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THERMO-MECHANICAL FRACTIONATION OF GREEN BIOMASS

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Abstract: Green biomass can efficiently be separated into a fiber rich fraction and a green juice with the TAMD process. This process combines a mechanical pressing and a moderate heating of the walls of the apparatus. This work aims to investigate the influence of the biomass water potential on the kinetic and to identify the optimal processing conditions. If the TAMD process is used as a pre-treatment for fodder dewatering, 70°C and 300 kPa are the optimum conditions to reach a high separation yield (+25.5% compared to current processes) in short times. For biorefining purpose, a lower wall temperature is required.

Key words: Dewatering, Process Intensification, Alfalfa, Water Potential, Extraction Yield

1. Introduction

As the ruminant livestock sector becomes less attractive to farmers and much of the lands under grass are unsuitable for alternative arable use, a surplus of grassland biomass is predicted in many regions of Europe [1, 2]. Green biorefineries (GBR) were identified as potential sustainable tools for processing these 'nature-wet' raw materials. GBR focus on valuable intermediates or end-products, derived from the processing of a fiber-free extract containing the protoplasmic fluid - the green juice – or a fiber-rich solid fraction - the press cake. Among the most promising derivative products from the green juice, let us quote speciality feeds for pigs and hens recovered from the green protein phase [3, 4], white proteins for foams, foam stabilizer or films recovered from the soluble protein phase [5], aminium lactate produced by fermentation of the deproteinizied green juice [6], polylactic acid [7] or even heat and power [8] coming from lactic acid and water soluble extracts used as raw material in a fermentation process. Fibres are usable as foodstuff for animals [9, 10] but can also be used as blow-in insulation products and insulation boards [11], as materials (pots and mulch boards) used in horticulture [12] or as combustion pellets [8, 11]. Several biorefining initiatives are reported or scheduled in many European countries [2, 5, 13, 14] while techno-economic studies aim to establish the conditions of profitability of GBR systems [5, 12, 15].

Mechanical fractionation is usually the first unit operation in a green biorefinery plant. So far, green juice extraction involves mechanical pulping to disintegrate the cell walls followed by pressing [16], possibly with multistage addition of water and expression following the first pressing [17]. Refiner and screw press are usually used. Successful separation with an extruder press is also reported [18]. Between 40 and 60% of the total available water, 50% of

the total amount of lactic acid and 23% of the total available amount of proteins go into the press juice while 95% of the total available fibers are recovered in the press cake [16, 18, 19]. Higher water separation yield can be achieved through process intensification. Intensification of mechanical dewatering can take several forms: simultaneous application of an electric field [20, 21, 22, 23], superimposition of ultrasounds [24, 25] or with heat supply. Combination of pressure and temperature can be envisaged during the filtration stage only (if any), the expression stage only, during both regimes, as a pre- or a post-treatment [26, 27, 28, 29, 30, 31]. Various processes were experimented at lab and pilot scales for paste-like products, like sewage sludge, and biomaterials, like lignite and bagasse. But, most of the time, the operating conditions lead to a vaporization of the solvent. For instance, the Mechanical Thermal Expression (MTE) process, first investigated in the mid 1990s by Strauss and coworkers [27, 28] and recently extended by Hoadley and coworkers in Monash University [30, 32], comprises a preheating step prior to filtration and consolidation in its current design. The processing temperature is set above the normal boiling point of the water. To prevent evaporation, the process is held under a sufficient back pressure. Consequently, upon exposure to atmospheric pressure, a flash evaporation occurs and contributes to further moisture reduction. The main part of the moisture reduction was attributed to the collapse of the internal material porosity [33, 34]. If thermal mechanical dewatering method is not a brand new technology, the operating conditions of the thermally assisted mechanical dewatering (TAMD) process [35] and its use for the mechanical fractionation of the herbaceous biomass [36, 37] are quite innovating. The TAMD process operates at low pressure (usually 300 kPa) with a moderate heating of the press walls ($T_{wall} \leq 80°C$). The TAMD process proved to be especially efficient for green biomass. For instance, up to 83% of the inherent liquid fraction can be removed from alfalfa. The energy consumption of thermally assisted mechanical dewatering processes ranged from 5 to 30% of the latent heat

of vaporization depending on the operating conditions and the biomass [30, 36, 38]. For alfalfa dehydration, the energy consumption of the TAMD process was assessed to 205 kJ/kg of the recovered juice, that is to say 8.5% of the latent heat of vaporization of water [36]. In a great number of countries, thermal drying of the fresh or pre-wilted fodder is an alternative method of forage conservation. Even for pre-wilted fodder, the main weakness of this industry is the high energy consumption of the dryers, inducing high production costs (compared to soya production costs for instance) but also high carbon footprint. The inherent energy requirement for drying is thus significantly reduced (-46%) when the feed is dewatered with the TAMD process [39], thanks to the increase of the dry solid content of the feed at the dryer inlet. In addition, implementation of the TAMD process prior to drying could contribute to turn existing dried fodder processing plants into biorefineries, diversifying the alfalfa products and increasing the sales turnover. So far, mechanical pressing is only used to extract the juice for poultry feeding in a few alfalfa drying factories.

