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Standardization of Process for Chia Germination

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Abstract

Chia seed stands in the front list of super foods, because of its unique nutritional properties and health benefits. Chia seeds are rich in omega 3 fatty acids, protein, fibre and minerals. In the current study, germination of white and black chia seeds was carried out. Raw chia seeds were sprinkled with water, allowed to stand at room temperature, incubated at 24 °C for time duration of 18, 24, 36, 48 and 54 hours with distilled water, warm distilled water at 45 °C and 2 per cent sugar solution with warm distilled water at 45 °C. The highest germination percentage of the treatments were selected for analysis of α amylase and vitamin C. The highest content of α amylase, vitamin C of specific time interval and sensory evaluation were carried out. The result revealed that for different time durations, the treatment with distilled water (T1) for white chia and with warm distilled water (T2) for black chia seeds were found to give highest germination percentage and both with T1 were highly accepted for sensory evaluation. Amylase activity was found to be highest at 36 hour's time duration having 1.24 and 1.20 mg/100g for white and black variety, respectively. Vitamin C content was highest for white and black variety at 36 hours having 80.67 and 66.67 mg/100g, respectively. Findings concluded that germination of chia enhanced α amylase and vitamin C content.

Keywords : Chia seeds, Germination, Vitamin C, α - amylase

The search for novel, high quality source of protein, fat, dietary fibre and antioxidant property has been attaining popularity in developing countries for meeting the challenges of malnutrition on one side and prevention and control of non-communicable diseases through diet on the other side. All the super foods are gaining popularity due to their nutraceutical properties (Din *et al*., 2021). Millet and chia are gluten free, have low glycemic index and chia mucilage can be used as fat replacement (Hiregoudar and Mamatha, 2021). Out of 900 species of genus *Salvia,* only *Salvia hispanica* can be grown domestically (Chaitanya *et al.*, 2022).

Due to increased consumer awareness on healthy food, chia, quinoa and millets are in demand. Quinoa and millets are explored for value added products, however not much of the products available using chia seeds. Chia is having lot of health benefits and gaining sufficient attention from consumers. To meet the demand, more value-added products from high nutrient rich foods like chia is gaining popularity. Due to climatic change in present days, chia is suitable as it can grow in adverse weather and low nutrient content soils. The use of chia in diet can be considered beneficial in the prevention and treatment of risk factors related to life style diseases such as diabetes and cardiovascular diseases, which are leading cause for fatality. Therefore, crops with multi utility, prominent nutrient composition and user-friendly processing methods are needed (Kilewela *et al*., 2021).

Germination is an inexpensive and effective method for improving the overall nutritional quality of any grains as it enhances the digestibility and reduces anti nutritional factors (Chavan and Kadam, 1989 and Ghorpade and Kadam, 1989). Germination increases the total phenolic content including γ-aminobutyric acid, the protein and insoluble dietary fibre increase,

whereas soluble dietary fibres and lipid component decreases (Nadtochii *et al*., 2019). The data available on different processing techniques used for super food is scanty. Keeping this in the background, present study was undertaken with the aim of standardization of process protocol for chia seeds germination.

Material and Methods

White and black seeded chia were procured from Kilaru Naturals, Hyderabad, Andhra Pradesh, India.

Germination of chia seeds : The germination of chia seeds are shown in flow chart (Fig. 1).

Place the filter paper onto sterilized Petri-dish on both sides by cutting into shape
↓
Put 100 chia seeds on petridish
↓
Sprinkle 4 ml water and wet the filter paper on both sides
↓
Treatments: T1 - distilled water, T2 - warm distilled water at 45 ^{0}C and T3 - 2% sugar solution with warm distilled water at 45 ^{0}C
↓
Germinate chia seeds for different time interval 18, 24, 36, 48 and 54 hours at 24 ^{0}C
↓
Record germination percentage and radical length using graph paper
↓
Best germinated chia were selected for sensory evaluation, Vitamin C and α amylase activity

Fig. 1: Flow chart of germination of chia seeds

Estimation of vitamin C content during germination : The method for determination of ascorbic acid was used with slight modification given by Harris and Ray (1935) used for germinated chia samples. Ascorbic acid reduced 2, 6-dichlorophenol indophenol dye to a colourless leucobase and gets oxidised to dehydro ascorbic acid changing the dye colour to pink.

