Micro-pressing of rapeseed (*Brassica napus* L.) and *Arabidopsis thaliana* seeds for evaluation of the oil extractability

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Abstract: *Pressing is a crucial step in the crushing process of rapeseed seeds, regarding its major effect on the oil extraction yield, the energy consumption and the quality of the meal. In order to study and model in a rigorous way the behaviour of rapeseed seeds, and the oil extraction during pressing, the potential of a micro-pressing technique using a instrumented micro press adapted to quantities of seeds as low as 10 g for rapeseed and 3 g for* Arabidopsis thaliana *was examined and discussed. Using a phenomenological model, data from the pressing process and the material behaviour (compressibility modules) were obtained with a good precision, highlighting small differences between samples. The well-known positive effect of the temperature on the oil extraction yield was confirmed with* A. thaliana. *Micro-pressing of ground and cooked rapeseed seeds did not lead to the results usually reported in the literature for continuous pressing. The results strongly suggest that the performance of the static micro-pressing is related to the macro-and micro-structure of seeds and is less sensitive to the moisture than continuous pressing. Further experiments are needed to confirm that the micro-pressing could be an effective tool for predicting the extractability of oil and therefore, contribute to plant breeding programmes in the future.*

Key words: *micro pressing, oil extractability, rapeseed,* Arabidopsis thaliana

Introduction

From 2006 to 2008, the capacity of the crushing industry in France increased from 3.7 to 6.2 million tons per year to meet the growing demand of oil for manufacturing bio diesel. Rapeseed represented 63% of oilseeds crushed in France in 2007, compared to soybeans (9%) and sunflower (28%). The large quantities crushed and the difficulties still encountered in the process for rapeseed highlight the improvement of the oil extractability as an important issue. The expected benefits concern energy savings, yield and quality of products (oil and cake) and more generally, the cost of seed processing. The process of industrial crushing for rapeseed implements several sequential treatments: flaking, cooking, pressing, solvent extraction and desolventisation. The pressing stage can be considered as critical since its efficacy to improve the solvent extraction depends to several factors such as moisture, maturity, composition, thermal or mechanical pre treatment (flaking, cooking) of the seeds. In addition, the quality of the expeller cake obtained after pressing will affect the solvent extraction since the mechanical strength and the porosity are crucial for the efficacy of the solvent percolation (Laisney, 1984). The control of pressing is then a major issue in the efficiency of the crushing process.

The optimization of pressing in terms of industrial production remains difficult because the crushing plants are equipped with screw presses with continuous flow capacities of several tons per hour. As a consequence, it is very expensive or virtually impossible to assay at such scales, various experimental conditions or new plant material when available in small quantities. The aim of this work is to consider the potential of the pressing technology operating at a micro-scale (micro-pressing) to provide a tool to evaluate the pressing ability of small quantities of seeds. These data could then be used during breeding programmes. Many studies have been conducted from small presses with continuous flow or not, to find and isolate the effects of several operating parameters. These systems were used to record deformation profiles of volume at constant pressure, which could then be modelled (Lanoiselle and Bouvier, 1994).

In this work, the goal was to apply the micro-pressing technique on the seeds of rapeseed on one hand and those from *Arabidopsis thaliana*, a model *Brassicaceae*, on the other hand. The characteristics of the pressing were determined using a mathematical model and the adequacy of the micro-pressing technique to study the expression of oil from rapeseed seeds and *A. thaliana* were questioned. One genotype of *A. thaliana* and two genotypes of rapeseed were tested as well as, for rapeseed, the effect of cooking and grinding pre-treatments.

doi: 10.1684/ocl.2010.0300

Article disponible sur le site http://www.ocl-journal.org ou http://dx.doi.org/10.1051/ocl.2010.0300

Materials and methods

Plant material

Seeds of wild-type A. thaliana (ecotype Columbia, Col) were multiplied at INRA Rennes (France). Arabidopsis plants were routinely grown in a greenhouse (16-h photoperiod, 10-15 °C night/20-25 °C day temperature) on Traysubstrat 092 (Klasmann-Deilmann, Bourgoin Jallieu, France). Seeds were harvested when maturity was completed.

