STUDY OF THE CHARACTERISTICS OF CULTIVATED VARIETIES OF SUNFLOWER, REGARDING THE PRODUCTION OF HIGH QUALITY SUNFLOWER MEAL WITH DEHULLING PROCESS

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ABSTRACT

Dehulling sunflower seeds, before crushing, increases the protein content in the meal up to 36%, whereas a cake obtained without dehulling contains 27-29% protein. The quality of sunflower seeds directly impacts the possibility of obtaining a high protein meal. The purpose of this study was to assess the varietal effect on the protein content and the hullability of sunflower seeds. Genetic effect was studied with seed samples from a network of variety evaluation trials in France during the two years. The protein content in seeds was expressed as a percentage of Defatted Dry Matter. Hullability was obtained by measuring the initial weight of the seeds and the weight of extracted hulls, removed by a laboratory dehulling equipment. Other measured characteristics were oil content, seed size, crude fibre content. Significant differences between varieties for protein content were observed within the medium early/medium late group in 2013 (from 33.2% to 41.3%), as for hullability (from 3.7% to 14.7%). As a consequence, the potential protein content of their dehulled meals also ranged widely (34-44%). Crude fibre content was closely correlated to hullability. An equation was established to estimate the protein content of dehulled sunflower meal as a function of protein content and crude fibre content in seeds. The protein content of sunflower seeds proved to be the key characteristic determining the quality of sunflower meal. Genetic selection, which allowed great improvements in the oil content and fatty acid composition, should therefore also help to improve the quality of sunflower meal.

Key words: Sunflower, Hullability, Protein, Variety

INTRODUCTION

Sunflower seed processing produces 2 principal co-products: oil, mainly for human consumption, and meal, for animal feed. Two main variants exist in the crushing industry: oil extraction from whole seeds and oil extraction from partially dehulled seeds. In the first process, the resulting meal is of low protein content (27-29%). In the second process, the resulting meal has a higher protein content (36% protein content is a standard quality for this type of meal) and reduced fibre. The second process is highly developed in Eastern Europe and Argentina. In France, until recently, dehulling was carried out in only one oil mill which has a limited dehulling capacity offering only a modest improvement in meal protein content. Capacity has begun to develop since 2013, with a larger factory now partially dehulling prior to crushing.

Dehulling offers 2 advantages:

- The higher protein and lower fibre content meal has an increased economic value on the animal feed market. Peyronnet *et al.* (2012) demonstrated that the interest price of a 36% protein content meal (i.e., that maximum price at which it remained competitive) was 70% of the soybean meal price, whereas for a 29% protein meal it was only 43%.

- The hulls removed can be used as an energy source for steam production in a high-performance biomass boiler. Rising energy costs and environmental concerns have led to a growing interest within the crushing industry for using hulls as energy source instead of fossil fuels (Tostain *et al*., 2012).

In the 1980s, energy prices were very high; towards the end of the decade and the early 1990s, research was undertaken in France to prepare the crushing industry for greater use of the dehulling process. Genetic studies were carried out concerning the ease with which hulls could be removed from sunflower seeds (hullability). These studies showed that this characteristic could be introduced through breeding programmes. Cultivars producing seeds with a smaller hull mass, higher oil content but a good hullability offered the most promise for improving the quality of sunflower meal. Such genotypes were rare, but a recurrent selection programme could be used to increase the frequency of favourable genes (Denis and Vear, 1996). Although all this work constituted a favourable basis for the growth of sunflower dehulling in the French crushing industry, the technology was not developed. In France, the oil mill that only recently reintroduced dehulling actually abandoned the technique in the early 1990s, the context being one where the oil content of the new cultivars was improving but hullability was decreasing, resulting in considerable losses of oil from the hull fraction. At that time moreover, boiler technology for burning hulls had not achieved an adequate level of efficiency. So, the economics were against dehulling. As a consequence, sunflower breeding has ignored the characteristic of hullability, and likewise protein content.

The supply of vegetable protein to livestock is now a matter of political concern. Oilseed meals are an attractive source of proteins. Moreover, sunflowers have the advantage of containing no anti-nutrients or toxic components. Increasing the protein content in sunflower meal would therefore be advantageous. The quality of seeds, notably the protein content expressed as a percentage of Defatted Dry Matter (DDM) and their hullability, determines the potential protein content of the resulting meal. It has been shown that the most profitable way to reach a set requirement of protein content in the meal is to produce seeds with high protein content as a percentage of DDM, in order to require extraction of only a minimum amount of hulls (Dauguet *et al.*, 2015).

