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# Optimisation of Clove Basil and Sweet Basil Seeds Mucilages Extraction for Utilisation as Functional Ingredients

B Neeharika* & K G Vijayalaxmi

Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bengaluru 560 065, Karnataka, India

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The hydrothermal extraction of clove basil and sweet basil seeds mucilages were optimised using response surface methodology for maximum yield, water absorption, swelling capacities, minimum water solubility and syneresis. The optimum conditions for extraction of clove basil seeds mucilage were 67.34°C, 2.51 h and water-to-seed ratio of 80:1 while of sweet basil seeds mucilage were 57.01°C, 1.31 h and water-to-seed ratio of 70.66:1, which provided yield of 32.53, 26.30% respectively with water absorption capacity of 383.42, 474.34 g/g, water solubility capacity of 0.11, 0.70%, swelling capacity of 16.29, 13.23 mL/mL and syneresis of 0.50, 0.19% respectively. The experimental values of both mucilages extracted under optimum conditions were very close to predicted values indicating models were adequate. These extracted basil seed mucilages can be utilized as fat replacers, gelling agents, texture improvers, stabilizers, emulsifiers, thickening agents, prebiotics, laxatives, hypolipidemic and hypoglycemic agents to enhance the functionalities of incorporated foods.

**Keywords:** Functional parameters, Hydrocolloid, *Ocimum* seeds, Response surface methodology

## Introduction

The clove basil (*Ocimum gratissimum* L.) and sweet basil (*Ocimum basilicum* L.) are herbaceous plants with culinary and therapeutic applications attributed to their aromatic compounds. They belong to the *Lamiaceae* family and are indigenous to tropical and subtropical regions of southern Asia and Africa.[1] Both the clove basil and sweet basil seeds extrude gelatinous mucilage when immersed in aqueous solutions. The mucilage-producing ability made them suitable for use in traditional drinks (sharbat) and frozen desserts (falooda) for aesthetic purpose.[2]

Plant-based mucilages can be utilised as potential functional food ingredients owing to their laxative, prebiotic, hypolipidemic, anti-hyperglycaemic, antioxidant and antibacterial effects.[3] Basil seeds mucilage contained significant amounts of soluble fibre (96.6%) and has a huge potential as a functional ingredient for enriching foods.[4] In recent years, food industries are exploring vegan hydrocolloids for the development of innovative food products with unique sensory and nutritional characteristics. Basil seeds mucilages could serve as a novel source of hydrocolloids with broad-spectrum applications in the food industry as fat replacers, texture improvers, thickening agents, gelling agents, stabilizers and emulsifiers.[5]

The investigation on extraction of functional hydrocolloids from novel plant-based sources for their utilisation in food products has been a research trend. However, the efficiency of their extraction was influenced by multiple factors such as extraction temperature, time and water to raw material ratio *etc*. These parameters could affect the exhibited functional characteristics of mucilages either independently or interactively. In such conditions, where multiple factors and interactions affect the desired variables, Response Surface Methodology (RSM) acts as an effective tool for optimizing the extraction process.[6]

Other researchers[6–8] have also hydrothermally extracted mucilages from *Ximenia americana* seeds, Thai hoary basil seeds, Kashmiri sweet basil seeds under conditions optimised for better yield. However, not much research has been carried out on the optimisation of clove basil and sweet basil seeds mucilage extraction conditions based on their affected functional parameters to promote their utilisation in novel food products development. Henceforth, to address the gap between demand for vegan hydrocolloids and utilisation of basil seeds as a source of functional hydrocolloid, the protocols were standardised using RSM for mucilage extraction from

*Author for Correspondence
E-mail: bneeharika05@gmail.com

clove basil and sweet basil seeds in a cost-effective manner with optimised yield and functionalities.

## Materials and Methods

### Sample Collection and Preparation

The clove basil and sweet basil seeds were procured from the local markets of Kadapa district, Andhra Pradesh, India. The seed samples were pooled, cleaned manually and freed from broken seeds, other variety seeds and inert matter. The obtained pure seeds were stored in airtight containers until further use.

### Experimental Design

The study aimed to optimise the mucilage extraction process from clove basil and sweet basil seeds for better yield and functional properties. Henceforth, RSM was employed to study the effect of independent variables of hydrothermal mucilage extraction process from basil seeds *viz.* temperature ($X_1$), time ($X_2$) and distilled water-to-seed ratio (v/w) ($X_3$) on the responses *viz.* yield ($Y_1$), water absorption index ($Y_2$), water solubility index ($Y_3$), swelling index ($Y_4$) and syneresis ($Y_5$) of mucilage. The design expert software of version 8.0.6 was employed for statistical analysis and to generate the experimental designs.

The Box-Behnken Design (BBD) was employed to generate the response surfaces using fewer experimental runs than a normal factorial technique. The BBD decreased the number of treatment combinations required to avoid extremes and helped in utilisation of time and material resources efficiently. Each independent variable had three levels, which were coded as −1, 0 and +1 corresponding to low, mid and high levels.[9] A total of 17 different combinations (including five replicates of centre point) were chosen in random order according to BBD configuration for three factors. The maximum and minimum values for extraction temperature were 50°C and 80°C, where extraction time ranged from 1 to 3 h with water-to-seed ratio between 50:1 and 80:1.

### Model Fitting and Validation

The $R^2$ (coefficient of determination) is the proportion of variation in response attributed to model rather than to random error. It has been suggested that for a well-fitted model, the $R^2$ should not be < 0.95.[(10)] For the model fitting of variations in responses *i.e.,* dependent variables, the sequential sum of squares was analysed. The analysis showed that adding cubic terms significantly improved the models of all responses. Therefore, the second-order polynomial equations with extended cubic interactions were employed. These models can be referred to as reduced cubic models. The Eq. (1) depicted the response function (Y):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{133} X_1 X_3^2 + \beta_{223} X_2^2 X_3 + \beta_{233} X_2 X_3^2 \quad \ldots (1)$$

where $\beta_0$ is constant, $\beta_1$, $\beta_2$, $\beta_3$ are linear coefficients, $\beta_{12}$, $\beta_{13}$, $\beta_{23}$ are interaction coefficients, $\beta_{11}$, $\beta_{22}$, $\beta_{33}$ are quadratic coefficients and $\beta_{133}$, $\beta_{223}$, $\beta_{233}$ are cubic coefficients.

