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# Traditional processing methods for quality enhancement of indigenous basil seeds and formulation of functional flours

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The changing food habits and lifestyle led to consumption of faulty diets with increased prevalence of life style diseases in India. This has spurred health consciousness among food consumers and enhanced the demand for functional foods. The indigenous underutilised clove and sweet basil seeds being rich source of fibre (36.23, 28.85%), protein (9.16, 8.55%), polyphenols (17.28, 17.71 mg GAE/g extract) and antioxidants (266.13 and 344.63 mg TE/g extract), exhibited vast potential for formulation of functional flours. Traditional processing methods such as roasting, fermentation and germination have significantly enhanced the nutritional and antioxidant properties of both the seeds. Among them, germination was found to be ideal processing technique with relatively higher fibre, protein, total mineral, phenolic contents, antioxidant capacity and less fat content. Henceforth, processing of basil seeds in a traditional way could significantly enhance their quality and promote their utilisation as functional ingredients for designing healthy foods.

**Keywords**: Antioxidants, Basil seeds, Functional flours, Nutrients, Processing

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The present fast paced era with drastic urbanisation, economic and scientific development led to dramatic shift in food habits and life style of Indians. Often food consumers failed to choose right food and ended up consuming easily available unhealthy foods that led to development of enormous lifestyle diseases[1]. As per World Health Organization (WHO) reports, the prevalence of lifestyle diseases exponentially rose across India with 14 million people being affected annually. Further, the COVID-19 pandemic raised the health consciousness amongst people and much attention is being paid for functional foods with health promoting bioactive constituents.

The genus *Ocimum* (commonly known as 'Basil' or 'Tulsi'), native to tropical Asia and likely to have originated in India was known for its curative potential. The *Ocimum gratissimum* (clove basil or wild basil) and *Ocimum basilicum* (sweet basil or common basil) are underutilised aromatic culinary herbs that are widely grown in warm and tropical areas of India. They have been utilised in traditional medicines (Unani, Ayurveda and Siddha) since ancient times due to their stomachic, antioxidant, antiviral, antimicrobial, analgesic, anti-inflammatory, antidiabetic, antistress and antipyretic properties[2]. According to WHO, about 80% of patients in India still rely on traditional medicines[3]. India features ultimate annual production of more than 250-300 tons basil against world production of 500 tons. Basil is cultivated throughout India in the states *viz.*, Assam, West Bengal, Bihar, Uttar Pradesh, Madhya Pradesh, Maharashtra, Jammu, Andhra Pradesh and Telangana over an area of 25,000 ha essentially for purpose of seeds and aromatic leaves[4].

Basil seeds are edible and being rich source of dietary fibre, protein, polyphenols, flavonoids and other antioxidants, they exhibited enormous potential for utilisation as functional ingredients in formulation of designer foods. The seeds are generally added as whole to fruit-based beverages, ice creams and as milled to bakery products for aesthetic, functional and nutritional purposes[5]. The minimal processing techniques such as soaking, roasting, fermentation and germination have been traditionally utilised since ancient times for enhancing the quality of seeds by reducing antinutrients, improving bioavailability of nutrients and synthesis of bioactive components[6].

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The processing of basil seeds can be helpful in enhancing their quality and promote their utilisation as functional ingredients. Till date, not much work has been reported on the effect of processing on quality of basil seeds. Henceforth, present study was carried out to assess the impact of traditional processing methods such as soaking, roasting, fermentation and germination on nutritional and antioxidant properties of indigenous basil seeds for the formulation of functional flours.

## Materials and Methods

### Sample collection and preparation

The clove (*O. gratissimum*) and sweet basil (*O. basilicum*) are widely distributed in the tropical areas of Andhra Pradesh (13.6288°N - 14.4674°N latitude and 78.8241°E - 79.4192°E). Till date, ethnic and indigenous tribes of Andhra Pradesh and rural people depended on them for primary health care[7]. In the present study, the clove and sweet basil seeds were procured from local farmers of Kadapa district of Andhra Pradesh, India. The seeds were cleaned manually and obtained pure seeds were pooled and utilised for further investigation (Plate 1).

### Traditional processing of basil seeds

The traditionally used minimal processing techniques such as soaking, roasting, fermentation and germination of basil seeds were employed to develop functional flours that can be utilised for the formulation of designer foods with health promoting ingredients for disease prevention.