The dehydration yield depends mainly on the processing temperature [36] while the kinetic depends on the processing temperature and sometimes on the applied pressure [40]. Especially, at low processing temperature ($T_{wall} < 70°C$), the processing pressure has a crucial influence on the moment when the juice begins to flow out. The latency time seems to be correlated with the hydric status of the plant: the driest the biomass, the longer is the waiting period. For the transition from batch to continuous processing, the interaction between the plant water status and the processing conditions needs to be clarified. The present study aims (1) to clarify the role of the plant water status on the juice extraction kinetics, (2) to suggest some dewatering mechanisms and (3) to identify the optimal processing conditions for dewatering purpose and/or for biorefining purpose. After a description of the experimental device and analytical protocols, the influence of the processing conditions on the dewatering

kinetics, the water extraction yield and the green juice composition are analyzed in section 3. Finally, the optimal conditions for dewatering and biorefining purposes are given as concluding remarks.

2. Materials and methods

2.1 Thermally assisted mechanical dewatering press

At the laboratory scale, the TAMD process consists of a compression cell inserted CARVER® hydraulic press (Carver Inc., Wabash, United State), which has a maximum pressing capacity of 14 800 kPa and provides the pressure required to progress a downward expression. The cell (Figure 1) includes a compressive piston, a hollowed cylindrical vessel, a filter medium and a collector. The compression chamber has a diameter of 148 mm and a maximum height of 60 mm. In spite of its low mechanical resistance, Teflon™ was selected as constitutive material of the vessel walls to minimize the frictions with the piston. Consequently, a stainless steel external jacket is added to ensure the mechanical resistance of the unit. The cell is fitted with a planar metal medium, with square pores of side 300 μm. To investigate the influence of a heat supply, three electric resistances are inserted in the upper part of the compressive piston, made of copper to reduce thermal inertia. Each resistor can supply up to 350 W. A temperature sensor, set in the piston, is used for the temperature regulation of the copper block. The accuracy of the sensor with its acquisition line is estimated at ±0.03°C.

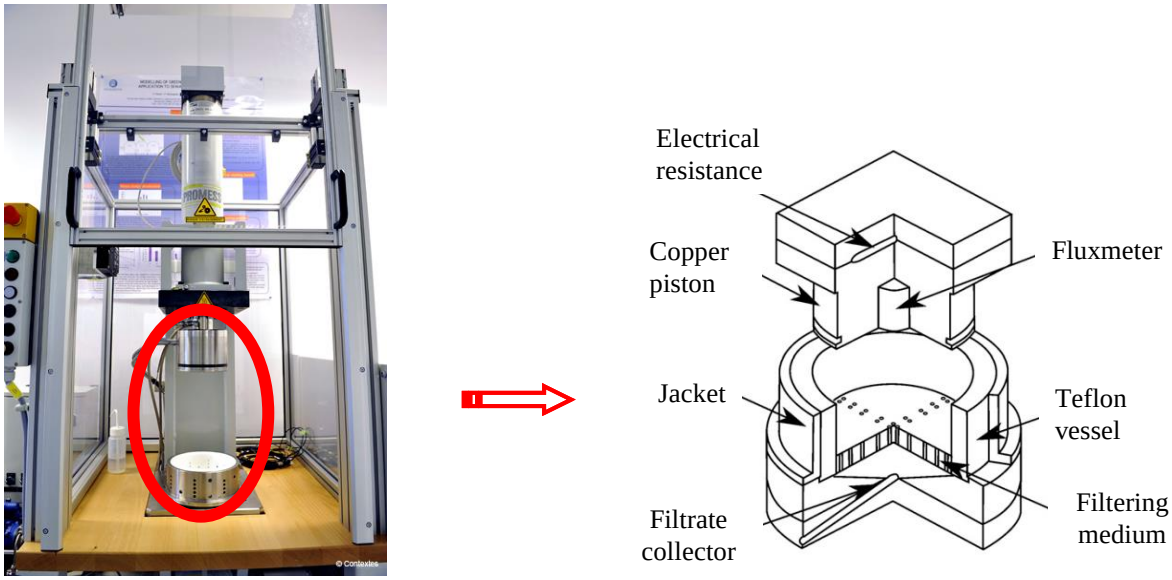

Figure 1 – TAMD process

2.2 Feed material and TAMD procedure

Alfalfa, also known as Lucerne in Europe, was selected as raw material for the present study. With a dedicated area of about 30 million hectares worldwide and an estimated annual production [41] of 436 million tons, alfalfa is the major forage crop in temperate regions. Indeed, this perennial legume, in the pea family Fabaceae, has the highest feeding value of all common hay crops, with high protein content and highly digestible fiber. Fresh alfalfa (Medicago sativa L.) was field-chopped manually. Samples were taken at weekly intervals between mid-May and mid-June. The fresh alfalfa was immediately transported to the laboratory and stored at 4°C in a tight container within 1h after the harvest. Dewatering experiments were carried out in the two days following the harvest.

