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Abstract

As the outermost layer of many oleaginous seeds, mucilage can be extracted by different shear forces. At lab scale, ultrasound is an effective extraction method and is associated here with hydrodynamic forces on a higher scale. In the example of camelina mucilage, an ultrasonic tubular reactor and a recirculating pump are used separately and combined with a response surface methodology. With a seed concentration of 10% in water, mucilage extraction yields of 6.9 ± 1.5 % and 6.6 ± 0.3 % are obtained after 55' of pulsed ultrasonic treatment with a 3L ultrasonic tubular reactor and a lab scale (100 mL) ultrasonic probe, respectively. High flow rate recirculation (100L/min) used alone allows yields of more than 10%. Extraction efficiency then depends directly on the rheological behavior of the medium and allows a high seed concentration (up to 15%). The combination of ultrasounds and high flow rate circulation reveals a high importance of ultrasound on protein composition and rheological behavior of mucilage while extraction yield, protein and ash content are mainly controlled by circulation time.

Keywords

Hydrodynamic, Rheology, Upscaling, Polysaccharides, Protein, Surface shear

1. Introduction

Having been excluded from food circuits for hundreds of years, Camelina sativa is still underappreciated. However, it cumulates very advantageous agronomical behavior as a low input, short growing time (D.H. Putnam et al., 1993), high resistance to cold (Zubr, 1997) and pests (Conn et al., 1988). Besides, its seed is generally rich in omega-3 fatty acids. Hence, several applications are gradually opened to exploit these characteristics. For example, Camelina oil can be used as a biofuel even in aeronautic sector (Borah et al., 2019; Moser, 2010; Murphy, 2011). As other well-known seeds (in particular flaxseed and mustard), Camelina is rich in soluble fibers known as mucilage, concentrated in the outermost layer of the seed. If non extracted, these fibers remain in the extraction meal and can slow fat assimilation (Cherian, 2012), which can be a disadvantage for some animals feeding. Once extracted, these fibers can be valorized in human consumption, mucilages easily finding pharmaceutical applications. Camelina mucilage can also be used for technical applications, for example as a tacking agent for hydromulch (Vaughn et al., 2013) and it also demonstrated film forming properties (Qi et al., 2016). The objective of this study is to develop a scalable method of mucilage extraction allowing its recovering at high yield while keeping intact the other parts of the seed. Once hydrated, mucilage distends, breaks the mucilaginous cells and gradually expands outside. It is generally constituted of an adherent part, strongly bound to the seed and a more loosely adsorbed one. According to the extraction processes, mucilage dry matter can finally represent 7 to 10% of the mass of the seed (Sarv et al., 2017; Zubr, 2010) and is mainly composed of polysaccharides (~80%) but also proteins (10-15%) and minerals (~6%) (Sarv et al., 2017; Taylor et al., 1847). The polysaccharides are composed of arabinose, galactose, glucose, rhamnose, xylose (Li et al., 2016; Sanchez, 2014) while the acid, pectin-like part is mainly in the form of Gal-rhamnogalacturonan (North et al., 2014). If a gentle stirring of the seeds in water can allow the extraction of the less adherent parts,

stronger conditions are required to achieve a high extraction yield. Different stirring methods are then available to achieve this goal. Ultrasounds have been successfully employed to extract polysaccharides for a variety of plants. The creation of cavitation bubbles and their subsequent collapse generate high spots with very high temperature and pressure, able to break the bonds between the seed coat and the mucilage. Hence, amplitude of the ultrasounds, extraction time and temperature must be correctly chosen to avoid extraction of undesired compounds and mechanical disruption of the seeds. However, when this process has to be scaled up, the challenge is to maintain sufficient surface shear on the seeds. The energy concentration allowed with ultrasonic probes at laboratory scale is rarely found in high volume processes, large ultrasonic baths being unable to generate such a volume power. An ultrasonic reactor, with high power transducers along a small diameter cylinder may be an alternative device. Another way to exert an efficient surface shear is the use of hydrodynamic forces through a high flow rate fluid circulation pump. Hence, a 3L ultrasound tubular reactor was compared to a laboratory probe and the effect of hydrodynamic forces on mucilage extraction was evaluated without ultrasounds and combined with them through an experimental design.