#### *Development of soaked and roasted basil seeds flours*

Basil seeds when soaked in water, the outer pericarp was swollen and produced considerable amount of mucilage. The raw clove and sweet basil seeds were soaked in distilled water (1:40 w/v) for 4 h and 2 h respectively, drained of excess water, dried at 60°C for 4 h, milled into fine flours of 75 µ particle size[8]. The roasting of raw clove and sweet basil seeds was done in an open pan at 105°C for 3 min and 115°C for 5 min respectively with continuous stirring beyond which resulted in charring. The seeds were cooled, milled into fine flours of 75 µ particle size[9].

#### *Development of fermented basil seeds flours*

The raw clove and sweet basil seeds were allowed to ferment naturally with distilled water (1:40 w/v), curd (1:3 w/w) and honey (1:1 w/w) at 37°C in a BOD incubator for 18 h for clove basil seeds and 30 h for sweet basil seeds. Fermented seeds were drained of excess water, dried at 60°C for 4 h, milled into fine flours of 75 µ particle size[10].

#### *Development of germinated basil seeds flours*

The basil seeds were prechilled at 4°C for 2 days to reduce germination inhibitors and then allowed to germinate at 35°C in a BOD incubator for 60 h for clove basil seeds and 48 h for sweet basil seeds (Plate 2). The germinated basil seeds were dried at 60°C for 4 h and milled into fine flours of 75 µ particle size[11].

### Determination of ideal processing method for basil seeds flours formulation

The developed unprocessed and processed (soaked, roasted, fermented and germinated) basil seed flours (Plates 3 and 4) were assessed for their proximate composition (moisture, ash, protein, fat, crude fibre, carbohydrates and energy contents)[12], total phenols content[13] and total antioxidant capacity by DPPH radical scavenging assay[14] to determine ideal processing method. For determination of total phenols content and total antioxidant capacity, bioactive

Clove basil plant | Clove basil seeds | Sweet basil plant | Sweet basil seeds

Plate 1 — Basil plants and seeds


Plate 2 — Soaked and germinated basil seeds

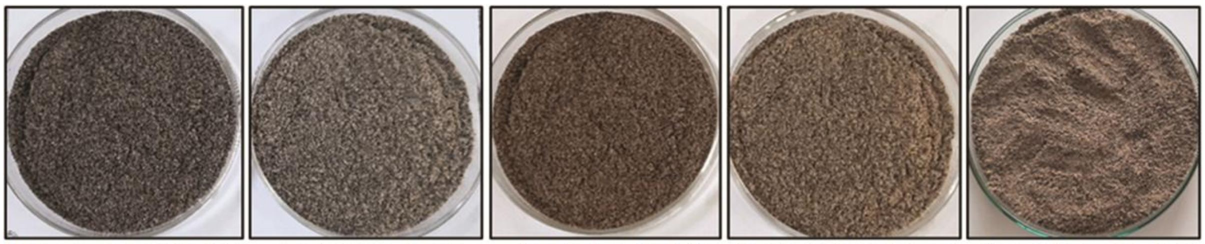


Plate 3 — Clove basil seeds flours

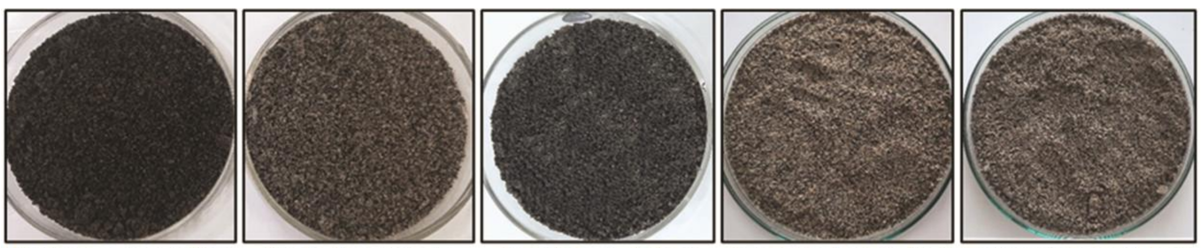


Plate 4 — Sweet basil seeds flours

constituents of basil seeds flours were extracted using 80.0% ethanol and distilled water as solvents.

