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Research Article

The Effect of Germination Process on the Characteristics of Chia (*Salvia hispanica* L.) Seeds

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Abstract:

In recent years, the usage of chia (*Salvia hispanica* L.) seeds has increased due to their high nutritional and medicinal value. This study was performed to shed some light on the effect of the germination process on the physicochemical characteristics, nutritional value and phytochemicals of the seedling. The effect of crowding of chia seeds during planting was also studied. The results display that the degree of crowding of seeds during germination for 5 days had no actual effect until crowding of 100 seeds/250 ml, as the degree of seed germination was affected after that by the degree of crowding. As for physical properties, chia sprouts had higher volume (7.54 cm^3), weight (2.69 g) and true density (2.80 g/cm^3), but smaller bulk density (0.34 g/ml) than those of seeds. The chia sprouts also contain higher moisture (13.18%), proteins (25.13%), ash (5.88%) and dietary fibers (21.17%) than those of the raw seeds. On the other hand, they contain lower crude fat (22.65%), nitrogen-free extract (12.04%) and total carbohydrates (33.24%). It is interesting to note that the biomass of the chia sprouts can provide the human body with different secondary metabolites and nutrients with low calories (352.48 Kcal/100 g) compared with the chia seeds.

1. Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous plant, originally from Southern Mexico and Northern Guatemala. It belongs to the order Lamiales, mint family Labiate, subfamily Nepetoideae, and genus Salvia (Ciau-Solís et al., 2014). Chia can grow up to 1.0 m tall and has oppositely arranged leaves. Chia flowers are small (3-4 mm) with tiny corolla and fused flower parts that contribute to the high self-pollination rate. Its seed's color varies from black, grey, and black spotted to white, and the shape is oval with sizes ranging from 1 to 2 mm (Bresson et al., 2009; Cahill, 2003). The seeds contain some active substances that have anti-inflammatory, antithrombotic, and antitumor properties (Ali et al., 2012; Jeong et al., 2010). The consumption of chia seeds could decrease cholesterolemia (Ayerza and Coates, 2005), modulate glycemic and insulinemic responses (Chicco et al., 2009), improve intestinal function, play an important role in protecting the body from cardiovascular diseases (Vuksan et al., 2008).
The germination of edible seeds and grains is an old habit and ideal method followed by the ancient Egyptians to transform hard seeds into soft ones (Abdallah, 2008). This process improves nutritional value, reducing anti-nutritional factors and accumulating bioactive compounds to prevent chronic diseases (Ijarotimi and Keshinro, 2013). The sprouts are normally produced by soaking, draining, and leaving seeds until they germinate without the use of chemical fertilizers or pesticides (Oates, 2014). As a result, the functional properties of the seeds increase (Ijarotimi and Keshinro, 2013) including vitamins, minerals, proteins, and enzymes.
Despite the benefits of sprout chia seeds, there is little literature about the chemical composition and nutritional value of the sprouts as a functional food. Therefore, this work is performed to accumulate some information about the changes in the nutritional value of dry seeds compared with their sprouts.

2. Materials and methods

Materials

Chia seeds (*Salvia hispanica* L.) were supplied from the Agriculture Research Centre, Toukh, Qalyubia Governorate Egypt during the 2021 season.
All chemicals used in this work were analytical grade purchased from El-Gomhouria Pharmaceutical Company and El-Nasr Pharmaceutical Chemical Company, Egypt.

Methods description

2.1. Determination of physical characteristics of Chia seeds and sprouts

Physical properties of chia seed including the volume (cm^3) of dry and wetted seeds using a 100 ml-gradual cylinder, weight (g) using digital accurate balance and density (g/cm^3) were calculated according to Cresswell and Hamilton, (2002)=and El-Raie et al. (2004) as follows:

Actual density (g/cm^{-3}) = (Volume of 1000 seeds (cm^3)/(Mass of 1000 seeds (g)

Bulk density (g/cm^3) = (Mass of 1000 seeds (g)/ (Volume of 1000 seeds (ml)

Bulk density is defined as weight of grains per unit volume, often expressed as g/ml, and is a good index of structural changes (Sreerama et al., 2009)

2.2. Calculation of germinated seeds crowding and determination of shoot and root criteria

Chia seeds were washed with tap water followed by distilled water. Then they were sterilized in 0.7% (w/v) sodium hypochlorite solution for 30 min at room temperature (Pasko et al., 2009). The sterilized seeds were germinated in plastic pots (having 250 cm^3) in crowding patterns 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 700 and 1000/pot which they moistened with distilled water whenever needed. The germinated seeds were calculated after 5 days of planting using standard procedures described in AOSA (1983). Growth criteria (e.g. weight in mg, length in cm for both shoot and root) of chia sprouts were determined. Chia sprouts were dried in an electric oven at 70°C. The dried sprouts were ground with an electric mill (Braun grinder, model Ksm2, Germany), sieved through 0.45 mm sieves, packaged in paper bags, and stored till further analysis.

