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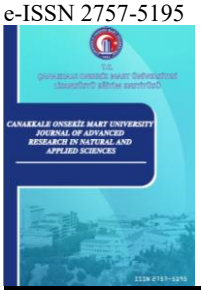

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Optimization of Mucilage Removal from Cress Seeds (*L. sativum*)

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Research Article

Abstract − In this study, optimum removal conditions of the cress seeds mucilage by a chemical method were determined. Moreover, proximate analysis (moisture, fat, protein, ash and total carbohydrate) of the cress seeds was carried out. The independent process variables were sodium bicarbonate concentration (0-0.5 M), solvent-to-seeds ratio (25-75 ml/g) and soaking time (3-9 hours). A central composite design having 20 points for given independent variables was used and the optimization of the process conditions was done by desirability function approach. The results showed that proximate analysis results of the cress seeds were in accordance with the previous reports in literature, and fat and protein percentages were found to be higher than 20%. The mucilage removal study results showed that higher concentrations of sodium bicarbonate enhanced the mucilage removal. Also, the solubility of the seed coat polysaccharides of the cress seeds increased at higher soaking times, resulting in better mucilage removal. The optimum chemical mucilage removal conditions were determined as 0.43 M of sodium bicarbonate concentration, 75 ml/g of solvent-to-seeds ratio and 8.96 hours of soaking time. At these conditions, total carbohydrates content (determined using Anthrone method) in the final extract was predicted as 2.47 g/ml. Verification tests were carried out at the optimum conditions and there was no statistical difference between the experimental (2.64±0.43 mg/ml) and the predicted values.

Keywords *– Cress seeds, desirability function, mucilage, optimization, sodium bicarbonate*

1. Introduction

Cress (*Lepidium sativum* L.) is an annual erect herbaceous plant belonging to the *Brassicaceae* family and growing up to 30-50 cm height (Behrouzian, Razavi, & Phillips, 2014; Sharma & Agarwal, 2011). The leaves of the *L. sativum* are generally consumed freshly and used as a garnish in the human diet. The roots of the plant mainly used for the treatment of some diseases such as syphilis and tenesmus (Paranjape & Mehta, 2006). Even though *L. sativum* leaves and roots have economic value, its cultivation is mainly done for the seeds of the plant (Mohammed Ali, 2013). The cress seeds have an oval shape and brownish-red colour, and the bulk densities of the seeds were reported as 740-760 kg/m^3 (Mathews, Singhal, & Kulkarni, 1993; Razavi, Bostan, Niknia, & Razmkhah, 2011). The cress seeds have a bitter taste and strong odour, and can be used as a traditional medicine for the diseases such as diarrhoea, scurvy, leprosy, asthma and splenomegaly. The proximate analysis for the main compounds of the cress seeds was reported as 22-25% protein, 14-27% lipids, 33-54% carbohydrates and 1-4% ash (Mohammed Ali, 2013; Mulla & Ahmed 2019). The carbohydrates of the cress seeds mainly composed of non-starch polysaccharides (>90%). Moreover, the cress seeds have a mucilage portion of 6.5 to 15% (Divekar & Mohan, 2010; Karazhiyan, Razavi, & Phillips, 2011).

In the food industry, the polysaccharides obtained from plant materials can be used as coatings and edible films because of their environmentally friendly, non-toxic and biodegradability properties (Beikzadeh, Khezerlou, Pilevar, & Mortazavian, 2020). Among these polysaccharides, mucilage derived from plants and seeds has been used as active ingredients in the formulation of functional and nutraceutical products because of its health benefits, functional and physicochemical properties. Mucilage is defined as a complex polymeric polysaccharide and mainly composed of carbohydrates with highly branched structures that consist of monomer units of