The economic focus remains on sunflower oil (about 700-800€/t in 2015, as compared with approximately 180-200€/t for non-dehulled meal and 250-280€/t for 36% protein meal). Hence, breeding has always been centred on obtaining varieties combining high yield and high oil content. These are the 2 criteria that are currently taken into account in the registration of new sunflower varieties; protein content is not a criterion in the registration of new sunflower varieties and nor is it measured in the official trials. Studies have shown that soil and climatic conditions exert a greater influence on protein content than genetics (Nel, 2001; Oraki *et al.*, 2011; Dauguet *et al.*, 2015). This can be explained by the fact that breeding programmes have not searched for variability in protein content. No relationship has been observed between oil content and protein content as a percentage of DDM (Dauguet *et al.*, 2015). So, the independence of these 2 features would suggest that there is considerable scope for improving the protein content of the defatted fraction without penalizing oil content.

Hullability increases with the size of seed and decreases with their oil content; these are varietal characteristics and so genetic improvements of hullability might be considered (Baldini *et al.*, 1994; Denis *et al.*, 1994; Evrard *et al.*, 1996; Nel, 2001; Sharma *et al.*, 2009; Dauguet *et al.*, 2015).

In a previous study (Dauguet *et al.*, 2015), we examined seed samples taken from a wide network of farmers' fields in South West France, looking at 3 varieties, over 2 years (2 varieties each year). Both protein content and hullability were found to be influenced by the environment, with water stress having a substantial effect. Some differences between cultivars could be identified, affecting protein content and hullability. In contrast, the influence of agricultural practices such as nitrogen fertilization could not be established. In order to improve meal quality, and the competitiveness of sunflowers in the food chain, boosting the protein content of sunflower seeds through breeding would be very beneficial, so long as there was no negative effect on oil content and hullability remained adequate.

The objective of the present study, designed in close collaboration with Terres Univia, the French oil and protein crops inter-branch organization, was to improve knowledge of the sunflower cultivars traded on the French market, in particular with regard to their characteristics that impact the possibility of producing good quality meal: seed protein content as a percentage of DDM and hullability. We studied genetic and climatic effects on these characteristics, with samples from a network of varietal evaluation trials, during 2 consecutive years to evaluate the variability in of marketed sunflower varieties for these or other characteristics not taken into account in breeding programmes. We also measured crude fibre content in order to study its correlation with hullability, and the possibility of evaluating hullability using this more simple analytical result rather than employing laboratory dehulling equipment.

MATERIALS AND METHODS

Samples: Seed samples were collected from the Terres Inovia experimental network. This is constructed each year to evaluate the performance of varieties marketed in France (agronomic performance such as yield and diseases resistance; quality traits of the seeds such as oil content and Thousand Seed Weight). For the purposes of this study, additional analyses were performed on the seed samples to measure their protein content, hullability and crude fibre content. The varieties studied were oleic and linoleic types in 2 maturity groups (early or medium early/medium late). Each year, the Terres Inovia experimental network includes about 30 variety trials for each maturity group.
The seed samples collected for this study came from 2012 and 2013. Each year, we collected samples from several experimental locations, from regions where sunflowers are commonly cultivated: South-West, West and Central France (see Figure 1).

Each year, we studied the protein content of 5 to 7 different varieties in each maturity group, in the 8 different trial locations. For hullability and crude fibre analyses, the number of varieties studied was reduced to 2 per maturity group, as the measurements were time-consuming and costly.

In order to investigate the variability of profiles for protein content and hullability, we also studied each year a larger number of varieties (12 and 16) in only 2 or 3 trials (see Table 1).

Overall, the data set included 275 samples of sunflower seeds, with 40 different sunflower varieties, at 23 different locations (see Table 1).

