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Ultrasonication as a highly efficient method of flaxseed mucilage extraction

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ABSTRACT

Three methods of aqueous extraction of flaxseed mucilage (magnetic stirring, microwaves and ultrasounds) were compared and tested, from whole flaxseeds at a concentration of 5% (w/v) and a temperature of 50°C. While microwaves are less efficient than a magnetic stirring, ultrasound-assisted extraction showed the highest mass transfer coefficient and a higher order kinetic. 7% of the seed mass were extracted after only 30 min of treatment.

Ultrasound assisted extraction decreases the intrinsic viscosity of the mucilage from 12.5 dL/g (for magnetic stirring) to 6.2 dL/g, and the weight-average molecular weight of the largest polysaccharides from 1.5×10^6 Da to 0.5×10^6 Da, whilst having a limited impact on protein content and monosaccharide composition.

1. Introduction

One of the remarkable features of flaxseed is its high mucilage content; located in the outermost layer of the seed's hull, flaxseed mucilage represents from 3 to 9% of the total seed (Fedeniuk and Biliaderis, 1994). It is composed of 50–80% of carbohydrates, 4–20% of proteins and 3 to 9% of ashes (Cui, 2001; Oomah et al., 1995). The polysaccharides are composed of galacturonic acid (21–36%), xylose (19–38%), rhamnose (11–16%), galactose (12–16%), arabinose (8–13%) and glucose (4–6%) (Fedeniuk and Biliaderis, 1994; Oomah et al., 1995). They are present in an acidic, pectin-like, rhamnogalacturonan I (Naran et al., 2008; Cui et al., 1994) form and a neutral (galacto)-arabinoxylan form, both at differing ratios according to the cultivar (Fedeniuk and Biliaderis, 1994; Erskine and Jones, 1957; Hunt and Jones, 1962). The acidic polysaccharide can be divided into different fractions: a minor one with high molecular weight (6.5×10^5 g/mol (Warrand et al., 2003) to 1.5×10^6 g/mol (Qian et al., 2012)); and a major one with a lower molecular weight (1.7×10^4 – 3.41×10^5 g/mol). The neutral (galacto)-arabinoxylan is also polymeric with a Mw of 1.2×10^6 g/mol (Warrand et al., 2003)– 1.5×10^6 g/mol (Qian et al.,

2012) and is responsible for the high viscosity (Cui et al., 1994) and shear-thinning behavior in solution.

Flaxseed mucilage has several potential applications. In food, it is generally used as a texturing agent due to its thickening ability (Dev and Quensel, 1989; Susheelamma, 1989). In cosmetic and pharmaceutical applications, it can be used as a lubricant for skin surface and even as artificial mucus (O'Mullane and Hayter, 1993). It can prevent intestinal inflammation and is of interest as an agent for decreasing blood glucose and blood cholesterol (Thakur et al., 2009; Mitra and Bhattacharya, 2009) when included in food. It has a similar rheometrical behavior to guar gum (Fedeniuk and Biliaderis, 1994) and Arabic gum (Barbary et al., 2009; Mazza and Biliaderis, 1989; Bemiller et al., 1993) and a high water absorption capacity has also been noted (Wanasundara and Shahidi, 1997).

Due to its high thickening capacity, however, its presence can be limiting for any potential aqueous processing of the seeds. Hence, it is often better to use dehulled seeds, or to remove the mucilage prior to any processing of the seeds. The extraction is generally achieved with hot water (Mazza and Biliaderis, 1989) and at low seed concentration. Some simple basic or acid treatments are also known (Singer et al., 2011) although drawbacks of these methods are higher concentrations of proteins in the mucilage and their subsequent denaturing. Thus, to help in the extraction process, enzymes have been (Wanasundara and Shahidi, 1997; Wu et al., 2010) applied.

To improve the extraction yield of polysaccharides from plants, many authors use non-classical heating modes such as microwaves and ultrasounds. Microwaves have shown large improvements in

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the extraction yields, for example in the polysaccharides of black nightshade (Chen et al., 2005). Ultrasonic-assisted extraction, on the other hand, is the most used technique in this field. They have been employed in the extraction of polysaccharides from the roots of valerian (Hromádková et al., 2002), *Salvia officinalis* (Hromádková et al., 1999), Korean pine kernel (Chen and Zhang, 2007), lime (Toma et al., 2001) and *Zyziphus jujube* (Li et al., 2007). In addition, microwave and ultrasonic assisted extraction can also be used together, as in the extraction of pectin from grapefruit (Bagherian et al., 2011). As previously noted, however, longer treatment times and higher temperatures can lead to degradation of the polysaccharides. (Bagherian et al., 2011; Liu et al., 2013).

