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THE TWIN-SCREW EXTRUSION TECHNOLOGY, AN ORIGINAL AND POWERFUL SOLUTION FOR THE BIOREFINERY OF SUNFLOWER WHOLE PLANT

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ABSTRACT: The objective of this study was to evaluate the feasibility of an aqueous process for the biorefinery of sunflower whole plant using a twin-screw extruder. Aqueous extraction of oil was chosen as an environment-friendly alternative to the solvent extraction. The extruder was used to carry out three essential unit operations: grinding, liquid/solid extraction, and liquid/solid separation. Wringing out the mixing was effective. However, drying of the cake meal was not optimal. Lixiviation of cotyledon cells was also incomplete. Extraction efficiency depended on operating conditions: screw rotation speed, and input flow rates of whole plant and water. In the best conditions, oil yield was 57%. Residual oil content in the cake meal was 14%. These conditions leaded to the co-extraction of proteins, pectins, and hemicelluloses. The corresponding protein yield was 44%. Oil was extracted in the form of two oil-in-water emulsions. These hydrophobic phases were stabilized by phospholipids and proteins at interface. An aqueous extract containing part of the water-soluble constituents, mainly proteins and pectins, was also generated. As a mixture of fibers and proteins, the cake meal was molded by thermo-pressing. Panels produced had interesting mechanical properties in bending. The obtained fractions may have applications as bases for industrial products.
Keywords: sunflower, twin-screw extruder, aqueous extraction process, biorefinery, emulsion, biomaterial.

1 INTRODUCTION

Sunflower (*Helianthus annuus Linnaeus*) is cultivated for its seeds' high oil content which represents up to 80% of its economic value. The industrial processes for oil production consist of four successive stages: trituration, pressing, extraction of the residual oil using hexane and refining [1, 2]. The extraction yields are close to 100% with very good oil quality. However, the use of hexane to remove oil from the oily cake meal is an increasingly controversial issue and could be prohibited due to its carcinogenicity [3]. Consequently, numerous solvents have been considered, including water.

1.1 Aqueous extraction process

Water is an interesting alternative medium for extraction of oil from various oil-bearing materials such as sunflower [4, 5]. In the aqueous extraction process, the oil, being immiscible with water, separates readily from the extract. The fine crushing of the seeds is the first stage in cell disruption that facilitates the diffusion of the soluble compounds and the oil release. Liquid/solid separation by centrifugation produces three fractions: the hydrophobic phase (oil-in-water emulsion), the hydrophilic phase and the insoluble phase [2, 6, 7]. The oil is then recovered after demulsification by alcohol extraction. Besides, aqueous extraction of oil can be regarded as a process primarily aimed at solubilizing proteins which results in the release of the oil [2].

The oil and protein yields are respectively 86% and 85% when the aqueous extraction is carried out in the following operating conditions: dispersion of the ground seeds in water inside a batch reactor, extraction by simple stirring at room temperature, 45 min for the extraction duration, 1:10 for the ratio of the seeds to the water, 10 for pH [4]. As oil bodies are located inside the cotyledon cell, oil yield depends on cell disruption during the extraction process, and a fraction of the oil remains trapped in the cellular matrix.

1.2 Twin-screw extrusion

It is also possible to conduct the aqueous extraction process from whole seeds in a twin-screw extruder that enables an efficient mechanical lysis of the cells [7]. Co-penetrating and co-rotating twin-screw extruders are most common [8]. A very wide choice of screw elements is available. The screw elements affect different functions such as conveying, heating, cooling, shearing, crushing, mixing, chemical reaction, liquid/solid extraction, liquid/solid separation, and drying [9].

The screw profile (or screw configuration) is defined by the arrangement of different characteristics of screw elements (pitch, stagger angle, and length) in different positions and spacings. It is the main factor influencing performance (product transformation, residence time distribution, and mechanical energy input) during extrusion processing [10-13].

The forward pitch screws mainly ensure conveying action. The monolobe paddles exert a radial compression and shearing action on the matter but have limited mixing ability. In combination with forward pitch screws, the bilobe paddles exert significant mixing, shearing, conveying, and axial compression actions on the matter. The bilobe paddles are favourable to intimate mixing required in the liquid/solid extraction of soluble constituents in the cell structure. Finally, the reversed pitch screws carry out intensive shearing and considerable mixing on the matter, and exert a strong axial compression in combination with forward pitch screws [9]. The reversed pitch screws are frequently used to place pressure on the matter, which is essential for the separation of liquid and solid phases by filtration.

When twin-screw extruder is used to extract the oil from whole seeds according to an aqueous extraction process, liquid/solid separation requires the addition of wheat straw used as a lignocellulosic residue upstream from the filtration zone [7]. The best oil yield obtained is only 54.5% of the total lipid content of the seed and the residual oil content of the cake meal is approximately 30%, partly due to incomplete cell lysis within the seed. Another factor in incomplete oil extraction is the techno-

logical limits of the twin-screw extruder that does not enable a full separation of liquid and solid phases, even with the maximum fiber inlet flow.

During the aqueous extraction process, the oil is extracted in the form of oil-in-water emulsions. After high-pressure homogenisation, the oil droplets have an average size of approximately 3 μm. Their stability is ensured by the presence at the interface of natural surface-active agents co-extracted during the process, the phospholipids and proteins. Their demulsification using a mix of ethanol and diethyl ether (3/1) makes possible to isolate the oil extracted during the process. This process also allows the production of a protein extract.