2. Materials and methods

The seeds are from the “Celine” variety. They come from different french farmers gathered within the cooperative CAVAC (La Roche Sur Yon, France, https://www.coop-cavac.fr) associated with INRA (Institut National de la Recherche Agronomique, Versailles, France).

2.1. Mucilage extraction and preparation

At laboratory scale, the ultrasound probe has a 13mm diameter and can deliver a power of 500W at its maximal amplitude with a frequency of 22 kHz (SONICS Vibracell 500). The sonication was performed in pulsed mode in a 125mL pl astic beaker containing 100 mL of

a suspension of camelina seeds in water. The probe was placed 2 centimeters below the surface. After treatment, the medium was filtrated on a plastic sieve (1mm x 1mm mesh).

Freeze-drying of the extracts was performed in a Cryo-Rivoire device, in which the samples were cooled to -40°C (0.5°C/min until 5°C, 0.1°C/min until -20°C, 0.5°C/min until -40°C), then held under a vacuum around 0.2 mbar whilst they warmed 20°C.

The ultrasound tubular reactor, LIXEA FORMULATOR C80-500-PI was realized by SINAPTEC (France). It is composed of 4 ultrasound generators of 500W, each connected to a ring of 8 transducers around the 3.1 L cylinder with a DN80 section. The power of the generators can be modulated through the software NEXTGEN 2001 (v. 1.0.6.0). A flow of compressed air circulates to cool the generators. An asymmetric temporizer Broyce Control M1ARM was also added to work in pulsed mode. In batch mode, the medium was stirred with an IKA Eurostar Control motor, temperature and motor couples being recorded along the treatment time through COMTOOLS software (v.2.4.4.0). A stirring paddle was expressly manufactured for this application

In recirculation mode, a pump Grundfos CR4-60, with a nominal flow rate of 100L/min was linked to the reactor and the temperature was measured within the recirculating pipe. At the end of the treatment, the mucilage was recovered by filtration of the medium on two successive metallic sieves (1mm x 1mm and 0.5mm x 0.5 mm mesh).

The extraction yield of the mucilage was based on the dry matter of the extract with the formula:

$$Y(\%) = 100 \times \frac{m_{muc.} \times DM}{m_S} \quad \textbf{(Equation 1)}$$

With $m_{muc:}$ mass of the mucilage extract (g). The mass of the mucilage extract can be approximated with the mass of the initial water phase minus the mass of water absorbed by

the seeds (this last value is obtained with the measurement of the dry matter of the treated seeds).

DM: Dry matter percentage of the mucilage extract (g/100g)

m_s*:* mass of the seeds (g)

2.2. Characterization

The dry matter was determined by heating a sample of the mucilage extract in an oven at 103°C until a constant mass is reached. The dry matter determination was repeated twice in order to achieve a representative value.

Ionic chromatography was used to analyze the monosaccharides of the mucilage. A HPIC Dionex ICS 3000 DC-EG with a column Carbo-PAC PA1 and a post-column filled with NaOH 300mM is linked to an autosampler AS3000. The device is controlled with the Chromeleon software (Dionex 1996-2006 Version 6.80 SR15). Detection is allowed with an AgCl based electrochemical cell. 1.25 mL of a sulfuric acid solution (72% m/m) were first dropped on the sample then 13.5 mL of water were added before heating at 100°C in hermetically sealed tubes for different durations. The end of hydrolysis was provoked by the addition of 3.6 mL of NaOH 32% (m/m) and the sample was diluted (1/50) with ultra-pure water. An external calibration curve was realized with a standard solution of monosaccharides. For each hydrolysis duration, the concentration of each monosaccharide was determined and the maximal value was recorded, some monosaccharides requiring longer durations to be released and some being degraded with hydrolysis time.

The Lowry method was used for protein determination, using 96-well microplates with the titration kit “Pierce Modified Lowry Protein Assay Kit” from Sigma-Aldrich (St Quentin-Fallavier, France). A Bovine Serum Albumen (BSA) solution was prepared with 10 different

concentrations and freeze-dried mucilage extracts were solubilized in distilled water with a dry matter concentration around 1mg/mL. 40µL of each standard and sample solution were pipetted in a 96-well microplate, each well being at least once repeated. Then, 200µL of the Lowry reagent were added in each well. After 30 seconds of moderate agitation (200 rpm) and 10 minutes at rest, 20 µL of a Folin-Ciocalteu reagent were added. The microplate was again stirred with the same conditions and incubated 30 minutes at ambient temperature. The absorbance of the wells was then measured at 750nm. A calibration curve was realized with the 10 different concentrations of BSA and allowed the measurement of the protein concentration (in eq. BSA) of the samples.