Estimation of α amylase activity during germination of chia : The α-amylase activity was assayed using the method of Bernfeld (1955) for germinated chia.

Plate 1: Germinated white chia

Plate 2: Germinated black chia

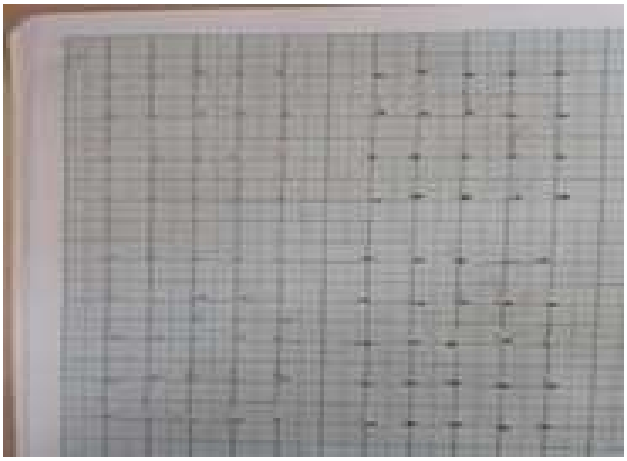

Plate 3: Measurement of length of radical on graph paper

Sensory Analysis

Sensory analysis of germinated chia was carried out by twenty-one semi-trained panelists using 9-point hedonic scale and scores were recorded for appearance, colour, texture, taste, after-taste, flavour and overall acceptability (Meilgaard *et al.*, 1999).

Statistical Analysis

The data was subjected to analysis of variance (ANOVA) for testing the significance of variation in

Plate 4: Different treatments of chia germination

Plate 5: Sensory evaluation of germinated chia seeds

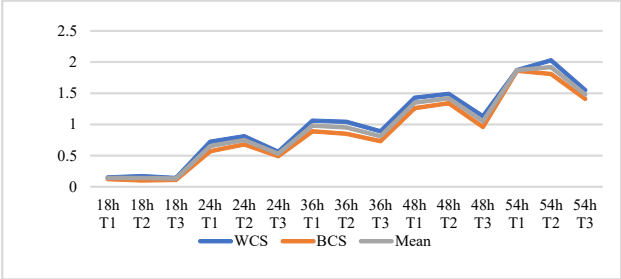

Fig. 2 : Length of radical (cm) of germinated Chia seeds

Germinated at different time interval 18h, 24h, 36h, 48h and 54h (h: hour) and three treatments (T1: Distilled water, T2: Warm distilled water at 45 ^{0}C and T3: 2 per cent sugar solution with warm distilled water at 45 ^{0}C)

WCS: White chia seeds; BCS: Black chia seeds

germination percentage, vitamin C, α amylase and sensory evaluation. Mean values were calculated and compared at 5 per cent significance level, one way factor and multiple comparisons were determined using the software OPSTAT (CCS, Haryana Agricultural University, Hisar, India).

Results and Discussion

Germination Percentage of Chia Seeds

Germination is the development of a plant from a seed or spore after a period of dormancy. Among the factors affecting seed germination, substrate and water availability stand out because of their direct influence. In the germination test, an appropriate substrate must be used to provide sufficient amounts of water for soaking the seeds, besides serving as a support for germinated seedlings (Nadtochii *et al*., 2020). Thus, the choice of the substrate type should consider the size of the seed, its requirement in terms of water, light sensitivity and substrate for the development and evaluation of seedlings (Paiva, 2016). In this present investigation (Table 1), white chia seeds and black chia seeds were germinated at different time intervals - 18h, 24h, 36h, 48h and 54h with three different treatments: T1- distilled water, T2- warm distilled water at 45 °C and T3- 2 per cent sugar solution with warm distilled water at 45 °C. The results from the table 1 revealed that germination percentage of white and black chia seeds at different time interval showed significant difference. 18h, 24h and 54h was found to be significantly higher in