Besides wheat straw, it is also possible to add sunflower depithed stalk upstream from the filter to enable the liquid/solid separation [7]. Consequently, it seems promising to introduce the whole plant instead of the only seeds at the entrance of the twin-screw extruder to simplify the extraction process of the oil with water. At the same time, some of the by-products from stalks and heads (pectins…), largely described and characterized by Maréchal and Rigal [14], would also become potentially extractable.

This study aimed to show that a co-rotating twin-screw extruder could be used for the biorefinery of sunflower whole plant in a single step, including its thermo-mechanical fractionation and the aqueous extraction of sunflower oil and other water-soluble by-products of sunflower culture. It also aimed to evaluate the potential of the fractions obtained after treatment of the extract and the raffinate's one for industrial applications.

2 MATERIALS AND METHODS

2.1 Materials

All trials were carried out using a single batch of sunflower whole plant (La Toulousaine de Céréales, France). Whole plant was previously dried (50°C, 48 h) and crushed using a hammer mill (Electra VS 1, France) fitted with a 15 mm screen. The average moisture content of the whole plant was 8.2%. The lipid and protein contents were 26.8% and 10.7%, respectively (e.g. Table I). All solvents and chemicals were analytical grade and were obtained from Sigma-Aldrich, Fluka, Prolabo and ICS (France).

Table I: Chemical composition of the sunflower whole plant from the batch used for experimental, and cake meal from trial 4 (% of dry matter).

	Sunflower whole plant	Cake meal from trial 4
Minerals	6.5	5.9
Lipids	26.8	14.3
Proteins	10.7	7.3
Cellulose	23.9	32.0
Hemicelluloses	7.8	14.8
Lignins	9.1	10.7
Pectins	7.0	-

2.2 Thermo-mechanical fractionation in the twin-screw extruder

Experiments were conducted with a Clextral BC 45 (France) co-penetrating and co-rotating twin-screw extruder. The extruder had seven modular barrels, each 200 mm in length, and different twin-screws which had

segmental screw elements each 50 and 100 mm in length (e.g. Fig. 1). Four modules (modules 3, 4, 5 and 7) were heated to 80°C by thermal induction and cooled by water circulation. A filter section consisting of six hemispherical dishes with perforations 1 mm in diameter was outfitted on module 6 to enable the filtrates to be collected. Screw rotation speed (S_S), the sunflower whole plant feed rate (Q_S), and the barrel temperature (θ_c) were monitored from a control panel.

T2F, trapezoidal double-thread screw; C2F, conveying double-thread screw; C1F, conveying simple screw; DM, monolobe paddle-screw; BB, bilobe paddle-screw; CF1C, reversed screw. The numbers following the type of the screw indicate the pitch of T2F, C2F, C1F and CF1C screws, and the length of the DM and BB screws.
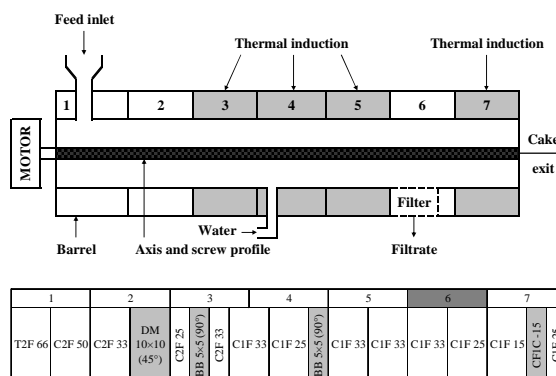
Figure 1: Schematic modular barrel and screw configuration of the Clextral BC 45 twin-screw extruder used for thermo-mechanical fractionation of sunflower whole plant ($\theta_c = 80°C$).

Sunflower whole plant was fed into the extruder inlet port by a volumetric screw feeder (Clextral 40, France) located in module 1. Deionised water was injected using a piston pump (Clextral DKM K20-2-P32, France) at the start of module 4 (e.g. Fig. 1). The only screw profile tested in this study (e.g. Fig. 1) was already successfully used for direct aqueous extraction of oil from whole sunflower seeds [7]. The trituration zone was located in modules 2 and 3. It consisted of a succession of 10 monolobe paddles and 5 bilobe paddles, 5 cm apart. The extraction zone was situated in modules 4 and 5 and was composed of a second series of 5 bilobe paddles. The reversed pitch screws were positioned in module 7, immediately downstream from the filtration module, to press the liquid/solid mixture.

The extruder was left to function for 20 to 25 minutes before any sampling to ensure the stabilization of the operating conditions. Such conditions include feed flows of sunflower whole plant and water, temperature and current feeding the motor. Upon achieving steady operation, the filtrate and the cake meal were immediately collected over a period of 20 minutes to avoid any variability of the outlet flow rates. Sample collection time was determined with a stopwatch. For each test, sample collection was carried out once. The filtrate and the cake meal were weighed.

The oil yield was calculated according to the following formula:

$$R_L = \frac{(Q_S \times L_S) - (Q_C \times L_C)}{(Q_S \times L_S)} \times 100$$

R_L is the oil yield based on the residual oil content of

the cake meal (%), Q_S the inlet flow rate of the sunflower whole plant (kg/h), Q_C the flow rate of the cake meal (kg/h), L_S the oil content in the sunflower whole plant (%), and L_C is the oil content in the cake meal (%).