Ash content was determined by heating a freeze-dried sample for 3 hours in a muffle furnace at 600°C. This measure was repeated at least once.

Cryo-SEM observations were realized with a MEB Quanta 250 FEG FEI with a Quorum PP3000T module. Samples were cooled in slush nitrogen, fractured and metalized with platinum prior to observation.

2.3. Statistical analysis

The data were submitted to one-way ANOVA All the results are presented as mean values with uncertainties calculated with student coefficients at a 95% confidence level according to the number of degrees of freedom (measurements are usually twice repeated).

The experimental design was constructed and analyzed with NEMRODW 2000 (Mathieu D, Nony J, Phan-Tan-Luu R. NEMROD-W software. LPRAI; Marseille: 2000). Defining different independent variables and their levels of variation, the Doehlert formalism could be used and a matrix of experiments was constructed. The observed responses were then regressed with a second order polynomial model:

$$Y_k = \mathbf{b_0} + \sum_i \mathbf{b_i X_{i,k}} + \sum_i \mathbf{b_{ii} X_{i,k}^2} + \sum_i \sum_{j \neq i} \mathbf{b_{ij} X_{i,k} X_{j,k}} \quad \textbf{(Equation 2)}$$

Where Y_k is the calculated response value at the k^{th} experiment, $X_{i,k}$ is the coded variable i for the k^{th} experiment, b_0 is the intercept term, b_i are the main coefficients for each variable, b_{ii} are the squared coefficients and b_{ij} are the interaction terms. The pertinence of the model was determined with the usual Fisher-Snedecor test, comparing model and residual variances while its validity (or descriptive importance) was determined with the comparison of the residual variance with the experimental one. Using Doehlert matrix and the calculated response values, response surface curves were plotted considering the variation of two independent variables, the other being fixed.

3. Results and Discussion

3.1. Physical characterization of the seeds

Camelina seeds were first characterized. Dry matter of the seeds was measured: DM = 93.46 ± 0.02 %

Measuring the evolution of the mass of a collection of seeds while new individuals are added, the average mass of a seed was determined as m = 1.36 ± 0.05 mg. By adding a known mass (5g) of Camelina seeds to a fixed volume of ethanol (5mL), it was possible to measure the volume increase and deduce the average volume of a seed: V_{seed}=1.20 ± 0.02 mm^3.

Once hydrated, seeds are separated each other by the mucilaginous layer. The seed and its deployed mucilaginous layer can occupy a decupled volume.

Cryo SEM analysis allowed the observation of the endosperm and the seed coat. The aspect of the mucilage is similar to *Arabidopsis thaliana* (Western et al., 2000). The mucilaginous layer at the surface of the testa has a thickness of 20-40µm (Figure 1a). The observation of the surface reveals the columella and surrounding mucilage (Figure 1b). After hydration,

mucilage is extracted above the columella (Figure 1c) then after 55 minutes of ultrasonic treatment (as detailed above), the mucilage seems to be totally extracted (Figure 1d).

3.2. Mucilage Extraction by cavitation in batch mode

With a simple mechanical stirring (1000 rpm) of the camelina seeds in water (10% w/w) during 55 minutes at ambient temperature, the mucilage extraction yield is limited at 4.5 ± 0.1 %. Mucilage extraction by cavitation is a method which could increase this value. This method has ever been used for several seeds: flaxseed (Fabre et al., 2015), arabidopsis (Zhao et al., 2017), chia (Castejón et al., 2017). Lab-scale probes are generally used, which allows a high energy concentration on a low diameter probe. For instance, using an ultrasonic probe of 13mm diameter, a pulse mode of 4 (20s OFF/5s ON), a volume of 100mL and the same seed concentration of 10%, the yield increases after 55 minutes to 6.6 ± 0.3 %. However, these probes are not adapted to high volumes. Large ultrasonic baths allow a good ultrasonic effect in the vicinity of the transducers but it gradually vanishes and the total volumetric power is generally very low. For example, with a 500W ultrasonic transducer linked to a 13mm probe, the maximal recommended volume is 250mL which allows a volumetric power of 2000W/L while a classical 100W cleaning ultrasonic bath of 3L only delivers 33W/L.

