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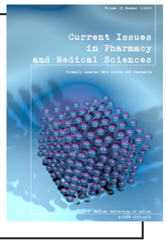

Chia sprouts and *microgreens* as a new nutraceutical raw materials and their health-promoting impact in modern dietetics

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ABSTRACT

Plant sprouts are one of the most important forms of functional foods (FOSHU – Food For Specified Health Use), crucial in nutraceutical diets based on so-called "healthy food". The health-promoting effect of sprouts is due to their rich chemical composition and high nutritional quality compared to standard crop raw materials. Recently, many scientific studies have pointed to the medicinal and therapeutic effects of chia seeds (*Salvia hispanica*), but there is still a lack of research on the composition and biological properties of chia sprouts. In addition to chia sprouts, chia *microgreens* (microleaves) are becoming prominent in the food industry. This paper reviews the literature data on research on chia sprouts and *microgreens*. The process of sprouting chia seeds has been proven to boost their nutraceutical properties by increasing their content of protein, dietary fiber, vitamins and mineral salts. In addition, sprouting contributes to the enhancement of antioxidant potential by increasing the production of polyphenolic compounds from the phenolic acid group and flavonoids. Single studies also prove the antimicrobial properties of chia sprout extracts against Gram-negative (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacterial strains. The paper is the first comprehensive review of the latest scientific information on the comparison of chia: dry seeds, sprouted seeds, sprouts and *microgreens*.

INTRODUCTION

Currently, a growing public interest in leading a healthy lifestyle is noted. The interest of the food industry is focused on meeting the increasingly demanding nutritional needs of consumers. The popularization of novel foods, referred to as functional foods (FOSHU – Food For Specified Health Use) is gaining prominence as foods with medicinal and therapeutic properties [1-5].

Nowadays, plant-based raw materials are particularly important in preventing and supporting the treatment of many civilization diseases. The increasing prevalence of diet-related diseases is making dietary patterns based on greater consumption of plant-based products increasingly popular. It is recommended to abandon the Western style of eating (*Western diet*) identified with the consumption of large amounts of animal products, saturated fats and refined sugars in favor of a dietary pattern based on increased consumption of plant-based products [6,7]. In this regard, the Mediterranean Diet (*MedDiet*) is gaining popularity, which is now an established and recommended dietary pattern increasingly chosen by the public. Adherence to the *MedDiet* is associated with scientifically proven improvements in overall health and body function [8]. *MedDiet* is defined as a diet low in saturated fatty acids and high in unsaturated fatty acids [9]. *MedDiet* recommends consuming plenty of vegetables, fruits, fiber-rich foods, nuts, vegetable oils and pulses, including sprouts, and less consumption of animal products [2,10-14]. In this aspect, sprouts and microgreens (*microleaves*) are a particularly valuable, universal and timeless part of the diet. Their consumption can make a significant contribution to diversifying the diet and enriching it with health-value nutrients [15-17]. In addition to the well-known and popularly used sprouts or *microgreens* of various crop plants in the diet, a particular alternative may be those extracted from the *Salvia hispanica* (chia) species. Moreover, chia seeds are sold as food products and are generally available on the market, while chia sprouts and *microgreens* have not been commercialized yet [18].

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According to Regulation (WE) No. 208/2013, a sprout is a product that is obtained during the germination of seeds carried out in water or other medium, which is harvested before the development of the proper leaves, intended for consumption as a whole, including the seed [19]. However, the American Association of Cereal Chemists (AACC) with the acceptance of the opinion of the U.S. Department of Agriculture (USDA) have specified the concept of „sprouted seeds," which are defined as malted or sprouted seeds containing all the original bran, germ and endosperm, which should be whole grains as long as the sprout growth does not exceed the length of the grain and the nutritional values have not been reduced. These grains should be classified as malted or sprouted whole grains [20]. In contrast, *microgreens* are a relatively new class of edible vegetables defined as tender, immature greens extracted from the seeds of vegetables, herbs or grains, including local varieties and wild species. The term refers to young seedlings that have germinated and are at the cotyledon or very young first leaf stage [13,21]. A wide range of herbs (e.g., basil, cilantro), vegetables (e.g., radish, broccoli), and flowers (e.g., nasturtium) are being cultivated today as so-called *microgreens*. Microgreens are a new culinary ingredient that is being used to improve an extensive range of foods, both from a sensory and health perspective [22,23].

Sprouts have been known and used since ancient times. The Egyptians practiced the sprouting process as early as 3000 B.C., and sprouts were an essential part of their culinary history [24]. In China, crop sprouts have been used for 5,000 years as a popular ingredient in regional dishes. Initially, only cereal and legume sprouts were used, but over time, the health and therapeutic potential of sprouts from other plants, including alfalfa and broccoli, also began to be exploited [25]. Since the 1980s, there has been a significant increase in interest in the consumption of seed sprouts in eastern countries, due to their special health-promoting properties [16]. Sprouts are a very attractive resource because they are a valuable source of protein, minerals (especially calcium, magnesium) and vitamins (mainly A, E, C and B group) [16,26]. In addition, sprouts contain high concentrations of plant secondary metabolites, especially polyphenols with strong antioxidant potential [27,28]. Due to the high nutritional value and many desirable sensory qualities, sprouts are an important ingredient in many foods, including tortillas, confectionery, breakfast cereals, salads and gluten-free products [29-32].

In terms of chia sprouts, based on the results of preliminary scientific studies, it is suggested that a mixture of dry seeds, sprouted seeds and green sprouts may be beneficial in the food industry due to the variable content of antioxidant vitamins (E, C) depending on the developmental stage. This treatment allows the effective use of the composition of antioxidant components [33].

The popularity of microgreens cultivation and interest in microgreens is of recent note, hence few scientific studies on chia concern to *microgreens*. Scientific papers are systematically appearing that analyze the nutritional composition of *microgreens* extracts of more common plant species. These studies prove that the content of bioactive substances, including compounds with antioxidant properties in microgreens is higher compared to the mature plant [22,34]. The increased interest in *microgreens* was strengthened by initial scientific findings which proved that *microgreens* can contain 4 to as much as 40 times more nutrients and key vitamins compared to a common plant raw material [21].