If a potential flaxseed biorefinery is to be realized, it will be critical to have easily manipulable seeds and a high quality final product. Thus, high mucilage extraction yields and high mucilage purity are highly desired. Herein, we present a comparison of classical, microwave and ultrasonic-assisted extraction techniques, including extraction kinetics, composition and intrinsic viscosity of the mucilage obtained.

2. Materials and methods

2.1. Materials and reagents

Flaxseeds used are the Niagara variety coming from Linea, France. Sodium tetraborate, m-hydroxybiphenyl, sulfuric acid, sodium hydroxide and monosaccharides standards were purchased from Sigma–Aldrich, France. Polysaccharides standards were purchased from Polymer Laboratories, USA.

2.2. Mucilage extraction

Classical heating for mucilage extraction was performed in a water bath at 50 °C with magnetic stirring. The seeds were immersed in the water once the desired temperature was reached.

Microwave extraction was performed at 2450 MHz in a multimode oven MARSX from CEM instruments, with HP500 closed digestion reactors. The temperature was controlled and measured by an optical probe inserted into the reactor.

Ultrasonic extraction was performed using a Sonics Vibracell Probe – 500 W with a frequency of 22 kHz. The sonication was performed at different amplitudes in a 100 or 150 mL plastic beaker with a 13 mm diameter probe placed 2 cm below the surface. The device was used in a pulsed mode (i.e. 1 s on; 5 s off, etc.).

The mucilage was recovered by filtration of the sample on a plastic mesh (1 mm × 1 mm) assisted with a water aspirator. The mucilage concentration was obtained by determining the dry matter after heating the sample in an oven at 103 °C until weight stabilization. Extraction yield is expressed as:

$$Y = 100 \cdot \frac{C_m \cdot V_{mr}}{m_s} \quad (1)$$

where C_m is the dry matter content of the mucilage recovered (g/dL). V_m is the volume of the recoverable mucilage (dL). m_s is the mass of the seeds (g). The dry matter determination was repeated twice on one or two different samples in order to achieve a representative value.

Freeze-drying of the extracts was performed on a Cryo-Rivoire device, to which the samples were cooled to –40 °C at a rate of 0.5 °C/min, then held under a vacuum of 0.3 mbar whilst they warmed 20 °C.

2.3. Mucilage analysis

Kinematic viscosity was measured with capillary Cannon-Fenske viscosimeters in a 40 °C thermostated bath. The

measurement was repeated several times on two different samples. Intrinsic viscosity was calculated by measuring the viscosities obtained at different mucilage concentrations. It is defined as:

$$\lim_{c \rightarrow 0} \left(\frac{1}{c} \frac{\eta - \eta_0}{\eta_0} \right)$$

where η is the viscosity of the solution. η_0 is the viscosity in the absence of mucilage and c is the concentration of mucilage (g/mL). The methods of Solomon and Gottesman (1968), Huggins (1942) and Kraemer (1938) were used for the calculation.

Ash content was determined by heating a freeze-dried sample for 3 h in a muffle furnace at 600 °C. This measure was repeated at least once. Protein content was analyzed by the Kjeldahl method using a Kjeltac 8400 automatic analyzer, and a standard conversion factor (nitrogen to protein) of 6.25. Each sample was first concentrated by freeze-drying in order to get measurable quantities of nitrogen and each measurement was repeated at least once.

Monosaccharides distribution was obtained by a HPLC DIONEX ICS 3000 DC-EG with a Carbo-Pac PA-1 column, a post-column with NaOH 300 mM and an automatic sampler AS3000. The device is equipped with a pulsed electrochemical detector. 100 mg of freeze-dried mucilage is first hydrolyzed with 1.25 mL of a solution of H₂SO₄, 72% (w/w) then 13.5 mL of water is added and the mixture heated at 100 °C in closed tubes for different durations. 3.6 mL of NaOH 32% (w/v) is added to stop hydrolysis and the sample is diluted 50 times with ultra-pure water prior to analysis. Samples and monosaccharides standards used for calibration are injected two times. Results consider maximum concentration value according to hydrolysis duration.