The mechanical fractionation protocol was as follows. About 200 g of fresh alfalfa were chopped into 5-cm pieces and introduced at room temperature into the TAMD device. At t=0, a constant pressure was applied through the compressive piston, previously heated at the selected operating temperature (30, 50 or 70°C). The biomass was progressively separated

into a green juice and a press cake. The filtrate recovered in the collector flowed out into a container laid on the computer interfaced balance. The mass of filtrate was recorded at set time intervals of 1 s. At the end of the experiment, the press cake was weighed. Traditionally, juice extraction involves mechanical pulping followed by pressing. To assess the performances of the TAMD process, some samples were macerated in a blender and dewatered at 300 kPa and ambient temperature. In the following, 'Pulp' will indicate the minced alfalfa while 'Raw' will refer to the chopped alfalfa.

2.3 Plant water status characterization

Oven-drying at 105°C for 24h is used for the determination of the dry basis moisture content, W. According to literature [42], moisture content often fails as an indicator of the plant water status and the concept of water potential, ψ_w, has proven to be more useful in understanding water movement in plants. Plant cell consists of a gel-like substance, called the protoplasm, bounded on the outside by the plasmalemma, a selectively permeable membrane, and the cell wall. Difference in solute concentrations across the plasmalemma (generally a dilute solution on the outside and a concentrated solution on the inside) causes net flow of water toward the side with lower water concentration. ψ_w, expressed in Pa, is thermodynamically defined according to equation (3):

$$\psi_w = \frac{\mu_w - \mu_0}{\overline{V}_w} \qquad \text{(eq. 1)}$$

where μ_w is the chemical potential of water in the solution (kJ/mol), μ_0 the chemical potential of pure water at the same temperature (kJ/mol) and $\overline{V}_w$ is the partial molar volume of water (m^3/mol).

Rather than the thermodynamic definition, the following relation is usually preferred:

$$\psi_w = \psi_s + \psi_p + \psi_m + \psi_g \qquad \text{(eq. 2)}$$

Where:

- ✓ ψ_s, called the osmotic potential, represents the effect of all dissolved molecules (as long as they do not precipitate);
- ✓ ψ_p, the pressure potential or turgor pressure, takes into account the effects of all external pressures build up in the cell liquid thanks to the walls (usually positive in a living plant);
- ✓ surface effects resulting from the interaction between water and porous solids are included in ψ_m, called the matrix potential;
- ✓ and ψ_g represents gravity effects. It can be neglected as long as plant heights are lower than 1m.

Usually, ψ_s and ψ_m are negative and ψ_p does not compensate them so that water in plants generally has a negative ψ_w. Increasing solute concentration lowers the water potential while applying an external pressure above atmospheric or hydrating the wall matrix increases ψ_w. ψ_w determines the direction of water exchanges when a water potential difference exists between the two sides of the plasmalemma: water runs out towards more negative ψ_w.

Pressure chambers are the most widely used tools for measuring ψ_w. Excising a leaf opens the xylem to the atmosphere. As a result, the xylem solution retracts to cross walls where sufficiently small pores exist to prevent solution from retracting farther. Pressurizing the tissue increases the water potential and forces water to return to its initial position. Pressure chambers are based on this concept [43]. A leaf is sealed into the top of the chamber, in such

a way that a small amount of the xylem extends outside through the top (Figure 2). Gas is introduced into the chamber, increasing the pressure in the chamber and uniformly pressurizing the tissue. This raises the protoplasm water potential above that of the xylem and water flows inside the xylem. At equilibrium, when the xylem solution forms a stationary flat film without any excess on the cut surface, the pressure of the gas (P_{gas}) counteracts the tension exerted on the xylem solution. The xylem being part of the apoplast formed by the continuum of cell walls of adjacent cells as well as the extracellular spaces, the negative of P_{gas} is thus a measure of the apoplast matrix potential, $\psi_m(a)$:

$$\psi_m(a) = -P_{gas} \qquad \text{(eq. 3)}$$

In the apoplast, ψ_p can be ignored. The water potential is thus reduced to:

$$\psi_w(a) = \psi_s(a) + \psi_m(a) \qquad \text{(eq. 4)}$$

Now, if we admit that the osmotic potential of the xylem is low and negligible, the gas pressure is appreciably equal to the water potential of the apoplast [44]. Finally, the water potential in the apoplast being almost always the same as in each protoplasm, the water potential of the tissue is given by the negative of the gas pressure:

$$\psi_w(a) = \psi_w(p) - P_{gas} \qquad \text{(eq. 5)}$$

The more dehydrated the tissue, the more pressure is required.

Measurements of the water potential were performed with a conventional pressure chamber (Sols Mesure, Elancourt, France). The plant water status is measured within the hour following the picking up at the field. In order to prevent any evolution of the water potential, the alfalfa is bagged into a hermetic and opaque container. The tip of the stem is cut as straight as possible with a sharp blade to make the observation easier. Young alfalfa stems are

rather soft. When sealed into the chamber, the highly flexible stem of alfalfa automatically releases some water at the cut surface. This first exhaust of water is eliminated by absorption with a tissue. Then, nitrogen is introduced in the pressure chamber. The precision of the measure is also highly depending on the nitrogen bottle outlet pressure. If this latter is set high from the beginning, the rate will be out of control in the chamber and the water potential might be exceeded. The recommended experimental procedure is to increase the pressure little by little and to wait about 15 s between two pressure increments so as to make sure not to overestimate the value. This technique is not expected to give an accurate value but rather an average of the whole sample. For a given alfalfa batch, at least five different samples were measured each time.

