

ORIGINAL ARTICLE

Mucilage from *Plantago ovata* Forsk seed: Optimization of extraction process conditions and investigation of some functional properties

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Cite as: Assi, A. G., Kalkan, E., & Maskan, M. (2024). Mucilage from *Plantago ovata* Forsk seed: Optimization of extraction process conditions and investigation of some functional properties. *Brazilian Journal of Food Technology*, 27, e2024017. <https://doi.org/10.1590/1981-6723.01724>

Abstract

Psyllium mucilage was extracted from *Plantago ovata* Forsk and functional properties were investigated. Concerning this objective, Response Surface Methodology based on a 4-factor 3-level Box-Behnken design was used. The independent variables were as follows: extraction temperature (50-80 °C), extraction time (60-120 min), water-to-*Psyllium* seed ratio (50/1-100/1), and water pH (4-10). On the other hand, the dependent variables were decided as EY (extraction yield), SC (swelling capacity), EC (emulsion capacity), and ES (emulsion stability). Optimum extraction conditions based on numerical optimization were 79.99 °C extraction temperature, 60.02 min extraction time, 99.99:1 water-to-seed ratio, and 7.38 water pH. Within these optimum conditions, 29.54% EY, 25.47 mL g⁻¹ SC, 68.39% EC, and 76.61% ES were predicted. The results indicated that *Psyllium* mucilage can be used as a promising additive as a stabilizing agent in food formulations.

Keywords: *Psyllium*; Optimization; Extraction yield; Emulsifying properties; Swelling; Functional properties.

Highlights

- The extraction yield and functional properties of *Psyllium* mucilage were investigated
- Extraction parameters had a profound impact on *Psyllium* mucilage
- Outstanding functional properties could make *Psyllium* mucilage ideal for the industry

1 Introduction

Natural polysaccharides have drawn increasing interest due to their multifunctional bioactivities in recent decades (Tudu & Samanta, 2023). Although the first academic research focused on the source of the mucilages and qualitative testing, the popularity of mucilages increased between 1991 and 2000, with between 18 and 37 publications appearing each year. Since 2008, the curiosity about mucilages has increased steadily, with more than 140 articles published in 2020 (Dybka-Stępień et al., 2021). *Psyllium* mucilage



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stands as a highly beneficial polysaccharide due to its biodegradability, easy availability, and digestibility. It can be used in a variety of applications including functional edibles, dietary supplements, nutraceuticals, pharmaceuticals, medicinal applications, cosmetic formulations, and the food industry. The term “psyllium” often refers to over 200 species of *Plantago* plants. These species are predominantly found in Bangladesh, Pakistan, Iran, and India's temperate zones, which make these areas perfect for growing the plant. India is currently the world's top producer and supplier of plantago husks and seeds. The United States of America (USA), on the other hand, purchases the most, 8,000 thousand tonnes annually (Franco et al., 2020).

Only two species of *Plantago* (*P. ovata* Forsk. and *P. psyllium*) are grown for their seed coats, which are in demand by the cosmetic and pharmaceutical industries. A natural source of dietary fibre and functional ingredients is the mucilage extracted from the seed coat (Kumar et al., 2017). Moreover, the husk is separated from the seed during the milling process and comprises a mucilaginous membrane, and accounts for approximately 30% of the seed's weight. *Psyllium* seed has a shelf life of about 6 months under normal storage circumstances. It has a moisture content of 4.22% to 12.55%, a protein content of 0.94% to 7.12%, an ash content of 3.4-12%, and a total carbohydrate content of 30.33-84.98% (Franco et al., 2020). According to some reports, carbohydrates are the main element in *Psyllium* seed husk's composition. Additionally, *Psyllium* seed husk is made up of a unique highly branched arabinoxylan, a polymer with high arabinose and xylose content (Noguerol et al., 2022). Xylose (50.3-56.72 g 100 g⁻¹) and arabinose (20.7-21.96 g 100 g⁻¹) are predominantly found. The next sugars are glucose (0.64-2 g 100 g⁻¹), galactose (3.76-2.8 g 100 g⁻¹), mannose (0.4-1.1 g 100 g⁻¹), and rhamnose (1.1-1.5 g 100 g⁻¹). Also, mucilages are present at a level of 20% to 25% (Franco et al., 2020; Ziemińchód et al., 2019).

Most commonly, *Psyllium* is grown for its mucilage content which has a white fibrous structure. *Psyllium* seed husk are used as a raw material for producing *Psyllium* mucilage. Mucilage is a sort of clear, colourless, gel-forming material produced by plants. It is purified by precipitating alcohol or acetone in an aqueous solution and then drying (Kumar et al., 2017). *Psyllium* seed coat produces a kind of gel-like mucilage with a high water retention capacity of around 80 times its weight. Due to its exceptional mucilage properties, seed husk finds a wide range of applications in the food sector as a thickener. These physical properties, such as clear, colorless, gelling, stabilizing, thickening, and its structure, which qualifies it as a natural dietary fiber, could demand for prompt rise in the utilization of *Psyllium* by the food processing industry. The use of *psyllium* husk in food products has been allowed by the Food and Drug Administration (FDA) and is considered as safe (Franco et al., 2020).

As repeatedly mentioned in the literature, *Psyllium* can be used as a food additive to increase shelf life and customer satisfaction (Santos et al., 2021). Studies have demonstrated that *Psyllium*'s gel-forming ability along with functional qualities makes it a suitable ingredient for utilization in food preparation. Therefore, an improvement in shelf life could result from a reduction in water activity (Franco et al., 2020). *Psyllium* not only boosts health, but it also improves the volume (Fratelli et al., 2021), structure, texture (Souza et al., 2020a), acceptability (Aldughpassi et al., 2021), antioxidant activity (Blassy & Abdeldaiem, 2024), and anti-staling (Filipčev et al., 2021) qualities of food products. Oat-based gluten-free cookies were formulated to contain 0%, 2.5%, and 5% *Psyllium* husk and it was found that the incorporation of 5% *Psyllium* husk increased the functional properties, overall acceptability, and total fibre content up to 19.41% (Khan et al., 2021). Similarly, the preparation of dietary prebiotic sponge with different percentages of *Psyllium* husk was investigated (Beikzadeh et al., 2016). According to the results obtained, the highest overall acceptability regarding sensory evaluation was obtained with 5% *Psyllium*, and the overall acceptability of the diet sponge cake was improved with 7.5% *Psyllium*. Additionally, *Psyllium* husk powder greatly improved emulsion stability and oxidative stability with a 0.6% addition and was a potential emulsifier for stable oil-in-water emulsions (Fu et al., 2022). In another study, the incorporation of *Psyllium* husk into gluten-free pasta with an equal amount of (50%) *Psyllium* gel and rice flour resulted in improved cooking and textural quality characteristics, higher healthy fiber content, and even increased antioxidant activity (Fradinho et al., 2020). In the pharmaceutical industry, mucilages can be derived from many sources and used as thickeners, disintegrants, bioadhesive agents, emulsion stabilizers, and binders to formulate mucoadhesive nasal gel,

colon-targeted tablets, controlled-release matrix tablets, and fast-dissolving tablets (Kamel et al., 2020). Therefore, mucilage was also employed as a biopolymer for tissue engineering applications a decade ago and evaluated as a scaffold for tissue regeneration or new tissue developments.

Response Surface Methodology (RSM) is a set of mathematical and statistical instruments that are used to design, enhance, and optimize complex processes, as well as to shed light on the relationships between one or more dependent and independent variables. RSM is especially successful in demonstrating the interaction between various variables of hydrocolloid extraction by employing 3D graphic surface plots (Bukhari et al., 2022; Goswami et al., 2022). The use of this strategy saves the cost and time for large-scale operations. Various researchers have used plant-based mucilage extraction using RSM for *Mimosa pudica* L. seed mucilage (Bukhari et al., 2022) for *Ximenia americana* L. seed mucilage (Bazezew et al., 2022), for sepestan fruit mucilage (SFM) (Kaykha et al., 2022), and for chia mucilage (Orifici et al., 2018).