2.3. Determination of chemical composition

Moisture content using an electric oven at 105±5°C (method no. 934.01), total nitrogen following Kjeldahl method (method no. 950.36), ether extract (method no. 935.38) using petroleum ether (60-80°C) in a Soxhlet apparatus, ash content in a muffle furnace at 450-500°C (method no. 930.22), and crude fiber (method no. 950.37) were performed as described in AOAC (2005). Total carbohydrates and nitrogen-free extract (NFE) (available carbohydrates) were calculated (as dry weight bases) according to Aurand (2013) using the following equations:

Total carbohydrates (TC% dry bases) = 100 – (Protein% + Lipid% + Ash%)

Nitrogen free extract (NFE%) = Total carbohydrates % - Crude fiber %

Calculation of energy value was carried out using the Atwater factor of 4.0 Cal/g for both protein and carbohydrate while it was 9.0 Cal/g for fat (FAO/ WHO, 2002).

2.4. Determination of some phytochemical constituents

Chlorophyll a, chlorophyll b, and carotenoids of fresh chia sprout were determined according to the method of Metzner et al. (1965). While total phenolic content in both chia seed and sprout was quantitatively determined as described by Jindal and Singh (1975). Determination of flavonoids was determined according to the modified method by Chang et al. (2002) Saponin was estimated according to the method described by Hiai *et al.* (1975). Total alkaloids were measured quantitatively according to the method described by Harbone (1973). Vitamin C was titrimetrically determined using sodium salt of 2, 6 dichlorophenolindophenol as an internal indicator according to (method no 967.21) AOAC (2005).

2.5. Statistical Analysis

Data are represented as the mean and standard error ± (M±SE) of successful three trials calculated by Microsoft Excel. Analysis of Variance (ANOVA) was applied to evaluate the significance of variances due to the used treatments and the differences between means were further tested using the Dunce's Squire Multiple Range Test (Steel and Torrie, 1980).

3. Results and discussion

Physical properties of chia seeds and sprouts

Data in Table (1) shows that the biomass of 1.0 g chia seed is significantly increase ($p<0.05$) reaching to 2.69 g for fresh sprouts. The weight, volume, actual and bulk density of 1000 chia seeds are 1.47 g, 2.02 cm^3, 1.36 g/cm^{-3} and 0.70 g/ml, respectively. These results are in close to the results of Mohammed et al. (2019) who found that the mean weight of 1000 chia seeds varied from 1.1855 to 1.2043 g and bulk density from 0.598 to 0.718 g/cm^3. These values are amplified for sprout except the bulk density (0.34 g/ml) which is significantly lower ($p<0.05$) than that of the seed (0.70 g/ml). This result could be explained by the large size of chia sprouts leading to the presence of higher interspaces between its units providing higher volumes that are reflected in the weight gain. On the other hand, the interspaces between chia seeds are small, which negatively affects the size of 1000 seeds.

Table (1): Physical characteristics of chia (*Salvia hispanica* L.) seeds.

Property	Chia seeds	Chia sprouts
Volume of 1000 seeds (cm^3)	2.02±0.00[b]	7.53±0.00[a]
Weight of 1000 seeds (g)	1.47±0.00[b]	2.68±0.01[a]
Actual density (g/cm^{-3})	1.37±0.00[b]	2.81±0.00[a]
Bulk density (g/ml)	0.72±0.00[a]	0.35±0.00[b]

Values are means ± standard error (M± SE) of successful three trials. In a row, means having the same superscript letters are not significantly different at 0.05% level (P>0.05).

Effect of crowding on the growing chia seeds:

The effect of crowding on the germination rates of chia seeds was studied in terms of weight (g) and length (cm) of both shoots and roots. The obtained results display that the germination ratio differed significantly ($p<0.05$), as the number of sprouts gradually decreased with increasing the number of planting seeds per area (Fig. 1). The number of seeds planted per area unit is significantly ($p<0.05$) influential to the point (ratio of 1000 seeds/unit area) that increasing one seed may markedly reduce the germination ratio at regression line is R2 (0.9655).