Seeds from Brassica napus Goéland (winter type variety) and Westar (spring type variety) were produced in the fields by INRA Rennes in 2006 for Goéland and 2008 for Westar. Both genotypes were double low varieties (i.e., zero erucic acid and low glucosinolate content). For Goéland, four seed lots were prepared in response to the following pre treatments: the FGS lot (fresh ground seeds) was ground in a blade mill (type IKA), the CWS lot (cooked whole seeds) was cooked in an oven at 110 °C for 90 min, the GCS lot (ground and cooked seeds) was ground and cooked, and the FWS lot (fresh whole seeds) did not get any treatment.

MicroPress

The device consisted of a cell linked to a pressing texturometer (Ta.Hdi Stablesmicrosystems, Surrey, UK). The texturometer was used as a source of strength and was equipped with a force capacity sensor of 4903 N (corresponding to a pressure of 100 bar) which an accuracy of 10 N allows to know the force applied during pressing and a step by step motor to monitor the movement of the piston (accuracy 0.001 mm) during compression.

Temperatures between ambient and 80 °C were applied by a heated collar on the pressing cell *(figure 1)*. The capacity of the pressing cell was approximately 10 g of seeds, but smaller quantities were tested due to the low amount of seeds available for some samples. Because the seeds of A. thaliana were very small *(table 1)*, the press was adjusted to the level of mesh sizes of the frits.

Measurements of pressing yields were obtained from weighing seeds before pressing, then from fractions of oil extracted and pressing cakes. Because of the occurrence of oil losses in dead volumes, the most accurate measurements were obtained from the mass of the pressing cakes. Depending on the type of seeds, the assays were performed at room temperature (~20 °C) or 50 °C. The speed of the piston prior to the phase of constant pressure pressing was set at 0.1 mm/s, the pressure record at 100 bar and the pressing time at 1 hour. For each sample tested, two replicates were performed.

Pressing modelling

The pressing experimental curves representing the piston displacement as a function of pressing time were modelled using the phenomenological model of equation (1) developed by Lanoiselle *et al.* (1996).

$$h = \frac{h_\infty}{\sum_{i=1_0,1,\ldots,n} \frac{1}{G_i}} \left[\sum_{i=1_0,1,\ldots,n} \frac{1}{G_i} \left(1 - e^{-v_i t}\right) \right] \qquad (1)$$

This model considers the oilseed cake as a sum of volumes nested inside one another, each having a resistance to the flow of oil. Thus, the pressing can be seen as the consolidation of three volumes (intra cellular, extra cellular and extra particle). The method used for this model involves compressibility modules (G_1, G_2 and G_3) associated with each type of volume. An additional module (G_{1o}) is used to represent the primary consolidation of the cake, i.e. the occupation of the entire volume by the solid and the liquid (Savoire *et al.*, 2008). *Figure 2* shows the gradual decomposition of an experimental pressing curve with the different compressibility modules given by the model represented by exponential functions. We note that the parameters with indexes 1o to 3 describe successively different parts of the curve. The combination of these four phases allows a complete description of the experiment. Depending on the type of seed, the pressing time used may not allow to reach the final phase of consolidation. In this case, the model only needs three exponentials, instead of four, to describe the experimental behaviour.

Micro-pressing tests

On rapeseed: to get closer to industrial conditions for pressing rapeseed, the tests were conducted at 50 °C using the heating regulated device fitting the Micro Press. The study focused on: a) the evaluation of the