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L-arabinose, D-xylose, D-galactose, L-rhamnose, and galacturonic acid (Tosif et al., 2021). The composition of the cress seed mucilage (77.03% carbohydrate) was reported as follows: 38.9% mannose, 19.4% arabinose, 8% galacturonic acid, 6.8% fructose, 6.7% glucuronic acid, 4.7% galactose, 1.9% rhamnose and 1% glucose (Behrouzian et al., 2014). The molecular weight of the cress seed mucilage was determined as 540 kDa (Karazhiyan et al., 2011). Polyuronide chains of the cress seed mucilage interact with water and cellulose micelles can be dispersed, and hydrated cellulose micelles scattered with hydrated polyuronide chains create a gel network (Sharma & Agarwal, 2011). Because of its functional and physicochemical properties, the cress seed mucilage can be used as a thickener in the food industry. On the other hand, gum and mucilage formations of seeds can be challenging for the extraction process of biomaterials present in the seeds because of interactions with water. The polysaccharides in the seed coat can make it difficult to separate proteins from an aqueous medium (Wanasundara & Shahidi, 1997). Extraction of proteins from plant seeds can be enhanced by removal of mucilage prior to the extraction process. Moreover, mucilage removal prior to the aqueous oil separation from the mustard seeds (Balke & Diosady, 2000) and Omega-3-rich oil from chia seeds (Castejón, Luna, & Señoráns, 2017) significantly reduced the water requirement. Hence, removing conditions of mucilage should be determined.

Removal of mucilage from the seeds coat can be carried out by enzymatic and chemical methods. Chemical method which is soaking of seeds in sodium bicarbonate ($NaHCO_3$) is generally preferred because of its ease of use and lower price than enzymatic treatment. $NaHCO_3$ increases the solubility of coat polysaccharides and makes it easier to remove the sticky mucilage interactions with the seed coat. Marambe, Shand, & Wanasundara (2008) used $NaHCO_3$ to remove mucilage from flaxseeds prior to protein extraction. They mixed flaxseeds with 0.5 M $NaHCO_3$ (1:8 w/v) at 50°C for 1 hour and solubilized mucilage was removed by filtering. In another study of Marambe, Shand, & Wanasundara (2013), similar conditions were used for the mucilage removal from flaxseed coat. Dash, Kumar, Kumari, & Malik (2021) prepared a demucilaged and defatted flaxseed meal to prepare flaxseed protein isolate. Flaxseed mucilage was removed by stirring the flaxseeds with 0.5 M $NaHCO_3$ (1:8 w/v) at 40°C for 1 hour, and the collected seeds were filtered and washed several times to remove residual mucilage. Tehrani, Batal, Kamalinejad, & Mahbubi (2014) used similar conditions to remove mucilage from flaxseeds. Timilsena, Adhikari, Barrow, & Adhikari (2016) also used $NaHCO_3$ to remove mucilage from Australian chia seeds prior to obtain protein isolate. They mixed chia seeds with 0.5 M $NaHCO_3$ (1:10 w/v) at room temperature for 18 hours. Then the dispersion was filtered, washed with water five times to remove residual mucilage. A detailed study was carried out by Wanasundara & Shahidi (1997) to remove mucilage from flaxseeds and they used different concentrations of $NaHCO_3$ (0.05 and 0.10 M), different soaking times (3, 6, and 12 hours) at a seed to solvent ratio of 1:10 (w/v). Wanasundara & Shahidi (1997) reported that a considerable amount of flaxseed coat mucilage was removed by the usage of $NaHCO_3$. As far as we know, even though its high protein and mucilage content, there is no study for the removal of the cress seed mucilage by a chemical method in literature. In this study, the proximate analysis of the cress seeds was carried out and the optimum conditions of the cress seed mucilage removal by a chemical method were determined.

2. Materials and Methods

2.1. Material

Cress seeds were provided from a local market in Tokat province. The seeds were harvested in July 2022. The location of the harvested seeds was in the Black Sea region having the geographical coordinates of 39° 51′ north and 35° 27′ east, and the elevation above sea level was 623 m. The cress seeds were screened and combed out to remove impurities. The seeds were stored in dark and at room temperature for further analysis.