To upscale mucilage extraction, we introduced a low diameter ultrasonic tubular reactor with transducer rings placed regularly along the cylinder. The device used in this study allows an intermediate volumetric power of about 650W/L. It was first used with a mechanical stirrer in order to homogenize the ultrasound effect on the entire medium. The stirring rate was fixed at 1000 rpm to avoid sedimentation while seeds concentration (m/V) and ultrasound amplitude were 10% and 100% (corresponding to a power of 2000 W), respectively. Pulse mode was set at 4 (20s OFF/5s ON) to remain at temperatures below 60°C. Different samplings of around 5mL were made at different intervals. Each sample was immediately centrifuged (10s at 1350xg) with a low volume centrifuge (Wisd Wisespin CF-10) device to easily obtain an

aqueous supernatant phase and measure its refractive index and dry matter. After 55 minutes of ultrasonic treatment, the entire solution was rather filtrated to obtain a mucilage extract and partially demucilaginated seeds. Refractive index and dry matter evolutions from 0.1 to 0.9% can be correlated with the relation (14 points):

$$RI = 0.1283 \times DM + 1.3325 \quad R^2 = 0.9902 \qquad \textbf{(Equation 3)}$$

The extraction yield obtained with the ultrasonic tubular reactor (6.9 ± 1.5 %) can be compared to the value obtained with the ultrasound probe (6.6 ± 0.3 %). Measuring the refractive index of the aqueous phase at different intervals, it appears that the two ultrasonic methods give similar results (Figure 2). Considering a second order extraction kinetic model, the following equation can be postulated:

$$\frac{dc}{dt} = k.(C_{eq} - C)^2 \qquad \textbf{(Equation 4)}$$

k: second order extraction constant ($m^3.kg^{-1}.min^{-1}$), C: dry matter concentration (kg/m^3) and C_{eq} : dry matter concentration at equilibrium. Equation 4 can be simplified and the following expression can be plotted with good regression coefficients:

$$\frac{t}{c} = A + B.t \qquad \textbf{(Equation 5)}$$

With $A=\frac{1}{k.C_{eq}^2}$ and $B=\frac{1}{C_{eq}}$.

k=0.0086 $m^3.kg^{-1}$, C_{eq}=9.29 g/L, R^2=0.9926 with the tubular reactor.

k=0.0105 $m^3.kg^{-1}$, C_{eq}=9.41 g/L, R^2=0.9913 with the US probe.

3.3. Mucilage Extraction by hydrodynamic forces

In order to create a fluid circulation with supplemental shear stress, it was decided to associate the ultrasound tubular reactor with a recirculating pump. A plastic pipe with an inner diameter of 2.8 cm and a length of 3.15m was placed between the pump and the reactor. To first

determine the influence of the shear stress induced by the recirculating pump on mucilage extraction, ultrasounds were deactivated.

The hydrodynamic characteristics of the flow must be taken into account. Cavitation induced by recirculation will be neglected even if it may occur within the pump as in the vicinity of different obstacles or section changes in the water path. The precise determination of the influence of a shearing medium on a visco-elastic layer bound to a solid structure would require very complicated models. However, it may be interesting to evaluate the different forces acting on the seeds. The Reynolds number of the flow must first be determined:

$$Re_L = \boldsymbol{\rho}.\frac{\boldsymbol{V}.\boldsymbol{\phi}}{\boldsymbol{\eta}} \quad \textbf{(Equation 6)}$$

It depends on the diameter of the pipe ϕ (m), the fluid velocity V (m/s), its density ρ (kg/m^3) and its dynamic viscosity η (Pa.s). The diameter of the pipe is 28mm. The flow rate gives a fluid velocity of 2.7 m/s. Concerning the viscosity, as mucilage solutions have a shear-thinning behavior, it will depend on the shear rate. An approximated value of the shear rate is given by the formula:

$$\dot{\boldsymbol{\gamma}} = \frac{\boldsymbol{4Q}}{\boldsymbol{\pi}.\boldsymbol{r^3}} \quad \textbf{(Equation 7)}$$

Considering a nominal flow rate of 6 m^3/h, a value of 773 s^{-1} is obtained.