The adequacy and fitness of these models were tested by Analysis Of Variance (ANOVA) and $R^2$. To visualise the relationship between the response and experimental levels of each factor, the fitted polynomial regression equations were expressed graphically as three-dimensional response surface and two-dimensional contour plots. Finally, the optimisation of independent variables of extraction was done for better yield and functionalities of basil seed mucilages.

### Hydrothermal Extraction of Mucilages from Basil Seeds

The mucilages were extracted by adding distilled water to basil seeds (50:1 to 80:1 v/w) maintained at temperature of 50–80°C and continuously stirred using a magnetic stirrer under reflux conditions during extraction period of 1–3 h. Later, the mucilages were separated from basil seeds by passing the slurries through 150 µm mesh sieve (A.S.T.M. no. 100) and pressing with a rubber spatula on the mesh screen. The separated mucilages and seed suspensions were dried at 45°C for 12 h in a tray drier. The adhered mucilages from dried seeds were separated by rubbing them over a 150 µm mesh sieve. Finally, the obtained dried mucilages were pooled, finely ground and their weights were recorded.

### Yield of Basil Seeds Mucilages

The basil seed mucilages obtained from various experimental runs were weighed and their yields were calculated using Eq. (2).[6]

$$\text{Mucilage yield} = \frac{\text{Weight of extracted mucilage after drying (g)}}{\text{Weight of basil seeds taken for extraction (g)}} \times 100 \quad \ldots (2)$$

### Water Absorption and Solubility Capacities of Basil Seeds Mucilages

The aqueous dispersions (0.2% w/v) of basil seed mucilages of different experimental runs were made in pre-weighed centrifuge tubes, allowed to stand at room temperature for 30 min and centrifuged at 6000 rpm for 20 min at 20°C to obtain clear supernatants, which were decanted into petri dishes of known weights. The Water Absorption Capacity (WAC) of basil seed mucilages was the weight of suspension obtained after the removal of supernatant per unit of original weight.[11]

$$\text{WAC (g/g)} = \frac{\text{Weight of sediment (g)}}{\text{Weight of sample taken (g)}} \quad \ldots (3)$$

The supernatants in petri dishes were oven-dried at 105°C until they reached constant weights. The Water Solubility Capacity (WSC) of basil seed mucilages was the weight of dry solids in supernatant expressed as the per cent original weight of the sample.

$$\text{WSC (\%)} = \frac{\text{Weight of dissolved solids in the supernatant (g)}}{\text{Weight of the sample taken (g)}} \times 100 \quad \ldots (4)$$

**Swelling Capacity of Basil Seeds Mucilages**

The basil seed mucilages of various experimental runs were added to a definite volume of water (0.2% w/v) and left undisturbed at room temperature. The volume occupied by the mucilages was measured every hour until they reached constant volume. Then the supernatants were decanted and the volume of the final swollen gels was noted. The swelling index of basil seed mucilages was calculated using following Eq. (5) given by [12].

$$\text{SC (mL/mL)} = \frac{\text{Final volume of swollen sample (mL) -Initial volume of sample (mL)}}{\text{Initial volume of sample (mL)}} \quad \ldots (5)$$

**Syneresis of Basil Seeds Mucilages**

The aqueous dispersions (0.5%) of basil seed mucilages were made in pre-weighed centrifuge tubes, heated in water bath at 90°C for 30 min. The samples were stored for 2 days at 4°C and then centrifuged at 3000 rpm for 15 min at 20°C. The syneresis was measured as the percentage of water released after centrifugation.[4]

## Results and Discussion

**Experimental Data**

The variations in yield and functional properties (WAC, WSC, SC, syneresis) of clove basil and sweet basil seeds mucilages with respect to change in temperature, time and water-to-seed ratio of hydrothermal mucilage extraction process was depicted in Table 1. The clove basil and sweet basil seeds mucilage yields from different experimental runs ranged from 26.10–36.27% and 19.35–33.77% respectively. The basil seeds mucilages yield were much higher than that of flax seeds (5.56–6.54%)[13], chia seeds (6.97–11.6%)[14] and quince seeds (11.58%)[15]. Further, it was higher than the reported mucilage yield (7.86–20.5%) of Kashmiri sweet basil seeds extracted hydrothermally at 40–91°C for 1.6–3.3 h with a water-to-seed ratio of 18:1–77:1.[(6)] The difference in mucilage yield was due to variability among the composition of seeds grown under different geographical conditions across the world and the extraction conditions employed.[13]

The amount of water absorbed by the mucilages could greatly influence the texture and viscosity of incorporated food products. The Water Absorption Capacity (WAC) of clove basil and sweet basil seeds mucilages from different experimental runs ranged from 341.43–401.22 g/g and 281.04–492.93 g/g respectively. Basil seeds mucilages exhibited phenomenal WAC as larger amounts of water was held within the capillary structures of polysaccharide matrix especially by the soluble fibres through hydrogen bonding.[4] The WAC (g/g) of basil seeds mucilages were much higher than that of durian seeds (139.5–274.0)[16], karaya seeds (67.0)[17] and *Eruca sativa* seeds (13.91)[18] mucilages. The high water holding capacity could make the basil seeds mucilages as suitable fat replacers for designing low-fat or fat-free food products.[8]