Table 1: Cultivar distribution according to year and locations

Year	Maturity group	Trial locations (postal code of French department)	Cultivars
2012	Early	Antoigné (79), Frozes (86), Levroux (36), Saint Branchs (37), Rhodon (41), Maslacq (64)	ES Biba, Vellox, Extrasol, ES Balistic, ES Ethic
		Saint-Martial (16), Vibrac (17)	ES Biba, Vellox, Extrasol, ES Balistic, ES Ethic, Ullys, Fydgi, Volltage, ES Violetta, P64LL41, ES Atletic, SY Valeo
	ME/ML	Virson (17), Loudun (86), Vicq / Nahon (36), Lévignac (31), Tané (32), Le Saumont (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO
		Vibrac (17), Duras (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Breha, Sherllok, Dougllas, Mobill, SY Edenis, ES Akustic, NK Adagio, LG5625, ES Tektonic, ES Unic
2013	Early	Vibrac (17), Levroux (36), Meung sur Loire (45), Ivoy le pré (18), Maslacq (64)	ES Biba, Vellox, Extrasol, SY Valeo, ES Violetta, Fydgi
		Antoigné (79), Triaize (85), Trouy (18)	ES Biba, Vellox, Extrasol, SY Valeo, ES Violetta, Fydgi, SY Sanbala, P63LL78, LG5377, Bering, MAS83R, ES Lumina, ES Columbella, SY Revelio, ES Atletic, ES Balistic
	ME/ML	Antoigné (79), Benet (85), Lévignac (31), L'Isle Jourdain (32), Montagnac Auvignon (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Dougllas, ES Tektonic
		Pompertuzat (31), Tané (32), Duras (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Dougllas, ES Tektonic, Clloser, Meddia CS, LG5687HO, SY Explorer, LG5528, SY Edenis, ES Akustic, LG5625

ME/ML=Medium early/medium late

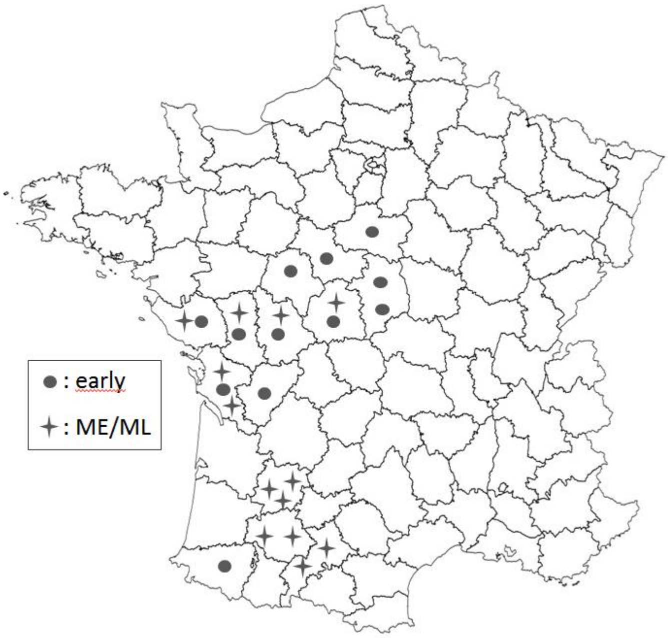

Figure 1. Location in France of the selected sunflower variety trials in 2012 and 2013, by maturity group.

Chemical analyses: For each seed sample collected, the oil content was assessed by Nuclear Magnetic Resonance (NF EN ISO 10565) and expressed as a percentage at marketing standard (9% moisture content and 2% impurities level), as commonly used in varietal trials. Protein content was assessed by the Dumas method (NF EN ISO 16634-1) and expressed as a percentage of Defatted Dry Matter (DDM) or Dry Matter (DM). The crude fibre content was measured by the Weende method (NF V03-040 with previous oil extraction by hexane), and was expressed as a percentage of Defatted Dry Matter (DDM) or Dry Matter (DM). The expression of results as a percentage of DDM, for protein or crude fibre content, has an obvious interest from an end-user perspective, as it gives information on the content that would be obtained in the meal, after oil extraction.
An indicator of the seed size, the Thousand Seeds Weight (TSW), was measured on clean dry grain (0% moisture).
While each sample was analysed for it oil and protein content and TSW, the crude fibre content was assessed in only 2 varieties in each maturity group each year.
All of these analyses were carried out at Terres Inovia's Analysis Laboratory in Ardon.
Hullability determination: What we refer to as "hullability" was obtained by measuring the initial weight of the seeds and the weight of extracted hulls, removed by a standard procedure: Hullability (%) = (mass of extracted hulls (g))/ (mass of initial seeds (g)).
Seed hullability is affected by water content (Sharma et al, 2009). Since the seeds had been stored at various levels of humidity, they were taken out of cold storage and placed in Petri dishes that were then left open for 48 hours, to facilitate equilibration of water content prior to dehulling. The water content of the seeds was low, as they had previously been dried slightly to favour long-term storage: about 5.5-6.0% (mean moisture 5.7%) and sufficiently uniform (standard deviation 0.7%) to permit a comparison of hullability.

