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INSIGHTS INTO MICROGREENS PHYSIOLOGY

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Abstract

In recent years people have a substantial interest for the consumption of fruits and vegetables characterized by a high content of bioactive substances. It is known that these are beneficial not only because they provides the necessary nutrients for human body, but, also have important effects on health. From this point of view, microgreens represent a new class of vegetables that can be considered as "functional foods". Although they have a short life cycle, one of the possibilities of manipulation of the bioactive compounds biosynthesis is to know that species physiology during germination, during the growth, as well as during postharvest. This review presents a general overview on some technological measures that may influence microgreens physiology, based on few recent research works carried out on these topics. There are reviewed data on: 1. beneficial effects of pre-sowing treatments; 2. the lights effects on microgreens physiology (in terms of quantity, but mostly quality of light) concerning the growth process, as well as accumulation of bioactive compounds; 3. measures to influence microgreens post-harvest physiology, to avoid the incidence of some microorganisms, to extend shelf life and to maintain their nutritional quality. Despite microgreens short life cycle, technological measures applied based on species physiology understanding are undoubtedly intended to result in increased the productivity, to obtain healthy products, with lower prices.

***Key words**: microgreens, physiology, bioactive compounds.*

INTRODUCTION

In recent years people have a substantial interest for the consumption of fruits and vegetables characterized by a high content of bioactive substances. It is known that these are beneficial not only because they provides the necessary nutrients for human body, but, also have important effects on health (Beceanu, 2008; Galaverna et al., 2008; Mahima et al., 2013).

Microgreens are a new class of edible vegetables (Pinto et al., 2015), a very specific type which includes seedlings of edible vegetables, herbs or other plants, ranging in size from five to ten centimeters long (including stem and cotyledons) (Xiao et al., 2012).

Over the past few years, microgreens have gained popularity as a new culinary trend, being served as an edible garnish to embellish a wide variety of others dishes or a new salad ingredient (Frank and Richardson, 2009; Hedges and Lister, 2009; Chandra et al., 2012; Xiao et al., 2012; Kou et al., 2013; Pinto et al., 2015) thanks to their greater nutritional benefits (Chandra et al., 2012; Pinto et al., 2015).

Today, more attention is given to a healthy nutrition to prevent certain diseases (Márton et al., 2010). Microgreens are considered as "functional foods" which are food products that possess particular health promoting or disease preventing properties, that are additional to their normal nutritional values (Xiao et al., 2012). Nowadays, demand for these products is growing rapidly (Janovská et al., 2010; Samuoliené et al., 2012) and consumption is growing given their particular characteristics: unique color, rich flavor and appreciable content of bioactive substances (Brazaitytė et al., 2013; Kou et al., 2014; Sun et al., 2014; Brazaitytė et al., 2015a). These are also classified as a good source of minerals in the human diet (Pinto et al., 2015). However, it should be noted that due to the high price and high perishability, microgreens are not currently available to commercial terms in chain stores.

This crop has a quick production cycle (two to three weeks) and occupies very little space in greenhouse production (Kopsell et al., 2012;

Viršilė and Sirtautas, 2013). However, shorten the production cycle and thus reduce greenhouse production cost is one of the major goal of the current researches (Murphy and Pill, 2010). Another issue addressed was that the impact of the biotic stress agents on sprouts and microgreens, through the necessity of obtaining disease-free products (Pill et al., 2011; Xiao et al., 2014). The need to extend their shelf life is also a recent concern to researchers (Berba and Uchanski, 2012; Sasuga, 2014). Preharvest (Kou et al., 2014) or post-harvest (Lee et al., 2009) treatments can be effective means to achieve this objective (Kou et al., 2014).

The purpose of this review is to make a brief insight into microgreens physiology based on the recent research works carried out in this field. There are reviewed data on : 1. pre - sowing applied treatments as beneficial effects for seeds germination physiology; 2. lights effects on microgreens physiology (in terms of light quantity, but mostly light quality) referring to growth process, as well as accumulation of bioactive compounds; 3. measures to influence post-harvest physiology, to avoid the incidence of some microorganisms, to extend shelf life and to maintain microgreens quality.