The protein yield was calculated according to the following formula:

$$R_P = \frac{(Q_S \times P_S) - (Q_C \times P_C)}{Q_S \times P_S} \times 100$$

R_P is the protein yield based on the residual protein content of the cake meal (%), P_S the protein content in the sunflower whole plant (%), and P_C is the protein content in the cake meal (%).

The energy consumed by the motor was determined according to the following formulas:

$$P = U \times I \times \cos\varphi \times \frac{S_S}{S_{max}}$$

P is the electric power supplied by the motor (W), U the motor's operating voltage (U = 460V), I the current feeding the motor (A), $\cos\varphi$ the theoretical yield of the extruder motor ($\cos\varphi = 0.95$), and S_S and S_{max} are the test speed and maximum speed (600 rpm) of the rotating screws (rpm), respectively.

$$SME = \frac{P}{Q_S}$$

SME is the specific mechanical energy consumed by the motor per unit weight of sunflower whole plant (W h/kg).

Sample collection was followed by two different experimental investigations for trial 5. First, the residence time distribution (RTD) of the two phases in the twin-screw extruder was determined using an erythrosin tracer technique. Then, the extruder was suddenly stopped and opened in order to observe the inside matter location.

The erythrosin tracer was chosen by N'Diaye [15] for its neutral characteristics with respect to the process and for the good colorization of most of the vegetable matter, itself often highly colored. Some crushed sunflower seeds were colored with erythrosin and then directly introduced into the extruder. The colored matter injection was small enough in mass (5 g) and time (< 2 s), compared to the inlet flow rates and to the mean residence times, to be considered as a Dirac pulse. Samples were collected every 10 s at the filtrate and cake meal outlets of the apparatus during 10 min.

The filtrate samples were stored in a 4°C room until being analyzed. The cake meal samples were dried (105°C, 24 h) and ground in a micro-grinder to homogenise the color and to eliminate large size particles which could disturb measurements.

Furthermore, the quantity of colorant in samples was determined in the referential CIE L*a*b* using a Data Color ACS ICS (Switzerland) spectrocolorimeter. The a* color values measured were used to quantify the red color coming from erythrosin. These results were the average of five consecutive measurements.

The RTD data were examined through typical distribution versus time plots. RTD was defined as:

$$E(t) = \frac{C(t)}{C(t_0) \times \frac{t_1 - t_0}{2} + \sum_{i=1}^{n-1} C(t_i) \times \frac{t_{i+1} - t_{i-1}}{2} + C(t_n) \times \frac{t_n - t_{n-1}}{2}}$$

C(t) is the tracer concentration in each sample, and Δt is the sampling period.

2.3 Treatment of the filtrate

The filtrate from trial 4 was treated according to the separation process described in Fig. 2. Four fractions were produced. First, the filtrate was treated by pressure filtration (bolting cloth, 60 μm) to remove the foot (solid particles driven through the filtering sieve). The supernatant was then treated by high-pressure homogenisation (300 bar, two cycles) (APV 1000, Denmark) to obtain smaller and more regular oil droplets in the oil-in-water emulsions [6], and the homogenate was centrifuged ($3000 \times g$, 10 min, 10°C). Centrifugation revealed three liquid phases: (i) the higher hydrophobic phase, (ii) the hydrophilic phase, and (iii) the lower hydrophobic phase.
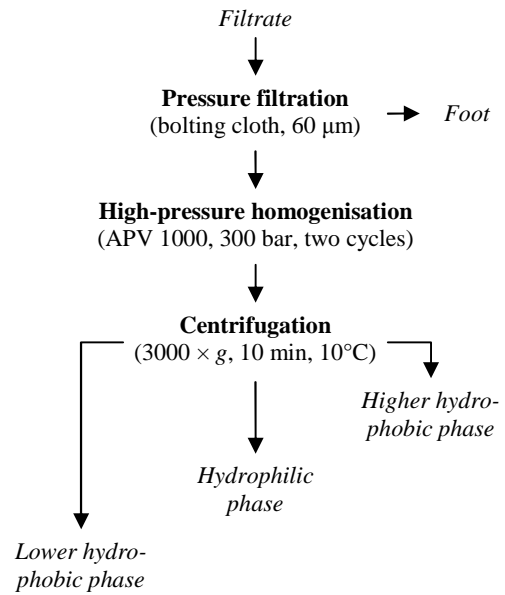

Figure 2: Schematic diagram for the treatment of the filtrate from trial 4.

2.4 Analytical methods

The moisture contents were determined according to the French standard NF V 03-903. The mineral contents were determined according to the French standard NF V 03-322. The oil content of the solids was determined according to the French standard NF V 03-908. The oil content of the hydrophobic phases was calculated after demulsification of the oil-in-water emulsions using a mix of ethanol and diethyl ether (3/1) [6]. The oil content of the hydrophilic phase was calculated by liquid/liquid extraction using a mixture of chloroform and methanol (1/2) according to the method developed by Bligh and Dyer [16]. The protein contents were determined according to the French standard NF V 18-100. An estimation of the three parietal constituents (cellulose, hemicelluloses, and lignins) contained in the solids was made by the ADF-NDF method of Van Soest and Wine [17, 18]. The pectin contents were estimated using the quantitative determination of galacturonic acid according to the colorimetric method of Blumenkrantz and Asboe-Hansen [19]. The non pectic sugar contents were estimated using the quantitative determination of total sugars according to the colorimetric method of Dubois *et al.* [20], and with calibration starting from xylose solutions. All determinations were carried out in duplicate.

