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ORIGINAL PAPER

Optimization of the mucilage extraction process from mallow (*Malva sylvestris* L.) leaves using response surface methodology and its application as an egg replacer in gluten-free vegan noodles

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Abstract

Mallow (*Malva sylvestris* L.) is an eco-friendly, nutrient-rich wild plant with potential in food applications due to its widespread availability and low raw material cost resulting from its natural growth. In particular, the mucilage derived from mallow presents a promising natural alternative to animal-based ingredients such as eggs in food formulations. Considering the increasing demand for gluten-free (GF) and vegan nutrition in recent years, it is important to introduce these types of foods to the sector. The present study focused on optimizing the extraction parameters of mallow (*Malva sylvestris* L.) mucilage to improve its yield and functional characteristics. Furthermore, the extracted mucilage was applied as an egg substitute in the formulation of GF and vegan noodles prepared with chickpea flour. Optimization was carried out through Response Surface Methodology (RSM), and the optimal extraction conditions were established as a raw material to water (RM/W) ratio of 1/26.3, an extraction temperature (ET) of 62.9 °C, and an extraction time of 56.3 min. Using mallow mucilage produced under optimum conditions instead of eggs in GF noodles increased ash values while decreasing cooking time, water absorption, volume increase, fat and L*, a*, b* values. It was determined that total phenolic (TP) content and antioxidant activity (AA) values were higher in the sample with 3% mallow mucilage compared to the control. Consequently, it was determined that the nutritional values were largely preserved, particularly in the sample with 3% mucilage addition. Using mallow mucilage, as an egg substitute in the GF noodle formulation resulted in a product with high nutritional value that meets the criteria accepted by panellists.

Keywords Mallow · *Malva sylvestris* L. · Plant mucilage · Sustainability · Optimization · Gluten-free and vegan noodles

Introduction

Owing to the combined effects of population growth, escalating food requirements, economic volatility, global epidemics, rapid urbanization, and climate change, the pursuit of sustainability in food production has gained remarkable attention in recent years. Wild edible plants are increasingly recognized as promising sources of nutrient-dense and functional foods, offering potential benefits for both human health and environmental sustainability. Mallow (*M sylvestris* L.), a wild edible plant from the Malvaceae family, grows widely in Europe, Asia, and North Africa. Traditionally used for food and medicinal purposes, it is environmentally friendly, economical, biocompatible, and sustainable [1]. Mallow is a perennial herb with purple flowers, rich in protein, vitamin C, anthocyanins, and minerals such as iron, calcium, and phosphorus. It exhibits antioxidant, antibacterial, antifungal, anti-inflammatory, and antidiabetic properties. The plant contains mucilage, a plant hydrocolloid composed of sugars and uronic acids, known for its protective and softening effects [2]. Due to concerns about the toxicity and carcinogenicity of synthetic polymers, interest in sustainable plant polymers like mucilage has increased. The

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properties of mucilage depend on its source and extraction conditions. RSM is widely used to optimize mucilage extraction efficiently by reducing experimental trials [3].

Environmental, cultural, and socio-economic factors play a decisive role in determining food consumption and consumer habits. Gluten-related disorders (celiac disease, gluten intolerance, etc.) affect approximately 5% of the global population, and GF diets are also preferred by consumers who believe that GF foods aid in weight loss. Since most GF products are made from GF flours and starches, their nutritional value, taste, and quality tend to be low, and their variety is limited. Additionally, the demand for vegan diets is rising due to an increasing number of people seeking sustainable and healthy lifestyles. However, individuals following vegan diets for extended periods may experience health issues related to imbalances. These issues are often associated with high fiber intake and deficiencies in vitamins such as B_{12} and D, as well as minerals including iron, zinc, and calcium [4]. Therefore, it is essential to introduce a diverse range of GF and vegan foods with enhanced nutritional value to the sector.

Noodles are widely consumed worldwide, especially in Asia and the Americas, and hold cultural significance in Türkiye. Traditional Turkish noodles are made from flour, water, salt, and eggs [5]. To improve their nutritional value, various grain or legume flours can be used. Chickpea flour, a GF ingredient rich in protein, essential minerals, vitamin D, and dietary fiber, is a suitable raw material for GF noodles. A study examining GF noodles made with different legume flours found that noodles produced with chickpea flour had the most preferred sensory and functional properties [6]. Based on these findings, chickpea flour was used in this study to address the low nutritional value, a key challenge in GF and vegan foods. In addition, chia seeds and flax seeds are among the most frequently reported plant-based sources as egg substitutes in the literature. The mucilage of these seeds can partially mimic the binding and structuring functions of egg due to their high WHC and gelling properties [7]. However, the characteristic aroma and colour of these raw materials, along with the formation of excessively rigid textures associated with high viscosity, can limit formulation flexibility in some food products. In this context, mallow (*M. sylvestris* L.) mucilage can be considered as a promising plant-based egg substitute due to its comparable hydrophilic and gelling properties, its relatively neutral sensory profile, and its ability to form an effective structure at low concentrations.

Research has shown that mucilage obtained from mallow (*M. sylvestris* L.) leaves has not been used as an egg substitute in any food product, and no studies have optimized its extraction process. The aim of this study was to determine the optimal mucilage extraction conditions from mallow (*M. sylvestris* L.) leaves and to produce GF noodles with improved nutritional, physical, and sensory properties by using mallow mucilage extracted under optimal conditions as an egg replacer in a GF noodle formulation. Based on this aim, the study was conducted under the hypothesis that mucilage obtained by optimizing the extraction conditions of mallow (*M. sylvestris* L.) has the potential to provide technological functions that can substitute for those of egg (binding and structure formation) in GF noodle systems. Consequently, the incorporation of optimized mucilage into egg-free GF noodle formulations was expected to influence the cooking, textural, and sensory properties of the final product.

Materials and methods

Material

The mallow plant (*M. sylvestris* L.) used in this study was collected from the fields in Torbalı district of İzmir, Türkiye. The leaves of mallow plant were sorted and washed within 24 h. It was dried in a tray dryer (Armfield Ltd. UOP8, UK) at 30 °C with an air flow rate 1 m/s until less than 10% moisture remained. Subsequently, the dried material was milled using a laboratory-scale grinder (Waring 8011, USA), then screened through a 250 µm aperture and stored in glass jars. Chickpea flour, corn starch, salt and eggs used in noodle production were sourced from a retail supplier in İzmir, Türkiye.

Extraction of mucilage from mallow leaves

Dried and ground leaves were soaked with distilled water as given in the experimental design (1:10 − 1:40) for 2 h and kept at room temperature. According to the experimental design, the sample was heated in a water bath (Memmert WNB, Germany) at temperatures changing from 40 to 100 °C for 30 to 90 min with continuous stirring, followed by cooling to room temperature for 2 h (Table 1). The resulting sediment was separated through filtration using muslin cloth. The viscous filtrate was then reduced by evaporation under vacuum at a temperature 60–65 °C using an evaporator (Heidolph Hei-VAP Value, Germany). To induce mucilage precipitation, two volumes of absolute alcohol were added. The mucilage was subsequently dissolved in distilled water and subjected to centrifugation (Hettich Universal 320, Germany). To facilitate further precipitation, alcohol

Table 1 Independent variables and their levels for optimization of extraction conditions of mallow mucilage

Factors	Independent variables	Variable levels		
		-1	0	1
x_1	RM/W ratio (w/v)	1:10	1:25	1:40
x_2	ET (°C)	40	70	100
x_3	Extraction time (min)	30	60	90

was added again. The precipitated mucilage was isolated, rinsed with alcohol, and dried in an oven at 50 °C before being converted into powder (Fig. 1). It was then sieved with a 200 µm sieve and made ready for analysis [8].