2.2. Proximate Analysis

Moisture, fat, protein, ash and total carbohydrate percentages of the cress seed samples were determined and given in Table 1. The moisture content of the cress seeds was determined by a drying oven (Memmert UFE 600, Germany) at 103±2°C for 4 hours (AOAC, 2000). The fat content (%) of the cress seeds was determined using the Soxhlet apparatus according to the modified method of AOAC, only n-hexane (Sigma Aldrich, Germany) was used as the extraction solvent instead of petroleum ether (AOAC, 2000). The % protein content of the cress seeds was determined by Kjeldahl protein determination method. The samples were placed to the heater (Behrotest® InKjel M, Germany) and digested at 25% power of the device for the first 1 hour and 100% for the next 3 hours (~400°C). The distillation process (Behrotest® Distillation Unit S1, Germany) was

completed in 6 minutes using 32% NaOH (Sigma Aldrich, Germany). The distillates were titrated with 0.2 N HCl (Sigma Aldrich, Germany), and the conversion factor of 6.25 was used to convert total nitrogen to percentage protein (AOAC 2000). To determine the ash content (%) of the cress seeds, a muffle furnace (Protherm PLF 115M, Turkey) was used. The samples placed in the muffle furnace were burned at 200°C for 1 hour, at 600°C for 3 hours, and at 900°C for 6 hours, and the ash contents of the cress seeds were determined by weighing the incinerated samples (AOAC, 2000; Özcan & Al Juhaimi, 2011). The total carbohydrate amount (%) of the cress seeds was calculated by the difference method. Total amount of moisture, fat, protein and ash percentages of the cress seeds were subtracted from 100 to give total carbohydrate content (Albakry et al., 2022; Eknayake, Jansz, & Nair, 1999; Khoddami, Ghazali, Yassoralipour, Ramakrishnan, & Ganjloo, 2011).

2.3. Mucilage removal process and experimental design

Three independent variables were chosen for the mucilage removal from the cress seeds namely $NaHCO_3$ concentration (M), solvent-to-seeds ratio (v/w), and soaking time (hours). A central composite design was used for the mucilage removal processes and $NaHCO_3$ concentration was 0-0.5 M, solvent-to-seeds ratio was 25-75 v/w and soaking time was 3-9 hours in the design. Distilled water was used at the points where $NaHCO_3$ concentration was zero. The cress seeds were mixed with the solvent at 400 rpm for different soaking times as specified in the design. Afterwards, the samples were taken into 50 ml centrifuge tubes and centrifuged at 9000 rpm for 10 minutes. After the supernatant was removed, 0.1 N HCl (half of the volume used in the mucilage removal process) was added to the samples and the mixture was left for 5 minutes. The samples were passed through a 0.212 mm sieve (to remove the seeds and mucilage) and were washed 5 times with distilled water. The seed samples were dried at 45°C for 6 hours, and mixed with distilled water for 6 hours having initial temperature of 100°C (distilled water:seeds 10:1). Following that, the volume of the filtered extract was completed to the initial volume and the amount of carbohydrates (including mucilage) in the extract was determined by modified Anthrone Total Carbohydrate Determination (Chen & Vaidyanathan, 2013; Loewus, 1952). In the experimental design, the expression of total carbohydrate in the final extract was given in response, and the optimization process was performed for the lowest values. All analysis results are shown in Table 2.

2.4. Anthrone Total Carbohydrate Determination

A modified Anthrone method of Chen & Vaidyanathan (2013) was used to determine the total carbohydrate of the mucilage-containing extracts. 1 ml of 75% H_2SO_4 was added to 0.5 ml of extract and mixed with vortex. Then, 2 ml of Anthrone solution (0.5 g Anthrone was dissolved in 10 ml of ethanol and the total volume was made up to 250 ml with 75% H_2SO_4) was added to the samples and the samples were mixed again using vortex. The absorbance values of the samples kept at 100°C for 15 minutes were measured at 578 nm. To determine the total carbohydrate, D-glucose was used as the standard and D-glucose solutions were prepared at concentrations of 20, 40, 60 and 80 µg/ml. The absorbances of the standard solutions at different concentrations were determined by applying the same analysis steps for the prepared standard solutions.

2.5. Statistical Analysis

For the regression analysis of the response, the model was used for total carbohydrate in the final extract (mg/ml) given with Equation (2.1).

$$\text{Total carbohydrate (mg/ml)} = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_{ji} \quad (2.1)$$

$$k=1, 2, 3$$

Here, β_0, β_i and β_{ii} indicate the constants, k is the number of independent variables, X_i is the i^{th} independent variable, β_{ij} is the j^{th} coefficient of i^{th} observation, and X_{ji} indicates the j^{th} independent variable of i^{th} observation.

