

## Optimised Ultrasound-Assisted Alkaline Extraction and Rheology of Chia Mucilage with Co-Solutes

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### ABSTRACT

Chia mucilage is a promising food additive with applications in food systems. However, its attachment to seeds hinders the extraction and utilisation of the mucilage. Therefore, the study aims to optimise chia mucilage extraction using ultrasound and alkaline treatment to enhance yield, hue angle, and apparent viscosity and evaluate its rheological behaviour in the presence of co-solutes (sucrose and sodium chloride). Chia seeds were dispersed in distilled water, and the suspension was modulated to various pH values (7-9) in an ultrasound bath at varying temperatures (50-70 °C) and duration (30-60 minutes). Response Surface Methodology identified the optimum conditions (67.2 °C, 32.7 minutes, pH 9), yielding 79.62% with a hue angle of 74.79° and viscosity of 0.82 Pa.s. The optimised (M2) chia mucilage solution (CMS) was validated and showed higher yield

and hue angle than the macerated (M1) samples. Rheological evaluation with incorporation of sucrose (0-20%) and NaCl (0-250mM) showed non-Newtonian pseudoplastic behaviour, with 20% sucrose exhibiting the lowest  $k$  value in M1, yet the highest in M2. The relatively stable consistency coefficient value in M2, despite the addition of sucrose from 1 to 20%, indicates the suitability of ultrasonication and alkaline pH during the extraction process. Moreover, the addition of salt exerted a significant negative effect on the apparent viscosity of M1 samples while exerting the opposite effect on M2 CMS. These findings demonstrate that ultrasound-

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assisted alkaline extraction improves both yield and functional stability of chia mucilage, and its rheological properties were strongly influenced by sucrose and salt concentrations, indicating strong potential as a functional food hydrocolloid for diverse food formulation applications.

*Keywords:* Alkaline extraction, chia mucilage, co-solutes, optimisation, sonication, sonication time, sonication temperature

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## INTRODUCTION

The food hydrocolloid market is expected to grow significantly, focusing on sustainability, health benefits, and sensory perceptions (Saqib et al., 2022). Natural mucilages, also known as hydrocolloids, are more appealing than synthetic thickeners, such as xanthan and guar gum, owing to their cost-effectiveness, advanced biocompatibility, and non-toxicity (de Campo et al., 2017; Timilsena et al., 2015). According to Coorey et al. (2014) water and oil retention capacities and emulsion stability of chia mucilage (CM) were higher compared to commercially used guar gum. This makes chia mucilage an excellent foam stabiliser, emulsifier or thickener in the food industry (Coorey et al., 2014; Muñoz et al., 2012).

Previously, conventional extraction of chia mucilage was optimised in ranges; extraction temperature (70-85 °C), water: seed ratio (14-40:1), pH 8 and extraction time (2-2.4 h) using centrifugation, vacuum filtration, as well as drying processes (Campos et al., 2016; Chavan et al., 2019; Muñoz et al., 2012). Ultrasonication, a promising environmentally friendly treatment, has proven to be efficient for the extraction and modification of hydrocolloids, attributing to its high reproducibility, lower extraction time for highly efficient extractions and effective extraction of tightly bound hydrocolloids (Silva et al., 2022). According to Maran and Priya (2014), ultrasound creates cavitation bubbles that facilitate breaking of the matrix of the seed connecting to the desired product, hence encouraging mass transfer from matrix to solvent, which yields superior quality of material in less time.

The extraction yield, physicochemical and also functional properties of hydrocolloids, including chia, *Hypytis suaveolens* (L.) Poit, and *Salvia macrosiphon* can be highly affected by sonication conditions such as time, temperature, pH, seed: water ratio and sonication power (Farahnaky et al., 2013; Morales-Tovar et al., 2020; Silva et al., 2022). The optimisation of sonication conditions (Morales-Tovar et al., 2020; Silva et al., 2022) are necessary to make the ultrasound-assisted extraction (UAE) procedure more efficient and cost-effective (Hedayati et al., 2021), as well as to produce a greater yield of the desired product without compromising the necessary properties of hydrocolloids.

For industrial applications, real food formulations typically require the incorporation of additional ingredients, for instance, sugar and salt. The functionalities of food polymers within food systems are influenced by their interactions with sugars, salts and proteins

(Dickinson, 2003). Various studies have examined the impact of hydrocolloids, including quince seed, sage seed, balangu seed, qodume shahri seeds and *Hyptis suaveolens* L. seed and its interactions with salts and sugars (Farahmand et al., 2021; Koocheki et al., 2013; Pérez-Orozco et al., 2019; Yousefi et al., 2014).

Despite the promising functional properties of chia mucilage, its strong adherence to the seed coat limits efficient extraction using conventional methods, which are often time-consuming and may compromise quality. To the best of our knowledge, few studies have utilised UAE in the extraction of chia mucilage (Saporittis et al., 2023; Silva et al., 2022; Urbizo-Reyes et al., 2019), as current extraction approaches do not adequately address the optimisation of process conditions under combined ultrasound and alkaline environments, especially in a solution form without any drying step conducted. With the integration of ultrasound-assisted extraction with alkaline pH conditions, the yield and functional properties of chia mucilage could be enhanced while maintaining its structural integrity.

In addition, the behaviour of chia mucilage in the presence of common food co-solutes such as sugars and salts remains largely unexplored. Furthermore, there is a strong possibility that the optimised mucilage will exhibit stable and desirable rheological behaviour in the presence of co-solutes, which is critical for predicting its performance in real food systems. Determining this behaviour is significant in ensuring consistency, functionality, and adaptability of chia mucilage across varying formulation conditions. A key challenge lies in balancing process intensification with the preservation of its functional characteristics. Thus, this study aimed to optimise the integrated ultrasound-assisted alkaline extraction (UAE) of chia mucilage solution (CMS) with response surface optimisation without drying and evaluate the impact of the addition of sugar and salt on the rheological characteristics of the solution. By gaining insights into these factors, we can better utilise chia mucilage as a sustainable and functional ingredient in various food formulations.