Analytical methods

The extraction yield (EY) was determined by calculating the ratio of the weight of the powdered mucilage to the initial weight of the mallow leaves used. For analyses of the water holding capacity (WHC) and oil holding capacity (OHC) of mucilage, one gram of mucilage sample was mixed with 10 ml of distilled water or sunflower oil and vortexed for 2 min. The mixture was centrifuged at 4500 rpm for 30 min (MSE Mistral 1000, UK). After removing the supernatant, the remaining residue was weighed. WHC and OHC of mucilage were calculated using Eq. 1 and Eq. 2, respectively [8].

$$WHC = \frac{Weight\ of\ wet\ sample - Weight\ of\ dry\ sample}{Weight\ of\ dry\ sample} \quad (1)$$

$$OHC = \frac{Weight\ of\ wet\ sample - Weight\ of\ dry\ sample}{Weight\ of\ dry\ sample} \quad (2)$$

WHC and OHC were selected as key functional parameters to evaluate the potential of mallow mucilage to mimic specific technological roles of egg in GF noodle formulations. In such systems, egg primarily contribute to structure formation, binding, and stabilization. Their foaming capacity is more relevant to aerated products and is not critical in non-aerated matrices such as noodles. Accordingly, WHC was considered indicative of the water-binding and dough-structuring ability required for matrix cohesion. In contrast, OHC was used to assess the capacity to interact with and retain lipid components within the formulation. Similar functional roles of egg proteins, including their contributions to structure, emulsification, and stabilization depending on the food system, have been reported in the literature [9].

Experimental design and statistical analysis

In this study, RSM was used to optimize the extraction of mallow (*M. sylvestris* L.) leaf mucilage. The experimental data required to develop mathematical models relating the responses to the variables were collected using Central Composite Design (CCD). Three independent variables were chosen for optimization: the RM/W ratio, ET and extraction time. The lower and upper bounds for these variables are provided in Table 1. Mucilage extraction from mallow leaves was carried out in 20 trials, and these trials were performed in random order.

In optimizing the extraction conditions of mallow mucilage, three responses were used: EY, WHC and OHC of the mucilage. Optimum extraction conditions were determined as conditions that simultaneously provide maximum EY, maximum WHC and maximum OHC according to the "desirability function" method. The study design and obtained data were analyzed using Design Expert 13.0 software. Regression analysis of the data led to the development of a model for each response, as outlined in Eq. 3. In the model, R is the response value, $ß_0$ is a constant, $ß_i$ is linear, $ß_{ii}$ is quadratic and $ß_{ij}$ is interaction constants, and X values are independent variables.

$$R = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_{ii}^2 + \Sigma\beta_{ij} X_i X_j \quad (3)$$

As a result of the optimization, the optimum RM/W ratio, ET and extraction time were determined, and 5 repeated validation trials were carried out on the mucilage obtained under optimum extraction conditions. A one-way analysis of variance (ANOVA) was performed, which revealed no significant difference between the experimental data from the mucilage obtained under the optimal extraction conditions and the data predicted by the mathematical model generated by the software, thereby confirming the accuracy of the optimized extraction conditions.



Fig. 1 Mucilage solution obtained from mallow (*M. sylvestris* L.) leaves

GF vegan noodle production

GF vegan noodles were formulated using mallow mucilage extracted under optimum extraction conditions, based on EY, WHC and OHC responses. While egg was used in the control GF noodles, mallow mucilage solution extracted under optimum conditions was used instead of egg in the GF vegan noodles. GF noodle formulations are given in Table 2.

Chickpea flour, corn starch and salt were mixed in a bowl to prepare control sample. Eggs and water were added to the mixture to form the dough. In the GF noodle samples, 1, 2 and 3% mucilage were added instead of egg. Chickpea flour, corn starch and salt were mixed in a bowl. Mucilage weighed at the specified ratio, was mixed with an amount of water equivalent to the total eggs used in the control sample until a homogeneous mucilage solution was formed, which was then added to the dry ingredients. The fixed amount of water determined in the formulation was added and kneaded until a medium hard dough was obtained. The resulting dough was left to rest at 25 °C for 15 min before being rolled out using a rolling pin and then processed through a noodle rolling machine (New Life, Türkiye) for preliminary thinning. The dough was thinned gradually to 0.8 mm using the 2nd, 3rd and 4th setting of the dough thinning section of the machine. The dough was flattened into a thin layer, cut into 4 cm wide rectangles, and cut into strips using a thick cut noodle section on the noodle machine. The noodles were dried at 25 °C for 2 days and stored in glass jars at +4 °C (Fig. 2).

Chemical analysis

Moisture [10], ash [11], fat [10] and protein [10] analyses were conducted on the mallow mucilage and GF noodle samples, following the methods specified in the respective method numbers: 925.10, 08 − 01, 945.16 and 968.06. The carbohydrate content (CC) was calculated using the difference method [12].

TP content and AA analysis

To perform TP content and AA of the mallow mucilage and GF noodle samples, 5 g of mallow mucilage and 7 g of ground samples from GF noodles were extracted using 10 ml of 80% (v/v) methanol in a water bath (Memmert WNB, Germany) set at 50 °C, with continuous agitation at 150 rpm for 75 min. Following the extraction, the mixture was centrifuged (Hettich Universal 320, Germany) at

Table 2 GF noodle formulations

Sample[1]	Chickpea flour (g)	Corn starch (g)	Salt (g)	Water (g)	Egg (g)	Mucilage solution (g)
C	90.0	10.0	0.5	50.0	30.0	-
MN1	90.0	10.0	0.5	50.0	-	30.0 (1 g mucilage+29 g water)
MN2	90.0	10.0	0.5	50.0	-	30.0 (2 g mucilage+28 g water)
MN3	90.0	10.0	0.5	50.0	-	30.0 (3 g mucilage+27 g water)

[1]C: GF control noodles, *MN1* GF vegan noodles with 1% mucilage, *MN2* GF vegan noodles with 2% mucilage, *MN3* GF vegan noodles with 3% mucilage