Table 1

Germination percentage of Chia seeds

Treatments	WCS	BCS
18hT1	64.67 ± 0.34 ^{j}	36.67 ± 0.33 ^{k}
18hT2	60.67 ± 0.67 ^{k}	30.33 ± 0.33 ^{l}
18hT3	41.67 ± 0.89 ^{l}	27.33 ± 0.33 ^{m}
24hT1	79.67 ± 0.33 ^{g}	50.33 ± 0.33 ^{j}
24hT2	69.33 ± 0.67 ^{h}	58.67 ± 0.33 ^{h}
24hT3	67.67 ± 0.33 ^{i}	53.67 ± 0.33 ^{i}
36hT1	92.33 ± 0.33 ^{e}	77.67 ± 0.34 ^{f}
36hT2	96.67 ± 0.34 ^{bc}	79.33 ± 0.33 ^{e}
36hT3	90.00 ± 0.00 ^{f}	73.33 ± 0.33 ^{g}
48hT1	96.33 ± 0.33 ^{bcd}	81.67 ± 0.34 ^{d}
48hT2	96.67 ± 0.34 ^{bc}	87.67 ± 0.34 ^{a}
48hT3	95.00 ± 0.57 ^{d}	83.33 ± 0.33 ^{c}
54hT1	98.33 ± 0.33 ^{a}	87.33 ± 0.33 ^{a}
54hT2	97.33 ± 03 ^{ab}	87.33 ± 0.33 ^{a}
54hT3	95.67 ± 0.67 ^{cd}	86.33 ± 0.33 ^{b}
Mean ± SD	82.77 ± 0.69	66.73 ± 0.31
F Test	*	*
C.D. 5%	1.440	0.656
SE(m)	0.494	0.225
C.V.	1.034	0.585

Note: Values are expressed as mean ± SD of three determinations. Germinated at different time interval- 18h, 24h, 36h, 42h and 54h (h: hour)

WCS: White chia seeds; BCS: Black chia seeds; Three treatments; T1: Distilled water; T2: Warm distilled water at 45 ^{0}C and T3: 2 per cent sugar solution with warm distilled water at 45 ^{0}C.

treatment 1 *i.e.* distilled water followed by T2 *i.e.* warm distilled water for 36 and 48 hours in white chia. With reference to black chia seeds, there was increasing trend of germination percent from 24 to 54 hours in T2 with warm distilled water has given at 24h (58.67), 36h (79.33), 48h (87.67) and 54h (87.33).

The difference in germination percentage between white and black chia seeds may be due to higher percent of anti-nutritional factors in black chia might have hindered the germination at early stage and reached maximum at 54 hours. Therefore, black chia seeds need more time duration to germinate as against to white chia seeds. They also exhibited best at warm distilled water T2. Germination showed

TABLE 2
Vitamin C (mg/100g) of germinated chia seeds

Sample	0h	18h	24h	36h	48h	54h
GWCS	50.67 ± 0.67	53.33 ± 0.67	66.67 ± 0.67	80.67 ± 0.67	66.67 ± 0.67	52.67 ± 0.67
GBCS	39.33 ± 0.67	40.67 ± 0.67	52.67 ± 0.67	66.67 ± 0.67	52.67 ± 0.67	40.67 ± 0.67
Mean ± SD	45 ± 0.94	47 ± 0.94	59.67 ± 0.94	73.67 ± 0.94	59.67 ± 0.94	46.67 ± 0.94
F Test	*	*	*	*	*	*
C.D. 5%	2.688	2.688	2.688	2.688	2.688	2.688
SE(m)	0.667	0.667	0.667	0.667	0.667	0.667
C.V.	2.566	2.457	1.935	1.567	1.935	2.474

Note: Values are expressed as mean ± SD of three determinations.
GWCS: Germinated white chia seeds; GBCS: Germinated black chia seeds
The highest per cent germination treatment was selected for vitamin C content estimation at different time interval.

more effective result in reducing trypsin inhibitor activity, tannin, polyphenols and phytic acid than other cooking treatments (Ramakrishna *et al*., 2006).