Various mucilage extraction methods (conventional or ultrasound, microwave, enzymatic assisted) in combination with different conditions such as temperature, time, pH of the solvent, effect of co-solutes, ratio of solvent to seed, precipitating agents, and drying methods were studied in previous works (Amiri et al., 2021; Chiang & Lai, 2019; Ma et al., 2021; Pereira et al., 2019). The aqueous extraction of mucilage from flaxseed, for example, was conducted to examine the impact of solvent pH on the extraction yield (EY). The largest yield was achieved at a pH of 6.75, while the highest quality mucilage was obtained at a pH of 9.69. However, the use of ethanol as a precipitating agent was not entirely successful in the elimination of any impurities (Rocha et al., 2021). In another study, the effects of two different temperatures and precipitating agents on the EY of *Hibiscus rosa-sinensis* Linn. mucilage were studied. The EY increased by 5% at 50 °C compared to 25 °C, and by 2% to 3% when precipitated with acetone against ethyl alcohol (Vignesh & Nair, 2018).

The few studies on *Psyllium* mucilage contained its usage in food systems as an additive. Optimization is needed to achieve high mucilage yield with better emulsifying/stabilizing properties to be used in food systems. Optimal conditions and models which describe the extraction process are recommended. The information is vital for determining the extraction processing conditions for industrial production of high quality mucilage. Therefore, the purposes of this study were: (1) to examine the effect of extraction temperature, extraction time, water-to-seed ratio, and water pH on the extraction yield (EY), swelling capacity (SC), emulsion capacity (EC), and emulsion stability (ES) of *Psyllium* mucilage, (2) to determine the optimum extraction conditions for psyllium mucilage by using RSM.

2 Materials and methods

2.1 Material and reagents

As a raw material, *Psyllium* seeds (*Plantago ovata* Forsk) were acquired from a local market in Gaziantep, Turkey in September 2021. Sunflower oil was also obtained from a marketplace in Gaziantep, Turkey. It must adhere to the Codex Alimentarius norms and/or criteria listed below. The technical properties are as listed below: maximum of 0.3% free fatty acid referred as oleic acid, 10 milliequivalent O₂ kg⁻¹ (w w⁻¹) of peroxide value, 4.0 to 7.6 of palmitic acid (C16:0), 2.1 to 6.5 of stearic acid (C18:0), 14.0 to 71.8 of oleic acid (C18:1) and 18.7 to 74.0 of linoleic acid (C18:2). The pure acetone employed was of analytical grade and purchased from Sigma Aldrich Co (St. Louis, MO).

2.2 Sample preparation

A laboratory blender (Blender 8011ES Model HGB2WTS3, 400 W) was used to completely grind the *Psyllium* seeds after approximately 2 min of homogenization.

2.3 Extraction of mucilage

For each experimental run, 50 g of psyllium seed was used. *Psyllium* seed husk mucilage was isolated from whole seeds using an altered format of the procedure described by Souza et al. (2020a). Homogenized *Psyllium* seeds were taken to extract its mucilage according to the experimental design given in Table 1 and 2. In fact, 0.1 mol L⁻¹ hydrochloric acid (HCl) or sodium hydroxide (NaOH) solutions were used to regulate pH. Mucilage was extracted manually using distilled water at water to *Psyllium* seeds ratio between the range of 50:1 to 100:1 (mL g⁻¹), a water pH between the range of 4 to 10, an extraction period between the range of 60 to 120 min, and an extraction temperature between the range of 50-80 °C. Before adding the seeds, the water was heated to a specified experiment temperature. The homogenized seeds and water mixture were seated in a water bath with constant stirring to keep the temperature consistent and for a more uniform dispersion during the extraction time. Subsequently, the mucilage-water mixture was then filtered with a double-layered white muslin cloth to remove all unwanted residues. The filtrate was then mixed with the same amount of acetone to precipitate the mucilage. In a rotary evaporator (IKA RV 10 digital, IKA, Germany) set at 50 °C, precipitated mucilage was concentrated. Acetone-free precipitated mucilage was put into a tray and frozen at -70 °C for two days. To sublimate ice into vapor directly, the frozen sample was dried in a freeze dryer (CHRIST, Alpha 1-4 LSC, Osterode am Harz, Germany) for two days. Given that acetone has a boiling point of 56 °C, operating the rotary evaporator under vacuum at 50 °C guaranteed that all of the acetone is removed. Also, after additional freeze-drying, even traces of acetone in the mucilage is not expected. Dried *Psyllium* mucilage samples were kept in a glass jar with a lid in the refrigerator until used. The analyses were repeated twice.

2.4 Moisture content analysis

0.75 g of *Psyllium* seeds was obtained and examined by a moisture analyser (Mettler Toledo MJ 33, USA) based on the infrared method. The moisture content of freeze-dried mucilage sample was also examined too (Khodabux et al., 2007).

2.5 Extraction yield

EY was calculated as the weight of *Psyllium* mucilage powder (dry basis) produced after freeze drying divided by the weight of homogenized *Psyllium* seeds (dry basis) utilized in the extraction process using Equation 1 (Halász et al., 2022).

$$EY(\%) = \frac{\text{Dried } Psyllium \text{ husk mucilage powder(g)}}{\text{Homogenized } Psyllium \text{ husk seed (g)}} \times 100 \quad (1)$$

2.6 Swelling capacity

The SC of *Psyllium* mucilage was determined, in the current study, according to the method of Bhatia & Ahuja (2013) with some modifications. First, 0.25 g of *Psyllium* mucilage was placed in a graduated cylinder with a volume of 10 mL. The sample's initial volume (V₁) was recorded. In order to make sure of proper dispersion, 5 mL of water was added, agitated effectively for 30 seconds, and left to stand for 24 hours. Later, a sufficient amount of water was added to equalize 10 mL and gently mixed for 30 seconds before being allowed to stand for another 48 hours. The volume occupied by the swollen mucilage was measured and recorded (V₂). Based on Equation 2, SC was determined in (mL g⁻¹).

$$SC(\text{mL g}^{-1}) = \frac{V_2 - V_1}{0.25} \quad (2)$$

2.7 Emulsifying properties

The methodology given by Bukhari *et al.* (2022) was applied with slight modifications to determine the EC and ES. A sample of 0.25 g of mucilage was weighed and dissolved in 10 mL of water for EC. Subsequently, 10 mL of sunflower oil was poured into the suspension and homogenized with homogenizer Ultra-Turrax (IKA, T18 basic, Germany) at $1100 \times g$ for 3 to 4 min. The emulsion was centrifuged for 5 min at $7200 \times g$ at 25 °C. To generate the emulsion for ES, it was heated in a water bath for 30 min at 80 °C. In the final, the height of the emulsified layer and the height of the whole layer were measured and compared. By using Equations 3 and 4, the volumes of EC and ES were calculated as a percentage of EC and ES.

$$EC(\%) = \frac{\text{Height of emulsified layer}}{\text{Height of whole layer}} \times 100 \quad (3)$$

$$ES(\%) = \frac{\text{Height of emulsified layer after heating}}{\text{Height of whole layer}} \times 100 \quad (4)$$

2.8 Experimental design, modeling, optimization, and statistical analysis

In this research, RSM was applied in this study to investigate the influence of four independent variables (extraction temperature, extraction duration, water-to-seed ratio, and water pH) on four dependent variables (EY, SC, EC, ES).

The extraction of *Psyllium* mucilage was optimized using a Box-Behnken Design (BBD) statistical experiment layout with four independent variables at three stages. The detection of extraction condition ranges was clarified by preliminary research. Thus, a total of 29 runs, including five center points, were used. There were three distinct levels for every single independent variable: +1, 0, and 1. Responses in this current research cover EY, SC, EC, and ES. Extraction temperature (°C), extraction time (min), seed-to-water ratio and, water pH were the independent variables A, B, C, and D, respectively. Table 1 displays the independent variables and their levels. Also Table S1 exhibits the experimental design with coded and actual variable levels. Analysis of variance (ANOVA) was the technique utilized to evaluate the results. All of the independent variables remained within their particular ranges while the responses were maximized. The significance of the regression coefficients was evaluated as well using the F test, which was used for verifying the statistical significance of models at $p \leq 0.05$.