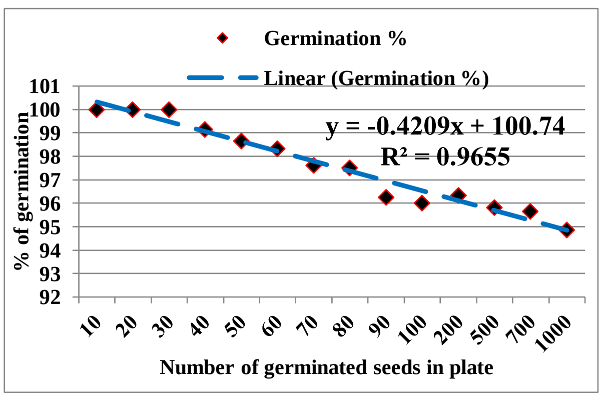

Fig. (1): Effect of number planting chia seeds/area unit on the germination ratio and its regression line

The weight of the roots and shoots of the seedling at the maximum number of seeds planted (1000 seeds/area) was 0.03% of the weight of the roots and the shoots growing at a crowding level of 10 seeds/area under the same growth conditions (Fig. 2). Therefore, planting seeds collectively at a ratio of more than 1000 seeds/unit area has a significant negative ($p<0.05$) effect on the growth and weight of sprouted shoots. The same trend is also observed for the length of shoots and germinating roots (Figs 4 and 5), where the length of sprouts (cm) at the maximum number of the seed (1000 seeds/studied area) is about 5 cm, while it is 6 cm for sprouts when 10 seeds grow under the same conditions.

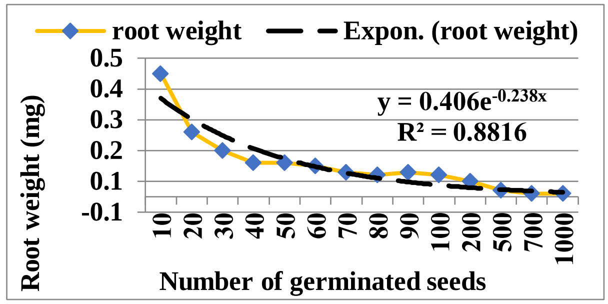

Fig. (2): The weight of the root of chia sprouts under different numbers of seeds/studied area and their regression line

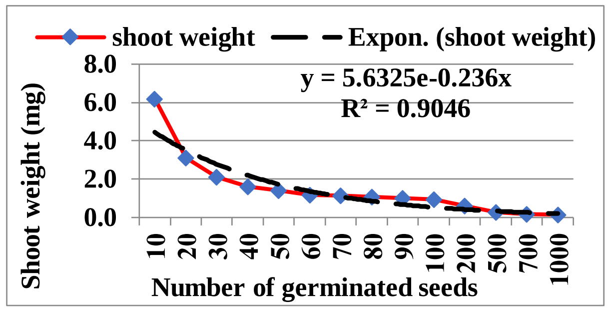

Fig. (3): The weight of shoot of chia sprouts under different numbers of seeds/studied area and their regression line

The length of the germinated sprout shoots and roots were significantly different ($p<0.05$). Since they progressively decreased but the decrement in sprout length was not as much as that in the sprout weight Fig. (4). The sprout shoot length at the maximum number of seeds (1000 seeds/studied area) is about 0.6 of those sprouts in the same area containing 10 seeds, while sprout root length at the maximum number of seeds (1000 seeds/studied area) is about 0.5 of those sprouts in same area containing 10 seeds.

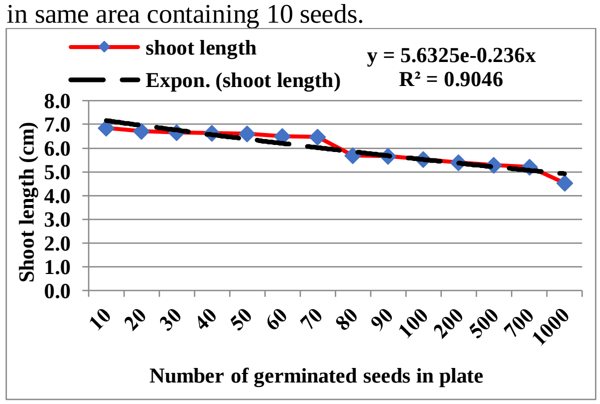

Fig. (4): Length (cm) shoot of chia sprouts under different numbers of seeds/studied area and their regression line.