MEASURES TO INCREASE GREENHOUSE PRODUCTION AND REDUCE COST PRICE

Growing microgreens is a relatively simple process, which does not require much time, energy and experience (Franks and Richardson, 2009). However, attention should be given to each step and besides there are necessary research to improve this type of culture, in terms of productivity, nutritional quality and last but not least the cost of production. Even if microgreens life cycle is very short, certain applied measures for improving seeds germination (as germination faculty, as well as germination velocity) are welcome, to determine a more rapid stabilization and to promote vigorous seedlings. To shorten the greenhouse production and thus the production cost, Murphy and Pill (2010) conducted a series of experiments on microgreen arugula (*Eruca vesicaria* subsp. *sativa*) grown in peat -lite (a soilless medium). Regarding pre-germination treatments results have revealed that seed incubation in exfoliated vermiculite (1.12 g seed in 157 g vermiculite) moistened with 2 g H_20 g^{-1} dry weight vermiculite for 1 day at 20 ^{0} C resulted in a 21% increase in shoot fresh weight by 14 days after planting, as against untreated seed before sowing. Pre-germinated seed germination was 81.5% and at the time of sowing the radicle already had an average length of 2 mm. In terms of sowing density it has proven that high density caused an increase in shoot fresh weight m^{-2} at 10 days after planting, compared to using a lower density. Regarding fertilization, the experiments showed that the most economical measures that have induced an increase in fresh weight m^{-2} were those based on daily use of 150 mg N L^{-1} or daily solution fertilisation that contained 75 mg N L^{-1} plus a pre-plant media incorporation of 1,000 mg N L^{-1} from $Ca(NO_3)$;

Impact of seed treatment was experienced as well for others species: radish (*Raphanus sativus*), kale (*Brassica napus* var. *pabularia*), and amaranth (*Amaranthus tricolor*) (Lee and Pill, 2005). Pretreatment for 3 days in vermiculite (at 12 ^{0} C and -1.0 MPa), removing from the vermiculite and then dried the seeds to the initial weight (before sowing) although not greatly influenced germination faculty, had a positive impact on germination velocity at all studied species. The emergence of radish and kale was faster than untreated seeds although 13 days after planting there has not been an increase of shoot dry weight. In contrast, the amaranth shoot dry weight was increased. If seeds have been exposed to a two-step primed treatment: 3 days at 50% H_20 and then germination 1 day in vermiculite at 150 % H_20, the shoot dry weights increased by 20, 49, and 84% for radish, kale, and amaranth, respectively, as against to those from non-treated seeds.

Also, Lee et al. (2004) examined some possibilities to advance the establishment of table beet or chard (*Beta vulgaris* L.) for greenhouse microgreens production. Germination of seeds in fine grade vermiculite and sowing the germinated seeds with vermiculite mixture caused a more rapid seedlings emergence. If before this process seeds were primed and soaked with hydrogen peroxide, there has been no progress in terms of emergence advancement or growth. On the other hand, germinating the seeds in shallow (4 cm deep) vermiculite (150% initial water, 1 seed : 3 vermiculite dry weight ratio, 27 ^{0}C) for 2 days (table beet) or 3 days (chard) resulted in 0.33-fold and 2.79-fold greater shoot fresh weight, respectively, at 11 days after planting than was achieved by sowing untreated seeds.

LIGHT INFLUENCE ON MICROGREENS PHYSIOLOGY AND THEIR NUTRACEUTICAL QUALITY

One of the possibilities to manipulate the physiology of the plant, including bioactive compounds is controlling environmental conditions (Murchie et al., 2011; Jones, 2014).

Light is one of the main external factors absolutely necessary for the photosynthetic organisms, as it is a source of energy and information from the environment (Murchie and Niyogi, 2011; Fortunato et al., 2015). All oxygenic photosynthetic organisms need strategies for maintaining the balance between efficient light harvesting, photochemistry and photoprotection from excess light (Goss and Lepetit, 2015; Quaas et al., 2015). Light intensity and its quality not only influence the rate of photosynthesis in plants, but also the accumulation of different organic compounds (in terms of their quantity and quality), including production of secondary plant compounds (Murchie et al., 2011; Brazaitytė et al. 2015 a,b).