One-sample t test for the validation of the optimized conditions was carried out using the SPSS 21.0 (IBM, USA) package program. ANOVA, response surface graph and optimization study were done using the Design Expert 7.0 (Stat-Ease Inc., USA) package program.

3. Results and Discussion

The proximate analysis results of the cress seeds were given in Table 1. The component found in the highest amount in cress seeds was carbohydrate. Fat and protein contents were over 20%, which make cress seeds an economically valuable product. The proximate analysis results were in accordance with the literature. Gokavi, Malleshi, & Guo (2004) reported the composition of the cress seeds as the following: 4.14% moisture, 27.48% fat, 22.47% protein, 4.65% ash and 41.25% carbohydrates. Zia-Ul-Haq et al. (2012) carried out a compositional study for the *Ipomoea hederacea* Jacq. and *Lepidium sativum* L. seeds. They reported that the moisture, fat, protein, ash and carbohydrate contents of the *Lepidium sativum* L. seeds as 3.92%, 28.03%, 24.18%, 4.25% and 32.87%, respectively. Our results were in accordance with the reported results in literature. However, the difference between the values reported in this study and the proximate analysis results found in literature can be associated with the agronomic practices and climatic/geological conditions.

The results for the mucilage removal from the cress seeds are shown in Table 2. The total carbohydrate values in the final extract were ranged between 2.46 and 5.69 mg/ml. The aim of the study was to minimize the carbohydrate content in the final extract. Hence, it was observed that lower total carbohydrate values in the final extract were obtained when 0.5 M $NaHCO_3$ was applied. On the other hand, the total carbohydrate values in the final extract increased when the distilled water was used as solvent solely. The effect of the $NaHCO_3$ as a mucilage removal agent is observed by the obtained data. It is a known fact that polysaccharides have high affinity to water, and the polysaccharides can be dissolved in water by continuous hydration and the changes in the intramolecular binding of the polysaccharides. Polysaccharides' amorphous regions which are not possessing intermolecular H-bonds can be available for hydration. Therefore, the solubility of the polysaccharides having no possibility of the intermolecular association will be increased. During soaking process in $NaHCO_3$ medium, depolymerization of the polysaccharides can be enhanced because of the alkali nature (Wanasundara & Shahidi, 1997). The presence of the $NaHCO_3$ in the medium increases the solubility of the polysaccharides than that of water alone by this mechanism. Solvent-to-seeds ratio is another independent variable for our design and it was observed that the increment in the solvent-to-seeds ratio decreased the total carbohydrate values in the final extract, removed more mucilage. Soaking time also affected the mucilage removal process, higher soaking times led to more mucilage removal possibly because of the higher number of depolymerized polysaccharides. To the best of our knowledge, there is no study in the literature about optimization of mucilage removal from cress seed coats to compare our results.

Table 1
Proximate analysis results for the cress seeds

Component	Value (%)
Moisture	7.47 (±0.03)
Fat	21.58 (±0.26)
Protein	26.37 (±1.38)
Ash	5.42 (±0.37)
Total carbohydrate*	39.16 (±0.51)

**Total carbohydrate of the seeds was calculated by difference method.*

Table 2
The experimental design and analysis results

Run	$NaHCO_3$ (M) (X_1)	Solvent-to-seeds (v/w) (X_2)	Soaking time (h) (X_3)	TC (mg/ml)
1	0	75	9	3.51 (±0.06)
2	0	25	9	4.43 (±0.03)
3	0.5	50	6	2.76 (±0.06)
4	0.5	75	3	3.33 (±0.04)
5	0.25	50	6	3.06 (±0.02)
6	0.25	50	3	3.94 (±0.07)
7	0	75	3	4.53 (±0.03)
8	0.25	50	6	3.31 (±0.07)
9	0.5	25	3	3.85 (±0.03)
10	0.25	50	6	3.14 (±0.01)
11	0.25	50	6	3.24 (±0.03)
12	0	25	3	5.69 (±0.20)
13	0	50	6	4.26 (±0.04)
14	0.25	50	6	3.23 (±0.11)
15	0.25	25	6	3.80 (±0.06)
16	0.25	50	6	3.25 (±0.03)
17	0.25	50	9	2.84 (±0.02)
18	0.25	75	6	2.92 (±0.16)
19	0.5	25	9	3.48 (±0.03)
20	0.5	75	9	2.46 (±0.07)