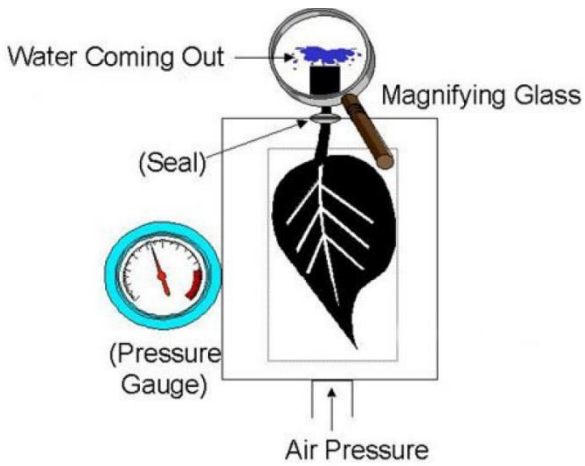

Figure 2 - Pressure Chamber

2.3.3 Total nitrogen and crude protein of the green juice

As pressing generates a nitrogen-rich liquid fraction, likely to be processed in a biorefinery plant or requiring to be treated before being discharged in the environment, the total nitrogen in the green juice has been analyzed with a TNM-1 unit for TOC-V_{CSH} instrument (Shimadzu, Marne La Vallée, France). The juice was kept in a fridge if the analyze was carried out during the day or frozen to be analyzed later. Two replicates at least are made for

each measure. A factor of 6.25 is used to convert the total nitrogen into crude protein (CP). Consequently, crude protein includes both true protein and non-protein nitrogen.

2.3.5 Cell structure

The plant tissues were fixed with 2.5% glutaraldehyde (25%) in a 0.05M sodium-cacodylate buffer for an hour. After rinsing and ambient-air drying, the samples were cut into 30µm-thick sections with a microtome Microm HM 255 (Thermo Scientific, Saint Herblain, France). Observations were then carried out thanks to the optic microscope LEICA DMRB whose magnifying power goes from x100 to x630.

3. Results and discussion

Alfalfa batches were sampled during three consecutive weeks (June 8th, June 15th and June 21st). This corresponds to the first regrowth period after the first agricultural harvest. Because of the weather conditions, the three batches appreciably have different moisture contents and water potentials (Table 1). And, as can be seen on Table 1, these two physical properties are not correlated.

	W (d.b)	$\lvert \Psi_W \rvert$ (kPa)
8 June	4.71	850 ± 50
15 June	4.46	350 ± 50
21 June	3.93	600 ± 100

Table 1 - Initial moisture content and water potential of the alfalfa samples

The dewatering kinetics were investigated for two wall temperatures, 30 and 50°C, and three processing pressures, 300, 500 and 1 000 kPa. The results achieved with alfalfa sampled on

June 8th are described in details in the following. For a wall temperature set at 30°C (Figure 3), no water is removed from the press cake as long as the applied pressure remains significantly lower than the water potential. When the applied pressure exceeds the water potential, water runs out almost immediately. For the intermediate pressure ($P_{applied} = 500$ kPa), six minutes are required before a small amount of juice flows out of the press cake. In the plant, water moves down a water potential gradient, from root to leaf. As a result, water potential in growing tissues is less than the one in the mature tissues located in the bottom part of the plant. Fulfilling a representative sampling is thus rather difficult and the five measurements performed give only an order of magnitude of the average water potential. The water potential of some leaves was probably lower than the applied pressure of 500 kPa, what would explain the partial dewatering.