Galacturonic acid content was measured via the Blumenkrantz titration method (Blumenkrantz and Asboe-Hansen, 1973). Samples are hydrolyzed 5 min at 100 °C with a solution of sodium tetraborate in sulfuric acid. After cooling the samples, a basic solution of three methoxybiphenyl is added and absorption is recorded at 520 nm. Concentration is obtained with a calibration curve obtained with different galacturonic acid standards.

Gel permeation chromatography was realized on a Dionex Summit ASI100 injection autosampler with a P580 Pump and a GPC oven with a column PL Aquagel OH 40 8 μm + PL Aquagel OH 30 8 μm thermostated at 40 °C and coupled to a VARIAN 350 RI detector. The eluent was ultra-pure water with a flow rate of 0.8 mL/min and the temperature was fixed at 40 °C. The softwares used are Chromeleon and Seccial. Polysaccharides standards (Mw from 0.75 kDa to 788 kDa) were used for calibration.

2.4. Expression of the results

The results are expressed with the formula:

$$X = \bar{x} \pm t \times \frac{\sigma}{\sqrt{n}}$$

where \bar{x} is the calculated experimental average value, σ is the standard deviation of the measurements and n is the number of measurements. t is the student coefficient at a 68% confidence level for the expression and representation of the results. However, a 95% confidence level was used and indicated to denote significant differences.

3. Results and discussion

3.1. Classical heating

Different parameters are known to influence the aqueous extraction of vegetable products. Temperature, dilution, pH, time, heating and stirring modes are the key factors that can exert an action on the release of hydrosoluble compounds. As indicated in

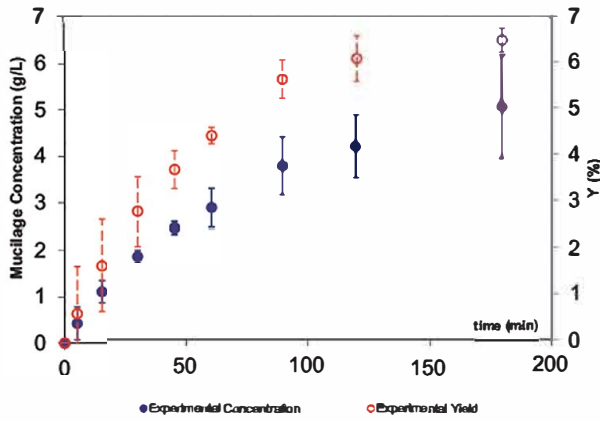


Fig. 1. Kinetic profile of mucilage extraction (50°C, 250 rpm).

the literature (Ziolkovska, 2012), a high temperature allows a better extraction of mucilage, however, it increases protein and ash contents (Barbary et al., 2009) and can induce protein denaturation. Hence our extraction was performed at a mild temperature of 50°C. Dilution is also important to avoid high viscosity in the medium. The viscosity of the medium increases with seed concentration and the mucilage is not as easily recovered at low than at high dilution. In this case, a standard concentration of 5% of seeds (w/v) in water is preferred. Concerning pH, strong acid solutions are known to enhance mucilage extraction but can deteriorate seeds and cause complete hydrolysis of their mucilage. Neutral pH conditions have no influence on the solubility of polysaccharides but can increase the solubility of proteins (Martínez-Flores et al., 2006). Thus, between pH 4 and 10, we do not observe any change in the mucilage yield at 50°C after 30 min of extraction. This may indicate that the proteins are still not fully hydrated.

3.1.1. Kinetic approach

Considering the mucilage extraction curves (Fig. 1), we assume that the loss rate of mucilage from the seeds follows a first-order model, being proportional to the difference between the concentration in the mucilage cells and the concentration in the extractant.

Mucilage, being a surface-coated compound, exhibits a mass-transfer area equal to that of the outer surface area of the seeds, S (m^2).

$$-V_s \frac{dC_s}{dt} = kS(C_s - C_w) \quad (2)$$

V_s is the initial volume of mucilage cells which surrounds the flaxseed (m^3).

C_s is the concentration of mucilage in the cells on the surface of the flaxseed (kg/m^3).

C_w is the concentration of mucilage in the extractant (kg/m^3).

k is the mass-transfer coefficient (m/s).

The law of conservation of mass means:

$$V_w(C_w - C_{w0}) = V_s(C_{s0} - C_s) \quad (3)$$

V_w is the volume of extractant (m^3).

C_{s0} is the concentration of mucilage in the cells at $t = 0$.

C_{w0} is the concentration of mucilage in the extractant at $t = 0$.