Concerning the particular Reynolds number, it is calculated with the formula:

$$Re_p = \boldsymbol{\rho}.\frac{\boldsymbol{U}.\boldsymbol{d_p}}{\boldsymbol{\eta}} \quad \textbf{(Equation 8)}$$

$U = V_f - V_p$ is the relative velocity of the particles (V_f : velocity of the fluid, V_p : velocity of the particle), d_p is the characteristic dimension of the particles.

Several forces can be exerted on the particles by the circulating fluid. The drag force is certainly the most important. It can be generally expressed as:

$$F_D = \boldsymbol{C_D}.\left(\frac{\rho.U^2}{2}\right).\boldsymbol{S_p} \quad \textbf{(Equation 9)}$$

C_D is the drag coefficient and S_p is the section of the particle perpendicular to the flow direction. The drag coefficient has different expressions according to the flow type and the particle shape. In the case of a Stokes flow (Re_p <1) around a spherical particle:

$$C_D = \frac{\boldsymbol{24}}{\boldsymbol{Re_p}} \text{ and } F_D = \boldsymbol{3\pi.\eta.U.d_p} \quad \textbf{(Equation 10)}$$

For a higher Reynolds number (1<Re_p<1000) the expression becomes:

$$C_D = \frac{\boldsymbol{18,5}}{\boldsymbol{Re_p^{0,6}}} \text{ and } F_D = \boldsymbol{18.5\rho^{0,4}.U^{1,4}.S_p{}^{0,4}.\eta^{0,6}} \quad \textbf{(Equation 11)}$$

The force is inversely proportional to the particular Reynolds number and will increase with the density, relative velocity, size of the particle and viscosity of the medium.

The relative velocity U of the particles strongly determines the amplitude of the shear stress encountered. It is yet not easy to obtain this value. Within whirlpools or boundary layers, the particle velocity can strongly decrease and U can be close to the fluid velocity.

If the seed is considered as an ellipsoidal structure, with a length around 2mm and a diameter around 1mm, it is possible to use an empirical drag coefficient of 0.3 (Çengel et al., 2017) to approximate the value of this force at approximately 1 mN.

Within the ultrasonic tubular reactor, with a much higher diameter, the velocity of the fluid is strongly decreased and maximum drag forces are inferior to those exerted within the pipe. If the centrifugal force is not taken into account (this force directly induces the movement of the fluid and it is hypothesized that seeds are not constrained within the centrifugal pump), the

main forces exerted on the particles will take place in the pipe. If the mucilaginous layer is considered, the volume occupied by a particle and its mucilage is strongly increased. This layer can't be considered as totally elastic but the section of the particles to be considered in the calculation of the forces may be increased. The new dimension of the particles can lead to forces reaching much higher values.

As drag force directly depends on viscosity, in order to change its value, different percentages of seeds were tested: 1%, 5%, 10% and 15%. Several samplings were performed at different extraction times to determine the evolution of the rheological properties and extraction yield with time and seeds concentration. The heat exchange within the pump and mechanic energy dissipation of the fluid induce a temperature increase which is independent on the seed percentage. Starting from ambient temperature, a temperature of 48-52°C was reached after 55 minutes of treatment.

With seeds at a concentration of 1%, the aqueous phase never develops an elastic behavior, as revealed by oscillatory rheometry and the viscosity, at a shearing rate of 773 s^{-1} reaches a value of 2.10 mPa.s after 10 minutes and do not change while dry matter concentration increases (Figure 3a). This low viscosity induces a high particular Reynolds number which is superior to 1000 and turbulence may occur. After 55 minutes of extraction, an extraction yield of 10.51 ± 1.8 % is achieved.

At a value of 5%, an elastic behavior appears at the end of the extraction time, with a loss factor remaining inferior to 1 (G'>G'') until approximately 2.5% of deformation. Particular Reynolds number remains inferior to 1000 and Equation 11 can be considered. Viscosity quickly increases in accordance with dry matter at the beginning of extraction then remains stable or decreases (Figure 3b). An extraction yield of 9.4 ± 0.4 % is obtained.