$NaHCO_3$: sodium bicarbonate, TC: Total carbohydrate in the final extract

The effects of the process variables on the total carbohydrate values in the final extract of the cress seeds were shown in the analysis of variance (ANOVA) table (Table 3). The model was significant (p<0.05) and the lack of fit value was not significant statistically (p>0.05). It is important to obtain non-significant lack of fit value, because lack of fit is one of the most important criteria required for the generated models to be able to explain the experimental data with high accuracy (Myers & Montgomery, 1995). In this study, the statistically insignificant value of lack of fit (p>0.05) showed the success of the generated model. Results showed that all independent variables had significant effect on the total carbohydrate values in the final extract values. Moreover, the quadratic effects of the all-model parameters independently affected the model significantly. Also, the interaction of $NaHCO_3$ concentration – soaking time had statistically significant effect on the model (p<0.05) (Table 3). On the other hand, the interactions of the $NaHCO_3$ concentration – solvent-to-seeds ratio and solvent-to-seeds ratio – soaking time did not significantly affect the given model (p>0.05) (Table 3). Statistical results of the model were also shown in Table 3. The coefficient of determination (R^2) value was >0.98 which indicated that the generated model was adequate and high proportion of variability was explained by the data. Also, the closeness of the R^2 and adj-R^2 values showed the goodness of the models and it was a proof that only statistically significant terms were included in the generated models (Table 3). The predicted residual error sum of squares (PRESS) and coefficient of variation (C.V., %) values were small enough to demonstrate the suitability of the model for the given data.

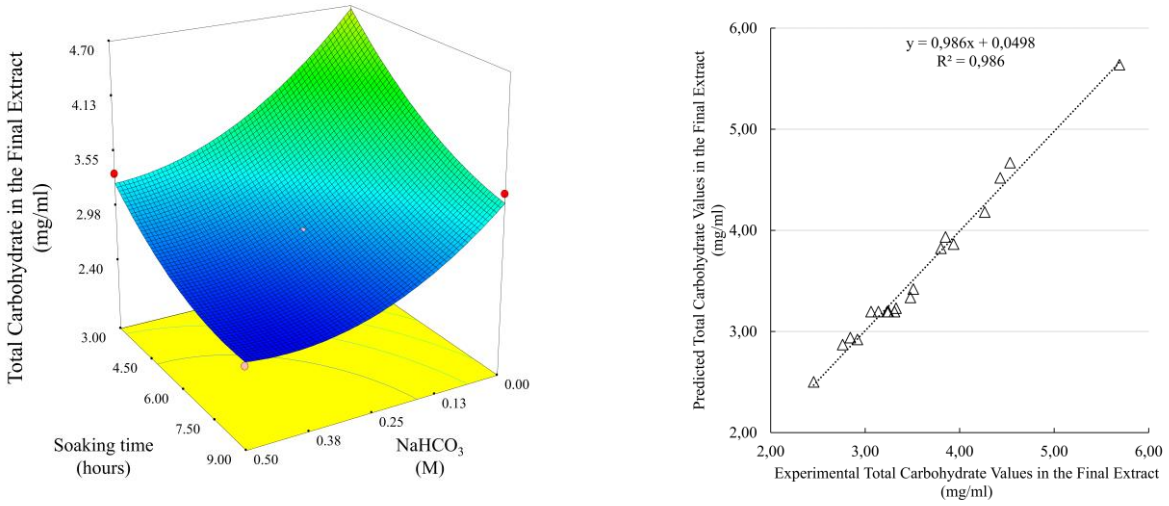
Table 3
ANOVA table and statistical parameters