Therefore we can assume that $C_{w0} = 0$

Then:

$$V_w C_w = V_s(C_{s0} - C_s) \quad (4)$$

In addition, we will assume that after 180 min of treatment, all of the mucilage has been removed from the surface of the flaxseeds, and that $C_w = C_s = C_{weq}$.

$$1 - \frac{C_w}{C_{weq}} = \exp\left(-\frac{kS(1+\beta)t}{V_s}\right) \quad (5)$$

where $\beta = V_s/V_w$

$$\log\left(1 - \frac{C_w}{C_{weq}}\right) = -k^* \cdot t \quad (6)$$

Plotting $\log(1 - C_w/C_{weq})$ versus t , a regression coefficient of 0.9987 is obtained with $k^* = 0.0065$.

$$k^* = \frac{0.43kS(1+\beta)}{V_s} \quad (7)$$

The ratio V_s/S can be approximated as the thickness of the mucilage layer. This thickness is never constant, as it can vary from 0 to more than 80 μm . By microscopic observation on different cross sections of flaxseeds at a magnification of $\times 1000$ on several seeds, we found an average thickness of 27 μm .

$$K = \frac{e_s \cdot k^*}{0.434(1+\beta)} \quad (8)$$

with the dilution used here, β can be neglected. $k \sim 4 \cdot 10^{-7}$ m/s.

The extraction kinetic is thus quite slow. Even if the mucilage concentration in the extractant increases with time, its high viscosity makes its withdrawal from the seeds complicated.

After 3 h, we obtain a mucilage concentration of 5.0 g L^{-1} , which corresponds to an extraction yield of 6.5%. Limitations of the yield can be explained by low recovered mucilage volumes (<80% of initial extractant volume) due to difficulties in the filtration of the seeds when the mucilage concentration and viscosity become important.

3.2. Use of microwave technology

Carbohydrates are compounds far less dielectric than water and so we can consider that any microwave effect would be mainly due to the agitation of the water molecules. Collision between water and flaxseed's carbohydrates may also help solubilizing them.

We use pressurized reactors with a temperature control. Thus the microwaves could be used to heat the sample and/or to maintain its temperature. To test the presence of a microwave effect, we compared the dry matter extracted after 30 min at 50°C in a normal oven with the dry matter extracted after 30 min with many different profiles (fast, slow with different ramps durations to reach 50°C, pulsed with different on/off time ratios at 50°C) of heating modes in a microwave oven at 1200 W.

Pulsed modes gave the best results of mucilage extraction as a compromise between temperature and the time of microwave action. A slight but significant enhancement of the extraction yield of mucilage was achieved (pulsed modes gave an extraction yield of $2.11 \pm 0.28\%$ compared to $1.23 \pm 0.49\%$ with a classical oven at a 95% confidence level). However, this effect is identical or inferior to the one produced by magnetic stirring ($2.11 \pm 0.28\%$ compared to $2.82 \pm 0.73\%$ at a 95% confidence level). Although microwave extraction offers a viable method to quickly increase the temperature of the medium in order to perform subsequent processes, it does not show a significant effect on the overall extraction yield of mucilage.

3.3. Use of ultrasonic technology

Thus, we found that at 20% of the maximum amplitude of the sonotrode, the extraction yield after 30 min (5 min of effective

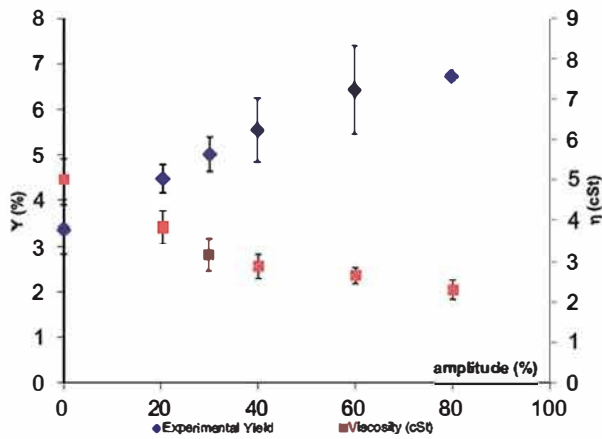


Fig. 2. Evolution of the extraction yield and kinematic viscosity of the extractant with the amplitude of ultrasounds (after 30 min of treatment in pulsed mode).

ultrasonic treatment), is $4.5 \pm 1.0\%$ compared to $4.4 \pm 0.3\%$ after 1 h of extraction via magnetical stirring. Increasing the amplitude further to 80% gives an extraction yield of $6.75 \pm 0.08\%$, which is equivalent to that obtained after 3 h of magnetical stirring treatment $6.48 \pm 0.28\%$ (Fig. 2).

