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Grewia mollis bark powder impact on the clarification of *Mbayeri* sorghum wort

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ABSTRACT

A study was undertaken on the clarity of *Mbayeri* sorghum wort. *Grewia mollis* has not, to our knowledge, been used to clarify sorghum wort. Therefore, it was described alongside malted and brewed *Mbayeri* sorghum from a physicochemical standpoint. *Grewia mollis* was incorporated into the design using a Box-Behnken model with three parameters (wort/*Grewia mollis* powder ratio, stirring speed, and stirring time) to clarify the wort. On the acquired worts' physicochemical studies, statistical and mathematical modeling was employed. Statistical methods such as bias and accuracy factors, among others, were utilized to validate the developed models. According to the results of the physicochemical investigation, *Mbayeri* sorghum was suitable for brewing. The same holds true for the classification of *Grewia mollis*, which was shown to be appropriate for the clarifying method. During mathematical modeling, it was determined that the ratio of wort to *Grewia*, stirring speed, and stirring time had a substantial effect on the selected physicochemical criteria (responses). Multi-response optimization performed according to a specified specification, which includes minimizing pH and titratable acidity and maximizing Brix, color, turbidity, while decreasing sugar, soluble protein, and polyphenol content, yielded the following results: pH, 5.2; turbidity, 224 NTU; brix, 12.97 °P, colour, 46 ASBC; reducing sugars, 94.03 mg/mL; soluble protein, 502.42 mg/L; polyphenols, 54.21 mg/L; and titratable acidity, 1.2 g/L H_2T. The optimal blend of 0.25 g/L *Grewia mollis* powder, 45 rpm, and 60 minutes yielded this result. These characteristics suggest that *Grewia mollis* could be used as a brewing filter assist.

1. Introduction

In 2020, Cameroon produced approximately 1,215,377 t of sorghum out of a total of 3,733,377 t of cereal (FAOSTAT, 2022). It represented almost one-third of the nation's cereal output. Sorghum is the primary cereal ingested by the majority of people in northern Cameroon, who frequently endure recurrent famines (Desobgo et al., 2011; Desobgo and Nso, 2013). Bread, couscous, dumplings, fermented and unfermented porridges were typical sorghum-based cuisines in Africa and Cameroon. The production of this crop has quadrupled over the past two decades, from 420,000 t in 2000 to 1,217,377 t in 2020 (FAOSTAT, 2022). This increasing demand is attributable to Bili-increased Bili's output. It was the ideal grain for brewing traditional African brews and was regarded as Africa's grain of the 21st century (Taylor, 2003). Bili-Bili is a typical Cameroonian beer manufactured and drank primarily in the country's northern region. In the competitive environment of multinational corporations, sorghum was the best substitute for malted barley when producing beer (Davana and Revanna, 2021). Unlike barley, however, the absence of straw in sorghum grain was until recently seen as a significant obstacle to the use of sorghum in the production of light and lager beers. This indicates the difficulty conventional brewers have in clarifying this beer (Goode et al., 2002; Goode and Arendt, 2003; Nso et al., 2003). Due to its foggy look, *Bili-Bili* was sometimes referred to as an opaque beer. Typically, filtering agents such as Irish moss, isinglass, gelatin, and fish glue were added to beer to diminish its cloudiness. Their price may be a constraint. *Grewia mollis*, a naturally occurring coagulant/flocculant that has already been utilized for water purification, was a less expensive, more practical, and more accessible choice for traditional and industrial brewers (Ngounou et al., 2021). *Grewia mollis*, a common shrub or tree in the Sudano-Sahelian region, is also present in Cameroon and Nigeria. Some regional recipes call for the dried and pulverized inner bark of the stem as a thickener (Muazu et al., 2009). Particularly in the Adamawa region of Cameroon, the powder is used as a binder in the preparation of fried maize cakes. In Nigeria, it has been used to produce soups and Hausa-named native pastries called "Kosai" by crushing it and combining it with bean flour (Emeje et al., 2008). Mucilage, a naturally occurring polysaccharide, has been associated with the functional properties of *Grewia* powder (Nep and Conway, 2011). Since the eventual goal is to replace existing clarifying agents, it would be best to use the powder as-is initially, assuming it does not pose any issues

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during wort treatment. Production, quality, structure, and bioactive properties of plant polysaccharides can vary depending on the extraction method employed (Chen et al., 2018; Ghori et al., 2017). Determining the operating conditions for the use of *Grewia mollis* powders in clarifying and evaluating its effect on some physicochemical properties of *Mbayeri* sorghum wort was the purpose of this study.

2. Material and methods

2.1. Biological material

In January 2022, the *Grewia mollis* bark was acquired in the northern region of Cameroon, specifically in Guider's town, while the *Mbayeri* sorghum cultivar was purchased at the little market of the city of Ngaoundere.

2.2. Grewia mollis characterization

Utilizing a procedure developed by the Association of Official Analytical Chemists, the moisture, ash, fat, total soluble solids, and protein levels were determined (AOAC, 2006).

The total phenolic content was calculated using the Folin-Ciocalteu reagent (Meda et al., 2005). A methanol/water solution (1:1 v/v) was used to create samples, which were then filtered through a 0.45 m disc filter at a concentration of 0.05 g/mL. In a ratio of 1:25:25, the filtrate was mixed with sodium carbonate solution (75 mg/mL) and Folin-Ciocalteu reagent solution (1:10 v/v). After one hour of incubation at room temperature in the dark, absorbance was measured using a spectrophotometer (Spectro UV-VIS Dual Beam UVS-2800, Labomed, Inc., USA) at a wavelength of 760 nm. Various amounts of gallic acid were utilized to produce a standard curve with detection and quantification limits of 6.38 and 19.34 mg/L, or almost 14.0 to 112.0 mg/L.

2.3. Mbayeri sorghum characterization

Before malting, the grain was subjected to a number of physicochemical studies, including those for moisture content, germination capability, germination energy, thousand-corn weight, and protein content. All of these analyses were conducted using the standard ASBC technique (ASBC, 2009).

2.4. Sorghum malting

One kilogram of the sorghum cultivar's grain was washed three times with three liters of distilled water to eliminate dust and contaminants. The grains were steeped in 3 L of distilled water at room temperature (25°C) for 48 hours with three 12-h water changes. Germination was conducted in Heraeus D-63450 (Kendro laboratory product, Hanau, Germany) for four days at a temperature of 25°C, with watering every six hours. The malt was subsequently dried for four days at 40°C with the CKA 2000 AUF dryer. The malted sorghum was then removed of its rootlets and stored.