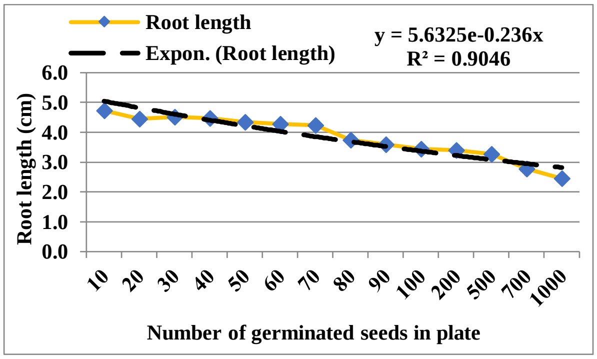

Fig. (5): Length (cm) root of chia sprouts under different numbers of seeds/studied area and their regression line.

These results are in the line with Abdul Raouf (2014), who stated the inhibitory effects of crowding germination ratio (number of seeds/ planting area) of alfalfa seeds had a remarkably negative effect on the number of sprouts and the length of both shoots and roots.

Chemical composition of chia seeds and sprout:

The chemical composition of chia seeds as affected by the germination process is displayed in Table (2). The germination process leads to improving the nutrient components of the sprouts.

Moisture content:

The sprouts of chia seeds have significantly ($p<0.05$) higher contents of moisture (13.18%) compared with the seeds (7.25%). Amankwah et al. (2009) reported that the removal of moisture increased concentrations of nutrients and can make some nutrients more available.

Crude protein:

The sprouts of chia seeds had significantly ($p<0.05$) higher protein (28.91%) compared with the seeds (22.70%). This is because germination is a biotechnological process that involves the activation of metabolic enzymes like proteinases. This process can release some amino acids and peptides and allow for the synthesis or use of additional amino acids and peptides to build new proteins. The germination process is recommended as a technological method for enhancing the nutritional quality of legumes and other seeds because it can improve the nutritional quality of proteins Sibian et al. (2017).

Ether extract:

The sprouts of chia seeds had significantly ($p<0.05$) lower ether extract (26.09%) compared with the raw seeds (35.75%). where the fat content decreases with an increase in the time of germination. This is because fat was used as the major source of carbon for seed growth (Bau et al., 1997). Hahm et al. (2008) also suggested that fatty acids are oxidized to carbon dioxide and water to generate energy for germination.

Ash:

The highest ash content (6.78%) was observed for germinated chia flour and the lowest (4.50%) was for chia seed flour. The observed decrease in the ash content of chia seed flour might be due to the leaching of minerals during steeping and washing (Ahmadzadeh and Prakash, 2007). The chia sprout has 13.82% of fibers higher than the raw one. Also, the NFE content of seeds is higher by (15.59%) than in sprouts.

Carbohydrates:

The carbohydrate contents of germinated chia flour are 38.21%. The lowest carbohydrate was reported for chia flour (37.04%). Numerous variables, including the temperature, the process of hydrating dried seed, the amount of oxygen and other ingredients in the steep media as well as other conditions, can affect how seeds germinate and affect their carbohydrate components. These elements have a significant ($p<0.05$) impact on respiration and the synthesis or breakdown of seed carbohydrates. The nutrient's utilization as a readily available energy source during sprouting may be the cause of the sprouted chia flour's lower carbohydrate content (Atlaw and Kumar, 2018) on the other side increase in protein and other nutrients. So, the nutritional value of the chia sprouts is enhanced and improving its nutritional quality. These results are in full agreement with the results obtained by Sibian et al. (2017).

Energy

The total energy of chia sprouts is (405.80 Kcal/100 g) which is lower than that of seed (474.95 Kcal/100 g). Thus, sprouts can provide the human body with different nutrients with lower calories.
This data agrees with Ixtaina et al. (2008) who reported chia seed is an underutilized pseudocereal with a high amount of nutrients that helps to improve high-density lipoprotein (HDL) in humans and protects from heart attack and stroke.

Table (2): Effect of the germination process for 5 days on the chemical composition of chia seeds and their sprouts (g/100 g dry weight bases)

Constitutes	Chia Seeds	Chia Sprouts
Moisture (%)	7.25±0.08b	13.18±0.01a
Crude Protein (%)*	22.70±0.02b	28.91±0.03a
Ether extract (%)*	35.75±0.05a	26.09±0.09b
Ash (%)*	4.50±0.04b	6.78±0.02a
Crude fiber (%)*	21.46±0.10b	24.39±0.04a
Nitrogen-free extract (NFE) (%)*	15.59±0.12a	13.82±0.04b
Total carbohydrate (%)	37.04±0.04b	38.21±0.08a
Total energy (Kcal/100 g)	474.95±0.21a	405.80±0.61b

Values are mean ± standard error (M±SE) of successful three trials. *: On dry weight basis
In a row, means having the same superscript letters are not significantly different at 0.05% level (p>0.05).

