

## Standardization of germination conditions for nutritional enhancement of underutilized basil seeds

B Neeharika<sup>1\*</sup>, KG Vijayalaxmi<sup>2</sup> & Shamshad Begum S<sup>2</sup>

<sup>1</sup>ICAR-NIRCA-Krishi Vigyan Kendra, Kandukur-523 105, Andhra Pradesh, India

<sup>2</sup>University of Agricultural Sciences, Bengaluru-560 065, Karnataka, India

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The current study aimed at determining the ideal conditions for the germination of clove basil and sweet basil seeds to enhance their nutritional and sensorial quality. Prechilling of seeds at 4°C for 2 days followed by germination at 35°C in continuous light for 60 h for clove basil seeds and for 48 h for sweet basil seeds were found to be ideal. Under these conditions, clove basil and sweet basil seeds exhibited desirable  $\alpha$ -amylase activity (9.27 U, 10.95 U), vitamin C content (14.66 mg/100 g, 19.52 mg/100 g) and overall sensorial acceptability (8.14, 8.24) along with germination percentage of 20.67%, 84.33%, respectively. Further, germination significantly improved dietary fibre (56.45 to 61.26 g/100 g, 48.46 to 52.83 g/100 g), calcium (603.60 to 646.80 mg/100 g, 568.40 to 598.80 mg/100 g), magnesium (270.72 to 291.36 mg/100 g, 261.36 to 276.24 mg/100 g), phosphorus (69.25 to 85.29 mg/100 g, 106.68 to 122.73 mg/100 g), zinc (4.17 to 4.77 mg/100 g, 6.95 to 7.05 mg/100 g), copper (2.13 to 2.21 mg/100 g, 1.87 to 1.95 mg/100 g) and manganese (4.34 to 4.42 mg/100 g, 1.88 to 2.08 mg/100 g) contents of clove basil and sweet basil seeds.

**Keywords:**  $\alpha$ -Amylase, Basil sprouts, Dietary fibre, Minerals, Vitamin C

The *Ocimum gratissimum* (clove basil or wild basil or shrubby basil) and *O. basilicum* (sweet basil or great basil or common basil) are important economical species cultivated across India for medicinal and culinary purposes<sup>1</sup>. The vernacular names of clove basil included ban tulusi (Hindi), van tulusi (Gujarati), ajavala (Marathi), vanatulasi (Kannada), mahabeera (Telugu), elumichamtulasi (Tamil) and kattutulasi or kattuthrithavu (Malayalam) while that of sweet basil included babui tulusi or tukmaria or sabja (Hindi), sabje (Gujarati), bhutulasi or sabza (Marathi), kaamakasturi or kaamaggare (Kannada), bhootulasi (Telugu), tirunitru or chapchavitai (Tamil) and pach-cha-pushpam (Malayalam)<sup>2</sup>. Despite diverse climatic and ecological conditions, basil is widely cultivated across the country with a reasonable yield<sup>3</sup>. Though several studies have been carried out on the phytoconstituents and medicinal value of basil leaves, research on the nutritional and therapeutic aspects of the edible seeds of basil is limited to date.

Basil seeds are aromatic, dark brown to black in colour, small in size, oval in shape with dimensions ranging between 2.31-3.11 mm length, 1.30-1.82 mm

width, 0.99-1.34 mm thickness<sup>4</sup>. Germination or sprouting is a traditional food process that has been widely practiced to activate the metabolic machinery of seeds and promote their nutritional, functional and sensory profiles in a natural way<sup>5</sup>. Sprouted grains are whole grains consisting of all of the original bran, germ and endosperm with sprout growth not exceeding the kernel length and nutrient values not diminished<sup>6</sup>. Controlled germination is desirable when the sprouted grains are intended to be used for food applications as prolonged germination results in increased hydrolytic enzyme activities and negatively impacts their functionality, nutritional quality and sensorial acceptability<sup>7</sup>. Hence, the present investigation was carried out with the objective of standardizing the ideal conditions for germination of basil seeds in order to enhance their nutritional and sensory quality.