A conical divider was used to produce 4 identical subsamples of approximately 15g from the primary sample. Three replicates were used in the dehulling test; the 4th was used to measure water content. Employing a method determined by a previous study (see Dauguet et al., 2015), the weighed samples were passed 3 times through the laboratory dehulling equipment, a Techmachine, at 2 000 revolutions per minute (rpm). This is equivalent to limited or moderate dehulling in an industrial dehulling process which would result in 10% hull extraction, whereas 15% hull removal is current practice in industry.

After sorting using laboratory sorting equipment, the various fractions (kernels, whole seeds, fines and hulls) were weighed (to the nearest 0.01 g). The percentage of extracted hulls was taken from the average of 3 replicates. Water content was assessed from the difference in seed weight before and after 15 hours in an oven at 103°C (NF V03-909).

Hullability also was measured only for 2 varieties in each trial, except for the trials with a wider range of varieties studied (2 or 3 trials each year in each maturity group) where hullability was measured for each cultivar.

Statistical analyses: Data were analysed using analysis of variance (ANOVA). F-test and differences were evaluated via the Student-Newman-Keuls Test (software SAS 9.4). The coefficients of determination, and associated probability (Student) were also established using SAS software. Shapiro-Wilk tests were performed to check the normality of the residuals; homoscedasticity was verified visually. *Calculation of the protein content in sunflower meal post-dehulling:* Given the defined quality characteristics of sunflower seeds (protein content, hullability), we developed a formula to estimate for each sample the protein content of the dehulled meal. In this way, we were able to assess the potential of a particular variety to produce meal of the required quality. This formula is based on measured values: initial seed protein content and the degree of hullability (percentage of extracted hulls), as well as assumptions regarding oil and moisture content of the meal, and the protein, oil and moisture content of the sunflower hulls. These assumptions were based on a yearly study of meal quality in the French crushing industry (Terres Inovia’s unpublished results from a particular factory) and from an online database on feedstuffs (Feedipedia) for parameters on hulls.
Assumptions:

A = Oil content in meal in raw matter (RM) = 1.2%

B = Moisture content in meal = 11.5%

C = Protein content in hulls in RM = 6%

Moisture content in hulls = 10%

Oil content in hulls in RM = 2%

X = mass of removed hulls (g/100g seeds)

Formulae:

D = Defatted Dry Matter (DDM) of seeds = 1 – (moisture content of seeds) – (oil content of seeds on NMR)

E = Protein content of seeds (%DDM) = protein content of seeds (%DM)/ (1 - oil content of seeds (%DM))

F = Defatted Dry matter of the hulls = 1 – (moisture content of hulls) – (oil content of hulls) = 88%

G = Protein content of non-dehulled meal (% RM) = $E * (1 - A - B) * \left(\frac{D}{1-A-B}\right)$

<u>H = Protein content of extracted hulls (% RM) = X * C</u>

<u>I = Protein content of dehulled meal (%RM) =</u> $\frac{G-H}{D-X*F} * (1 - A - B)$

RESULTS AND DISCUSSION

Analysis of variance

The results were aggregated by maturity group and by year, see Tables 2 and 3. Here, we assessed the influence of location and cultivar on the seed characteristics in 8 trials.

Table 2: Results for the Early group cultivars grown in eight trial locations (mean by cultivar, t comparison tests at 5% and levels of significance of ANOVA of seed components).

Year	Factor		Oil content (% at marketing standards)	Thousand Seeds Weight (g DM)	Protein content (% DDM)	Protein content (% DM)	Crude fibre (% DDM)	Crude fibre (% DM)	Hullability (% extracted hulls)	Calculated protein content in dehulled meal (% RM)
2012	Cultivar	ES Biba	46.1 (B)	42.4 (BC)	34.5 (A)	16.7 (B)				
		Vellox	47.9 (A)	39.6 (C)	36.2 (A)	16.8 (B)	28.3 (B)	13.5 (B)	11.0 (B)	38.9 (A)
		Extrasol	45.4 (B)	45.4 (AB)	35.5 (A)	17.4 (B)	30.3 (A)	15.0 (A)	15.5 (A)	41.5 (A)
		ES Balistic	42.9 (C)	46.3 (A)	35.9 (A)	18.6 (A)				
		ES Ethic	46.0 (B)	42.4 (BC)	34.5 (A)	16.7 (B)				
	Level of significance	Location	***	***	***	***	**	**	NS	NS
		Cultivar	***	***	NS	***	***	***	**	NS
2013	Cultivar	ES Biba	48.1 (C)	49.7 (C)	32.4 (AB)	14.9 (AB)				
		Vellox	52.3 (A)	50.2 (C)	33.3 (A)	13.8 (B)				
		Extrasol	47.7 (C)	57.9 (A)	33.5 (A)	15.6 (A)				
		SY Valeo	48.2 (C)	50.4 (C)	31.3 (AB)	14.4 (B)	35.5 (A)	16.6 (A)	10.2 (A)	33.1 (A)
		ES Violetta	47.7 (C)	55.6 (AB)	30.9 (B)	14.3 (B)				
		Fydgi	50.7 (B)	52.1 (BC)	32.9 (AB)	14.2 (B)	35.2 (A)	15.3 (B)	9.6 (A)	34.7 (A)
	Level of significance	Location	***	***	**	**	NS	NS	*	NS
		Cultivar	***	***	**	**	NS	*	NS	NS