At a seed concentration of 10%, mucilage quickly gains an elastic behavior with a flow point increasing from 5 to 40% of deformation at the end of extraction time. The dry matter evolution follows three steps (Figure 3c): a quick increase until 10' then a plateau and a new evolution from 30 minutes to the end. Viscosity follows dry matter within the first 30 minutes then remains stable while dry matter strongly increases. The extraction yield is 9.1 ±0.3 %.

At a concentration of 15%, viscosity follows the dry matter until the very end of the extraction (Figure 3d). As observed for the other seeds concentration, a decrease in viscosity is observed while dry matter still increases. Particular Reynolds number decreases from 210 to 35 and hydrodynamic forces become gradually important and may change the conformation or the size of the polymers (polysaccharides and polypeptides). The extraction yield is 11.5±1.4 % and it is noteworthy that it seems to be even better than at lower seeds concentration. With the recirculating pump, extraction kinetic is different from that observed with ultrasound assisted treatments and seems to follow three steps (Figure 3d): a quick increase until 10 minutes of extraction then a plateau until 30 minutes and a new linear increase. This seems to obey a second order (Table 1) then zero order extraction kinetic and may correspond to a quick extraction of non-adherent mucilage then a slower extraction of the adherent one as internal compounds. It seems to correlate to stronger ultrasound treatments as observed in ultrasound assisted flaxseed mucilage extraction (Fabre et al., 2015).

Table 1. Parameters of the second order extraction kinetic in hydrodynamic extraction (first 30 minutes)

Seeds percentage	Ceq (kg/m^3)	k (m^3/kg)	R^2
5%	4.0	0.06	0.9958
10%	6.0	0.11	0.9987
15%	11.8	0.03	0.9900

During mucilage extraction, drag force may change due to an increase then decrease of fluid dynamic viscosity and of the thickness of the mucilaginous layer. Besides, mucilage dissolution induces a rheological behavior whose elastic component gradually increases,

which may improve force transmission but also decrease the relative velocity of the particles. It can then be predicted that mucilage extraction is directly linked to its rheological behavior. With ultrasound assisted extraction, high viscosities decrease the mechanical efficiency of ultrasound waves and diffusion rates of molecules, resulting in a decrease of the extraction yield with seed concentration. On the contrary, in hydrodynamic extraction, as long as the pump succeeds in developing a high flow rate, the increase of the dynamic viscosity with the percentage of seeds induce an increase of the hydrodynamic forces exerted on the seeds and the extraction yield can reach high values even with high seed concentration. The absence of ultrasounds allows a high viscosity during a longer time and a less pronounced hydrolysis of polysaccharides. If the filtration of the extract is more difficult, this method can yet be a good way to extract surface compounds from seeds or other materials at high load values. At a seed concentration of 15%, 30 minutes allow a good extraction yield. Increasing extraction time, the extraction yield reach values up to 11,5% but the medium contains seeds debris.

3.4. Combination of ultrasound and hydrodynamic forces

Different experiments were conducted associating ultrasounds and high flow rate recirculation. To discriminate the effect of both ultrasounds and recirculation on the extraction yield but also on the composition and rheological properties of the mucilage extracted, an experimental design was conducted. To allow a moderate viscosity and an ultrasound effect, the seed concentration was fixed at 10%.

With a constant flow rate and volume, three independent parameters were modified:

- Extraction time
- Ultrasound amplitude
- Ultrasound pulsation

The extraction time varied from 5 to 55 minutes, the amplitude of the ultrasounds varied from 10% to 70% and the pulse mode (p=time off/time on) was evaluated from p=2 to p=6.

The following experiments were conducted (Table 2).

Table 2. Variables and parameters of the conducted experiments according to Doehlert formalism

EXP.	Time (min)	Amplitude (%)	t_{off}/t_{on}	Time Coded value X1	Amplitude Coded value X2	t_{off}/t_{on} Coded value X3
1	55	40	4	1	0	0
2	5	40	4	-1	0	0
3	42,5	70,3	4	0.5	0.866	0
4	17,5	9,7	4	-0.5	-0.866	0
5	42,5	9,7	4	0.5	-0.866	0
6	17,5	70,3	4	-0.5	0.866	0
7	42,5	50,1	6	0.5	0.288	0.817
8	17,5	29,9	2	-0.5	-0.288	-0.817
9	42,5	29,9	2	0.5	-0.288	-0.817
10	30	60,2	2	0	0.577	-0.817
11	17,5	50,1	6	-0.5	0.288	0.817
12	30	19,8	6	0	-0.577	0.817
13	30	40	4	0	0	0
14	30	40	4	0	0	0
15	30	40	4	0	0	0

If ultrasounds can accelerate the temperature increase, it never exceeded 55°C.