The solubility and swelling power can be the reliable descriptors of water absorption index of basil seeds mucilages. The WSC of clove basil and sweet basil seeds mucilages from different experimental runs ranged from 0.07–1.27% and 0.19–71.59% respectively. They were comparatively lower than the WSC of *Cordia myxa* (93.27 ± 5.63%)[19] and *Eruca sativa* seeds (28.5–88.8%)[18] mucilages. The low WSC of basil seed mucilages was due to their ability to form gels by holding larger amounts of water.[20]

The Swelling Capacity (SC) denoted the degree of mucilage hydration and high SC was indicative of weaker binding forces among the mucilage molecules. The SC of clove basil and sweet basil seeds mucilages from different experimental runs varied from 14.33–16.98 mL/mL and 3.86–15.0 mL/mL respectively. The values were similar to the reported SC values of mucilages from Italian flax seeds (8.0–21.0 mL/mL)[21], psyllium seeds (4.0–19.0 mL/mL)[22] and sweet basil seeds from Mumbai (9.16–15.6 mL/mL).[23]

Table 1 — Effect of temperature, time and water-to-seed ratio on dependent variables of basil seeds extracted mucilages

| Exp. no. | Temp. (°C) ($X_1$) | Time (h) ($X_2$) | Water: Seed ($X_3$) | Clove basil seeds mucilage | | | | | Sweet basil seeds mucilage | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Yield (%) | WAC (g/g) | WSC (%) | SC (mL/mL) | Syneresis (%) | Yield (%) | WAC (g/g) | WSC (%) | SC (mL/mL) | Syneresis (%) |
| 1 | 50 | 1 | 65:1 | 29.56 | 371.66 | 0.13 | 16.83 | 0.50 | 27.67 | 474.39 | 0.20 | 11.25 | 0.50 |
| 2 | 80 | 1 | 65:1 | 28.24 | 371.61 | 0.20 | 16.73 | 0.30 | 27.71 | 464.68 | 0.19 | 8.09 | 0.30 |
| 3 | 50 | 3 | 65:1 | 27.91 | 371.52 | 1.13 | 14.33 | 2.00 | 26.67 | 318.98 | 22.78 | 3.86 | 0.01 |
| 4 | 80 | 3 | 65:1 | 32.44 | 374.23 | 0.67 | 14.67 | 1.80 | 27.73 | 365.39 | 8.82 | 12.33 | 1.00 |
| 5 | 50 | 2 | 50:1 | 29.01 | 341.43 | 0.20 | 15.50 | 0.80 | 22.55 | 365.34 | 7.44 | 5.58 | 0.00 |
| 6 | 80 | 2 | 50:1 | 29.74 | 349.80 | 0.20 | 15.33 | 0.90 | 24.76 | 355.59 | 15.77 | 6.50 | 0.10 |
| 7 | 50 | 2 | 80:1 | 33.57 | 371.55 | 0.40 | 15.00 | 1.20 | 31.19 | 431.81 | 7.16 | 5.43 | 0.20 |
| 8 | 80 | 2 | 80:1 | 28.31 | 369.43 | 0.13 | 16.33 | 0.40 | 29.68 | 348.90 | 17.88 | 5.17 | 0.00 |
| 9 | 65 | 1 | 50:1 | 27.93 | 364.47 | 0.07 | 16.67 | 0.20 | 19.35 | 492.93 | 1.20 | 5.25 | 1.20 |
| 10 | 65 | 3 | 50:1 | 29.02 | 349.83 | 1.27 | 14.67 | 1.60 | 21.90 | 403.26 | 9.09 | 10.00 | 0.00 |
| 11 | 65 | 1 | 80:1 | 26.10 | 349.82 | 0.13 | 16.00 | 0.60 | 25.55 | 465.34 | 0.76 | 9.00 | 0.50 |
| 12 | 65 | 3 | 80:1 | 36.27 | 371.62 | 0.13 | 15.67 | 1.00 | 33.77 | 281.04 | 71.59 | 9.00 | 0.00 |
| 13 | 65 | 2 | 65:1 | 27.76 | 398.37 | 0.07 | 16.97 | 0.10 | 23.95 | 430.65 | 5.58 | 15.00 | 0.00 |
| 14 | 65 | 2 | 65:1 | 27.75 | 399.62 | 0.20 | 16.98 | 0.05 | 23.95 | 430.66 | 5.49 | 14.67 | 0.01 |
| 15 | 65 | 2 | 65:1 | 27.77 | 398.56 | 0.10 | 16.97 | 0.10 | 23.96 | 430.53 | 5.39 | 14.33 | 0.00 |
| 16 | 65 | 2 | 65:1 | 27.77 | 398.44 | 0.14 | 16.96 | 0.10 | 23.95 | 430.54 | 5.67 | 14.52 | 0.01 |
| 17 | 65 | 2 | 65:1 | 27.75 | 401.22 | 0.07 | 16.97 | 0.05 | 23.96 | 430.57 | 5.31 | 14.48 | 0.02 |
| | | | Mean | 29.23 | 373.72 | 0.31 | 16.03 | 0.69 | 25.78 | 407.09 | 11.19 | 9.67 | 0.23 |
| | | | $SD_m$ | 2.56 | 19.60 | 0.37 | 0.96 | 0.64 | 3.58 | 58.72 | 16.77 | 3.98 | 0.37 |
| | | | $SE_m$ | 0.01 | 1.22 | 0.06 | 0.01 | 0.03 | 0.00 | 0.06 | 0.15 | 0.25 | 0.01 |
| | | | CV (%) | 0.03 | 0.33 | 17.86 | 0.04 | 3.98 | 0.01 | 0.02 | 1.30 | 2.61 | 3.69 |

Note: WAC: Water absorption capacity; WSC: Water solubility capacity; SC: Swelling capacity.