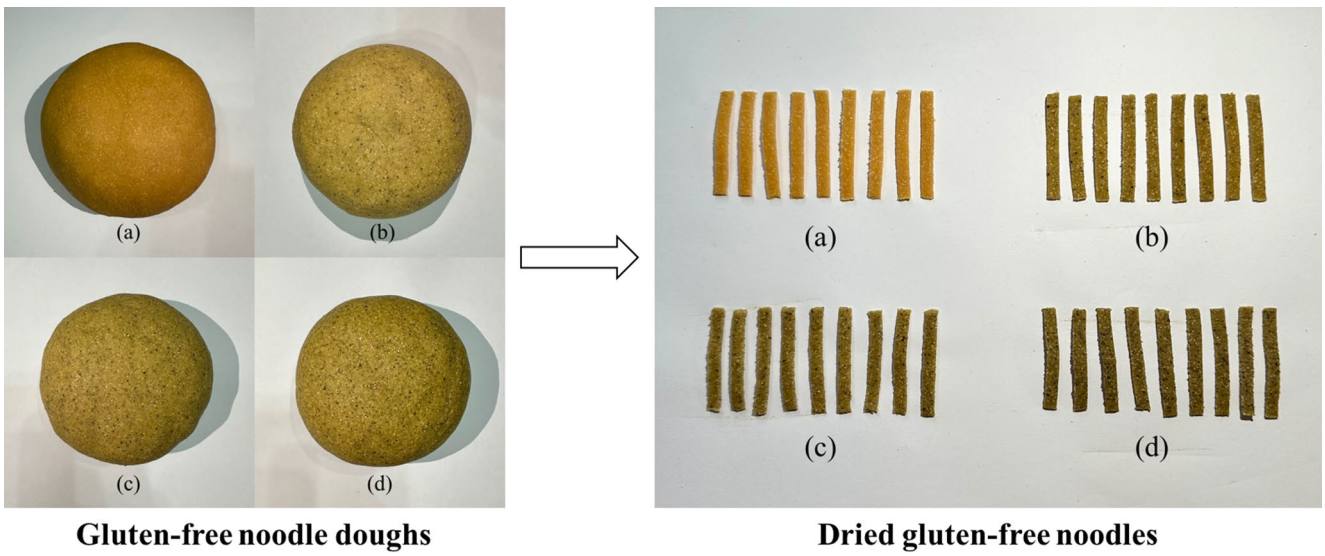


Fig. 2 The doughs and GF noodles with different concentrations of mucilage. **a** C: GF control noodles, (**b**) MN1: GF vegan noodles with 1% mucilage, (**c**) MN2: GF vegan noodles with 2% mucilage, (**d**) MN3: GF vegan noodles with 3% mucilage

4000 rpm for 15 min at 4 °C. The supernatant mixed with 80% (v/v) methanol to reach a final volume of 10 mL.

TP content was determined with minor modifications to the Folin-Ciocalteu procedure outlined by Xu and Chang [13]. To 250 µl Folin-Ciocalteu reagent, 3 ml of ultrapure water was added and combined with 50 µl of the sample extract. Following the addition of 750 µl of 7% Na_2CO_3 solution, the reaction mixture was kept in dark for 8 min. Thereafter, 950 µl ultrapure water was incorporated and the solution was maintained in darkness for 2 h to allow the reaction to proceed. The absorbance of the resulting solution was recorded at 765 nm with a UV-VIS spectrophotometer (Agilent Cary 60 UV-VIS, USA). Standard curves were constructed from gallic acid solutions of appropriate concentrations, and the TP content of the samples was expressed as mg gallic acid equivalent (GAE)/100 g dry matter (DM).

DPPH and ABTS analyses were performed to determine AA. AA analysis by ABTS method was carried out following the procedure outlined by Re et al. [14] and Cemeroğlu [15], with minor modifications. A 7 mM ABTS solution (3960 µl), containing 2.45 mM potassium persulfate, was added to 20, 40 and 60 µl of the sample extracts, and the mixtures were kept in the dark for 6 min. The absorbance was determined at 734 nm with a spectrophotometer (Agilent Cary 60 UV-VIS, USA). The AA of the noodles was calculated as µmol Trolox equivalent (TE) per gram of DM based on the inhibition percentage values and the standard curve constructed with Trolox standard solutions. AA analysis using DPPH method was carried out with slight modifications to the procedure outlined by Cemeroğlu [15]. A 0.1 mM DPPH solution (1970 µl) was added to 15, 30 and 45 µl of the sample extracts. The mixtures were left in darkness for 20 min and, the absorbance values were determined at 515 nm with a spectrophotometer. The AA of the samples was calculated as µmol TE per gram of DM, based on the inhibition percentage values and the standard curve generated from Trolox standard solutions.

Physical analysis of GF noodles

Cooking time The AACC 66-50.01 method [11] was used to determine the cooking time of GF noodle samples. For this purpose, 15 noodle samples were added to boiling water. After 7 min, a noodle was taken with forceps and pressed between glass plates. This process was continued with one-minute intervals until the opaque, uncooked part in the middle of the crushed noodles disappeared. The time from the beginning until this moment was considered the cooking time.

Water absorption To determine water absorption, 25 g of GF noodles were boiled in 250 ml of distilled water for the optimal cooking time. After cooking, the noodles were drained and weighed. Water absorption was determined by calculating the ratio of the difference in weight between the cooked noodles and the raw noodles to the weight of the raw noodles [11].

Volume increase To assess the volume increase of GF noodle samples, the noodles were cooked and drained following the procedure used in the water absorption analysis. After waiting for 2 min, they were taken into a measuring cylinder containing distilled water and the volume of overflowing water was recorded. The ratio of the difference in volume between the cooked and raw noodles to the volume of the raw noodles was used to determine the volume increase [16].

Colour analysis Colour measurements of GF noodle samples were implemented using Konica Minolta (CR-400, Japan). Colour values are given as L* (brightness), a* (a+ (redness) and a- (greenness)) and b* (b+ (yellowness) and b- (blueness)) [16].

Texture analysis The hardness of GF noodles was assessed using the Stable Microsystems TAXTPlus Texture Analyzer (United Kingdom). In this analysis, the A/LKB-F knife was used [17]. Five cooked noodle samples placed side by side, were cut using a 5 kg load cell and a specially designed plastic knife with a thickness of 1 mm, and the hardness of the noodles was measured in N.

Sensory analysis Sensory analysis of GF noodle samples was performed using the ranking test with the participation of 15 trained panellists, who had prior experience working with noodle products. Ethical approval for the sensory evaluation in this study was obtained from the Ethics Committee of Ege University, Faculty of Engineering (Protocol No: 2955; Meeting No: 08/15; 27 June 2025). GF noodle samples were cooked for the designated cooking times and then cooled for 2 min. The noodles were presented on flat white plates, and each was assigned a random three-digit code consisting of non-consecutive numbers. The trained panellists evaluated the noodle samples based on taste, hardness, colour and overall acceptability [18].

Statistical analysis

Design-Expert 13.0 software was used to determine the optimum mallow mucilage extraction conditions. SPSS version 25 (IBM, USA) was employed to perform statistical analysis of the experimental results. Analysis of variance (ANOVA) was used to evaluate group differences, and Duncan's multiple range test was subsequently performed to determine significance levels. A confidence level of 95% was used to determine statistical significance. During the evaluation of the results obtained from the ranking tests conducted as part of the research, the sensory analysis outcomes

of the GF noodles were assessed using ranking analysis and the Friedman test, in accordance with the guidelines of ISO 8587/2006 [19].