#### Statistical analysis

The obtained results were statistically analysed by ANOVA and Duncan's multiple range test using IBM SPSS Statistics 20 at a confidence level of 99.0%. The Pearson correlation calculation was done using Microsoft Excel 2021.

## Results and Discussion

#### Proximate composition of processed basil seeds flours

The proximate composition of unprocessed and processed (soaked, roasted, fermented and germinated) clove and sweet basil seed flours was given in Table 1. The moisture, ash, protein, fat, fibre, carbohydrate and energy contents of unprocessed clove and sweet basil seeds flours were 9.07%, 4.04%, 9.16%, 14.27%, 36.23%, 27.24%, 274.00 Kcal/100 g and 8.20%, 5.36%, 8.55%, 19.61%, 28.85%, 29.43%, 328.44 Kcal/100 g respectively. Statistically significant difference was observed between the clove and sweet basil seed flours for proximate composition at $p \leq 0.01$. The findings were on par with the results reported by other researchers[15,16] where moisture, ash, protein, fat and fibre contents of Indian basil seeds ranged between 8.9-9.6%, 5.0-7.7%, 9.4-14.8%, 13.8-33.0% and 22.6-41.6%. The proximate composition of basil seeds may have varied in accordance to geographical location, altitude, soil properties, environmental conditions, agronomic management and degree of absorption of water. The interaction between seed variety and processing method employed had significantly influenced the proximate composition of basil seeds flours at 1.0% level.

The processed basil seeds flours exhibited less moisture content than unprocessed basil seeds flours. Soaking and fermentation have significantly decreased the moisture content of clove and sweet basil seeds flours by 45.13, 30.81% and 44.63, 30.79% respectively due to subsequent drying of seeds before pulverising them into fine flours. Roasting has significantly decreased the moisture

Table 1 — Proximate composition of raw and processed basil seeds flours

| Seeds | Processing method | Moisture (%) | Ash (%) | Protein (%) | Fat (%) | Crude fiber (%) | Carbohydrate# (%) | Energy## (Kcal/100 g) |
|---|---|---|---|---|---|---|---|---|
| Clove basil | Unprocessed | 9.07±0.01 | 4.04±0.01 | 9.16±0.01 | 14.27±0.01 | 36.23±0.01 | 27.24±0.03 | 274±0.05 |
| | Soaked | 4.98±0.01 | 4.15±0.00 | 10.30±0.01 | 10.23±0.01 | 37.88±0.01 | 32.46±0.02 | 263±0.04 |
| | Roasted | 2.04±0.01 | 4.73±0.01 | 7.32±0.01 | 16.27±0.01 | 33.77±0.01 | 35.87±0.04 | 319±0.01 |
| | Fermented | 6.28±0.01 | 4.66±0.01 | 10.82±0.01 | 11.61±0.01 | 38.68±0.01 | 27.96±0.02 | 260±0.03 |
| | Germinated | 4.13±0.00 | 4.87±0.01 | 11.96±0.01 | 13.02±0.01 | 39.76±0.01 | 26.26±0.02 | 270±0.05 |
| Sweet basil | Unprocessed | 8.20±0.01 | 5.36±0.01 | 8.55±0.01 | 19.61±0.01 | 28.85±0.01 | 29.43±0.03 | 328±0.03 |
| | Soaked | 4.54±0.01 | 5.72±0.01 | 8.90±0.01 | 15.78±0.01 | 29.61±0.01 | 35.45±0.04 | 319±0.03 |
| | Roasted | 1.10±0.01 | 5.96±0.01 | 8.37±0.01 | 22.56±0.01 | 25.60±0.01 | 36.41±0.03 | 382±0.04 |
| | Fermented | 5.67±0.01 | 5.82±0.01 | 10.12±0.01 | 17.72±0.01 | 29.69±0.01 | 30.97±0.02 | 324±0.05 |
| | Germinated | 2.94±0.01 | 6.02±0.01 | 12.22±0.01 | 18.19±0.01 | 31.52±0.01 | 29.06±0.03 | 329±0.04 |
| | Mean | 4.89 | 5.14 | 9.77 | 15.93 | 33.16 | 31.11 | 307 |
| | $SE_m$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 |