MATHERIALS AND METHODS

A proper search strategy helps to correctly define the appropriate search term and identify the subject databases sought in order to collect a satisfactory amount of scientific literature. Eligible literature was selected according to inclusion and exclusion criteria. We pre-defined the inclusion/exclusion criteria to exclude publications that were not empirical or were not documents or guidelines (e.g., commentaries, letters, book reviews). We decided that the spectrum of main interests must include all aspects of *S. hispanica*, dealing with botany, ethnopharmacology, geobotany, phytochemistry and pharmacology in the broadest sense. The search databases for this review were SCOPUS, PubMed/MEDLINE, Web of Science (SCI-EXPANDED), Wiley Online Library, Taylor & Francis Online, Google Scholar, REAXYS Database, Science Direct/ELSEV-IER, EBSCO Discovery Service (EDS) and Cosing (Cosmetic Ingredients Database). They were searched systematically for articles published from 1950 to 2023. The following syntax was used: TITLE-ABS-KEY in addition to a combination of the following keywords: "*Salvia*" or "*Salvia hispanica* L." or "Sage" or "Salba" or "chia" or "chia seeds" or "Spanish sage" or "*Salviae hispanicae semen*" or "phytochemistry" or "functional food" or "nutraceutical" or "*Lamiaceae*". Search terms were used separately or in limited combinations, according to the requirements or limitations of the database used.

NUTRACEUTICAL IMPORTANCE OF SPROUTS

Over the past several years, there have been significant advances in technology aimed at developing methods to enhance the health and nutritional properties of edible plant sprouts. Scientists are striving to develop modern methods that would increase the synthesis of valuable secondary metabolites in sprouts [35-37]. The most popular strategies to raise the production of phytonutrients in sprouts include using ionized water as an elicitor, treating seeds with 3% sucrose, using lighting with different wavelengths of light, seed conditioning (e.g., controlled hydration of seeds throughout the germination process), biofortification (e.g., soaking seeds in a solution of $FeSO_4 \times 7H_20$ before germination) [38-44]. The germination process itself increases the bioavailability of active compounds in the germinated seeds and contributes to a favorable change in macronutrient content [35,45,46]. During germination, there is basically an increase, in sprouts, compared to dry seeds, in the content of bioactive compounds: essential amino acids, dietary fiber, phenolic compounds and minerals (mainly calcium) [47-49]. All changes that occur in the composition of germinating seeds are related to swelling and seed germination [50]. Soaking the seeds conditions their swelling before germination. A crucial stage is seed germination, which is often closely monitored. Typically, seed germination takes place in the dark, in a temperature range from10 to 20°C [26]. Germination time for most crop species is from 3 to 5 days [51]. Many factors are observed to affect the quality of sprouts such as temperature, light conditions, soaking time, germination time and moisture content. These are significant factors that are the main predictors of morphological and physiological changes in seeds during the germination process. Morphological changes include delayed germination, reduced germination rate. Physiological changes, include accumulation of reactive oxygen species (ROS), hardening of the cell membrane, protein instability and a number of metabolic disorders [52-56]. The germination process reduces the content of non-nutritive compounds such as phytates, oxalates, trypsin inhibitors, while positively increasing the digestibility of proteins and the bioavailability and content of health-promoting phytochemicals such as γ-aminobutyric acid (GABA) and natural antioxidants including, in particular, polyphenolic compounds [57,58]. In addition, there is an improvement in the bioavailability of calcium and iron. The reduction of non-nutritive compounds promotes the enhancement of the sensory properties (mainly taste) of sprouts. Numerous scientific studies conducted on different types of crop sprouts have shown that sprouts during the germination process enhance their nutritional and medicinal properties such as antioxidant, anti-inflammatory, anti-diabetic, hypolipemic and anti-cancerogenic activities [12,13,59] the interest in fresh, ready-to-eat, functional food, such as microscale vegetables (sprouted seeds and microgreens). As an example, are scientific researches which has been conducted on sprouts of common, well-known plants (e.g., broccoli and alfalfa), but there is little scientific data on chia sprouts or *microgreens* and their medicinal properties.

SALVIA HISPANICA – CHARACTERISTICS OF THE SPECIES

In recent years, the exploitation of chia seeds, which are used in the food, pharmaceutical and cosmetic industries, has been gaining popularity [60,61]. Chia seeds are extracted from the *Salvia hispanica* L. (chia) species of Lamiaceae family (Figure 1) [62-64]. The plant is indigenous to areas of present-day southern Mexico and northern Guatemala. Today, its cultivation on an industrial scale is carried out in many areas of the world, including South America (Argentina, Bolivia, Brazil, Ecuador, Colombia, Paraguay, Peru), Central and North America (Guatemala, Honduras, Mexico, Nicaragua and Panama). In the last few years, chia has been produced in middle- and high-income countries, mainly in Argentina, Brazil, Mexico and in the USA [62,65].


A B C

Figure 1. *Salvia hispanica*; A - plant; B - seeds; C - sprouts

Chia seeds phenomenon is attributed to their rich chemical composition and high nutritional value, which determine their beneficial effects on the body. They are a valuable source of essential fatty acids. The quantitatively dominant are α-linolenic acid (ALA), belonging to the Omega 3 acids and linoleic acid (LA) classified to Omega 6. In addition, they supply plant protein, which contain a set of essential amino acids (arginine, leucine, phenylalanine, lysine, valine, isoleucine, threonine, methionine, histidine, tryptophan). Chia seeds also deliver dietary fiber, especially the water-insoluble fraction, which accounts for about 85-93%, with the remaining amount going to the water-soluble fiber fraction [66,67]. The widespread use of chia seeds is also determined by the presence of valuable vitamins and macro- and micronutrients. Macronutrients include phosphorus, magnesium, potassium, sulfur, sodium, and calcium, while micronutrients include zinc, manganese, copper, molybdenum, selenium, and iron [60,67]. Chia seeds are also a remarkable source of bioactive compounds especially antioxidant polyphenols, such as simple phenolic acids (cinnamic acid, ferulic acid, gallic acid, *p*-coumaric acid, caffeic acid), depsides (rosmarinic acid and chlorogenic acid), flavones (apigenin), flavonols (kaempferol, quercetin, myricetin, and rutoside), isoflavones (daidzein, glycitein, genistein, genistein), and falavan-3-ols (catechin, epicatechin) [60,67-69]. Scientific research proves the wide spectrum of therapeutic and healing effects of chia seeds on the body. Among other, they exhibit antioxidant [47,68, 70-72], antidiabetic [73-75] and hypotensive activities [76,77]. Chia seeds are a very popular and valuable resource with a wide range of uses, thus the rather low popularity and use of chia sprouts and *microgreens* is noteworthy.

