FEED-A-GENE

Adapting the feed, the animal and the feeding techniques to improve the efficiency and sustainability of monogastric livestock production systems

Deliverable D1.1

New parameters for use of soybean and rapeseed products in feed production

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Classified, as referred to in Commission Decision 2001/844/EC - Cl	

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1. Summary

Objectives

The objective of this document is to provide a description of the production of novel feed protein products from rapeseeds or soybeans grown in Europe. This work was undertaken to investigate the potential of alternative European feedstuffs to increase their production in quantity and quality in Europe, thereby helping Europe to reduce its dependency on imported protein sources. The present deliverable is based on activities in WP1 task 1.1 but has strong links with the activities in task 1.3 to task 1.5.

Rationale:

Task 1.1 focuses on the processing of European grown rapeseeds and soybeans to obtain products with high protein content and/or suitable for the feed market. A process employing extrusion-pressing or flaking-pressing cooking was used to produce expeller soybean meals from European cultivated soybeans. The impact on the quality of the soybean product of the initial dehulling of beans was evaluated and the different steps in the process were followed.

Besides that, innovative and improved process stages of solvent extraction of rapeseed oil have been investigated. The classical process of rapeseed cake involves defatting with hexane, treatment with aqueous ethanol under vacuum to eliminate soluble carbohydrates, and drying with ethanol. New successive steps of oil extraction using hydro-ethanol / ethanol / hexane were investigated. The goas was to concentrate the protein content, limit the energy required for desolvation compared to that of hexane, and ensure a good removal of anti-nutritional factors.

Lastly, an upgrading of conventional rapeseed meal was undertaken by a biological method. The goals were to increase the protein content of the meal and to limit the content of anti-nutritional factors (i.e., glucosinolates).

Teams involved:

IFIP

TERRES INOVIA & OLEAD (previously CETIOM)

HAMLET PROTEIN

DLO

Species and production systems considered: all animal species and countries in Europe

2. Introduction

During months 1 to 36 of the Feed-a-Gene project, a study was carried out to determine the influence of different processes on the nutritional quality of expeller soybean meals. A 2 x 2 factorial design based on extrusion or cooking processes in combination with dehulling and pressing was used to produce four partly-defatted soybean meals with low residual trypsin inhibitor activity. The effects of process factors (preparation, temperature) and variations of flow rate/speed and specific mechanical energy were monitored.

Previous authors have highlighted the effect of operating parameters (i.e., screw rotation speed, temperature, and back pressure) and raw material (i.e., seed species, variety, water content, and pre-treatment) on process performance (oil yield and press capacity). For a given bean or seed origin, the main factors influencing the process are pressure, temperature, and moisture content.
Industrial pressing of oilseeds is realized using continuous screw presses. The presses are fed with crude or pre-treated seeds. Types of pre-treatments (e.g., thermal pre-treatment, size reduction, mechanical sieving) differ for various seed species (Carr, 1997). Each type of seed pre-treatment provides its own advantage. Dehulling is used to separate the oil-rich almond from hulls containing little oil and to eliminate anti-nutritional factors that have a negative impact on the animal. Crushing and flaking promote the solvent extraction step by changing the cake permeability. Cooking provides several advantages: moisture conditioning of seeds, oil viscosity reduction, increasing plasticity of seed, breaking of cell walls, protein clotting by denaturation, sterilization and deactivation of thermosensitive enzymes, and destruction of thermolabile toxic components (Dunford 2008; Laisney 1992).

A laboratory study of the successive steps of oil extraction with hydro-ethanol / ethanol / hexane has been carried out. The main goal of this work was to verify that the hydro-alcoholic solvent solubilizes in hexane to carry out a succession of extractions by ethanol-water followed by hexane to concentrate the protein in the meal (to obtain a protein content of at least 60%) while limiting the energy requirement for desolvation compared to that of hexane. For this, the work focused on the study of partitioning components (lipids, non-lipid dry matter, volatile components) between the cake and the miscella during successive washes and depending on the chosen solvent combination.