| | Moisture F value | Moisture CD at 1.0% | Ash F value | Ash CD at 1.0% | Protein F value | Protein CD at 1.0% | Fat F value | Fat CD at 1.0% | Crude fiber F value | Crude fiber CD at 1.0% | Carbohydrate F value | Carbohydrate CD at 1.0% | Energy F value | Energy CD at 1.0% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed variety (S) | * | 0.01 | * | 0.01 | * | 0.01 | * | 0.01 | * | 0.01 | * | 0.03 | * | 0.04 |
| Processing method (P) | * | 0.02 | * | 0.02 | * | 0.02 | * | 0.02 | * | 0.02 | * | 0.05 | * | 0.06 |
| S × P | * | 0.02 | * | 0.03 | * | 0.02 | * | 0.03 | * | 0.03 | * | 0.07 | * | 0.08 |

Note: #Calculated by difference method. ##Determined by computation. Values expressed as mean ± standard deviation of three determinations. $SE_m$: Standard error of mean, CD: Critical difference. * Significant difference at $p \leq 0.01$, NS: Non-significant difference.

content of clove and sweet basil seeds flours by 77.52 and 86.57% respectively. Further, germinated clove and sweet basil seeds flours have exhibited reduced moisture content by 54.51 and 64.13% respectively due to subsequent drying and mild roasting of seeds before pulverisation.

The processing of basil seeds had significantly enhanced the ash (total mineral) content of flour compared to unprocessed ones might be due to decline in antinutritional factors and increment in minerals bioavailability. Soaking and roasting have significantly improved ash content of clove and sweet basil seeds flours by 2.94, 17.10% and 6.73, 11.18% respectively at $p<0.01$. Similar findings were reported by other researchers[17], where soaking enhanced the ash content of garden cress seeds by 2.48%. While fermentation and germination have significantly improved ash content of clove and sweet basil seeds flours by 15.45, 20.78% and 8.68, 13.48% respectively due to activation of endogenous enzymes in seeds that promoted break down of antinutritional components and released bound minerals.

The soaking, fermentation and germination of clove and sweet basil seeds have significantly improved protein content of flours by 12.42, 18.15, 30.57% and 4.10, 18.43, 43.00% respectively compared to unprocessed flours. The increment was due to enhanced metabolic activity of seeds promoting protein synthesis, digestibility and total amino acids content of flours. While, roasting had significantly declined the protein content of clove and sweet basil seeds flours by 20.06 and 2.04% respectively at $p<0.01$. Other researchers[18] have also reported a significant negative association between the protein content of kernels and roasting temperature or duration. Roasting had significantly decreased the protein content of kernels regardless of roasting temperature or duration due to denaturation of proteins and participation of produced amino acids in Maillard reactions for characteristic colour and flavour development.

However, soaking, fermentation and germination of clove and sweet basil seeds have significantly decreased fat content of flours by 28.29, 18.62, 8.73% and 19.53, 9.64, 7.27% respectively compared to unprocessed flours due to increased lipolytic activity. Similar findings were reported by[19,20] where soaking and germination have significantly improved the protein content (35.1 and 7.08%) and reduced fat content (4.6 and 19.85%) of fenugreek seeds flours. Roasting had significantly enhanced fat content of clove and sweet basil seeds flours by 14.06 and 15.02% respectively as application of dry heat promoted release of fat due to damage of lipoprotein membranes surrounding lipid bodies[17].

Further, soaking, fermentation and germination of clove and sweet basil seeds have significantly improved fibre content of flours by 4.56, 6.75, 9.75% and 2.64, 2.90, 9.25% respectively at $p<0.01$ due to formation of mucilage upon hydration that mainly constituted of soluble dietary fibre. Likewise, other researchers[17] also reported increment in fibre and protein contents of garden cress seeds by 5.03 and 2.10% respectively due to mucilage formation and fibre-protein complex formation, a possible chemical modification induced by soaking. The increase in fibre content during germination was also due to synthesis of structural polysaccharides like cellulose, hemi-cellulose and lignin to promote radicle and shoot elongation. While, roasting had significantly declined fibre content of clove and sweet basil seeds flours by 6.78 and 11.26% respectively due to breakdown of mucilage forming hydrophilic polysaccharides. Likewise, other researchers[17] also reported decline in fibre and protein contents of garden cress seeds by 2.29 and 1.93% respectively.