3.3.1. Kinetic approach

To study the kinetic evolution of the extraction we choose to work at an amplitude of 60%, in order to maintain a stable temperature during the extraction process. The data suggests that the mucilage extraction can be divided into two steps (Fig. 3). A first equilibrium state is reached after 30 min of extraction and a further increase of the extract concentration is then observed, leading to an extraction yield of $13.5 \pm 0.05\%$.

If we test for a second order rate kinetics assuming a rate proportional to the square of the concentration of mucilage in the extractant, simplified as:

$$\frac{dC_w}{dt} = k'(C_{weq} - C_w)^2 \quad (9)$$

k' is the second-order extraction rate constant ($\text{m}^3 \text{kg}^{-1} \text{min}^{-1}$). This equation can be resolved by using Peleg's (1988) model:

$$\frac{t}{C_w} = A + B \cdot t \quad (10)$$

with $A = 1/k'C_{weq}^2$ and $B = 1/C_{weq}$

Plotting t/C versus t (where $t = 1-30$ min), we obtain a regression coefficient of $R^2 = 0.9917$.

We can deduce $C_{weq} = 5.3 \text{ g L}^{-1}$. $k' = 0.031 \text{ m}^3 \text{ kg}^{-1} \text{ min}^{-1}$.

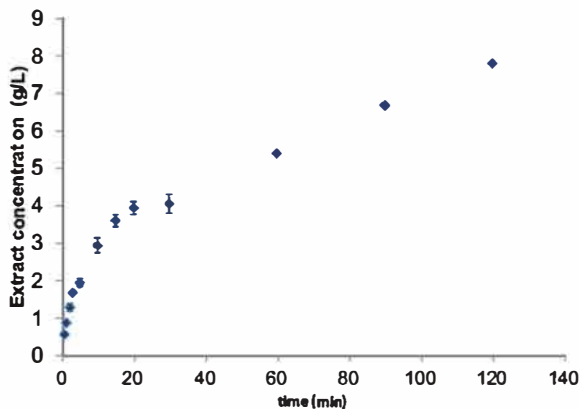


Fig. 3. Evolution of the extract concentration with time of ultrasonic treatment at an amplitude of 60% at 50°C.

Thus, ultrasound-assisted extraction of mucilage follows a second order rate kinetics for the first 30 min of extraction. The second step of mucilage extraction demonstrates a zero-order rate kinetics. Therefore, plotting concentration versus time (where $t = 30-120$ min), we obtain $k = 0.039 \text{ m}^3 \text{ kg}^{-1} \text{ min}^{-1}$ and $R^2 = 0.9994$. Therefore, we can presume that, after 30 min, the mucilage layer has vanished and inner material (proteins, fibrous particles, etc.) are now desorbing into the extractant via ultrasonic-assisted osmosis.

To explain the higher extraction rate and kinetic order of ultrasound assisted mucilage extraction, different phenomena have to be taken into account. While an increase of the extraction yield is responsible for an increase of the viscosity during classical heating extraction, viscosity gradually decreases with time and amplitude of ultrasounds (Fig. 2). Hence, diffusion is not hindered by viscosity and a higher volume is recovered after an ultrasonic treatment. When immersed in water, mucilage cells begin to swell and the cell wall becomes more fragile. With ultrasounds, a strong mechanical stress occurs and its rupture is favored and uncoated molecules are quickly released.

Then, ultrasounds, through their thermomechanic action, the high energy dissipated by the collapse of cavitation bubbles and subsequent microjets, desorb surface coated molecules. They can also alter the structure of large molecules such as polysaccharides. The measured power at the different amplitudes, as indicated by the device, is from 7 to 83 W from 20 to 80% and the energy provided to the system by ultrasounds after 30 min is from 1.3 kJ to 17 kJ. Considering the activation energy of ultrasonic degradation of different polysaccharides as reported in literature (26.5 kJ/mol for *Lycium barbarum* polysaccharide (Zhu et al., 2010), 52.13 kJ/mol for a red algae (Zhou and Ma, 2006), 65.8 kJ/mol for Arabinan (Shatalov and Pereira, 2005)) polysaccharides degradation is expected. To determine more precisely this phenomenon, the intrinsic viscosity of the extracted mucilage was calculated.