It is important that the heat treatment of rapeseed meal is not too strong to avoid reducing protein solubility and the lysine content. However, the disadvantage of a mild process could result in an insufficient removal of glucosinolates. This could be obtained by using a biological treatment of the meals. While the technology has been proven for soybean meal, such a novel process has to be developed to improve the value of rapeseed protein.

3. Results

3.1 Evaluation of the effects of dehulling and thermal treatments for production of expeller soybean meal

An experimental work was conducted at the OLEAD 'Oilseed–protein crops technology platform' (Pessac, France) on the production of expeller soybean meal (SBM) using different processes.

Origin of beans

The non-GMO soya (varieties Ecudor, Euralis Semences, Lescar, France) was grown near Toulouse, France in accordance with industry specifications (CC Sojadoc vs 19) and yielded around 3000 kg/ha. They were harvested in September 2015 and a batch (4200 kg) was bought by Cetiom and Ifip and delivered in November 2015 and January 2016 (N°585/SO/P15 and 585-2/SO/P15).

Characteristics are detailed in Table 1 and Figure 1. The oil content had a regular value, whereas the protein content (44%) was rather high compared to the mean result for the 2015 harvest (40.7%, Terres Inovia, 2015), or the average values in the animal feed database (39.6%; feedipedia.org). This high protein content can be related to the high thousand-kernel-weight and the high yield resulting from good grain filling conditions at the end of the crop. The trypsin inhibitor value was only 25 units/mg.

Table 1 Chemical composition (% of DM) of European soybeans

		Crude Fiber	Water content	Oil soxlhet	Proteins Kjeldahl	KOH sol. Proteins	Solubility, %	Trypsin Inbitor activity, TIU /mg
EAAP batch	Farmer values				41.8			
	Analysis	5.6	13.4	20.5	44.3	42.1	95.0	25
France 2015 Survey, Terres Inovia			13.2	21.4	40.7			
Feedipedia base		6.2	11.3	21.4	39.6			

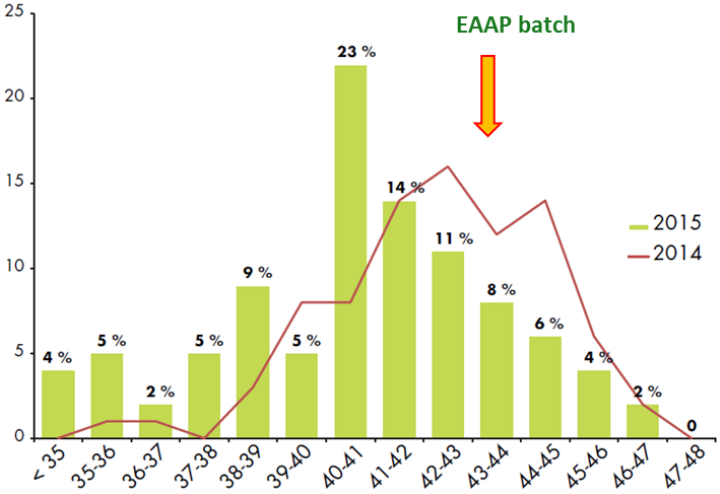

Figure 1 Protein (%) of French harvest soybeans in 2014 & 2015

Source : Enquête Soja à destination de l'alimentation animale – Récolte 2015 (Terres Inovia)

Methods for process

The schematic diagram of the processes is shown in Figure 2.

