The twin-screw extrusion technology, an original solution for the extraction of proteins from sunflower and alfalfa

Ph. Evon[*, 1, 2], D. Colas[1, 2], P.Y. Pontalier[1, 2], L. Rigal[1, 2]

[1] Université de Toulouse; INP; LCA (Laboratoire de Chimie Agro-industrielle); ENSIACET, 4 Allée Emile Monso, 31432 Toulouse Cedex 4, France

[2] INRA; LCA (Laboratoire de Chimie Agro-industrielle); 31432 Toulouse Cedex 4, France

Keywords: Twin-screw extrusion, Protein extraction, Sunflower, Alfalfa.

Twin-screw extrusion has been used for the protein extraction from sunflower and alfalfa. Thermo-mechanical fractionation and aqueous extraction are conducted simultaneously to collect separately a liquid extract and a solid raffinate.

From sunflower whole plant, squeezing in the reversed screws is favored by the fibers abundance in the stalk, and it enables L/S separation. Protein yield is 44%, in the best conditions, and lipids are partly co-extracted. Water-soluble proteins are in an aqueous extract and in two O/W emulsions due to their surface-active properties. Hence, the oil is co-extracted in the form of emulsions stabilized by proteins at interface.

Proteins can be collected in the aqueous extract by isoelectric precipitation. Emulsions are usable for oil production. Their demulsification with ethanol produces a precipitate rich in proteins with low denaturation level.

From alfalfa whole plant, the highest protein yield in the aqueous extract (called green juice) is 25% when water is added in the extruder. Water-soluble and water-insoluble green juice proteins can be fractionated thanks to L/L extraction in sunflower oil and ammonium sulfate precipitation.

Some other molecules are co-extracted (polysaccharides, phenolic compounds). They can be purified with ultrafiltration or chromatography.

---

* Corresponding author. Tel.: + 33 (0)5 62 44 60 80; fax: + 33 (0)5 62 44 60 82.
E-mail address: Philippe.Evon@ensiacet.fr (Ph. Evon).

The twin-screw extrusion technology, an original solution for the extraction of proteins from sunflower and alfalfa

Ph. Evon [1,2], D. Colas [1,2], P.Y. Pontalier [1,2] and L. Rigal [1,2]

[1] *Université de Toulouse; INP; LCA (Laboratoire de Chimie Agro-industrielle);*

ENSIACET, 4 Allée Emile Monso, F-31029 Toulouse, France

[2] *INRA; LCA (Laboratoire de Chimie Agro-industrielle);*

F-31029 Toulouse, France

LCA Laboratoire de Chimie Agro-Industrielle

Unité Mixte de Recherche INRA / INP - ENSIACET (UMR 1010)