## MATERIALS AND METHOD

### Chia Seed Collection and Preparation

Black chia seeds (*Salvia hispanica* L.) were provided by The Chia Company (Victoria, Australia). Distilled water was used for all solution preparations. Sodium hydroxide (analytical grade) was obtained from R&M Chemicals (Malaysia). Sucrose and sodium chloride (analytical grade) were purchased from Chemiz (Malaysia).

### Chia Mucilage Solution (CMS) Extraction

Chia seeds were hydrated in distilled water at a ratio of 1:40 (seed-to-water) with constant stirring for 2 h (Orifici et al., 2018). Following that, the dispersion was subjected to ultrasound and pH treatment as described below. After treatment, the samples were vacuum filtrated using cheesecloth and a chia mucilage solution (CMS) was obtained.

Conventionally extracted CMS was prepared as a control with a similar seed-to-water ratio and stirring time (maceration only) to evaluate the differences between the optimised and conventionally extracted samples.

The gel solutions were kept at 4 °C till further analysis. According to the preliminary studies, the yield of the mucilage solution was greater at this seed-to-water ratio (1:40) compared to other seed-to-water ratios (1:10-50) and the hydration period was fixed at this limit as chia mucilage gel reaches its full potential after 2 hours of immersion according to Muñoz et al. (2012). pH values were fixed based on the preliminary studies, as well as the observations from Capitani et al. (2015) and Muñoz et al. (2012) in which, at higher pH values (8-9), higher chia mucilage yield values were observed due to the increased solubility of chia mucilage at alkaline conditions, allowing the mucilage to be extracted easily.

### ***Optimisation of CMS using Ultrasound and Alkaline Treatment***

To fix the optimal condition of ultrasound and alkaline parameters, the impact of three independent variables including pH ( $X_1$ : 7, 8, 9), sonication time ( $X_2$ : 20, 45, 60 minutes) and sonication temperature ( $X_3$ : 50, 60, 70 °C) on the dependent variables (Y), extraction yield (%), hue angle (°) and apparent viscosity (Pa.s) were analysed through RSM using Minitab software. The hydrated chia mucilage samples were treated with ultrasonication using an ultrasonic bath (Elmasonic S100H, Germany) operating at a frequency of 37 kHz and with a recirculation bath.

Experimental design included a three-level, three-factor design, comprising a randomised 15 experimental runs, in which the centre point was repeated three times.

For each response, ANOVA was performed to identify the significant model terms. The experimental design matrix by the BBD and the corresponding experiment values are tabulated in Table 1.

The response optimiser plot in Minitab was utilised to establish the optimal factor levels, with targets defined for all variables and responses. Under recommended optimum conditions, the adequacy of the model was verified by conducting experiments (Bazezew et al., 2022).

### ***Yield Analysis***

Yield percentage (%) of CMS was calculated as the ratio of the final mass after vacuum filtration to the initial mass after dispersion, multiplied by 100.

### ***Colour***

Colour parameters were identified using a colourimeter (Konica Minolta, Model No. CR-410, Japan). Measurements were obtained in the  $L^*a^*b^*$  scale, and the hue angle value was calculated (Campos et al., 2016).

Table 1

*Box Behnken Design (BBD) with experimental values for the extraction of mucilage*

Sample	pH (A)	Sonication Time (min, B)	Sonication Temperature (°C, C)	Yield (%)	Hue Angle (°)	Apparent Viscosity, $\eta$ (Pa.s)
1	7	60	60	78.7417	74.4606	0.4205
2	8	30	50	80.7917	45.0000	0.2379
3	7	45	70	82.6750	70.2239	0.1485
4	8	60	50	75.7500	64.5821	0.3966
5 <sub>c</sub>	8	45	60	80.3250	64.9439	0.4395
6 <sub>c</sub>	8	45	60	78.4000	52.6333	0.4389
7	8	60	70	77.0000	72.1157	0.1493
8	9	60	60	75.5500	73.6476	0.5067
9	7	45	50	77.5500	64.3793	0.5727
10	9	30	60	80.0417	65.0809	0.5034
11	8	30	70	82.2250	72.8476	0.0849
12	9	45	70	77.9250	75.5878	0.7658
13	9	45	50	83.7000	54.2356	0.6689
14 <sub>c</sub>	8	45	60	76.4750	63.4349	0.5412
15	7	30	60	84.1250	59.1986	0.2641

Note. c = Centre point

## Rheology

The rheological measurements were carried out in a dynamic controlled stress rheometer (Haake RS600, ThermoElectron, Germany). For the apparent viscosity, the PP35 Ti plate was used with measurements executed under a shear rate range of 0 to 300 s<sup>-1</sup> within 3 minutes at 25 ± 1 °C. For the addition of co-solutes, a PP60 Ti plate was used. CMS solutions were allowed to equilibrate at 25 °C for 3 minutes at 3 s<sup>-1</sup> and then followed by a linear increasing shear rate from 1 to 100 s<sup>-1</sup> in 3 minutes. As suggested by previous studies, the power law model was used to obtain the measurements (Capitani et al., 2015; Timilsena et al., 2015).

## Addition of Co-Solutes in CMS

Addition of co-solutes such as sucrose and sodium chloride was evaluated in CMS extracted using two different methods, macerated (M1) and optimised (M2) CMS. The macerated CMS was previously used as a control to evaluate the difference between the conventionally extracted and the optimised CMS. To reiterate, M1 samples were not subjected to any ultrasound or pH treatment while M2 samples underwent the optimised treatment determined at pH 9, 32.7 minutes at 67.2 °C based on high yield, hue angle and viscosity.