CONTENT OF NUTRACEUTICAL COMPONENTS IN CHIA SPROUTS

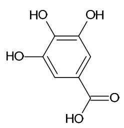
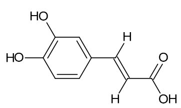
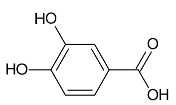
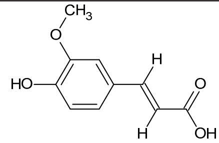
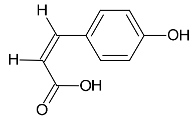
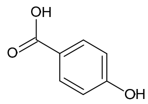
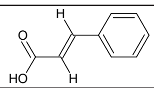
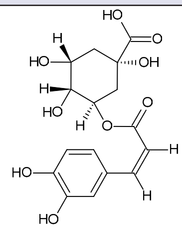
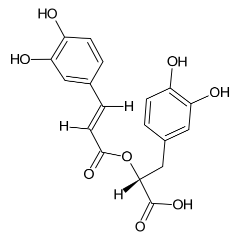
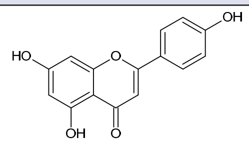
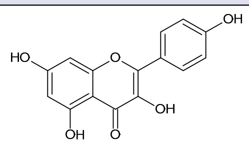
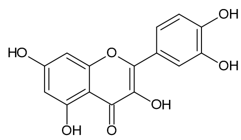
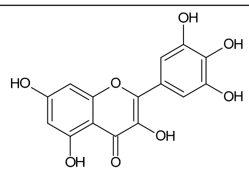
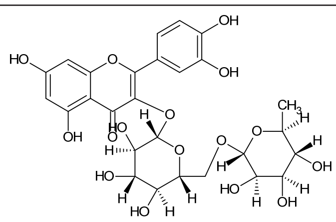
Chia seeds are the main raw material extracted from *S. hispanica*, but according to current scientific research, compared to seeds, sprouts also have high nutritional value, making them an interesting plant raw material with high potential for use in the agri-food industry [78].

Chia sprouts are a plant product that is rich in numerous phytochemicals with high health-promoting potential, such as phenolic acids (cinnamic acid, ferulic acid, gallic acid, caffeic acid, protocatechuic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, depsides (rosmarinic acid and chlorogenic acid) and flavonoids (apigenin, kaempferol, quercetin) (Table 1). The quantitatively dominant phenolic acid in sprouts is protocatechuic acid (0.80 mg/g DW) and rosmarinic acid (0.60 mg/g DW). Chia sprouts are also a source of carotenoids. Among the carotenoids identified in chia sprouts are carotenes (β-carotene) and xanthophylls (neoxanthin , violaxanthin, antheraxanthin, lutein and zeaxanthin) [18] (Table 1, Figure 2). In addition, another advantage of sprouts is the high content of GABA, which is a compound with strong anti-inflammatory, anti-diabetic and anti-cancer properties [47,78-81]. An additional benefit of the phytochemical profile of chia sprouts is that they contain low levels of anti-nutritional compounds, which include mainly phytates and oxalates [82].

Table 1. Comparison of the content of selected active ingredients in chia seeds and sprouts

Compounds	Chia seeds	Chia sprouts	References
Vitamins (mg/g DW)			
Vitamin C	1.60 14.2	nd* 4.3	[65] [33]
Vitamin E	0.50	nd	[65]
Vitamin B1	0.62	nd	[65]
Vitamin B2	0.17	nd	[65]
Vitamin B3	8.83	nd	[65]
Phenolic acids (mg/g DW)			
Gallic acid	0.05 0.01 1.15 4.26	nd 0.08 nd nd	[83] [79] [70] [84]
Caffeic acid	0.28 0.30-0.68 2.74 3.09 12.54	0.50 nd nd nd nd	[79] [85] [70] [86] [84]
Protocatechuic acid	0.170	0.80	[79]
Ferulic acid	0.112 3.59	0.34 nd	[79] [84]
p-Coumaric acid	nd* 0.02 2.60	0.24 nd nd	[79] [87] [84]
p-Hydroxybenzoic acid	0.01	0.22	[79]
Cinnamic acid	0.04	0.16	[79]
Depsides (mg/g DW)			
Rosmarinic acid	0.32 65.40 92.67	0.60 nd nd	[79] [84] [70]
Chlorogenic acid	0.03 0.47 4.59-10.20	0.34 nd nd	[79] [86] [85]
Flavones (mg/g DW)			
Apigenin	0.02	0.43	[79]
Flavonols (mg/g DW)			
Kaempferol	nd	0.16	[79]
Quercetin	0.08	0.40	[79]
Carotenes (µg/g DW)			
β-Carotene	nd	100	[18]
Xanthophylls (µg/g DW)			
Neoxanthin	nd	150	[18]
Violaxanthin	nd	150	[18]
Antheraxanthin	nd	15	[18]
Lutein	nd	1180	[18]
Zeaxanthin	nd	25	[18]

nd – no data

Chemical compound	Structural formula
Phenolic acids	
Gallic acid	
Caffeic acid	
Protocatechuic acid	
Ferulic acid	
p-Coumaric acid	
p-Hydroxybenzoic acid	
Cinnamic acid	
Depsides	
Chlorogenic acid	
Rosmarinic acid	
Flavones	
Apigenin	
Flavonoles	
Kaempferol	
Quercetin	
Myricetin	
Rutoside	