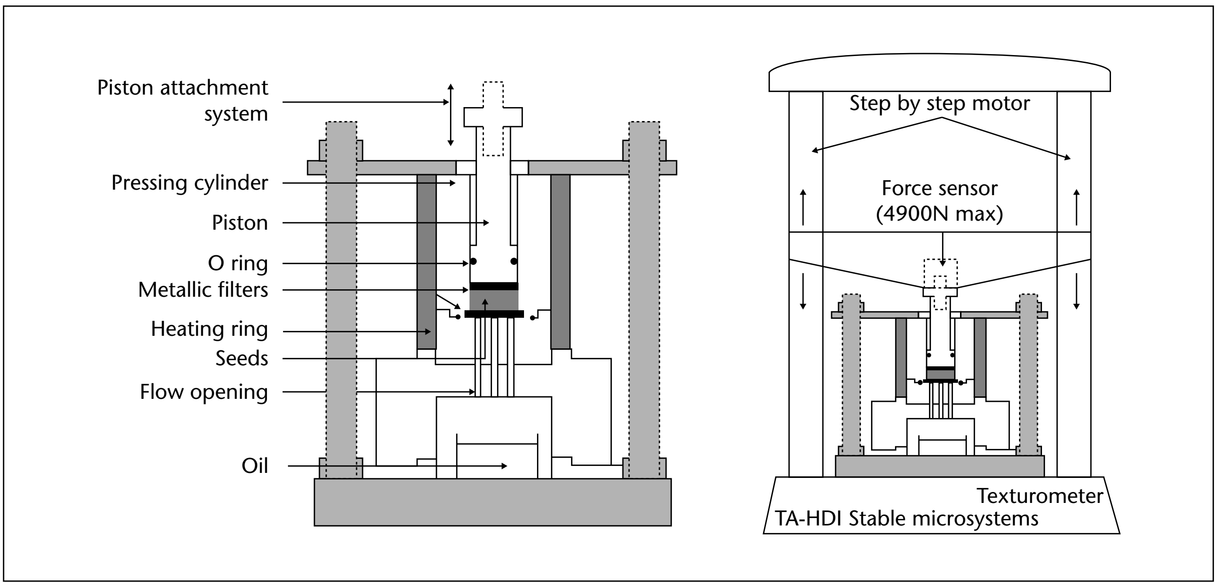

Figure 1. *Diagram of the pressing cell*

repeatability of the tests and the uncertainty of the oil extraction yield, b) the comparison of different pre-treatments (grinding and cooking) of the seed, c) comparison of seeds from two different genotypes (Westar and Goéland) without grinding and cooking pre-treatment.

On *Arabidopsis thaliana* seeds: the tests consisted in adapting the equipment (frits) to the seeds of *A. thaliana* which particularity is to be very small, and then compare the behaviour of seeds of two different populations (Columbia harvested in 2007 and 2008).

Table 1. *Characteristics of seeds from rapeseed (*Brassica napus *L.) and* Arabidopsis thaliana*.*

Seeds	*Brassica napus* L.	*Arabidopsis thaliana*
Mass (mg)	~ 5	~ 0.02
Size (mm)	2-3	0.4-0.5
Shape	Spherical	Flat
Oil content (%)	~ 45	30-40

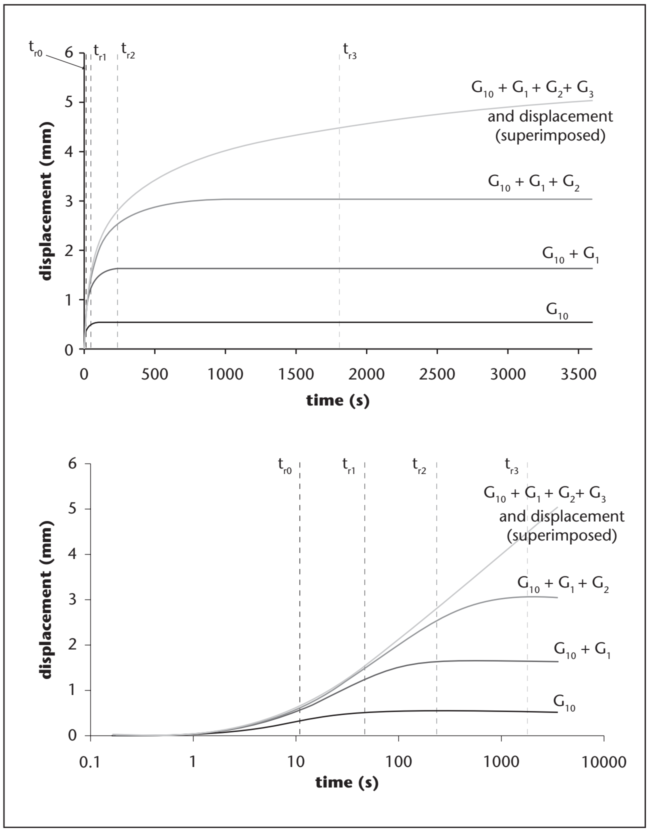

Figure 2. *Successive decomposition of experimental modelling curves (top: linear scale; bottom: log scale). Taking into account the four compressibility modules, the piston displacement can be linearly represented on a logarithmic scale*