2.5. Mbayeri sorghum brewing

The malted sorghum was milled to a 0.7 mm thickness. Five kilograms of flour were weighed and added to a Braumeister along with twenty liters of 45°C water. For 30 min during the proteolytic phase, the medium was agitated at this temperature to prevent floc formation. Next, the starch was gelatinized by draining the supernatant (15 L) and heating the remaining material at 95-98°C for 40 min on a gas plate. Following gelatinization and cooling to 60-65°C, the amylolytic stage commenced, and the supernatant and gelatinized starch were reintroduced into the Braumeister at 65°C. At this temperature, stirring lasted 1 h and thirty minutes. The following process was saccharification, which included raising the temperature to 72°C for one hour. The mash was finally mixed and chilled to 25°C.

Table 1
Range of different factors

Factors	Interval of factors
Mass/volume ratio	1/1000 – 1/10000
Stirring time (min)	5 – 60
Stirring speed (rpm)	0 – 200

2.6. Clarification methods

2.6.1. Hot test

The cloudy wort was added to five jars for the coagulation/flocculation experiments, which were then carried out in a 100°C water bath. The mixture was stirred using a spatula after the powdered *Grewia mollis* bark was added. The jars were removed from the water bath after 10 min, and turbidity measurements were taken every five minutes.

2.6.2. Cold test

In a subsequent phase, before adding the bark, a Jar test agitator was employed to conduct the cold test.

2.7. Wort characterisation

The extract, titratable acidity, pH, polyphenols, soluble proteins, color, and turbidity were all assessed using standard ASBC techniques (ASBC, 2009). This equation was utilized to determine the efficacy of turbidity elimination:

$$\%Turbidity = \frac{Turb_i - Turb_f}{Turb_i} \times 100 \quad (1)$$

Where $Turb_i$ and $Turb_f$ are the initial and final turbidity (EBC), respectively.

2.8. Experimental design

When constructing an experimental design (Table 1), the mass/volume ratio, stirring time, and stirring speed were evaluated in order to investigate the interaction between the various parameters (these factors were chosen based on the preliminary tests).

2.9. Choice of experimental responses

The experimental responses used were Brix, pH, reducing sugar concentration, soluble protein content, total polyphenol content, and titratable acidity. These seven reactions served as criteria for evaluating the effect of *Grewia mollis* on the quality of the worts. Table 2 displays the experiment matrix. Three factors comprised the experimental matrix of a Box Behnken response surface design: mass-to-volume ratio (x1), stirring time (x2), and stirring speed (x3).

2.10. Modeling

The criteria for selection were those that promoted flocculation/coagulation of suspended particles and aided in wort clarifying. Adjustments were made using a Box-Behnken experimental design with three parameters. The experimental design represented in the matrix was the result of the selected factors. Physical and biological relevance led to the selection of these components. In actuality, time was an essential part of clarity, since it permitted the largest extraction yields possible within a given time limit. The rate of agitation would maximize the polymer extraction from *Grewia mollis* bark. Using the mass of *Grewia mollis* powder, we would be able to establish the optimal quantity for enhancing the clarity of a volume of wort.

The subsequent mathematical models took into account the coded variables (Eq. 2). These mathematical models employed polynomials

Table 2
Experience matrix

Experiences Order of trials	Factors Mass/volume ratio (m/v) X_1		Stirring speed (rpm) X_2		Stirring time (min) X_3	
	Coded variable	Real variable	Coded variable	Real variable	Coded variable	Real variable
1	0	1/5500	-1	0	-1	5
2	1	1/10000	0	100	1	60
3	1	1/10000	1	200	0	32.5
4	0	1/5500	1	200	-1	5
5	1	1/10000	0	100	-1	5
6	1	1/10000	-1	0	0	32.5
7	-1	1/1000	0	100	1	60
8	0	1/5500	0	100	0	32.5
9	0	1/5500	1	200	1	60
10	-1	1/1000	-1	0	0	32.5
11	0	1/5500	0	100	0	32.5
12	0	1/5500	0	200	0	32.5
13	0	1/5500	-1	0	1	60
14	-1	1/1000	0	100	-1	5
15	-1	1/1000	1	200	0	32.5

with several variables. The model's factors consisted of first-degree factors (x_1, x_2, and x_3), second-degree factors (x_1^2, x_2^2, and x_3^2), and interactions (x_1x_2, x_1x_3 and x_2x_3). If the probability (p) was less than or greater than 0.05, these factors were deemed statistically significant.

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum \sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

With β_0: the constant, ε: the error, and the βj βjj and βij were the coefficients of the model and y: the response.

The model's validity was determined by comparing theoretical and observed values. Ross, (1996) provided the following equations for the polarisation factor, Bf, and the polarized accuracy factor, Af1:

$$B_f = 10^{\frac{1}{n}\sum_{i=1}^{n} \log\left(\frac{y_{i,cal}}{y_{i,\exp}}\right)} \quad (3)$$

$$A_{f1} = 10^{\frac{1}{n}\sum_{i=1}^{n} \left|\log\left(\frac{y_{i,cal}}{y_{i,\exp}}\right)\right|} \quad (4)$$

The perfect predictive model leads to: $Af_1 = B_f = 1$. The acceptable predictive model was: $0.75 < B_f$ or $Af_1 < 1.25$ (Dalgaard and Jørgensen, 1998). The software Minitab 21 was used to generate the models and the statistics, while OriginLab 2022 was used to plot the graphs.

2.11. Optimization

This optimization aimed to find a satisfactory compromise for each response. Consequently, the objective was to locate the combination that matched all of the criteria needs adequately. The objectives of this specification were to obtain a wort that could ferment rapidly, to have wort with standard hues, and to benefit from the antioxidant impact of the polyphenols while minimizing turbidity, so reducing the wort's opacity and thereby clarifying it. After establishing these conditions, the Minitab 21 software was utilized to identify the optimal triplet that satisfied them. Using the same software, the theoretical results for each of the four responses were determined. Utilizing the optimal theoretical combination, the wort was clarified. The powder of *Grewia mollis* and the wort were placed in the freezer for further analysis to ascertain the wort's characteristics. Prior to this clarification, the wort was brought to room temperature (25°C). The protein content, color, polyphenol content, and turbidity were evaluated physicochemically. In addition, the content of extract, titratable acidity, pH, and reducing sugar was assessed.

