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Impact of Soaking and Germination Time on Nutritional Composition and Antioxidant Activity of *Nigella Sativa*

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Abstract

Indian traditional dishes make use of *Nigella sativa* seeds because of its distinctive aroma and taste but its application is restricted due to its pungent flavour which can be overcome by various methods of processing such as roasting, soaking, germination etc. Soaking and germination have positive impact on the nutritional, sensorial and phytochemical attributes of *Nigella sativa*. The current study was carried out to standardize the optimum soaking and germination conditions with improved nutritional, sensorial and phytochemical attributes of seeds. *Nigella sativa* seeds were soaked for different time duration (0 hr, 6 hr, 12 hr and 18 hr) followed by germination for 0, 7, 9 and 11 days. The soaked and germinated samples were exposed to a temperature of 50ºC for drying till the constant moisture was obtained and analysed for various physiochemical, nutritional and sensory attributes. All the attributes under study were significantly affected by soaking and germination as compared to the control. Comparatively, except the moisture content all the phytochemicals and nutritional attributes decreased significantly from the control sample. Based on the qualitative attributes, soaking done for 18 hours along with 9th day of germination was observed to be the best and can be considered as the optimum condition to maintain the phytochemical to the safe level as well as reducing the antinutritional factors which can further be used for value addition.

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Introduction

Black cumin (*Nigella sativa*) also known as "Kalonji" in South Asian countries, is a good source of nutritionally essential components including proteins, carbohydrates, fatty acids and appreciable amount of minerals.[1] It also contains variety of health beneficial ingredients, including thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, thymol etc. which regards *Nigella sativa* seed as a valuable remedy for a number of ailments including asthma, bronchitis, rheumatism and related inflammatory diseases.[2] Due to its unique composition it possesses the pungent flavour and utilized traditionally in wide range of food products such as pickles, coffee, bread recipes etc.[3] Recently researchers took a shift to utilize these seeds by incorporating them in various food products such as tea, coffee, pickles, bread recipes and savoury dishes.[3] But its pungency is an obstacle in its way of utilization in food industry which can be decreased by various food processing methods including roasting, soaking, germination etc. In our earlier attempt, a technology for roasting of *Nigella sativa* seeds have been developed using specified roasting condition i.e., 180 ^{o}C for 20 minutes, but still the application for soaking and germinating is lacking which is the need of the hour. Therefore, the present study was carried out to fulfill the research gap.

Germination of seeds results in alteration of various biochemical and nutritional parameters that contributes in enhancing its nutritional status and results in favourable as well as advantageous impact on human health. The process incorporated is inexpensive and effective which involves utilization of seed reserves to supply energy during respiration and synthesis of new cells before embryo development, enhance digestibility and increase nutritive values thereby reducing phytic acid and tannin content in the seed.[4] Soaking of the seeds in water must precede germination. Traditional processing methods (soaking and germination) initiate curtailing the bitterness of the seeds and thus, making it feasible to incorporate in various recipes. But those methods are still not standardized which is the current demand of the present era.

Material and Method

Nigella sativa seeds were procured from the local market of Jalandhar, India. Seeds were cleaned and were soaked in distilled water in the ratio 1:3 (w/v) at room temperature for different time durations (0 hr, 6 hr, 12 hr and 18 hr). The excess water was drained and humid condition was maintained for seeds to germinate at room temperature at 28 ± 2º C for 0, 7, 9 and 11 days respectively. Untill the constant moisture content was achieved, seeds were dried at 50ºC in a tray drier (Labfit, India) in trays (37.7×15.4) with the feed rate of 150 g/m^2. Raw seeds i.e., without any treatment served as a control. Super mill grinder was used to obtain the flour of all the samples. Airtight containers were chosen to store the respective samples following grinding and sieving for further analysis of nutritional, physicochemical, sensorial attributes.