The swelling of mucilages was due to entanglement of polysaccharide chains and intra- plus inter- molecular hydrogen bonding between the polysaccharide and water resulting in entrapment of larger amounts of water within the macromolecular chains.[22]

The syneresis of clove basil and sweet basil seeds mucilages from different experimental runs varied from 0.05–2.00% and 0.00–1.20% respectively. It was lower than the reported syneresis value (1.27%) of sweet basil seeds mucilage by other researchers[24] due to variations in extraction conditions. The lower syneresis values represented better freeze-thaw stability of basil seeds mucilages and thus can be utilised in the formulation of frozen food products.

#### Model Fitting and Validation

The independent and dependent variables of clove basil and sweet basil seeds mucilage extraction process were fitted to a second-order polynomial equations with extended cubic interactions to minimize the variations in responses and make the models significant ($R^2 > 0.95$). The magnitude of regression coefficients reflected the level of influence of independent variables and their interactions on the dependent variables (Table 2).

The $R^2$ values for clove basil seeds extracted mucilage yield, WAC, WSC, SC and syneresis were 1.0000, 0.9990, 0.9944, 1.0000 and 0.9995 respectively while for the sweet basil seeds extracted mucilage were 1.0000, 1.0000, 1.0000, 0.9990 and 0.9999 respectively. These $R^2$ values provided a measure of variability in the observed response values that can be explained by the experimental factors and their interactions. The closer the $R^2$ values approach unity, the better the empirical model fits the actual data. The results of this study fitted well to actual data as the $R^2$ value was closer to unity.

By keeping one independent variable constant at the centre point and varying the other two independent variables within the experimental range, the 3D response surface plots and 2D contour plots were drawn to describe the individual and cumulative effects on responses.

#### Effect of Independent Variables on Mucilage Yield

##### *Clove Basil Seeds*

Initially, when the extraction was done for a lesser duration (1 h), the temperature rise had not increased the clove basil seeds mucilage yield. However, the combined effect of an increase in temperature from

Table 2 — Coefficients of reduced cubic models

| | Clove basil seeds mucilage | | | | | Sweet basil seeds mucilage | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yield | WAC | WSC | SC | Syneresis | Yield | WAC | WSC | SC | Syneresis |
| Intercept | 137.5161 | −381.0697 | 0.1160 | −37.9413 | 26.7122 | −54.9440 | 2732.0958 | −1112.8240 | −150.1301 | 43.9770 |
| A- Temperature | −2.7393 | 9.8847 | −0.0975 | 0.6145 | −0.3374 | −1.4307 | −19.1410 | 12.1540 | 0.7419 | −0.3107 |
| B- Time | 32.3753 | −152.3110 | 0.3675 | 8.5411 | −6.4233 | 64.0613 | −1041.5835 | 416.5988 | 53.3556 | −16.0171 |
| C- Water: seed | −2.7708 | 16.8777 | 0.032 | 1.4337 | −0.5730 | 3.5235 | −76.5154 | 30.6131 | 2.6100 | −1.1736 |
| AB | 0.0975 | 0.0460 | −0.1325 | 0.0073 | −5.34E−017 | 0.0172 | 0.9354 | −0.2325 | 0.1939 | 0.0198 |
| AC | 0.0679 | −0.0462 | −0.0675 | −0.0072 | 0.0019 | −0.0003 | 1.1645 | −0.3153 | 0.0436 | 0.0082 |
| BC | -1.2594 | 2.7720 | −0.3000 | -0.2829 | 0.1633 | −1.9039 | 15.1771 | −9.8296 | −1.5029 | 0.3409 |
| $A^2$ | 0.0047 | −0.0619 | 0.1245 | −0.0034 | 0.0023 | 0.0120 | −0.1332 | −0.0135 | −0.0186 | 0.0002 |
| $B^2$ | −1.7558 | 33.1086 | 0.2920 | −0.3746 | 0.6558 | −4.0990 | 232.2998 | −59.7084 | −6.1285 | 1.2639 |
| $C^2$ | 0.0239 | −0.1488 | −0.0080 | −0.0123 | 0.0051 | −0.0224 | 0.5537 | −0.2218 | −0.0162 | 0.0086 |
| $AC^2$ | −0.0006 | 0.0003 | 0.0300 | 0.0001 | −2.22E−005 | −2.97E−005 | −0.0096 | 0.0024 | −0.0003 | −0.0001 |
| $B^2C$ | 0.0382 | −0.7102 | −0.3025 | −0.0028 | −0.0017 | 0.0752 | −3.4931 | 1.0040 | 0.0706 | −0.0133 |
| $BC^2$ | 0.0097 | 0.0052 | −0.0675 | 0.0025 | −0.0013 | 0.0131 | −0.0214 | 0.0528 | 0.0088 | −0.0021 |
| $R^2$ | 1.0000 | 0.9990 | 0.9944 | 1.0000 | 0.9995 | 1.0000 | 1.0000 | 1.0000 | 0.9990 | 0.9999 |
| Adjusted $R^2$ | 1.0000 | 0.9961 | 0.9775 | 0.9999 | 0.9982 | 1.0000 | 1.0000 | 0.9999 | 0.9960 | 0.9995 |

Note: WAC: Water absorption capacity; WSC: Water solubility capacity; SC: Swelling capacity