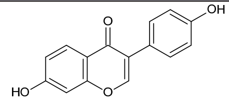
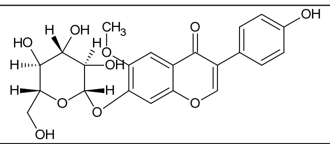
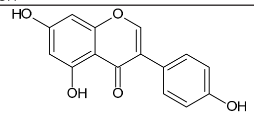
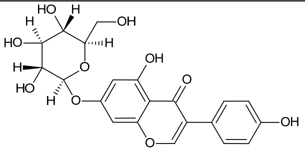
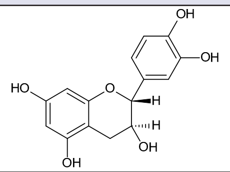
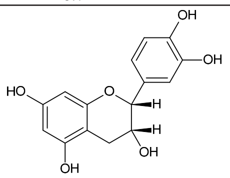
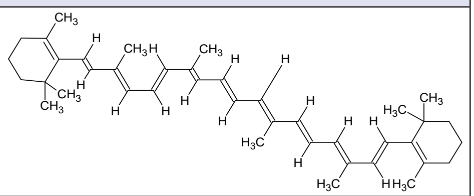
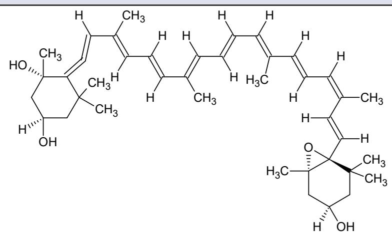
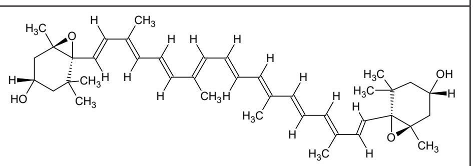
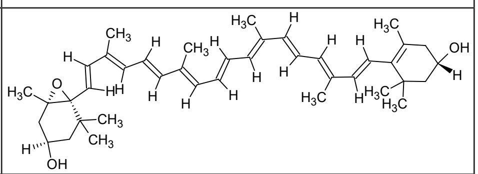
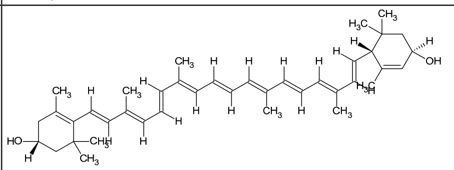
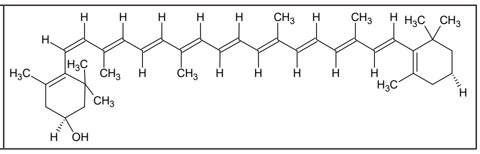
Izoflavones	
Daidzein	
Glicitin	
Genistein	
Genistin	
Flavan-3-ols	
Catechin	
Epicatechin	
Carotenes	
β-Carotene	
Xanthophylls	
Neoxanthin	
Violaxanthin	
Antheraxanthin	
Lutein	
Zeaxanthin	

Figure 2. Chemical structures of the main polyphenolic compounds found in chia seeds and sprouts

CHIA SPROUTS IN THE LIGHT OF THE LATEST SCIENTIFIC RESEARCH

According to the literature review performed, research conducted on chia sprouts and microgreens focuses on studying their phytochemical profile and determining antioxidant and antimicrobial activity.

The team of Bermejo *et al*. (Departament de Biologia Evolutiva, Ecologia e Ciències Ambientals/Spain) examined the chemical composition of chia sprouts. The study focused on determining the content of selected metabolites: β-carotene, neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll a and chlorophyll b using high-performance liquid chromatography (HPLC). For this purpose, samples were extracted with methanol. In addition, the effect of applying various plant growth and development regulators (PGRs) on increasing β-carotene production in chia sprouts was evaluated. The authors analyzed the composition of chia seeds before germination (day 0), soaked seeds maintained under dark conditions (day 2), seeds germinated in the dark (day 3), etiolated sprouts exposed to light for 30 min (day 6) and green sprouts exposed to light for 48 h (day 8). The researchers observed that the composition of *S. hispanica* sprouts changed during the developmental stages observed in the experiment. After qualitative analysis, neoxanthin, violaxanthin and antheraxanthin were not detected in the seeds on day 0. A significant increase in the content of these xanthophylls was observed on days 6 and 8 in sprouts exposed to light. On day 6, the content of neoxanthin was ca. 150 µg/g DW, the amount of violaxanthin was ca. 150 µg/g DW, and the content of antheraxanthin was ca. 30 µg/g DW. On day 8, the researchers observed an increase in neoxanthin content, was approximately 170 µg/g DW. Moreover they found a slight decrease in violaxanthin (150 µg/g DW) and antheraxanthin content (15 µg/g DW). In addition, the content of carotenoids and chlorophyll was negligible in the seeds before germination. On days 6 and 8, carotenoid and chlorophyll levels were elevated in sprouts exposed to light. On day 6, the chlorophyll a content in the sprouts was ca. 400 µg/g DW, while the chlorophyll b content was ca. 170 µg/g DW The highest increase in chlorophyll content was found on day 8, this time the level of chlorophyll a was ca. 1200 µg/g DW, and chlorophyll b was ca. 500 µg/g DW The level of β-carotene on days 6 and 8 remained at the same level and was ca. 100 µg/g DW Light increased the production of the main carotenoids found in *S. hispanica* sprouts. In general, chlorophyll a was the quantitatively dominant chlorophyll in *S. hispanica* sprouts, while lutein was the dominant carotenoid. In addition, the study evaluated the effect of applying different PGRs and stress agents to assess the increase in β-carotene production. Several treatments were used, including the addition of abscisic acid (ABA), methyl jasmonate (MeJa), methyl salicylate (MeSa), Promalin® (P), and combinations of P+ABA, P+MeJa and P+MeSa. Promalin® is a commercial product containing 1.9% gibberellins – GA4 and GA7 and 1.9% cytokinins 6-benzyladenine – BA (w/v). A concentration of 100 μM of each PGRs was used. Application of MeSa at a concentration of 100 μM resulted in an increase in β-carotene content (by 235%) compared to the control (244.54 mg/g DW *vs* 104.07 mg/g DW). In addition, application of MeSA caused a significant increase in antheraxanthin content (29.88 μg/g DW *vs*. 15.49 μg/g DW). In contrast, ABA application caused a significant decrease in the content (84.7%) of β-carotene compared to the control. After the application of ABA, the content of neoxanthin in *S. hispanica* sprouts decreased compared to that in the control sample (36.87 μg/g DW *vs*. 152.22 μg/g DW). The study proved that exogenous supply of MeSa can promote an increase in β-carotene content, so this PGR can be used for large-scale in chia seed biofortification. In addition, the study also proved that under 48 hours of light exposure (direct light from fluorescent lamps together with indirect sunlight in the laboratory, PAR 270 $\mu mol/m^2/s$), the increase in β-carotene content was accompanied by an increase in anteraaxanthin, which is a carotenoid with high antioxidant potential (Table 2) [18].