Different concentrations of sucrose (0, 1, 5 and 20%) and NaCl (0, 10, 50 and 250mM) were added to the freshly extracted mucilage solutions, under constant magnetic stirring for 10 min. A total of two controls, one for each method (M1 and M2) without any addition of co-solutes (0% or 0mM), were prepared. The respective control samples were used as the baseline for evaluating the effects of the various sucrose and NaCl concentrations on the mucilage solutions extracted using different methods. The concentration of sucrose and salt was selected according to Yang et al. (2020) and Yousefi et al. (2014)'s studies, respectively.

### Statistical Analysis

The statistical analyses were executed using Minitab 19. A two-way ANOVA with Tukey's test was carried out to distinguish the effect of method type and varying concentrations of sucrose and salt. Modelling of rheological data was performed using Microsoft Excel 16 (Solver).

## RESULT AND DISCUSSION

### Optimisation of US-Assisted Alkaline Mucilage Solution Extraction Parameters and Model Fitting for Response Surface

Table 2 displays the linear, quadratic, interaction terms and statistical parameters ( $R^2$ , Adj- $R^2$ , p- and f-values) after removing the effect of non-significant factors for the process variables. The ANOVA analysis shows that lack of fit was not significant ( $p > 0.05$ ) and the high  $R^2$  values demonstrate the model's suitability in predicting the responses (yield, hue angle and apparent viscosity).

#### *Effect of Independent Variables on Mucilage Yield*

After removing the non-significant terms, the linear effect of sonication time and the interaction effects of pH and sonication temperature had significant effects ( $p < 0.05$ ) on the extraction yield (Table 2).

The regression equation of mucilage yield ( $Y_1$ ) is as in Equation 1:

$$Y_1 = 75.2 - 6.9X_1 - 0.207X_2 + 1.39X_3 + 1.368X_1^2 - 0.00068X_2^2 + 0.006956X_3^2 + 0.0149X_{12} - 0.2725X_{13} - 0.00031X_{23} \quad [1]$$

Table 2  
*Analysis of variance (ANOVA) for fitting the model of yield, hue angle, and apparent viscosity*

Source	Response Variables		
	Yield (% $, Y_1$ )	Hue Angle ( $^{\circ}$ , $Y_2$ )	Apparent Viscosity, $\eta$ (Pa.s, $Y_3$ )
Linear			
$x_1$	0.201	0.981	0.004*
$x_2$	0.001*	0.009*	0.124
$x_3$	0.644	0.001*	0.017*
Quadratic			
$x_{11}$	0.122	0.055	0.016*
$x_{22}$	-	0.278	0.005*
$x_{33}$	-	-	0.125
Interaction			
$x_1 x_2$	-	-	0.345
$x_1 x_3$	0.006*	0.111	0.016*
$x_2 x_3$	-	0.048*	0.549
$R^2$	0.82	0.89	0.95
Adj- $R^2$	0.72	0.77	0.86
p-value	0.002*	0.007*	0.009*
f-value	9.83	7.86	10.81
Lack-of-fit (p-value)	0.625	0.956	0.361

Note. \*Significant at  $p < 0.05$ .  $x_1$  to  $x_3$  denote pH, sonication time, and temperature, respectively;  $x_1^2, x_3^2$  and  $x_1x_2, x_1x_3, x_2x_3$  represent quadratic and interaction terms, respectively

Contour plots generated by the model in Figure 1 (a-c) represent the correlation between independent variables and yield. The combined effect of pH and sonication time affected the chia yield, as depicted in Figure 1a. A decrease in yield can be seen when sonication time increased from 30 to 60 minutes at pH 7 and 9. Similarly, an increase in pH to 9 led to a declining yield at all time ranges. The highest value of pH (9) and sonication time (60 minutes) at 60 °C resulted in the lowest yield (75.55%). Meanwhile, the lowest pH (7) and sonication time (30 mins) at 60 °C yielded the highest at 84.13%. Higher yield value at pH 7 could be from hydrolysis, in which certain insoluble polysaccharide fractions could have been converted to soluble ones (Karazhiyan et al., 2011).

Maran and Priya (2014) explained that the cavitation effects of the ultrasonic waves accelerate the swelling as well as the hydration. Hence, the number of cells exposed to the extraction medium (aqueous) increases and causes disruption. Hence, polysaccharides are easily released into the medium, and the yield is enhanced in a shorter time. A longer sonicating time leads to overexposure to ultrasound treatment, causing structural and polysaccharide ruin. Moreover, this leads to the loss of soluble mucilage components

during the extraction process, resulting in lower overall yields compared to shorter sonication times (Urbizo-Reyes et al., 2019). Therefore, increasing the sonication time is both time-consuming and an unreasonable situation from an economic point of view (Şahin & Şamlı, 2013).

Likewise, as seen in Figure 1b, at pH 7, the yield greatly rose from 77.55 to 82.68% at rising temperatures, while giving an opposite result at pH 9. As the interaction between pH and sonication temperature has a significantly ( $p < 0.05$ ) negative effect (Table 2) on yield, it can be deduced that a higher yield can be obtained at a higher temperature at pH 7 and a lower temperature at pH 9. As chia mucilage contains a huge quantity of uronic acids, a lower extraction rate can be obtained under acidic conditions (Balke & Diosady, 2000). Hence, at alkaline pH, a higher yield is observed.