Vegetables are designated as healthy foods of the millennium or nutraceutical foods of the century (for reviews see Rahal et al., 2014). Microgreens contain higher concentrations of bioactive compounds such as vitamins, minerals, and antioxidants, than mature greens (Janovská et al., 2010; Xiao et al., 2012).

According to Kopsell and Kopsell (2006) one important class of phytochemicals is the carotenoids. Recent studies of Brazaitytė et al. (2015) from the view point of light intensity and its quality influence emphasized that *Brassicaceae* microgreens accumulated more total carotenoids in the case of 330–440 µmol m^{-2} s^{-1} wavelengths levels, as against to 220 µmol m^{-2} s^{-1} , considered as a normal one.

On the other hand, changes of carotenoids content can be achieved by changing of the light spectral composition, relative to the species. Thus, in mustard, supplementation with green light determines an increase in lutein / zeaxanthin and beta- carotene. In the case of other species (red pak choi and tatsoi), the standard blue, red, and far- red light are favourable.

As stated Brazaitytė et al. (2015b), additional application of UV-irradiation for basal lighting with light emitting diodes may lead to an improvement in antioxidant characteristics at microgreens depending on the species. A significant beneficial effect was induced in the case of wavelengths of 366 nm and 390 nm at a photon flux density of 12.4 µmol m^{-2} s^{-1}.

Short-term pre-harvest supplemental 638-red lighting on *Perilla frutescens* (L.) Britton (an annual herbaceous edible and medical plant of *Lamiaceae* Lindl. family naturally growing in the East Asia) on the one hand may reduce the quality of plants, due to the increase in major antioxidants content (e.g. anthocyanins and acid ascorbic) and on the other hand the decrease of nitrate (Brazaitytė et al., 2013). The authors did not show any effect of treatment on DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity and flavonols index, while α-tocopherol content decreased.

Sirtautas and Samuoliené (2013) noticed that the red light-emitting diode (LED) and 24 h photoperiod effect for nitrate and antioxidant contents red baby leaf lettuce is variety – dependant and proper lighting strategies should be selected seeking to cultivate lettuce with optimal contents of phytochemical compounds. However, light spectra and photoperiod are suitable tools seeking to create mild photo stress for plants, with the aim to enhance the contents of antioxidant phytochemicals. In the case of borage (*Borago officinalis* L.) Viršilė and Sirtautas (2013) showed that 440 µmol m^{-2}s^{-1} LED illumination is a recommendable light intensity for microgreens production with optimal growth and nutrient contents. Lower irradiance levels result in significant accumulation of nitrates, decreased fresh weight and elongated hypocotyls, when the highest investigated 545 µmol m^{-2} s^{-1} photosynthetic photon flux density level exceeds plant tolerance and is associated with the decreased growth parameters and slightly lower antioxidant phytochemical contents.

As regard as the impact of supplementary short-term red LEDs lighting on the antioxidant properties of microgreens, Samuoliené et al. (2012) established that natural antioxidant compounds varied in function of the studied species. Thus, the gradual decline of this activity was registered, from a maximum value determined at pea (then lower values for broccoli, borage, mustard, amaranth, basil, kale, beet, parsley, in the order presented here), to very low values for tatsoi. Differentiation was recorded in terms of content in phenols due to additional red light assuring, so variable content was noticed, with an increase (from 9.1% to 40.8% in the tatsoi mustard) or a decrease (with 14.8% at amaranth) .

An obvious variability was also noted in terms of ascorbic acid and anthocyanins content. For example, ascorbic acid in amarantus increased by 79.5%, while in basil it decreased by 53.9%. The amount of anthocyanins increased significantly in broccoli (45.1%), decreased markedly in borage (51.8%), while in basil it was not significantly influenced.

For *Brassicaceae*, Samuoliené et al. (2013) noticed that, the best conditions for growth and nutritional quality (higher leaves, higher content of anthocyanins, phenolics, antioxidant activity, lower nitrate content) were at 320-440 μmol cm $^{-2}$ s^{-1}. In the case of 545 μmol cm $^{-2}$s^{-1} there has not been a significant positive impact on these indicators.