In their latest study, the same team expanded their research and determined the chemical composition of seeds (dry and sprouted) and chia sprouts profiling them to increase the content of vitamins with antioxidant potential. To improve the vitamin E and vitamin C content of chia sprouts, they tested the use of different substances with the same regimen as in the previous study. To evaluate the antioxidant vitamin content of dry and sprouted seeds and chia sprouts, five samples were taken at different developmental stages: before germination (day 0), soaked seeds kept in dark conditions (day 2), seeds germinated in the dark (day 3), etiolated sprouts exposed to light for 30 min (day 6) and green sprouts exposed to a prolonged light stimulus for 48 h (day 8). The germination process began on day 0 and ended on day 8, when the chia sprouts reached the optimal developmental stage and were ready for consumption. To evaluate the influence on the antioxidant and vitamin content of chia sprouts, the addition of abscisic acid (ABA), methyl jasmonate (MeJA), methyl salicylate (MeSA), Promalin® (P), P + ABA, P + MeJA, P + MeSA and P + ABA + MeJA + MeSA, among others, were tested. In order to analyze the content of vitamin E and C, spectrophotometric assays were performed which showed that the process of sprouting chia seeds caused a 5-fold decrease in vitamin E content compared to dry seeds (23 μg/g DW *vs*. 120 μg/g DW). Vitamin E was mainly composed of; 95.5% γ-tocopherol, the content of which, along with δ-tocopherol, gradually decreased over the duration of germination. It was observed that α-tocopherol content grew as germination time increased, with the highest value found in green sprouts. However, these were still insignificant amounts, compared to the γ-tocopherol content of the seeds. After 2 days, the vitamin C content of the sprouted seeds (76.8 μg/g DW) was 5 times higher compared to dry seeds (14.2 mg/100 g DW) and as much as 17.5 times higher compared to chia sprouts (4.3 mg/100 g DW). The highest percentage of vitamin C was found on the 3rd day of germination. After the application of PGRs, an increase in vitamin E production was observed in chia sprouts, especially after the application of ABA and the combination of P + ABA + MeJA + MeSA and P + ABA. The highest efficiency in activating vitamin E synthesis was found after the application of ABA, under the influence of which the vitamin E content of chia sprouts increased 3 times. An increase in production was found for γ-tocopherol and δ-tocopherol, but no positive effect was noted for α-tocopherol. The study proved that the application of PGRs enhanced the nutritional value of the sprouts by increasing vitamin E content. In addition, it was found that germination process significantly contributed to an increase in vitamin C content (Table 2) [33].

The team of Gómez Velázquez *et al*. (Centro Universitario de Los Lagos/Mexico) examined the effects of chemical elicitation (induced by salicylic acid (SA) and hydrogen peroxide (H_2O_2)) applied to chia sprouts on the total content of phenolic compounds and flavonoids and their antioxidant potential in *in vitro* conditions. In addition, researchers evaluated their antioxidant capacity in serum and urine samples of rats with induced obesity. During the study, the rats were fed a high-fat, high-fructose diet (HFFD), which was supplemented with additional elicited and non-elicited chia sprouts. Analysis of the elicited chia sprouts showed that there was an increase in total phenolic compounds (TPC) (1.5-fold), total flavonoid content (TFC) (2-fold) and induced inhibition of DPPH (1-(2, 6-dimethylphenoxy)-2-(3, 4-dimethoxyphenylethylamino propane hydrochloride) (1.5-fold). Application of SA (at concentrations of 0.1 and 1 mM) and treatments with H_2O_2 (at concentrations of 10 and 20 mM) increased TFC by 37-40% compared to non-elicited chia sprouts. For TFC, the highest values were obtained with H_2O_2 (10 mM), followed by SA (0.1 and 1 mM). Elicitation contributed to the enhanced antioxidant capacity of chia sprouts. Treatment of sprouts with high concentrations of SA (1 and 2 mM) and all concentrations of H_2O_2 (10, 20, 30 mM), resulted in the highest values for the DPPH assay, which increased significantly (82-86%) compared to non-elicited sprouts. On the other hand, the use of 1 mM SA resulted in the highest values (61%) for the ABTS test (using 2,2'-azobis(3-ethylbenzothiazoline-6-sulfonate), which were higher compared to 2 mM SA (38%) and all analyzed H_2O_2 concentrations (29-37%) compared to non-elicited sprouts. To evaluate the antioxidant effects of sprouts in obese rats, chia sprouts treated with SA at 1 mM and H_2O_2 at 20 mM were selected. Dietary supplementation of HFFD with *S. hispanica* sprouts treated with SA at a concentration of 1 mM resulted in an increase in antioxidant capacity and a decrease in oxidative stress in rat serum. The DPPH test result for rats fed a standard diet without the addition of sprouts showed that the antioxidant capacity (mmol TX trolox equivalent/L) was 1.8 mmol TX/L, while the addition of SA elicited sprouts to the diet at a concentration of 1 mM caused an increase to 2.4 mmol TX/L. The result for the ABTS test in the serum of rats fed the HFFD diet was 9.7 mmol TX/L, while the diet enriched with SA elicited chia sprouts at a concentration of 1 mM was 12.6 mmol TX/L. The study proved that compared to non-elicited sprouts, SA (1 mM) elicited chia sprouts conditioned increased phenolic compounds and antioxidant capacity and improved obesity-related oxidative stress in the serum of rats (Table 2) [88].

The team of Calvo-Lerma *et al*. (Instituto de Ingeniería de Alimentos para el Desarrollo/Italy) characterized the chemical composition of chia sprouts and analyzed the digestibility of proteins, lipids, and performed an analysis of polyphenolic compounds and calcium bioavailability in dry and sprouted chia seeds using an *in vitro* digestion model simulating the different stages of food digestion. The pre-digestion assays found that chia sprouts contained more of the following compared to seeds: proteins – 0.229 *vs*. 0.201 g/g DW, carbohydrates – 0.644 *vs*. 0.472 g/g DW Compared to dry seeds, chia sprouts are also more abundant in calcium (7.26 *vs*. 6.46 mg/g DW). The polyphenol content is also higher in sprouts than in seeds (2.87 *vs*. 1.78 mg gallic acid equivalent (GA eq.)/g DW). However, they contain less lipids compared to seeds: 0.097 *vs*. 0.325 g/g DW. The researchers also determined total antioxidant activity using the DPPH spectrophotometric assay. They found higher activity for sprout extracts compared to seeds (5.69 *vs*. 3.49 mg trolox equivalent (TX eq.)/g DW). The above parameters were also determined in the bioavailable fraction after *in vitro* digestion under standard and modified intestinal conditions. The modified intestinal conditions were simulated at an intestinal pH of 6 and a bile salt concentration of 1 mM, while standard digestion was simulated at an intestinal pH of 7 and a bile salt concentration of 10 mM. It was found that the bioavailability of calcium significantly decreased under all conditions tested. Under standard intestinal conditions, the calcium concentration in chia sprouts was 0.15 mg/g DW, while under altered conditions it was 0.32 mg/g DW.