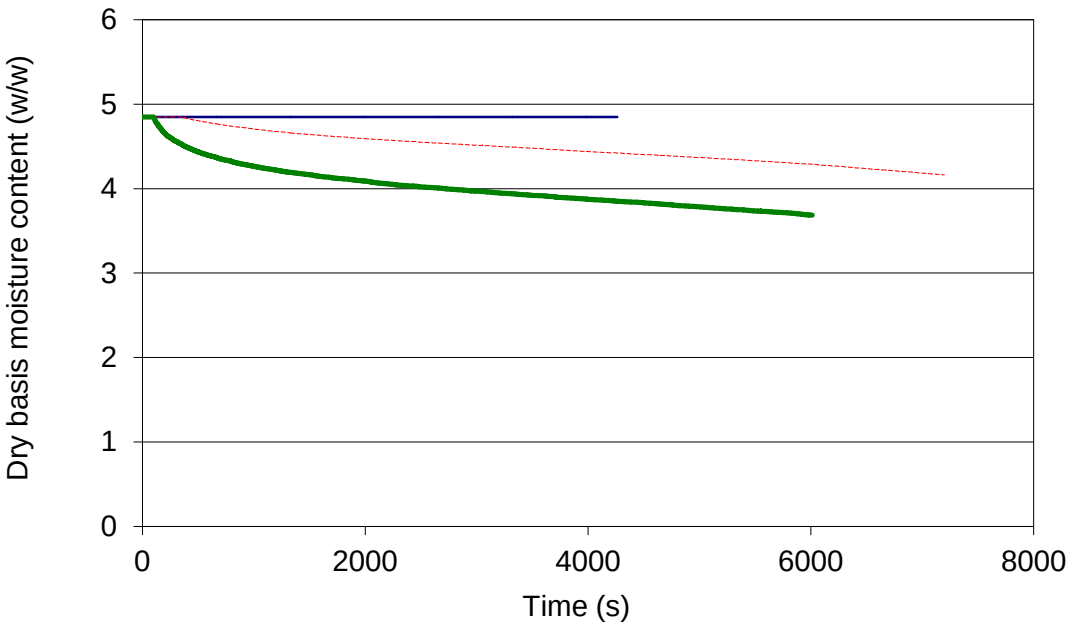

Figure 3 – Influence of the processing conditions on the dewatering kinetics for $\psi_w = 850$ kPa : $T_{wall} = 30°C$ and $P_{applied} = 300$ kPa (—), $P_{applied} = 500$ kPa (- -), $P_{applied} = 1\,000$ kPa (—)

Cell to cell mass transport through the symplasm, the cytoplasmic continuum created by the plasmodesmata, is the main pathways for alfalfa mechanical dewatering, as can be seen on

Figure 4. Applying an external pressure increases the protoplasm water potential above that of the xylem and water flows inside the xylem. Since the plasmalemma is water permeable, the juice extruded from the cut end of the stem is almost pure water. Consequently, the solute concentration in the cells near the xylem increases and the water potential decreases. A water potential gradient appears locally, inducing a water movement by osmosis from the adjacent turgid cells towards those partially dehydrated or plasmolyzed.

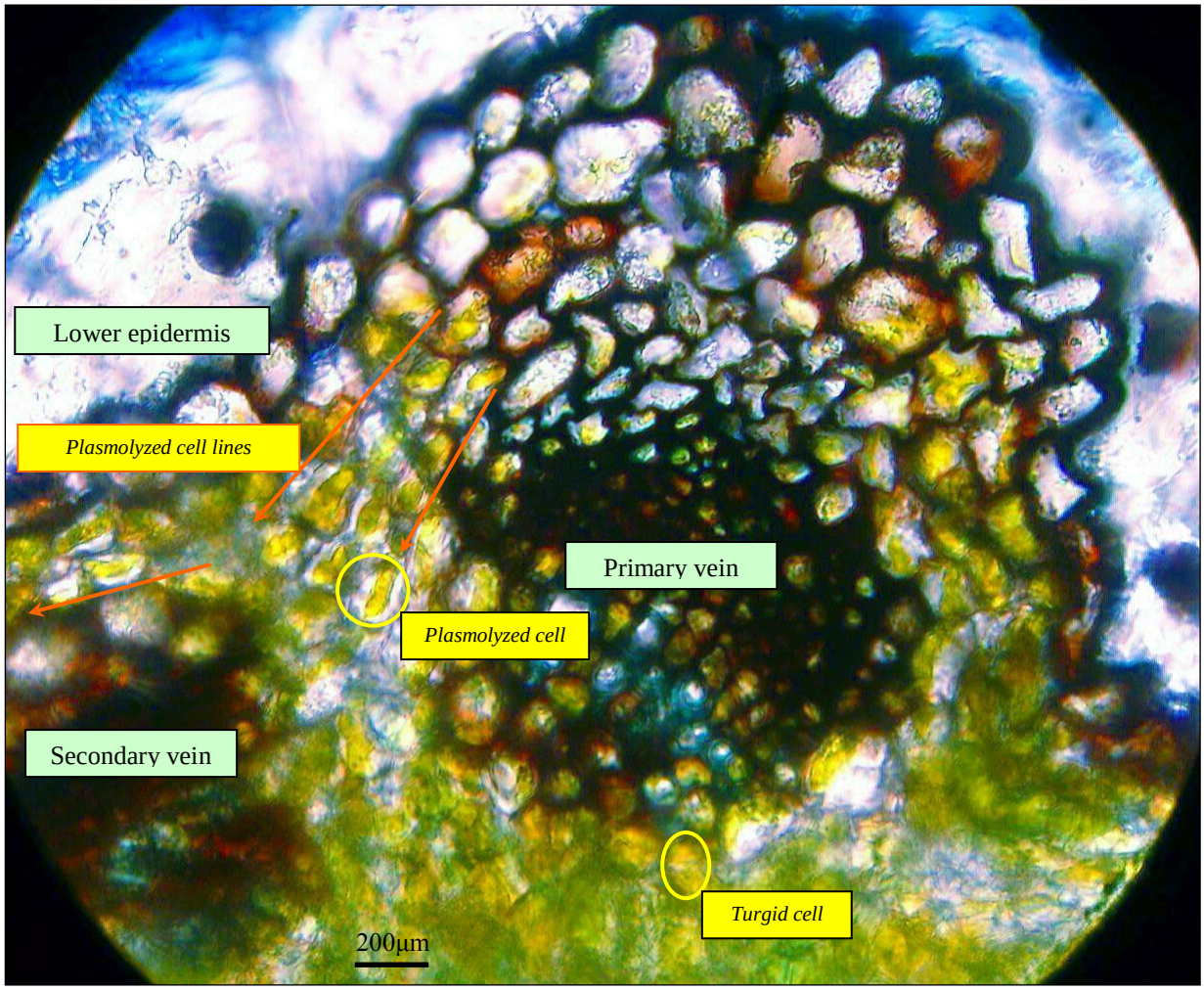

Figure 4 - Photograph of the vegetable cells after fractionation in the TAMD process

In the absence of any preliminary trituration of the biomass, which would aim at splitting the cell walls to recover hydrophobic proteins bound to the cell walls, this mechanism explains the low CP concentration of the green juice produced with chopped alfalfa (referred as 'Raw'

in Table 2). For the minced alfalfa (referred as ‘Pulp’ in Table 2), the shearing induced by the blender breaks the cellular walls and releases parietal proteins, enhancing significantly the CP content of the green juice.