2.5 Optical microscopy

Oil droplets in hydrophobic phases were observed with an optical microscope (Nikon Eclipse E 600, Japan). The distribution of the oil droplets size was estimated using the Lucia G software (Japan) by manual measurement of the diameter of the 200 droplets on the image.

2.6 Measurement of the oil droplets size

The hydrophobic phases were analyzed using a laser light scattering technique Mastersizer 2000 (Malvern Instruments) for characterization of oil droplets size distribution. The light source was red laser (He-Ne, 632 nm). Before analysis, hydrophobic phases were diluted in deionised water with 1:10 dilution factor.

2.7 Rheology of the hydrophobic phases

Steady shear viscosity tests were conducted using a TA Instruments AR 2000ex (USA) controlled stress rheometer. Emulsion viscosity (η) was measured at 25°C with cone-plate geometry (40 mm diameter, 3°59 angle). Herschel-Bulkley power-law model was used to analyze the emulsion flow curves:

$$\tau = \tau_c + \left(k \times \gamma^n \right)$$

τ is the shear stress (Pa), τ_c the yield stress (Pa), k the consistency coefficient (Pa s^n), γ the shear rate (s^{-1}), and n is the power-law exponent.

2.8 DSC analysis

Differential Scanning Calorimetry (DSC) analysis was used to evaluate the denaturation level of proteins in dry centrifugation pellets obtained after demulsification of hydrophobic phases and in cake meal from trial 4 [21]. The study was performed on a PerkinElmer (USA) Pyris 1 power compensation calorimeter fitted with an intracooler cooling system. The purge gas used was nitrogen of analytical quality at a flow rate of 20 mL/min. Temperature and energy calibration was carried out with indium ($T_f = 156.6$°C) and deionised water ($T_f = 0$°C) before the beginning of the tests.

All analyses were performed with hermetic 60 μL stainless steel capsules fitted out with O-rings resistant to an internal pressure of 40 bar (PerkinElmer, USA). Reference cell was empty. They were carried out at a heating speed of 20°C/min from 25°C and stopped at 200°C. Before analysis, sunflower whole plant and cake meal were equilibrated in climatic chamber (60% RH, 25°C) during three weeks whereas centrifugation pellets obtained after demulsification of hydrophobic phases were dried (105°C, 24 h). The sample mass was around 10 mg and all measurements were done in triplicate. Peak integration was realized with a sigmoid base line.

2.9 Thermo-pressing and mechanical properties in bending of the panels produced

The cake meal from trial 4 was molded by thermo-pressing between two aluminium plates covered with grease-proof paper, using a MAPA 50 (Pinette Emidecau Industries, France) heated hydraulic press. The moisture content of the cake meal was 4.4%. The panels produced were 130 mm × 130 mm squares.

A 5-kN H5KT (JFC, France) universal testing machine fitted with 100 N load cell was used to assess the flexural properties of the test specimens according to the French standard NF EN 310. The test specimens were 130 mm long and 30 mm wide. Their thickness was measured at three points with an electronic digital sliding caliper having a 0.01 mm resolution, and the mean value (t) was recorded to calculate their volume and section. All specimens were weighed to calculate mean apparent density (d).

These bars were then used to measure flexural properties in bending of the material, including stress at break (σ_f) and elastic modulus (E_f). The test speed was 3 mm/min and the grip separation was 100 mm. Test specimens were cut, and equilibrated in climatic chamber (60% RH, 25°C) during three weeks before being tested.

3 RESULTS AND DISCUSSION

3.1 Thermo-mechanical fractionation in the twin-screw extruder

The twin-screw extruder was used for its capacity to crush the whole plant whilst pressing and mixing as for direct aqueous extraction of oil from whole sunflower seeds [7]. The observation of the inside matter location made possible by the sudden shutdown of the extruder in the case of trial 5 (e.g. Fig. 3) indicated that the 10 monolobe paddles and the first series of 5 bilobe paddles reduced significantly the size of the solid particles and released the oil from the seeds. The second series of 5 bilobe paddles extended the mechanical action of seed trituration and promoted the intimate mixing of liquid and solid, thus achieving better contact between the extraction solvent and cells.
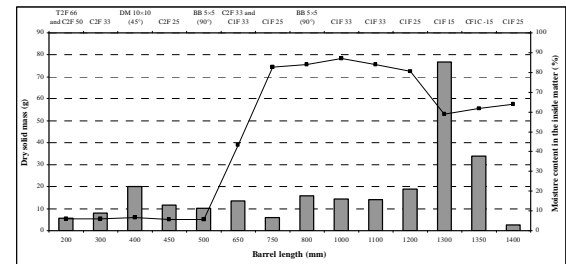

Figure 3: Twin-screw extruder opening experiment results for dry solid mass (▮) and moisture content (▪) in the inside matter in the case of trial 5.

A strong accumulation of solid matter was observed in the reversed screws (CF1C -15) and in the conveying simple screws located upstream (C1F 15). The moisture content in the inside matter decreased rapidly in C1F 15 conveying simple screws. It appeared that the compression of the liquid/solid mixture at this place allowed the liquid/solid separation. CF1C screws pushed part of the mixture upstream against the general movement in the extruder. This counter pressure allowed the mixture above the metal filter to be dried and ensured the efficiency of the liquid/solid separation. The natural abundance of fibers in sunflower stalk (e.g. Table II) explained the appearance of the matter compression. This result indicated that the whole plant fractionation did not require the addition of a lignocellulosic residue upstream from the pressing zone, as it was for seed fractionation [7], leading to the simplification of the aqueous extraction process.