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means per year within a column followed by the same letter are not significantly different ($P < 0.05$)

Table 3: Results for the ME/ML group cultivars grown in eight trial locations (means by cultivar and levels of significance of ANOVA of seed components)

Year	Factor		Oil content (% at marketing standards)	Thousand Seeds Weight (g DM)	Protein content (% DDM)	Protein content (% DM)	Crude fibre (% DDM)	Crude fibre (% DM)	Hullability (% extracted hulls)	Calculated protein content of dehulled meal (% RM)
2012	Cultivar	NK Kondi	44.9 (A)	41.1 (B)	35.3 (A)	17.5 (C)				
		Kapllan	44.9 (A)	42.7 (AB)	36.1 (A)	17.9 (BC)				
		Extrasol	43.6 (AB)	46.0 (A)	36.7 (A)	18.7 (AB)	29.1 (A)	14.8 (B)	16.8 (A)	43.7 (A)
		DKF3333	43.2 (B)	45.8 (A)	36.1 (A)	18.6 (AB)	30.0 (A)	15.4 (A)	15.3 (A)	41.4 (A)
		LG5656HO	42.3 (B)	44.1 (AB)	36.3 (A)	19.0 (A)				
	Level of significance	Location	***	***	***	***	**	**	**	NS
		Cultivar	***	*	NS	**	NS	*	NS	NS
2013	Cultivar	NK Kondi	47.9 (A)	49.1 (D)	35.5 (C)	16.4 (C)	36.8 (A)	17.4 (A)	9.7 (B)	37.3 (B)
		Kapllan	48.2 (A)	52.4 (CD)	38.0 (AB)	17.5 (B)				
		Extrasol	46.6 (B)	56.3 (B)	37.2 (ABC)	17.8 (AB)				
		DKF3333	46.9 (B)	51.0 (CD)	37.7 (ABC)	17.9 (AB)				
		LG5656HO	44.5 (C)	49.8 (CD)	37.4 (ABC)	18.7 (A)				
		DOUGLLAS	47.6 (A)	60.1 (A)	39.0 (A)	18.0 (AB)				
		ES TEKTONIC CL	46.0 (B)	53.7 (BC)	36.2 (BC)	17.6 (B)	36.1 (A)	17.9 (A)	14.4 (A)	41.8 (A)
	Level of significance	Location	***	***	***	***	*	**	*	*
		Cultivar	***	***	**	***	NS	NS	***	**

NS: non-significant; ∗$P < 0.05$; ∗∗$P < 0.01$; ∗∗∗$P < 0.001$

Means per year within a column followed by the same letter are not significantly different ($P < 0.05$)

ANOVA were performed on the parameters studied using location and cultivar as explicative factors (see Tables 2 and 3). The impact of location was significant, except on hullability and crude fibre content in the early group and for the calculated protein content of the dehulled meal. This impact might be attributable to different meteorological and soil conditions affecting plant growth.

Cultivar was the principal factor affecting oil content, seed size (TSW) and percentage protein of DM. It did not however systematically affect the protein content of DDM: the variability for each variety was high (see Figure 2). Significant differences between varieties for protein content as a percentage of DDM were observed only in 2013: ES Violetta was significantly lower than Extrasol and Vellox (30.9%

versus 33.5 and 33.3%) within the early group. Within the ME/ML group, NK Kondi and ES Tektonic had significantly lower protein contents than Dougllas (35.5% and 36.2 % versus 39%). For this parameter, in 2012 the differences between locations were greater than the differences between cultivars.