Antioxidant activity was found both in common and tartary buckwheat microgreens. High levels of flavonoids, carotenoids, and α-tocopherol were detected as well. Higher amount of flavonoids was detected in tartary buckwheat microgreens. No significant differences were detected between common and tartary buckwheat microgreens in content of phenolic acids. Microgreens of both common and tartary buckwheat represent potential nutritional sources of alternative vegetable in the Czech Republic (Janovská et al., 2010). All the vegetables (microgreens, sprouts and leafy greens) of both varieties of buckwheat can be regarded as a potent source of phenolics (rutin, quercetin, vitexin, isovitexin, orientin isoorientin and chlorogenic acids) and has high antioxidant activities. Nepalese strain buckwheat vegetables contain high phenolics with higher biological (antioxidant and α-glucosidase inhibition) activity and can be used as an alternative food. Therefore, mass production of more and more buckwheat food products should be encouraged and included in the daily diet, which would help the people to prevent diabetes and many other diseases caused by the free radicals (Sharma et al., 2012).

That microgreens are suitable sources for bioactive substances was also demonstrated by Sun et al and (2013) from studies conducted on five species of *Brassica* vegetables. Considering the use of advanced techniques, such as ultrahigh-performance liquid chromatography photodiode array multistage high-resolution mass spectrometry it was possible to identify 164 polyphenols: anthocyanins (30), flavonol glycosides (105) and hydroxycinnamic and hydroxybenzoic acid derivatives (29). It was also revealed that the phenolic profile was more complex and also the types of substances present in microgreens were varied unlike mature plants.

The lack of scientific data about the nutritional content of microgreens prompted Xiao et al. (2012) to undertake extensive research on concentrations of ascorbic acid, carotenoids, phylloquinone, and tocopherols in 25 commercially available microgreens. Red cabbage, cilantro, garnet amaranth, and green daikon radish had the highest concentrations of ascorbic acids, carotenoids, phylloquinone, and tocopherols, respectively. In comparison with nutritional concentrations in mature leaves (USDA National Nutrient Database), the microgreen cotyledon leaves possessed higher nutritional densities.

Increased nutritional value of microgreens can be also achieved by light management as demonstrated results of Kopsell et al. (2012). The authors' conclusion was that simple applications of short duration high light resulted in biochemical shifts in xanthophyll cycle pigment concentrations in the microgreens, most notably increases in zeaxanthin (characterized by antioxidant and protective effects of vision) may have beneficial effects for the human diet. Thus, the xanthophyll cycle pigments (zeaxanthin + antheraxanthin + violaxanthin) (known in plants as having a major role in the dissipation of excess light absorbed energy) by mustard (*Brassica juncea* L ' Florida Broadleaf ') microgreens exposure to increased light intensity before tissue harvest resulted in increased concentration in zeaxanthin levels. Treatment with 463 μmol photons $m^{-2} s^{-1}$ after the appearance of the first true leaves (light treatment had accumulated 36 h during the photoperiod) resulted in a significant decrease in chlorophyll (a and b) content, β - carotene, and neoxanthin, which indicated the incidence of stress. Lutein content remained unchanged, while the concentration of zeaxanthin and antheraxanthin increased.

Also, management of LED lighting technology through preharvest, short-duration blue light acted to increase important phytochemical compounds influencing the nutritional value of broccoli microgreens (Kopsell and Sams, 2013). At 13 days after sowing broccoli plantlets were treated for 5 days before being harvested using: 1) red and blue LED light (350 μmol $m^{-2} s^{-1}$); or 2) blue LED light (41 μmol $m^{-2} s^{-1}$). Determinations done on biological material highlighted that short-term treatment before harvest with blue light significantly increased shoot tissue β-carotene, violaxanthin, total xanthophyll cycle pigments, glucoraphanin, epiprogoitrin, aliphatic glucosinolates, essential micronutrients of copper, iron, boron, manganese, molybdenum, sodium, zinc, and the essential macronutrients of calcium, phosphorus, potassium, magnesium, and sulphur.