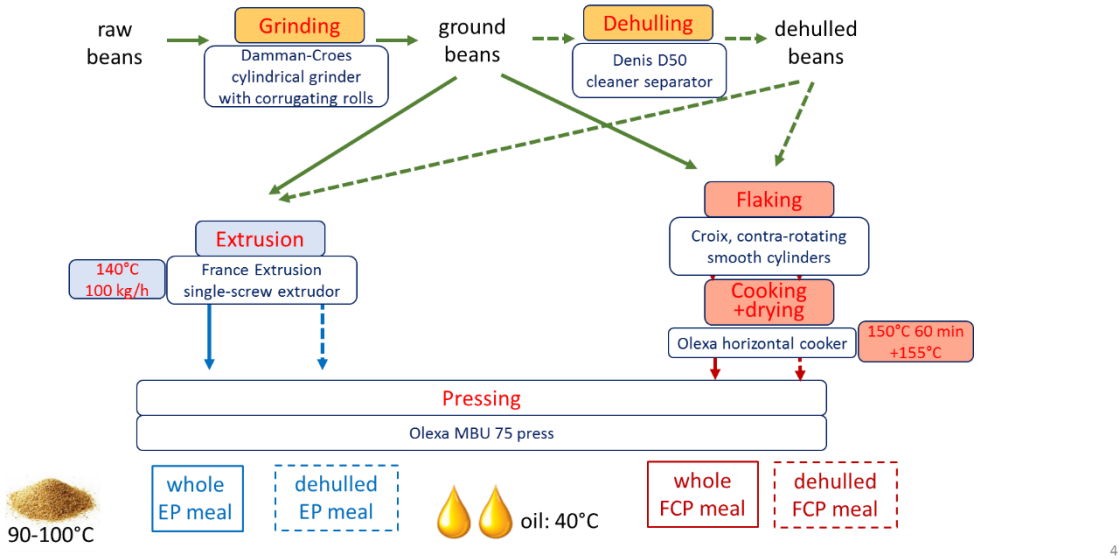

Figure 2 Schematic diagram of the soybean process.

Grinding/Dehulling

First, soybeans (4.2 T) were ground in a cylindrical grinder with corrugating rolls (Damman-Croes, Roeselare, Belgium) with a setting of the nip between the rollers as for kernels. Around 55% of the ground product (2.3 T) was then dehulled with a cleaner-separator (D50, Ets Denis, Brou, France). Soybean almonds rich in oil were separated from hulls and shells. The quantity balance of the dehulling is detailed in Table 2.

Table 2 Material balance of the dehulling operation

Initial quantity, kg	Seed almonds, kg	Hulls, kg	Losses, kg
2300	1899	384	17

Flaking-Cooking (FCP)

A flaking was operated for both products (dehulled or not) to increase the surface area. The gap of the contra-rotating smooth cylinders (Croix) were set to obtain flakes without almond residue. The flakes were then cooked (horizontal cooker, Olexa, Arras, France) at 150°C during 60 minutes.

Extrusion (EP)

The two products (dehulled or not) were extruded using single-screw extrusion (FEX1 France extrusion) at 140°C at around 100 kg/h.

Press operation

All beans were then pressed using a single screw press (MBU 75, Olexa, arras, France) with a barrel diameter of 180 mm and a theoretical capacity of 400 kg/h for cold-pressed rapeseed. The half-compressing arrangement was chosen (least compressing arrangement with addition of two ring-cones in the exudation zone) to slow the progression of the cake.

Nutritional composition analysis

Raw beans and SBM products were analyzed at COFRAC accredited laboratory of Terres Inovia (Ardon, France) for moisture (ISO 665 for beans and 771 for meals, respectively), crude protein (ISO 5983-2), crude fat (NF V03-908 for beans and ISO 22630 for meals), crude fibre (NF V03-040), and KOH protein solubility (NF ISO 14244). Trypsin inhibitor contents [1 TIU/mg = 1.9 trypsin inhibitor activity (TIA) mg/g] (TIU/mg) were analyzed at a COFRAC accredited commercial laboratory (InVivo Labs, France) using the AOCS Ba 12-75 SN method.

Table 3 Chemical composition of the raw soybeans and of processed expeller soybean meals

	Dry Matter (%)	*Oil (%)*	*Oil % DM)*	*Protein (%)*	*Protein (% de-oiled DM)*	*Protein solubility KOH (%)*	*Crude Fiber (%)*	*Trypsin inhibitors (TIU/mg)*
Raw soybeans	86.6	17.8	20.5	38.4	55.7	95.0	4.8	25
EP-dehulled	93.9	4.8	5.2	52.3	58.8	75.9	2.9	3.5
EP-whole seeds	94.2	4.6	4.9	50.1	56.0	70.2	5.5	2.6
FCP-dehulled	92.3	5.9	6.4	50.5	58.4	88.8	3.2	7.6
FCP-whole seeds	91.3	7.8	8.6	46.6	55.8	82.0	5.1	3.6

As showed in Table 3, extrusion allowed a higher oil extraction than cooking (residual oil: 4.9 vs 8.6 g/100 g of dry matter (DM) in whole EP and FCP meals and 5.2 vs 6.4 g/100 g of DM in dehulled EP and FCP meals, respectively).

