Original article
DOI: 10.14529/food220308

MICROGREENS AS A RICH SOURCE OF IMMUNOMODULATORY FUNCTIONAL COMPONENTS FOR THE PREVENTION OF COVID-19

***A.J. Othman*[1]**, *osman.a@rea.ru*
***L.G. Eliseeva*[1]**, *eliseeva-reu@mail.ru*
***T.A. Santuryan*[1]**, *santuryan.TA@rea.ru*
***P.G. Molodkina*[1]**, *molodkina.PG@rea.ru*
***A.M.Y. Kadi*[2]**, *ammarka89@gmail.com*
[1] *Plekhanov Russian University of Economics, Moscow, Russia*
[2] *South Ural State University, Chelyabinsk, Russia*

Abstract. SARS-CoV-2 the pathogenic agent of the ongoing pandemic of coronavirus disease has a strong transmission capacity through inhalation of air droplets leading to diseases ranging from asymptomatic to fatal. COVID-19 can cause multi-organ disorders in which inflammatory progression and oxidative stress caused by the storm release of cytokines play crucial roles. Microgreens are newly emerging superfood products in developed countries due to their high content of immunomodulators and antioxidants. consumption of fresh microgreens, shoots of many vegetables have been gaining broad popularity as a culinary trend due to its intense flavor and aroma along with its density of micro- and phytonutrients. In this study, we analysed thirteen variety microgreens content of ascorbic acid, total antioxidant capacity, total polyphenols, and chlorophyll content. We outline that fortifying salad dishes with microgreens can provide human's organism with good portion of phytonutrients as an additional barrier for securing our immunity. The results demonstrated in this paper indicate to the fact that the tested microgreens are capable to provide a means for consumer-access to larger quantities of nutrients per portion of even less than 100 grams' plant biomass including immunomodulating antioxidants, polyphenols and ascorbic acid of which are considered of critical importance for human's immune system. Inducing microgreens to our daily salads and meals could be an easy possible way to fortify our immunity and most probably would increase the effectiveness of the fight against new coronavirus (SARS-CoV-2) and probably his other mutated variants.

Keywords: microgreens, phytochemicals, ascorbic acid, total antioxidants capacity, total phenolic contents

For citation: Othman A.J., Eliseeva L.G., Santuryan T.A., Molodkina P.G., Kadi A.M.Y. Microgreens as a rich source of immunomodulatory functional components for the prevention of COVID-19. *Bulletin of the South Ural State University. Ser. Food and Biotechnology*, 2022, vol. 10, no. 3, pp. 74–82. DOI: 10.14529/food220308

Научная статья
УДК 637.049 + 557.161.22
DOI: 10.14529/food220308

МИКРОЗЕЛЕНЬ КАК БОГАТЫЙ ИСТОЧНИК ИММУНОМОДУЛИРУЮЩИХ ФУНКЦИОНАЛЬНЫХ КОМПОНЕНТОВ ДЛЯ ПРОФИЛАКТИКИ COVID-19

А. Осман[1], *osman.a@rea.ru*
Л.Г. Елисеева[1], *eliseeva-reu@mail.ru*
Т.А. Сантурян[1], *santuryan.TA@rea.ru*
П.Г. Молодкина[1], *molodkina.PG@rea.ru*
А.М.Я. Кади[2], *ammarka89@gmail.com*
[1] *Российский экономический университет им. Г.В. Плеханова, Москва, Россия*
[2] *Южно-Уральский государственный университет, Челябинск, Россия*

Аннотация. SARS-CoV-2, патогенный агент продолжающейся пандемии коронавирусной болезни, обладает высокой способностью к передаче через вдыхание капель воздуха, что приводит к заболеваниям в диапазоне от бессимптомных до смертельных. COVID-19 может вызывать полиорганные расстройства, в которых решающую роль играют прогрессирование воспаления и окислительный стресс, вызванный бурным высвобождением цитокинов. Микрозелень является недавно появившимся суперпродуктом в развитых странах из-за высокого содержания в ней иммуномодуляторов и антиоксидантов. Потребление свежей микрозелени, побеги многих овощей приобретают широкую популярность в качестве кулинарного направления из-за их интенсивного вкуса и аромата, а также плотности микро- и фитонутриентов. В этом исследовании мы проанализировали содержание аскорбиновой кислоты в тринадцати сортах микрозелени, общую антиоксидантную способность, общее количество полифенолов и содержание хлорофилла. Подчеркнем, что обогащение салатных блюд микрозеленью может обеспечить организм человека хорошей порцией фитонутриентов в качестве дополнительного барьера для защиты нашего иммунитета. Результаты, продемонстрированные в этой статье, указывают на то, что протестированные микрогрины способны обеспечить доступ потребителей к большему количеству питательных веществ на порцию даже менее 100 граммов растительной биомассы, включая иммуномодулирующие антиоксиданты, полифенолы и аскорбиновую кислоту, которые считается критически важным для иммунной системы человека. Добавление микрозелени в наши ежедневные салаты и блюда может быть простым способом укрепить наш иммунитет и, скорее всего, повысит эффективность борьбы с новым коронавирусом (SARS-CoV-2) и возможно, его мутировавшими вариантами.