Table 2. The effect of the germination process and the use of various factors influencing the chemical composition, antioxidant and antimicrobial activity of chia sprouts – review of studies comparing the sprout and seed extracts

Indicated ingredients	Influencing factors	Differences/Results	References
β-carotene, neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll a, chlorophyll b	germination process, light conditions (brightness/darkness)	• germination process and exposure to light increased the content of all xanthophylls in sprouted seeds compared to dry chia seeds before germination • light increased the production of the main carotenoids and chlorophylls in *S. hispanica* sprouts	[18]
vitamin C, vitamin E	germination process, light conditions (brightness/darkness), PGRs: ABA, MeJA, MeSA, Promalin® (P), P + ABA, P + MeJA, P + MeSA and P + ABA + MeJA + MeSA	• germination process contributed to increase in vitamin C content in sprouted seeds • application of PGRs resulted in increase in vitamin E content, especially after the application of ABA and the combination of P + ABA + MeJA + MeSA and P + ABA	[33]
TPC, TFC, antioxidant activity in *in vitro* conditions.	SA and H_2O_2	• elicitation results in increase of TPC and TFC values in elicited sprouts compared to chia seeds • for TFC, the highest values were obtained after H_2O_2 (10 mM), followed by SA (0.1 and 1 mM) • elicitation contributed to the antioxidant capacity of chia sprouts and resulted in the highest inhibition values in the DPPH assay compared to non-elicited sprouts	[88]
proteins, carbohydrates, lipids, calcium, antioxidant activity	*in vitro* digestion model simulating the different stages of food digestion	• chia sprouts contain more proteins, carbohydrates, calcium, polyphenols and less lipids compared to dry seeds • total antioxidant activity was higher for sprout extracts compared to dry chia seeds extract • the simulated digestion process led to an increase in the bioavailability of polyphenols, both in the seeds and chia sprouts • digestion under standard intestinal conditions resulted in higher extraction of polyphenols than under altered conditions	[48]
proteins, amino acids dietary fiber (water soluble and water insoluble fraction), fatty acids	different temperature conditions and germination time	• germination under optimized conditions (temperature: 21°C, germination time: 157 h) result in a significant increase in protein and amino acids content compared to dry seeds • the content of fatty acids decreased in the seeds after the germination process • germination process positively increased the content of dietary fiber of the water-insoluble fraction and caused a decrease in the content of water-soluble fraction	[47]
TPC, TFC, antioxidant and antimicrobial activity	lack of influencig factors/ no influencing factors	• TPC increased during germination, reaching the highest value in 7-day-old sprouts, after that time TPC was decreased • the quantitatively dominant phenolic acids in the chia sprout and seeds extracts were protocatechuic acid, rosmarinic acid and caffeic acid • catechin was determined to be particularly high both in the dry seed and sprout extracts • the increase in germination duration resulted in higher IC_{50} values for DPPH and ABTS assays • antimicrobial activity was the strongest for 7-day-old sprout extracts in terms of zones of inhibition and minimum growth inhibitory concentration against all bacterial strains tested compared to chia seed extract • chia sprouts extract exhibited the highest antimicrobial activity against *E. coli* and *S. aureus* strains compared to the dry chia seed extracts	[79]
accelerate germination	different light conditions (continuous light, continuous darkness, and alternating light/dark (8 hours light/ 16 hours dark)) and temperature ranges (constant temperatures: 20, 25, 30 and 35°C and two variable ones, in ranges: 20-30 and 25-30°C for 8 and 16 hours)	• reduced percentage of germination in constant darkness and alternating light/darkness combined with constant temperature conditions • chia seeds were found to be indifferent to varying light conditions, as germination occurred after both light and dark stimulation	[89]
protein, lipids, dietary fiber, vitamin C, TPC, TFC	lack of indicated influencing factors	• germination process alters the nutrient content and antioxidant activity of sprouted chia seeds • as germination time increased, the protein content decreased • the highest content of lipids was found at 48 h of germination, and similarly, in subsequent hours of the germination process their content decreased • germination process increase TPC, TFC, amino acid, dietary fiber and vitamin C content in sprouted seeds compared to dry chia seeds	[90]

The calcium content of the seeds was higher compared to the sprouts and was 3.82 mg/g DW under standard conditions, while it was 3.77 mg/g DW under modified conditions. The simulated digestion process led to an increase in the bioavailability of polyphenols, both in the seeds and chia sprouts. For chia sprouts under standard intestinal conditions, the polyphenol content was 4.41 mg GA eq./g DW while under modified conditions it was 3.55 mg GA eq./g DW. Under standard conditions for chia seeds, the polyphenol content was 1.81 mg GA eq./g DW and under modified conditions it was 1.51 mg GA eq./g DW. Digestion under standard intestinal conditions resulted in higher extraction of polyphenols than under altered conditions. The antioxidant activity of the extracts after digestion decreased more under changed conditions than under standard conditions. Regarding sprouts under standard conditions, antioxidant activity was 4.21 mg TX eq./g DW), while under modified conditions it was much lower at 2.23 mg TX eq./g DW In chia seeds, the total antioxidant activity under standard conditions was 1.17 mg TX eq./g DW, and 1.03 mg TX eq./g DW under altered conditions. The researchers found that sprout digestion significantly enhanced proteolysis, but prevented lipolysis, thus sprout consumption conditions improved protein digestibility (Table 2) [48].