Results and discussion

Chemical and technological properties of mallow (*M. sylvestris* L.) mucilage

Analytical results of moisture, ash, fat, TP content, AA and colour analyses performed on mallow (*M. sylvestris* L.) leaf mucilage are given in Table 3. Based on the analytical findings, the moisture content (MC) of mallow leaf mucilage is 2.60%. Typically, the MC of natural gums and mucilage is expected to be less than 15% [8]. The value found in the study is lower than the desired value for natural gums and mucilage. Microbial spoilage is prevented due to the low MC. The ash content (AC) of mallow mucilage was calculated as 0.85%. This value is approximately 1.5% in xanthan gum, 1.2% in arabic gum and 11.9% in guar gum, which are frequently used industrially. Yeole et al. [20] reported an AC of 26.25% for mucilage obtained from the fruit of mallow (*M. sylvestris*). In contrast, Munir et al. [8] found an AC of 15.6% in mucilage obtained from mallow leaves of the species *Malva parviflora*. In a study by Koocheki et al. [21], the AC of *Lepidium perfoliatum* seed mucilage was determined as 0.18%. The fat content (FC) of mallow mucilage is 0.61%. This value is lower than that reported for root mucilage of *Malva verticillata*, another mallow species (1.68%), by Korir and Salim [22], but higher than the FC of mucilage from *Salvia macrosiphon* seeds (0.17%) reported by Farahnaky et al. [23]. The protein content (PC) is higher than that of mallow root mucilage (3.42%) reported by Korir and Salim [22] and mallow leaf mucilage (13.55%) reported by Munir et al. [8]. However, it is lower than the PC of quince mucilage (20.90%) reported by Fekri et al. [24] and okra mucilage (20.30%) reported by Adetuyi and Dada [25]. Considering that one of the main components of plant mucilage is proteins, the determination of protein in mallow mucilage is consistent with the literature. The CC of mallow mucilage was determined as 76.8%. This value is lower than the CC of mucilage found in *Lepidium perfoliatum* seeds (88.23%) reported by Koocheki et al. [21]; and higher than the CC of mucilage found in cress seeds (43.51%) reported by Karazhiyan et al. [12]. The TP content of mallow mucilage was determined as 1.04 mg GAE/100 g DM. In a study examining the mucilages of okra (*Abelmoschus esculentus*) and molehiya (*Corchorus olitorius*), *Talinum triangulare*, TP contents were determined as 4.81, 2.98 and 3.50 mg GAE/g, respectively [25]. According to ABTS and DPPH analyses, the AA of mallow mucilage was found as 0.46 µmol TE/g DM and 0.35 µmol TE/g DM, respectively. The AA value measured by ABTS was determined as 1.30 µmol TE/g DM in the aqueous fraction of *M. sylvestris* extract [26]. A study found that the TP and AA of plants decrease, particularly at temperatures above 60 °C [27]. In the present study, since the mucilage ET from mallow leaves was above this threshold (62.9 °C), it is believed that the TP and AA of mucilage may have decreased due to the temperature. When the colour analysis results of the mallow leaf mucilage were examined, it was seen that the lightness value known as L* is 49.03, the greenness known as -a* is -1.84 and the yellowness known as b* is 11.46. The colour analysis results are consistent with the L*, a* and b* values of mallow powder of the species *Malva aegyptiaca* L. (55.38, -7.02 and 13.39, respectively) [28].

The literature review indicates that the chemical compositions of plant mucilage varies due to several factors, including the mucilage source, the existence of different species within the same plant, the region where the plants grown and the time of collection, and the conditions under which the mucilage is extracted.

Table 3 Analysis results of mallow (*M. sylvestris* L.) leaf mucilage

Moisture (%)		2.60 ± 0.01
Ash (%)1		0.85 ± 0.12
Fat (%)1		0.61 ± 0.06
Protein (%)1		19.14 ± 0.08
Carbohydrate (%)1		76.80 ± 0.22
TP content (mg GAE/100 g DM)		1.04 ± 0.04
AA (µmol Trolox/g DM)	ABTS Method	0.46 ± 0.01
	DPPH Method	0.35 ± 0.02
Colour	L*	49.03 ± 0.02
	a*	-1.84 ± 0.01
	b*	11.46 ± 0.06

[1]Ash, fat, protein and carbohydrate analysis results are given in dry matter terms

Model fitting to optimize mucilage extraction from mallow leaf

Studies have shown that selecting the appropriate extraction method and conditions is crucial for producing mucilage obtained from plants. ET, time and seed: water ratio are important parameters to produce plant mucilage efficiently. Therefore, RM/W ratio, ET and time were selected as independent variables for the optimization of mallow mucilage extraction used as egg replacer in GF noodles. RSM and CCD were used to optimize mallow mucilage extraction. Three responses, EY, WHC and OHC, were used as dependent variables in the optimization. Table 4 presents the outcomes of the analyses conducted according to the experimental design developed using CCD. The addition of quadratic terms to the EY, WHC and OHC responses significantly improved the models. A

Table 4 Mallow mucilage extraction conditions ratios and responses obtained from CCD

Exp. Number	RM/W ratio (w: v)	ET (°C)	Extraction time (min)	EY	WHC	OHC
1	1:10	40	30	5.57	4.70	3.37
2	1:40	40	30	6.12	5.02	3.17
3	1:10	100	30	4.93	4.82	2.95
4	1:40	100	30	5.92	5.18	2.24
5	1:10	40	90	5.70	4.51	3.12
6	1:40	40	90	6.51	4.98	3.04
7	1:10	100	90	5.45	4.73	2.79
8	1:40	100	90	6.37	5.10	2.15
9	1:10	70	60	5.95	5.09	3.39
10	1:40	70	60	6.99	5.43	3.11
11	1:25	40	60	8.25	5.19	3.46
12	1:25	100	60	6.61	5.23	2.75
13	1:25	70	30	8.61	5.32	3.16
14	1:25	70	90	9.85	5.18	2.76
15	1:25	70	60	8.87	5.48	3.25
16	1:25	70	60	9.54	5.64	3.02
17	1:25	70	60	8.88	5.56	3.12
18	1:25	70	60	9.76	5.69	3.18
19	1:25	70	60	8.92	5.75	3.16
20	1:25	70	60	9.31	5.71	3.08

Table 5 ANOVA table showing the effects of linear, quadratic, and interaction terms on each response individually (X_1: RM/W ratio, X_2: ET, X_3: Extraction time)

	EY	WHC	OHC
Model	<0.0001	<0.0001	<0.0001
X_1	0.0179	0.0006	<0.0001
X_2	0.0889	0.1123	<0.0001
X_3	0.1033	0.1848	0.0046
$X_1 \times_2$	0.6949	0.8631	0.0018
$X_1 \times_3$	0.8919	0.6471	0.4712
$X_2 \times_3$	0.7480	0.8631	0.6195
X_1^2	<0.0001	0.0125	0.4204
X_2^2	0.0008	0.0039	0.0956
X_3^2	0.1758	0.0099	0.0011
Lack of Fit	0.2065	0.2689	0.3368
R^2	0.9565	0.9417	0.9632

second-order polynomial model was identified as the most appropriate for predicting EY, WHC, and OHC. The effects of RM/W ratio, ET and time on these responses were evaluated through analysis of variance using the Design Expert software. Table 5 summarizes the statistical significance of individual factors, their interactions, and quadratic terms. The resulting model should be significant for the regression model due to lack of fit. The regression model was found to be statistically significant at the 99% confidence level, while the lack of fit was not significant at the 95% confidence level for all three responses (EY, WHC and OHC) (Table 5). R^2 values for EY, WHC and OHC responses were determined as 0.9565, 0.9417 and 0.9632, respectively. High R^2 values indicate that most of the variation is explained by the models.