Ключевые слова: микрозелень, фитохимикаты, аскорбиновая кислота, общая антиоксидантная способность, общее содержание фенолов

Для цитирования: Othman A.J., Eliseeva L.G., Santuryan T.A., Molodkina P.G., Kadi A.M.Y. Microgreens as a rich source of immunomodulatory functional components for the prevention of COVID-19 // Вестник ЮУрГУ. Серия «Пищевые и биотехнологии». 2022. Т. 10, № 3. С. 74–82. DOI: 10.14529/food220308

Introduction

On 11th of March, 2020, worldwide pandemic due to the wide spread of COVID-19 was announced by World Health Organization. To date (June 2022), the number of infected people around the world already reached 548,395,651 cases and caused 6,356,8905 deaths. Coronavirus (COVID-19) by classification belongs to single-stranded RNA family. Highly contagious, can cause respiratory, gastrointestinal, hepatic, and neurologic diseases. [1]. In addition, the development of vaccinations along with pharmacological therapies, many societies are recently paying more attention to the alternative therapy like fortified products, superfood [1, 2]. Dietary supplements and nutraceuticals as possible way for either curing or preventing SARS-CoV-2 infection considering them useful strategy. COVID-19 and many bioactive substances, which are naturally present in foods, have widely displayed potent antioxidant activities [3]. It is reasonable to consider the potent effect of immunomodulating phytonutrients having antioxidant properties, oxidative stress and inflammation-induced endothelial dysfunction as relevant suspected remedies for supporting our immune system against infections [4]. Luteolin, daidzein, apigenin, amentoflavone, quercetin and gallocatechin gallate are known active polyphenols in vivo obtained from plants that have been clearly highlighted in the literature over the past decades. They exhibit antiviral activity due to their molecular behavior of inhibiting the proteolytic activity of SARS-CoV 3C-like protease, which is mainly responsible for virus replication [2]. Polyphenols from plants are strong antioxidants, antitumor, antibacterial, antifungal and anti-inflammatory [3, 4]. Among them, quercetin, luteolin, tannic acid, gallocatechin gallate and daidzein exhibit remarkable antiviral activity by suppressing the proteolytic activity of 3C-like protease SARS-CoV and MERS-CoV, an enzyme that plays a key role in viral replication [5–7]. A detailed study on polyphenols in five different types of Brassica microgreens revealed relatively high concentrations of bioactive immunomodulators directly related to a large number of human health benefits. The proposed identification revealed a total of 164 polyphenolic compounds, including 105 flavonol glycosides, 30 anthocyanins and 29 derivatives of hydroxybenzoic and hydroxycoric acids. They concluded that microgreens of the Brassica species can be considered as potential sources of food polyphenols even when consumed in small portions [8]. Microgreens are also rich in corresponding amounts of β-carotene (provitamin A), α-tocopherol (vitamin E), phylloquinone (vitamin K1), ascorbic acid and their precursors. Researchers study and compare their vitamin content with their mature plants [9–11]. The study [12], in Nigeria showed that 74 % and 64 % of patients with COVID-19 were deficient in vitamins C and E, respectively, while 58 % were deficient in vitamin A. A cross-comparative study was carried out on patients infected with coronavirus symptoms who were admitted to an isolation center. Physicians working with COVID-19 patients are well aware of the importance of stopping the systematic inflammatory storm of cytokines caused by inhalation of reactive oxygen species [13, 14]. Antioxidant drugs such as N-acetylcysteine, ascorbic acid, quercetin, polyphenols, fat-soluble vitamins and glutathione have proven themselves well in previous clinical studies of respiratory diseases such as pneumonia and influenza [15–18]. About 1 billion people are considered chronically malnourished and about 2 billion more suffer from a deficiency of essential trace elements, both phytonutrients and zoonutrients, according to the World Health Organization (WHO) in 2020 [19, 20]. Microgreens are a recent trend of fresh food, gaining popularity and increased attention. They are considered as a potential enrichment of the human diet. Here we specifically comment on some literature that link microgreens with the treatment and prevention of oxidative stress and inflammation, which play a key role in the progression of COVID-19 symptoms [21, 22]. Others call microgreens "functional foods" that have properties that promote health and disease prevention, and are the latest food miracle food [23–25]. Getting the aimed health benefits of microgreen products given their ease of growing indoors or even at home in pots has glowed consumers and attracted in growing them at home. The influence of different types of cultivation methods for microgreens on the content of bioactive components have not been comprehensively assessed [26].