OUTLINE

1. Introduction.

2. Materials and methods.

   2.1. Thermo-mechanical fractionation in the twin-screw extruder.

   2.2. Chemical composition of the vegetable matters.

3. Results and discussion.

   3.1. Thermo-mechanical fractionation of the sunflower whole plant in the twin-screw extruder.

   3.2. Thermo-mechanical fractionation of the alfalfa whole plant in the twin-screw extruder.

4. Conclusion.

OUTLINE

1. Introduction.

2. Materials and methods.

2.1. Thermo-mechanical fractionation in the twin-screw extruder.

2.2. Chemical composition of the vegetable matters.

3. Results and discussion.

3.1. Thermo-mechanical fractionation of the sunflower whole plant in the twin-screw extruder.

3.2. Thermo-mechanical fractionation of the alfalfa whole plant in the twin-screw extruder.

4. Conclusion.

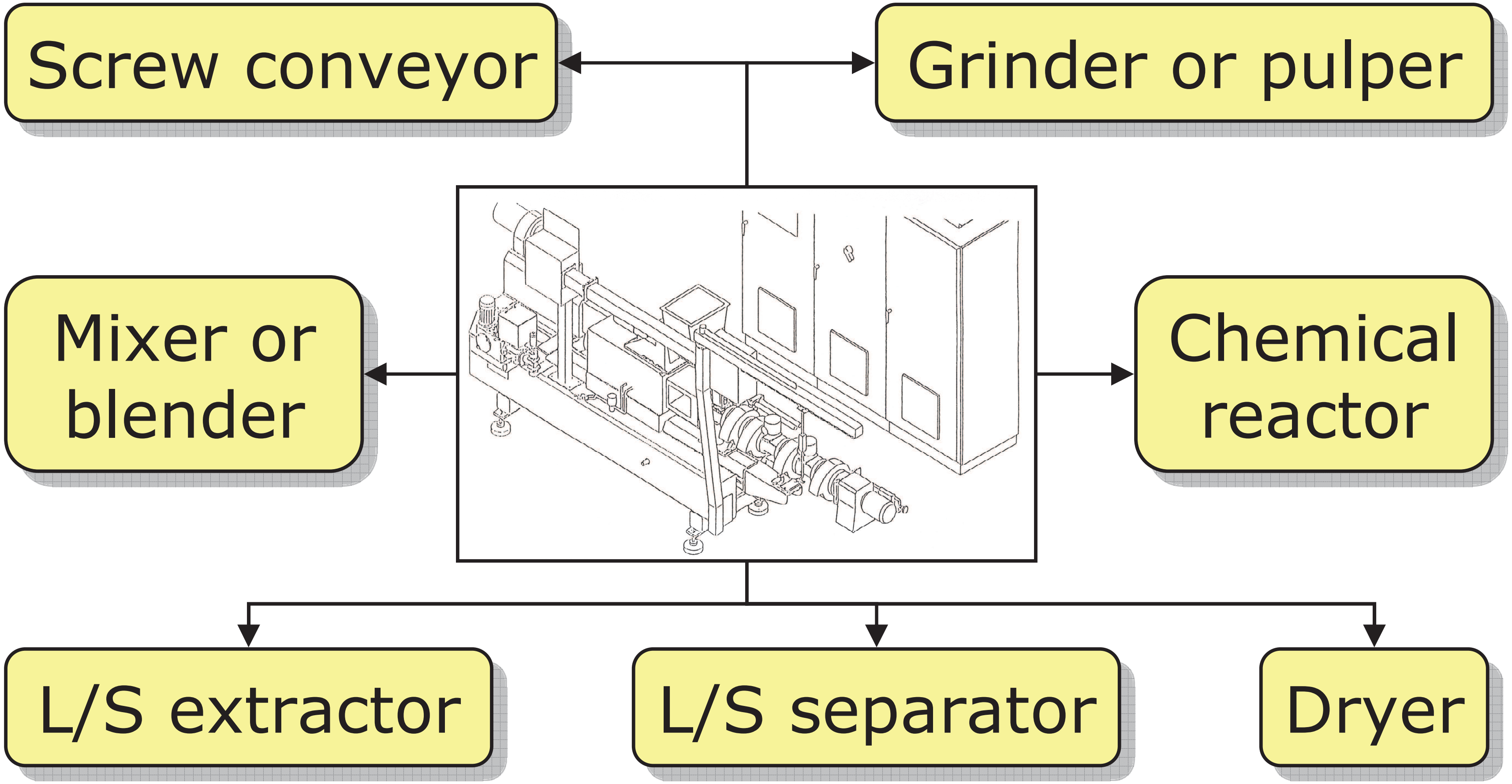
TWIN-SCREW EXTRUDER FRACTIONATION

- In classical fractionation processes, installations are specially designed for each operation.
- Perfecting a reactor-extractor is essential:
  - … to combine actions:
    - ***- Thermal actions,***
    - ***- Mechanical actions,***
    - ***- Chemical actions.***
  - … to compact basic operations.

- **Because of its <u>compactness</u> and its <u>flexibility</u>, the twin-screw extrusion appears to be an interesting technology for the vegetable fractionation (i.e. L/S extraction and L/S separation).**

- **It behaves like a <u>thermo-mechano-chemical reactor</u>.**

TWIN-SCREW EXTRUDER FRACTIONATION



SOME EXAMPLES OF VEGETABLE FRACTIONATION

- Extraction of hemicelluloses from lignocellulosic matters:
  - Sorghum [1],
  - Poplar [2-3],
  - Wheat straw and wheat bran [4].
- Extraction of xylans and arabinoxylans from wheat bran [5].
- Extraction of pectic substances from the pith of sunflower stalk [6].

[1] **Manolas**, **C.**, Fractionnement du sorgho à fibres : extraction et caractérisation des hémicelluloses de la moelle, étude des matériaux composites. *Thèse de Doctorat*, INP, Toulouse (1993).

[2] **N'Diaye**, **S.**, **Rigal**, **L.**, **Goyette**, **C.** and **Vidal**, **P.**, Extraction of hemicelluloses from poplar using twin-screw reactor: influence of the main factors. *Developments in Thermochemical Biomass Conversion*, IEA Bioenergy, **1**, 756-764 (1996).

[3] **N'Diaye**, **S.** and **Rigal**, **L.**, Factors influencing the alkaline extraction of poplar hemicelluloses in a twin-screw reactor: correlation with specific mechanical energy and residence time distribution of the liquid phase. *Bioresource Technology*, **75**, 13-18 (2000).

[4] **Maréchal**, **P.**, Analyse des principaux facteurs impliqués dans le fractionnement combiné de pailles et de sons de blé en extrudeur bi-vis : obtention d'agromatériaux. *Thèse de Doctorat*, INP, Toulouse (2001).

[5] **Rigal**, **L.**, **Ioualalen**, **R.** and **Gaset**, **A.**, Procédé pour obtenir un extrait de son désamylacé, un raffinat et un matériau obtenu à partir de ce procédé. *Brevet WO 9831713* (1998).