Results and discussion

Test validity

The repeatability of measurements of micro-pressing parameters was tested on seeds of *A. thaliana (table 2)* and rapeseed *(table 3)* with several trials in the same conditions. The yield of oil extraction is measured by weighing the seeds then the pressing cake. The uncertainty on the performance is mainly due to non recovery of the entire cake before weighing and to the absorption of the oil by the cake when the pressure of the piston is released. These two errors are partially balanced by each other and the resulting error is approximately 0.1 g, corresponding to 1.5% of the mass of cake from rapeseed (~7 g) and 3% of the mass of cake from rapeseed of *A. thaliana* (~3.5 g).

Micro-pressing of rapeseed seeds

Micro pressing experiments were conducted on rapeseeds seeds (Goéland) which oil content was 49% of dry matter. The oil extraction yields (expressed as the ratio oil/seed) ranged from 13.0% to 26.5% corresponding to 27.7% to 56.7% of the total oil content of the seed *(table 4)*. Assays confirmed that the thermal pre treatment of the seeds influences the moisture content and the extraction yield. Cooking without crushing had the greatest effect since the yield decreased almost twice. Grinding associated with cooking brought this performance to a value close to the yield obtained with untreated seeds (WFS), but significantly lower. The grinding alone, when not followed by heating, did not cause any effect on the yield which remained the same as on untreated seeds.

This experiment shows the ability of micro-pressing to highlight the change of the properties of rapeseed induced by treatments prior to pressing. The cooking treatment usually improves the performance of pressing (Singh *et al.*, 2002) because of the alteration of oleosomes (which store oil as discrete droplets stabilized by a half membrane containing specific structural proteins, oleosins). In this experiment, the extraction yield is reduced by the cooking treatment; a hypothesis

Table 3. *Mean values and standard deviations of the modules compressibility measured in twelve micro-pressing experiments on rapeseed (45 bar, 50 °C, 55 min; tests spread over a month).*

Compressibility module	Mean (bar)	Standard deviation (bar)	Relative Standard deviation (%)
G_{1o}	509	22	4.3
G_1	353	21	5.9
G_2	314	20	6.4

Table 2. *Values (bar) of the compressibility modules measured in three experiments of micro-pressing with* Arabidopsis thaliana *seeds (100 bar, 50 °C, 60 min; tests spread over a day).*

Compressibility module	Test 1	Test 2	Test 3	Mean	Standard deviation	Relative Standard deviation (%)
G_{1o}	429.9	421.7	419.4	423.6	5.5	1.3
G_1	728.6	768.2	745.5	747.4	19.9	2.7
G_2	945.9	984.3	1002.7	977.6	29.0	3.0
G_3	926.6	932.4	988.3	949.1	34.0	3.6

of such behaviour could be the lost of moisture which modifies the plasticity of the material and makes inefficient the pressing at 100 bar. In the case of ground seeds, the cooking-drying treatment had only a slight effect, the plasticity of the material being ensured by the grinding.

Difference observed between our experiments and published results obtained on a continuous pressing process (Singh *et al.*, 2002; Singh and Bargale, 2000) could be explained by the facts that:

a) micro-pressing is done in batch in a static press with a limited pressure (100 bar) and not in a continuous flow press where shear is provided and moisture has a great effect on the plasticity of the conveyed material and the plug (cooking leads to a lower content of moisture and a higher hardness of the material);

b) the seeds were not pressed directly after the cooking treatment and the storage led to a cooling and then to the loss of the temperature effect on the oil viscosity.

