the physiological role of carotenoids is not limited to their participation in the transfer of energy to chlorophyll molecules. The key role of carotenoids in living organisms is that they protect body cells from the negative effects of free radicals. Another advantage of these biologically active substances is the fact that they are able to accumulate in certain tissues of the body, thus creating a protective effect.

For example, a carotenoid such as lutein, accumulates in the human retina - while reducing the risk of dystrophy of the so-called macula lutea. The great importance of carotenoids lies in their A-provitamin activity. It is known that the human body cannot independently synthesize the

vital vitamin A, but assimilates it along with plant-based foods. In the human body, carotenoids contribute to maintaining water balance, calcium transport through membranes, the functioning of olfactory receptors and chemoreceptors, and form complexes with proteins.

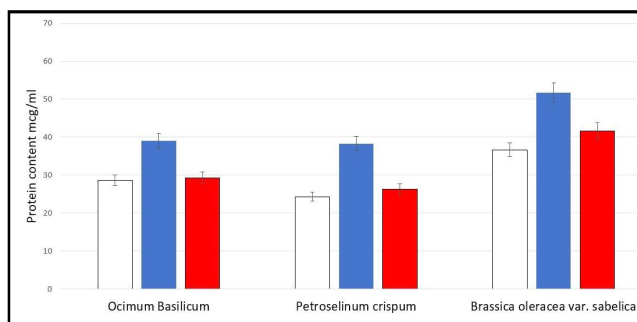
Li and Kubota reported an increase in the content of anthocyanins, xanthophylls and β -carotene in lettuce leaves (*L. sativa* "Red Cross") growing under blue and red LED lighting compared to cold white fluorescent lamps (Li & Kubota, 2009). Similar results have been demonstrated by other authors who emphasize the importance of blue LED lighting in the synthesis of secondary metabolites, such as phenolic compounds and carotenoids in lettuce (Konrad & Nieman, 2014). In contrast, Martineau *et al.*, did not find significant differences in the concentration of carotenoids in lettuce when grown under blue and red LED lighting compared to HPS lamps (Samuolienė *et al.*, 2013). In this regard, we studied the effect of light quality on the accumulation of carotenoids in the studied plant species. Based on the data presented, it was found that for Kale cabbage, blue light had a positive effect on the content of carotenoids. So, the leaves of Kale cabbage grown under blue fluorescent lamps contained a significantly larger amount of carotenoids compared to other plants. Data from other samples did not have statistically significant deviations ($P < 0.05$) in the content of carotenoids. Thus, Kale cabbage grown in the blue part of the spectrum was distinguished by the highest number of carotenoids compared with other plant species studied by us.



The accumulation of proteins in the studied species, depending on the different light spectrum

Proteins are complex organic compounds consisting of amino acids (over 80), of which 22 are most common in foods. Proteins perform many vital functions in the human body:

- serve as material for the construction of cells, tissues and organs, the formation of enzymes and most hormones, hemoglobin and other compounds;
- form compounds that provide immunity to infections;



- participate in the process of assimilation of fats, carbohydrates, minerals and vitamins.

Unlike fats and carbohydrates, proteins do not accumulate in the reserve and are not formed from other nutrients, being an indispensable part of food. With a lack of proteins, serious disturbances in the functioning of the endocrine glands, blood composition, weakening of mental activity, a slowdown in the growth and development of children, and a decrease in resistance to infections occur. As a source of energy, proteins are of secondary importance because they can be replaced by fats and carbohydrates.

We see that the blue part of the spectrum has a significant effect on the increase in the concentration of proteins in the studied species. In this study, the protein content of Kale cabbage is higher than that of the other two species.

Thus, when growing in closed ground in the three studied plant species, it was found that the blue part of the spectrum plays a significant role in the accumulation of secondary metabolites that exhibit antioxidant effects and are necessary components for food.

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