### Materials and Methods

#### Procurement of basil seeds

The seeds of clove basil (*Ocimum gratissimum*) and sweet basil (*O. basilicum*) were purchased from local markets in Kadapa, Andhra Pradesh, India. The seeds were cleaned manually, freed from broken seeds, other varieties of seeds and inert matter. The

\*Correspondence:  
E-mail: b.neeharika@icar.gov.in

obtained pure seeds were pooled and utilized for further investigation.

#### Determination of suitable conditions for germination of basil seeds

The germination trials were conducted with 100 seeds in petridishes lined with Whatman No. 1 filter papers and moistened with distilled water. The germination conditions included: without prechilling (T<sub>1</sub>: continuous darkness and 25°C; T<sub>2</sub>: 16/8 h light/dark photoperiod with an alternating temperature of 18/25°C) and with prechilling at 4°C for 2 days followed by germination under continuous light (T<sub>3</sub>: at 30°C; T<sub>4</sub>: at 35°C) to reduce the germination inhibitors and promote rapid germination. The seeds were observed daily for radicle and plumule emergence and the count of germinated seeds was taken until the maximum germination percentage was attained<sup>8</sup>. The sprouting parameters *viz.* germination capacity, speed and degree of germination were assessed to determine the suitable conditions for better germination of basil seeds.

Germination capacity represents the proportion of total seeds germinated and is expressed as a percentage. The non-germinated seeds represented dry seeds that were not in contact with water. The seeds in which cotyledons emerged before the radical were considered abnormally germinated<sup>9</sup>. The speed of germination of basil seeds was assessed using parameters *viz.* mean germination time (MGT), first day of germination (FDG), last day of germination (LDG), time spread of germination (TSG), germination rate index (GRI), coefficient of velocity of germination (CVG) and germination index (GI)<sup>10</sup>. The degree of germination of basil seeds was assessed using parameters *viz.* Timson index (T) and Maguire's index (T<sub>mod</sub>)<sup>11</sup>.

#### Standardization of germination duration for basil seeds

Clove basil seeds were allowed to germinate for 48, 60 and 72 h and sweet basil seeds for 24, 36 and 48 h under standardized conditions. The ideal duration for germination was determined based on  $\alpha$ -amylase activity<sup>12</sup>, vitamin C content<sup>13</sup> and sensory attributes. For sensory evaluation, the germinated seeds were dried in a tray dryer at 60°C for 4 h, roasted until a pleasant aroma emerged and made into fine powders. A semi-trained panel (n=30) from the Department of Food Science and Nutrition, University of Agricultural Sciences, Bengaluru evaluated the powders for sensory attributes *viz.* appearance,

texture, flavour, taste and overall acceptability on a nine-point hedonic scale<sup>14</sup>.

#### Nutritional quality of germinated basil seeds

The non-germinated and germinated basil seeds under standardized conditions were assessed for dietary fiber content and mineral composition<sup>13</sup>.

#### Statistical analysis

All experiments were conducted in replicates and the results obtained were presented as the mean  $\pm$  standard deviation (SD). The treatment means were compared for significant differences by one-way analysis of variance (ANOVA) and the means were separated using Duncan's multiple range test at the 1% level using SPSS 20.0 (IBM, NY, USA). The Pearson correlation calculation was done using Microsoft Excel 2021.

## Results and Discussion

#### Determination of suitable conditions for germination of basil seeds

##### *Germination capacity of basil seeds*

The maximum germination percentage attained by the clove basil and sweet basil seeds under four different conditions during the trial period of seven days was 1.0, 0.3, 11.7, 28.3 % and 14.3, 20.3, 68.3, 92.7 %, respectively, with a maximum value under T<sub>4</sub> condition for both seeds. The percentage of abnormally germinated seeds under four different conditions during the last day of the germination trial was 0.0, 0.3, 0.3, 0.0 % and 0.3, 7.3, 0.3, 0.0 % for clove basil and sweet basil seeds, respectively. The percentage of non-germinated seeds under four different conditions during the last day of germination trial was 99.0, 99.3, 88.0, 71.7 % and 85.3, 72.3, 31.3, 7.3 % for clove basil and sweet basil seeds, respectively, with a minimum value under T<sub>4</sub> condition for both seeds (Fig. 1).