Table 1. Independent variables and their levels were used in the response surface design.

Independent variables	Variable Level Codes			
	Code	- 1	0	1
Extraction temperature temperature	A	50	65	80
Extraction time	B	60	90	120
Water-to-seed ratio	C	50	75	100
Water pH	D	4	7	10

The responses were optimized using numerical and graphical (3D plots) optimization techniques from Design-Expert version 13 (Minneapolis, USA). The experiments were carried out in a random order to lessen the influence of undetectable variability in observed responses due to external factors. The *Psyllium* mucilage extraction process was optimized by increasing EY, SC, EC, and ES.

3 Result and discussion

3.1 Data analyses and model fitting

Response Surface Methodology was carried out to assess the impact of four independent variables [extraction temperature (A); extraction time (B); water to *Psyllium* seed ratio (C); and water pH (D)] on four responses (EY, SC, EC, and ES). The regression model graphs illustrated the impacts of process variables using linear, quadratic, and 2FI equations. Table 2 includes the results of 29 distinct experimental runs, according to BBD. While the *P*-value of ANOVA is significant, i.e. ≤ 0.05 , it demonstrated that the BBD model is successfully employed to data in order to optimize extraction conditions.

Table 2. Results of responses obtained from four-factor, three-level BBD.

Run	Independent Variables				Responses			
	A	B	C	D	EY (%)	SC (mL) g ⁻¹	EC (%)	ES (%)
1	65	60	75	10	23.81	15.42	49.99	71.43
2	65	90	50	10	23.40	18.41	54.23	74.97
3	80	60	75	7	27.63	22.73	58.75	81.25
4	50	90	100	7	25.05	18.38	57.14	77.14
5	50	90	75	10	21.67	15.70	47.14	85.14
6	65	60	100	7	27.40	22.79	77.49	82.50
7	50	90	50	7	23.71	14.50	63.51	81.94
8	65	90	75	7	26.90	21.86	52.49	81.25
9	80	90	75	4	28.84	19.79	48.75	63.64
10	65	120	75	10	24.59	16.81	64.23	67.41
11	65	120	50	7	26.69	22.60	60.02	77.14
12	80	90	50	7	29.19	20.69	43.64	68.75
13	65	60	50	7	25.87	20.69	55.02	81.75
14	65	90	75	7	30.09	23.70	55.13	72.22
15	65	90	75	7	29.29	20.70	67.51	77.49
16	50	90	75	4	23.56	11.52	75.01	67.50
17	65	90	75	7	27.50	20.80	62.86	78.57
18	80	120	75	7	32.36	26.53	52.78	64.20
19	50	60	75	7	20.15	14.88	57.14	81.43
20	80	90	75	10	27.80	20.90	51.74	75.04
21	50	120	75	7	24.87	17.80	67.50	76.70
22	65	90	75	7	24.63	22.39	59.90	75.03
23	65	120	75	4	27.57	21.60	38.64	67.50
24	65	90	100	10	31.93	22.63	42.86	71.78
25	65	90	50	4	21.47	19.65	57.50	66.25
26	65	120	100	7	27.51	24.90	37.61	64.31
27	65	60	75	4	24.59	15.79	50.01	62.86
28	80	90	100	7	29.60	27.61	43.42	62.83
29	65	90	100	4	25.20	22.80	75.14	41.43

Regression analysis and ANOVA were applied to fit the model and test the statistical significance of the terms. Table S2 displays the model summary and ANOVA of the results. The greater the R^2 value, the better

the empirical data matches the actual data. The mission of P -values is to control the statistical significance of each parameter. P -values ≤ 0.05 demonstrate that model terms are significant, and a lower P -value shows that the attributed parameter is more significant.

The response EY was analyzed as a function of linear terms because a linear model was proposed. The response EC was evaluated using linear and interaction terms since the 2FI model was found to be statistically significant. SC and ES responses were assessed using linear, quadratic, and interaction terms for the independent variables water to seed ratio, extraction temperature, and extraction time.

Regression analysis and ANOVA were applied to adjust the model and measure the statistical significance of the terms. The model's suitability for clarifying the relationships between variables is indicated by the higher R^2 value. The R^2 values for EY, SC, EC, and ES were 0.5926, 0.8951, 0.5743 and 0.8015, respectively. The insignificance of the lack of fit ($p > 0.05$) indicates that the proposed approach works well for predicting responses within the range of the experiment. The F -value and P -value were used as indicators of statistical significance and regression coefficient significance, in that order (Kaykha *et al.*, 2022). Temperature and water-to-seed ratio were significant independent variables (P -value < 0.0001 and 0.0318 , respectively) in ANOVA. About these findings, EY demonstrated the greatest value of 32.36% under the conditions of a maximum temperature of 80 °C, maximum time of 120 min, water-to-seed ratio of 75:1, and water pH of 7. A^2 and D^2 were considerably effective in SC, in addition to all linear variables. The linear term of temperature had the highest mean square and the lowest P -value (less than 0.0001). Accordingly, the lowest SC of 11.52 mL g⁻¹ was obtained at the lowest temperature of 50 °C, while the maximum SC of 27.61 mL g⁻¹ was obtained at the highest temperature of 80 °C. The interaction between time and water pH had a major effect on EC, as shown by the lowest P -value of 0.0164. Lastly, the most important variable on ES among all linear terms and D^2 was the linear term of water pH. In a similar vein, the favorable influence of water pH on ES was evidenced with the highest ES of 85.17% achieved at the highest water pH level.

3.2 Extraction yield

According to ANOVA results (Table S2), the linear model is statistically significant ($p \leq 0.05$) for EY with F -value of 8.73 and P -value of 0.0002. The ANOVA table also shows that the only significant factors are the extraction temperature (A) and the water-to-seed ratio (C). Other terms and a lack of fit were not significant according to the analysis of variance results ($p > 0.05$). Fortunately, a non-significant 0.92 F -value lack of fit ($p > 0.05$) implies that the model is properly fitted to the yield data and that an associated response element should be involved in the regression (Bukhari *et al.*, 2022). According to the sum of squares, the order of significance for the independent variables is as follows: extraction temperature > water to seed ratio > extraction time > water pH.

The EY in the current study ranged from 20.15% to 32.36%. These findings were discovered at the midpoints of these parameters, with the same water-to-seed ratio and water pH. Nevertheless, the yield rose from the lowest value to the greatest value when the temperature jumped from 50 to 80 °C and the extraction period was extended from 60 to 120 min. The highest EY was achieved at the maximum points of the extraction temperature and time ranges, whereas the water/seed ratio and water pH ranges were at the midpoints. According to optimum extraction conditions (79.99 °C extraction temperature, 60.02 min extraction time, 99.99:1 water to seed ratio and 7.38 water pH) based on numerical optimization, EY was predicted to be 29.54%. This finding demonstrates that a very small drop in yield (32.36 to 29.54%) can result in large time savings in the extraction process, and hence in operational expenses.