The processed clove and sweet (except germinated) basil seeds flours exhibited significantly higher carbohydrate content than unprocessed flours at $p<0.01$. Soaking, roasting and fermentation improved carbohydrate content by 19.16, 31.68, 2.64% and 20.46, 23.70 and 5.23% respectively for clove and sweet basil seeds flours. As carbohydrate content of flours was calculated by difference method, the decreased moisture, fat contents on soaking and fermentation, decreased moisture, protein and fat contents on roasting resulted in increased values for carbohydrate content. The germinated clove and sweet basil seeds flours exhibited significantly lower carbohydrate content by 3.59 and 1.27% respectively due to increased α-amylase activity that promoted break down of complex carbohydrates into simpler and more absorbable sugars.

The calorific value of clove and sweet basil seeds flours decreased with soaking, fermentation treatments by 3.98, 5.25% and 2.74, 1.39% respectively while enhanced with roasting treatment by 16.50 and 16.36% respectively due to altered fat content. Germination had slightly reduced calorific value of clove basil seeds flour by 1.44% while of sweet basil seeds flour was improved by 0.11%. As total energy content of flours was determined by computation, the change in protein, fat and carbohydrate contents upon processing resulted in altered calorific content.

The processed basil seeds flours with better nutritional profile (high fibre, protein, mineral contents and low-fat content) can be utilised for fortification of regularly consumed foods such as pasta, beverages, bakery products *etc.* Other researchers[9] have also reported that the incorporation of roasted basil seeds had improved the protein, fat, energy, fibre and ash contents of idlis. Further,[21] reported that idlis made with 20% basil seeds exhibited low glycaemic index (48.09) and high fibre, resistant starch contents beneficial for obese, diabetic and cardiac disorder populations.

**Total phenols content of processed basil seeds flours**

The total phenols content of ethanol and aqueous extracts of unprocessed and processed (soaked, roasted, fermented and germinated) clove and sweet basil seeds flours in terms of gallic acid equivalent (GAE) was depicted through a heat map (Fig. 1A). The ethanol extracts of basil seeds flours exhibited higher phenols content than aqueous extracts. The total phenols content of ethanol extracts of clove and sweet basil seeds flours was 17.28 and 17.71 mg GAE/g respectively while of aqueous extracts was 3.92 and 4.38 mg GAE/g respectively. The soaking of basil seeds had significantly reduced the total phenols content of clove and sweet basil seeds flours by 11.69 and 24.51% respectively for ethanol extracts and by 47.88 and 28.03% respectively for aqueous extracts due to leaching of phenols into soaking medium.

The roasting, fermentation and germination have improved phenols content of clove basil seeds flours by 8.43, 3.51 and 21.67% respectively for ethanol extracts and by 55.05, 18.40 and 100.00% respectively for aqueous extracts. While roasting, fermentation and germination have improved phenols

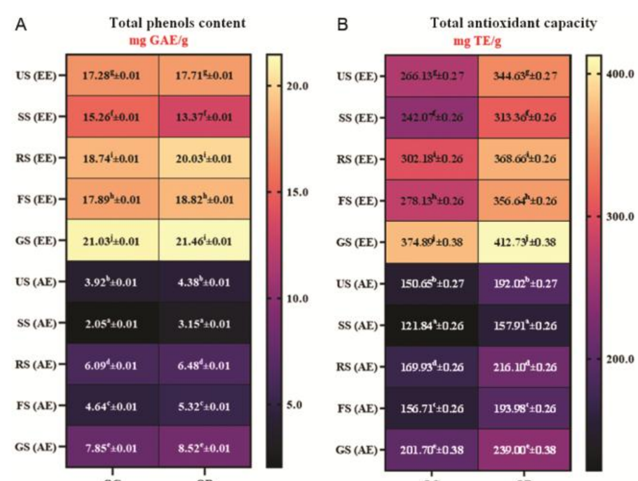


Fig. 1 — Heat maps depicting total phenols content and total antioxidant capacity of unprocessed and processed basil seeds extracts Note: Values presented as mean ± standard deviation of two determinations. Means within the same column followed by different superscripts differed significantly at $p \leq 0.01$. GAE: Gallic acid equivalent, TE: Trolox equivalent, OG: *O. gratissimum* seeds, OB: *O. basilicum* seeds, US: Unprocessed seeds, SS: Soaked seeds, RS: Roasted seeds, FS: Fermented seeds, GS: Germinated seeds, EE: Ethanol extract, AE: Aqueous extract

content of sweet basil seeds flours by 13.10, 6.28 and 21.19% respectively for ethanol extracts and by 48.03, 21.61 and 94.60% respectively for aqueous extracts. Other researchers[19,22] have also reported similar findings, where roasting and germination of fenugreek seeds have improved the phenolic content of flours by 5.99-6.82 and 45.56-77.975 respectively.