[6] **Maréchal**, **V.** and **Rigal**, **L.**, Characterization of by-products of sunflower culture: commercial applications for stalks and heads. *Industrial Crops and Products*, **10**, 185-200 (1999).

SOME EXAMPLES OF VEGETABLE FRACTIONATION

- Extraction and molecular transformation of proteins with vegetable origin:
  - Industrial sunflower oilcake [7],
  - Cornmeal [8].
- Co-extraction of sunflower oil and sunflower proteins according to an aqueous extraction process, starting from:
  - … the seeds [9],
  - … the press cake [10].

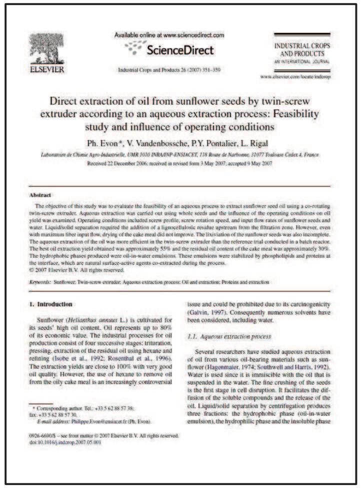
[7] **Silvestre**, **F.**, **Rigal**, **L.**, **Leyris**, **J.** and **Gaset**, **A.**, Colle à l'eau à base d'extrait protéique végétal et procédé de préparation. *Brevet EP 0997513* (1999).

[8] **Wasserman**, **B.P.**, **Wen**, **L.** and **Chan**, **K.**, Molecular transformations of starch and protein during twin-screw extrusion processing of cornmeal. *Food Extrusion Science and Technology*, *Dekker M. Inc. Ed.*, *New York* (1992).

[9] **Evon**, **Ph.**, **Vandenbossche**, **V.**, **Pontalier**, **P.Y.** and **Rigal**, **L.**, Direct extraction of oil from sunflower seeds by twin-screw extruder according to an aqueous extraction process: feasibility study and influence of operating conditions. *Industrial Crops and Products*, **26 (3)**, 351-359 (2007).

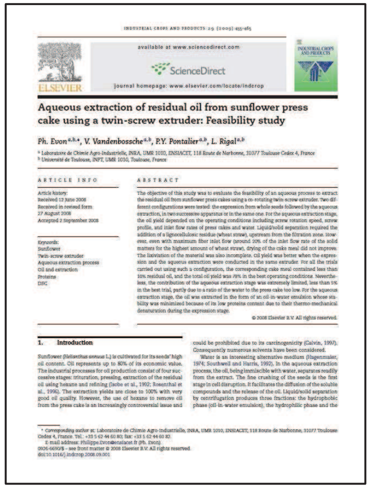
[10] **Evon**, **Ph.**, **Vandenbossche**, **V.**, **Pontalier**, **P.Y.** and **Rigal**, **L.**, Aqueous extraction of residual oil from sunflower press cake using a twin-screw extruder: feasibility study. *Industrial Crops and Products*, **29 (2-3)**, 455-465 (2009).

[9]



Direct extraction of oil from sunflower seeds by twin-screw extruder according to an aqueous extraction process: Feasibility study and influence of operating conditions

[10]



Aqueous extraction of residual oil from sunflower press cake using a twin-screw extruder: Feasibility study

OUTLINE

1. Introduction.

2. Materials and methods.

2.1. Thermo-mechanical fractionation in the twin-screw extruder.

2.2. Chemical composition of the vegetable matters.

3. Results and discussion.

3.1. Thermo-mechanical fractionation of the sunflower whole plant in the twin-screw extruder.

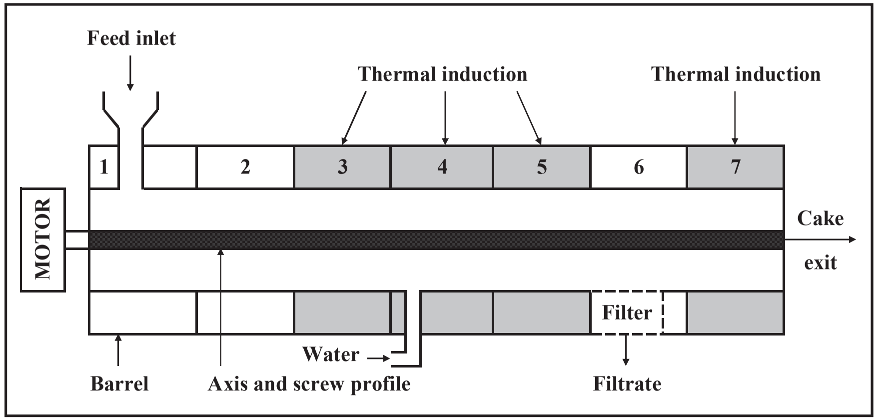
3.2. Thermo-mechanical fractionation of the alfalfa whole plant in the twin-screw extruder.