Effect of extraction conditions of mucilage from mallow leaf on EY, WHC and OHC

Among the extraction parameters, the RM/W ratio exhibited both linear and quadratic effects on EY that were statistically significant ($p<0.05$) as shown in Table 5. In addition, ET demonstrated a significant quadratic influence on EY ($p<0.05$). Another condition, extraction time, was determined to have no statistically significant effect on mucilage EY ($p>0.05$). Additionally, the interaction effects between the RM/W ratio and ET, the RM/W ratio and extraction time, and ET and extraction time did not significantly affect mucilage EY ($p>0.05$). The counter plots of the calculated extraction conditions effect on mucilage EY are given in Fig. 3. It was determined that the mucilage extraction process performed under the conditions of 1:25 RM/W ratio, 70 °C ET and 90 min extraction time (experiment 14) had higher EY (9.85%) (Table 4). According to the results of experiment 3, the lowest mucilage EY (4.93%) was obtained when it was performed at the highest ET, lowest RM/W ratio and time.

In a study conducted by Nazir et al. [29], the mucilage EY obtained from basil (*Ocimum basilicum* L.) seeds increased with the increase in extraction time. Extraction time provides more contact of the raw material with water. Mucilage EY was positively affected by a significantly longer extraction time [29]. Moderate temperatures facilitate polysaccharide dissolution by improving water penetration into the plant matrix. With the help of temperature, mucilage is easily released and extraction efficiency increases.

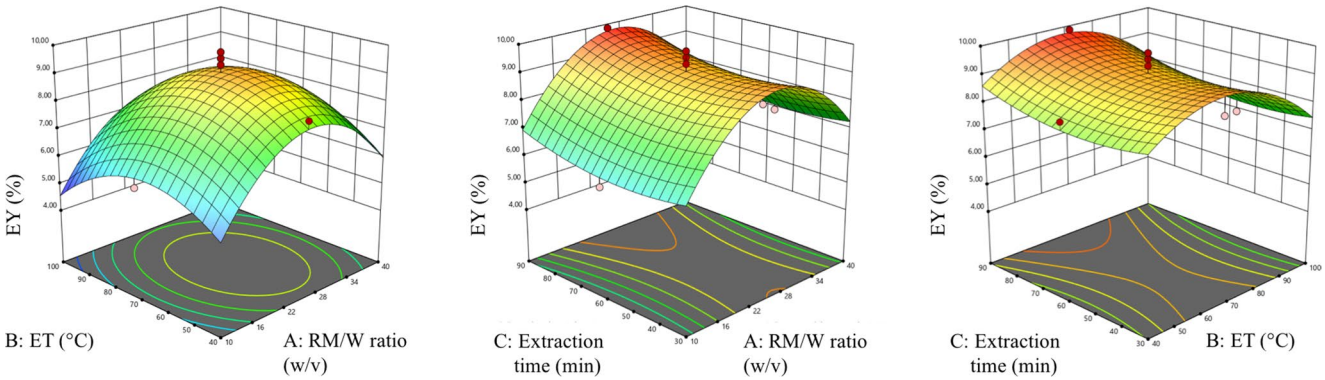


Fig. 3 The calculated effect of extraction conditions on EY of mucilage

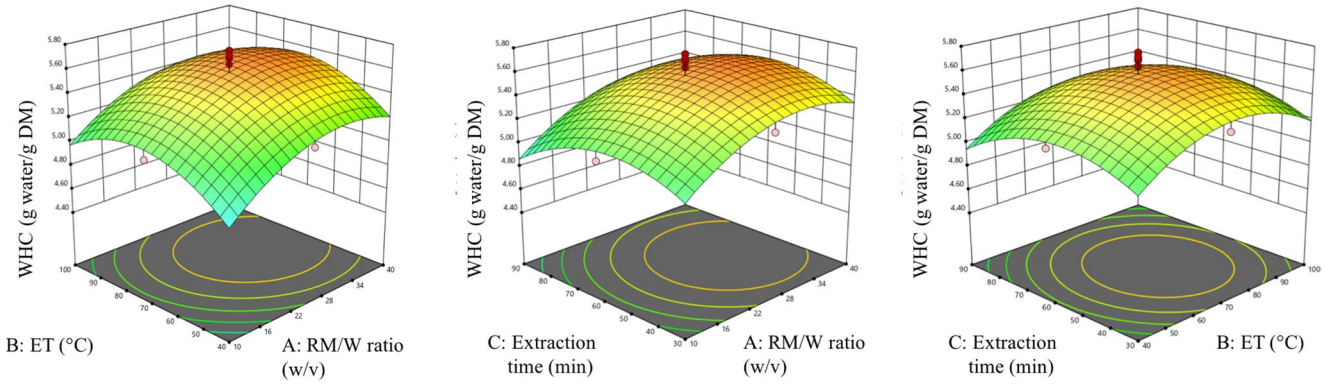


Fig. 4 The calculated effect of extraction conditions on WHC of mucilage

Nevertheless, at elevated temperatures, there is a decline in the efficacy of mucilage due to the degradation of polysaccharides [12]. Mucilage efficiency increases as the water volume in contact with the raw material increases [29]. Koocheki et al. [3], determined that mucilage was obtained with higher efficiency from *Alyssum homolocarpum* seeds with the increase in the water volume in contact with the raw material. As the volume of water in contact with the raw material increases, the extraction efficiency is positively affected since there is more liquid acting as the driving force for mucilage secretion from the raw material. Muñoz et al. [30] concluded that the two criteria with the highest impact on extraction efficiency in mucilage extraction from chia seeds were temperature and water: seed ratio.

The RM/W ratio had a statistically significant influence on WHC, with both linear and quadratic terms being significant ($p<0.05$). Moreover, ET and time showed significant quadratic effects on WHC ($p<0.05$). The interaction effects of the RM/W ratio with ET, RM/W ratio with time and ET with time did not significantly affect WHC of the mucilage ($p>0.05$) (Table 5). Response surface plots showing the change in WHC of mucilage depending on extraction conditions are given in Fig. 4. It was determined that mucilage had a higher WHC (5.75 g water/g DM) in the extraction carried out under the conditions of RM/W ratio 1:25, ET 70 °C and time 60 min (experiment 19) (Table 4). According to the results of the 5th experiment, the lowest WHC (4.51 g water/g DM) was obtained when the extraction was carried out at the highest time, lowest RM/W ratio and ET.