Physicochemical Analysis

The concentration of moisture and ash of various samples i.e., control, soaked and germinated seed flour was analyzed as described in AOAC.[5] Estimation of total phenol was done by following Folin Ciocalteu method given by Singleton and Rossi[6] and was expressed as Gallic acid equivalents in mg per g dry weight (mg GAE/g DW). Antioxidant activity (DPPH free radical scavenging activity) was determined by following the method given by Brand-Williams *et al.*,[7] and was expressed as percent inhibition. Method described by Sadasivam and Manickam[8] was followed for colorimetric determination of tannin content and was expressed as tannic acid equivalents in mg per g dry weight (mg TAE/g DW). Carbohydrate content was analyzed colorimetric by phenol sulphuric method.[8] Protein content (nitrogen × 6.25) was estimated in the form of nitrogen using Kjeldhal apparatus (Kel Plus; Pelican Equipments, India).[5] Total flavonoid content was determined by a colorimetric method as described previously .[9] Phytic acid estimation of all the samples was estimated using the procedure outlined by Rusydi *et al.*,[4]

Sensory Analysis

Sensory analysis of all the samples was carried out using a nine point hedonic scale as described by Amerine *et al.*,[10] Samples were evaluated at 29 ± 2^{o}C room temperature for colour, taste, aroma and flavour attributes by a panel of 10 semi-trained members from Department of Food Technology and Nutrition, Lovely Professional University, Phagwara, India. The mean score of all attributes was used to draw overall acceptability of the product.

Table 1: Effect of different soaking and germination time on the quality characteristics of *Nigella sativa*

Treatments	Moisture (%) (wet basis)	Ash (%)	Phenolic content (mg GAE/100g DW)	Antioxidant activity (%)	Tannin (mg/100g)	Carbohydrate (%)	Protein (%)	Flavonoid (mg/100g)	Phytic acid (mg/100g)	Overall Acceptability
Control	8.14±0.02*,a**	2.72±0.02[a]	154.80±8.03[a]	84.27±4.03[a]	68.59±1.03[a]	39.50±0.03[a]	21.66±0.6[a]	12.83±0.03[a]	64.08±1.04[a]	4.2±0.4[a]
6 hour soaking 0 day germination	8.38±0.07[b]	2.67±0.06[b]	115.51±8.05[b]	74.39±4.02[b]	67.83±1.02[b]	30.15±0.04[b]	22.42±0.5[b]	9.50±0.05[b]	46.71±1.02[b]	5.1±0.3[b]
12 hour soaking 0 day germination	8.90±0.04[c]	2.67±0.02[c]	107.41±8.02[c]	73.97±4.06[c]	67.54±1.05[c]	30.11±0.06[c]	23.73±0.3[c]	9.02±0.02[c]	46.53±1.03[c]	5.6±0.1[c]
18 hour soaking 0 day germination	9.27±0.04[d]	2.65±0.04[d]	104.71±7.01[d]	73.91±3.01[d]	59.50±1.04[d]	30.07±0.07[d]	24.68±0.1[d]	7.94±0.04[d]	47.52±1.07[d]	5.7±0.2[d]
6 hour soaking 7 day germination	8.29±0.08[e]	2.63±0.06[e]	110.54±8.04[e]	67.98±4.04[e]	65.67±1.06[e]	29.34±0.01[e]	22.94±0.3[e]	9.35±0.02[e]	45.09±1.08[e]	6.3±0.5[e]
12 hour soaking 7 day germination	10.71±0.01[f]	2.63±0.01[f]	101.36±7.06[f]	67.15±4.06[f]	62.33±1.01[f]	29.17±0.05[f]	22.21±0.2[f]	7.71±0.01[f]	45.99±1.01[f]	6.8±0.4[f]
18 hour soaking 7 day germination	12.36±0.06[g]	2.60±0.01[g]	94.23±7.04[g]	67.33±3.02[g]	64.47±1.02[g]	29.13±0.01[g]	24.52±0.7[g]	7.02±0.03[g]	44.46±1.02[g]	6.8±0.5[g]
6 hour soaking 9 day germination	9.69±0.02[h]	2.58±0.07[h]	95.67±6.07[h]	65.58±4.07[h]	61.36±1.08[h]	29.35±0.04[h]	20.74±0.6[h]	8.39±0.06[h]	44.19±1.05[h]	7.1±0.3[h]
12 hour soaking 9 day germination	12.70±0.07[i]	2.55±0.04[i]	94.95±8.05[i]	65.93±3.01[i]	60.74±1.01[i]	29.11±0.02[i]	19.69±0.8[i]	7.68±0.07[i]	43.65±1.04[i]	7.4±0.3[i]
18 hour soaking 9 day germination	13.58±0.05[j]	2.53±0.03[j]	89.27±7.05[j]	65.77±3.02[j]	59.51±1.07[j]	29.08±0.06[j]	19.48±0.4[j]	6.50±0.06[j]	42.39±1.06[j]	7.9±0.3[j]
6 hour soaking 11 day germination	12.91±0.02[k]	2.55±0.01[k]	91.57±6.01[k]	62.90±3.04[k]	53.28±1.05[k]	28.95±0.06[k]	14.70±0.2[k]	7.64±0.03[k]	42.93±1.01[k]	6.3±0.5[k]
12 hour soaking 11 day germination	13.81±0.01[l]	2.50±0.06[l]	90.20±6.03[l]	62.75±3.03[l]	50.10±1.03[l]	28.61±0.03[l]	13.91±0.1[l]	6.32±0.02[l]	37.17±1.06[l]	6.2±0.5[l]
18 hour soaking 11 day germination	14.09±0.06[m]	2.44±0.02[m]	85.37±6.05[m]	62.62±3.01[m]	44.29±1.02[m]	28.55±0.05[m]	11.13±0.3[m]	6.23±0.01[m]	35.10±1.03[m]	6.3±0.5[m]