This study compares the content of immunomodulators and antioxidants, including the total amount of phenols, ascorbic acid and the total amount of chlorophylls, phytochemicals of microgreens grown on mineral wool substrates (mineral wool pads).

Materials and methods

Experimental design

Thirteen varieties of microgreens were grown in a closed climate chamber (phytotron ISR 01), developed by the Institute of Development Strategies, Moscow, Russia. The experiment was conducted at the Plekhanov Russian University of Economics at the Department of Commodity Science and Expertise on May 29, 2021. All microgreens were germinated for 12 days on a substrate material (pads) made from mineral rockwool, in total 100 seeds were sown in each growing pad. After harvesting, the cotyledon stems were cut with sterile scissors as close as possible to the substrate for growth.

Total antioxidant capacity

Total antioxidants capacity in the analysed microgreens samples were determined using a coulometric analyzer (EXPERT-006, Russia Federation). The essence of this method is based on generating bromine ions from the buffer solution upon application of 50 mA electrical current inside an analytical electric-cell.

At the beginning, 50 mL of the buffer the solution containing (0.1 M sulfuric acid and 0.2 M potassium bromide) was poured into a glass becher, then the electrodes were lowered and the generator circuit was switched on. An aliquot of the test sample (Exactly 1000 mg of the freshly macerated microgreen sample) were added to the electric-cell. All through the reaction process, bioactive components with antioxidant properties will react with the excess of the generated bromine. The titration point was determined when the initial value of the indicator potential was reached. After the mixing time, the device automatically filtered the outflow of bromine, which was numerically equal to the amount of antioxidant substances introduced into the aliquot. Results expressed in milligrams of Rutin equivalents (mg R.E/g) fresh microgreen sample [27, 42].

Total phenols content

Folin-Chokalteu method was used to determine the total phenolic contents in microgreens samples. A 0.05 g of the dry sample was grounded for 5 min with 2 mL of ice-cold 95 % methanol using ice-cold mortar and pestle. extraction of phenolic compounds was conducted during 45 minutes at 45 °C after subsequent centrifugation for 2 minutes at a rotation speed of 15,000 rpm, from the resulting samples of the extract were taken with a volume of 75 µl, adding to them 75 µl of the Folin-Chokalteu reagent and with careful stirring, after 3 minutes, we added 0.15 cm^3 of a 20 % sodium carbonate solution and 1.2 cm^3 of distilled water, then closed the tap lid. The samples were kept at room temperature for 1 hour. Samples of the extract were taken with a volume of 75 µl, adding to them 75 µl of the Folin-Chokalteu reagent and with careful stirring, after 3 minutes, we added 0.15 cm^3 of a 20 % sodium carbonate solution and 1.2 cm^3 of distilled water, then closed the tap lid. The samples were kept at room temperature for 1 hour. The optical density of the microgreens samples were calculated at 725 nm wavelength. Total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of dry mass of sample using gallic acid calibration curve (R2 = 0.978) [28, 42].

Vitamin C (ascorbic acid) content

The content of ascorbic acid (the free form of vitamin C) was determined by capillary electrophoresis system (Lumex-Drops 105M., Russia). In experimental conditions carried out under positive polarity of high voltage +20 kV silicon capillary (total length 75 cm and internal diameter of the capillary 50/60 microns), buffer was used: sodium tetraborate 10.0 mm, 40.0 mm, pH 9.2, Injection of the sample under pressure $mbar^{-1}$. Detection was carried out at wavelengths of 254 nm or 200 nm, at 24.5 °C. Exactly 5 g of the fresh sample was diluted to 100 cm^3 and kept in the dark for 10 minutes with periodic shaking, then filtered and centrifuged twice at 16,000 rpm to precipitate any impurities. The supernatant was replaced into a device for analysis [27, 29].