3.4.1. Extraction yield

Comparing the yields of the different experiments, it appears that experiments 10 and 12 to 15, with 30 minutes of extraction time, have very similar yields. Experiments 4, 6, 8 and 11 with 17.5 minutes of extraction have also similar yields. 30 minutes of mucilage extraction without ultrasonic treatment allow an extraction yield just hardly inferior to an extraction with. After 55 minutes, the yields are a bit more different.

According to the Doehlert matrix, a model can be postulated:

$$Y=\mathbf{4.7}+\mathbf{2.6}t+0.2A-0.1P+0.5t^2+0.6A^2+0.8p^2+0.2tA-0.2tP \quad \textbf{(Equation 12)}$$

Significant parameters are shown in bold. The variance analysis, comparing calculated and experimental values, indicates a good representability of the above equation (P_1=1.15) while the comparison of repeated experiments gives a good validity of P_2=79.5.

The response surface representation for the extraction yield (Figure 4a), at constant pulse, shows an evolution mainly based on extraction time, despite different ultrasound amplitudes.

3.4.2. Rheological behavior at a concentration of 1%.

Solubilizing freeze-dried mucilage extracts at 1% in water (m/v), rheological tests were performed in rotational and oscillatory modes. With the first mode, it appears that all the mucilage solutions have a non-Newtonian, shear-thinning behavior, as observed elsewhere (Sanchez, 2014). Among the most correlated rheological models, the simplest is Sisko with a regression coefficient superior to 0,999.

$$\boldsymbol{\sigma = a.\dot{\gamma} + \dot{b}.\dot{\gamma}^{p}} \quad \textbf{(Equation 13)}$$

With

$\sigma : shear\ strain\ (mPa.s),\ \dot{\gamma} : shear\ stress\ (s^{-1}),\ a, b, p : constants$

It is possible to model the viscosity measured at a shear stress of $50s^{-1}$.

$Y=\mathbf{78.3-41.9t}-10.6A+1.5p-5.1t^2+\mathbf{54.3A^2}-5.8p^2-12.4tA+5.7tp+30.3Ap$ **(Equation 14)**

P_1=1.71; P_2=79.2.

Considering the response surface (Figure 4c), the viscosity seems to depend on both ultrasound amplitude and extraction time. The second order effect of ultrasound amplitude means that both low and high ultrasound amplitudes increase the viscosity of mucilage. Ultrasounds can decrease viscosity with the hydrolysis of macromolecules, but can also increase it with a better solubilization of proteins and polysaccharides.

With the oscillatory mode, mucilage solutions were submitted to different amplitudes of deformation, at a frequency of 1Hz. All solutions have a predominant elastic behavior until 30 to 90% of deformation. At a deformation of 0,5%, they are all within their linear viscoelastic region and it is possible to observe their behavior at different frequencies (from 0,01 to 1 Hz). All remain elastic in this zone with a constant gap between storage (G') and loss modulus (G''). At a concentration of 1%, this elastic behavior was expected as it has ever been observed at 0,5% from 0,1 to 10 Hz and at 0,1% at higher frequencies (1-10 Hz) (Li et al., 2016).

The loss factor (G''/G') can be modeled with the following equation:

$$Y=\mathbf{0,45+0,07t}+0,02A-0,01p+0,03t^2+0,03A^2-0,02p^2+\mathbf{0,06tA}-0,05tp+0,03Ap \quad \textbf{(Equation 15)}$$

$P_1=1,23$; $P_2=85.8$

According to the response surface (Figure 4d), at short extraction times, a high ultrasound amplitude allows a lower loss factor, perhaps due to the solubilization of large macromolecules. On the contrary, at long extraction times, high amplitude increases the loss of elasticity of the mucilage solution.