		CP (g/L)	
30°C	Raw, P=300 kPa	**-**	-
	Pulp, P=300 kPa	**25.5**	*0.17*
	Raw, P=500 kPa	**4.92**	*0.02*
	Raw, P=1 MPa	**4.51**	*0.03*
50°C	Raw, P=300 kPa	**12.69**	*0.04*
	Raw, P=500 kPa	**12.52**	*0.11*
	Raw, P=1 MPa	**10**	*0.09*
70°C	Raw, P=300 kPa	**9.98**	*0.13*

Table 2 - Influence of the processing conditions on the crude protein content in the juice (median value in bold and standard deviation in italic).

For a wall temperature set at 50°C (Figure 5), the flow is like previously immediate if the applied pressure exceeds the water potential. When the applied pressure is lower, a latency period appears. The period is all the more long as the applied pressure is low. An increase in the applied pressure results in a decrease of the press cake macro-porosity and thus an enhancement of the diffusive heat transfer. The rise in temperature of the press cake is faster when a higher stress is applied on the press cake. This induces an increase of the osmotic pressure, conventionally calculated with the van’t Hoff equation [42] and enhances mass transfer. Of course, the final moisture content is independent of the pressure applied (asymptotic behavior for long times). Furthermore, phospholipids constituting the membrane become more fluid as temperature increases. This affects the permeability of the cell membrane, allowing substances, that wouldn’t usually do so, to leave the cell more easily. Thus, without any preliminary maceration, the CP concentration of the green juice increases from 4.92 g/L at 30°C to 12.52 g/L at 50°C for an applied pressure of 500 kPa (Table 2). The same tendency is observed for the highest applied pressure.

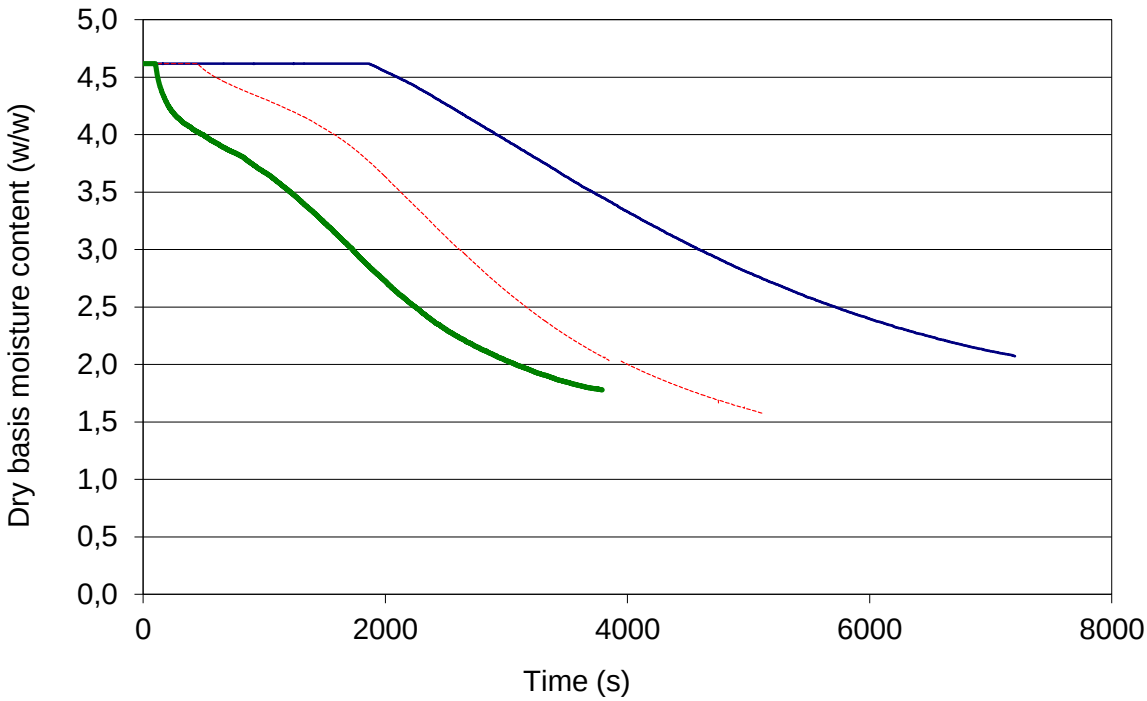

Figure 5 - Influence of the processing conditions on the dewatering kinetics for $\psi_w = 850$ kPa and $T_{wall} = 50°C$ and $P_{applied} = 300$ kPa (—), $P_{applied} = 500$ kPa (- -), $P_{applied} = 1\,000$ kPa (—)