Chlorophyll a and b content

The content of chlorophylls (a + b) and carotenoids were determined spectrophotometrically using acetone solvent to extract the pigmentations from leaf sample using the method and the Lichtenthaler and Wellburn equations [30, 31]. A precisely weighted 0.5 g of fresh plant leaf sample was taken and macerated with 10 ml of 85 % acetone solvent to extract the pigments. The homogenized sample mixture was then centrifuged at 10,000 rpm for 10 minutes at room temperature. The infusion liquid was separated and 0.5 ml of it was mixed with 4.5 ml of acetone. The mixture of solutions were analyzed for chlorophyll-a, chlorophyll-b and total carotenoid content in a spectrophotometer at wavelengths of 663 nm, 646 nm and 470 nm.

Results and Discussion

The analysis of the content of health-promoting phytochemicals including ascorbic acid, total phenolic compounds, and total antioxidant capacity revealed relatively high concentrations in the studied microgreens considering the dietary reference intake (RDI) for the average person per 100 grams. Recently, natural diets rich in phenolic compounds with high antioxidant activity have fostered the interests for nutritionists and food scientists [32, 33]. Phenolic compounds are considered as potent electron donors due to their (OH^-) groups which incapable it for directly contribution them to antioxidant action [34, 35]. Researching in old and recently published scientific papers, phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden. Among the abundant phenolic compounds in the analyzed microgreens in our study are cinnamic acid, chlorogenic acid, caffeic acid, and chicoric acid [36–39]. It is well known that ascorbic acid is capable of improving the function of leucocytes (chemokinesis and chemotaxis), the production of lysosomal enzyme, promoting the production of phagocytosis, generating the reactive oxygen species, regulation of antibody-response, leveling up the production of interferon [43–47]. These effects were monitored in patients either receiving ascorbic acid intra-venously or taking 1000 mg orally on daily basis.

Researchers in Wuhan, China, are investigating the effectiveness of using 12000 mg of intra-venously dose of patients with severe pneumonia who are COVID19 positive [48]. Another study launched in Italy for using 10000 mg IV as well.

All these factors show the importance of the analysed phytocompounds containing in microgreens which lead us to suggest them as relevant superfood for consumption on daily basis adding them to culinary and salads. In this study, the maximum total phenolic content of the microgreen's plants was found in Amaranth red (Amaranthus hypochondriacus L.) followed by Kale (Brassica napus L.), Red radish (Raphanus sativus L.), daikon (Raphanus sativus L.) recording 1391.5 ± 41, 1103.3 ± 6.8, 872.8 ± 16.8, 822 ± 13.2 mg GAE/g of the dry weight (DW) respectively. Taking into account the important role of antioxidants for providing healthy nutrition for the consumer, we analysed the total antioxidant capacity in the studied microgreens (Table 1).The

Table 1

Average phytochemical contents (n=5, (standard deviation)), for hydroponically grown mature and microgreens

Commercial name	International scientific name (lat.)	Family	Total ascorbic acid (mg/100 g)	Chlorophyll a and b, (mg/100 g)	Total Anti-oxidant capacity, (mg R.E/g) FW	Total phenols, (GAE/g) DW
Arugula	*Eruca sativa Mill.*	Brassicaceae	46.1 ± 0.12	74.3 ± 4.2	28.22 ± 1.2	688.4 ± 14.8
Sango radish	*Raphanus sativus L.*	Brassicaceae	94.21 ± 0.1	36.3 ± 3.67	18.30 ± 0.9	521.2 ± 22.9
Red radish	*Raphanus sativus L.*	Brassicaceae	92.5 ± 0.09	52.2 ± 2.2	17.6 ± 0.3	822 ± 13.2
Amaranth red	*Amaranthus hypochondriacus L*	Amara-nthaceae	144. 7 ± 1.3	42.1 ± 2.21	23.52 ± 0.21	1391.5 ± 41
Green basil	*Ocimum basilicum L*	Lamiaceae	64.05 ± 1.1	36.24 ± 1.89	16.15 ± 0.2	655.9 ± 9.5
Red basil	*Ocimum basilicum L*	Lamiaceae	96.8 ± 0.08	31.72 ± 1.41	17.47 ± 1.3	872.8 ± 16.8
Daikon radish	*Raphanus sativus L.var. longipinnatus*	Brassicaceae	82.3 ± 0.44	28.70 ± 2.04	14.1 ± 1.9	663.6 ± 22.6
Japanese Cabbage – Mizuna Green	*Brassica rapa L. ssp.nipposinica*	Brassicaceae	32.3 ± 0.05	25.66 ± 2.34	8.92 ± 0.8	402.9 ± 24.9
Japanese Cabbage - Mizuna Red	*Brassica rapa L. ssp.nipposinica*	Brassicaceae	40.5 ± 0.78	30.5 ± 3.1	12.0 ± 1.5	478.06 ± 7.2
Pea lentiles	*Pisum sativum L.*	Fabaceae	53.4 ± 0.0	33.6 ± 4.5	15.1 ± 2.2	821.2 ± 19.2
Chinese cabbage-Pak choy	*Brassica rapa subsp. chinensis*	Brassicaceae /Cruciferae	45.2 ± 0.2	28.8 ± 2.63	11.66 ± 3.6	620.2 ± 18.8
Watercress	*Nasturtium officinale.*	Brassicaceae	30.6 ± 0.7	23.76 ± 1.6	10.3 ± 1.7	519.6 ± 14.7
Kale – Russian red	*Brassica napus L.*	Brassicaceae	66.8 ± 0.9	56.7 ± 4.6	24.34 ± 2.7	1103.3 ± 6.8