Table 3
Physicochemical characteristics of the bark of *Grewia mollis* and viability test of unmalted *Mbayeri* sorghum

Chemical composition	bark (*Grewia mollis*)	*Mbayeri* sorghum
Moisture (%)	9.8 ±0.1	8.1 ± 0.0
Ash (%)	7.4 ±0.1	/
Proteins (%)	12.6 ±0.1	12.2 ± 0.6
Lipids (%)	1.9 ±0.7	/
Total sugars (%)	35.3 ±0.1	/
Total polyphenols (%)	17.2±0.1	/
Germinative capacity (%)	/	98 ± 0.1
Germinative energy (4 mL) (%)	/	98.4 ± 1.1
Germinative energy (8 mL) (%)	/	97 ± 0.9
Thousand corn weight (g)	/	43.3 ± 0.1

3. Results and discussion

3.1. Proximate analysis of Grewia mollis and viability test of Mbayeri sorghum cultivar

The chemical composition of *Grewia mollis* powder, expressed as a percentage of dry matter, is shown in Table 3. Water, ash, protein, lipids, total sugars, and polyphenols were 9.8 ± 0.1%, 7.4 ± 0.13%, 12.6 ± 0.11%, 1.9 ± 0.7%, 35.3 ± 0.10%, and 17.8 ± 0.11%, respectively, according to this table. All of these data fell within the ranges specified in the literature for a variety of *Grewia* species: 6.30-8.71% for ash, 12.91-18.8 % for protein, 2.64-3.86% for lipids, and 28.6-40.1% for total sugars (Muhammad et al., 2021; Nep and Conway, 2011; Panyoo et al., 2014; Pradip, 2020). The total polyphenols content achieved was greater than what Zia-Ul-Haq et al., (2013) found, which was between 0.95 to 2.05%. This could be attributable to the species or the geographical location. The powdered bark had a low moisture level and was composed of sugars and other substances. High levels of total sugars in the sample of bark suggested the existence of polysaccharide gum. Due to the amount of sugars, Panyoo et al. (2014) were driven to extract the polysaccharide-rich *Grewia* gum. *Grewia mollis* gum included the neutral monosaccharide carbohydrates glucose, rhamnose, galactose, arabinose, and xylose (Nep and Conway, 2011; Panyoo et al., 2014). The protein content was ordinary whereas the ash amount was high. This was likewise noted by Panyoo et al., (2014).

The moisture level of unmalted *Mbayeri* sorghum, which was 8.12 ± 0.02%, was within the acceptable limit for grain preservation, as shown in Table 3. In fact, this figure should go below or equal 13% (Hough et al., 2012). Germinative capacity, germinative energy (4 mL),

Table 4
Summary of the physicochemical characteristics of the malted *Mbayeri* wort after mashing

Characteristics	Wort
Extract (°P)	12.9 ± 0.6
Turbidity (EBC)	356 ± 2
Turbidity (NTU)	1463 ± 1
Colour (ASBC)	49.1 ± 0.5
Reducing sugars (mg/mL)	108.2 ± 1.3
Soluble protein (mg/L)	548.5 ± 0.9
Polyphenols (mg/L)	61.5 ± 9.2
pH	4.4 ± 0.4
Titratable acidity (meq g/100 g DM H_2T)	1.5± 0.5

and germination energy (8 mL), with respective values of 98 ± 0.15 %, 98.41 ± 1.15 %, and 97 ± 0.96 %, were within the EBC-Analysis-Committee, (1998). Therefore, the grains were suitable for malting. The weight of 1000 grains, 43.36 ± 0.13 g, fell within the range determined by Desobgo et al. (2013). Protein content was 12.26 ± 0.57 %. According to these data, *Mbayeri* sorghum is suitable for malting and brewing.

3.2. Physicochemical characteristics of the wort after mashing

The observed extract in Table 4 was 12.9 ± 0.60 °P. Indeed, it was the most important factor to consider when determining the brewing capability of wort (Briggs et al., 2004). Because they are the principal source of energy for yeast metabolism, reducing sugars (108.2 ± 1.3 mg/mL) were required for alcoholic fermentation in brewing. Moreover, proteins are important to a successful fermentation process. Therefore, the protein level was crucial not only for the fermenting capacity of the yeast, but also for the flavor of the beer. The *Mbayeri* wort was appropriate for fermentation into sorghum beer, according to Table 4. It was essential to take note of the extremely high turbidity value and confirm the opacity of the sorghum wort as mentioned in the literature (Embashu et al., 2019; Kayode et al., 2011). Therefore, it was vital that the wort be clarified.

3.3. Wort clarification using Grewia mollis

3.3.1. During wort boiling

Molecular instability of polysaccharides, possibly by hydrolysis, at this temperature (100°C) would account for the observed increase in Plato, turbidity, color, reducing sugars, soluble proteins, and polyphenols with increasing concentrations of *Grewia mollis* or decreasing ratios (Table 5). In addition, *Grewia mollis* gums would experience a mass loss between 30 and 140°C. This may be attributable to the loss of structural and adsorbed water from biopolymers (Kittur et al., 2002; Vendruscolo et al., 2009) or to the desorption of structural water from polysaccharides. The results preclude the use of *Grewia mollis* during the boiling of wort. The decline in sorption capacity as temperature rises can be rationalized as follows: Athletic activities were exothermic. Consequently, an increase in temperature should diminish sorption. The solubility of the solute changes as the temperature changes. At elevated temperatures, the size of the sorbent decreases, resulting in a less porous sorbent with fewer bonding sites, which decreases the distribution ratio values (Alessandro, 2001).

3.3.2. After wort boiling

Table 6 contains the physicochemical analyses of *Mbayeri* wort purified with *Grewia mollis* bark powder. The response surface methodology was used to statistically model the following physicochemical characteristics: color (ASBC), soluble protein content (mg/L), polyphenol content (mg/L), and turbidity (EBC). As indicated in Table 7, the mathematical models obtained were all second-order with interactions and validated (Dalgaard and Jørgensen, 1998). Additionally, a factor was considered important if its probability was less than 0.05 (Table 8).