*Mean ± SD (n=3)

** Different lowercase case superscripts in the same column indicate the significant difference ($p<0.05$)

Statistical Analysis

GraphPad Prism (La Jolla, CA, USA) (version 5.01) software was used for the statistical analysis of the data and the results were expressed as means ± SEM. Differences between the means were tested for the statistical significance using a one-way ANOVA and followed by Bonferroni post hoc test and the significance level was set at 5% ($P \leq 0.05$). To get comparative comprehensive overview of effect of soaking and germination on the physicochemical attributes of *Nigella sativa* flour; rescaled cluster analysis of data was performed. The output obtained was plotted as a dendrogram and the interpretation of data was made - accordingly.

Results and Discussion

Effect of Soaking and Germination on Physico-chemical Attributes

Soaking is the process of softening and saturating the spice by immersing in water thereby decreasing the amount of anti-nutrients and increasing the enzymatic activity which will further initiate germinat-

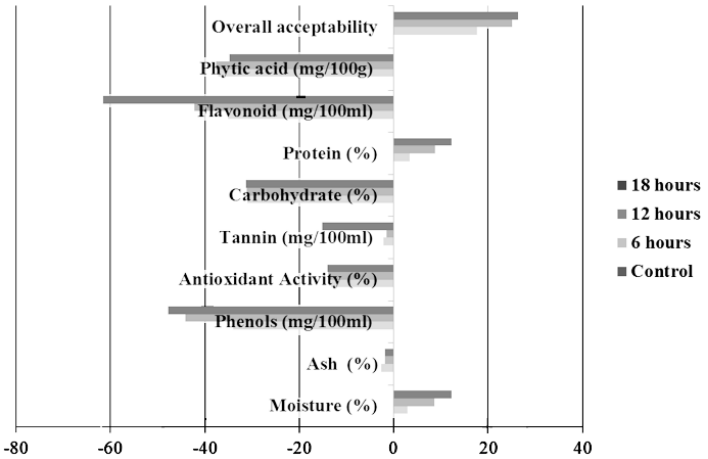

Fig. 1: Effect of soaking time (hours) on nutritional, physiochemical and sensorial attributes of *Nigella sativa* as compared to control

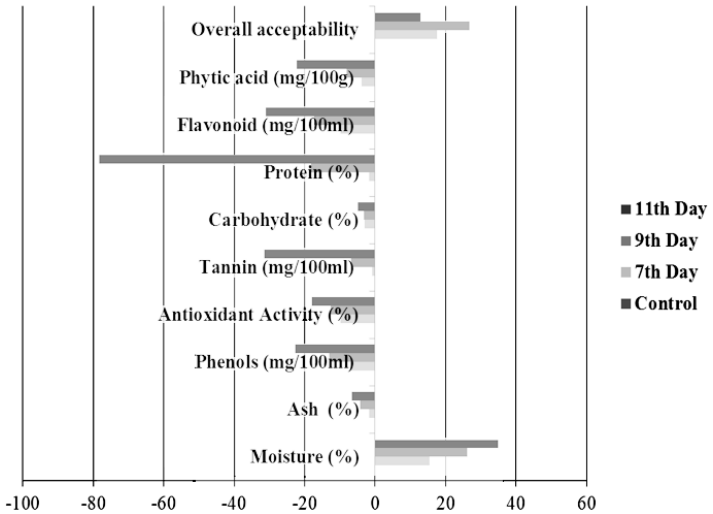

Fig. 2: Effect of germination time (days) on nutritional, physiochemical and sensorial attributes of *Nigella sativa* as compared to control

ion. Impact of soaking and germination on nutritional, physicochemical and sensorial attributes of the flour formulated by soaking and germinating *Nigella sativa* is tabulated in Table 1. Almost all the variables under study decreased significantly as compared to the control except the moisture, protein and overall acceptability, which increased during soaking (Figure1) whereas, increase in moisture and overall acceptability was observed in case of germination (Figure 2).