##### *Speed and Degree of Germination of Basil Seeds*

The speed and degree of germination of clove basil and sweet basil seeds under employed germination conditions are as depicted in (Table 1). The MGT measured the time taken for seeds to germinate but it was oblivious to the uniformity of germination. The TSG measured the length of time lapse between the first and last germination events of seeds taken for trial<sup>10</sup>. The GRI indicated the germination percentage of seeds per day while the CVG measured the rapidity of seeds in attaining the final germination percentage.

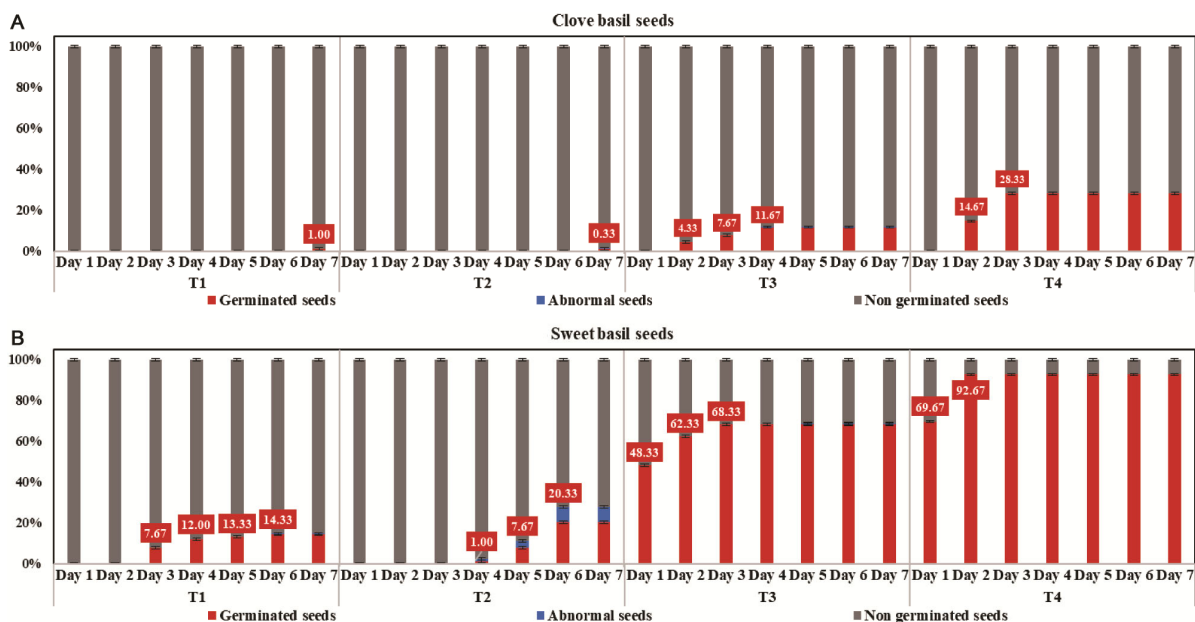


Fig. 1 — Germination capacity of basil seeds under different conditions. Note: Data presented is the mean ± standard deviation of three determinations. T1: Continuous darkness and 25°C, T2: 16/8 h light/dark and alternating temperature (18-25°C), T3: Prechilling at 4°C for 2 days, germination under continuous light and 30°C, T4: Prechilling at 4°C for 2 days, germination under continuous light and 35°C

Table 1 — Speed and degree of germination of basil seeds under different conditions