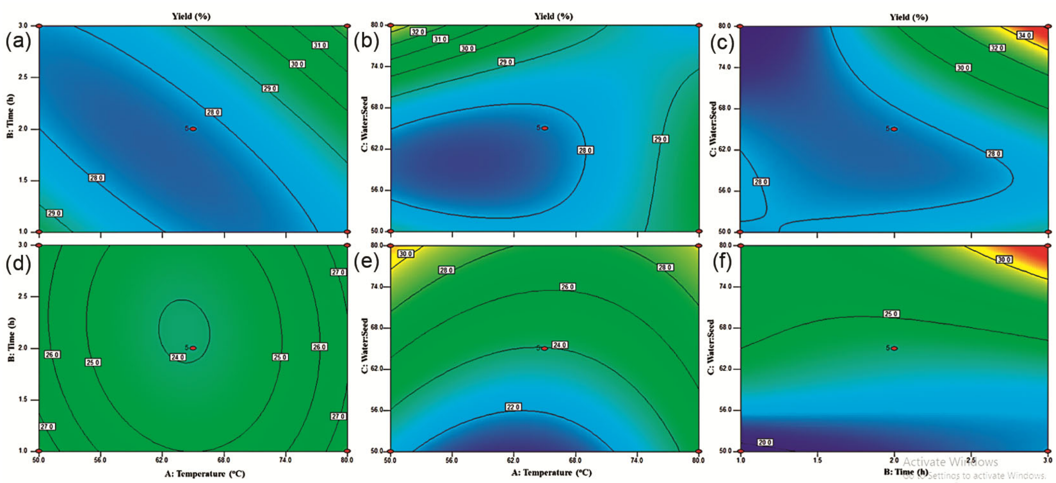


Fig. 1 — Contour plots depicting interactive effects of independent variables on basil seeds extracted mucilage yield, a & d: Effect of temperature and time on yield, b & e: Effect of temperature and water: seed on yield, c & f: Effect of time and water: seed on yield

50–80°C and extraction time from 1–3 h led to an increase in the mucilage yield from 29.6% to 32.4% (Fig. 1a). Thus, the temperature and time synergistically influenced the efficiency of mucilage extraction by allowing better penetration of water into seed, solubilizing the compounds and promoting the release of mucilage from seed pericarp.

At lower temperature (50°C), the increase in water-to-seed ratio from 50:1 to 80:1 had non-linearly increased the mucilage yield from 29.0% to 33.6% due to the availability of more water that acted as a driving force for the mucilage to extrude out of seeds. However, there was no increase in mucilage yield with the rise in water/seed ratio at higher temperatures (80°C), as the aqueous medium became stickier with heat decreasing the efficiency of extraction (Fig. 1b).

Further, the rise in extraction time from 1 h to 3 h and water-to-seed ratio from 50:1 to 80:1 significantly improved the mucilage yield from 27.9% to 36.3% (Fig. 1c). The high water-to-seed ratio made the slurry less viscous and the increased extraction time promoted better mass transfer of water into seed, thus releasing out water-soluble hydrocolloids from seeds contributing to efficient extraction of mucilage. Henceforth, the dependence of clove basil seeds mucilage extraction yield on independent factors can be rated as extraction time > water-to-seed ratio ~ extraction temperature.

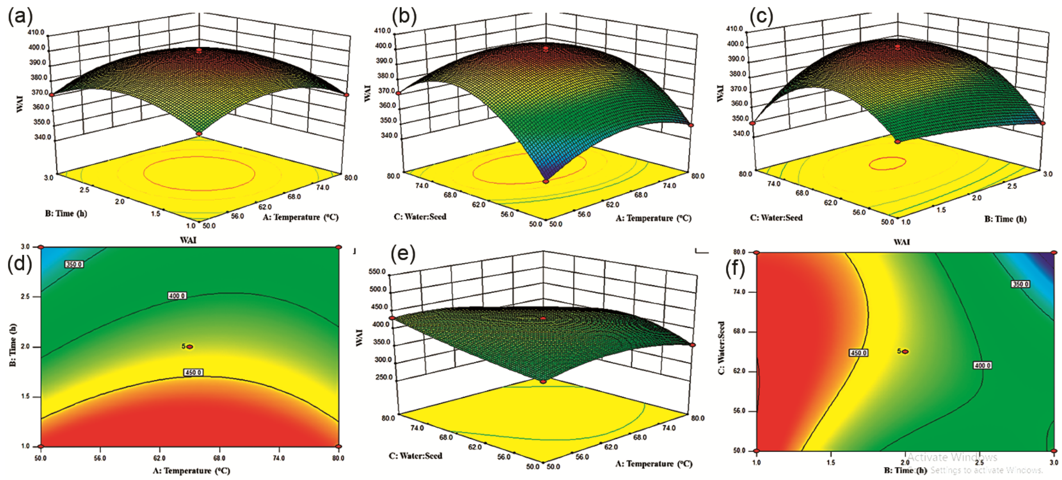


Fig. 2 — Response surface and counter plots depicting interactive effects of independent variables on water absorption index (WAI) of basil seeds mucilage

***Sweet Basil Seeds***

As depicted in Fig. 1d, at shorter extraction duration (1 h), the temperature rise had not increased the sweet basil seeds mucilage yield. A slight increase in the mucilage yield from 26.7% to 27.7% was observed with an increase in temperature from 50–80°C when the extraction was done for a longer duration (3 h). The Fig. 1e depicted that both at lower (50°C) and higher temperatures, the increase in water-to-seed ratio from 50:1 to 80:1 has increased the mucilage yield from 22.6% to 31.2% and from 24.8% to 29.7% respectively. Similarly, as the water-to-seed ratio increased from 20:1 to 60:1, the extraction yield increased due to an increase in driving force for the mass transfer of mucilage from *Eruca sativa* seeds.[18] The Fig. 1f showed that the rise in time from 1h to 3 h and water-seed ratio from 50:1 to 80:1 had significantly improved the mucilage yield from 19.3% to 33.8%.

The prolonged extraction duration and availability of more water promoted the complete hydration of sweet basil seeds polysaccharides and their release into the surrounding medium. The extraction temperature had the least influence on mucilage yield, as the aqueous medium did not become much stickier with the increase in heat in case of sweet basil seeds. Likewise, increased extraction temperature did not have a significant effect while the increased extraction time and water-to-seed ratio had significant positive linear effects on the extraction yield of *Lepidium sativum* seeds mucilage.[25] Henceforth, the dependence of sweet basil seeds mucilage extraction yield on the independent factors can be rated as extraction time > water-to-seed ratio > extraction temperature.