The dehulling step resulted in an increase of almost 3 g/100 g of the protein content (58.8 and 58.4 g/100 g for dehulled EP and FCP meals, respectively, and 56.0 and 55.8 g/100 g for whole EP and FCP meals, respectively, on a fat-free DM basis).

The KOH protein solubility was increased by dehulling for the EP (70 vs. 76%) and FCP processes (82 vs. 89%, for whole and dehulled meals, respectively). Trypsin inhibitor (TI) values were 2.6, 3.5, 3.6, and 7.6 TIU/mg for whole and dehulled EP and FCP meals, respectively. The lower dryer outlet temperature measured for the dehulled beans compared to whole beans (90 vs. 97°C) may explain the lower TI inactivation for the dehulled FCP meal *(See in Annex the Cooking table for Product 1: Dehulled FCP meal).*

Quality of protein

The relative protein digestibility of the products was determined by DLO as the rate and maximum hydrolysis of peptide bonds using the effect of hydrolysis on the pH of the solution.

The degree of hydrolysis of the intact raw soybeans and the four oil extracted meals is presented in Figure 3. The curve characteristics and the initial pH of the solution (before pH was brought to pH 8.0) is provided in following two Tables with and without the raw soybeans included in the analysis.

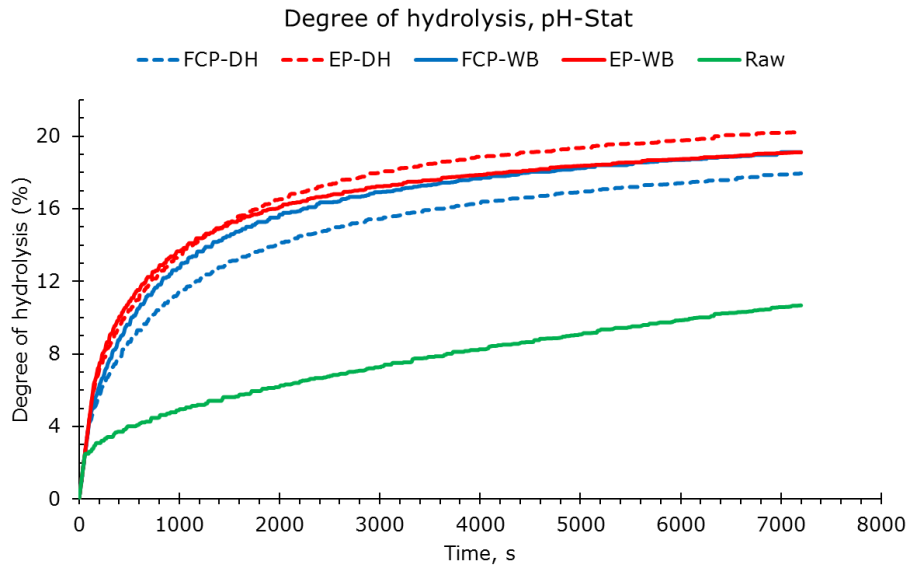

Figure 3 Rate of in vitro hydrolysis of raw soybean and extracted soybean meal extruded (E) or flaked (FC) or dehulled (D) or whole (W)

Table 4 Parameters describing the degree of hydrolysis of raw soybean and extracted soybean meals.

	Soybean	FCP-DH	EP-DH	FCP-WB	EP-WB	SEM	P-value
Rate k ($\times 10^{-6}$)	46.1^{a}	80.0^{b}	82.0^{b}	87.1^{b}	115.9^{c}	0.290	<0.001
DH max.	12.38^{a}	19.10^{b}	21.52^{d}	20.29^{c}	19.90^{bc}	0.319	<0.001
Initial pH	5.99^{a}	6.84^{b}	7.00^{b}	6.97^{b}	7.06^{b}	0.093	<0.001

FCP-DH = flaking-cooking process of dehulled beans; EP-DH = expanding process of dehulled beans; FCP-WB = flaking-cooking process of whole beans; EP-DH = expanding process of whole beans

Table 5 Parameters describing the degree of hydrolysis of extracted soybean meals.