Phytochemicals content of chia seeds as affected by the germination process:

The total chlorophyll and its fractions as well as carotenoids in the germinated seeds (sprouts) is present in Table (3). The results reveal that fresh chia sprout contains 1.51 mg/g chlorophyll a and 0.82 mg/g chlorophyll b, in addition to 0.47 mg/g carotenoids.

Table (3): The content of chlorophyll and carotenoids in fresh chia sprouts

Pigment	Value (mg/g)
Chlorophyll a	1.51±0.08
Chlorophyll b	0.82±0.03
Chlorophyll (a/b)	1.83±0.04
Carotenoids	0.47±0.01
The rate of total chl/car	4.97±0.12
Total (chl+car)	2.81±0.12

Values are means ± standard error (M±SE) of successful three trials.

The total phenolic contents of chia seeds and sprouts were found to be 1.96 mg GAE/g and 2.33 mg GAE/g, respectively (Table 4). This result is lower than that Marineli et al. (2014) results, who reported the total phenolic contents of white and black chia seeds were 3.52±0.08 and 3.42±0.06 mg GAE/g defatted seeds, respectively. However, close to the results of Martinez-Cruz and Paredes-Lopez, (2014). Who mentioned that the total phenolic content of Chilean chia seeds was 0.94±0.06 mg GAE/g and 1.64±0.08 mg GAE/g for the Mexican chia seeds.

The germination process of chia seeds leads to an increase in the concentration of total phenolic substances in its sprouts, as their concentration increased from 1.96 mg GAE/g in the seeds to 2.34 mg GAE/g in the sprouts within 5 days of germination at the room temperature. This result is consistent with Beltran-Orozco et al. (2020), who reported that the amount of total phenolic substances in Mexican chia seeds increases from 0.977 mg GAE/g to 2.936 mg GAE/g after 4 days of germination at 21°C. The differences in total phenolic content in chia seeds may be related to the source of the seeds including agriculture conditions and the harvested time (Ayerza and Coates, 2004; Ayerza, 2010; Ayerza, 2013) beside of the phenolic extraction method (Scapin et al., 2016).
The total flavonoids of chia seeds and sprouts are 0.51 mg/g and 1.16 mg/g, respectively (Table 4). These results are in agreement with Beltran-Orozco et al. (2020) who reported that the nutritional total flavonoids contents of Mexican dry chia seeds increased from (0.358 mg QE/g) to (1.06 mg QE/g) after 4 days of germination. Also, total saponins of chia seeds and sprouts are 0.026 mg/g and 0.058 mg/g, respectively (Table 4). As suggested by Osbourn (1996) increase of saponin levels during germination could be explained by their implication in the defiance system of the plant.
Referring to Table (4) the alkaloid content in chia seeds is 0.042 mg/g. which is significantly (p<0.05) higher than that of sprouts (0.034 mg/g). However, chia seeds contain significantly higher (p<0.05) ascorbic acid than that of sprouts. It was reported that during germination, the respiration process is triggered by the ascorbic acid (Ahmad and Pathak, 2000).

Table (4): Phytochemicals of chia seeds as affected by germination process.

Phytochemicals	Chia seeds	Chia sprouts
Phenolic compounds (mg GAE/g)	1.960±0.02^{b}	2.340±0.03^{a}
Flavonoids (mg QE/g)	0.510±0.01^{b}	1.160±0.02^{a}
Saponins (mg /g)	0.026±0.01^{b}	0.058±0.05^{a}
Alkaloids (mg /g)	0.042±0.00^{a}	0.034±0.00^{a}
Ascorbic acid (mg /g)	0.610±0.00^{b}	1.440±0.02^{a}

Values are means ± standard error (M±SE) of successful three trials
In a row, which means having the same letters are not significantly different at 0.05% level (p>0.05).

4. Conclusions

The results show that the degree of crowding of seeds during germination for 5 days had no actual effect until crowding of 1000 seeds/250 ml (studied area unit), as the degree of seed germination was affected after that by the degree of crowding. As for physical properties, chia sprouts had higher volume, weight, and true density, but smaller bulk density than those of seeds. The chia sprouts also comprehend higher moisture, proteins, ash, and dietary fibers than those of the seeds. On the other hand, they contain lower crude fat, nitrogen-free extract, and total carbohydrates. The biomass of the chia sprouts can provide the human body with different secondary metabolites and nutrients with low calories compared with the raw seeds.

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