The nutritional value of microgreens was also demonstrated by Pinto et al. (2015), who carried out a comparative study as regard as microgreens and mature lettuces mineral profile. Studies have led to excellent results, not only in scientific terms but rather in terms of practical utility. Microgreens had higher content of Ca, Mg, Fe, Mn, Zn, Se and Mo than mature lettuces, although the former possessed higher N, P and K content. Therefore, microgreens may be considered a good source of minerals. In addition, very low content of NO_3 justifies their recommendation as being safety in the human diet, especially for children, in order to complete their requirements for minerals.

HEALTHY MICROGREENS WITH A LONGER SHELF LIFE

Microgreens senesce rapidly after harvest and have typically a very short shelf-life (1-2 days) at ambient temperature, due to the sudden disruption of plant growth at a very early stage (Guo and Gan, 2012; Xiao et al., 2014).

At broccoli microgreens (Sun et al., 2015), obtaining a high production and extend shelf life has been demonstrated to be possible by applying preharvest treatment with 10 mM calcium chloride. Metabolome analysis obtained by ultra-high-performance liquid chromatography with mass spectrometry led to the conclusion that glucosinolates were the main group of chemical compounds in the treatment variant and the amount of aliphatic and indolic glucosinolates was significantly higher compared with control.

Extending shelf life (by optimize postharvest handling conditions) is a permanent goal of all those who produce and sell horticultural products of any kind. There are concerns on this topic (Berba and Uchanschi, 2012: Chandra et al., 2012; Kou et al., 2013; Kou et al., 2014; Xiao et al., 2014). It should be noted that, recently Sasuga (2014) patented a method for providing a microgreens product with significantly longer shelf life than most other microgreens products, is claimed, where the product microgreens product has a shelf life of at least 10 days.

Researches carried out by Xiao et al. (2014) using radish seeds inoculated with *Escherichia coli* O157: H7 or O104: H4 and *E. coli* populations on harvested products (sprouts and microgreens) highlighted the followings: the proliferation of the bacteria was done in the case of both products, but on microgreens bacteria was significantly reduced. During sprouting, *E. coli* O157: H7 and O104: H4 has reached levels of 5.8-8.1 log cfu / g and 5.2-73 log cfu / g, respectively, depending on the inoculation level (1.5-4.6 log cfu / g and 0.8-43 log cfu / g on radish seeds, respectively), while for microgreens values ranged from 0.8 to 4.5 log cfu / g and from 0.6 to 4.0 log cfu / g, respectively. At table beet (*Beta vulgaris* L.) (,of 'Early Wonder Tall Top') in order to biological control of the fungus *Pythium aphanidermatum* (Edson) Fitzp., Pill et al.

(2011) experienced the influence of the seeds treatment by using equal amounts of antagonistic fungus *Trichoderma harzianum* Rifai strain KRL-AG2 G41 and *T. virens* strain G-41 (*ThTv*) at 0 , 0.25 , 0.50 , 0.75 or 1.00 mg per seed ball. Four days before planting, the peat-lite was inoculated with *P. aphanidermatum* at 0, 0.5 and 1.0x the rate that resulted in 96% damping-off when non-*T. harzianum* -*T. virens*-treated dry seed balls were sown in peat-lite containing 1.0 *P. aphanidermatum*. Decreasing incidence of damping-off with increasing *T.hartzianum*-*T.virens* application to seed balls or growth media was associated with increasing shoot fresh weight m^{-2} at 14 days after planting, a response attributable to increased percentage plant survival and not to a *ThTv* growth-promoting effect.

Post-harvest tah tasai Chinese cabbage young leaf vegetable were washed in cold (5^{o} C) and warm (25^{o} C) chlorinated water (0, 50 or 100 mg L $^{-1}$ free chlorine for 90 sec), then packaged in polypropylene film bag and stored for 8 days at 15^{o} C. Chlorinated water treatment at 5^{o} C had a more beneficial effect on visual quality, weight loss, SPAD (greenness) value change than 25^{o} C chlorinated water treatment. No significant difference was found in aerobic plate count on the surface of microgreens after 3-day storage period. Chlorinated water either at 5^{0} C or 25^{0} C with 50-100 mg L^{-1} free chlorine significantly reduced aerobic plate count during the initial period of storage (up to 2 days) (Lee et al., 2009).