						P-values		
	FCP-DH	EP-DH	FCP-WB	EP-WB	SEM	Process (P)	Dehulling (DH)	P x DH
Rate k ($\times 10^{-6}$)	80.0^{a}	82.0^{a}	87.1^{a}	115.9^{b}	0.316	0.001	<0.001	0.003
DH max.	19.10^{a}	21.52^{c}	20.29^{b}	19.90^{ab}	0.274	0.006	0.455	<0.001
Initial pH	6.84	7.00	6.97	7.06	0.069	0.114	0.198	0.605

FCP-DH = flaking-cooking process of dehulled beans; EP-DH = expanding process of dehulled beans; FCP-WB = flaking-cooking process of whole beans; EP-DH = expanding process of whole beans

The results in Figure 3 and Table 4 demonstrate a lower rate and maximum degree of hydrolysis of intact raw soybeans compared to all four oil extracted meals. This effect may be related by the much higher trypsin inhibitor activity in the raw soybeans (25 TIU/mg) compared to the extracted meals (2.6-7.6 TIU/mg).

The results in Figure 3 and Table 5 demonstrate an interaction between processing and dehulling on the rate and maximum degree of hydrolysis. The rate of hydrolysis (k) was higher for the meal from extrusion processing of whole beans compared to the other products and not different for the other three products. The maximum degree of hydrolysis was higher in meal from extrusion processing than from flaking cooking when soybeans were dehulled before processing, but not when whole soybeans were processed. Dehulling increased the maximum degree of hydrolysis in meal from extrusion processing, but decreased the degree of hydrolysis in meal from flaking cooking. It can be speculated that the latter was related to the higher trypsine inhibitor activity in FCP-DH (7.6 TIU/mg) compared to FCP-WB (3.6 TIU/mg), but it is not clear whether the trypsine inhibitor activity plays a role at these low levels. On the other hand, dehulling may increase the maximum degree of hydrolysis if we assume that the protein in hulls is less digestible than protein in beans.

3.2 Experimental work on the processing of rapeseed meal

Olead and Terres Inovia have begun work on improving the technological methods to extract oil from rapeseeds and process the rapeseed meals.

Conventional proteins concentrates are made by two successive steps, the first one in hexane for oil, the second one in aqueous ethanol to extract the sugars while keeping the proteins in an insoluble state. This protocol is likely to be little efficient for rapeseed, because rapeseed proteins are more heat sensitive than those of soybean. The goal of the study was to propose a new combination of polar and apolar solvents to extract both the fats and sugars of the dehulled rapeseed without intermediate desolventization.

A polar solvent, which is difficult to evaporate, was employed as a first extraction step, followed by the apolar hexane. Starting with ground dehulled seeds to obtain a press cake at 18% of oil, the extractions were done with either 79% ethanol followed by 96% ethanol, and finished by hexane (OP2), or with a simplified succession of 76% ethanol and hexane (OP1; Figure 4). Only the OP2 combination was able to reach easily a low oil content.