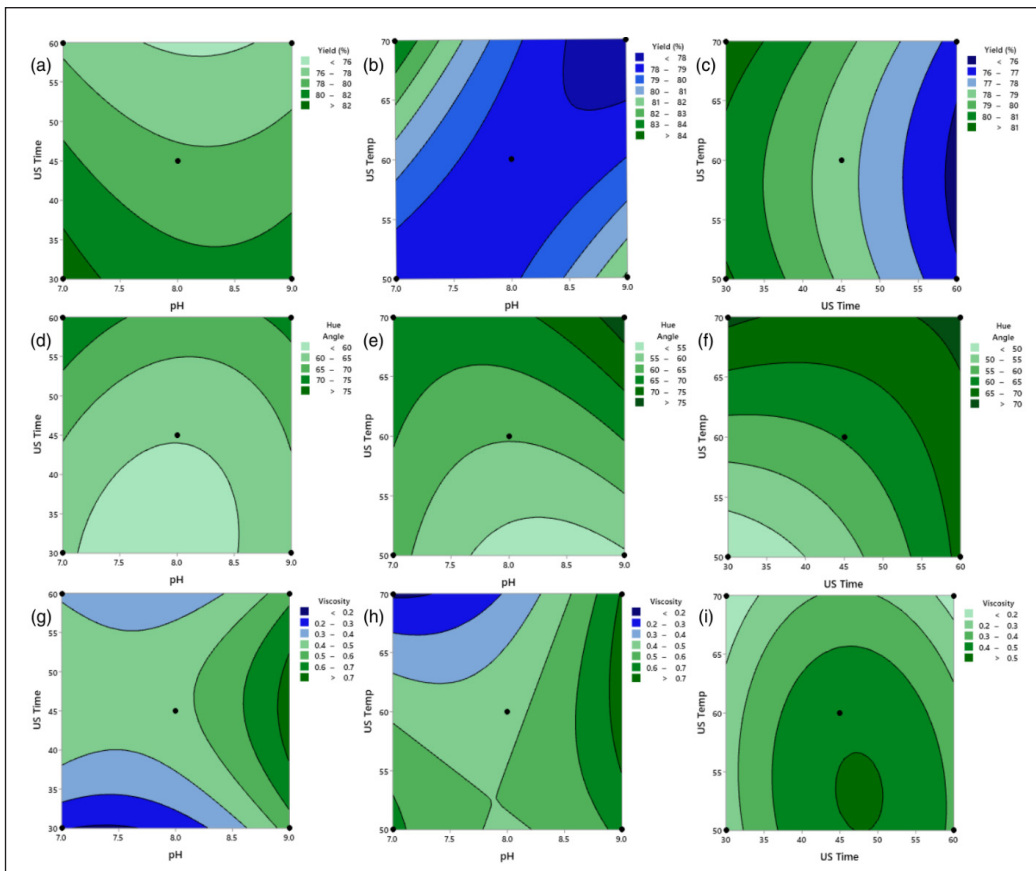


Figure 1. Contour plots of extraction yield (a-c), hue angle (d-f) as well as viscosity (g-i) explaining the relationship of pH and ultrasonication time (a, d, g - sonication temperature = 60 °C), pH and sonication temperature (b, e, h - sonication time = 45 minutes) and sonication time and temperature (c, f, i - pH = 8)

A decline is noticed as sonication time increases at all temperatures, i.e., 50 to 70 °C (Figure 1c). Conversely, as the temperature rises from 50 to 70 °C, a slight increase in yield can be seen both at 30 and 60 minutes. This correlation strongly indicates the favourable effect of sonication temperature on mucilage yield. The collective effect of temperature and time can be explained by the mass transfer effect, in which the mucilage diffuses at a higher rate. The effect of time will be more pronounced at higher temperatures (50-65 °C), so a higher extraction time can cause detrimental effects in the polysaccharides' structure and decrease the yield.

### ***Effect of Independent Variables on Hue Angle***

Linear effect of sonication time and temperature, as well as the interactive effects between pH and sonication time, had significant effects ( $p < 0.05$ ) on hue angle (Table 2). Koocheki et al. (2009) suggested that the colour of the mucilage may be attributed to the leaching of natural pigments or tannic substances from the coat of chia.

The regression equation for hue angle ( $Y_2$ ) is given as in Equation 2:

$$Y_2 = 426 - 100.0X_1 + 2.22X_2 - 1.58X_3 + 5.11X_1^2 + 0.0118X_2^2 + 0.0065X_3^2 - 0.112X_{12} + 0.388X_{13} - 0.0339X_{23} \quad [2]$$

As shown in Figure 1d, while the hue angle considerably rose at 30 minutes, only a slight decline was noticed at 60 minutes. Conversely, a significant rise in hue angle was observed as sonication time increased from 30 to 60 minutes, both at pH 7 and 9. At pH 8, when sonication time was held steady at 45 minutes, the hue angle ranged from 52.63 to 64.94°. Koocheki et al. (2009) found that at acidic conditions and a higher water-to-seed ratio, the colour of the *Lepidium perfoliatum* gum was almost yellow. This explains that the pigments were highly soluble in alkaline conditions or dissolved at lower pHs. The gradated pigment in a lower water-to-seed ratio reached saturation, and thus the hue angle did not change with pH.

Meanwhile, a linear increase in hue angle was observed when sonication temperature increased at both pH 7 and 9 (Figure 1e). The highest hue angle (75.59°) was obtained at the highest pH (9) and sonication temperature (70 °C). The hue angle obtained in this study (65.51-71.46°) is lower than that of chia mucilage powder (84.03°) but higher than that of *Lepidium perfoliatum* powder (60.76°), indicating that this mucilage possesses a yellow colour (Campos et al., 2016; Koocheki, Taherian, et al., 2009). Studies conducted on white mustard seed mucilage (Balke & Diosady, 2000) and *P. flexuosa* seed gum (Ibañez & Ferrero, 2003) concluded that a highly coloured product was obtained at a higher temperature and alkaline medium, respectively.

### *Effect of Independent Variables on Apparent Viscosity*

As seen from Table 1, all the samples display  $n < 1$ , implying the characteristics of a shear-thinning liquid of a non-Newtonian behaviour and the values of apparent viscosity,  $\eta$ , were obtained spanning between 0.08 and 0.77 Pa.s. The apparent viscosity was significantly ( $p < 0.05$ ) affected by the linear effects of pH and sonication temperature, quadratic effects of pH and time, as well as the interaction between pH and temperature, as referred in Table 2.