3.3.2. Intrinsic viscosity

Intrinsic viscosity, in mL/g is the expression of the hydrodynamic volume occupied by the molecules. It depends on the molecular conformation (linear, branched) but also on the rigidity of the polymer chains. It is correlated with the molecular weight through the Mark-Houwink-Sakurada relationship (Gómez-Díaz et al., 2008).

$$[\eta] = K(M_w)^\alpha \quad (11)$$

where K and α are parameters specific to the couple polymer-solvent. Alpha is a function of polymer geometry, related to chain flexibility.

Intrinsic viscosity can be calculated through different methods at high dilution:

Solomon method:

$$[\eta] = \frac{[2(\eta_{rel} - 1 - \ln \eta_{rel})]^{1/2}}{c} \quad (12)$$

where c is the concentration, in g/dL . η_{rel} is the relative viscosity. The advantage of this equation is that it is a one-point measure and does not need several dilutions of the medium.

Kraemer method:

$$\frac{\ln \eta_{rel}}{c} = [\eta] - k_k[\eta]^2 c \quad (13)$$

$$[\eta] = \lim_{c \rightarrow 0} \left(\frac{1}{c} \ln \frac{\eta}{\eta_0} \right) \quad (14)$$

Huggins method:

$$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \quad (15)$$

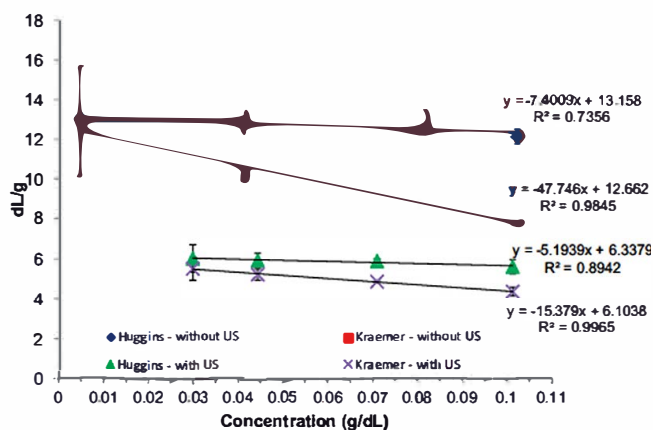


Fig. 4. Kraemer and Huggins plots for ultrasonicated (60% amplitude, 30', 50°C) and magnetically stirred sample (250 rpm, 50°C).

$$[\eta] = \lim_{c \rightarrow 0} \left(\frac{\eta_{sp}}{c} \right) \quad (16)$$

where η_{sp} is the specific viscosity.

Kraemer and Huggins methods, on the other hand, need the values of the viscosity measured at different mucilage concentrations (Fig. 4).

If intrinsic viscosity does not depend on concentration, it depends on the ionic strength of the solvent. This is particularly true for polyelectrolytes in solution as the electrostatic repulsions can expand the shape of the molecules. The intrinsic viscosity of flaxseed gum, for example, is superior in deionized water than in NaCl or CaCl₂ (Regina Kuhn et al., 2011). To determine precisely the effect of ultrasounds on the intrinsic viscosity, mucilage extracts were first freeze-dried and tested at different concentrations in ultra-pure water (from 5 to 100 mg/dL).

We obtain similar results with the three calculation methods (Table 1), with the ultrasonicated sample exhibiting an intrinsic viscosity almost half that of the sample extracted by classical stirring (6.23 ± 1.02 dl/g compared 12.52 ± 1.83 dl/g). This result demonstrates that ultrasounds have an effect on the hydrodynamic volume of the mucilage molecules.

If we analyze the molecular weight profile of the mucilage by gel permeation chromatography we can observe different polysaccharides of high molecular weight (Fig. 5). When ultrasounds are applied, very large polysaccharides, greater than 1.5 × 10⁶ Da desorb first during the initial 2 min. After 3 min of ultrasonic treatment, a profile similar to polysaccharide extraction after 30 min of a classical heating is obtained. With longer ultrasonic times, degradation becomes gradually more visible. After 30 min of ultrasonic treatment, we obtain a molecular weight centered around the value of 0.5 × 10⁶ Da instead of 1.5 × 10⁶ Da for a magnetically stirred sample. These results agree with the intrinsic viscosity decrease of the samples according to the Mark-Houwink equation.