Roasting resulted in disruption of cell structure and development of Maillard reaction products that promoted release of bound phenols with reactive oxygen species scavenging capacity. Fermentation promoted biosynthesis of phenolics, structural breakdown of cell walls liberating phenols and increased activity of β-glucosidase that hydrolysed phenolic glycosides for release of free phenols[23]. Germination promoted biosynthesis, bioaccumulation and degradation of polymerized polyphenols especially hydrolysable tannins and hydrolysis of other glycosylated flavonoids[22].

A daily intake of phenols of ~200 mg/day reduced disease risk and enhanced human health. Phenolic compounds were readily absorbed through intestinal tract walls and served as antioxidants to avert cell damage from free radicals[24]. In such scenario, processed basil seeds flours can be utilised potentially for development of functional foods with better phenolic profile.

#### Total antioxidant capacity of processed basil seeds flours

The total antioxidant capacity of ethanol and aqueous extracts of unprocessed and processed (soaked, roasted, fermented and germinated) clove and sweet basil seeds flours in terms of trolox equivalent (TE) was depicted through a heat map (Fig. 1B). The ethanol extracts of basil seeds flours exhibited better antioxidant ability to scavenge DPPH radicals than aqueous extracts. The total antioxidant capacity of ethanol extracts of clove and sweet basil seeds flours was 266.13 and 344.63 mg TE/g respectively while of aqueous extracts was 150.65 and 192.02 mg TE/g respectively.

The soaking of basil seeds had significantly reduced total antioxidant capacity of clove and sweet basil seeds flours by 9.04 and 9.07% respectively for ethanol extracts and by 19.12 and 17.76% respectively for aqueous extracts due to decline in total phenolic content. The roasting, fermentation and germination have improved total antioxidant capacity of clove basil seeds flours by 13.54, 4.51 and 40.87% respectively for ethanol extracts and by 12.80, 4.02 and 33.88% respectively for aqueous extracts. While roasting, fermentation and germination have improved total antioxidant capacity of sweet basil seeds flours by 6.97, 3.48 and 19.76% respectively for ethanol extracts and by 12.54, 1.02 and 24.47% respectively for aqueous extracts due to enhanced phenolic content. Similar findings were reported by other researchers[19], where roasting and germination of fenugreek seeds have improved the antioxidant activity of flours by 76.80 and 308.29% respectively.

Roasting promoted generation of Maillard reaction products such as melanoidins and Amadori rearrangement products that possessed ability to chelate metals and scavenge oxygen free radicals. Fermentation induced breakdown of cell walls that led to liberation or synthesis of various antioxidant compounds[25]. Germination promoted synthesis of antioxidant vitamins (vitamin C) and other antioxidant compounds (phenols, flavonoids) and enhanced total antioxidant capacity of flours. The total antioxidant capacities of clove and sweet basil seeds flours were positively correlated with their total phenolic contents at $p<0.05$ ($r = 0.9665$ and 0.9934 respectively). Thus, processing technique and extraction solvent employed have greatly influenced total phenolics content and antioxidant capacity of basil seeds extracts.

### Conclusion

Minimal processing of indigenous basil seeds helped in significant enhancement of their nutritional and antioxidant profile that can be beneficial in promoting health and disease prevention. Among different processing techniques employed, germination was found to be ideal processing method for development of functional flours with comparatively better fibre, protein, mineral, phenolic contents and antioxidant capacity and reduced fat content. The formulated flours can be efficiently utilised as functional ingredients for development of designer foods for health-conscious food consumers from novel traditional sources.

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### Conflict of Interest

There is no conflict of interest declared by the authors.

### Authors' Contributions

NB has presented the idea, carried out research work, wrote manuscript and performed data analysis. VKG and SBS have supervised the research work.

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