The range of *Psyllium* mucilage EY presented in this study (20.15-32.36%) is in line with the outcomes of Kalkan & Maskan (2023) for EY of okra mucilage (16.92-32.19%) which is extracted at temperatures ranging from 25 to 80 °C for 1 to 7 hours, with a 4:1 to 10:1 water-to-okra ratio. The RSM-BBD model's suitability for EY data was probed to check the values of R^2 , adjusted- R^2 , and predicted- R^2 . The difference

between R^2 (0.5925) and adjusted- R^2 (0.5246) was 0.0679, while the difference between R^2 (0.5925) and predicted- R^2 (0.4162) was 0.1763. This study established that RSM-BBD is a suited model for mucilage extracted from *Psyllium* yield data. In contrast to R^2 , adjusted- R^2 only accounts for variation caused by significant terms. The difference between adjusted- R^2 and predicted- R^2 is less than 0.2 in the current investigation, indicating that the model is adequate. Furthermore, non-significant terms are not eliminated from the predicted equation to ensure the accuracy of the equation. The relationship between EY and independent variables of extraction temperature, water-to-seed ratio, extraction time and water pH in terms of coded variables are given in Equation 5.

$$\text{EY (\%)} = 26.3069 + 3.03667 A + 1.17917 B + 1.3625 C + 0.163333 D \quad (5)$$

Response surface 3D plots for the effects of independent variables on the yield is given in Figure 1.

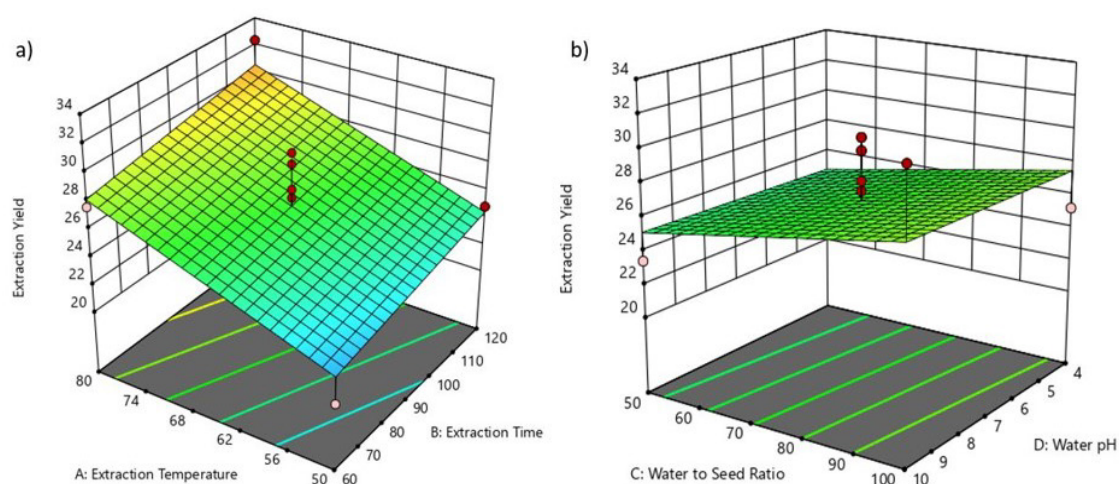


Figure 1. Interaction effect of temperature and time (a), water to seed ratio, and water pH (b) on EY (Third and fourth parameters are kept at the center point for each plot).

Figure 1a exhibits the movements in EY concerning temperature and time while maintaining a constant water-to-seed ratio and water pH. As shown, the EY increases proportionally to the increase in temperature. Two theories are provided that help to explain the tendency. First, increasing the temperature of the solvent increases the solubility of polysaccharides. As a consequence of this, the polysaccharide's diffusion coefficient rises. A high diffusion coefficient leads to a bigger mass of polysaccharides extracted from seeds to water, increasing yield. Second, when the temperature rises, the seeds evolve into less viscous aspects, which increase the amount of mucilage released into the water (Bukhari et al., 2022; Kaykha et al., 2022). Additionally, the primary component of *Psyllium* seed husk is arabinoxylan which is reported to be weakly acidic (Naran et al., 2008) or neutral (Ren et al., 2020). Increasing the extraction temperature of arabinoxylan from 20 °C to 95 °C elevated its EY from 29% to 50%. Similarly, arabinoxylan yield from corn fiber was higher at 60 °C than at 40 °C. The bonds between arabinoxylan and the cell wall matrix are unable to be disrupted under mild conditions (such as, below 100 °C) as reported by Zhang et al. (2014).

According to Campos et al. (2016), EY of the chia mucilage was the highest at high temperature, 80 °C. Bazezew et al. (2022) described a study that was conducted under the circumstances of extraction temperature (15-110 °C), extraction time (0.5h to 6h), and water-to-seed ratio (10:1-100:1) to enhance the conditions for isolating mucilage from *X. americana* seeds. In contrast to the present investigation, EY was elevated until a temperature of 69 °C, at which point it began to decline. Likewise, the yield of mucilage extracted from *Descurainia sophia* (L.) Webb ex Prantl seeds rises to a temperature of 94.3 °C before it sharply drops afterward due to the structure of the polysaccharides being hydrolysed (Golalikhani et al.,

2014). The EY, protein content, emulsifying properties, and color of the mucilages are primarily influenced by the temperature and length of the extraction process. In terms of yield, raising temperatures generally enhance extraction yield to some extent; however, further increases cause yield to decline. Increased yield is induced by the ability of water to penetrate seeds more easily due to temperature effects, and mucilage is simply dispersed and freed. Conversely, greater extraction temperatures produce mucilage hydrolysis, resulting in a lower yield (Nazir et al., 2017). EY's dependence on extraction temperature could also be explained by an increase in mucilage solubility. Many researchers have discovered that the solubility of mucilage increases as the temperature increases (Souza et al., 2020a). This phenomenon can be attributed to the breakdown of hydrogen bonds between polysaccharide chains at elevated temperatures, leading to the ingestion of OH groups into water (Antigo et al., 2020). Consequently, the increase in mucilage solubility could be attributed to the presence of protein compounds that were extracted concurrently with mucilage during extraction and were not completely removed. The variation in solubility may be attributed to the disparity in the purity of the extracted gums under different extraction conditions (Souza et al., 2020a). *Psyllium* polysaccharide was determined to be insoluble in acetone, alcohol, ether, chloroform, diethyl ether, and ethyl acetate. However, it does form a gel when exposed to an aqueous environment, such as water and buffers (Rao et al., 2013). Previous works proposed that the drying process modifies the amount of galactose, glucose, arabinose, and xylose in the chemical structure of polysaccharides, which could explain the drastic changes in solubility. The presence of arabinose impacts water solubility because the molecule with high arabinose binds more water and becomes more soluble (Souza et al., 2020a; Sternemalm et al., 2008).

As illustrated in Figure 1a, extraction time had no effect on EY, contrary to observations from Morales-Tovar et al. (2020). Although, Karazhiyan et al. (2011) suggested that increasing the extraction time can increase the EY up to a certain degree, doing so for a prolonged exposure may lead to structural modifications of the polysaccharides. According to Nazir et al. (2017), as the interaction period between seed and solvent is extended, the yield of mucilage increases with extraction time. Similarly, raising the water-to-seed ratio to 45:1 enhanced EY, which subsequently began to decline as the ratio was increased. As demonstrated in Figure 1b, when the extraction temperature and duration were held constant, the water-to-seed ratio boosted the EY. This is a result of extra available liquid, which enhances the driving force of mucilage out of the seeds as claimed by Koocheki et al. (2009a). Furthermore, contrary to much research, water pH had no apparent effect on yield. For example, according to Golalikhani et al. (2014), a study using four parameters and pH points of 4.0, 5.5, 7.0, 8.5, and 10.0 found that the EY of *Descurainia sophia* mucilage was highest at pH 7.55 and dropped as pH rose. Behbahani et al. (2017) created a setup for extracting mucilage from *Plantago* major seed and produced the maximum yield, 15.18% when the pH was 6.8. They also stated that this straight-line trend of pH was nonsignificant.

3.3 Swelling capacity

In the presence of water, mucilage creates a sticky solution or gel, which has the potential to be utilized by the food industries as a thickener, gelling agent, and emulsion stabilizer (Amaral et al., 2018; Hussain et al., 2019). For instance, the taste and texture of the gluten-free products were superior due to gelling and high water-holding capacity of chia mucilage. Also, adding 2% chia mucilage delayed the melting of ice creams and had the potential as an emulsifier and stabilizer for ice cream. Flaxseed mucilage can be employed as a thickening in a food system (Chiang et al., 2021; Puligundla & Lim, 2022).