4. Conclusion.

THERMO-MECHANICAL FRACTIONATION IN THE TWIN-SCREW EXTRUDER

- Experiments are conducted with a Clextral BC 45 (France) co-penetrating and co-rotating twin-screw extruder:
  - Distance between the two screw shafts centres = 45 mm.
  - Screw diameter = 56 mm.
  - 7 standard barrel modules (total length = 140 cm).
  - A filter (filtration module) in sixth position.

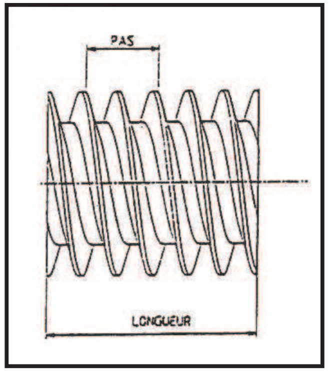
Schematic modular barrel



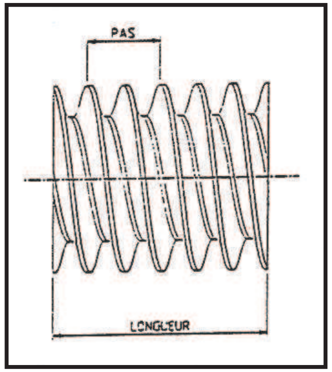
THERMO-MECHANICAL FRACTIONATION IN THE TWIN-SCREW EXTRUDER

Forward pitch screws

T2F: trapezoidal double-thread screws



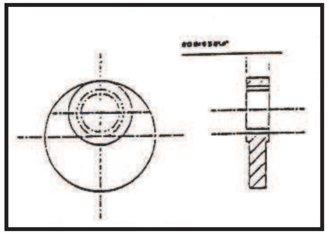
C2F: conveying double-thread screws



Conveying action

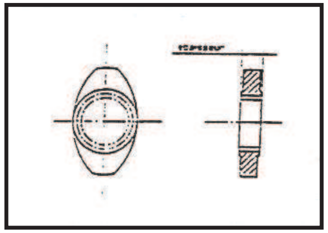
Paddle-screws

MAL 0 (or **DM**): monolobe paddle-screws



(i) Shearing
(ii) Radial compression
→ ***Mechanical lysis of the cells***

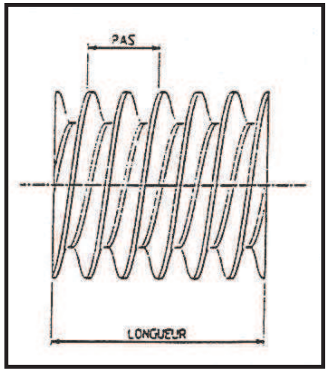
MAL 2 (or **BB**): bilobe paddle-screws



Intimate mixing
→ ***Required in the L/S ex-traction of soluble constituents***

Reversed screws

CF2C: Reverse pitch double-thread screws



(i) Shearing
(ii) High axial compression
→ ***Enable the L/S separation***

OUTLINE

1. Introduction.

2. Materials and methods.

2.1. Thermo-mechanical fractionation in the twin-screw extruder.

2.2. Chemical composition of the vegetable matters.

3. Results and discussion.

3.1. Thermo-mechanical fractionation of the sunflower whole plant in the twin-screw extruder.

3.2. Thermo-mechanical fractionation of the alfalfa whole plant in the twin-screw extruder.

4. Conclusion.

CHEMICAL COMPOSITION OF THE VEGETABLE MATTERS

Helianthus annuus L.

- **Oleic sunflower whole plant**

(15 mm homogenate)

(La Toulousaine de Céréales, France):

8.2% of moisture content. – 26.8% DM of oil content; **10.7% DM of protein content;** 40.9% DM of fibers content (cellulose, hemicelluloses, and lignins); 7.0% DM of pectin content.

Medicago sativa L.

- **Alfalfa whole plant**

(5 cm chopped pieces)

(Angers, France):

62.1% of moisture content. – 8.2% DM of ash content; **23.5% DM of protein content;** 35.4% DM of fibers content (cellulose, hemicelluloses and lignin).

DM = dry matter.