overall content of total antioxidant capacity ranged between 8.92 ± 0.8 in Japanese cabbage (Mizuna red), up to 23.52 ±0.21 and 24.34 ± 2.7 of the fresh weight (FW) in Kale (*Brassica napus L.*) and Amaranth red (*Amaranthus hypochondriacus L.*), respectively. Moreover, it was found that the content of ascorbic acid, the free form of vitamin C were high in all the microgreens samples of this study comparing to the average contents in their mature plants. As shown in (Table 1), the highest content of ascorbic acid was found in Amaranth red (*Amaranthus hypochondriacus L.*) 144. 7 ± 1.3 mg/100 g while the lowest content was recorded in Watercress (*Nasturtium officinale*) both of which compromising 52 to 135 % of the recommended daily intake from vitamin C in healthy adults as mentioned previously by researchers [40, 41].

Conclusion

The results demonstrated in this paper signpost that the tested microgreens are capable to provide a means for consumer-access to larger quantities of nutrients per portion of even less than 100 grams plant biomass including immunomodulating antioxidants, polyphenols and ascorbic acid of which are considered of critical importance for human's immune system. We suggest that inducing microgreens to our daily salads and meals could be an easy possible way to fortify our immunity and most probably would increase the effectiveness of the fight against new coronavirus.

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Information about the authors

Ali J. Othman, Senior Lecturer, Academic Department of Commodity Science and Commodity Examination / laboratory of plant products expertise, Plekhanov Russian University of Economics, Moscow, Russia, osman.a@rea.ru

Ludmila G. Eliseeva, Dr. Sci. (Engin.), professor, Academic Department of Commodity Science and Commodity Examination / laboratory of plant products expertise, Plekhanov Russian University of Economics, Moscow, Russia, eliseeva-reu@mail.ru

Tatyana A. Santuryan, Senior Lecturer, Academic Department of Commodity Science and Commodity Examination / laboratory of plant products expertise, Plekhanov Russian University of Economics, Moscow, Russia, santuryan.TA@rea.ru

Polina G. Molodkina, head of department, Academic Department of Commodity Science and Commodity Examination / laboratory of plant products expertise, Plekhanov Russian University of Economics, Moscow, Russia, molodkina.PG@rea.ru

Ammar M.Y. Kadi, Post-graduate student at the Department of Food and Biotechnologies, South Ural State University, Chelyabinsk, Russia, ammarka89@gmail.com

Информация об авторах

Осман Али, старший преподаватель кафедры товароведения и товарной экспертизы, Российский экономический университет им. Г.В. Плеханова, Москва, Россия, osman.a@rea.ru

Елисеева Людмила Георгиевна, доктор технических наук, профессор кафедры товароведения и товарной экспертизы, Российский экономический университет им. Г.В. Плеханова, Москва, Россия, eliseeva-reu@mail.ru

Сантурян Татьяна Анатольевна, старший преподаватель кафедры товароведения и товарной экспертизы, Российский экономический университет им. Г.В. Плеханова, Москва, Россия, santuryan.TA@rea.ru

Молодкина Полина Григорьевна, профессор, ассистент кафедры товароведения и товарной экспертизы, Российский экономический университет им. Г.В. Плеханова, Москва, Россия, molodkina.PG@rea.ru

Кади Аммар Мохаммад Яхья, аспирант кафедры «Пищевые и биотехнологии», Южно-Уральский государственный университет, Челябинск, Россия, ammarka89@gmail.com

The article was submitted 20.01.2022
Статья поступила в редакцию 20.01.2022