The study of the pressing profiles and the calculation of the compressibility modules for these four types of samples showed that the kinetics of oil extraction, as illustrated by the displacement of the piston, were very different depending on the pre-treatment applied *(figure 3)*. For GCS the curve ended in a plateau illustrating the phenomenon of consolidation while WFS, WCS and especially GFS did not seem to have reached this plateau of consolidation. Different values of h_∞ (height of the cake theoretically achievable in a pressing of infinite duration) ranged from 2.15 mm (WCS) to 5.46 mm (GFS) were also observed *(table 5)*.

This difference in behaviour during pressing was also highlighted by the compressibility modules. The cooking treatment tended to increase the compressibility modules while grinding affected mainly the parameter G_{1o} (primary consolidation of the extra particle volume). The combination of cooking and grinding did not change G_{1o} from simple cooking. On the other hand, it reduced the compressibility modules of extra particle and extra cellular volumes. Interestingly, the global compressibility module G_g decreased as performance increased and seemed to characterize the ease of oil extraction *(table 5)*.

Since the maximum yield was achieved with whole or ground fresh seeds (WFS, GFS), the next experiments were carried out in the simplest way, without grinding and cooking treatments.

The comparison of oil extraction yields obtained with samples from varieties “Westar” and “Goéland” showed large differences *(table 6)*. They could be explained by the genetic effect, the harvest year (2006 for Goéland and 2008 for Westar) and the composition (moisture and oil content). The measurements of the global compressibility modules were found to be of the same order of magnitude in both cases (200 bar for Westar and 211 bar for Goéland).

The micro-pressing method is then a method capable to discriminate oilseeds for their behaviour in pressing.

Micro-pressing of A. thaliana *seeds*

Micro-pressing seeds of a wild-type ecotype from *A. thaliana* demonstrated the ability of this technique to study the behaviour of such seed material. With the micro-press used, 0.3 to 0.9 g of oil was extracted from seeds initially containing 42.9% which corresponds to extraction of 40 to 74% of total oil within the seeds.

Seeds of wild *A. thaliana* harvested in 2007 and 2008 were tested to assess oil extraction yields and compressibility modules *(table 7)*. Yield

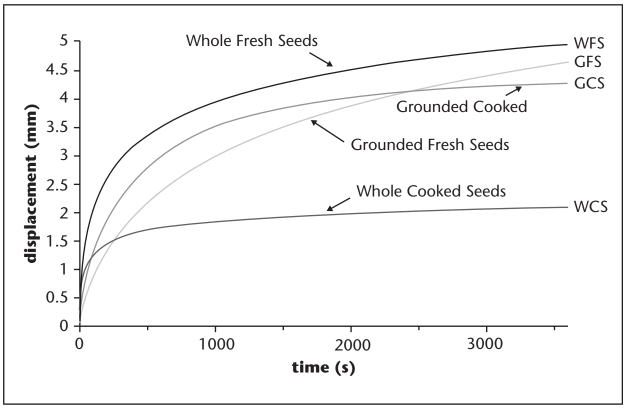

Figure 3. *Pressing profiles of the different rapeseed samples*

Table 4. *Oil extraction yields (%) after micro-pressing of rapeseed seeds (Goéland) treated by grinding and/or cooking (oil extraction yield = mass extracted oil/mass seeds).*

Treatment	Sample		Moisture (%)	Extraction yield %		
				Test 1	Test 2	Mean (1)
None	Whole fresh seeds	WFS	7,60	24.8	26.5	25.7[a]
Cooking	Whole cooked seeds	WCS	3,36	13.6	13.0	13.3[b]
Grinding	Ground fresh seeds	GFS	7,30	25.0	25.6	25.3[a]
Grinding and cooking	Ground cooked seeds	GCS	3,47	22.2	22.4	22.3[c]

(1) yields with different letters are significantly different.