3.3.3. Influence of ultrasounds on monosaccharides composition

To determine whether ultrasounds have an effect on the composition of the mucilage extracted after 30 min of treatment, the

Table 1
Differences of intrinsic viscosity (dl/g) between ultrasonicated (60% amplitude, 50°C, 30') and magnetically stirred sample (250 rpm, 50°C).

Methods	With ultrasounds	With conventional stirring
Solomon	6.24 ± 1.28	11.73 ± 2.01
Huggins	6.34 ± 1.03	13.16 ± 1.94
Kraemer	6.10 ± 0.74	12.66 ± 1.55
Average	6.23 ± 1.02	12.52 ± 1.83

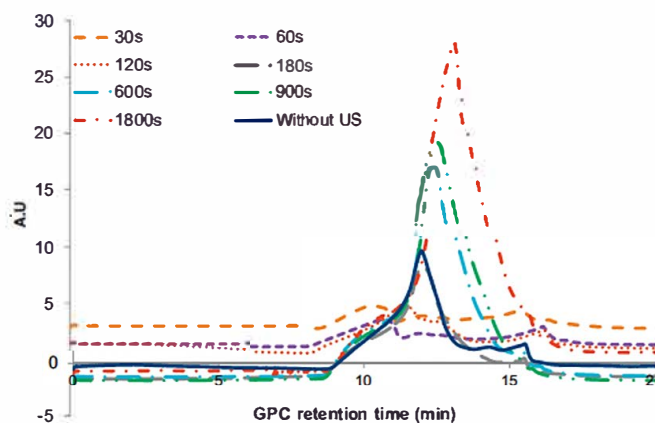


Fig. 5. GPC profiles of mucilage extracts at different times of ultrasonic treatment.

monosaccharide distribution of the mucilage was analyzed with different amplitudes of ultrasounds. In addition, the polysaccharides distribution was also determined after 2 h of an ultrasonic treatment at high amplitude (80%) (Fig. 6).

The two major monosaccharides of the mucilage are xylose and arabinose, two pentoses which can find applications in green chemistry, as in the production of furfural (Bergez-Lacoste et al., 2014.). At lower amplitudes (between 0 and 40%), the desorption of the mucilage polysaccharides is not complete and thus may explain the observed differences in the nature of the polysaccharides extracted.

In the first 30 min of extraction, we observe a slight decrease in fucose and rhamnose relative to a slight increase in arabinose and galactose. After a long treatment with an amplitude of 80%, glucose concentration largely increased while xylose and arabinose concentrations decreased. As fucose and rhamnose are monosaccharides of acidic polysaccharides whereas arabinose and galactose are monosaccharides of neutral polysaccharides, it seems then that acid polysaccharides are first extracted followed by neutral polysaccharides and, finally, non-mucilaginous polysaccharides. The increase of the ratio (arabinose + galactose)/rhamnose with ultrasonic treatment can also suggest an increase in the neutral sugar side chain size of polysaccharides (Liu et al., 2013).

To determine more precisely the effect of ultrasounds on the acid fraction of polysaccharides, we analyzed the content of galacturonic acid (Fig. 7). This analysis was performed with the Blumenkrantz titration method. High variations for low amplitude samples may be due to a lower solubility of mucilage solution. The content in galacturonic acid first largely increases with amplitude and then decreases. It seems then that galacturonic acid is the first monosaccharide extracted which could explain its lower

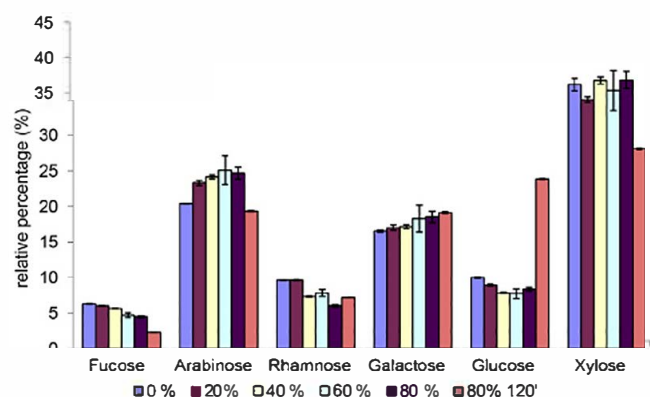


Fig. 6. Monosaccharides composition of the mucilage extract after 30 min of ultrasonic treatment with different amplitudes and after 120' at an amplitude of 80%.