Length of Radical

The length of radical was measured with the help of graph paper. The length of radical increased with increase in time interval (Fig. 2).

Vitamin C Content

Vitamin C is a natural antioxidant, has immense benefits. Germination usually increases the vitamin C content of the grains. The vitamin C content was studied for different time interval of white and black chia (Table 2). The vitamin C content of white chia at 0h, 18h, 24h, 36h, 48h and 54h were 50.67, 53.33, 66.67, 80.67, 66.67 and 52.67 mg/100g respectively. The first three time interval *i.e.* 0h, 18h and 24h, vitamin C content gradually increased. At 36h, it reached the peak point and then decreased at 48 and 54h. Vitamin C content of germinated white chia seeds was found maximum at 36h (80.67 mg/100g). Similarly, for black chia, vitamin C content at different time - 0h, 18h, 24h, 36h, 48h and 54h were 39.33, 40.67, 52.67, 66.67, 52.67 and 40.67 mg/100g respectively. The vitamin C content were maximum for germinated black chia seeds at 36h (66.67 mg/100g). The results of statistical analysis revealed non-significant difference. Srujana *et al*. (2019) studied on germinated quinoa effect on vitamin C which range from 4.21 to 78.26 mg/100g at different time interval 4 to 60 hours. From the vitamin C content analysis, it is evident that 36h is the best to get maximum vitamin C than the rest of the time duration. Silva *et al*. (2020) studied on germination of soybean and found that vitamin C content of germinated soybean is 61 per cent higher than non germinated soybean.

α - Amylase Content

The amylases are commonly distributed throughout the plant, but abundantly in the germ and pericarp of the grains. During germination, amylases migrate to regions that are rich in starch, proteins and lipids where they can initiate hydrolytic processes to generate energy (Delcour and Hoseney, 2000).

α amylase on germination are activated and start to break down starch into small sugars making it more digestible (Helland *et al*., 2002). So, the germinated products are suitable for weaning food and for geriatric people. The α- amylase content was observed at different time interval for white and black chia (Table 3). The white chia contains α- amylase content at 0h, 18h, 24h, 36h, 48h and 54h were 1.06, 1.08, 1.22, 1.24, 1.20 and 1.19 respectively and for black chia were 1.01, 1.04, 1.19, 1.20, 1.18 and 1.14 mg/100g respectively. The α-amylase content was highest at 36h for both for germinated

TABLE 3
α Amylase (mg/100g) content of germinated chia seeds

Sample	0h	18h	24h	36h	48h	54h
GWCS	1.06	1.08	1.22	1.24	1.20	1.19
GBCS	1.01	1.04	1.19	1.20	1.18	1.14
Mean ± SD	1.03	1.06	1.20	1.22	1.19	1.16
F Test	*	*	*	*	*	*
C.D. 5%	0.021	0.013	0.013	0.013	0.013	0.013
SE(m)	0.005	0.003	0.003	0.003	0.003	0.003
C.V.	0.882	0.545	0.479	0.472	0.485	0.494

Note: Values are expressed as mean of three determinations
GWCS: Germinated white chia seeds; GBCS: Germinated black chia seeds
The highest germinated treatment were selected for α amylase content at different time interval

TABLE 4
Sensory evaluation of processed chia seeds (Germination)