The inlet flow rate of whole plant required for an efficient liquid/solid separation depended on both liquid/solid ratio at feeding and screw rotation speed. When liquid/solid ratio was high (4.1), it appeared that

the oil recovery increased with the screw rotation speed (e.g. Table III; trials 1 and 5). As liquid/solid ratio was similar for trials 1 and 5, it seemed that the difference did not come from the liquid/solid separation but mainly from the mechanical action on the cells. Residual oil content of the cake meal was only 13.1% for the highest value of the screw rotation speed (60 rpm) (e.g. Table III; trial 5) instead of 26.8% in whole plant (e.g. Table I). However, the energy consumed by the motor was higher for trial 5 (128.5 W h/kg for SME). At the same time, the mass content of the foot in the filtrate was not negligible (6.5%), corresponding to 7.1% of the dry matter from whole plant. This result confirmed the strong mechanical lysis of the cells inside whole plant in the case of trial 5. It also explained the significant decrease of oil yield after foot elimination (53.2% for R_L' instead of 64.9% for R_L). The same phenomenon was also observed for protein yield (46.3% for R_P' instead of 54.9% for R_P).

Table II: Distribution of cellulose, hemicelluloses, and lignins in the various organs of the sunflower whole plant (% of dry matter).

Organs	Cellulose	Hemi-celluloses	Lignins
Seed [7]	12.5	6.9	4.7
Kernel [7]	1.7	1.5	0.6
Hull [7]	42.6	16.1	21.5
Head [14]	19.6	11.0	6.3
Stalk	44.6	17.1	18.4
Depithed stalk	46.6	18.0	19.3
Pith in the stalk	28.1	1.3	11.8

When screw rotation speed was similar (34-39 rpm), the influence of liquid/solid ratio presented an optimal value (e.g. Table III; trials 2, 3 and 4). When this ratio was high (3.9), the mechanical lysis of solid matter was lower and liquid/solid separation was less efficient. On the contrary, the oil extraction was limited by the mass transfer when liquid/solid ratio was too low (3.0). Trial 4 was conducted with a medium value of the liquid/solid ratio (3.5). It revealed that the aqueous extraction process was much more efficient (e.g. Table III; trial 4). Residual oil content of the cake meal was only 14.3%, and the foot of the filtrate represented only 4.4% of the dry matter from whole plant. Oil yield (R_L') was even higher than for trial 5 (56.6% instead of 53.2%). On the contrary, a slight decrease of protein yield (R_P') was observed (43.6% instead of 46.3% for trial 5).

As a conclusion, it appeared that the thermo-mechanical action of the twin-screw extruder was efficient because it allowed the recovery of almost 60% of the oil with quite short extraction time. Indeed, mean residence times of the filtrate and the cake meal were respectively 147 s and 261 s for trial 5 (e.g. Table III). A large part of this action seemed to occur at the end of the extruder because the difference between these two mean residence times was about 2 min.

3.2 Characterization of the liquid phases in the filtrate

After foot removal, the extract from trial 4 was first treated by high-pressure homogenisation and then centrifuged (e.g. Fig. 2). Centrifugation leaded to the reorganisation of the liquid in three phases (e.g. Table IV and Fig. 4): (i) the higher hydrophobic phase, (ii) the hydrophilic phase, and (iii) the lower hydrophobic phase. Their densities were 0.974 ± 0.001, 1.009 ± 0.001 and 1.026 ± 0.001, respectively.

Table III: Results of the thermo-mechanical fractionation experiments conducted with the Clextral BC 45 twin-screw extruder and using the sunflower whole plant ($\theta_c = 80°C$).

	Trial				
	1	2	3	4	5
Operating conditions					
S_S (rpm)	32	39	39	34	60
Q_S (kg/h)	6.1	6.5	8.2	5.8	5.0
Q_W (kg/h)	25.0	25.2	24.9	20.5	20.3
Q_W / Q_S	4.1	3.9	3.0	3.5	4.1
C_F (kg/h rpm)	0.19	0.17	0.21	0.17	0.08
Filtrate					
Q_F (kg/h)	19.3	19.0	16.5	15.5	15.8
T_F (%)	-	-	-	4.5	6.5
Foot of the filtrate					
H_F (%)	-	-	-	66.1	68.3
L_F (% of DM [a])	-	-	-	22.6	44.3
P_F (% of DM [a])	-	-	-	14.1	12.8
Cake meal					
Q_C (kg/h)	11.8	12.7	16.6	10.8	9.6
H_C (%)	62.3	63.4	63.4	62.6	65.8
L_C (% of DM [a])	18.9	19.5	20.3	14.3	13.1
P_C (% of DM [a])	7.3	7.1	7.8	7.3	6.7
Oil yields (%)					
R_L	44.8	43.8	39.6	60.2	64.9
R_L'	-	-	-	56.6	53.2
Protein yields (%)					
R_P	46.1	48.3	41.3	49.3	54.9
R_P'	-	-	-	43.6	46.3
Energy consumed					
I (A)	28.9	24.1	29.3	23.3	14.8
P (W)	670.4	687.5	820.2	567.3	644.6
SME (W h/kg)	109.6	106.2	100.2	97.5	128.5
Residence time distribution					
τ_F (s)	-	-	-	-	147
τ_C (s)	-	-	-	-	261