Source	DF	Sum of Squares	F Value	p - Value
Model	9	1.17	78.18	< 0.0001
X_1	1	4.29	287.45	< 0.0001
X_2	1	2.03	136.04	< 0.0001
X_3	1	2.13	142.86	< 0.0001
X_1X_2	1	0.035	2.35	0.1563
X_1X_3	1	0.14	9.08	0.0130
X_2X_3	1	$8.6x10^{-3}$	0.57	0.4661
X_1^2	1	0.30	19.91	0.0012
X_2^2	1	0.085	5.67	0.0385
X_3^2	1	0.12	7.74	0.0194
Residual	10	0.015		
Lack of Fit	5	0.022	2.82	0.1398
Pure Error	5	$7.8x10^{-3}$		
Total	19			
R^2	*adj- R^2*	*Adequate Precision*	*PRESS*	*C.V. (%)*
0.9860	0.9734	36.29	1.96	3.44

X_1: $NaHCO_3$ concentration (M), X_2: Solvent-to-seeds ratio (v/w), X_3: Soaking time (h), DF: Degrees of Freedom, Adj- R^2: Adjusted R^2, PRESS: Predicted residual error sum of squares, C.V. (%): Coefficient of variation

The effect of the different process variables on the total carbohydrate values in the final extract was shown with response surface graph in Figure 1(a). Response surface graphs visually supported our findings, and it was observed that higher $NaHCO_3$ concentration lowered the total carbohydrate values in the final extract. At a constant rate of solvent-to-seeds ratio of 75 ml/g, it was clearly seen in the graph that the total carbohydrate values in the final extract decreased at higher soaking times. A second-order polynomial model was generated for the mucilage removal from the cress seeds and the total carbohydrate values in the final extract were minimized for obtaining optimum mucilage removal conditions (Equation 3.1). The relation between predicted and experimental data were shown in Figure 1(b) and the closeness of the predicted and experimental values was clearly demonstrated, which is another proof for the suitable model fitting.

$$\text{Total carbohydrate (mg/ml)} = 7.79-6.82X_1-0.046X_2-0.45X_3+0.17X_1X_3+5.26X_1^2+2.80\times10^{-4}X_2^2+0.02X_3^2 \quad (3.1)$$



(a) (b)

Figure 1. Mucilage removal of cress seed and TC values: (a) response surface graph at 75 ml/g solvent-to-seeds ratio, (b) the relation between predicted and experimental data

Mucilage removal process was subjected to numerical optimization to determine the optimum process conditions. We found out 4 different but quite similar optimization results and one of these results having the highest desirability (0.995) was chosen as the optimum point. The predicted optimum mucilage removal conditions were 0.43 M of $NaHCO_3$ concentration, 75 ml/g of solvent-to-seeds ratio and 8.96 hours of soaking time. Total carbohydrate value in the final extract at these conditions was 2.64±0.43 mg/ml. The optimum point verified for three times and the single sample t-test showed there was no statistically significant difference between experimental and predicted value (2.47 mg/ml) at the optimum point (p>0.05).

4. Conclusion

In this study, mucilage present in the coat of the cress seeds was removed by a chemical soaking process and the optimum removal conditions ensuring the lowest total carbohydrate values in the final extract were determined. Response surface methodology was used to optimize the mucilage removal conditions. Our results showed that $NaHCO_3$ presence in the medium enhanced the solubility of the mucilage found in the seed coat and the removal process of the mucilage was more efficient at higher concentrations of $NaHCO_3$. Solvent-to-seeds ratio and soaking time also affected the mucilage removal process. Because of having considerable amount of biomaterials, the cress seeds can be used as a valuable product in the food industry. To obtain biomaterials such as oils, protein and phenolic substances bound to the mucilage from the cress seeds, mucilage removal step is vital to prevent hindering the extraction of biomaterials. Our data can be used as a pre-treatment for the biomaterial extraction from cress seeds and further researches can be conducted to find out the effect of mucilage removal on the extraction yields of biomaterials.

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Author Contributions

Izzet Turker: Formal analysis, Data curation, Investigation, Writing - Original Draft

Hilal Isleroglu: Methodology, Conceptualization, Writing - Original Draft, Writing - Review & Editing, Supervision

Conflicts of Interest

The authors declare no conflict of interest.

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