Raw *Nigella sativa* seed contained moisture (8.14 ± 0.02%), ash (2.72 ± 0.02%), phenolic content (154.80 ± 8.03mg GAE/100gDW), antioxidant activity (84.27 ± 4.03%), tannin (68.59 ± 1.03 mg/100g), carbohydrate (39.50 ± 0.03%), protein (21.66 ± 0.6%), flavonoid (12.83 ± 0.03 mg/100g), phytic acid (64.08 ± 1.04 mg/100g) and overall acceptability (4.2 ± 0.4). Raw seeds had higher amount of ash, carbohydrates, antioxidant activity, phenolic content, tannins, flavonoid and phytic acid in comparison to soaked and germinated *Nigella sativa* seeds.

The moisture content of *Nigella sativa* flour depicts that the powder was dry enough i.e., ranged from 8.14 ± 0.02% to 14.09 ± 0.06%. Variation in moisture content with different soaking time might be the result of breakage in cell wall of seed thereby absorbing water rapidly. Similar results have been reported by Nonogaki *et al.*,[11] Fouad *et al.*,[12] and Handa *et al.*,[13] stating further that there is increase in number of cells within the seed during germination, thus making it gain more moisture. Soaking and germination decreased the ash content from 2.72 ± 0.02% to 2.44 ± 0.02% which might be due to the leaching of the minerals in the soaked water with increasing time duration.[14]

With the advancement in the soaking time, decrement in the phenolic, flavonoid and antioxidant content of flour was also observed i.e., from 154.80 ± 4.03 to 85.37 ± 6.05 GAE mg/100gm; 12.83 ± 0.03 to 6.23 ± 0.01 mg/100g and 84.27 ± 4.03% to 62.62 ± 3.01% respectively. Water-soluble phenols and other components are leached into the water when soaking and germination time was increased as supported by Deshpande *et al.*,[15] Igbedioh *et al.*,[16] Paramjyothi and Anjali.[17] Studies also support this decrease due to the presence of phenolics along with other bioactive components including vitamins and carotenoid[18-20] that can also affect the antioxidant activity of the samples. With increase in soaking