OUTLINE

1. Introduction.

2. Materials and methods.

2.1. Thermo-mechanical fractionation in the twin-screw extruder.

2.2. Chemical composition of the vegetable matters.

3. Results and discussion.

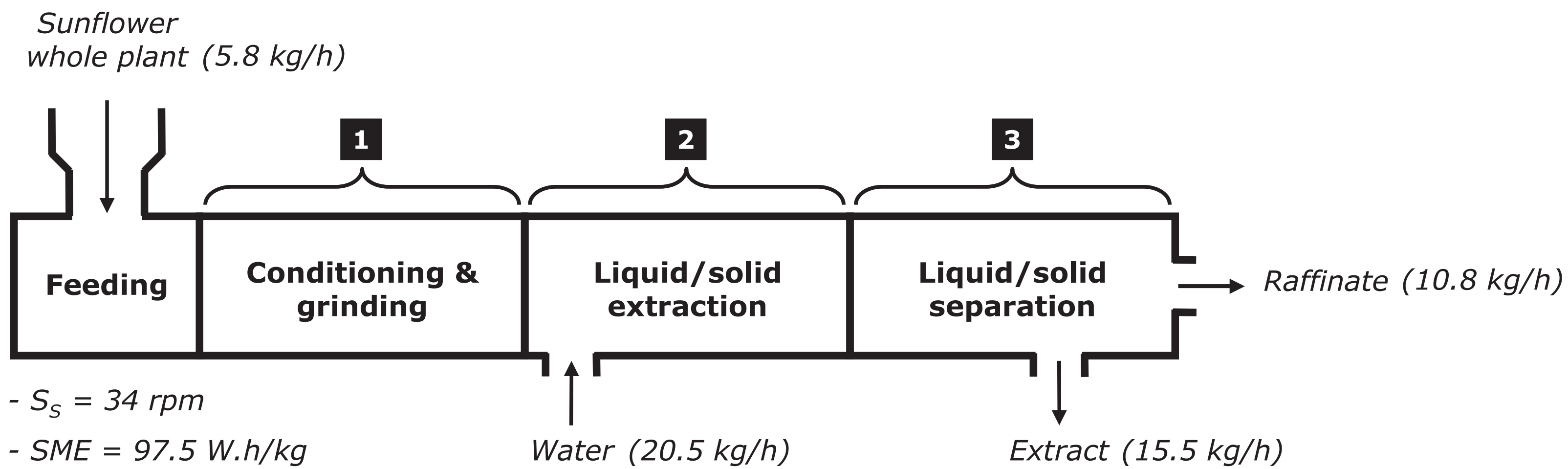
3.1. Thermo-mechanical fractionation of the sunflower whole plant in the twin-screw extruder.

3.2. Thermo-mechanical fractionation of the alfalfa whole plant in the twin-screw extruder.

4. Conclusion.

THERMO-MECHANICAL FRACTIONATION OF THE SUNFLOWER WHOLE PLANT IN THE TWIN-SCREW EXTRUDER

- Co-extraction of oil and proteins is effective with water starting from whole plant (80°C for the barrel temperature).
- After feeding, three essential unit operations are carried out in a single step and in a continuous mode:

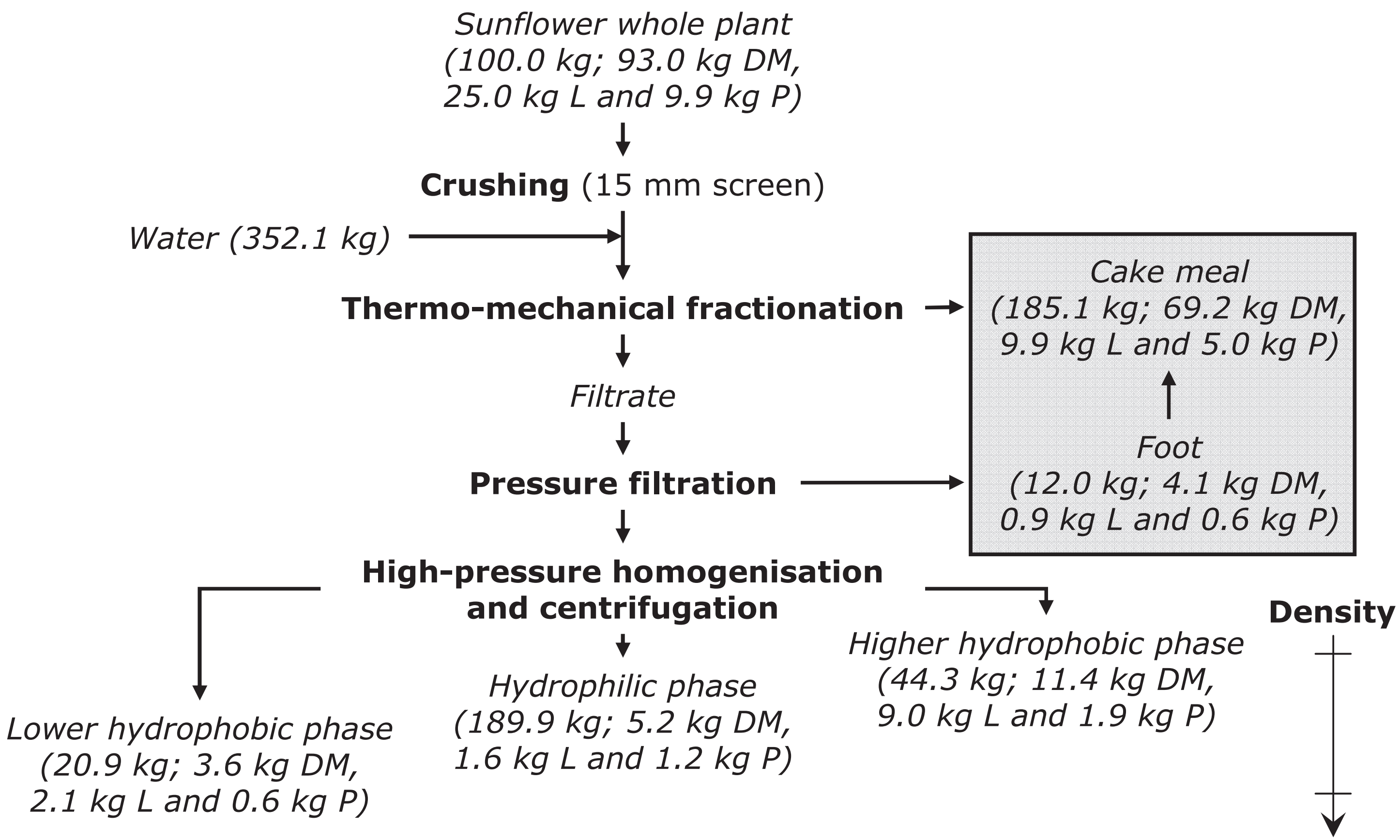


- Wringing out the mixing is favoured by the natural abundance of fibers in the sunflower stalk (until 80%).
- An extract (filtrate) and a raffinate (cake meal) are produced.

S_S = screw rotation speed, SME = specific mechanical energy.

LCA Laboratoire de Chimie Agro-Industrielle
Unité Mixte de Recherche INRA / INP - ENSIACET (UMR 1010)
INRA INP ENSIACET

MATTER ASSESSMENT FOR THE THERMO-MECHANICAL FRACTIONATION OF THE SUNFLOWER WHOLE PLANT



- Lipids are extracted in the form of two oil-in-water emulsions.
- Oil yield is 56.6%, and **protein yield is 43.6%**.

DM = dry matter, L = lipids, P = proteins.

DESCRIPTION OF THE HYDROPHOBIC PHASES

	Higher hydrophobic phase *(16.6% of the mass of the filtrate)*	Lower hydrophobic phase *(7.8% of the mass of the filtrate)*
Moisture (%)	*74.1%*	*80.3%*
Lipids (% of DM)	78.5%	58.5%
Proteins (% of DM)	**17.0%**	**15.9%**
Pectins (% of DM)	0.7%	10.2%
Non pectic sugars (% of DM)	0.8%	9.2%

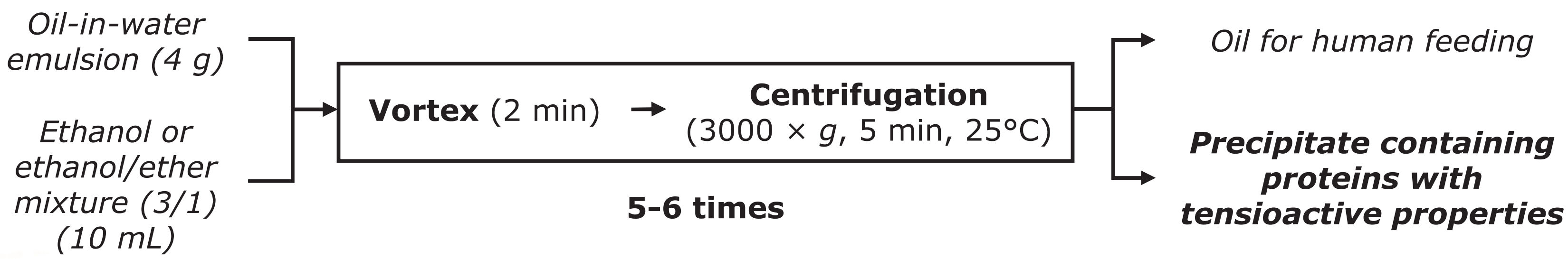
Higher hydro-phobic phase

- Stability of both hydrophobic phases is ensured by the presence at interface of surface active agents co-extracted during the aqueous extraction process:
  - Phospholipids.
  - **Proteins (albumins) from kernel**.
- Pectins (from pith in the stalk, and head) and non pectic sugars (hemicelluloses from depithed stalk) complete the dry matter of lower hydrophobic phase.

DM = dry matter.

APPLICATIONS OF THE HYDROPHOBIC PHASES

- Hydrophobic phases may have applications for **non food uses** in various fields as:
  - … the biolubricants market.
  - … the transport of active principles: odors, colors, bactericides, and antifungals.
  - … the treatment of surfaces with hydrophilic matter.
- The can be also used for **oil production** because their demulsification with ethanol is efficient.
- Oil recovery produces also a precipitate containing **tensio-active proteins**.