Q_W is the inlet flow rate of the water (kg/h). C_F is the device's filling coefficient (kg/h rpm); it is defined as the ratio of the inlet flow rate of the solid matter (Q_S) to the screw rotation speed (S_S). Q_F is the flow rate of the filtrate (kg/h). T_F is the mass content of the foot in the filtrate (%). H_F is the moisture content in the foot of the filtrate (%). L_F is the oil content in the foot of the filtrate (%). P_F is the protein content in the foot of the filtrate (%). H_C is the moisture content in the cake meal (%). R_L' (%) and R_P' (%) also take into account the oil and protein contents in the foot of the filtrate. τ_F and τ_C are the mean residence times of the filtrate and the cake meal (s), respectively. – [a] DM, dry matter.

Higher hydrophobic phase and lower hydrophobic phase were both oil-in-water emulsions. They represented respectively 16.6% and 7.8% of the total mass of the filtrate, with water contents of 74% and 80%. Their dry matter contents were mostly composed of lipids, corresponding to 36% and 8% of the oil in whole plant (e.g. Fig. 4). For both hydrophobic phases, the remainder contained proteins, phospholipids, and minerals. The presence of proteins is indicative of their role as natural surface-active agents in the emulsions. They represented 20% of the proteins in whole plant for the higher hydrophobic phase, and 6% for the lower hydrophobic phase. In the case of the lower hydrophobic phase,

pectins from pith in the stalk and head [14] and non pectic sugars represented also a large part of the dry matter (19%). Their presence could explain that the lower hydrophobic phase was denser than the two other liquid phases. The non pectic sugars, maybe hemicelluloses, could come from the stalk (e.g. Table II).

Table IV: Proportion and chemical composition of the three liquid phases from the filtrate obtained in trial 4 (S_S = 34 rpm, Q_S = 5.8 kg/h, Q_W = 20.5 kg/h, θ_c = 80°C).

	Higher hydro-phobic phase	Hydro-philic phase	Lower hydro-phobic phase
Flows (kg/h)	2.6 (16.6%)	11.1 (71.1%)	1.2 (7.8%)
Moisture (%)	74.1	97.3	80.3
Chemical composition (% of dry matter)			
Minerals	2.6	27.7	6.7
Lipids	78.5	30.6	58.5
Proteins	17.0	22.2	15.9
Pectins	0.7	11.7	10.2
Non pectic sugars	0.8	8.3	9.2
Extraction yields (%)			
Lipids [b]	36.0	6.4	8.4
Proteins [c]	19.7	11.6	6.2

[b] *In proportion to the total lipid content of the sunflower whole plant. –* [c] *In proportion to the total protein content of the sunflower whole plant.*

Demulsification of both hydrophobic phases was possible using a mixture of ethanol and diethyl ether (3/1) to recover the extracted oil. It also generated a precipitate with high protein content (79% of the dry matter for the higher hydrophobic phase, 38% for the lower hydrophobic phase). These proteins could be used for their tensioactive properties, and DSC measurements indicated that proteins from the higher hydrophobic phase were less denatured during the thermo-mechanical fractionation in twin-screw extruder than those from the lower hydrophobic phase. Indeed, the average enthalpy of the denaturation peak observed on the DSC scan of the dry centrifugation pellet obtained after demulsification of the higher hydrophobic phase was 4.1 J/g of dry protein while it was only 0.7 J/g of dry protein in the case of the lower hydrophobic phase, instead of 6.7 J/g of dry protein in the sunflower whole plant (e.g. Table V).

The surface of the oil droplets appeared to be fine and smooth when observed under the optical microscope for both hydrophobic phases. The Gauss-type distribution of the droplets size revealed an average size of the fatty globules of 1.1 ± 0.3 μm for the higher hydrophobic phase, and 1.4 ± 0.3 μm for the lower hydrophobic phase. These oil droplets size distributions were confirmed by the laser light scattering technique analysis: 1.1 ± 0.4 μm for the higher hydrophobic phase, and 1.2 ± 0.5 μm for the lower hydrophobic phase. Both hydrophobic phases were therefore relatively monodisperse, which indicates that oil droplets were not coalescing. These dispersed phases showed good stability over time after high-pressure homogenisation. After storage for 3 months at 5°C, only a thin layer of oxidation appeared on the surfaces but no coalescence process had been activated.