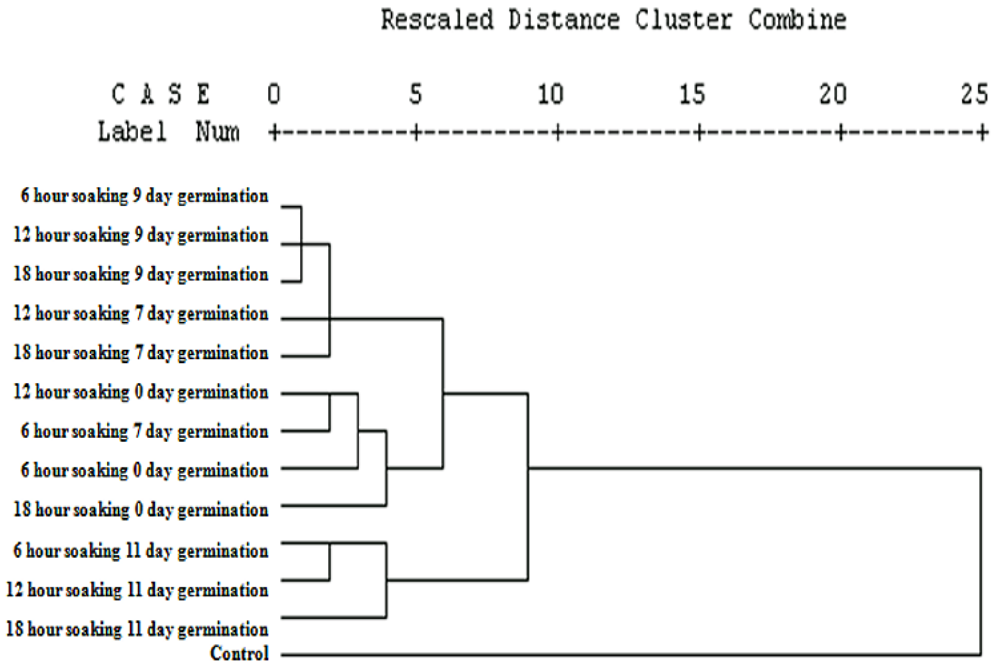

Fig. 3: Cluster analysis of different time intervals selected for soaking and germination of Nigella sativa

time, a significant decrease in tannin content (68.59 ± 1.03 to 44.29 ± 1.02 mg/100g) was observed. The decline might be due to extractability of these low molecular weight polyphenolic compounds in water.[13] Moreover binding of polyphenols with other organic substances such as carbohydrate or protein makes tannin unavailable.[15,21]

Carbohydrate content of *Nigella sativa* flour showed a decrease from 39.50 ± 0.03% to 28.55 ± 0.05% with increase in soaking and germination time which might be due to the breakdown and utilization of carbohydrates by the growing sprouts as the source of energy for the young seedlings.[22] During soaking, various complex compounds undergo biological breakdown into simpler compounds as proposed by Narsih and Harijono[23] and thus with enhancement in the soaking time a significant increase in total protein content was observed i.e., from 21.66 ± 0.6 to 24.68 ± 0.1%. Also, a drop in concentration of protein was observed with increase in germination time i.e., from 21.66 ± 0.6 to 11.13 ± 0.3% which might be the result of increased degree of protease activity during germination as described by Torres *et al.*,[24] and Pal *et al.*,[25] Soaking and germination of seeds is also responsible for the reduction in phytic acid content i.e., from 64.08 ± 1.04 to 35.10 ± 1.03mg/100g. The germination process originates the phytase activity which was negligible in the raw unprocessed seeds as suggested by El- Mahdy and El- Sebaiy[26] and reduction of phytic acid content in seeds after germination was followed by the increase in phosphatase activity.[27]

Soaking and germination improved the overall acceptability of the sprouted seed flour up to a significant level. With the enhancement in the soaking duration there was increase in the overall acceptability which might be the result of the leached tannins, phytic acids and other bitter components[28] thereby enhancing the sensory characteristics. Germination contributed in enhancing the sensorial characteristics up to the 9th day of germination which might be due to the enzyme production and other changes occurring during germination process.[13] Further increase in the germination time resulted in a sharp decrease of the overall acceptability which might be due to the detrimental effect of germination especially on sugars (a result of starch hydrolysis) and amino acids (a result of proteins hydrolysis) which leads to Maillard reaction and proceeded with deterioration of colour. Whereas, an abundant amount of free sugars and amino acids is another a strong reason of fermentation during long germination period and may be responsible for the deterioration of flavour and overall acceptability of the germinated mass.[29]

Cluster Analysis

The data obtained from physico-chemical analyses of soaked and germinated *Nigella sativa* was subjected to rescaled distance cluster analysis and the results are shown in Figure 3. It is evident from the figure that there was formation of various clusters based on the duration(time) for which Nigella seeds were soaked and germinated which reveals that there is a significant difference among the various treatments in comparison with the control sample (unprocessed). Thereafter, first cluster comprises of the samples germinated for 0, 7 and 9 days whereas, second cluster comprises of the samples germinated for 11 days, which indicated that among the various soaking and germination time, germination had a significant effect on the phytochemical potential of *Nigella sativa* seeds as compared to the soaking duration.

Conclusions

It is apparent from the present study that soaking and germination of *Nigella sativa* seeds exerted a significant effect on physicochemical, phytochemical and sensorial attributes. Undoubtedly, a significant decrease was observed in the various parameters i.e., the antioxidant activity, total phenols and flavonoids on soaking followed by germination, but a significant decrement was also noticed in the anti-nutritional factors including tannins and phytic acid which will definitely improve the processing quality of the *Nigella sativa* seed flour thereby further increasing its utilization in food industries. The optimized conditions i.e. 18 hours soaking and 9th day germination can be successfully used for soaking and germination at farm level or cottage scale industries which will definitely initiate the utilization of *Nigella sativa* seeds.

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