Seeds	Germination condition	MGT (day)	FDG (day)	LDG (day)	TSG (days)	GRI (%/day)	CVG	GI	T	T <sub>mod</sub>	
Clove basil	Without prechilling	Continuous darkness and 25°C	7.00 <sup>c</sup> ± 0.00	7.00 ± 0.00	-	-	0.14 <sup>a</sup> ± 0.00	14.29 <sup>a</sup> ± 0.00	4.00 <sup>a</sup> ± 0.00	0.40 <sup>a</sup> ± 0.00	0.40 <sup>a</sup> ± 0.00
		16/8 h light/dark and alternating temperature (18-25°C)	7.00 <sup>c</sup> ± 0.00	7.00 ± 0.00	-	-	0.14 <sup>a</sup> ± 0.00	14.29 <sup>a</sup> ± 0.00	4.00 <sup>a</sup> ± 0.00	0.40 <sup>a</sup> ± 0.00	0.13 <sup>a</sup> ± 0.23
	Prechilling at 4°C for 2 days	Continuous light and 30°C	2.97 <sup>b</sup> ± 0.05	2.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00	4.28 <sup>b</sup> ± 0.25	33.65 <sup>b</sup> ± 0.55	93.67 <sup>b</sup> ± 4.93	9.37 <sup>b</sup> ± 0.49	0.54 <sup>a</sup> ± 0.46
		Continuous light and 35°C	2.48 <sup>a</sup> ± 0.02	2.00 ± 0.00	3.00 ± 0.00	1.00 ± 0.00	11.89 <sup>c</sup> ± 0.26	40.29 <sup>c</sup> ± 0.29	241.33 <sup>c</sup> ± 4.93	24.13 <sup>c</sup> ± 0.49	0.85 <sup>a</sup> ± 0.00
	CD at 1%		0.07	-	-	-	0.49	0.86	9.56	0.96	-
	Sweet basil	Without prechilling	Continuous darkness and 25°C	3.69 <sup>c</sup> ± 0.05	3.00 ± 0.00	6.00 ± 0.00	3.00 ± 0.00	4.07 <sup>a</sup> ± 0.14	27.05 <sup>b</sup> ± 0.00	104.67 <sup>a</sup> ± 3.79	10.47 <sup>a</sup> ± 0.38
16/8 h light/dark and alternating temperature (18-25°C)			5.57 <sup>d</sup> ± 0.03	4.00 ± 0.00	6.00 ± 0.00	2.00 ± 0.00	3.70 <sup>a</sup> ± 0.11	17.94 <sup>a</sup> ± 0.00	110.33 <sup>a</sup> ± 3.21	11.03 <sup>a</sup> ± 0.32	0.54 <sup>a</sup> ± 0.01
Prechilling at 4°C for 2 days		Continuous light and 30°C	1.38 <sup>b</sup> ± 0.01	1.00 ± 0.00	3.00 ± 0.00	2.00 ± 0.00	57.39 <sup>b</sup> ± 0.54	72.70 <sup>c</sup> ± 0.55	657.67 <sup>b</sup> ± 5.51	65.77 <sup>b</sup> ± 0.55	0.96 <sup>c</sup> ± 0.00
		Continuous light and 35°C	1.25 <sup>a</sup> ± 0.01	1.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00	81.17 <sup>c</sup> ± 0.29	80.12 <sup>d</sup> ± 0.29	903.67 <sup>c</sup> ± 4.93	90.37 <sup>c</sup> ± 0.49	0.98 <sup>d</sup> ± 0.01
CD at 1%		0.08	-	-	-	0.87	1.11	12.20	1.22	0.01	

Note: Values expressed as the mean ± standard deviation of three determinations. Means within the same column followed by a common superscript do not differ significantly at  $P \leq 0.01$ . MGT: Mean germination time, FDG: First day of germination, LDG: Last day of germination, TSG: Time spread of germination, GRI: Germination rate index, CVG: Coefficient of velocity of germination, GI: T: Timson index, T<sub>mod</sub>: Maguire's index, CD: Critical difference.

The CVG values ranged from 0 to 100 with values approaching 100 indicating faster germination while values near 0 represented slower or inhibited

germination<sup>15</sup>. GI was the most comprehensive single value coefficient as it measured both the germination percentage and the speed of germination of seeds<sup>16</sup>.