The outcomes from ANOVA (Table S2) showed that the quadratic model is highly recommended with F -value of 8.54 and P -value of 0.0001 for SC. Also, quadratic terms of A^2 and D^2 were also found to be significant terms ($p < 0.05$). Extraction temperature (A) was the most effective term on SC with the lowest P -value (< 0.0001). It is followed by water-to-seed ratio (C) and extraction time (B) ($p < 0.05$). Nevertheless, other terms were not significant ($p > 0.05$). Between 11.52 mL g⁻¹ and 27.61 mL g⁻¹ of SC was detected in this investigation. The highest SC value of the *Psyllium* mucilage (27.61 mL g⁻¹) was obtained under the

80°C extraction temperature, 90 min extraction time, 100:1 water to seed ratio, and 7 water pH. The conditions with the lowest SC values (11.52 mL g⁻¹) were 50 °C for the extraction temperature, 90 minutes for the extraction duration, 75:1 water-to-seed ratio, and 7 for the water pH. Increasing water pH, extraction temperature, and water-to-seed ratio (during the same extraction period of 90 minutes) increased SC from minimum to maximum. With 2.30 *F*-value, lack of fit was not significant, as desired statistically. The results for *R*², Adjusted *R*², and Predicted *R*² are 0.8951, 0.7902, and 0.4611, respectively, indicating strong agreement with the suggested relationship. In general, the closer the *R*² number is to one, the more accurate the modeling. The predicted-*R*² is a measure of how well the model predicts the response value. The quadratic model is more accurate in displaying SC in this investigation.

The relationship between SC and independent variables of extraction temperature, water-to-seed ratio, extraction time and water pH in terms of coded variables are given in Equation 6. This relationship is also shown in Figure 2. Non-significant model terms are not excluded in order to protect the accuracy of the predicted equation.

$$SC \text{ (mL g}^{-1}\text{)} = 21.89 + 3.78A + 1.49B + 1.88C - 0.11D + 0.22AB + 0.75AC - 0.775AD + 0.05BC - 1.1BD + 0.27CD - 1.87A^2 - 0.4285B^2 + 1.22C^2 - 3.12D^2 \quad (6)$$

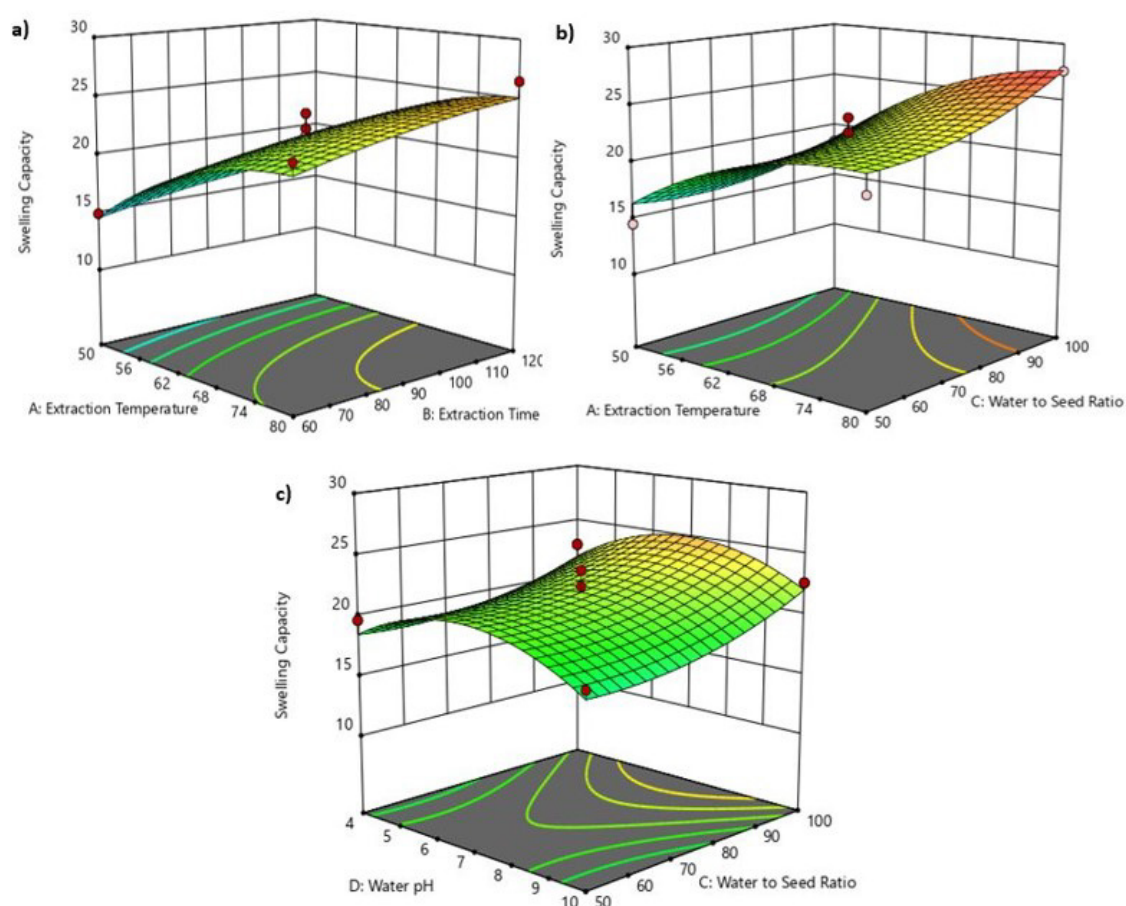


Figure 2. Interaction effect of temperature and time (a), temperature and water to seed ratio (b), water pH and water to seed ratio (c) on SC (Third and fourth parameters are kept at the center point for each plot).

As shown in Figure 2a, SC rose as extraction temperature and time increased. This observation is in line with the research of Kalkan & Maskan (2023). They observed that increasing the extraction time and temperatures increased the swelling capability of okra mucilage. Temperatures above 52.5 °C on the other hand, caused a

reduction in the SC of okra mucilage. Similarly, *Corchorus olitorius* L. mucilage was extracted using hot water, and its chemical, functional, and antioxidant properties were investigated. At 25 °C, 45 °C, and 65 °C the mucilages' swelling index was evaluated. They discovered that there appeared to be no noteworthy variation between 25 °C and 45 °C, although the swelling index was relatively low at 65 °C (Oh & Kim, 2022).

Figure 2b illustrates that increasing the water-to-seed ratio improves the SC of the psyllium mucilage. As reported by Jooyandeh & Samavati (2017), the presence of hydroxyl groups and protein substitutes in the structure of mucilage had a profound effect on water absorption capacity. Furthermore, Amiri *et al.* (2021) stated that water absorption promotes mucilage to expand and the intensity of mucilage is dependent on the type of mucilage. It is critical to emphasize the role that water absorption capacity plays in the creation as well as the processing of foods high in dietary fiber (Orifici *et al.*, 2018). In their investigation, Kaykha *et al.* (2022) discovered that the water absorption capacity of Sepestan fruit mucilage was greatly dependent on temperature and liquid to solid ratio. Initial increases in temperature and the water-to-solid ratio increased water absorption capacity, but further increases resulted in a decline because of the deterioration and dilution of these components in the current study.

As demonstrated in Figure 2c, SC was higher when the independent variable water pH was close to 7. However, the swelling capacity was adversely impacted by the tendency of water pH increase. For example, Basil (*Ocimum basilicum* L.) seed mucilage was extracted using a pH range of 5-9, a seed/water contact period of 4 to 8 hours, a temperature range of 35 °C -75 °C, and a seed/water ratio of 1:10 to 1:50. Basil seed mucilage tablets were developed by combining 125 mg mucilage, 75 mg cellulose, and 100 mg medicine, and their swelling behavior was assessed at two pH levels: pH 1.2 and 7.4 Swelling was found to be extremely minimal at pH 1.2; however, SC increased with pH 7.4 (Hasan *et al.*, 2023). Also, prior studies have shown that as pH and time increased, the swelling factor also expanded (Ziemichód *et al.*, 2019). According to Farahnaky *et al.* (2010), *Psyllium* solutions exhibit elastic behaviour and have the best functional qualities in the pH 4 to 7 range, making them an attractive option for food additives (Farahnaky *et al.*, 2010).