Similar behaviors (see Figure 6) were observed with the two other batches sampled on June 15th and 21st, respectively. For a wall temperature of 50°C, a very short latency delay is observed during the dehydration of the sample of June 21st when the applied pressure ($P_{applied} = 800$ kPa) exceeds slightly the biomass water potential ($|\psi_w| = 600 \pm 100$ kPa). At 70°C, water runs immediately out of the press cake, even for a low applied pressure ($P_{applied} = 300$ kPa). Because of the weather conditions, the absolute value of the water potential of the second batch, sampled on June 15th, was appreciably lower ($|\psi_w| = 350 \pm 50$ kPa) than that of the two other batches. As soon as a pressure is applied, water is removed and the applied pressure has no influence on the dewatering kinetic. Finally, as already

emphasized [37, 45], the amount of green juice recovered increases when the initial dry basis moisture content increases.

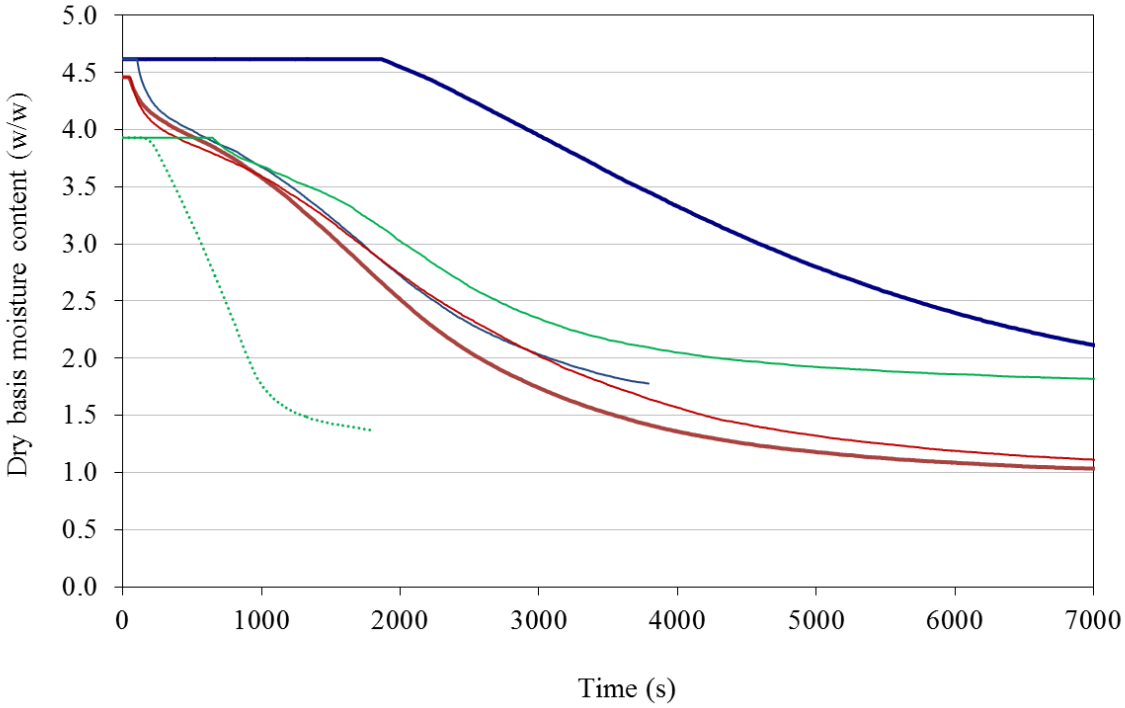

Figure 6 - Influence of the processing conditions and the water potential on the dewatering kinetics: $\psi_w = 850$ kPa, $T_{wall} = 50°C$ and $P_{applied} = 300$ kPa (—), $\psi_w = 850$ kPa, $T_{wall} = 50°C$ and $P_{applied} = 1000$ kPa (—), $\psi_w = 350$ kPa, $T_{wall} = 50°C$ and $P_{applied} = 300$ kPa (—), $\psi_w = 350$ kPa, $T_{wall} = 50°C$ and $P_{applied} = 500$ kPa (—), $\psi_w = 600$ kPa, $T_{wall} = 50°C$ and $P_{applied} = 800$ kPa (—), $\psi_w = 600$ kPa, $T_{wall} = 70°C$ and $P_{applied} = 300$ kPa (·····).

The temperature has a positive influence both on the extraction kinetic and on the total amount of juice extracted, as expected from former results [37]. According to figures 3, 5 and 6, the increase of the piston temperature significantly decreases the final biomass moisture content. The dry solid content of alfalfa sampled on June 8th reaches 37.7% after processing at 50°C and hardly 21.5% at ambient temperature. Figure 7 presents the evolution of the extraction yield according to time for the alfalfa batch sampled on June 8th and processing

conditions that do not induce a latency time. Experiments highlight that the flow is immediate beyond 70°C, whatever the pressure applied. The extraction yield is defined here as the ratio of the mass of green juice recovered to the mass of liquid intrinsically introduced with the raw biomass. Usually, it reaches 55% of the inherent liquid fraction available in the raw biomass. After 1h of pressing, the extraction yield reaches 69%, which represents an increase of 25.5% compared to current industrial processes. The enhancement is only of 11.8% at 50°C. On the other hand, the CP content of the green juice produced with the wall temperature set to 70°C decreases probably because of the denaturation of soluble proteins and others substances which appears around 60-77°C [46].