DESCRIPTION OF THE HYDROPHILIC PHASE

Hydrophilic phase

- The hydrophilic phase (aqueous phase) is the largest fraction of the extract (71.1% of the filtrate).
- It is an aqueous extract of the water-soluble constituents from whole plant:
  - **Proteins (albumins) from kernel**.
  - Pectins from pith in the stalk, and head.
  - Hemicelluloses from depithed stalk.

Moisture (%)	97.3%
Minerals	27.7%
Lipids (% of DM)	30.6%
Proteins (% of DM)	**22.2%**
Pectins (% of DM)	11.7%
Non pectic sugars (% of DM)	8.3%

DM = dry matter.

APPLICATIONS OF THE HYDROPHILIC PHASE

- Valorization of the hydrophilic phase is difficult because it is **much diluted**.

- Nevertheless, it would be potentially **recyclable for aqueous extraction**.

- It would be also possible to use it for the production of:
  - … pectins usable as gelling agents (after concentration, and alcoholic precipitation).
  - … **proteins with surface-active properties** (after concentration, and isoelectric precipitation).

DESCRIPTION OF THE CAKE MEAL

- The cake meal is first dried (105°C, 24 h) to make easier its conservation (< 5.0% for the moisture content after drying).

- It is a mixture of lignocellulosic fibres and **proteins (globulins) with thermoplastic properties**.

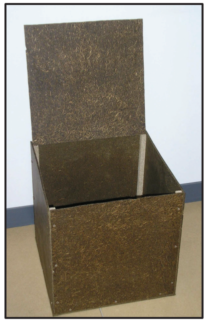
Moisture (%)	62.6% *(< 5.0% after drying)*
Lipids (% of DM)	14.3%
Proteins (% of DM)	**7.3%**
Cellulose and lignins (% of DM)	42.7%

DM = dry matter.

THERMO-PRESSING OF THE CAKE MEAL

- As a mixture of fibres and **proteins**, the cake meal is considered as a natural composite.
- Thermo-pressing is a promising molding operation for the manufacturing of **biodegradable and value-added agromaterials**.
- Panels have promising mechanical properties in bending compared with those of other industrial and experimental materials (Isorel®, laminated board, maize cob).

(i) Inter-layer sheet for pallets (panel)



(ii) Container (composter) *(assembly of panels)*

OUTLINE

1. Introduction.

2. Materials and methods.

2.1. Thermo-mechanical fractionation in the twin-screw extruder.

2.2. Chemical composition of the vegetable matters.

3. Results and discussion.

3.1. Thermo-mechanical fractionation of the sunflower whole plant in the twin-screw extruder.

3.2. Thermo-mechanical fractionation of the alfalfa whole plant in the twin-screw extruder.

4. Conclusion.

THERMO-MECHANICAL FRATIONATION OF THE ALFALFA WHOLE PLANT IN THE TWIN-SCREW EXTRUDER

- Extraction of proteins is investigated (i) with or without water addition, and (ii) with or without heating.

- The three essentials unit operations carried out are the same as for the sunflower whole plant :
  - Conditioning & grinding.
  - Liquid/solid extraction.
  - Liquid/solid separation.

- Optimum conditions for alfalfa protein extraction (based on an experimental design):
  - Barrel temperature: 50°C.
  - Water addition: 3.75 for the inlet L/S ratio.

MATTER ASSESSMENT FOR THE THERMO-MECHANICAL FRACTIONATION OF THE ALFALFA WHOLE PLANT

Alfalfa whole plant
(100.0 kg; 37.9 kg DM and 8.9 kg P)

↓

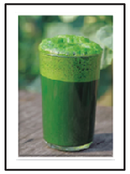
Chopping
(5 cm pieces)

Water (375 kg) →

↓

Thermo-mechanical fractionation

Filtrate (green juice)
(304.6 kg; 15.0 kg DM and 4.7 kg P)



Cake meal (solid fibrous residue)
(71.4 kg; 27.0 kg DM and 3.3 kg P)

- **Protein yield is 52.8%** in the green extract.

  ⇨ *Fractionation between hydrophilic and hydrophobic proteins.*

DM = dry matter, P = proteins.