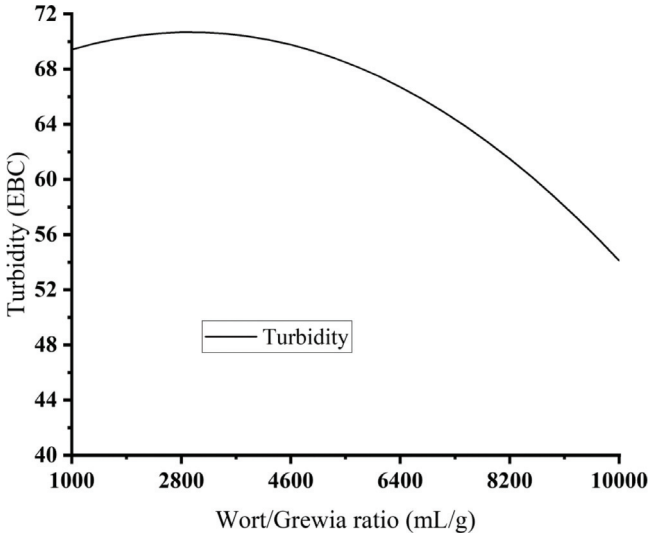

Fig. 1. Evolution of the turbidity as a function of wort/*Grewia* ratio (Stirring speed and stirring time fixed at 0 rpm and 5 min)

3.4. Effect of singular contribution

3.4.1. Effect of wort/Grewia mollis ratio (x_1)

The ratio of wort volume to *Grewia mollis* mass (x1) has only a significant influence on wort turbidity (whose model equation is documented in Table 7) and the quadratic effect (P = 0.031, Table 8). Fig. 1 demonstrates that wort turbidity decreases with increasing dilution (with decreasing *Grewia mollis*). Delelegn et al. (2018) also made this observation when treating river water with *Moringa* seed powder. With a high concentration of Moringa grain powder, the turbidity of river water did rise. The wort's turbidity was a measurement of the suspended colloidal particles. The coagulation/flocculation phenomenon could not occur if the stirring speed was zero and the time was five minutes. The immediate result was that the solid particles of *Grewia mollis* powder stayed suspended in the wort, hence increasing the turbidity.

3.4.2. Effect of stirring speed (x_2)

Mbayeri sorghum worts' turbidity, color, polyphenols, and proteins are strongly affected by the stirring speed (x2) (Table 8). Each model is described in Table 9. In fact, a decline in each of these physicochemical properties was found as stirring speed increased (Fig. 2).

Increasing the agitation speed would produce flocs from the *Grewia* powder and wort haze. *Grewia* biopolymer may favor adsorption and bridging effects, promote floc's tightly packed aggregate structure, and accelerate floc settling due to its large molecular mass (Li et al., 2006; Niu et al., 2013). The outcome was a reduction in turbidity.

When *Grewia* powder was added to the wort and the stirring speed was increased, the proteins would have generated charges similar to magnets, attracting mostly oppositely charged particles (Schwarz, 2001). The flocculation process happened when these proteins bound the charges and the wort particles collected into flocs. Due to the high molecular weight of *Grewia* gum, the flocs settled, reducing the amount of protein in the cleared wort.

Hot trub contained insoluble, denaturated proteins, complex polysaccharides, lipids, tannins, polyphenols, and various other minerals (Kühbeck et al., 2006). The rate of stirring facilitates contact between the haze and the flocculating ingredients in the *Grewia* powder. The decrease in polyphenols in cleared wort is justifiable given that they permit the settling of cloudiness.

Depending on the form and size of the molecules, phenolic chemicals affect the beer's color, flavor, froth, and chemical-physical stability. They are present in both the aleuronic layer and grain chaff. When

Table 5
Physicochemical characteristics of malted *Mbayeri* wort after introduction of *Grewia mollis* during boiling

Wort/*Grewia mollis* ratio (mL/g)	°Plato	Turbidity (EBC)	Colour (ASBC)	Reducing sugar (mg/L)	Soluble protein (mg/L)	Polyphenols (mg/mL)
500/1	14.5±0.6	1421±1	86.52±0.80	126.21±0.40	569.02±2.00	64.24±5.10
1000/1	14.1±0.2	954±0	77.11±0.30	114.04±0.70	551.15±0.80	61.72±3.70
5000/1	13.2±0.2	120±0	71.20±0.40	102.11±0.40	478.21±0.50	58.54±8.60
10000/1	12.2±0.3	98±1	58.14±0.70	103.26±0.40	428.43±0.60	51.24±1.20

Table 6
Physicochemical analysis of malted *Mbayeri* wort after clarification using *Grewia mollis*

x1	x2	x3	Colour (ASBC)	Protein (mg/L)	Polyphenols (mg/mL)	Turbidité (EBC)
0	-1	-1	56.8	479.5	39.5	70.61
1	0	1	58.1	490.8	40.8	55.36
1	1	0	67.7	501.4	50.4	55.34
0	1	-1	52.6	485.3	41.23	47.85
1	0	-1	57.6	491.3	41.3	59.73
1	-1	0	59.9	491.6	42.6	49.97
-1	0	1	58.8	491.5	41.5	48.51
0	0	0	68.1	500.8	50.72	70.35
0	1	1	57.1	489.8	39.8	48.45
-1	-1	0	68.6	501.3	51.3	69.50
0	0	0	67.4	502	50.1	72.73
0	0	0	64.7	499.4	49.4	68.57
0	-1	1	52.5	485.2	35.2	47.96
-1	0	-1	58	490.7	40.7	56.09
-1	1	0	59.5	493.2	42.2	48.60

Table 7
Mathematical models and validation criteria

Equations	R^2	Af1	Bf
$Y_{Colour}(ASBC) = 66.733 - 0.2x_1 - 0.112x_2 + 0.188x_3 + 0.283x_1^2 - 3.092x_2^2 - 8.892x_3^2 + 4.225x_1x_2 - 0.075x_1x_3 + 2.2x_2x_3$ (5)	0.9832	1.014	1.002
$Y_{Turbidity}(EBC) = 70.55 - 0.29x_1 - 4.73x_2 - 4.25x_3 - 6.75x_1^2 - 7.95x_2^2 - 8.88x_3^2 + 6.57x_1x_2 + 0.8x_1x_3 + 5.81x_2x_3$ (6)	0.9287	1.040	1.004
$Y_{proteins} = 500.73 - 0.2x_1 + 1.512x_2 + 1.312x_3 + 1.13x_1^2 - 4.99x_2^2 - 10.79x_3^2 + 4.47x_1x_2 - 0.33x_1x_3 - 0.3x_2x_3$ (7)	0.9611	1.022	1.001
$Y_{Polyphenols}(mg/L) = 50.073 - 0.075x_1 + 0.629x_2 - 0.679x_3 - 0.653x_1^2 - 2.795x_2^2 - 8.345x_3^2 + 4.225x_1x_2 - 0.325x_1x_3 + 0.717x_2x_3$ (8)	0.9654	1.021	1.002