WHC affects the nutritional and sensory properties of foods, especially their quality properties. WHC, which allows the retention of water lost during the cooking of foods, is important for preserving the flavor and increasing the technological properties of food. Mucilage, which have a good WHC in the food industry, can be used as stabilizers, emulsifiers, suspending agents, adhesives or binders. The low WHC of mucilage shows that they cannot form a strong gel. Studies have determined that factors such as the chemical structure of mucilage, pH, temperature and ionic strength affect WHC [31]. In mucilage extraction from mallow leaf, WHC of mucilage varies depending on RM/W ratio ($p < 0.05$). In a study conducted by Behbani et al. [32], it was observed that the WHC of the mucilage increased with the increase in the water: seed ratio in the extraction of mucilage from *Plantago major* seeds. However, it was concluded that the WHC of mucilage also depends on the ET ($p < 0.05$). In the study, the WHC of mucilage increased with the increase in ET up to a certain degree (62.5 °C) ($p < 0.05$). However, it was observed that this value decreased at higher temperatures ($p < 0.05$). Similarly, in another study, it was

determined that the ET had a significant effect on the WHC of mucilage. Initially, the WHC increased with increasing ET and liquid: solid ratio, but this value decreased at higher temperatures. Increasing temperature and liquid: solid ratio up to an optimum level enhances mucilage solubility, resulting in higher WHC. At excessively high temperatures and liquid-to-solid ratios, structural degradation and dilution effects reduce WHC [33]. The difference between the WHC of the mucilage obtained under different extraction conditions may vary depending on the polar hydroxyl groups and hydrodynamic interactions of the mucilage [34].

Analysis revealed that the RM/W ratio had a statistically significant linear effect on the OHC of mucilage in mucilage extraction from mallow leaf ($p<0.05$). OHC of mucilage was linearly and statistically significantly affected by ET ($p<0.05$). Extraction time had a statistically significant linear and quadratic effect on OHC of mucilage ($p<0.05$). At the same time, interaction effect of RM/W ratio and ET also affected OHC of mucilage ($p<0.05$). Interaction effects of the RM/W ratio with time and ET with time did not affect OHC of mucilage significantly ($p>0.05$) (Table 5). Response surface graphs showing the change in OHC of mucilage depending on extraction conditions are given in Fig. 5. It was determined that mucilage had higher OHC (3.46 g oil/g DM) in the extraction carried out under conditions of RM/W ratio of 1:25, ET of 40 °C and time of 60 min (experiment 11) (Table 4). According to the results of experiment 8, the lowest OHC (2.15 g oil/g DM) was obtained when the lowest RM/W ratio, highest ET and time were used.

OHC is one of the basic properties of hydrocolloids [31]. Mucilage with a good OHC are frequently preferred in the food and pharmaceutical industry. OHC is a common concern in certain foods, for example meat, bakery and cereal products. It enhances the flavor perception in the mouth and improves the distribution of the flavor. Furthermore, in food applications like mayonnaise and desserts, where oil content is a key factor in formulation, the incorporation of ingredients with high OHC is essential for maintaining oil retention within the product matrix. High OHC indicates a greater presence of apolar groups capable of holding oil. A study concluded that temperature has a significant effect on the OHC of mucilage. In the extraction of mucilage from Sepestan fruit, higher OHC results were obtained at lower temperatures. At the same time, the OHC of mucilage decreased with increasing temperature and liquid: solid ratio. It is thought that this decrease may be due to the effect of temperature on the chemical structure of hydrocolloids and the release of more non-polysaccharide compounds with increasing liquid: solid ratio [33].

This study investigated the effects of ET, extraction time, and RM/W ratio on EY, WHC, and OHC in mucilage extraction from mallow (*M. sylvestris* L.) leaves. EY is largely affected by temperature and solvent availability. Moderate temperatures and higher liquid-to-solid ratios facilitate polysaccharide release, while excessive temperatures can reduce yield due to structural degradation [3]. In contrast, WHC and OHC are directly related to the technological functionality of mucilage in food systems. High WHC promotes water retention and matrix cohesion, which is particularly important in GF doughs that lack a continuous gluten network [30]. OHC demonstrates the mucilage's ability to interact with lipid components and contribute to matrix stabilization and mouthfeel. This situation is frequently reported for plant-derived hydrocolloids used in cereal-based products. Accordingly, extraction-dependent variations in EY, WHC, and OHC provide a mechanistic basis for the functional performance of optimized mallow mucilage as an egg replacer in GF vegan noodle formulations.

Optimization and verification of predictive model

Optimal extraction conditions—including RM/W ratio, ET, and time—for obtaining mucilage from mallow leaves were established based on the evaluation of EY, WHC, and OHC of the extracted mucilage. Numerical optimization

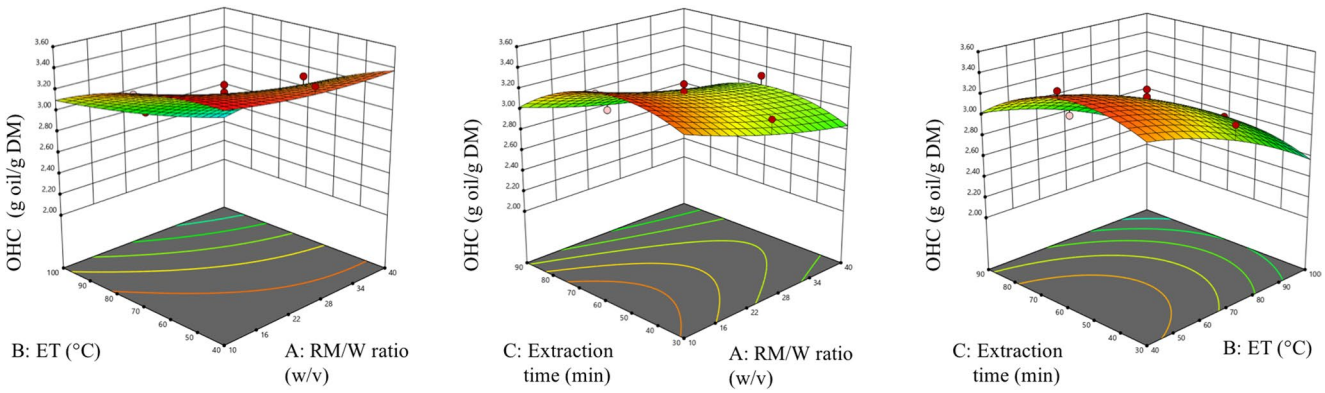


Fig. 5 The calculated effect of extraction conditions on OHC of mucilage

was applied for RM/W ratio, ET and time to determine the optimum extraction conditions. Using the desirability function approach, the optimal conditions for mucilage extraction were identified as an RM/W ratio of 1:26.3, an ET of 62.9 °C, and a time of 56.3 min. To validate the reliability of these optimized parameters, five independent experiments were conducted under these conditions. Table 6 presents the results of the verification trials. Statistical comparison between the experimental outcomes and the model-predicted values revealed no significant differences ($p > 0.05$), confirming the accuracy and predictive capability of the developed model. According to the results of the verification experiments, when mucilage extraction from mallow leaf was carried out under optimum extraction conditions; it was concluded that the model provided reliable estimations of EY, WHC and OHC, as the predicted values closely matched those obtained experimentally. A review of the literature indicated a lack of studies focused on optimizing the extraction parameters for mucilage obtained from *M. sylvestris* L. leaves. In this study, mucilage was obtained by optimizing the extraction conditions, including the RM/W ratio, ET and time from mallow (*M. sylvestris* L.) leaves.