Germination under  $T_4$  conditions resulted in lower FDG, MGT, TSG and significantly ( $P \leq 0.01$ ) higher GRI, CVG, GI values *i.e.*, 2.0 days, 2.5 days, 1.0 day, 11.9%/day, 40.3, 241.3 and 1.0 day, 1.3 days, 1.0 day, 81.2%/day, 80.1, 903.7 for clove basil and sweet basil seeds, respectively, indicating a faster rate of germination compared to other germination conditions. Further, germination under  $T_4$  conditions resulted in significantly ( $P \leq 0.01$ ) higher  $T$  and  $T_{mod}$  values *i.e.*, 24.13, 0.85 and 90.37, 0.98 for clove basil and sweet basil seeds, respectively, indicating a better degree of germination compared to other germination conditions. Other researchers<sup>17</sup> also reported that precooling sweet basil seeds at 10°C for four days reduced dormancy, promoted the physiological quality of the seeds, lowered the FDG value and improved seedling emergence.

Thus, prechilling of basil seeds at 4°C for 2 days followed by germination under continuous light at 35°C favoured for reduction of germination inhibitors, promoted rapid and higher degree of germination of both clove basil and sweet basil seeds and was considered the most suitable treatment for germination of basil seeds to carry out further investigation.

#### Standardization of germination duration for basil seeds

##### Germination percentage of basil seeds

The germination percentage of clove basil seeds at 48, 60 and 72 h of incubation was 14.67%, 20.67% and 28.33%, respectively, while that of sweet basil seeds at 24, 36 and 48 h of incubation was 69.67%, 84.33% and 92.67%, respectively. A significant correlation was noticed between germination duration and germination percentage of both clove basil ( $r = 0.9854$ ) and sweet basil seeds ( $r = 0.9621$ ).

##### Radicle and plumule lengths of germinated basil seeds

The box and whisker plots were drawn by taking radicle and plumule length measurements of ten

germinated basil seeds to observe their elongation behaviour at different incubation times (Fig. 2). In the plots, the top and bottom whiskers represented the range between maximum and minimum values. The boxes represented the interquartile range and were drawn from the lower quartile  $Q_1$  (25<sup>th</sup> percentile) to the upper quartile  $Q_3$  (75<sup>th</sup> percentile). The horizontal line drawn in the middle of the box represented the median (50<sup>th</sup> percentile) value. The range, median,  $Q_1$  and  $Q_3$  values for the radicle length of clove basil seeds were 0.15, 0.33, 0.30 and 0.35 cm, respectively, at 48 h of incubation, 0.30, 0.60, 0.50 and 0.65 cm, respectively, at 60 h of incubation and 0.35, 0.85, 0.71 and 0.89 cm, respectively, at 72 h of incubation. While the range, median,  $Q_1$  and  $Q_3$  values for the radicle length of sweet basil seeds were 0.18, 0.20, 0.16 and 0.30 cm, respectively, at 24 h of incubation, 0.23, 0.56, 0.51 and 0.60 cm, respectively, at 36 h of incubation and 0.38, 0.95, 0.86 and 1.10 cm, respectively, at 48 h of incubation. There was no plumule development for clove basil seeds till 48 h of germination while for sweet basil seeds till 72 h of germination. The range, median,  $Q_1$  and  $Q_3$  values for the plumule length of clove basil seeds were 0.25, 0.25, 0.20 and 0.30 cm, respectively, at 60 h of incubation and 0.24, 0.50, 0.45 and 0.57 cm, respectively, at 72 h of incubation.

##### $\alpha$ -amylase activity of germinated basil seeds

Germination significantly ( $P \leq 0.01$ ) improved the  $\alpha$ -amylase activity of clove basil seeds from 3.98 to 9.27 U with a peak value at 60 h incubation and thereafter declined to 6.59 U with the increase in germination duration to 72 h (Fig. 3A). Likewise, other researchers<sup>18</sup> depicted that the amylase activity of finger millet increased rapidly with germination upto 96 h and thereafter subsequently decreased. For sweet basil seeds, the  $\alpha$ -amylase activity was significantly ( $P \leq 0.01$ ) improved from 2.22 to 10.95