Table 8
Significance of factors

	Probability			
	Colour	Turbidity	Protein	Polyphenols
x_1	0.653	0.859	0.812	0.899
x_2	0.799	**0.028**	0.116	0.315
x_3	0.673	**0.040**	0.160	0.283
x_1^2	0.665	**0.031**	0.378	0.467
x_2^2	**0.004**	**0.017**	**0.008**	**0.020**
x_3^2	**0.000**	**0.011**	**0.000**	**0.000**
x_1x_2	**0.001**	**0.030**	**0.011**	**0.003**
x_1x_3	0.904	0.728	0.784	0.700
x_2x_3	**0.014**	**0.044**	0.800	0.409

amino acids and fermentable carbohydrates react, pigmented molecules called melanoidin are produced. Together with polyphenols, these compounds determine the final hue of wort and beer (Hodzić et al., 2007).

Grewia mollis powder, which aided in haze decantation by increasing stirring speed, decreased wort color due to the complexation of proteins that create haze by polyphenols.

3.4.3. *Effect of stirring time (x_3)*

The stirring time (x3) has a major influence on the turbidity, color, polyphenols, and proteins in *Mbayeri* sorghum worts (Table 8). All models are displayed in Table 7. A decline in these physicochemical properties was noticed when stirring duration increased (Fig. 3).

Regarding turbidity reduction, the clarification process was effective. The absorption was generated by the diffusion of wort components onto the surface of the sorbent. The extremely porous nature of sorbents (Osemeahon et al., 2016) and the particle size (powder) provide a large surface area for the sorption of wort components on the binding sites. All other components associated with the cloudiness of the worts (proteins, polyphenols, and color) underwent the same process and were adsorbed on the *Grewia mollis* powder.

Table 9
Physicochemical characteristics of optimized malted *Mbayeri* wort

Characteristics	Theoretical values (From models)	Experimental values
Colour (ASBC)	46	48.5±1.5
Turbidity (EBC)	56	57.6±2.2
Soluble protein (mg/L)	502.42	511±4
Polyphenols (mg/L)	54.21	52.3±2.9
Reducing sugars (mg/mL)	/	98.7±1.8
Titratable acidity (g/L)	/	1.2±0.1
pH	/	5.3±0.3
Plato (°P)	/	13.2±0.7

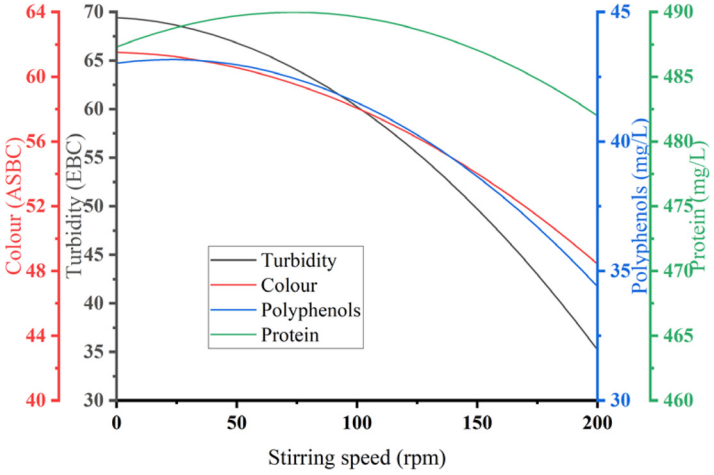

Fig. 2. Evolution of turbidity, colour, polyphenols and protein as a function of stirring speed (ratio wort/*Grewia* and stirring time fixed respectively at 1000 mL/g and 5 min)

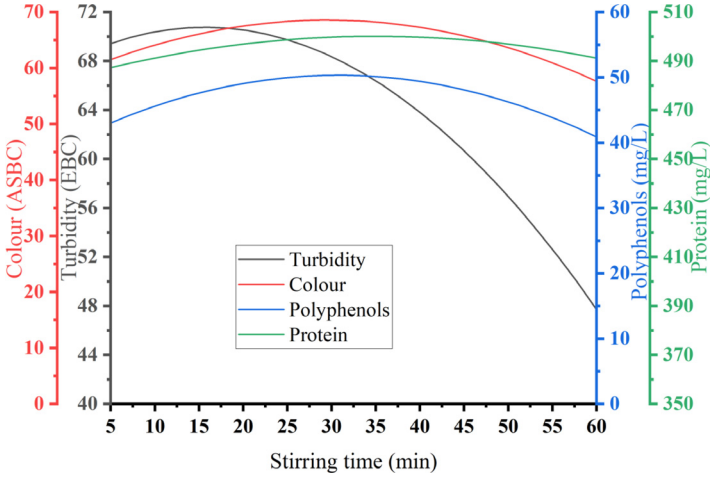

Fig. 3. Evolution of turbidity, colour, polyphenols and protein as a function of stirring time (ratio wort/*Grewia* and stirring speed fixed respectively at 1000 mL/g and 0 rpm)

It was required to allow sufficient time for the formation of particles large enough to be removed efficiently during the sedimentation process. In every clarifying process, including coagulation-flocculation activities, the time of macro flocs generation was a critical operating component (Wang et al., 2005).

3.5. Effect of interaction contribution

3.5.1. Effect of interaction wort/Grewia mollis ratio and stirring speed (x_1x_3)

The interaction x1 × 3 has a substantial effect on the turbidity, hue, polyphenols, and proteins of *Mbayeri* sorghum worts (Table 8).