For hullability, Vellox was significantly more difficult to dehull than Extrasol in 2012 (11% extracted hulls versus 15.5), and NK Kondi had also a lower hullability than Es Tektonic in 2013 (9.7% extracted hulls versus 14.4%). However, there was no significant difference between SY Valeo and Fydgi in 2013, or between Extrasol and DKF3333 in 2012, as the differences between locations were high (see Figure 3).

It is difficult to conclude from the results for crude fibre content. Crude fibre associated with higher hull content in seeds could be a favourable factor for hullability. For example, Extrasol had significantly higher crude fibre content (on DDM and on DM) than Vellox, which could be related to a better hullability, but, ES Tektonic showed better hullability than NK Kondi, although these 2 varieties had comparable crude fibre contents. This led to a conclusion that crude fibre content was not the unique factor affecting hullability. Seed size, hull structure and the phenomenon of adherence were probably important also.

The final aspect, the right hand column in Tables 2 and 3, a calculation of the potential protein content in meal that would be obtained after dehulling, based on protein content of the seeds and hullability, did not show significant genetic differences, except between NK Kondi and ES Tektonic, as the second had a better hullability and gave a richer meal. This parameter suggests that the protein content of meal could be quite high, above the standard level in high-protein meal (36%), since for some cultivars in some years it exceeded 40%. It was only in the early group in 2013 that the protein content was low for all the varieties, and hullability was moderate; so the calculated protein richness in the meal was less than the standard 36%. This highlighted the importance of initial protein content in seeds in the production of good quality meal.

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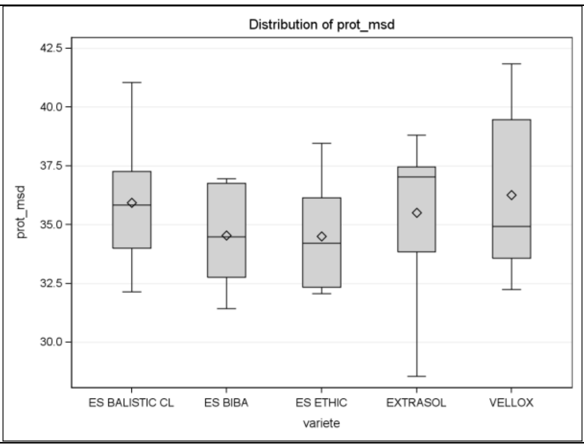

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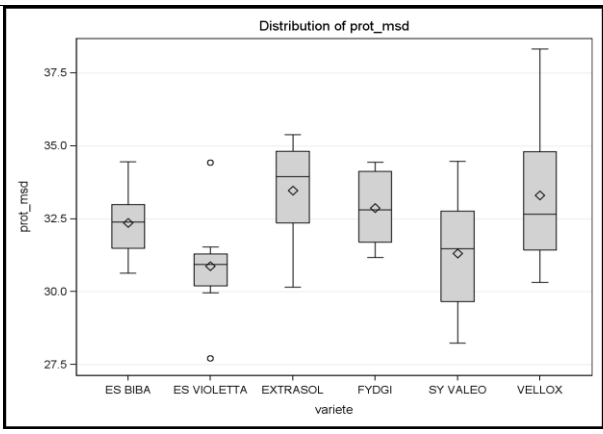

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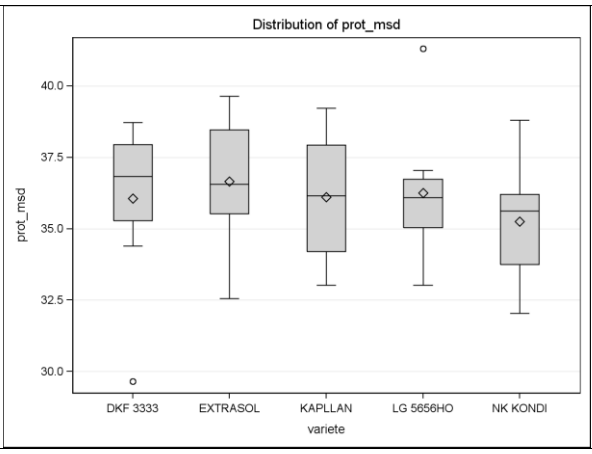
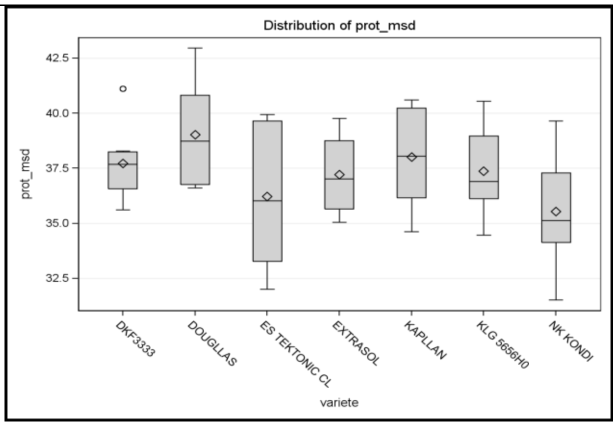
ME/ML		