3.4 Emulsifying properties

Polysaccharides, in comparison to proteins, exhibit a lesser ability for emulsification. This was related with hydrophilicity, their restricted flexibility, and uniform repetition of structural units. The main factors affecting the polysaccharide emulsion properties complicatedly depend on the structure of the sugar molecules, the remaining aqueous components of hydrophobic proteins, and the system's rheological characteristics. Understanding the physicochemical properties of polysaccharides improves our approach in areas such as food science and materials engineering (Tosif *et al.*, 2021).

The composition and chemical structure of mucilage are responsible for its exceptional technical qualities. The superior thickening and gelling properties arise from the intermolecular interactions between side groups of the polymer chain via the formation of hydrogen bridges or hydrophobic linkages. Furthermore, the mucilage structure's hydroxyl groups and protein equivalents are responsible for the high water holding capacity, the presence of non-polar molecules endorses oil holding capacity, and the simultaneous existence of hydrophobic and hydrophilic groups gives the mucilage an amphiphilic character. This supports its use in emulsions as a stabilizer. Additionally, the presence of proteins increases the viscosity, which facilitates the stabilization of emulsion systems (Goksen *et al.*, 2023). The emulsifying power of mucilage is attributed to the presence of proteins, especially the weakly polar amino acids (lysine, isoleucine, and tryptophan), carbohydrates that stabilise the emulsion by forming a barrier around the emulsion particles, methyl groups that provide hydrophobicity and the low lipid content of mucilage (Andrade *et al.*, 2015). Increased emulsifying efficiency can be achieved by combining proteins and polysaccharides synergistically. Commercial gums are typically constituted solely of polysaccharides, which enhance their hydrophilicity and surface tension, thereby lowering their emulsifying powers. mucilages exhibit distinctive emulsifying

properties as a consequence of their capacity to reduce surface tension, which is a result of the presence of proteins coupled with polysaccharides. According to the study conducted by Wu et al. (2015), the stability of the emulsion is impacted not only by polysaccharide concentration and conformation but also by, isoelectric point, and amino acid content.

EC describes a molecule's ability to stimulate the dispersion of two immiscible molecules. As opposed to this, ES refers to a molecule's capability to hold onto a dispersed molecule and avoid coalescence (Sangeethapriya & Siddhuraju, 2014). The relationship between emulsifying properties and independent variables of extraction temperature, water-to-seed ratio, extraction time, and water pH in terms of coded variables are given in Equation 7 and Equation 8. Based on the ANOVA results shown in Table S2, 2FI model was significant for EC ($p \leq 0.05$) with F -value of 2.43 and P -value of 0.0487. Additionally, the findings demonstrated that the interaction terms of extraction time-water to seed ratio (BC) and extraction temperature (A) were the significant terms on EC ($p \leq 0.05$). Comparing the P values given in Table S2, it can be seen that extraction time-water to seed ratio (BC) interaction has a greater effect than extraction temperature. However, the other terms were not significant ($p > 0.05$). According to the experiment, EC of psyllium mucilage was between 37.61% and 77.49%. As the lack of fit is non-significant, a model can fit the data well. The highest EC of the mucilage was detected under 65°C extraction temperature, 60 min extraction time, 100:1 water to seed ratio, and 7 water pH. The lowest EC was found at 65 °C of extraction temperature, 120 min of extraction duration, 100:1 water to seed ratio, and 7 of water Ph. The EC was substantially reduced by extending the extraction time from 120 to 60 minutes, as this result showed. R^2 values for this response was 0.5742. While considering the ES, according to ANOVA Table S2, the quadratic model was significant with F -value of 4.04 and P -value of 0.0067. Extraction temperature, extraction time, water-to-seed ratio, water pH and quadratic effect of water pH are the significant effect on ES ($p < 0.05$). However, other terms were not significant ($p > 0.05$). Results showed that water pH was the most significant term among other terms with a P value of 0.0017. Relying on the sum of squares, the effect of each independent variable on ES could be prioritized in the sequence that follows order: $D^2 > D > A > C > B$. Based on the results, ES of psyllium mucilage was changed from 41.43% to 85.14%. Lack of fit had no profound effect as it is desired statistically. The highest ES was determined at the following conditions: extraction temperature of 50 °C, extraction time of 90 min, water-to-seed ratio of 75:1, and water pH of 10. Conversely, the lowest ES was achieved at a temperature of 65 °C, an extraction period of 90 min, a water-to-seed ratio of 100:1, and a water pH of 4. The findings indicate that, for a constant extraction length, ES was considerably lowered from highest to lowest with increasing temperature, water-to-seed ratio, and water Ph. Coefficient of determination R^2 was 0.8015 since it is a good indicator that regression models were appropriate for predicting the behaviour of corresponding response. Non-significant and significant terms are displayed together in (Equation 7) and (Equation 8) to maintain the equations' correctness.

$$EC(\%) = 56.11 - 5.7A - 2.3B - 0.0183C - 2.9D - 4.08AB + 1.54AC + 7.71AD - 11.23BC + 6.4BD - 7.25CD \quad (7)$$

$$ES(\%) = 76.91 - 4.52A - 3.67B - 4.24C + 6.38D - 3.08AB - 0.28AC - 1.57AD - 3.4BC - 2.17BD + 5.4CD + 0.7835A^2 - 0.099B^2 - 3.56C^2 - 8.04D^2 \quad (8)$$

Figure 3 shows the effects of extraction temperature, extraction time, water to seed ratio, and water pH on the EC and ES of mucilage.

As it was clear from Figure 3a, an increase in extraction temperature and extraction time caused EC to decrease. Moreover, the water to seed ratio possesses an opposite influence to EC. The emulsion ability of *X. americana* seed mucilage was reported as 76% (Bazezew et al., 2022). This result is close to the value of the *Psyllium* mucilage in the current study. Another study Sangeethapriya & Siddhuraju (2014) revealed that *Zizyphus mauritiana* mucilage EC was $54.12 \pm 1.67\%$ which was lower than *Psyllium* mucilage EC value. EC of Nopal mucilage was lower $51.94 \pm 1.97\%$ than chia seed gel 61.5%. Differences in emulsion capacities were attributed to emulsifier qualities such as protein concentration (covalently or physically attached to the

polysaccharide), non-polar nature of their chemical groups, molecular weight, and viscosity (Cortés-Camargo et al., 2018).