**Effect of Independent Variables on WAC of Mucilage**

***Clove Basil Seeds***

The water holding capacity of mucilages greatly influences their yield, physical stability, sensory attribute and texture of incorporated foods. The WAC of clove basil seeds mucilage increased up to 65°C with the increase in extraction time up to 2 h with a peak value of 398.4 g/g and then decreased in parabolic manner (Fig. 2a). A similar trend was exhibited by the *Ximenia americana* seeds mucilage where the WAC was increased with the rise in temperature up to 69.46°C and thereafter decreased parabolically.[8]

The WAC of mucilage increased up to 65°C with the increase in water-to-seed ratio to 65:1 and then decreased in parabolic manner (Fig. 2b). Further, the WAC of mucilage increased up to 65:1 of water-to-seed ratio with the increased extraction time to 2 h and then decreased in parabolic manner (Fig. 2c). The difference in WAC of mucilage under different extraction conditions might be dependent on the changes in the polar hydroxyl groups present and the degree of hydrodynamic interactions.

***Sweet Basil Seeds***

The WAC of sweet basil seeds mucilage increased up to 65°C and attained a peak value of 430.7 g/g with the increase in extraction time up to 2 h or water-to-seed ratio to 65:1 and then decreased in parabolic manner (Figs. 2d–f). The mucilage extracted at 65°C for 2 h with a water-to-seed ratio of 65:1 was able to hold more water might be due to unfolding of constituent polysaccharides with a higher degree of ramification proportion that reduced the chain-to-

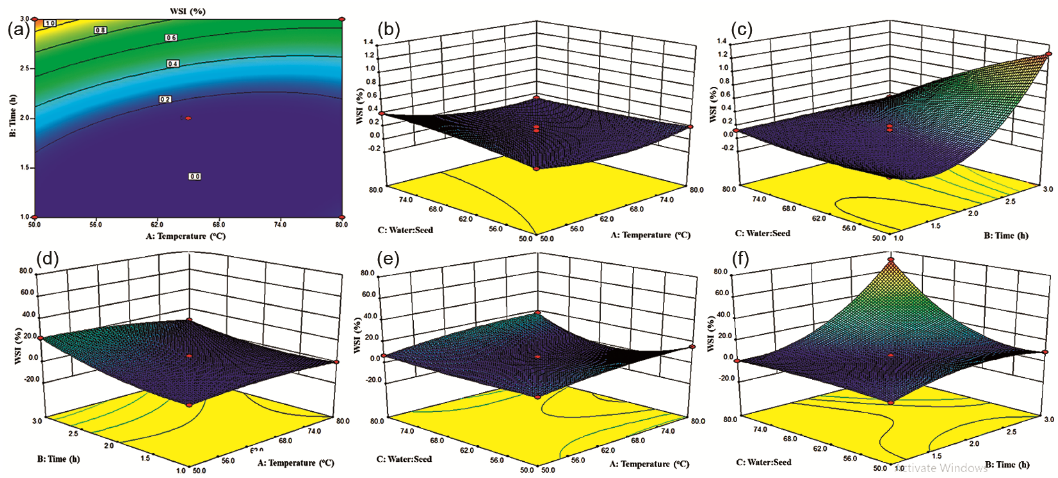


Fig. 3 — Counter and response surface plots depicting interactive effects of independent variables on water solubility index (WSI) of basil seeds mucilage, a & d: Effect of temperature and time on WSI, b & e: Effect of temperature and water: seed on WSI, c & f: Effect of time and water: seed on WSI

chain interaction and allowed water to interact easily with polysaccharide chains. Such mucilage with high water holding capacity can be utilised as a functional ingredient to formulate healthier foods.

#### Effect of Independent Variables on WSC of Mucilage

***Clove Basil Seeds***

The WSC of clove basil seeds mucilage increased from 0.1% to 0.7% with the rise in extraction temperature from 50–80°C and time from 1–3 h (Fig. 3a, supplementary material). Likewise, *Eruca sativa* seeds and *Plantago major* seeds mucilages extracted at higher temperatures exhibited more solubility due to the solubilisation of high molecular weight components that were not dissolved at lower temperatures resulting in mucilages with non-homogenous structures.[18,26]

The increase in water-to-seed ratio from 50:1 to 80:1 and extraction temperature from 50–80°C has resulted in a decrease of WSC of mucilage from 0.2% to 0.1% (Fig. 3b, supplementary material). This might be due to availability of liquid that acted as driving force of water insoluble impurities out of the seeds resulting in mucilage of less purity.

At lower water-to-seed ratio of 50:1, the WSC of mucilage had significantly increased from 0.1% to 1.3% with the increase in extraction time from 1–3 h. However, an opposing trend was noticed with the high water-to-seed ratio (Fig. 3c, supplementary material). The higher solubility of mucilage is indicative of high purity that might be additionally associated with other parameters such as particle size.[19]

***Sweet Basil Seeds***

The sweet basil seeds mucilage extracted at lesser duration (1 h), did not exhibit any significant change in WSC with the rise in temperature. However, the mucilage extracted for prolonged duration (3 h), had exhibited decrease in the WSC from 22.8% to 8.8% with the rise in temperature from 50–80°C (Fig. 3d). The increase in water-to-seed ratio (50:1 to 80:1) along with increase in extraction temperature (50°C to 80°C) or time (1 to 3 h) had improved the WSC of mucilage from 7.4% to 17.9% and 1.2% to 71.6% respectively (Figs. 3e and 3f, supplementary material). The extraction at higher temperatures with prolonged heat treatment and more water availability resulted in mucilage with more water insoluble impurities, reduced water holding and gelling capacities, thus exhibiting enhanced solubilisation in water.