The satisfactory stability of these emulsions was confirmed by steady shear viscosity tests. In both cases, the modelling of the emulsion flow curve with the Herschel-Bulkley power-law model (e.g. Table VI) revealed the existence of a yield stress (τ_c). For any lower shear stress value (τ), viscosity (η) of the emulsion was too high to enable its flowing. Viscosity then decreased regularly when the shear stress increased, but it was still higher than the viscosity of the two major constituents of the emulsions, water and sunflower oil. Yield stress was clearly higher for the lower hydrophobic phase (36.7 Pa instead of 2.1 Pa for the higher hydrophobic phase). Moreover, for the same shear stress value, its viscosity was also much more important than for the higher hydrophobic phase.
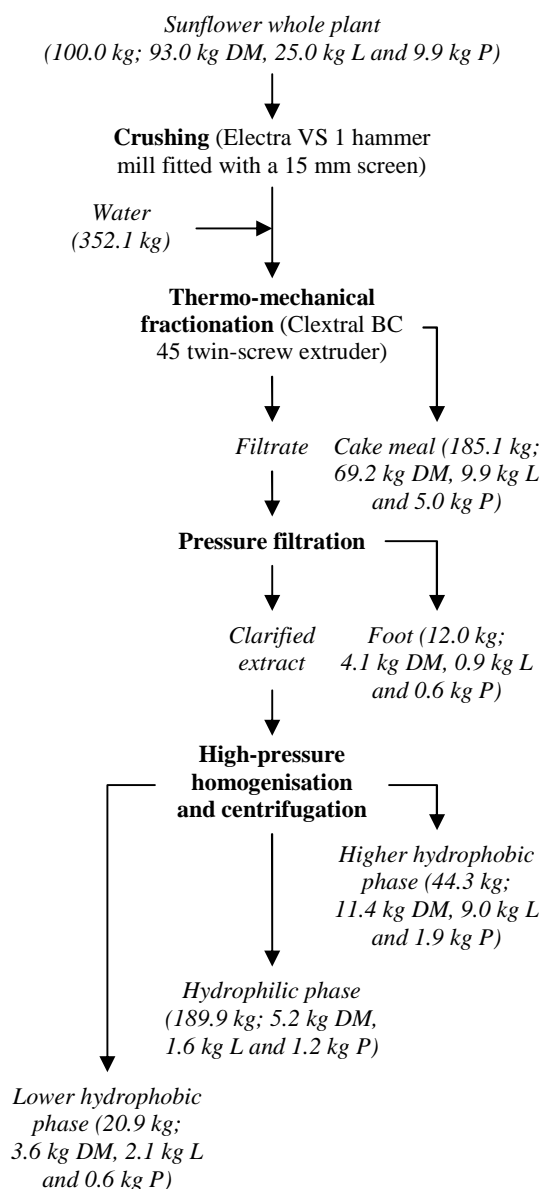

Figure 4: Matter assessment for thermo-mechanical fractionation of sunflower whole plant conducted with the Clextral BC 45 twin-screw extruder in the case of trial 4 (DM, dry matter; L, lipids; P, proteins).

The hydrophilic phase (aqueous phase) was the largest phase (71.1% of the filtrate) since the liquid/solid ratio for the extraction was close to 4. It represented 52% of the injected water (e.g. Fig. 4). It constituted an aqueous extract of the water-soluble constituents from

sunflower whole plant: (i) proteins from kernel, (ii) pectins from pith and head [14], and (iii) hemi-celluloses from stalk (e.g. Table II). The dry matter content was 3%, corresponding to 6% of dry matter in whole plant, 12% of proteins in whole plant, and 9% of pectins in whole plant. Lipids accounted for 31% of the dry matter, and their presence indicated that the separation between the three liquid phases was not optimal. The hydrophilic phase appeared to be incompletely separated from the higher hydrophobic phase. However, taking into account the low percentage of dry matter, these losses were minimal (only 6% of the oil in whole plant).

Table V: Average temperature, average enthalpy (ΔH) reported to the sample mass, to the mass of dry matter and to the mass of dry protein of the denaturation peak observed on DSC scans of sunflower whole plant, dry centrifugation pellets obtained after demulsification of hydrophobic phases and cake meal from trial 4, in pressure resistant pans.

	Sunflower whole plant	Higher hydro-phobic phase [d]	Lower hydro-phobic phase [d]	Cake meal from trial 4
Moisture (%)	7.1	Negli-gible	Negli-gible	7.9
Peak tem-perature (°C)	151.7	153.2	155.1	140.4
ΔH (J/g of sample)	0.66	3.21	0.26	0.07
ΔH (J/g of dry matter)	0.71	3.21	0.26	0.07
ΔH (J/g of dry protein)	6.65	4.05	0.68	1.00

[d] *Dry centrifugation pellets.*

Table VI: Rheological behaviour of higher hydrophobic phase and lower hydrophobic phase from trial 4 (emulsion flow curves analyzed with the Herschel-Bulkley power-law model).

	τ_c (Pa)	k (Pa s^n)	n
Higher hydrophobic phase	2.06	0.24	0.6960
Lower hydrophobic phase	36.65	2.47	0.4669

3.3 Characterization of the cake meal

The cake meal from trial 4 was relatively moist (63%). It was first dried to make easier its conservation. Before drying, it would have been also possible to add the foot of the corresponding filtrate to it. The cake meal contained notably cell debris from the kernel breakdown process. It had a porous structure and was largely composed of lignocellulosic fibers (e.g. Table I) coming principally from the depithed stalk (e.g. Table II). Actually, the cake meal was a lixiviated matter where soluble molecules (proteins, pectins…) and lipids were partly removed. At the same time, molecules from plant skeleton were not extracted. Nevertheless, 40% of the oil from whole plant and 51% of the proteins from whole plant remained trapped in the porous structure of the cake meal. DSC measurements indicated that the denaturation of these proteins was almost complete while it was not the case for proteins in the higher hydrophobic phase from the extract (e.g. Table V). The cake meal stayed a longer time inside the twin-screw extruder, in the rever-sed screws (CF1C -15) but also in the conveying simple screws located upstream (C1F 15), where the mechanical

action was the most intense. Consequently, proteins from the cake meal suffered a higher shearing, and they were denatured almost completely.