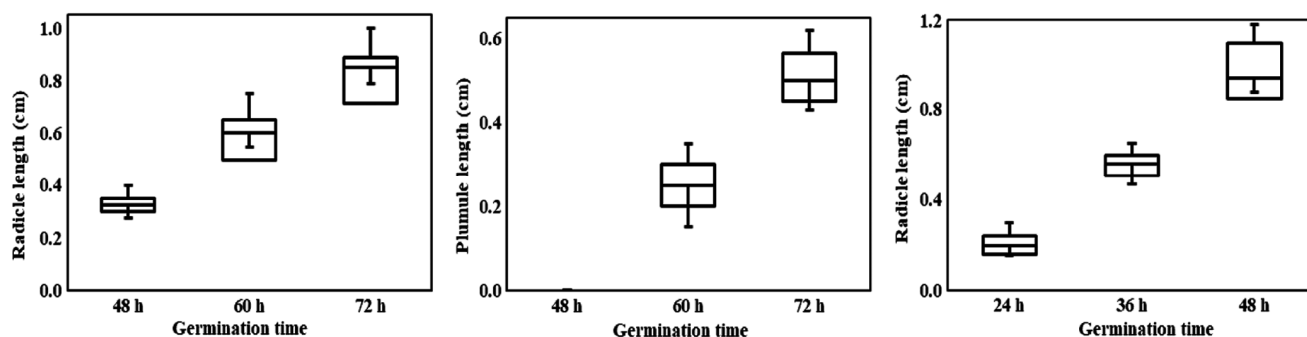


Fig. 2 — Box and whisker plots displaying radicle and plumule lengths of germinated basil seeds at different incubation times. Note: Values are often determinations

U with the increase in germination duration to 48 h (Fig. 3A). The increment in  $\alpha$ -amylase activity was due to an augmentation of gibberellic acid production by the embryo that diffused into surrounding aleurone layers and promoted rapid synthesis of hydrolytic enzymes for the breakdown of stored reserves for utilization by the growing embryonic axis<sup>19</sup>. However, the duration of germination is significant as extended germination reduced the hydrolytic enzyme activity as observed in the case of clove basil seeds due to the

depletion of stored reserves (especially starch) of the seeds.

*Vitamin C content of germinated basil seeds*

Germination significantly ( $P \leq 0.01$ ) improved the vitamin C content of clove basil seeds from 8.17 to 14.66 mg/100 g with a peak value at 60 h incubation and thereafter declined to 11.09 mg/100 g with the increase in germination duration to 72 h. While for sweet basil seeds, it was significantly ( $P \leq 0.01$ ) improved from 9.50 to 19.52 mg/100 g with the increase in germination duration to 48 h (Fig. 3B). The increment in the vitamin C content of seeds upon germination was due to the activation of an enzyme (L-galactono- $\gamma$ -lactone dehydrogenase) involved in the oxidation of L-galactono-1,4-lactone to ascorbic acid<sup>20</sup>. However, prolonged germination decreased the vitamin C content as observed in the case of clove basil seeds due to reduced hydrolytic enzyme activity and the unavailability of glucose for further conversion to vitamin C.

*Sensory parameters of germinated basil seed powders*

Findings revealed that the sensorial acceptance of clove basil seed powders significantly ( $P \leq 0.01$ ) improved with the increase in germination duration till 60 h and thereafter declined. While for sweet basil seed powders, the sensorial acceptance was significantly ( $P \leq 0.01$ ) improved with the increase in germination duration to 48 h. Thus, the powders of 60 h germinated clove basil seeds and 48 h germinated sweet basil seeds exhibited better sensorial acceptance with scores of 8.10, 8.05, 8.19, 8.14, 8.14 and 8.33, 8.14, 8.38, 8.29, 8.24 for appearance, texture, flavour, taste, overall acceptability, respectively, on the hedonic scale (Fig. 4).