#### Effect of Independent Variables on SC of Mucilage

***Clove Basil Seeds***

The SC of clove basil seeds mucilage increased up to 65°C, attained a peak value of 17.0 mL/mL with the increase in extraction time up to 2 h or water-to-seed ratio to 65:1 and then decreased in parabolic manner (Figs. 4a–c, supplementary material). The increased temperature resulted in weaker binding interactions between mucilage molecules and allowed the entrapment of larger amount of water molecules by promoting enlargement in mucilage chains.[27] The hydrocolloids with better water holding capacity will exhibit good gelation properties and in turn better swelling ability. However, the extraction conditions

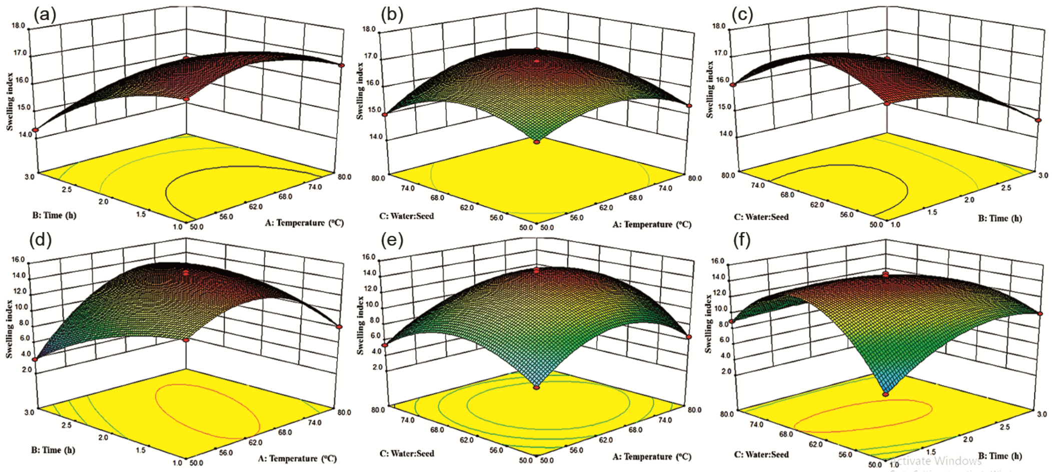


Fig. 4 — Response surface plots depicting interactive effects of independent variables on swelling index of basil seeds mucilage, a & d: Effect of temperature and time on swelling index, b & e: Effect of temperature and water: seed on swelling index, c & f: Effect of time and water: seed on swelling index

with higher extremes resulted in mucilage with reduced swelling ability. The prolonged high-temperature treatment caused the thermal decomposition and degradation of polysaccharides affecting the microporous structure and in turn water holding capacity of mucilage.[28]

***Sweet Basil Seeds***

The better swelling capacity of hydrocolloids could enhance the pore characteristics and texture of incorporated foods. The sweet basil seeds mucilage exhibited a similar trend of swelling ability as that of clove basil seeds mucilage under different extraction conditions. The SC of sweet basil seeds mucilage increased up to 65°C, attained a peak value of 15.0 mL/mL with the increase in extraction time up to 2 h or water-to-seed ratio to 65:1 and then decreased in parabolic manner (Figs. 4d–f, supplementary material).

**Effect of Independent Variables on Syneresis of Mucilage**

***Clove Basil Seeds***

The syneresis of frozen-thawed clove basil seeds mucilage decreased parabolically up to 65°C to a value of 0.1% with the increase in extraction time to 2 h or water-to-seed ratio to 65:1 and then increased with the rise in extraction conditions (Fig. 5a–c, supplementary material). The syneresis of mucilage decreased as its water binding capacity increased. Hence, the clove basil seeds mucilages from different experimental runs exhibited syneresis in an opposing trend compared to their water absorbing capacities.

***Sweet Basil Seeds***

The extent of syneresis among frozen-thawed mucilages varies depending on their polysaccharide composition. The extraction of sweet basil seeds mucilage for lesser duration (1 h) had shown a decline in the syneresis from 0.5% to 0.3% with the rise in extraction temperature from 50–80°C. While the extraction of mucilage for a longer duration (3 h) had shown an opposing trend by increased syneresis from 0.0% to 1.0% with the increase in extraction temperature from 50–80°C (Fig. 5d, supplementary material). Further, when the extraction was done with the water-to-seed ratio of 80:1, there was a decline in syneresis of mucilage from 0.2% to 0.0% with the rise in extraction temperature from 50–80°C. While the extraction of mucilage with water-to-seed ratio of 50:1, had shown a contradictory trend of increased syneresis from 0.0% to 0.1% with the rise in extraction temperature from 50–80°C (Fig. 5e, supplementary material).

The simultaneous increase in water-to-seed ratio from 50:1 to 80:1 and extraction duration from 1.0 to 3.0 h had significantly decreased the syneresis of mucilage from 1.2% to 0.0% which was highly desirable (Fig. 5f, supplementary material). The mucilages without syneresis upon freeze thawing can be utilised for incorporation into frozen desserts, spreads and yogurts as nutritious functional ingredients without affecting their storage stability.

**Optimisation and Verification**

The numerical and graphical optimisations were done for determining the optimum level of independent variables to obtain maximum yield with maximum water absorbing and swelling capacities, minimum water solubility index and syneresis upon freeze thawing. The graphical optimum region for