Gómez-Favela *et al*. (Universidad Autónoma de Sinaloa/Mexico) compared the content of protein, fatty acids, phenolic compounds between dry and germinated seeds using a combination of different temperature conditions and germination time to demonstrate that the germination process increases the content of bioactive compounds. Predictive models showed that a significant increase in protein content (20.89%) was observed after germination under optimized conditions (temperature: 21°C, germination time: 157h) compared to dry seeds. An inverse relationship was found for fatty acids, the content of which significantly decreased in the seeds after the germination process (by 55.31%). In addition, the germination process positively increased the content of dietary fiber of the water-insoluble fraction by 5.14%, while it caused a decrease in the content of water-soluble dietary fiber by 13.53%. The researchers also found that the germination process contributed to a significant increase in essential amino acids. Also, the content of phenolic compounds was increased in germinated seeds compared to dry seeds. The germination process increased free (+77.20%), bound (+22.06%) and total (+47.40%) phenolic content. The study proved that proper optimization of germination conditions is an effective strategy to increase protein content and polyphenolic compounds (Table 2) [47].

Abdel-Aty *et al*. (National Research Center/Egypt) evaluated the effect of the germination process of *S. hispanica* seeds on the total phenolic and flavonoid content, and on their antioxidant and antimicrobial properties. Phenolic compounds were analyzed using the HPLC method. DPPH and ABTS assays were used to estimate the antioxidant activity of the extracts. Antimicrobial activity was evaluated on three Gram-negative bacterial strains (*Escherichia coli* O157-H7 ATCC 51659, *Salmonella typhi* ATCC 15566 and *Pseudomonas aeruginosa* NRRL B-272) and one Gram-positive (*Staphylococcus aureus* ATCC 13565). The total phenolic and flavonoid content of dry chia seeds (1.41 mg GA eq./g DW and 0.20 mg catechin equivalent (CE eq.)/g DW respectively)) increased gradually during germination, reaching the highest value in 7-day-old sprouts (9.0 mg GA eq./g DW and 2.3 CE eq./g DW). These contents were respectively 6.4 and 11.5 times higher, compared to dry seeds. However, in the following days, ending the 10th day of germination, the content of phenols and flavonoids significantly decreased (6.8 mg GA eq./g DW and 1.3 mg CE eq./g DW). The total percentage content of flavonoids and phenols increased from 14.2% in dry seeds to 25.6% in 7-day-old sprouts. In addition, 12 phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, vanillic acid, ferulic acid, synapinic acid, *p*-coumaric acid, rosmarinic acid, cinnamic acid) and 4 flavonoids (3-flavanols: catechin, flavonols: kaempferol, quercetin, and flavones: apigenin) in concentrations ranging from 0.004 to 0.32 mg/g DW were identified. The quantitatively dominant phenolic acids were rosmarinic acid (0.320 mg/g DW), caffeic acid (0.280 mg/g DW) and protocatechuic acid (0.170 mg/g DW). On the other hand, 12 phenolic acids and 5 flavonoids were identified in the extract of 7-day-old chia sprouts, with concentrations ranging from 0.06 to 0.80 mg/g DW. In these extracts, *p*-coumaric acid and kaempferol were additionally identified, at concentrations of 0.24 and 0.16 mg/g DW, respectively. The quantitatively dominant phenolic acids in the chia sprout extracts were protocatechuic acid (0.80 mg/g DW), rosmarinic acid (0.60 mg/g DW) and caffeic acid (0.50 mg/g DW). In addition, catechin (0.140 mg/g DW *vs*. 0.45 mg/g DW) was determined to be particularly high in the dry seed and sprout extracts. Determinations of antioxidant potential by DPPH and ABTS assays showed low IC_{50} values for chia sprout extracts indicating their high antioxidant activity. The IC_{50} for dry seed extracts were 0.0216 and 0.012 mg GA eq./mL, and for 7-day chia sprout extracts were 0.0138 and 0.0045 mg GA eq./mL, for DPPH and ABTS assays, respectively. The increase in germination duration resulted in higher IC_{50} values for DPPH and ABTS assays, which increased to 0.0147 and 0.0061 mg GA eq./mL, respectively, at day 10 of germination. Analysis of the antimicrobial properties of dry chia seed extract and 7-day chia sprout extracts was conducted against four bacterial strains (*S. aureus*, *P. aeruginosa*, *E. coli* and *S. typhi*). Antimicrobial activity testing showed the strongest activity for 7-day-old sprout extracts in terms of zones of inhibition and minimum growth inhibitory concentration (range of values 0.40-0.65 mg/ml) against all bacterial strains tested compared to chia seed extract. Antimicrobial activity with zones of inhibition for 7-day-old chia sprouts was in the range of 15-23 mm, while in the dry seed extracts the range of inhibition against bacteria was 3-5 mm. The 7-day-old chia sprouts had high sensitivity to all tested bacterial strains (*S. aureus*, *P. aeruginosa*, *E. coli*, *S. typhi*). The chia sprout extracts showed the highest antimicrobial activity against *E. coli* and *S. aureus* bacterial strains and had higher values in terms of inhibition zones and minimum inhibitory concentration (3.5 and 4.0 mg/ml, respectively) compared to the dry chia seed extracts (Table 2) [79].

Pereira de Paiva *et al*. (Universidade Federal Rural do Semi-Árido/Brazil) conducted a study on the effect of different light conditions and temperature ranges on *S. hispanica* seed germination. They tested different types of light conditions: continuous light, continuous darkness, and alternating light/dark (8 hours light/16 hours dark). In addition, they tested the effect of temperature – constant temperatures: 20, 25, 30 and 35°C and two variable ones, in ranges: 20-30 and 25-30°C for 8 and 16 hours. The germination percentage and average seed germination time were evaluated. The progress of the germination process at constant temperatures was comparable to the variable temperatures. The researchers observed a reduced percentage of germination in constant darkness and alternating light/darkness combined with constant temperature conditions. *S. hispanica* seeds were found to be indifferent to varying light conditions, as germination occurred after both light and dark conditions, but the germination process was more efficient in the light. The highest average germination times were obtained under conditions of constant darkness regardless of temperature, ranging from 80 to 103 hours. On the other hand, under constant light and variable light/dark conditions, the shortest average germination time was obtained at constant 25 and 30°C and was respectively 68 and 62 hours, for variable temperature (25-30°C) it was 73.5 hours (Table 2) [89].