For polyphenols content (Fig. 4), an increase in wort was noted with an increase in *Grewia mollis* content (from 10,000 mL/g to 1,000 mL/g, which corresponds to 0.1 g/L to 1 g/L of *Grewia mollis* concentration) at stirring speeds ranging from 0 to 100 rpm. In addition, an increase in polyphenols and a decrease in *Grewia mollis* between 100 and 200 rpm. This could be attributed to the low agitation speed (0 to 100 rpm) during the first phase, which prevented the fixation of polyphenols on *Grewia mollis* powders. The increase in polyphenols would originate from the raw *Grewia mollis* powder, which includes polyphenols (Sambo et al., 2015) and would therefore bring some along with it. For the second phase, agitation speeds between 100 and 200 rpm would increase the exchange surface of the *Grewia mollis* powders, resulting in enhanced polyphenol adsorption and subsequent removal during decantation. Due to its high viscosity, *Grewia mollis* powder including gum will also blend more quickly. Consequently, the gum flocs were more stable. Perng and Bui, (2015) made this discovery while investigating the effect of agitation speed on the ability of reactive dyeing wastewater to decolorize Cassia fistula gum.

The same pattern was noted for color (Fig. 4). In fact, polyphenols were principally responsible for the color of sorghum worts, which resulted from the Maillard reaction triggered by kilning and wort boiling. Therefore, the same explanations for polyphenols would be relevant.

The protein trend was comparable to the hue (Fig. 4). In fact, the polyphenol/protein complex contributed to the cloudiness of the wort (Briggs, 1998; Kühbeck et al., 2006). Additionally, the hue was due to the polyphenols (Hough et al., 2012). Therefore, the complex would have the same adsorption as the color.

With increased stirring speed and a decrease in the wort/*Grewia mollis* ratio, turbidity decreased (Fig. 4). The synergy between these two forces allowed for a decrease in turbidity. In fact, the agitation speed would aid in the expansion of the exchange surface. Simultaneously, the fall in *Grewia mollis* amount would reduce steric hindrance and allow the adsorption of wort chemicals at all active sites (Perng and Bui, 2015).

3.5.2. Effect of interaction stirring speed and stirring time (x_2x_3)

Fig. 5 demonstrates that the turbidity and color of the worts drop dramatically (Table 8) as agitation speed and time increase. This is due to the fact that quick mixing was used in the coagulation process to spread the coagulant throughout the cloudy solution (Saritha et al., 2017). It is believed that the agitation speed is responsible for this synergistic effect, since it helps to expose the majority or all of the active sites in the *Grewia mollis* powder, so making the turbidity easier to absorb. In contrast, the agitation time maximizes the absorption of the turbidity components, including the polyphenols, and consequently the color.

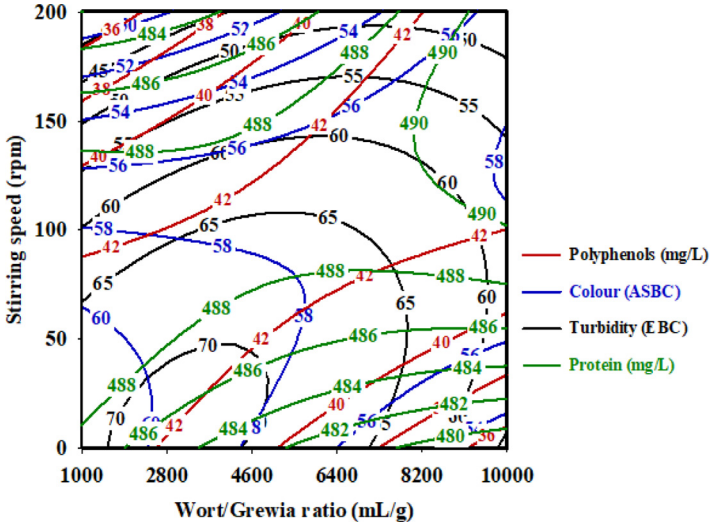

Fig. 4. Evolution of turbidity, colour, polyphenols and protein as a function of wort/Grewia ratio and stirring speed (stirring time fixed at 5 min)

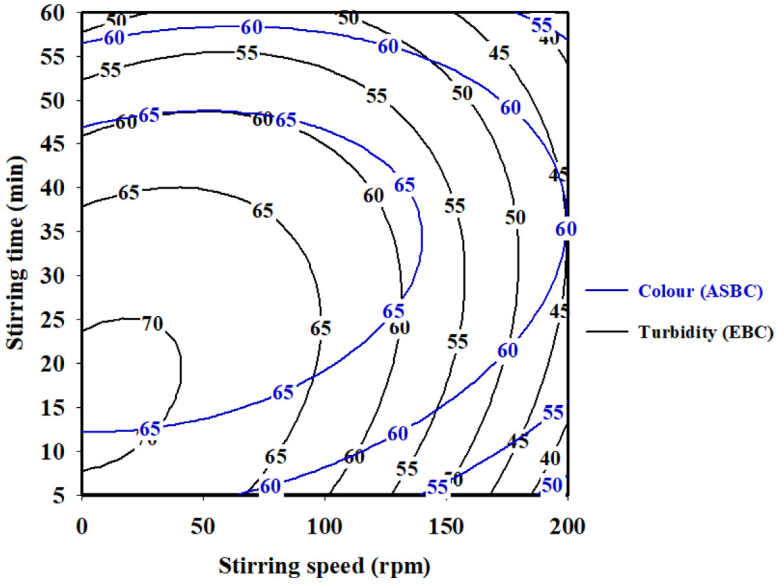

Fig. 5. Evolution of turbidity and colour as a function of stirring speed and stirring time (wort/Grewia ratio fixed at 1000 mL/g meaning 1 g/L)

According to other sources, frequent and gentle stirring promotes the formation and consolidation of flocs (Ndabigengesere et al., 1995).

3.6. Optimization

The following optimal trio was obtained via a multi-response optimization criterion in order to increase color, protein, and polyphenol content while limiting turbidity in order to produce an appetizing wort. The wort-to-*Grewia mollis* ratio was 4000 mL/g (0.25 g/L of *Grewia mollis*), and the stirring rate and duration were 45 rpm for 60 minutes. *Grewia* concentrations of 0.25 g/L were within the range reported in the literature when Moringa oleifera was used to clear various substrates (Villaseñor-Basulto et al., 2018). In addition, *Grewia*'s figure of 45 rpm fell within the range (40 rpm) determined by Arnoldsson et al. (2008) for Moringa water treatment using the jar test. Nevertheless, the optimal duration was longer than the 17-min estimate provided by the same authors. This may be due to the dissimilar nature of the suspended solids that should coagulate or flocculate. Table 9 displays the theoretical physicochemical attributes that came from this optimal theoretical value. After laboratory confirmation of these data, it was determined that the range of deviations from the theoretical results for turbidity, color, soluble proteins, and polyphenols were 2.85 %, 5.43 %, 1.70 %, and 3.55 %, respectively. As a result, theoretically optimal conditions for clarifying *Mbayeri* malt wort could be validated.