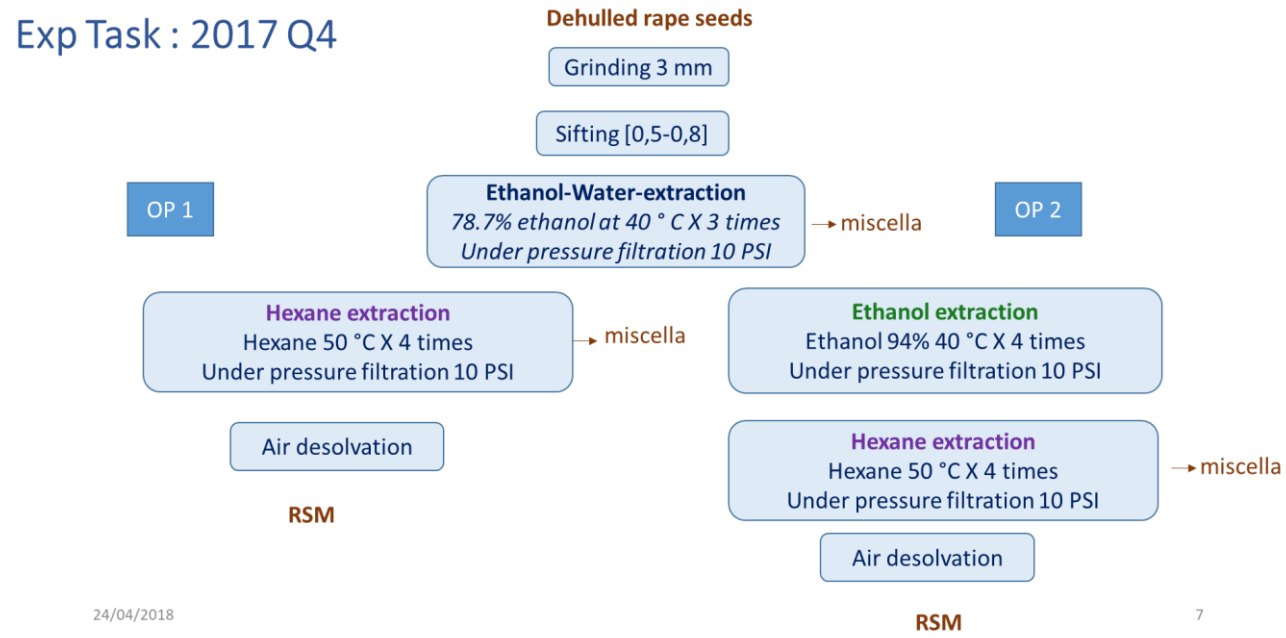

Figure 4 Desolvation processes tested for the rapeseed meal production

Table 6 Composition of RSM obtained in the Lab study

	Product	DM (%)	Oil (% DM)	CP (% DM)	GLS (µmol/g DM)
Dehulled rape seeds	31.OP1 Int	92,4	16,98	36,45	37,88
RSM OP1	90.OP1	84,5	0,79	51,18	7,50
RSM OP2	90.OP2	95,1	0,33	53,48	X
Miscella 1st washing with ethanol 80%	Miscella Lav.1 OP1	3,9	x	<LOQ	X

The composition results shown in Table 6 showed that both methods achieved a similar final oil yield. However, the final protein content of the RSM (51-53%) was lower than the target of 60% protein, partly because of a low initial protein content. Moreover, the glucosinolate extraction was also considered insufficient. Lastly, this step 1 of the work did not allow to experimentally evaluate the level of entrainment of ethanol by hexane.

3.3 Biological improvement of rapeseed meal

Ifip selected three rapeseed meals (RSM) processed at the beginning of 2016 from European grown and non-GMO rapeseeds from the main oilseed mills of Saipol-April group.

These samples were analysed by the COFRAC accredited Terres Inovia Laboratory (Ardon, France). The methods and results are shown in Table 7. Samples were also sent to Hamlet Protein for laboratory scale experiments to upgrade the nutritional value of the different European RSM sources.

Rapseed meal from Sète was chosen by Hamlet Protein for the pilot-scale production, because it was consistent with the high protein solubility and has a moderate glucosinolate content, indicating that the anti-nutritional factors were not totally removed.

Table 7 Chemical composition of the monitored RSM before selection (Winter 2016)

Origin	Water	Oil	Proteins	Protein KOH solubility %		GSL	Oil	Proteins	GSL	Trypsin inhibitors
method	Etuvage	Soxlhet	Kjeldahl	Kjeldahl		HPLC	Soxlhet	Kjeldahl	HPLC	
reference	ISO 771	ISO 22630	NF EN ISO 5983-2	Adapted from ISO 14244		NF ISO 10633-1	ISO 22630	NF EN ISO 5983-2	NF ISO 10633-1	AOCS Ba 12-75 - SN
	%	% in DM	% in DM	% inDM	% Total protein	µmol/g DM	(% crude)	(% crude)	µmol/g	TIU /mg
Sète	11.1	3.1	36.8	21.7	58.9	16.1	2.8	32.7	14.3	
Le Mériot	11.7	2.8	36.3	16.3	45.0	10.4	2.5	32.1	9.2	
Grand-Couronne	10.6	2.0	37.3	15.1	40.6	4.6	1.8	33.3	4.1	