The regression equation for apparent viscosity ( $Y_3$ ) is as in Equation 3:

$$Y_3 = 9.26 - 2.715X_1 + 0.1073X_2 - 0.0218X_3 + 0.1361X_1^2 - 0.000825X_2^2 - 0.000704X_3^2 - 0.00255X_{12} + 0.01303X_{13} - 0.000157X_{23} \quad [3]$$

Upon an increment in pH values, an increase in viscosity was observed at both 30 mins (0.26 to 0.50 Pa.s) and 60 minutes (0.42 to 0.51 Pa.s) (Figure 1g). The graphs also show that at pH 7 and 9, the viscosity showed a notable increase when the sonication time rose from 30 to 45 minutes. However, when the sonication time reached 60 minutes, the viscosity showed a slight decrease. It can be deduced that apparent viscosity peaks at 45 minutes and decreases when sonicated for a longer period of time.

The quadratic effect between pH and sonication temperature significantly ( $p < 0.05$ ) affected the mucilage viscosity as depicted in Figure 1h. Though viscosity values increased at both 50 and 70 °C as the pH increased, a substantial rise was noted, particularly at 70 °C, the values spanning from 0.15 to 0.77 Pa.s. For temperature variations, at pH 7, a temperature rise led to a decrease in viscosity values, while the opposite happened at pH 9. Notably, the highest viscosity (0.77 Pa.s) is obtained when both pH and temperature are at the highest range, at 9 and 70 °C.

A negative curvilinear trend was observed at increasing sonication time of mucilage extraction (Figure 1i). When the sonication temperature was set at the highest temperature (70 °C), and the shortest time (30 minutes) at pH 8, the viscosity was lowest at 0.08 Pa.s. Generally, when the sonication temperature reaches to 70 °C, parameters such as viscosity as well as surface tension of water (solvent) decrease, thereby decreasing the sonication effect (Zia et al., 2022). The above results suggest that the mucilage fibres were progressively separated from the seed and freed to the aqueous solution, hence the high yield and lower viscosity. This can be seen in run 11, which has a relatively high yield (82.23%) but contributed to the lowest viscosity (0.08 Pa.s). The study also suggests that a sonication temperature up to 65 °C gives a higher viscosity value, and further increase may cause a decline in the viscosity value.

Silva et al. (2022) discovered that a rise in stirring or sonication time led to a decrease in apparent viscosity and  $k$  value. 30 min sonication caused a drop in viscosity. This

statement is slightly contradictory to this study, as viscosity increased when the mucilage was sonicated for 45 minutes. This could be due to the differences in ultrasound type (probe and bath), as the ultrasound probe could allow for a more precise and localised delivery of ultrasound energy as opposed to the ultrasound bath, hence giving a more intense cavitation effect, leading to faster breakdown of molecular structures and reduction in viscosity (Chemat et al., 2011).

### Optimisation and Model Verification

In this study, 15 formulations were optimised by BBD based on a composite desirability (D) value of 0.7135. This optimisation study was planned for maximum extraction yield, hue angle and apparent viscosity in ultrasonicated alkaline chia mucilage. The predicted most desirable set of ultrasound extraction conditions was pH (9), sonication time (32.7 min) and temperature (67.2 °C) as presented in Table 3. Based on the suggested extraction conditions, the predicted favourable values attained were maximum extraction yield of 79.42%, maximum hue angle of 75.97° and maximum viscosity of 0.61 Pa.s (Table 4). Experimental validation was performed to confirm the adequacy and accuracy of the most desirable set of extraction conditions. When comparing the experimental to predicted results, a similar yield value was obtained, with slightly lower hue angle and higher apparent viscosity. Though one sample t-test indicated no significant differences ( $p > 0.05$ ) observed in all the responses to the predicted value. This has proven that pH 9, sonication at 32.7 minutes and 67.2 °C is the most desirable set of conditions for ultrasonic extraction of mucilage solution.

Table 3  
*Predicted optimal condition for CMS extraction*

Variables	Minimum	Maximum	Optimum
pH	7	9	9
Sonication Time (min)	30	60	32.7
Sonication Temperature (°C)	50	70	67.2

Table 4  
*Predicted and experimental values for the optimum extraction parameters*

Responses	Predicted Value	Experimental Values <sup>a</sup>	p-value
Yield (%)	79.4206	79.616 ± 0.421	0.507*
Hue Angle	75.9703	74.791 ± 0.611	0.079*
Apparent Viscosity (Pa.s)	0.6129	0.8182 ± 0.1072	0.08*

Note. <sup>a</sup>Mean (n = 3), \*Not significant at  $p < 0.05$

### Comparison Between Optimised and Control Extracted Mucilage Solution

The optimised mucilage solution sonicated at pH 9 for 32.7 minutes at 67.2 °C was compared with a macerated control sample in terms of extraction yield, colour parameters (hue angle, L\*, a\*, b\*) and rheological parameters such as  $\eta$  (apparent viscosity), k and n values (Table 5). This provides a reference on the impact of sonication on the mucilage solution compared to a non-sonicated sample.

The extraction yield of the optimised mucilage solution is significantly higher at 79.62% compared to the control, 75.89%. Several studies agree that ultrasound offers a greater yield than the conventional method, as seen in sage and chan seeds (Farahnaky et al., 2013; Morales-Tovar et al., 2020). Moreover, the optimised yield obtained in this study (79.62%) is much higher than the wet yield reported by Chaves et al. (2018) after centrifugation (18.25%). From centrifugation, only the gel is obtained, excluding the liquid and seeds, whereas vacuum filtration filters out the seeds only, leaving the mucilage in a liquid form. Hence, the higher yield in this study.