A comparison of the properties of cleared wort and starting wort revealed that, under optimal conditions, only turbidity fell significantly by 84.27 percent (Tables 9 and 4). This result falls within the range of the relevant literature. Guar gum was used to cleanse water, and turbidity was reduced by 88.1% (Mukherjee et al., 2013), whereas *Grewia* gum eradicated chromium from 47% to 98% (Kofa et al., 2019). The other characteristics did not considerably change. As a result, *Grewia mollis* powder substantially enhanced the clarity of wort by eliminating cloudiness.

4. Conclusion

Grewia mollis, a shrub native to the Adamawa region of Cameroon, was used to clarify and reduce the opacity of sorghum wort. Characterization of this substance revealed the existence of components that may allow this clarity to be achieved with a high sugar concentration, indicating the polysaccharide nature of the gum. The opacity (356 EBC) and the need for clarification were validated by the physicochemical parameters of the generated mash. The viability characteristics of *Mbayeri* sorghum permitted malting of this crop. Mathematical modeling re-

vealed the major impact of clarification-related elements (wort/*Grewia* ratio, stirring time, and speed, among others) on the wort's particular physicochemical features (turbidity, among others). With the parameters tuned, a turbidity reduction of approximately 84% was possible. This demonstrated the efficacy of *Grewia mollis* in treating opaque sorghum wort due to the absence of other nutrient leaching. This study emphasized opacity concerns and the possibilities for clarifying wort with inexpensive natural coagulants.

Ethical Statement

The authors declare that the study does not involve animals and humans

Declaration of Competing Interest

The authors declare that they have no conflict of interest

Data Availability

No data was used for the research described in the article.

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References

Alessandro, D. S. (2001). Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. A review. *Journal of Physical and Chemical Reference Data, 30*, 187.

AOAC. (2006). *Official methods of analysis of AOAC (Association of Official Analytical Chemists) International* (18 th Edn.). Gaithersburg, USA: Association of Official Analytical Chemists.

Arnoldsson, E., Bergman, M., Matsinhe, N., & Persson, K. M. (2008). Assessment of drinking water treatment using Moringa oleifera natural coagulant. *Vatten, 64*, 137.

ASBC. (2009). *Methods of analysis* (2009th ed.). St. Paul, MN: American Society of Brewing Chemists Inc..

Briggs, D. E. (1998). *Malts and malting* (1st ed.). London: Blackie Academic.

Briggs, D. E., Boulton, C. A., Brookes, P. A., & Stevens, R. (2004). Brewing: Science and practice. *Brewing: Science and practice* (1st ed). Elsevier Ltd.

Chen, G., Chen, K., Zhang, R., Chen, X., Hu, P., & Kan, J. (2018). Polysaccharides from bamboo shoots processing by-products: New insight into extraction and characterization. *Food Chemistry, 245*, 1113–1123.

Dalgaard, P., & Jørgensen, L. V. (1998). Predicted and observed growth of Listeria monocytogenes in seafood challenge tests and in naturally contaminated cold-smoked salmon. *International Journal of Food Microbiology, 40*, 105–115.

Davana, T. v., & Revanna, M. L. (2021). Development of beer from malted sorghum (Sorghum bicolor). *International Journal of Chemical Studies, 9*, 330–334.

Delelegn, A., Sahile, S., & Husen, A. (2018). Water purification and antibacterial efficacy of Moringa oleifera Lam. *Agriculture and Food Security, 7*, 1–10.

Desobgo, Z. S. C., Naponni, F. Y., & Nso, E. J. (2013). Caractérisation des moûts et bières du sorgho Safrari houblonnés avec Vernonia amygdalina et Nauclea diderrichii. *Int. J. Innov. Appl., 2*, 83–91.

Desobgo, Z. S. C., Nso, E. J., & Tenin, D. (2011). Use of the response surface methodology for optimizing the action of mashing enzymes on wort reducing sugars of the Madjeru sorghum cultivar. *African Journal of Food Science, 5*, 91–99.

Desobgo, Z. S. C., & Nso, J. E. (2013). Optimization of the Impact of Hitempase 2XL, Bioglucanase TX and Brewers Protease on the Turbidity of Madjeru sorghum cultivar wort. *International Journal of Applied Research & Studies, 2*, 1–10.

EBC-Analysis-Committee. (1998). *Analytica-EBC, The European reference methods for breweries by EBC*. Fachverlag Hans Carl.

Embashu, W., Iileka, O., & Nantanga, K. K. M. (2019). Namibian opaque beer: A review. *Journal of the Institute of Brewing, 125*, 4–9.

Emeje, M., Isimi, C., & Kunle, O. (2008). Effect of *Grewia* gum on the mechanical properties of Paracetamol tablet formulations. *African Journal of Pharmacy and Pharmacology, 2*, 1–6.

FAOSTAT, 2022. Crops and livestock products. (available at https://www.fao.org/faostat/fr/#data/QCL, accessed 21 April 2022).

Ghori, M. U., Mohammad, M. A., Rudrangi, S. R. S., Fleming, L. T., Merchant, H. A., Smith, A. M., & Conway, B. R. (2017). Impact of purification on physicochemical, surface and functional properties of okra biopolymer. *Food Hydrocolloids, 71*, 311–320.

Goode, D. L., & Arendt, E. K. (2003). Pilot Scale Production of a Lager Beer from a Grist Containing 50 % Unmalted Sorghum. *Journal of the Institute of Brewing, 109*, 208–217.

Goode, D. L., Halbert, C., & Arendt, E. K. (2002). Mashing Studies with Unmalted Sorghum and Malted Barley. *Journal of the Institute of Brewing, 108*, 465–473.

Hodzić, Z., Karahmetović, A., Saletović, M., & Šestan, A. (2007). Relationship between total polyphenols and colour values of wort and beer. *Journal of Engineering, 1*, 1584–2665.