Effect of using mallow mucilage as egg replacer on chemical, physical and sensory properties of GF noodles

As presented in Table 7, the MC of the GF noodle samples ranged between 10.61% and 10.72%. Statistical analysis indicated that the inclusion of mucilage did not lead to any significant variation in MC when compared to the control GF noodles ($p > 0.05$). In a study where chia mucilage was added to the noodles, the moisture of dry noodles was determined to be 6.53% [35]. The use of mallow mucilage as an egg replacer in GF noodles increased the AC ($p < 0.05$). In the study conducted by Naji-Tabasi et al. [36], the use of wild sage seeds with high mucilage content for the purpose of enrichment in pasta increased the AC. The use of mallow mucilage as an egg replacer in the GF noodle formulation caused a decrease in the FC of the noodles as expected ($p < 0.05$). It was observed that the FC of the control noodles was higher than the FC of the noodles with mucilage addition and that the FC increased as the mucilage addition rate increased ($p < 0.05$). In the study, the FC of the mallow leaf mucilage added to the GF vegan noodles as an egg replacer was determined as 0.61%. In addition, when the composition of the egg used in the control GF noodle sample was examined, it was known that the FC varies between 8.7 and 11.2% [37]. The higher FC of the control sample was attributed to the presence of egg, which contains substantially more fat than mallow mucilage ($p < 0.05$). It was determined that mallow mucilage had a significant effect on the PC of GF noodles ($p < 0.05$). The PC of GF noodles containing egg was found to be higher than other GF vegan noodles ($p < 0.05$). Although mallow mucilage, used as an egg replacer in GF noodles, has a high PC, its addition ratio to the formulation was low. Therefore, compared to the control sample, the PC of the mucilage-added noodles decreased. This decrease was approximately 11% in GF and vegan noodles compared to the control. Çakmakçı et al. [35], found lower protein values in noodles with 20% chia mucilage than the control. The PC of the control noodles was determined to be 14.97%, while the PC of the noodles with 20% chia mucilage added was found to be 14.14% [35]. Similarly, in this study, a decrease in PC was observed in the samples with added mallow mucilage compared to the control sample ($p < 0.05$). However, the PC of the noodles was found to be higher than reported in the literature. Although a reduction in PC was observed due to egg replacer, this outcome is expected in egg-free and vegan reformulation strategies. From a market standpoint, such products are intended for consumers seeking plant-based or allergen-free options, where maintaining product functionality and consumer acceptance is considered just as important as nutritional aspects. When the CC results calculated, it was determined that the carbohydrates amount decreased as the mucilage addition rate increased ($p < 0.05$). However, the CC of mucilage-added samples was higher than that of the control sample ($p < 0.05$). The TP content increased with the use of mallow mucilage as an egg substitute in GF noodles ($p < 0.05$). Results of the study revealed that the noodles with the highest TP content were 3% mucilage-added noodles (MN3) sample ($p < 0.05$). The ABTS results indicated a significant difference in terms of AA between control and mucilage-added noodles ($p < 0.05$). The AA of the noodle sample with 3% mucilage was higher than the AA of other noodle samples ($p < 0.05$). The DPPH

Table 6 Results of verification experiments

Response	Predicted value	Experimental value[1]	SE[2]	Difference	%Error[3]	*p* value
EY (%)	9.03	9.07 ± 0.48	0.1	-0.04	0.44	0.731
WHC (g water/g DM)	5.56	5.44 ± 0.12	0.05	0.12	2.21	0.066
OHC (g oil/g DM)	3.23	3.17 ± 0.09	0.04	0.06	1.89	0.200

[1]: The results were given as arithmetic mean ± standard deviation

[2]: SE: Mean standard error

[3]: % Error = $(|y_{exp} - y_{pre}|/y_{exp}) \times 100$

Table 7 Physical and chemical analysis results of GF noodles (The analysis results were given as arithmetic mean±standard deviation) (Different letters indicate statistical differences within column ($p<0.05$))

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	TP content (mg GAE/100 g DM)	AA with ABTS (µmol Trolox/g DM)	AA with DPPH (µmol Trolox/g DM)	Cooking time (minute)	Water absorption (%)	Volume increase (%)	L*	a*	b*	Hardness (N)
C	10.61±0.02^{a}	3.21±0.05^{a}	6.74±0.03^{d}	22.35±0.13^{b}	57.09±0.18^{a}	0.30±0.003^{c}	4.37±0.074^{e}	3.31±0.028^{e}	20.00±0.15^{d}	127.42±0.97^{d}	166.76±0.30^{d}	67.38±0.12^{d}	9.58±0.24^{d}	42.63±0.26^{d}	3.90±0.16^{a}
MN1	10.62±0.03^{a}	3.33±0.01^{b}	3.55±0.06^{a}	19.89±0.01^{a}	62.61±0.10^{d}	0.26±0.001^{a}	3.65±0.038^{a}	2.72±0.017^{a}	13.00±0.15^{c}	103.71±0.41^{a}	66.59±0.43^{a}	58.55±0.82^{c}	2.35±0.11^{c}	32.23±0.08^{c}	3.75±0.19^{a}
MN2	10.70±0.06^{a}	3.51±0.07^{c}	3.79±0.05^{b}	19.90±0.01^{a}	62.10±0.05^{c}	0.29±0.002^{b}	4.29±0.009^{b}	3.20±0.044^{b}	12.00±0.15^{b}	108.46±0.27^{b}	100.43±0.69^{b}	54.84±0.40^{b}	1.90±0.04^{b}	28.88±0.22^{b}	4.02±0.17^{a}
MN3	10.72±0.11^{a}	3.56±0.04^{c}	3.94±0.02^{c}	19.92±0.01^{a}	61.86±0.12^{b}	0.33±0.003^{d}	4.58±0.036^{d}	3.40±0.048^{d}	11.00±0.15^{a}	112.51±0.48^{c}	116.66±0.75^{c}	52.86±0.49^{a}	1.05±0.09^{a}	27.33±0.15^{a}	4.46±0.12^{b}

method revealed that the AA of the GF vegan noodle sample containing 3% mucilage was higher than that of other GF noodles ($p < 0.05$). It was observed that the AA of the control sample corresponded to the AA of the samples with 2% and 3% mucilage addition ($p < 0.05$). It was also noted that the AA exhibited a direct proportional increase with the increase in the mallow mucilage addition rate ($p < 0.05$). A study by Freire et al. [38] found that adding linseeds which is rich in mucilage, to pasta formulations resulted in an increase in both the TP content and AA of pasta [38].