Figure 2: Boxplots of cultivar effect on protein content (%DDM) showing the median (line in the middle), mean (diamond), interquartile range (box) and total range (whiskers) not including atypical values (circle symbols, where they exist)

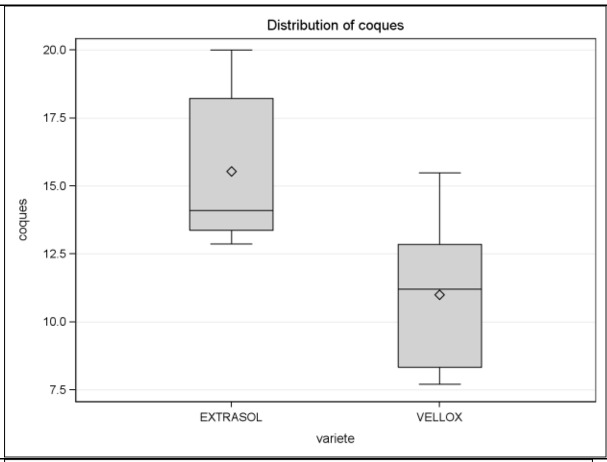
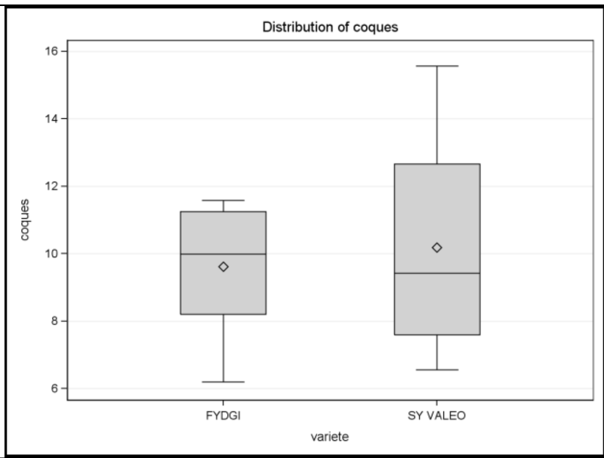
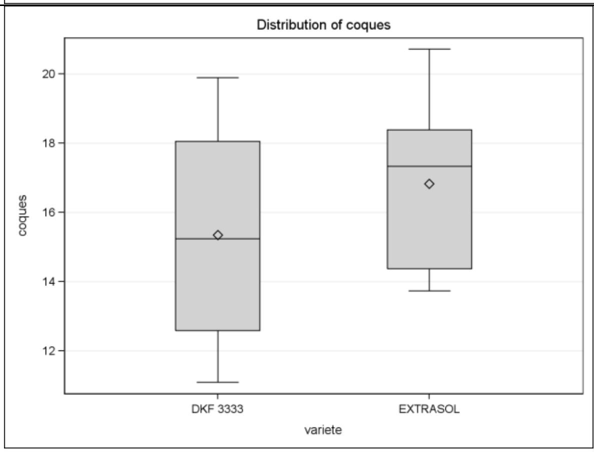
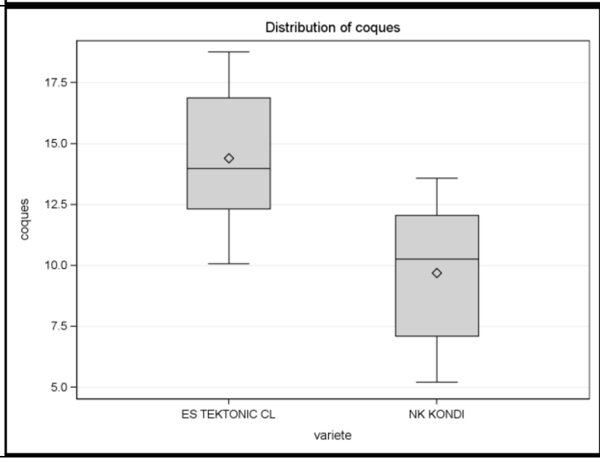
Group	2012	2013
Early		
ME/ML		

Figure 3: Boxplots of cultivar effect on hullability (% of extracted hulls) showing the median (line in the middle), mean (diamond), interquartile range (box) and total range (whiskers)

3.2. Year effect

Some trial locations and cultivars were constant in both years, which enabled the evaluation of the effect of year (including the climatic effect) (see Table 4):

- for the early group, 4 locations (Antoigné, Levroux, Vibrac and Maslacq) and 3 varieties (ES Biba, Vellox, Extrasol),

- for the ME/ML group, 3 locations (Duras, Tané, Lévignac) and 5 varieties (NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO).