Table 5. *Compressibility module and h_∞ values measured during the micro-pressing of rapeseeds pre treated by grinding and/or cooking (h_∞: height of the cake theoretically achievable in a pressing of infinite duration).*

Treatment	Seed sample		h_∞ (mm)	Compressibility modules (bar)			
				G_{1o}	G_1	G_2	G_g
None	Whole fresh seeds	WFS	5.26	1036	620	462	211
Cooking	Whole cooked seeds	WCS	2.15	1692	1312	1738	518
Grinding	Ground fresh seeds	GFS	5.46	3351	749	300	201
Grinding and cooking	Ground cooked seeds	GCS	4.64	1717	432	824	243

Table 6. *Extraction yield (in %) of oil by micro-pressing whole fresh rapeseed seeds from Goéland et Westar cultivars (oil extraction yield = mass extracted oil/mass seeds).*

Harvest year	Sample	Cultivar	Extraction yield %		
			Test 1	Test 2	Mean
2006	G1	Goéland	24.8	26.5	25.7
2008	W1	Westar	17.7	17.3	17.5
2008	W2	Westar	16.0	15.4	15.7

Table 7. *Micro-pressing of wild-type seeds of* A. thaliana *(Columbia): extraction yield of oil and compressibility modules values (oil extraction yield = mass extracted oil/mass seeds).*

Harvest year	Sample	Pressing temperature	Yield (%)	Compressibility modules (bar)				
				G_{1o}	G_1	G_2	G_3	G_g
2007	Col-1	20°C	10.2	120	786	880	1000	236
2008	Col-2	20°C	16.2	910	604	814	1050	203
2008	Col-2	50°C	29.8	812	480	620	800	162

differences by 6% between the two years were observed and we note that the higher the global compressibility module G_g, the lower performance of extraction. The seeds of the same variety harvested in 2007 and 2008 showed very different compressibility modules (G_{1o}) corresponding to the primary extra particle volume. These results demonstrated the strong impact of the nature of the raw material on pressing performance.

Pressing *A. thaliana* seeds at 20 °C and 50 °C was experimented to determine the effect of the temperature increase on the expression of oil and to compare results with experiments carried out on rapeseed seeds. Increasing the pressing temperature from 20 °C to 50 °C significantly improved the extraction efficiency *(table 7)* as reported by many authors in the literature (Singh and Bargale, 2000; Laisney, 1992; Khan and Hanna, 1983; Willems *et al.*, 2008). This increased performance was accompanied by a decrease in the global compressibility module (G_g) from 203 to 162 bar. If confirmed, this result could indicate that G_g is a good indicator of the oil extractability using pressing.

Conclusion

Our results showed that pressing of rapeseed and *Arabidopsis thaliana* seeds can be carried out at a micro scale (3 to 10 g) with a specific equipment. Despite the noticeable difference between seeds of rapeseed and *A. thaliana*, the mechanical part of the press (pressing cell and frit) was efficient to separate the oil from the cake. The micro-press measured the oil extraction yield and provided several data characterizing the pressing operation and the material behaviour. Results were obtained with enough precision for highlighting small differences between samples.

The modelling of the piston displacement allowed the determination of the values of different compressibility modules that could be related to the macro- and micro-structure of the cake. These measurements were found reproducible and sensitive to the nature of the seeds and their pre-treatment. The pressing profiles and the kinetics of the compressibility modules were obtained for rapeseed and for *A. thaliana* although their seeds characteristics (size, mass and shape) were very different.

The well-known effect of the pressing temperature on the oil extraction yield was confirmed with *A. thaliana*. Results obtained with the rapeseed seeds after cooking and grinding treatments showed that static micro pressing and continuous pressing are sensitive to different factors and that micro pressing is not able to directly simulate the global continuous pressing process used in industrial plants. Nevertheless, the hypothesis that micro pressing data are related to the macro- and micro-structure of the seeds could be used to explain an important part of the behaviour of the seeds when pressed. Research is in progress to merge data from micro pressing, chemical analyses, microscopy, continuous pressing at several scales in order to predict the oil extractability of rapeseed seeds. The added value of micro-pressing to breeding programmes should then increase in the future to evaluate the ability of cultivars to be easily crushed. It might be then possible to get insights into the effects of genetics on the oil extractability with the aim to minimize energetic inputs in the extraction process. ■

Acknowledgment. This work was supported by a grant from the Agence Nationale pour la Recherche (ANR) Génoplante "Genobodies" (GNP-05063G).

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