Comparing the colour parameters of the samples, the hue angle of the optimised sample is significantly higher, while a\* and b\* values were significantly lower. Moreover, no significant differences were observed in any of the rheological parameters between the control and the optimised sample. This is the opposite of Farahnaky et al. (2013) in which the apparent viscosity of the sage mucilage from the conventional extraction method was greater than that of the sonicating probe. To reiterate, the cavitation effect of the ultrasonic waves increases the yield of mucilage solution as well as elevates the dissemination of natural pigments from chia, leading to a higher hue angle, and the viscosity increases at alkaline conditions. Hence, ultrasound can be employed to facilitate the easy extraction of chia mucilage tightly bound to the seed, leading to functional, textural, and economic benefits. It allows for the incorporation of mucilage into an array of food products, aiding in their quality, appeal, and overall marketability.

Table 5  
*Experimental values for control and optimised mucilage*

Analysis	Control	Optimised
Yield (%)	75.892 <sup>b</sup> ± 0.667	79.616 <sup>a</sup> ± 0.421
Hue Angle (°)	72.749 <sup>b</sup> ± 0.598	74.791 <sup>a</sup> ± 0.611
L*	70.217 <sup>a</sup> ± 0.015	69.377 <sup>a</sup> ± 1.563
a*	1.8033 <sup>a</sup> ± 0.025	1.2767 <sup>b</sup> ± 0.015
b*	4.700 <sup>a</sup> ± 0.021	0.3833 <sup>b</sup> ± 0.161
Apparent Viscosity, $\eta$ (Pa.s)	0.7863 <sup>a</sup> ± 0.066	0.8183 <sup>a</sup> ± 0.107
k (Pa.s <sup>n</sup> )	0.0502 <sup>a</sup> ± 0.007	0.0489 <sup>a</sup> ± 0.004
n	0.5211 <sup>a</sup> ± 0.009	0.5211 <sup>a</sup> ± 0.010

Note. <sup>a</sup> Means in the same row with the same letters are not significantly different at p<0.05 (n = 3)

## Effect of Sucrose

Referring to Table 6, the Power Law parameters such as the flow behaviour index ( $n$ ), consistency coefficient ( $k$ ), apparent viscosity ( $\eta$ ) and  $R^2$  values.

The high  $R^2$  (0.95-0.97) denotes a good fit for the Power Law model (Marcotte et al., 2001). CMS's rheological behaviour was characterised based on the addition of sucrose, as well as how the differences in the method of extraction affect its fluid behaviour.

Table 6 shows that the concentration of sucrose and the interaction between the extraction method and the sucrose concentration had significant effects on  $k$ ,  $n$  and  $\eta$  values. The  $n$  values displayed a value of less than 1, hence the samples exhibit shear-thinning behaviour. A significant difference between the  $n$  value according to the method of extraction was observed only at 0%. The  $n$  value of M2 was significantly ( $p < 0.05$ ) higher than that of M1 at 0%, indicating that the sonicated CMS had a lower shear-thinning effect.

Table 6  
Effect of type of extraction (T), sucrose concentration (C) and their interactions (T × C) on Power Law parameters for chia mucilage solution

Analysis	C (%)	T		p-value		
		M1	M2	T	C	T × C
$k$ (Pa.s <sup>n</sup> )	0	0.104 <sup>aA</sup>	0.095 <sup>abA</sup>			
	1	0.075 <sup>abB</sup>	0.106 <sup>aA</sup>	ns	**	**
	5	0.103 <sup>aA</sup>	0.078 <sup>bB</sup>			
	20	0.043 <sup>bB</sup>	0.076 <sup>bA</sup>			
$n$	0	0.461 <sup>bB</sup>	0.505 <sup>bcA</sup>			
	1	0.505 <sup>abA</sup>	0.492 <sup>cA</sup>	ns	**	*
	5	0.517 <sup>abA</sup>	0.516 <sup>bA</sup>			
$\eta$ (Pa.s)	0	1.52 <sup>aA</sup>	1.048 <sup>bB</sup>			
	1	0.935 <sup>bA</sup>	1.219 <sup>aA</sup>	ns	**	**
	5	1.140 <sup>abA</sup>	0.875 <sup>cA</sup>			
$R^2$	20	0.393 <sup>cB</sup>	0.640 <sup>dA</sup>			
	0	0.970	0.961			
	1	0.963	0.959			
	5	0.972	0.953			
	20	0.962	0.965			

Note. \*Lowercase letters within a column denote no significant differences among sucrose-added samples. Uppercase letters within rows denote no significant difference between samples from different extraction methods at each concentration of sucrose. The control sample was prepared at 0%. (Mean = 2). <sup>1</sup>ANOVA: \*\* $p < 0.01$ ; \* $p < 0.05$ ; ns, not significant. T × C, the interaction between the type of extraction and the concentration of sucrose

A general increasing trend in  $n$  value was observed in both M1 and M2 upon increasing sucrose concentration. At 20% sucrose concentration, a pronounced ( $p < 0.05$ )  $n$  value was observed for both M1 and M2 (0.577 and 0.554, respectively). Hence, at increasing sucrose concentrations, the  $n$  value increased regardless of the extraction method, indicating a reduction in shear thinning behaviour of mucilage at these concentrations.

From this study, a greater flow behaviour index ( $n$ ) was obtained at increasing sucrose addition, indicating the solutions were less pseudoplastic. The addition of sucrose leading to a less pseudoplastic behaviour (higher  $n$  value) has been reported in solutions of chia, sodium alginate and *Alyssum homolocarpum* (Capitani et al., 2015; Koocheki et al., 2013; Yanes et al., 2002) and in Balangu (*Lallemantia royleana*) seed gum with glucose addition (Salehi et al., 2014). Sucrose has the ability to affect the structure and texture of gel due to its interaction with hydroxyl groups that stabilise the gel structure (Bayarri et al., 2004), hence making the gel structure more robust and less susceptible to deformation under shear stress.