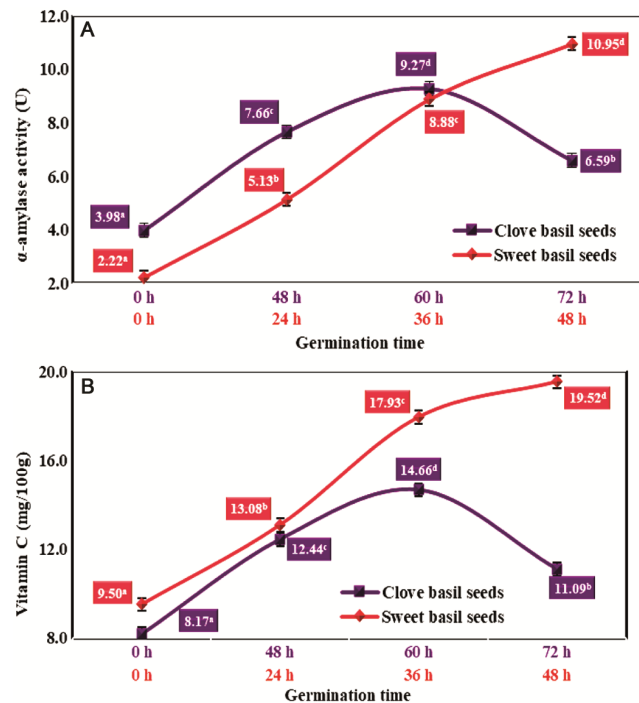


Fig. 3 — (A)  $\alpha$ -amylase activity of germinated basil seeds; and (B) Vitamin C content of germinated basil seeds. Note: Data presented is the mean  $\pm$  standard deviation of three determinations. Means followed by a common superscript do not differ significantly at  $P \leq 0.01$

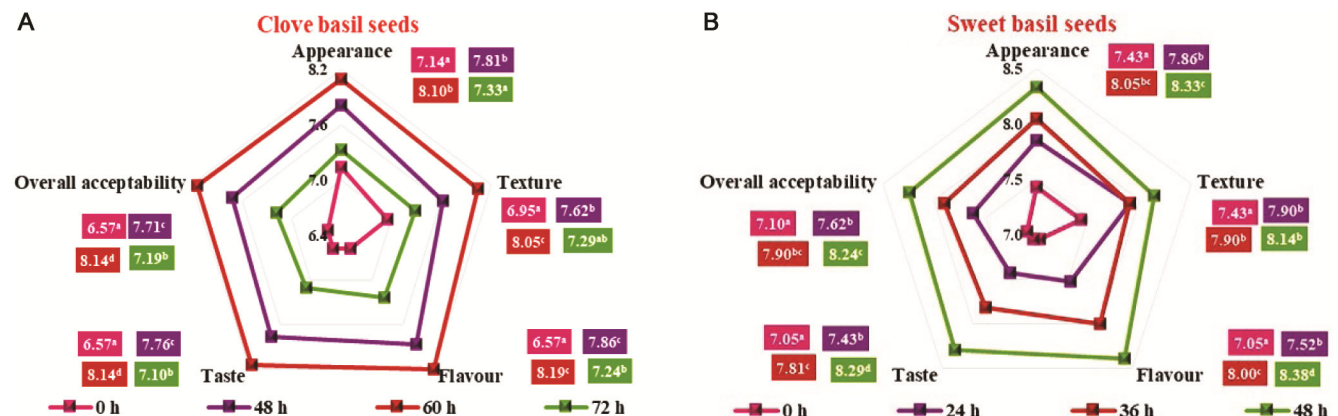


Fig. 4 — Sensory scores of germinated basil seed powders at different incubation times. Note: Data presented is the mean of thirty determinations. Means followed by a common superscript for a sensory attribute do not differ significantly at  $P \leq 0.01$

The germination and mild roasting of basil seeds promoted the development of sweet flavour, taste and texture due to maltose production from starch hydrolysis with increased amylolytic activity<sup>21</sup>. However, prolonged germination declined all the sensory attributes as observed for clove basil seed powders due to fall in amylolytic activity and high lipase activity generating free fatty acids associated with off flavour and bitter after-taste<sup>22</sup>. The  $\alpha$ -amylase activity was significantly correlated with texture ( $r = 0.9786$ ), flavour ( $r = 0.9865$ ), taste ( $r = 0.9783$ ) and overall acceptability ( $r = 0.9917$ ) of clove basil seed powder. While it was significantly correlated with the appearance ( $r = 0.9793$ ), flavour ( $r = 0.9981$ ), taste ( $r = 0.9873$ ) and overall acceptability ( $r = 0.9855$ ) of sweet basil seed powder. Thus, hydrolytic enzyme activity could greatly influence the sensory attributes of germinated seeds.