Hough, J. S., Briggs, D. E., Stevens, R., & Young, T. W. (2012). *Malting and brewing science: volume II hopped wort and beer*. Springer.

Kayode, A. P. P., Vieira-Dalodé, G., Linnemann, A. R., Kotchoni, S. O., Hounhouigan, A. J. D., van Boekel, M., & Nout, M. J. R. (2011). Diversity of yeasts involved in the fermentation of tchoukoutou, an opaque sorghum beer from Benin. *African Journal of Microbiology Research, 5*, 2737–2742.

Kittur, F. S., Harish Prashanth, K. v., Udaya Sankar, K., & Tharanathan, R. N. (2002). Characterization of chitin, chitosan and their carboxymethyl derivatives by differential scanning calorimetry. *Carbohydrate Polymers, 49*, 185–193.

Kofa, G. P., Nkoue Ndongo, G. R., Ngounou, K., Nsoe, M. N., Amba, E. v., & Ndi Koungou, S. (2019). *Grewia* spp. Biopolymer as low-cost biosorbent for hexavalent chromium removal. *Journal of Chemistry, 2019*.

Kühbeck, F., Schütz, M., Thiele, F., Krottenthaler, M., & Back, W. (2006). Influence of lauter turbidity and hot trub on wort composition, fermentation, and beer quality. *Journal of the American Society of Brewing Chemists, 64*, 16–28.

Li, T., Zhu, Z., Wang, D., Yao, C., & Tang, H. (2006). Characterization of floc size, strength and structure under various coagulation mechanisms. *Powder Technology, 168*, 104–110.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry, 91*, 571–577.

Muazu, J., Musa, H., & Musa, K. Y. (2009). Compression, mechanical and release properties of paracetamol tablet containing acid treated *Grewia* gum. *Journal of Pharmaceutical Science and Technology, 1*, 74–79.

B. Muhammad, Q., Saeed, A., Tariq, I., Muqeet, W., Ross, T., Tuba, E., & Zyta, M. (2021). The chemical composition and health-promoting effects of the *Grewia* species—a systematic review and meta-analysis. *Nutrients, 13*, 1–48.

Mukherjee, S., Pariatamby, A., Sahu, J. N., & sen Gupta, B. (2013). Clarification of rubber mill wastewater by a plant based biopolymer–Comparison with common inorganic coagulants. *Journal of Chemical Technology & Biotechnology, 88*, 1864–1873.

Ndabigengesere, A., Narasiah, K. S., & Talbot, B. G. (1995). Active agents and mechanism of coagulation of turbid waters using Moringa oleifera. *Water Research, 29*, 703–710.

Nep, E. I., & Conway, B. R. (2011). Physicochemical characterization of *Grewia* polysaccharide gum: Effect of drying method. *Carbohydrate Polymers, 84*, 446–453.

Ngounou, K., Bernard, M., Nkoue Ndondo, G. R., Patrice, K. G., Egbe, D. E., Sylvere, N. K., & Joseph, K. G. (2021). Physicochemical characterization of *Grewia* species biopolymer, a potential coagulation-flocculation agent. *Journal of Chemical Engineering and Materials Science, 12*, 64–72.

Niu, M., Zhang, W., Wang, D., Chen, Y., & Chen, R. (2013). Correlation of physicochemical properties and sludge dewaterability under chemical conditioning using inorganic coagulants. *Bioresource Technology, 144*, 337–343.

Nso, E. J., Ajebesone, P. E., Mbofung, C. M., & Palmer, G. H. (2003). Properties of Three Sorghum Cultivars Used for the Production of Bili-Bili Beverage in Northern Cameroon. *Journal of the Institute of Brewing, 109*, 245–250.

Osemeahon, S. A., Ifeoma, J. O., & Adama, H. M. A. (2016). Bioremediation of Wastewater with Immobilized Dargaza (*Grewia Mollis*): Effect of Some Physical Properties. *IOSR Journal of Applied Chemistry (IOSR-JAC), 9*, 37–43.

Panyoo, A. E., Boudjeko, T., Woguia, A. L., Njintang, Y. N., Gaiani, C., Scher, J., & Mbofung, C. M. F. (2014). Optimization of variables for aqueous extraction of gum from *Grewia mollis* powder. *Journal of Polymer, 2014*, 1–10.

Perng, Y. S., & Bui, M. H. (2015). The feasibility of Cassia fistula gum with polyaluminium chloride for the decolorization of reactive dyeing wastewater. *Journal of the Serbian Chemical Society, 80*, 115–125.

Pradip, P. S. (2020). The study on proximate composition of different species of genus *Grewia* from Western Maharashtra. *European Journal of Molecular & Clinical Medicine (EJMCM), 7*, 3919–3924.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology, 81*, 501–508.

Sambo, S.H., Olatunde, A., Shaltoe, S.M., 2015. Phytochemical screening and mineral analysis of *Grewia mollis* stems bark.

Saritha, V., Srinivas, N., & Srikanth Vuppala, N. v. (2017). Analysis and optimization of coagulation and flocculation process. *Applied Water Science, 7*, 451–460.

Schwarz, D., 2001. Water clarification using Moringa olifera. GATE-ESCHBORN- 17–20.

Taylor, J. R. N. (2003). Overview: Importance of sorghum in Africa. In *Afripro: Workshop on the Proteins of Sorghum and Millets: Enhancing Nutritional and Functional Properties for Africa* (pp. 1–21).

Vendruscolo, C. W., Ferrero, C., Pineda, E. A. G., Silveira, J. L. M., Freitas, R. A., Jiménez–Castellanos, M. R., & Bresolin, T. M. B. (2009). Physicochemical and mechanical characterization of galactomannan from Mimosa scabrella: Effect of drying method. *Carbohydrate Polymers, 76*, 86–93.

Villaseñor-Basulto, D. L., Astudillo-Sánchez, P. D., del Real-Olvera, J., & Bandala, E. R. (2018). Wastewater treatment using Moringa oleifera Lam seeds: a review. *Journal of Water Process Engineering, 23*, 151–164.

Wang, L. K., Hung, Y.-T., & Shammas, N. K. (2005). *Physicochemical treatment processes*. Springer.

Zia-Ul-Haq, M., Stanković, M. S., Rizwan, K., & de Feo, V. (2013). *Grewia* asiatica L., a food plant with multiple uses. *Molecules (Basel, Switzerland), 18*, 2663–2682.