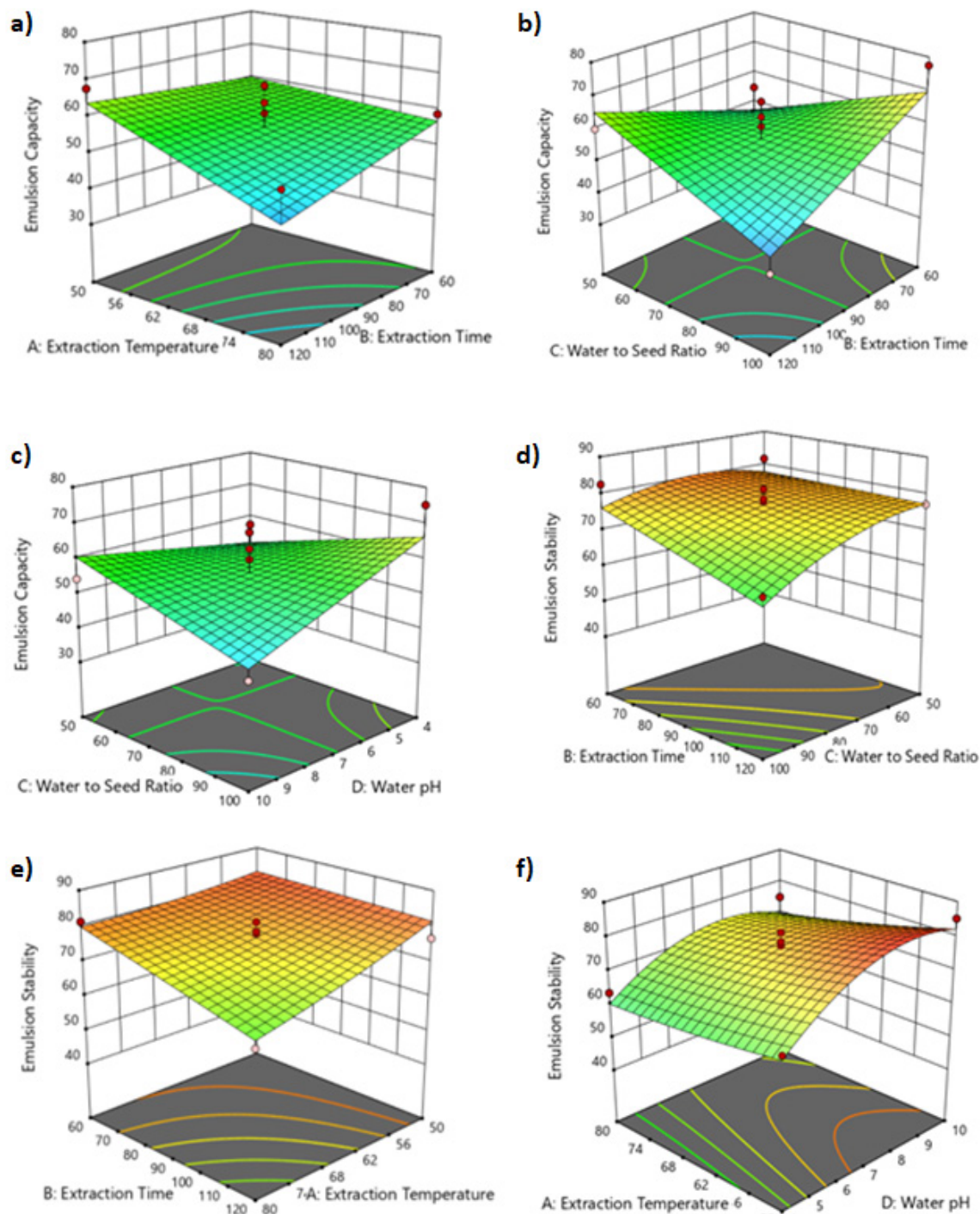


Figure 3. Interaction effect of temperature and time (a), time and water to seed ratio (b), water pH and water to seed ratio (c) on EC, an interaction effect of water to seed ratio and time (d), time and temperature (e), water pH and temperature (f) on ES (Third and fourth parameters are kept at center point for each plot).

It is illustrated from Figure 3b that at the lower extraction time and water-to-seed ratios, EC was higher. However, increasing of these factors caused a decreasing tendency on EC. It is seen from Figure 3c that as

the water pH increased, EC decreased. Corn gluten meal emulsion activity and stability were examined under the effect of pH (Wu, 2001). The study reported that from pH 3.2 to 6.2, both responses demonstrated were low in value. When the pH increases up to 6.5 and 6.9, emulsion activity increases remarkably. However, no further increase was observed in the emulsion activity of corn gluten meal at pH 9. Therefore, ES of corn gluten meal obtained the highest value at pH 7.8. Another study was conducted to compare two extraction methods for producing psyllium mucilage by water bath and ultrasound under the same extraction circumstances (Souza et al., 2020b). They claimed that the stability and capacity of the emulsion were unaffected significantly. For water bath extraction, these were $59.2 \pm 0.04\%$ and $92.0 \pm 0.08\%$, respectively. Similar to this, Orifici et al. (2018) found that the EC and ES of mucilage from chia seeds, were 54.67 ± 1.89 (mL 100 mL⁻¹) and 57.56 ± 0.31 (mL 100 mL⁻¹), respectively. These literature values are in accordance with the EC of *Psyllium* mucilage obtained in the current study (37.6% to 77.5%).

The variation of the ES with extraction temperature and extraction time at a constant water pH and water to *Psyllium* seeds ratio were represented in Figure 3d. As can be seen, higher extraction temperatures and times resulted in lowered ES of *Psyllium* mucilage which is very similar to many studies reported in the literature. For example, chia seed mucilage ES was reported to be highest when the extraction time was short and the extraction temperature was low. According to Arici et al. (2024), better emulsifying characteristics were usually the outcome of higher temperatures. This was clarified in accordance with Stoke's law, which states that the stability of an emulsion is raised as its viscosity increases. However, in this study and Koocheki et al. (2009b), as the temperature and time increased, the effect of stabilization of mucilage diminished. However, for constant extraction times, lowering the temperature and water-to-seed ratio improved the stability of the emulsion. According to Campos et al. (2016), chia mucilage ES ranged between 8.9% and 47.4% in their investigation which is lower than *Psyllium* mucilage stability value in the current study. Sharifian-Nejad & Shekarchizadeh (2019) investigated the EC and ES of polysaccharide from oleaster (*Elaeagnus angustifolia* L.) fruit. Oleaster polysaccharide emulsion activity was reported to be around 80% in emulsion with 5% sunflower oil addition. For stability, the emulsion was kept in the refrigerator for one month and reported to be completely stable. They found that extraction temperature has a profound effect on obtaining mucilage with good ES.

Figure 3e demonstrates that water to seed ratio has negatively influenced the ES. As the temperature increases the decrease in the ES showed itself more observable. Additionally, according to Behbahani et al. (2017), the relation between water to seed ratio was explained as increasing water to seed ratio up to 30:1 caused ES of *Plantago* major seed mucilage to decrease then, increased. However, Rostami & Gharibzadeh (2016), obtained high emulsifying activity and stability of Jujube polysaccharide white extracted by microwave assisted technique. They pointed out that high emulsion activity and stability were connected with the amount of proteinous materials because hydrophilic and hydrophobic side chains of proteins are responsible for the relation with gums chemically and physically. They stated that an increase in the concentration of polysaccharide increased emulsion activity and stability. Also, an increase in storage temperature from 4 to 25 °C decreased emulsion activity and stability.

Figure 3f showed that when the water pH increased, ES exhibited a remarkable increase up to around pH 7, then slightly increased. *Plantago* major seed mucilages were extracted and rheological properties were investigated by Behbahani et al. (2017). They stated that the interaction of pH and temperature influenced ES. In contrast to the present study, at low pH, ES dropped as temperature climbed. On the other hand, pH had no major influence at low temperatures ($p > 0.05$) on ES. An investigation to maximise the isolation of mucilage from Sepestan fruit was carried out and its ES was assessed (Kaykha et al., 2022). According to the research results, ES was significantly influenced by extraction temperature and liquid-to-solid ratio, which declined as temperature and pH increased. These literature observations are similar to our results. Lastly, Ma et al. (2017) evaluated the emulsification properties of *Dioscorea opposita* Thunb. mucilage and revealed that it lost its natural emulsifying properties following pH application ranging from 5.0 to 9.0. It is strongly

encouraged to utilize natural mucilage as a food ingredient since it exhibits better EC. From Table 3, mucilage from the various plant seeds clearly demonstrated that extraction methods, a plant source of mucilage, and extraction parameters have substantial effect on results.

Table 3. Relevant works on the extraction of mucilage, their methods, and main results as provided in the literature.