Beltran-Orozco *et al*. [2020] (Instituto Politécnico Nacional/Mexico) evaluated the nutritional composition of chia seeds and the effect of the germination process on the content of protein, lipids, dietary fiber, vitamin C, phenolic compounds and total flavonoids, as well as on protein digestibility and antioxidant activity. Studies have proven that the germination process alters the nutrient content of sprouted chia seeds. The initial percentage of protein in the dry seeds was 20.64% (20.66 g/100 g DW), while under germination, during the first 48 hours, the protein content of the germinated seeds increased by 13% (23.24 g/100 g). As germination time increased, the protein content decreased (72 h of germination – 22.16 g/100 g DW, 96 h of germination – 21.24 g/100 g DW). The highest content of lipids was found at 48 h of germination and reached 42.16 g/100 g DW, and similarly, in subsequent hours of the germination process their content decreased, successively at 72 h – 38.44 g/100 g DW, at 96 h – 30.98 g/100 g DW. Analyses showed a relatively low content of dietary fiber in dry chia seeds (16.6%), but germination promoted an increase in its content by about 46% after 4 days of germination. The highest content of dietary fiber compared to dry seeds was obtained after 96 h of germination (16.60 *vs*. 24.25 g/100 g DW). Analysis of changes in the content of vitamin C showed that it was undetectable in the extracts from the dry chia seeds, while the germination process from the second day resulted in an increase in its content and its amount steadily increased for the next 2 days. The content of vitamin C on the second day was 0.43 g/100 g DW, on the 3rd day it was 1.24 g/100 g DW, while on the 4th day it was 2.33 g/100 g DW. Various scientific studies show that seed germination induces an increase in amino acid content, but chia protein is low in tryptophan, which reduces its quality, so the researchers in this study also evaluated the effect of the germination process on tryptophan content. They obtained satisfactory conclusions. Germination caused an increase in tryptophan content by about 100% after 4 days of germination (day 0-2.51 g/100 g DW *vs*. day 4-4.84 g/100 g DW). Dry and germinated seeds were also evaluated considering protein digestibility. Protein digestibility decreased as germination progressed. The total amount of phenolic compounds in dry seeds was 97.7 mg GA eq./100 g DW, while after 4 days of germination this value increased 3 times (293.6 mg GA eq./100 g DW). The total flavonoid content of the dry seed extracts was 35.8 mg quercetin equivalents (QE eq.)/100 g DW while on day 4 it was 106.0 mg QE eq./100 g DW The antioxidant activity (μmol TX eq./100 g DW) of dry seed extracts determined by ABTS, DPPH and FRAP methods was 77.7, 41.1 and 72.3 μmol TX eq./100 g DW respectively. and as the germination process progressed, it increased by 105.1%, 101.6% and 87.7%, respectively, after 4 days of germination. In conclusion, the study proved that the germination process can be a beneficial method to promote the nutritional and nutraceutical values of chia seeds (Table 2) [90].

CONCLUSIONS

As the food industry sector continues to expand, food manufacturers are increasingly turning to innovative nutraceutical plant raw materials [91,92]. Plant-based diet therapy plays an important role in maintaining good health, so the use of functional foods is gaining popularity, and chia seeds, sprouts and *microgreens* are classified as modern , "healthy food" [93,94]. Chia seeds have been the subject of many scientific studies that have proven their broad spectrum of health-promoting effects on the body, exhibiting among others antioxidant, anti-inflammatory, anti-diabetic, hypolipemic and hypotensive effects. On the other hand, chia sprouts and *microgreens* are a newly discovered plant material that has not yet been fully explored, so all its health-promoting and medicinal properties are not known. *Microgreens* of cultivated plants are becoming an innovative part of the diet, moreover, scientific research proves that they not only affect favorably the sensory changes of the product, but also have an interesting bioactive and phytonutritional profile. So far, published scientific studies analyzing chia *microgreens* concern lipid profiling. Researchers are analyzing lipids extracted from chia microgreens and other common oilseeds (soy, flax, sunflower and canola) [95,96].

This review focuses on comparing chia seeds and sprouts from a nutritional and phytochemical perspective, as well as demonstrating their potential health-promoting effects. The paper reviews the literature on the germination process and chia sprouts, which are a novel plant material that has not yet been commercialized in the food market. Nowadays, the few scientific studies conducted on chia sprouts prove that both sprouted seeds and chia sprouts are classified as nutritionally interesting raw material as dry seeds. Seeds and chia sprouts are valuable sources of bioactive compounds, especially phenolic acids and flavonoids, but chia sprouts quantitatively contain more phenolic acids, including chlorogenic acid, ferulic acid, gallic acid, caffeic acid, protocatechuic acid, *p*-hydroxybenzoic acid and rosmarinic acid. The quantitatively dominant phenolic acids in chia seeds are rosmarinic acid and chlorogenic acid, while in sprouts are protocatechuic acid and rosmarinic acid. The improvement in the nutritional properties of chia sprouts is determined by the germination process, which is a method that allows the sprouts to increase their content of phenolic compounds, flavonoids and vitamins and minerals. In addition, germination increases the antioxidant and antimicrobial potential of sprouted chia seeds. So far, studies conducted on chia sprouts have provided satisfactory results, demonstrating that chia sprouts, like seeds, exhibit antioxidant activity. In addition, single studies also prove the antimicrobial properties of chia sprout extracts against Gram-negative (*E. coli*, *S. typhi*, *P. aeruginosa*) and Gram-positive (*S. aureus*) bacterial strains. Germination is an inexpensive and effective method that, in a short period of time, enables to obtain a raw material with more favorable nutritional properties compared to the standard raw material (seeds). Currently, various types of crop plant sprouts (including broccoli, buckwheat, alfalfa) are widely available on the food market and are a nutritious element enriching the diet with nutrients valuable from the health point of view, but sprouted seeds and chia sprouts are nowadays considered as a novel raw material, which has recently been the subject of scientific research and is gradually gaining popularity. In conclusion, the results of scientific work on chia sprouts suggest that they may be a potential plant raw material that can be produced on a large scale. Although chia sprouts are not very popular at present, their use in industrialized countries will undoubtedly increase in the immediate future.

AUTHOR CONTRIBUTIONS

Data collection, design of the study, analysis and interpretation of the data, drafting the manuscript, S.M. and A.S.; critical revision of the manuscript, S.M., H.E. and A.S. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ETHICAL STANDARDS

Not applicable.

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