EXAMPLE OF THE FRACTIONATION OF THE HYDROPHOBIC PROTEINS OF THE GREEN EXTRACT

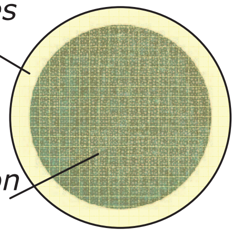
Green juice

Surface-active solvent: Tween 20 (polysorbate surfactant)

Centrifugation
(2500 × *g*, 20 min)

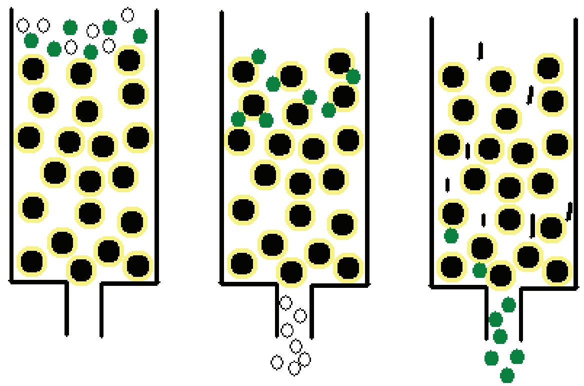
Clear brown juice (33.9% DM of protein content)

Liquid/liquid extraction
(in sunflower oil)



Macroporous adsorption resin conditioned in sunflower oil

CONTACT — WASHING — ELUTION



Hydrophilic proteins not retained (90% of total proteins)

Hydrophobic proteins released after elution (10%)

Surface-active agents production: food industry, cosmetics…

Green precipitate (13.9% DM of protein content)

Cake meal

Cattle feeding, bioethanol…

DM = dry matter.

OUTLINE

1. Introduction.

2. Materials and methods.

2.1. Thermo-mechanical fractionation in the twin-screw extruder.

2.2. Chemical composition of the vegetable matters.

3. Results and discussion.

3.1. Thermo-mechanical fractionation of the sunflower whole plant in the twin-screw extruder.

3.2. Thermo-mechanical fractionation of the alfalfa whole plant in the twin-screw extruder.

4. Conclusion.

CONCLUSION

- The feasibility of the extraction with water of proteins from (i) sunflower whole plant and (ii) alfalfa whole plant using a co-rotating twin-screw extruder is confirmed.

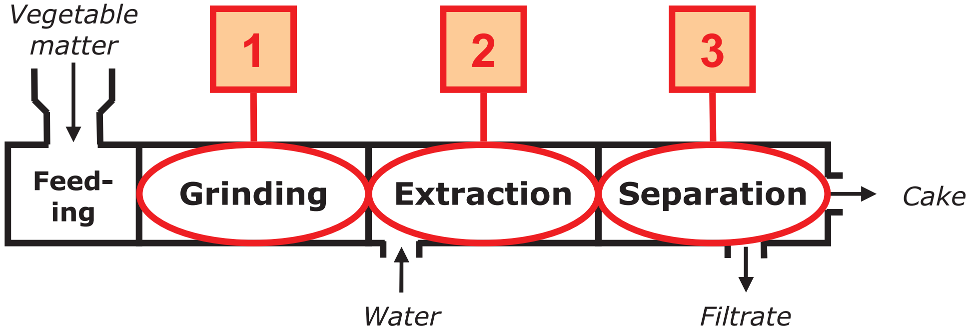
- Aqueous extraction is chosen as an environment-friendly extraction technique.

- The co-rotating twin-screw extruder behaves like a thermo-mechanical reactor.

- It is equipped with a filtration module to obtain separately an extract (filtrate) and a raffinate (cake meal).

CONCLUSION

- This only apparatus is used to carry out three essential unit operations in a single step and in a continuous mode:



- The obtained fractions containing proteins may have applications as bases for industrial products in various fields, not only for **food uses** but also for **non food uses**.

Thank you for your attention.

LABORATOIRE DE CHIMIE AGRO-INDUSTRIELLE
UMR 1010 INRA / INP-ENSIACET

Dr Philippe EVON
Ingénieur de Recherche / Research Engineer

. INSTITUT NATIONAL POLYTECHNIQUE .
ECOLE NATIONALE SUPERIEURE DES INGENIEURS EN ARTS CHIMIQUES ET TECHNOLOGIQUES

AGROMAT – Site de l'ENIT – 47 avenue d'Azereix – BP 1629 – 65016 Tarbes Cedex – France
Tél. : + 33 (0)5 62 44 60 80 – Fax : + 33 (0)5 62 44 60 82 – Portable : + 33 (0)6 88 09 40 41 – E-mail : Philippe.Evon@ensiacet.fr

The twin-screw extrusion technology, an original solution for the extraction of proteins from sunflower and alfalfa

Ph. Evon [1,2], D. Colas [1,2], P.Y. Pontalier [1,2] and L. Rigal [1,2]

[1] *Université de Toulouse; INP; LCA (Laboratoire de Chimie Agro-industrielle);*

ENSIACET, 4 Allée Emile Monso, F-31029 Toulouse, France

[2] *INRA; LCA (Laboratoire de Chimie Agro-industrielle);*

F-31029 Toulouse, France

LCA Laboratoire de Chimie Agro-Industrielle
Unité Mixte de Recherche INRA / INP - ENSIACET (UMR 1010)
INRA INP ENSIACET