Treatment	Appearance	Colour	Texture	Taste	After-Taste	Flavour	Overall-Accetability
WCS	7.39±0.19 ^{b}	7.40±0.19 ^{b}	7.14±0.18 ^{c}	6.85±0.19 ^{c}	6.92±0.18 ^{c}	6.96±0.19 ^{c}	7.11±0.16 ^{b}
BCS	7.19±0.24 ^{b}	7.23±0.27 ^{b}	6.98±0.21 ^{c}	6.92±0.19 ^{c}	6.86±0.19 ^{c}	7.02±0.19 ^{c}	7.03±0.19 ^{b}
GWC1	7.92±0.13 ^{a}	8.02±0.12 ^{a}	7.79±0.12 ^{ab}	7.71±0.16 ^{ab}	7.59±0.13 ^{ab}	7.69±0.14 ^{ab}	7.79±0.09 ^{a}
GWC2	7.71±0.12 ^{ab}	7.64±0.13 ^{ab}	7.19±0.17 ^{c}	7.29±0.18 ^{bc}	7.19±0.19 ^{bc}	7.09±0.17 ^{c}	7.36±0.12 ^{b}
GWC3	7.52±0.17 ^{ab}	7.50±0.17 ^{ab}	7.00±0.16 ^{c}	7.00±0.17 ^{c}	6.98±0.20 ^{c}	7.23±0.19 ^{bc}	7.20±0.14 ^{b}
GBC1	7.97±0.13 ^{a}	8.02±0.12 ^{a}	7.92±0.12 ^{a}	7.98±0.12 ^{a}	7.96±0.11 ^{a}	7.85±0.12 ^{a}	7.95±0.10 ^{a}
GBC2	7.38±0.19 ^{b}	7.33±0.19 ^{b}	6.98±0.18 ^{c}	7.09±0.19 ^{c}	7.07±0.16 ^{bc}	7.04±0.14 ^{c}	7.15±0.15 ^{b}
GBC3	7.28±0.19 ^{b}	7.42±0.20 ^{b}	7.31±0.23 ^{c}	6.98±0.20 ^{c}	7.11±0.21 ^{bc}	6.905±0.13 ^{c}	7.17±0.17 ^{b}
Mean ± SD	7.54±0.175	7.57±0.181	7.28±0.173	7.22±0.173	7.21±0.176	7.22±0.165	7.34±0.147
F Test	*	*	*	*	*	*	*
C.D. 5%	0.488	0.505	0.485	0.484	0.493	0.461	0.411
SE(m)	0.175	0.181	0.173	0.173	0.176	0.165	0.147
C.V.	10.596	10.921	10.898	10.967	11.209	10.458	9.181

Note: Values are expressed as mean ± SD of twenty-one determinations. WCS: White chia seeds; BCS: Black chia seeds; Germinated two varieties of white and black chia seeds keeping temperature and time constant at 24 ^{0}C for 36 hours

GWCST1- Germinated white chia seeds with distilled water; GWCST2- Germinated white chia seeds with warm distilled water at 45 ^{0}C; GWCST3- Germinated white chia seeds with 2 per cent sugar solution with warm distilled water; GBCST1- Germinated black chia seeds with distilled water; GBCST2- Germinated black chia seeds with warm distilled water at 45 ^{0}C; GBCST3- Germinated black chia seeds with 2 per cent sugar solution with warm distilled water

chia seeds. Studies shown by Srujana *et al*. (2019) on germinate quinoa showed similar amylase activity ranging from 0.15 to 1.48 mg/100g at different time interval 4 to 60 hours.

Sensory Evaluation of Germinated Chia Seeds

Any processing method is acceptable only when it gives a product of acceptable quality by the consumer.

The results obtained revealed (Table 4) that germinated white chia seeds with distilled water GCST1 had the highest mean scores for appearance (7.92), colour (8.02), texture (7.79), taste (7.71), after taste (7.59), flavour (7.69) and overall acceptability (7.79); while germinated black chia seeds with distilled water GBCT1 also scored the highest in all sensory parameters. Germinated white and black chia seeds with distilled water (T1) are highly significant with respect to sensory scores. There were better overall scores for processed germinated chia seeds than the raw chia seeds.

Germination is a simple processing method which enhances the nutrient content and sensory properties. Chia seeds can be germinated using distilled water for white and warm distilled water for black. Highest vitamin C and α amylase content was reported at 36 hours of germination. Panelist have accepted germinated chia seeds than the raw seeds.

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