A 3.3 ton batch of RSM processed by the Sète oil mill in October 2016 with European non-GMO seeds was bought by Ifip. Samples were analysed by NIR (Solevia Laboratory, Villefranche-de-Rouergue, France) and by wet chemistry (InVivo Labs, Chateau-Thierry, France). The results (Table 8) are in agreement with the expected values, except for protein solubility, but a different analytical method was used by the InVivo laboratory (see below). The GSL content was as expected.

Table 8 Chemical composition of the selected RSM before selection (October 2016)

Lab	Water	Oil	Proteins	Ash	Crude fiber	% KOH solubility of N	GSL	Total lysine	NDF	ADF	ADL	Tanins	GMO
	CE152/2000	Internal MGRA-H 15/02	NF EN ISO 16634	CE152/2000	Internal, CellF 13/02	Internal	Internal, glucenzy 01-06	CE152/2000	NF V18-122	NF V18-122	NF V18-122	SPM	PCR ISO 24276,
Invivo Labs	12.7	3.0	33.9	6.4	12.1	38.1	13.7	17.9	26.0	18.7	8.2	0.33	< 0.9%
	IR		IR		IR								
Solevial	12.2		32.6		14.1								

SPM : spectrophotometry

A successful pilot-scale upgrading of RSM was conducted by Hamlet Protein to conduct a feeding trial at IRTA (Table 9). The protein content in DM was increased by 4 points. For glucosinolates in the RSM prepared by Hamlet Protein, a total content of 16.9 µmol/g DM (i.e., 15.9 on a crude basis) was found with the HPLC method by the Terres Inovia laboratory. The value initially determined in the unprocessed RSM was 13.7 ± 2.7 µmol/g on a crude basis by InVivo with an enzymatic method. Because two different laboratories and methods were involved, these values can be considered as non-different.

Table 9 Chemical and nutritional composition of commercial RSM before and after biological treatment

	Dry matter (%)	*Crude fat (%)*	*Protein (%)*	*Protein (% in DM)*	*Ash (%)*	*Crude fiber (%)*	*GSL*
Basal RSM	87.8	1.8	34.1	38.8	6.4	12.0	13.7
Improved RSM	94.4	1.4	40.3	42.7	7.3		

Values are means of data provided by Ifip, Hamelet Protein and IRTA.

4. Conclusions

The difference in temperature during drying for one of the soybean batches was considered as an occasional event that can be easily controlled. It is concluded that all four processes based on either extrusion or cooking with or without dehulling produced good quality expeller soybean meals with 46-52 g/100 g crude protein, 4-8 g/100 g residual oil, low trypsin inhibitor content, and high digestibility values for protein and amino acids. In the future, the extruded-expelled SBM could be produced in medium-sized crushing plants from local and GMO-free soybean crops. This product could have interesting nutritional and economic values making their development possible in Europe.

The goals of the first experiment on improved rapeseed meal were not totally achieved, as the protein concentration was too low and the glucosinolates were insufficiently extracted. Laboratory experiments will be used to test the effect of the last hexane extraction on the facility of desolvation and will enable new extractions trials to improve the glucosinolate removal using aqueous solvents. Beside the experimental work, time will be dedicated to a model of prediction of the energy cost of aqueous ethanol extraction coupled with pure ethanol finishing and/or hexane finishing, and to the production of small batches for in vitro or in vivo evaluation of the nutritional value.