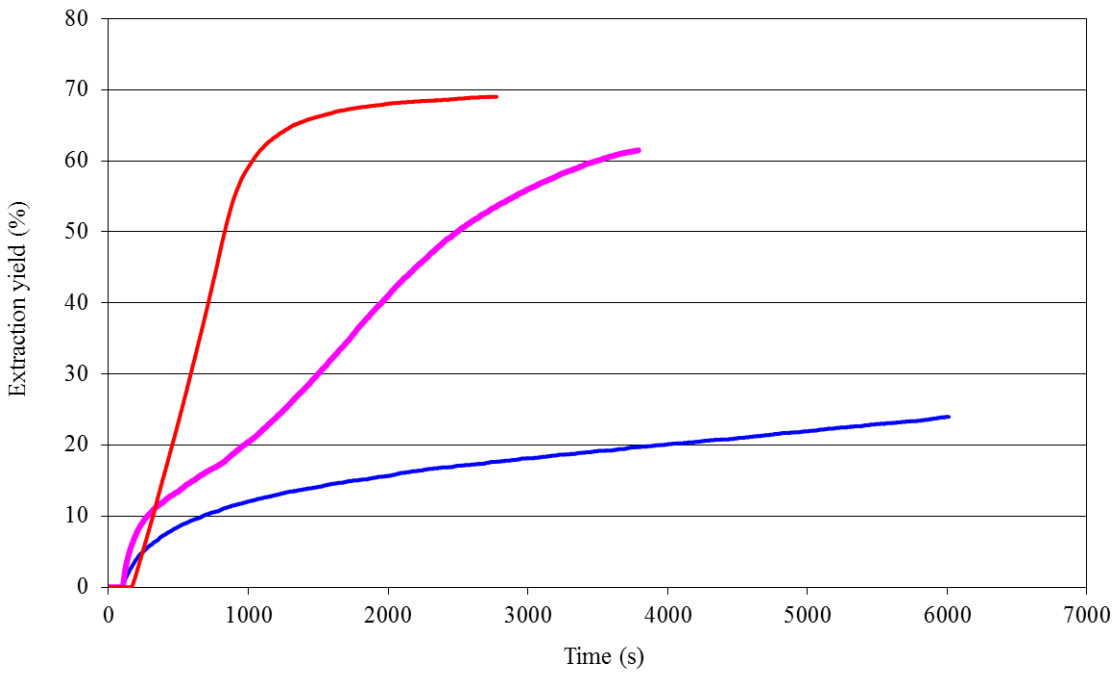

Figure 7 - Influence of the processing conditions on the dewatering yield for $\psi_w = 850$ kPa : $T_{wall} = 30°C$ and $P_{applied} = 1\,000$ kPa (—), $T_{wall} = 50°C$ and $P_{applied} = 1\,000$ kPa (—), $T_{wall} = 70°C$ and $P_{applied} = 300$ kPa (—).

4. Conclusions

High dewatering yield, 69% at 70°C and 61.5% at 50°C, can be achieved through thermal intensification of mechanical dewatering. This study confirms that water potential succeeds as an indicator of the plant water status. At 70°C, the juice flow is immediate, whatever the pressure applied. But, at lower wall temperature, the applied pressure must be adjusted to the water potential of the tissues to avoid a latency period before the juice flows out of the press cake. In the absence of any preliminary trituration of the biomass, the rise in temperature affects the permeability of the cell membrane, enhancing the CP content of the green juice. But if the temperature is too high, denaturation of soluble protein can occur and the CP concentration of the green juice decreases. To summarize, the TAMD process can operated in a conventional fodder drying facility as a pre-treatment for dewatering purpose. In the range of operating conditions investigated, 70°C and 300 kPa are the optimum conditions to preserve the quality of the press cake and reach high separation yield (+25.5% compared to the current process) in short times, reducing the inherent energy requirement for drying. The TAMD process can also be implemented for biorefining purpose. A lower wall temperature (50°C according to the present study) is required to improve the crude protein extraction. The major drawback is that the processing pressure should be adjusted to the water potential if the fodder is not sheared before pressing. Nevertheless, pressure chambers allow simple and fast measurements of the water potential even in the field environment. Knowing the optimal conditions for extraction (pressure, temperature and duration), the TAMD process scale-up is now considered. The objectives of the current work are to validate the proof of the concept in a continuous processing and to propose a mathematical model to predict heat and mass transfer in a thermally assisted mechanical dewatering process.

Nomenclature

a	Apoplast (-)
CP	Crude protein content (g/L)
p	Protoplast (-)
P	Pressure (kPa)
T	Temperature (°C)
$\bar{V}$	Partial molar volume (m^3/mol)
W	Dry basis moisture content (kg/kg)

Greek symbol

ψ	Potential (kPa)
μ	Chemical potential (kJ/mol)

Subscript

g	Gravity
gas	Gas
m	Matrix
p	Pressure
s	Osmotic
w	Water
wall	Copper piston
0	Pure water

Acknowledgements

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