As Hodges and Toivonen (2008) noticed, the two most important storage parameters for postharvest shelf life are storage temperature and atmospheric composition. For example, Kou et al. (2013) indicated that for buckwheat microgreens storage temperature significantly affected the changes in O_2 and CO_2 composition, tissue electrolyte leakage and microbial growth during storage. The authors suggested that buckwheat microgreens should be stored at 5^{0} C with moderately high O_2 (14.0-16.5 kPa) and moderately low CO_2 (1.0-1.5 kPa) content to maintain optimal quality and maximal shelf life. Another useful measure to extend the microgreens shelf life is controlling respiration rates (Berba and Uchanschi, 2012). Post-harvest experiments on arugula (*Eruca sativa*), radish (*Raphanus sativus*), and red cabbage (*Brassica oleracea* var. *rubra*) designed to lower respiration rates, increase shelf life and will allow for transportation to larger markets.

In order to increase biomass and delays senescence of broccoli microgreens, Kou et al. (2014) studied the effect of pre-harvest daily sprays (for 10 days) with calcium chloride solution (1, 10 and 20 mM) as compared to the control (water). After harvesting fresh-cut microgreens packaged in sealed polyethylene film bags were stored on headspace atmospheric conditions. Visual quality and tissue membrane integrity were evaluated on days 0, 7, 14, and 21 during 5 degrees C storage. It was found that treatment with 10 mM calcium chloride resulted in the increase of biomass by more than 50% and the calcium content was triplet, as against the control. Also, this treatment led to an increase in superoxide dismutase and peroxidase enzyme activity, decreased electrolyte leakage in the plant tissue, improved visual quality and reduced microbial growth during storage. Following research in Tan Tasai, Chinese cabbage (*Brassica campestris* var. *marinosa*) Chandra et al. (2012) reported that citric acid and ascorbic acid mixed solution (0.25 % w/v) in addition with ethanol spray (50%) can be used to replace chlorine used for washing microgreens and polyethylene film can provide more benefits for packaging microgreens.

Storage temperature, packaging film, and wash treatment were investigated by Xiao et al (2014) at daikon radish (*Raphanus sativus* L. var. *longipinnatus*) microgreens. Accordingly, studies conducted in dynamics during storage highlighted that storage temperature significantly affected package atmosphere, product quality and shelf life. The optimal temperature for storage of radish microgreens with no chilling injury was proved to be 1^{0}C. Film oxygen transmission rate significantly affected O_2 and CO_2 composition, but this did not significantly affect quality attributes during 28 days of storage at 1 ^{0}C. Chlorine wash treatment (100 mg L $^{-1}$) significantly reduced initial microbial populations.

As Artées-Hernández (2013) recently noticed, attention should be focused on “eco-innovative emerging alternative” to prolong the shelf life without losing quality characteristics specific to the fresh product.

CONCLUSIONS

Microgreens represent a new class of vegetables that can be considered as "functional foods". The manipulation of bioactive compounds biosynthesis and preserve nutritional quality after harvesting may be based on recent research works carried out on these topics so far, but mainly those that come as a necessity. The facts reviewed here indicate that there are promising results in terms of: 1. beneficial effects of pre-sowing treatments; 2. the lights effects on microgreens physiology (in terms of quantity, but mostly quality of light) concerning the growth process, as well as accumulation of bioactive compounds; 3. measures to influence microgreens post-harvest physiology, to avoid the incidence of some microorganisms, to extend shelf life and to maintain their nutritional quality.

Despite microgreens short life cycle, technological measures applied based on species physiology understanding are undoubtedly intended to result in increased the productivity, to obtain healthy products, with lower prices.

Undoubtedly, further studies are needed in terms of content in phytonutrients. These data may provide a scientific basis for evaluating nutritional values of microgreens and contribute to food composition database. Moreover, such results may be used as a reference for health agencies' recommendations and consumers' choices of fresh vegetables (Xiao et al., 2012).

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