1.1.1. Protein content

With a protein determination according to Lowry titration, the protein content is expressed as equivalent BSA as the calibration is realized with this protein (Bovine Serum Albumen). This titration is then mainly useful for comparative goals. It appears that the BSA equiv. content of the different mucilages can have important variations. A model can be developed with the following equation.

$$Y=\mathbf{9.5+2.5t+1.3A}-0.6P+\mathbf{3.3t^2}+0.3A^2+\mathbf{1.9P^2}+0.3t.A-2.3t.P\mathbf{-2.5A.p} \quad \textbf{(Equation 16)}$$

$P_1 = 0.815$; $P_2 = 77.1$

The response surface (Figure 4b) indicates a strong influence of ultrasonic amplitude at short extraction time while this influences later decreases. At high ultrasonic amplitude, long polymeric chains can be partially hydrolyzed, fostering the solubilization of protein extracts and increasing the content in hydrosoluble proteins.

The treatment time, with a high flow rate circulation of the medium has a dominant influence on mucilage extraction, ultrasounds allowing a small increase of the yield but having an influence on the rheological behavior and composition of the mucilage solution.

As treatment time remains the preponderant factor for mucilage extraction, it was interesting to determine its influence on the amino-acid composition of the proteins extracted, monosaccharide composition of polysaccharides, as well as the ash content of this mucilage.

1.1.1. Amino-acid composition

Amino-acid composition was determined for mucilages extracted with different conditions (Figure 5). Short reaction time favors threonine, serine, glycine, alanine and lysine. If the relative glutamic acid concentration is considered, particularly after 30 minutes of treatment, it appears that ultrasound power and total ultrasonication time (as revealed by pulsation values) have a strong influence.

1.1.2. Monosaccharides composition

Three extracts are compared (Figure 6), one obtained by simple aqueous mucilage extraction at mild mechanical stirring during 30 minutes, one obtained with 5 minutes of high flow rate recirculation with 40% ultrasound amplitude and a pulsation of 4, then one obtained with 42.5 min of high flow rate circulation with 30% ultrasound amplitude and a pulsation of 2.

If mucilages extracted with a short recirculation time or with mechanical stirring have similar monosaccharides relative compositions (the extraction yields are: 3.64 ± 0.05 % and 4.21 ± 0.07%, respectively), the mucilage obtained with a long recirculation time has a higher

glucose content and a lower arabinose concentration. The increase in glucose content may come from a partial extraction of cellulose which is generally linked to the most adherent fraction of mucilage, as already observed for *Arabidopsis thaliana* (Macquet et al., 2007).

1.1.3. Ash extraction yield

Ash extraction yield mainly depends on extraction time (Figure 7). Concerning ash concentration in the mucilage, it is between 5 and 8 % and it appears that the mucilage extracted with the shortest extraction time is the most concentrated in ashes (8.0 ± 0.1%). This indicates that minerals are mainly dispersed at the beginning of the treatment.

2. Conclusion

Ultrasounds are an ideal methodology to concentrate the shear stress at the surface of the seeds and decrease the extraction time. However, at a pilot scale, the use of ultrasonic probes is uneasy and ultrasonic transducers must act on a short distance. Pilot scaling can be accomplished by a tubular reactor. Used in continuous or recirculation mode, this method allows an extraction process which proceeds both by cavitation and hydrodynamic forces. High velocity fluid circulation seems to allow high extraction yields that can compete with those obtained with ultrasounds, the addition of an ultrasonic treatment allowing both high extraction yields and ease of filtration. Besides, ultrasound amplitude has an influence on protein content and rheological behavior of the mucilage. Playing with seeds concentration, ultrasound amplitude and extraction time, it would be possible to control the extraction yield and some physical and chemical properties of the mucilage in order to meet the needs of different food, pharmaceutical or technical applications.

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Figures captions

Figure 1. SEM photographs of a camelina seed a) cut of the seed coat with the mucilage layer b) Detail of the surface of a non-treated seed c) After hydration and partial release of mucilage d) After ultrasonic treatment in water.

Figure 2. Evolution of the refractive index of the mucilage extracts with treatment time.

Figure 3. Evolution of the dynamic viscosity at 773 s^{-1} and dry matter of mucilage extracted with a seed concentration of a) 1% b) 5% c) 10% and d) 15%.

Figure 4. Response surface graphs according to extraction time and ultrasound amplitude

Figure 5. Amino-acid composition of different mucilage extracts classified according to their relative glutamic acid content.

Figure 6. Monosaccharides composition of different mucilage extracts.

Figure 7. Evolution of ash extraction yield with treatment time according to different ultrasound amplitudes.