Hence, germination of clove basil seeds for 60 h and sweet basil seeds for 48 h under standardized conditions was found to be ideal for better  $\alpha$ -amylase activity, vitamin C content and the preparation of powders with better organoleptic properties.

#### Nutritional quality of germinated basil seeds

##### *Dietary fiber content of germinated basil seeds*

Germination significantly ( $P \leq 0.01$ ) improved the insoluble, soluble and total dietary fiber contents of clove basil seeds from 38.26 to 40.74 g/100 g, 18.19 to 20.52 g/100 g and 56.45 to 61.26 g/100 g, respectively, while that of sweet basil seeds from 31.26 to 34.51 g/100 g, 17.20 to 18.31 g/100 g and 48.46 to 52.83 g/100 g, respectively. This was due to mucilage formation upon hydration of seeds that mainly consisted of soluble fiber and the synthesis of new cell wall components for embryonic axis development that mainly consisted of insoluble fiber. Likewise, the germination of chia seeds for four days improved the total dietary fiber content by 46%<sup>23</sup>. The increase in dietary fiber content is desirable as regular consumption of dietary fiber rich foods was associated with a reduced risk of obesity, diabetes, cardiovascular diseases, gastrointestinal disorders and cancers. The soluble dietary fiber consumption significantly lowered the blood cholesterol levels and stabilized the blood glucose levels while the insoluble dietary fiber (IDF) consumption protected against colon cancer and inflammatory bowel disorders<sup>24</sup>.

##### *Mineral Composition of Germinated Basil Seeds*

Germination significantly ( $P \leq 0.01$ ) improved the calcium, magnesium, phosphorus, sulphur and sodium contents of clove basil seeds from 603.60 to 646.80

mg/100 g, 270.72 to 291.36 mg/100 g, 69.25 to 85.29 mg/100 g, 59.96 to 67.53 mg/100 g and 2.93 to 3.99 mg/100 g, respectively, while that of sweet basil seeds from 568.40 to 598.80 mg/100 g, 261.36 to 276.24 mg/100 g, 106.68 to 122.73 mg/100 g, 71.51 to 75.50 mg/100 g and 2.85 to 3.89 mg/100 g, respectively. Consumption of foods rich in calcium, magnesium and phosphorus was beneficial in combating stroke, osteoporosis, cardiovascular diseases, muscular diseases and type 2 diabetes<sup>25,26</sup>. Also, germination significantly ( $P \leq 0.01$ ) improved the zinc, copper and manganese contents of clove basil seeds from 4.17 to 4.77 mg/100 g, 2.13 to 2.21 mg/100 g and 4.34 to 4.42 mg/100 g, respectively, while those of sweet basil seeds improved from 6.95 to 7.05 mg/100 g, 1.87 to 1.95 mg/100 g and 1.88 to 2.08 mg/100 g, respectively. These microminerals are crucial for the proper functioning of the immune system<sup>27</sup>. Zinc, copper and manganese serve as cofactors for an antioxidant enzyme (superoxide dismutase) that scavenges free radicals and reduces the risk of inflammation-driven diseases<sup>28</sup>.

However, germination significantly ( $P \leq 0.01$ ) decreased the iron and potassium contents of clove basil seeds from 384.61 to 330.59 mg/100 g and 0.89 to 0.32 mg/100 g, respectively, while those of sweet basil seeds decreased from 3.28 to 594.41 to 480.19 and 1.60 mg/100 g, respectively. Similar findings were reported by other researchers<sup>29</sup> where the germination of chia seeds for seven days improved the sodium, calcium, copper and zinc contents by 94.29, 8.21, 5.34 and 13.82 %, respectively, due to increased enzymatic activities that promoted the release of minerals chelated with antinutrients. However, the potassium content decreased by 17.34% due to the leaching of some minerals during the germination process.

Thus, germination was found to be an ideal approach for the nutritional enhancement of underutilized basil seeds. The powders made from germinated basil seeds under standardized conditions can be utilized as functional ingredients for enrichment of regularly consumed foods. Further, the improved utilization of basil seeds in a similar way as that of basil leaves could benefit the farming communities by generating additional income.

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### Conflict of interest

All authors declare no conflict of interest.

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