Generally, a lower  $n$  value indicates higher shear-thinning behaviour (Koocheki, Mortazavi, et al., 2009) while a higher value creates a slimier mouth feeling (Szczeniak & Farkas, 1962). Hence, it is essential to use hydrocolloids possessing a low  $n$  value (Marcotte et al., 2001; Salehi et al., 2014). This is important especially in formulations of oil-in-water to inhibit drop separation but can allow the formulation to flow easily when poured (Taherian et al., 2007). Sucrose addition led to a similar increment in the  $n$  values of CMS, regardless of the extraction method.

The  $k$  values (0.043 to 0.106 Pa.s<sup>n</sup>) of the sucrose-added chia mucilage solution were significantly ( $p < 0.01$ ) affected by the sucrose concentration as well as the interaction between the type of extraction and the concentration. The consistency index ( $k$ ) was found to be significantly higher in M2 at the concentrations of 1 and 20% compared to M1, though the opposite was observed at 5%. An increase in sucrose concentration led to a significant decrease in  $k$  value in both M1 and M2. This indicates that higher sucrose concentrations significantly decrease the  $k$  value, producing a less viscous and easily flowing CMS that can be used in processes such as pumping, stirring or mixing processes.

Though in M1, the addition of 20% sucrose led to the lowest  $k$  value (0.043 Pa.s<sup>n</sup>) among all the formulations. Yet, at the same concentration, the  $k$  value of M2 was significantly higher (0.076 Pa.s<sup>n</sup>). Moreover, the addition of sucrose, even at the highest sucrose concentration (20%), did not have a significant decline in the M2 CMS samples, as opposed to M1, which showed a significant decline. Hence, the utilisation of ultrasonication and alkaline pH during the extraction process led to samples that exhibit a relatively stable consistency coefficient value despite the addition of sucrose from 1 to 20%.

The apparent viscosity ( $\eta$ ) of M1 samples and M2 samples was obtained from 0.393 to 1.520 Pa.s and 0.640 to 1.219 Pa.s, respectively. The addition of sucrose had a significant

effect on the  $\eta$  values. In both M1 and M2, the lowest viscosity was obtained at 20% sucrose level (0.393 and 0.640 Pa.s, respectively) compared to other formulations, though M2 was significantly higher than M1. The addition of sucrose at certain levels boosted the viscosity values compared to the control samples. In M1, though a significant decline in the  $\eta$  value was observed at increasing sugar, the addition of 5% boosted the  $\eta$  value comparable to the control sample. In M2, the addition of 1% sucrose in M2 significantly raised the viscosity value from 1.048 Pa.s to 1.219 Pa.s. However, further addition of sucrose caused the viscosity to decline even further. Hence, it can be concluded that the addition of sucrose significantly ( $p < 0.05$ ) decreased the apparent viscosity of CMS. However, an increase can be expected in CMS at lower sucrose levels (5% for M1 and 1% for M2), which will then decline above the optimum levels.

The viscosity of solutions containing gums of basil seed, guar and carboxymethyl cellulose (CMC) also reduced when 15% sucrose was added (BahramParvar & Razavi, 2012). It can be inferred that the presence of sugar disrupts the network structure of the hydrocolloids, leading to a decrease in viscosity. Contrastingly, studies have shown that the apparent viscosity of polysaccharides rises at increasing sucrose concentration of *Pereskia aculeata* Miller (OPN) mucilage and balangu seed gum (Amaral et al., 2019; Salehi et al., 2014). The effect of sugar (0-50 g/100 g) concentrations on the viscosity of pectin solutions was investigated (Kar & Arslan, 1999). The authors stated that the reduced viscosity of pectin was increased by glucose and maltose up to a concentration of 30%.

The effect of sucrose-hydrocolloid interaction on the flow characteristics of real products is contingent upon the product composition, the concentration of ingredients, and the rheological attributes specific to the hydrocolloid in question (Capitani et al., 2015; Yanes et al., 2002). To conclude, the sonicated CMS samples (M2) behaved better than M1 at higher sucrose levels (20%) by exhibiting significantly higher  $k$  and  $\eta$  values, indicating a greater consistency and viscosity value at these concentrations.

### Effect of Salt

Table 7 reports the rheological parameters of CMS that were extracted using M1 and M2, added with varying concentrations of salt. Similar to the addition of sucrose, the  $n$  values of chia mucilage solution display a value of less than 1, hence it confirms the shear-thinning behaviour of chia solution. Similar to sucrose, the extraction method had no significant effect on the rheological parameters; however, the salt concentration and the interaction between the salt levels and the extraction method yielded significance ( $p < 0.05$ ).

Based on the comparison between the extraction methods, at lower salt concentrations (0 and 10mM), the chia mucilage samples of M2 had significantly higher  $n$  value, while the opposite was observed at higher concentrations (50 and 250mM). This explains that M1 samples presented a stronger shear thinning effect (lower  $n$  value) in lower salt

Table 7

Effect of type of extraction (*T*), salt concentration (*C*) and their interactions (*T* × *C*) on Power Law (PL) parameters for chia mucilage solution (CMS)