3.4 Valorization of the different fractions

The hydrophobic phases could be used for oil production because their demulsification was efficient. The use of a mixture of ethanol and diethyl ether (3/1) to recover the extracted oil also produced a precipitate containing proteins with tensioactive properties, and with low denaturation level in the case of the higher hydro-phobic phase.

After stabilisation by high-pressure homogenisation, hydrophobic phases may also have direct industrial applications for non food uses in various fields as (i) the biolubricants market, (ii) the transport of active principles (odors, colors, bactericides, antifungals), and (iii) the treatment of surfaces with hydrophilic matter.

Value-adding of hydrophilic phase will be more difficult because it was much diluted. Nevertheless, it would be potentially recyclable for aqueous extraction in the twin-screw extruder. After concentration of organic substances with separation processes using membranes, it would be also possible to produce proteins and pectins separately. Proteins would be collected by isoelectric precipitation, and then used for their surface-active pro-perties. Pectins would be collected by alcoholic precipita-tion [2], or by spray drying after ultrafiltration concentra-tion. They are good gelling agents [14], and they would be usable in the food industry.

The cake meal would be suitable for use in animal feeds and for energy production in pellets burning furnaces. As the cake meal was a mixture of ligno-cellulosic fibers and proteins, it could be also considered as a natural composite that was processed successfully into biodegradable and value-added agromaterials by thermo-pressing.

During molding operation, macromolecular structure of the proteins was completely transformed due to their thermal sensibility, leading to their glass transition under the simultaneous effect of pressure and temperature. The reorganization of their structure allowed the mechanical aspect of the panels and gave to the agromaterial its flexibility, while the fibers entanglement also acted like reinforcement.

Mean apparent density of the material depended on the thermo-pressing conditions (e.g. Table VII). It was situated between 0.74 for panel 1 and 1.13 for panel 3. Its mechanical behaviour was evaluated by the flexural strength at break (σ_f) and the elastic modulus (E_f). These values increased simultaneously with temperature, pressure and time chosen for thermo-pressing operation. Higher stress at break (11.5 MPa) and higher elastic modulus (2.22 GPa) were therefore obtained for panel 5 (200°C, 320 kgf/cm^2, and 60 s for the thermo-pressing conditions). The corresponding mean apparent density was 1.04.

Because of their promising mechanical properties in bending compared with those of other industrial and experimental materials, panels could be used as inter-layer sheets for pallets in the handling and storage industry or for the manufacturing of biodegradable containers (composters, crates for vegetable gardening) by assembly of panels.

Table VII: Thermo-pressing conditions and mechanical properties in bending (σ_f, stress at break; E_f, elastic modulus) of the molded test specimens.

	Panel				
	1	2	3	4	5
Thermo-pressing conditions					
Temperature (°C)	160	160	160	180	200
Pressure (kgf/cm^2)	107	320	320	320	320
Time (s)	30	30	60	60	60
Flexural properties					
t (mm)	6.01	3.97	3.82	3.88	3.89
d	0.74	1.09	1.13	1.09	1.04
σ_f (MPa)	0.4	5.0	6.0	11.3	11.5
E_f (GPa)	0.10	1.07	1.28	2.11	2.22

4 CONCLUSION

Biorefinery of sunflower whole plant was carried out using the twin-screw extrusion technology in order to achieve simultaneously thermo-mechanical fractionation and aqueous extraction. A filter section was outfitted along the barrel of the extruder to collect separately an extract and a raffinate, in a single step and in a continuous mode. Natural abundance of fibers in sunflower stalk enabled the liquid/solid separation.

Twin-screw extruder could be a promising and cleaner alternate technology for sunflower oil processing. Nevertheless, the process efficiency was limited and the best oil yield was only 57% of the total lipid content of sunflower whole plant. The residual oil content of the cake meal was approximately 14%, partly due to an incomplete cell lysis within the seed. Another factor in incomplete oil extraction was the technological limits of the twin-screw extruder that did not enable a complete separation of liquid and solid phases.

The oil was extracted in the form of two different oil-in-water emulsions. Their stability was ensured by the presence at the interface of natural surface-active agents co-extracted during the process, the phospholipids and proteins. Pectins and non pectic sugars completed the dry matter of the denser emulsion (lower hydrophobic phase). For both hydrophobic phases, the demulsification was conducted using a mix of ethanol and diethyl ether (3/1). It made possible to isolate the oil extracted during the process. It also generated a precipitate with high protein content. Such proteins would be usable for their tensioactive properties. Because of their satisfactory stability over time, both dispersed phases may also have direct applications. They should become value-added fractions in various fields as the biolubricants market, the transport of active principles, and the surface treatment.

The process also allowed the production of the hydrophilic phase. This largest fraction in the filtrate was an aqueous extract of the water-soluble constituents from whole plant, mainly proteins and pectins. In addition to its recycling for aqueous extraction in the twin-screw extruder, the recovery of both proteins and pectins would be another interesting alternative. Proteins should be usable for their surface-active properties, and pectins as gelling agents.

The cake meal was a mixture of lignocellulosic fibers and proteins. Therefore, it could be considered as a natural composite. Its molding into agromaterials was carried out successfully by thermo-pressing. Panels had promising mechanical properties in bending. Conse-quently, it would be interesting to use them as inter-layer sheets for pallets or for the manufacturing of biodegradable containers.

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