The incorporation of mallow mucilage as an alternative to egg in GF noodles was found to significantly reduce the cooking time ($p < 0.05$). While the control sample required approximately 20 min of cooking, this time decreased to 11 min in the MN3 formulation, corresponding to a reduction of nearly 45% (Table 7) ($p < 0.05$). This pronounced decrease is technologically meaningful, as shorter cooking times may enhance process efficiency and reduce energy demand at production scale. From a consumer perspective, reduced cooking time may also enhance convenience, which is a critical quality attribute in GF products. The magnitude of this reduction indicates that mallow mucilage can provide functional performance comparable to egg in terms of cooking behaviour, enabling the product to achieve its target textural properties within a shorter cooking period. Water absorption and volume increase differed significantly between the control and mucilage-added GF noodles ($p < 0.05$). The study revealed that the control sample exhibited higher water absorption and volume increase compared to the other samples ($p < 0.05$). However, it was also observed that both water absorption and volume increased in parallel with the raising levels of mallow mucilage ($p < 0.05$), with MN1 showing the lowest values and MN3 demonstrating partial recovery in water absorption and volume increase values. It is known that the inclusion of eggs in noodle formulations leads to increased water absorption and, consequently, volume increase [39]. In line with this, our findings showed that the control sample containing egg had higher water absorption and volume increase values than the mucilage-containing noodle samples, which is consistent with the literature ($p < 0.05$). The colour values of the GF noodles are presented in Table 7. The results with the highest L*, a* and b* values were determined in the control noodles. The addition of mallow mucilage to the GF noodles led to a decrease in L*, a* and b* values ($p < 0.05$). This reduction became more pronounced with increasing mucilage levels. The decrease in b* and L* values, particularly in a*, can be associated with the replacement of egg and the incorporation of mallow mucilage into the formulation ($p < 0.05$). While the b* value was seen to be high due to the yellow colour of the egg in the control noodles, this value decreased due to

the increase in the mallow mucilage ratios and the absence of egg ($p < 0.05$). From a market perspective, colour is a critical quality attribute influencing initial consumer perception, especially in GF products where visual similarity to conventional egg-based noodles may affect acceptance. Therefore, the observed colour changes should be evaluated in relation to product positioning and consumer expectations within egg-free and plant-based formulations. Upon examining the texture analysis results, it was found that the addition of 3% mallow mucilage increased the hardness of GF noodles ($p < 0.05$). A statistically significant difference was observed between the MN3 sample and the other GF noodle samples ($p < 0.05$). The technological performance of mallow mucilage in GF noodle formulations may be related to its hydrocolloid structure and water-binding properties. In GF systems lacking a gluten network, hydrocolloids contribute to matrix stabilization by improving water retention and structural cohesion. In this study, the increase in hardness at higher mucilage levels may be attributed to the formation of a denser matrix structure caused by the interaction between mucilage polysaccharides and starch components. Similarly, the observed reduction in cooking time and changes in water absorption behaviour can be associated with the hydration characteristics and viscosity-enhancing effect of mucilage within the noodle matrix. These findings suggest that optimized mallow mucilage may partially mimic the technological functions of eggs in GF noodle systems by contributing to structural stability and improved cooking performance. The sensory evaluation results of GF noodles are given in Table 8. Adding mallow mucilage instead of egg to GF noodles positively affected the taste of the samples ($p < 0.05$). No statistical difference was observed between the GF noodle samples in terms of hardness properties ($p > 0.05$). In the texture analysis results, no statistically significant difference was found in terms of hardness between the control, MN1, and MN2 samples ($p > 0.05$). However, it was determined that the MN3 sample had a higher hardness value compared to the other samples ($p < 0.05$). In the texture analysis results, the increase in hardness of the noodles with 3% mallow mucilage added was not noticed by the panellists in the sensory evaluation. This finding suggests that the increase in instrumental hardness at higher mucilage levels did not negatively influence consumer-perceived texture, which is technologically relevant for maintaining acceptable product quality in egg-free GF formulations. No statistical difference was observed between the GF noodle samples in terms of colour properties ($p > 0.05$). It was determined that the noodles containing mallow mucilage were preferred over the control noodles ($p < 0.05$).

Table 8 Sensory analysis results of GF noodles (Different letters indicate statistical differences within column ($p < 0.05$))

Sensory Evaluation Criteria				
Sample	Taste	Hardness	Colour	Overall acceptability
C	20^{a}	33^{a}	41^{a}	23^{a}
MN1	41^{b}	36^{a}	30^{a}	40^{b}
MN2	46^{b}	41^{a}	38^{a}	47^{b}
MN3	43^{b}	40^{a}	41^{a}	40^{b}

While the present study offers comprehensive insight into extraction optimization and technological performance of mallow mucilage in GF noodle formulations, some aspects may be further advanced in future research. The current investigation primarily emphasized extraction efficiency and product-level physicochemical, cooking, texture, and sensory responses. A more detailed examination of the structural characteristics of the mucilage and its interaction mechanisms within the GF matrix would provide additional clarity regarding the functional behaviour observed. Although the sensory analysis yielded valuable information under controlled conditions, broader consumer-oriented evaluations could strengthen the assessment of market relevance. Furthermore, the storage stability and long-term quality evolution of mucilage-enriched noodles remain to be explored. While the extraction and application procedures demonstrated promising technological performance at laboratory scale, validation at pilot or industrial scale would be valuable to evaluate process feasibility, production efficiency, and economic sustainability. From an industrial perspective, the optimized extraction process may provide practical advantages due to the use of water-based extraction under moderate processing conditions. The extraction procedure does not require complex processing steps, which may support its applicability in industrial hydrocolloid production systems and facilitate potential scale-up with reduced energy input and process complexity. In addition, the use of mallow mucilage as a plant-based egg replacer is compatible with current market trends focused on vegan and allergen-free food formulations. Considering the increasing demand for GF products, the optimized mucilage may represent a promising natural alternative for improving the technological quality of egg-free cereal-based foods. Addressing these aspects in future studies may deepen mechanistic understanding and further support the practical applicability of mallow mucilage in sustainable GF systems.

Conclusion

This study demonstrates that mallow (*M. sylvestris* L.) mucilage is a sustainable and functional plant-based alternative to eggs in GF noodle formulations. The incorporation of mallow mucilage at a 3% level indicates its practical applicability in GF and vegan product development without adversely affecting technological quality. The sensory attributes of the resulting noodles were favourably evaluated by the panellists.

Moreover, the reduction in fat content observed following egg replacement, combined with the fact that the resulting noodles are naturally cholesterol-free, indicates additional nutritional advantages of mallow mucilage in GF and vegan products. Importantly, the findings of this study provide a basis for future research focusing on shelf-life assessment, structural and molecular characterization, and rheological properties, thereby supporting the broader utilization of mallow mucilage in innovative and sustainable food systems.

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Declarations

Ethical approval This study was approved by the Ethics Committee of Ege University, Faculty of Engineering (Protocol No: 2955; Meeting No: 08/15; Date of Approval: 27 June 2025).

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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