5. Appendix

Monitoring results of experimental production of expeller soybean meals

Product 1: Dehulled FCP meal (17-11-2016)

Cooking

Time h/mn	Cooker exit temperature (°C)	Steam flow (kg/h)	Cooker set temperature (°C)	Dryer set temperature (°C)	Dryer exit temperature (°C)
9 :00	82.0	10.0	155.0	155.0	91.0
10 :05	87.0	9.5	145.0	150.0	89.0
11 :35	89.0	9.5	145.0	150.0	91.0

Press operation

Time h/mn	Oil flow (kg/h)	Oil temperature (°C)	Cake flow (kg/h)	Cake temperature (°C)	Rotation speed (rpm)	motor power (Kw)	Barrel temperature (°C)
8 :55	29.5	35.0	185.0	99.0	15	9.5	-
10 :00	24.0	42.0	184.0	93.0	15	6.3	-
11 :30	23.0	44.0	182.0	103.0	13.5	6.0	-

Product 2 : Whole FCP meal (17-11-2016)

Cooking

Time h/mn	Cooker exit temperature (°C)	Steam flow (kg/h)	Cooker set temperature (°C)	Dryer set temperature (°C)	Dryer exit temperature (°C)
13 :20	90.5	9.5	148.0	155.0	96.1
14 :20	89.8	9.5	148.0	155.0	97.2
16 :10	90.2	9.5	148.0	155.0	97.4
17 :10	92.6	9.5	148.0	155.0	97.6

Press operation

Time h/mn	Oil flow (kg/h)	Oil temperature (°C)	Cake flow (kg/h)	Cake temperature (°C)	Rotation speed (rpm)	motor power (Kw)	Barrel temperature (°C)
13 :35	20.0	40.1	163.0	86.3	13.5	5.3	98.6
14 :20	19.5	39.8	150.5	86.1	13.5	5.3	105.2
16 :10	18.5	39.9	156.0	88.1	13.5	5.3	103.2
17 :10	18.0	39.7	132.0	89.0	13.5	5.3	107.3

Product 3 : Whole EP meal (18-11-2016)

Extrusion

Time h/mn	Flow rate (kg/h)	Flow rate index	Rotation speed (rpm)	Motor power (Kw)	Cake temperature (°C)	Last zone temperature (°C)
12 :10	96.8	1275	9	19.7	150.8	138.0
13 :40	-	1305	9	19.3	149.7	137.0
15 :00	-	1328	9	20.5	150.1	138.0
16 :30	-	1328	9	20.8	151.4	139.0

17 :00	-	1328	9	19.5	-	141.0
18 :15	-	1328	9	18.4	-	139.0
19 :00	-	1328	9	18.6	-	137.0

Press operation

Time h/mn	Oil flow (kg/h)	Oil temperature (°C)	Cake flow (kg/h)	Cake temperature (°C)	Rotation speed (rpm)	motor power (Kw)	Barrel temperature (°C)
12 :20	13.0	34.3	73.0	100.1	15	7.2	110.9
15 :30	14.0	34.8	75.5	99.8	15	5.3	114.4
17 :20	13.5	33.5	78.0	103.8	15	5.1	114.0

Product 4 : Dehulled EP meal (21-11-2016)

Extrusion

Time h/mn	Flow rate (kg/h)	Flow rate index	Rotation speed (rpm)	Motor power (Kw)	Cake temperature (°C)	Last zone temperature (°C)
10 :20	115	1375	9	25.7	152.8	141.0
11 :10	-	1375	9	25.7	152.6	140.0
12 :00	-	1375	9	25.0	-	141.0
13 :00	-	1375	9	24.8	-	142.0
14 :00	-	1375	9	23.7	-	142.0
15 :00	-	1375	9	-	-	141.0
16 :45	-	1375	9	-	-	139.0
17 :50	-	1375	9	-	-	141.0

Press operation

Time h/mn	Oil flow (kg/h)	Oil temperature (°C)	Cake flow (kg/h)	Cake temperature (°C)	Rotation speed (rpm)	motor power (Kw)	Barrel temperature (°C)
10 :15	16.5	31.6	98.5	77.4	15	5.0	77.9
11 :20	17.0	37.3	96.5	85.6	15	5.0	98.5
12 :20	22.5	41.0	96.0	85.0	15	5.0	105.6
14 :00	18.5	42.0	101.0	86.0	15	4.0	109.0

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