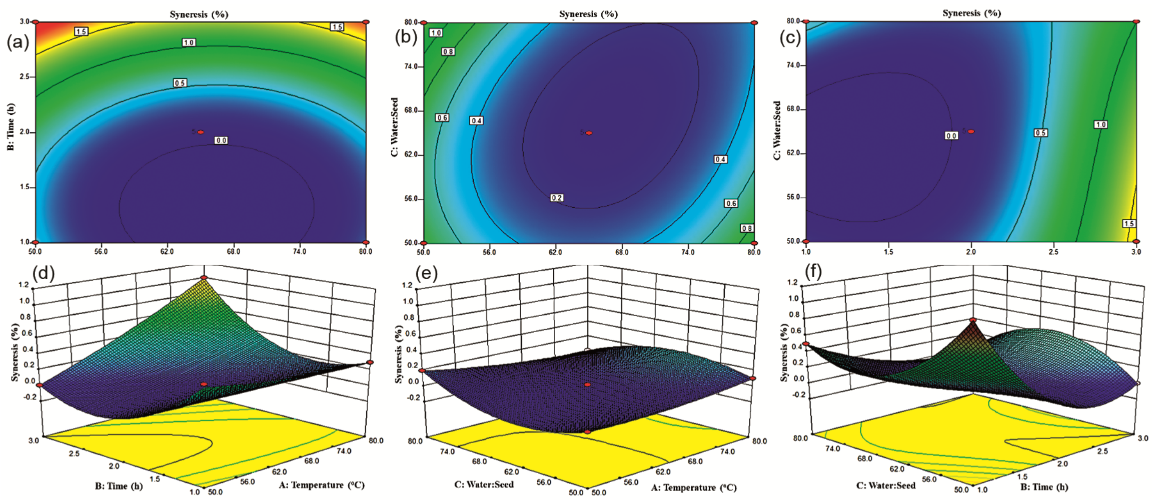


Fig. 5 — Counter and response surface plots depicting interactive effects of independent variables on syneresis of basil seeds mucilage, a & d: Effect of temperature and time on syneresis, b & e: Effect of temperature and water: seed on syneresis, c & f: Effect of time and water: seed on syneresis

extraction of clove basil and sweet basil seeds mucilages was obtained by superimposing contour plots of all the response variables as presented in Fig. 6. The adequacy of the models for predicting the optimum response values was tested by mucilage extraction from both clove basil and sweet basil seeds under optimum conditions and analyzing their response variables.

The optimum conditions for extraction of clove basil seeds mucilage were extraction temperature of 67.34°C, extraction time of 2.51 h and water-to-seed ratio of 80:1. These conditions could provide a mucilage with yield of 32.53% with water absorption capacity of 383.42 g/g, water solubility index of 0.11%, swelling capacity of 16.29 mL/mL and syneresis of 0.50%. The experimental values of mucilage extracted under optimum conditions were 32.49 ± 0.10, 383.39 ± 0.08, 0.13 ± 0.04, 16.29 ± 0.09 and 0.48 ± 0.04 corresponding to yield (%), WAC (g/g), WSC (%), SC (mL/mL) and syneresis (%) respectively. The experimental values were very close to the predicted values with statistically no significant difference between them at $p \leq 0.01$ indicating that the model was adequate.

Similarly, the optimum conditions for the extraction of sweet basil seeds mucilage were an extraction temperature of 57.01°C, extraction time of 1.31 h and water-to-seed ratio of 70.66:1. These extraction conditions could provide a mucilage with yield of 26.30% with water absorption capacity of 474.34 g/g, water solubility index of 0.70%, swelling capacity of 13.23mL/mL and syneresis of 0.19%. The

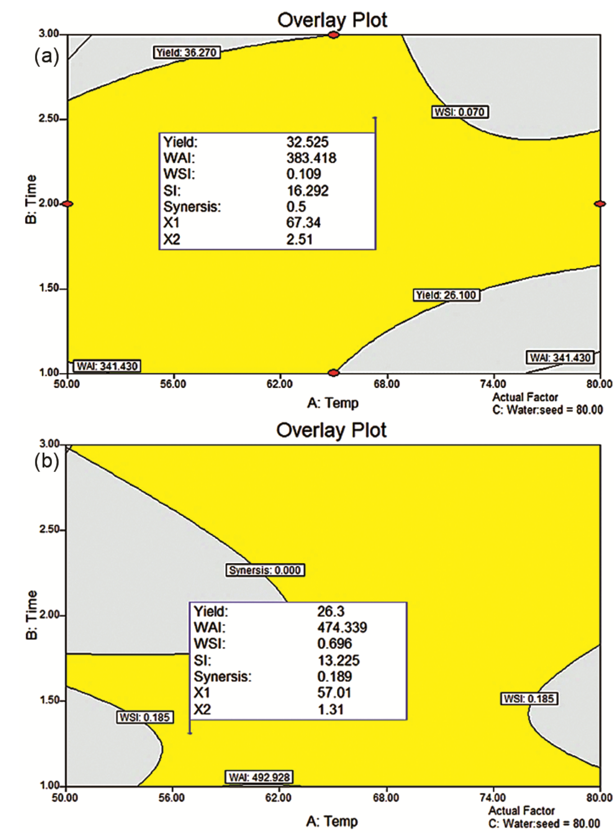


Fig. 6 — Optimised conditions for extraction of basil seeds mucilages with better yield and functionalities

experimental values of mucilage extracted under optimum conditions were 26.39 ± 0.05, 474.30 ± 0.11, 0.74 ± 0.02, 13.24 ± 0.02 and 0.21 ± 0.01 corresponding to yield (%), WAC (g/g), WSC (%), SC (mL/mL) and syneresis (%) respectively. The experimental values were very close to the predicted values with statistically no significant difference between them at $p \leq 0.01$ indicating that the model was adequate.

## Conclusions

The basil seeds mucilages can be utilised as functional vegan hydrocolloids that have huge potential in designing novel foods by food industries. However, conditions employed during hydrothermal extraction of these mucilages could greatly influence their exhibited functionalities. The response surface modelling depicted the individual and interactive effects of extraction conditions (temperature, time and water-to-seed ratio) on responses *viz.* yield, water absorption capacity, water solubility capacity, swelling capacity and syneresis of basil seeds mucilages. Henceforth, the numerical and graphical optimisations were done to extract basil seeds mucilages with better yield and functionalities. The experimental values of both the mucilages extracted under optimum conditions were very close to the predicted values. The extracted basil seeds mucilages being a very good source of soluble dietary fiber can be utilized as laxatives, hypolipidemic, hypoglycemic agents and prebiotics. Further, as fat replacers, gelling agents, texture improvers, stabilizers, emulsifiers and thickening agents they could offer splendid functionalities for incorporation in food formulations.

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## Conflict of interest

There is no conflict of interest declared by the authors.

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