Analysis	C (mM)	T		T	p-value	
		M1	M2		C	T × C
<i>k</i> (Pa.s <sup>n</sup> )	0	0.104 <sup>aA</sup>	0.095 <sup>aA</sup>			
	10	0.072 <sup>abA</sup>	0.088 <sup>abA</sup>	ns	**	*
	50	0.080 <sup>abA</sup>	0.065 <sup>bbB</sup>			
	250	0.047 <sup>bbB</sup>	0.080 <sup>abA</sup>			
0	0.461 <sup>bbB</sup>	0.505 <sup>aA</sup>				
<i>n</i>	10	0.396 <sup>cbB</sup>	0.484 <sup>aA</sup>	ns	**	**
	50	0.526 <sup>aA</sup>	0.506 <sup>abB</sup>			
	250	0.535 <sup>aA</sup>	0.409 <sup>bbB</sup>			
	0	1.520 <sup>baA</sup>	1.048 <sup>bcB</sup>			
$\eta$ (Pa.s)	10	2.313 <sup>aA</sup>	1.186 <sup>bbB</sup>	ns	**	**
	50	0.821 <sup>caA</sup>	0.848 <sup>caA</sup>			
	250	0.576 <sup>cbB</sup>	2.092 <sup>aA</sup>			
	0	0.970	0.961			
R <sup>2</sup>	10	0.975	0.961			
	50	0.953	0.955			
	250	0.959	0.962			

Note. \*Lowercase letters within a column denote no significant differences among sucrose-added samples. Uppercase letters within rows denote no significant difference between samples from different extraction methods at each concentration of sucrose. The control sample was prepared at 0%. (Mean = 2). <sup>1</sup>ANOVA: \*\*p<0.01; \*p<0.05; ns, not significant. T × C, the interaction between the type of extraction and the concentration of sucrose

concentrations (0 and 10mM), while M2 dominates at salt concentrations above 50mM. In M1, the addition of salt at 10mM gave a significantly lower *n* value, followed by significantly greater values at 50 and 250mM compared to the control. In contrast, no significant difference was spotted in M2 samples up to the addition of 50mM. However, the addition of 250mM significantly reduced the *n* value to 0.409. The effect of the addition of salt to *n* value behaved differently in M1 and M2 samples. The addition of salt caused a stronger shear-thinning effect in M2 samples, whereas the opposite for M1.

Assessing the influence of salt concentration on the viscosity of hydrocolloids is essential for identifying the hydrocolloid's role as a polyelectrolyte and for evaluating its functional and rheological characteristics (Koocheki, Mortazavi, et al., 2009). Electrostatic forces impact the arrangement of ionic polysaccharides and influence their physicochemical properties, including viscosity (Lin & Lai, 2009). The decreasing flow behaviour index (*n*) of M2 as the concentration of salt increases agrees with various studies (Salehi et al., 2014; Vardhanabhuti & Ikeda, 2006). Conversely, some studies found that *n* value increased

as salt concentration increased, similar to M1 extraction (Amaral et al., 2019; Koocheki, Mortazavi, et al., 2009).

Comparing the extraction methods, a significant difference in the  $k$  value was observed at higher salt concentrations, 50 and 250mM. At 50mM, M1 exhibited a higher  $k$  value than M2, and vice versa at 250mM. Besides that, a general decline in the  $k$  value can be observed at increasing salt concentration in both M1 and M2. In M1, the addition of 250mM significantly decreased the  $k$  value to 0.047, compared to M1's control sample. Meanwhile, in M2, at the same concentration (250mM - 0.080), the  $k$  value was not significantly different from its control sample (0.095). This indicates that sonicated CMS (M2) can exhibit higher viscosity despite the addition of salt at concentrations up to 250mM.

Based on the extraction methods, at lower salt concentrations (0 and 10mM), M1 yielded significantly higher viscosity ( $\eta$ ) values than M2, and at 250mM, M2 dominated. Firstly, in M1, the addition of salt was observed to peak (significantly) at 10mM, followed by a significant decline up to 250mM compared to the control. Whereas, for M2, the addition of 50mM salt decreased the  $\eta$  values to 0.848 Pa.s compared to the control sample (1.048 Pa.s). However, upon adding the highest salt concentration (250mM), the viscosity shot up to 2.092 Pa.s. This concludes that the addition of salt had a significant negative effect on the apparent viscosity of conventionally extracted mucilage (M1) while exhibiting a significant positive effect on the sonicated alkaline mucilage (M2).

The reduction in viscosity in M1 at increasing salt concentration could be due to the absence of intermolecular interaction (Koocheki et al., 2009). However, at a higher concentration, multivalent ions can facilitate interaction between chains, and thus the viscosity increases. Hence, explains the increase in the viscosity of M2 at 250mM salt concentrations.

It can be concluded that macerated (M1) and ultrasonicated (M2) CMS behave differently in the presence of salts due to the different extraction methods, with the usage of ultrasonication enabling CMS to display higher viscosity at higher salt concentrations. Hence, considering the interest of utilising CMS in the food industry as an emulsion stabiliser and fat replacer, in foods containing low salt concentrations (0-50mM), M1 CMS is better to be used due to its high  $\eta$ ,  $k$  and low  $n$ . However, for application in extreme salt concentrations (250mM), M2 behaves better as it is able to exhibit higher values and present a stronger shear-thinning effect with no significant effect on its yield stress values.

## CONCLUSION

The optimisation of uS ltrasound-assisted alkaline extraction significantly improved the extraction efficiency and functional properties of chia mucilage solution. Sonication temperature, extraction time, and pH collectively influenced the yield, colour, and viscosity of the mucilage, while the optimised sample demonstrated superior yield and hue angle

compared to the conventionally extracted sample. Rheological evaluation confirmed that the mucilage exhibited non-Newtonian pseudoplastic and shear-thinning behaviour, and its properties were significantly affected by sucrose and sodium chloride concentrations. The optimised mucilage showed greater rheological stability under higher co-solute concentrations, indicating its potential application as a sustainable food hydrocolloid in complex food systems. However, this study only evaluated the effects of sucrose and sodium chloride under controlled laboratory conditions, and the functionality of the mucilage was not assessed in actual food products or during storage. Therefore, future studies should investigate the performance of optimised chia mucilage in real food formulations, as well as evaluate the influence of other co-solutes, processing conditions, and storage stability on its physicochemical and rheological properties.

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