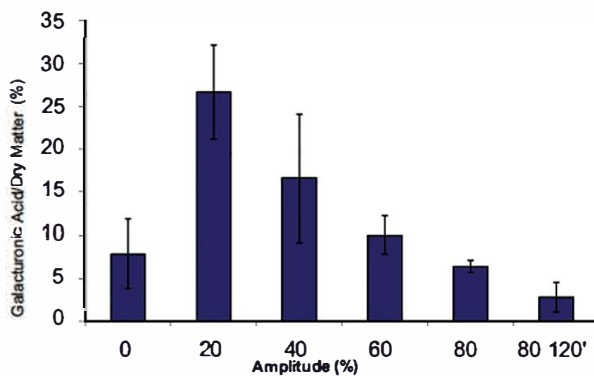


Fig. 7. Galacturonic acid concentration in the dry mucilage extract with ultrasonic amplitude and time.

Table 2
Ashes and proteins concentration (%) in the dry mucilage extracted with different amplitudes (30 min at 50°C).

Amplitude (%)	Ashes	Proteins [conversion factor = 6.25]
0	1344 ± 1.13	1.00 ± 0.40
20	8.96 ± 0.92	1.63 ± 0.33
40	7.24 ± 0.31	1.32 ± 0.42
60	7.00 ± 0.53	1.29 ± 0.40
80	7.52 ± 0.44	1.48 ± 0.26

concentration at high amplitude. At long ultrasonic treatment, the decrease of the galacturonic content is more pronounced, which could be explained by the release of non-mucilaginous molecules in the mucilage.

3.3.4. Influence of ultrasounds on protein and ashes content

Concerning the composition in ashes and proteins, a similar behavior was observed (Table 2). At low amplitudes, ashes represented a large amount of the extract and their concentration decreases while polysaccharides concentration increases. Protein variations are not significant, being inferior to the uncertainty at a 68% confidence level, which indicates that even at 80% of amplitude, endosperm proteins are not extracted. Protein release in the aqueous extractant media seems to be more dependent upon immersion time.

We can notice that protein and ash concentrations do not significantly increase with ultrasound amplitude. At long treatment time, ash concentration remains constant, even at 80% of amplitude. Protein concentration increases with treatment time (Table 3) but if the effect may be slightly more pronounced with ultrasounds, protein concentrations are not significantly different between ultrasonic extraction and magnetical stirring (at 30 min and 60 min at a confidence level of 68% and at 120 min at a confidence level of 95%).

Flaxseed proteins are generally smaller molecules than polysaccharides and are more concentrated in deeper layers of the seed. Their extractability depends on diffusion mechanisms along the seed volume. Acting at the surface of the seeds, ultrasounds do not increase the release rate of these molecules. At long ultrasonic times, however, the seeds begin to become destructured and proteins may therefore be more easily extracted.

Table 3
Protein concentration (%) in the dry mucilage at different treatment times.

Time (min)	US-amplitude 60%	Magnetically stirred
30	2.07 ± 0.46	1.8 ± 0.94
60	4.64 ± 0.28	4.04 ± 0.56
120	12.22 ± 0.18	10.83 ± 0.47

4. Conclusion

In testing and comparing our three chosen methods for the extraction of flaxseed mucilage: stirring, microwaves and ultrasounds, we have found that microwave assisted extraction to be the least efficient, as considerable energy is lost in the heating of the water molecules, in addition to the flaxseed carbohydrates not being agitated sufficiently to allow any dramatic increase in their extraction. By comparison we have shown that extraction via magnetical stirring or by ultrasounds is much more efficient and we have elucidated their kinetic parameters, demonstrating that the former exhibits first order kinetics while the latter shows second order kinetics with respect to mucilage concentration. Thus an extraction time of 30 min is sufficient to achieve quantitative extraction of the mucilage. Due to this shorter extraction time, the recovered mucilage contains a lower concentration of proteins and a higher concentration of the valuable pentoses. Higher ultrasonic amplitudes lead to an increased desorption of neutral polysaccharides, in addition to a decrease in their molecular weight. The resulting loss of viscosity observed when ultrasonic assisted extraction is employed makes any subsequent treatment much more feasible and is potentially the key to a flaxseed biorefinery where the ultrasonically extracted mucilage forms the starting material for a secondary biorefinery process. While its minor fractions of ashes, acid monosaccharides and proteins could find applications in the food, health or agriculture industries.

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