Reference	Source of mucilage	Extraction condition	Extraction method	Precipitation method	Drying method	Optimum Results
This study	Mucilage from <i>Plantago ovata</i> Forsk seed	Temperature: 50-80 °C, Time: 60-120 min, Water-to-seed ratio: 50:1-100:1, Water pH: 4-1.	Water extraction	By acetone	Freeze-drying	EY: 29.54% SC: 25.47 mL g ⁻¹ EC: 68.39% ES:76.61%
(Bukhari et al., 2022)	Mucilage from <i>Mimosa pudica</i> seed	Temperature: 30-90 °C, Time: 1-12 h, Water to seed ratio: 5:1-35:1 (w/v), Water pH: 1-10	Water extraction	<i>n</i> -Hexane and ethanol	Vacuum oven at 50 °C	EY: 10.66%
(Behbahani et al., 2017)	Mucilage from <i>Plantago major</i> seed	Temperature: 45-75 °C water to seed ratio: 20:1-60:1, water pH: 4-10	Water extraction	Extractor with a rotating plate	Oven at 45 °C	EY: 15.18%, ES: 67.4%, FS:88.4%, S: 97.36%, WAC: 39.74 g g ⁻¹ TPC:76.79 mg GAE g _{dw} ⁻¹ TFC:97.8 mg g ⁻¹ AC: 915.54 µg ml ⁻¹
(Kaykha et al., 2022)	Mucilage from Sepestan fruit	Temperature: 45-85 °C, Water to seed ratio: 5:1- 15:1 (mL g ⁻¹), Water pH: 4-7	Water extraction	-	Oven at 55 °C	EY: 8.83% S:27.3% WAC:28% FS:0.04% OAC:28% ES: 443.4% AC: 36.83%
(Nazir et al., 2017)	Mucilage from Basil seeds	Temperature: 40-91 °C, Time: 1.6-3.3 h, Water to seed ratio:18:1-77:1	Water extraction at pH:8	Rubber spatula	Conventional hot air oven at 50 C	EY:20.49%
(Bazewew et al., 2022)	Mucilage from <i>Ximenia americana</i> seed	Temperature: 50-80 °C, Time: 1.5-4 h, Water to seed ratio 20:1-40:1 (v/w)	Water extraction	Ethanol	Vacuum oven at 40 °C	EY:17.31% P:1.75% WAC:11.48 g g ⁻¹
(Yousuf & Maktedar, 2023)	Mucilage from quince seeds	Temperature: 30-60 °C, Time: 10-30 min, Water to seed ratio: 14.5:1- 44.5:1 1, Water pH:5-9	Water extraction	Electric mixing paddle	Oven at 45 °C	EY:16.17% ESI:84.62 min V = 1150.50 mPa s

EY: Extraction yield, SC: Swelling capacity, S: Solubility, FS: Foam stability, WAC: Water absorption capacity, OAC: Oil absorption capacity, AC: Antioxidant activity, TPC: total phenol content, P: Protein content, ESI: Emulsion stability index, V: Viscosity.

The present study reported a significantly high extraction yield, which was attributable to the influence of extraction conditions, drying techniques, and precipitation agents. While the temperature and water pH were consistent throughout all studies, there were variations in the extraction length and water-to-seed ratio. The findings demonstrated a substantial rise in the extraction yield as the ratio of water to seed was increased.

Kaykha et al. (2022) found that the ES was remarkably high at 443.4% when the temperature was 57.49 °C, the pH was 4, and the liquid-to-solid ratio was 6.27 mL g⁻¹. However, the ES of the present investigation and study done by Behbahani et al. (2017) exhibited a comparable level of similarity. Moreover, the temperature at which extraction takes place and the ratio of liquid to solid components have a significant impact on ES. Stokes' law provides clarification that the stability of an emulsion can increase as its viscosity increases (Behbahani et al., 2017). In addition to the current investigation listed in Table 3, Bukhari et al. (2022) and Bazezew et al. (2022) precipitated mucilage using an organic solvent. The impact of the precipitating agent on EY appears to be a consequence of the interactions between the polymeric chains of mucilage and the precipitating agent. Polysaccharides have varying polarity, and the addition of alcohols with different dielectric constants may reduce the dielectric constant of polysaccharide solutions, precipitating the mucilage. Hence, the morphology and microstructure of the dehydrated mucilage are contingent upon the technique employed for extraction, purification, drying, and grinding of the material (Mannai et al., 2023). Generally, the drying process can decrease the average molecular weight of mucilage. Freeze drying has a less harmful impact on the chemical and molecular structure of gums compared to other drying methods, since it minimizes thermal degradation. In comparison to other drying methods, freeze dried samples demonstrated greater emulsifying activity and emulsion stability than oven dried samples, most likely due to the low temperature utilized in this type of drying, which retains chemical and molecular structure. It is believed that the reduction of protein in *Psyllium* mucilage attributed to the oven drying procedure may have impacted the emulsifying activity of the mucilage (Antigo et al., 2020). Furthermore, the drying process had a major impact on the monosaccharide composition; xylose and arabinose contents were highest in freeze-dried mucilage and lowest in spray-dried mucilage. Moreover, oven-dried samples showed the best water holding capacity, while freeze-dried samples had the greatest oil holding capacity (Matsuhira et al., 2006; Tosif et al., 2021). The best emulsifying activities were produced by low-temperature ethanol precipitation (Andrade et al., 2020).

3.5 Optimization of extraction process variables

The most recently developed multivariate statistical method used in the optimization of food processes is called RSM. It combines both mathematical and statistical principles relying upon fitting a model to the data to define the behaviour of a data set to make statistical predictions. This method is employed in the development, design, and improvement of processes where a response or responses are affected by several variables. The term “independent variables” or “factors” refers to variables that can be altered independently from one another. An experiment's “runs” are the experiments that make it up. The term “dependent variables” or “responses” refers to response variables that are impacted by several independent variables (Reji & Kumar, 2022).

In this current experimentation, the optimization of extraction of *Psyllium* mucilage was carried out by maximizing all of the process variables which are EY, SC, EC and ES. Optimum extraction conditions based on numerical optimization were 79.99 °C extraction temperature, 60.02 min extraction time, 99.99:1 water-to-seed ratio, and 7.38 water pH. It was predicted that 29.54% EY, 25.47 mL g⁻¹ SC, 68.39% EC, and 76.61% ES would be obtained by applying these optimum conditions. The concept of desirability was defined as the conversion of all responses derived from separate measurement scales into an same scale of undesired desirability (also known as individual desirability) (Lafifi et al., 2019). The desirability value was determined as 0.803 in this study. It normally varies between 0 and 1. Furthermore, 0 represents undesirability, 1 represents perfect desirability, while below 0.3 is expressed as unacceptable. In this current situation, the value of 0.803 is corroborated by “Acceptable and outstanding” (Boateng, 2023).

4 Conclusion

In conclusion, this research has highlighted that extraction conditions have a profound impact on EY, SC, EC, and ES of *Psyllium* mucilage. The optimum extraction conditions were determined as 79.99 °C extraction

temperature, 60.02 min extraction time, 99.99:1 water to *Psyllium* seeds ratio, and 7.38 water pH. Under these optimum conditions, 29.54% EY, 25.47 mL g⁻¹ SC, 68.39% EC and 76.61% ES were achieved. Regarding these results, *Psyllium* mucilage was shown to possess high SC, EC, and ES, making it a viable substitute for low-fat emulsified meat products including sausages, patties, and nuggets, as well as baked goods. Because mucilage stabilizes the water-lipid interactions and reduces coalescence and separation. Based on its high stabilization, gel-forming, texturizing, and emulsifying properties, the interest of the food and nutraceutical industry in *Psyllium* mucilage as a promising additive is expected to increase continually over the years.

Acknowledgements

The authors would like to express their sincere thanks to all those who helped us in the successful completion of this research, for their scientific and important assistance and cooperation.

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Funding: None.

Received: Feb. 07, 2024; Accepted: July 28, 2024

Associate Editor: Gerson Teixeira Lopes.

Supplementary Material

Supplementary material accompanies this paper.

Table S1. Experimental design for extraction of *Psyllium* husk mucilage experiment with coded and actual variable levels.

Table S2. Model summary and analysis of variance of the dependent variables EY, SC, EC, ES

This material is available as part of the online article from <https://doi.org/10.1590/1981-6723.01724>