Table 4: Analysis of variance for year, location and cultivar effects on seed characteristics (means, t comparison tests at 5% and levels of significance)

Factors		N	Oil content (% at marketing standards)	TSW (g DM)	Yield (t/ha at marketing standards)	Protein content (% DDM)	Protein content (% DM)
Early	2012	12	47.5 (B)	45.4 (B)	3.80 (A)	35.8 (A)	16.7 (A)
	2013	12	49.3 (A)	55.3 (A)	3.57 (A)	33.3 (B)	14.9 (B)
	Year		*	***	NS	*	**
	Location		NS	NS	NS	**	**
	Cultivar		**	*	NS	NS	NS
ME/ML	2012	15	44.3 (B)	41.6 (B)	3.42 (B)	37.4 (A)	18.8 (A)
	2013	15	48.0 (A)	47.5 (A)	3.66 (A)	37.2 (A)	17.2 (B)
	Year		***	**	*	NS	***
	Location		**	NS	*	NS	NS
	Cultivar		**	NS	***	NS	**

NS: non-significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

Means within a column followed by the same letter are not significantly different ($P < 0.05$) with t test

It was rainier and less sunny in 2012 than in 2013. During 2013, water stress occurred after flowering, which led to lower yields in the South and West of France and in the country as a whole (respectively 2.38 t/ha in 2012, and 2.14 t/ha in 2013, according to public statistics). In the studied, this trend was observed for the early cultivar group, but it was not substantial (yield 2012 3.8 t/ha and yield 2013 3.57 t/ha); while the situation was the opposite for the ME/ML cultivar group with better yields in 2012 (3.66 t/ha) than in 2013 (3.42 t/ha). This is due to the fact that the varietal evaluation trials were grown in more optimal conditions than normal farmers' fields, and therefore were not representative of national sunflower production. The climatic effect influenced the oil content and seed size (TSW), higher in 2013 than in 2012, and percentage protein content of DM, higher in 2012, which was a consequence of lower oil content in 2012. For the percentage protein content of DDM, a significant difference was observed only in the early cultivar group, with higher levels observed in 2012 than in 2013.

The wider range of varieties

Each year, 15 cultivars were sampled and analysed in 2 (2012) or 3 (2013) locations for each maturity group, to obtain some idea of the diversity of cultivar profiles for protein content as a percentage of DDM and their hullability, and for their potential to produce good quality meal.

This larger panel of cultivars made it possible to assess the potential variability of the protein content in dehulled meals. The range for the calculated protein content of dehulled meal (see Table 5) is large, with 10 percentage points between the poorest and the best cultivars within ME/ML group in 2013 (for other maturity groups and years, the range was 7 to 9 points; the results are not presented here).

Table 5: Analysis of variance for seed components from 15 ME/ML group cultivars, grown in 3 locations in 2013 (means and level of significance of ANOVA on seed components)

Cultivar	Oil content (% at marketing standards)	TSW (g DM)	Protein content (% DDM)	Hullability (% extracted hulls)	Calculated protein content of dehulled meal (% RM)
CLLOSER	49.7 (A)	53.2 (ABC)	39.0 (AB)	7.3 (CD)	39.1 (ABCD)
MEDDIA CS	49.0 (AB)	47.1 (C)	41.3 (A)	3.7 (E)	38.6 (BCD)
KAPLLAN	48.5 (BC)	53.1 (ABC)	38.9 (AB)	10.3 (BC)	41.5 (ABC)
DOUGLLAS	47.6 (CD)	61.3 (A)	39.4 (AB)	11.0 (B)	42.5 (AB)
NK KONDI	47.4 (CD)	51.3 (BC)	36.1 (BC)	9.5 (BCD)	37.4 (BCD)
LG5687HO	47.2 (DE)	48.0 (C)	35.6 (BC)	8.6 (BCD)	36.2 (CD)
SY EXPLORER	46.9 (DEF)	51.4 (BC)	36.9 (ABC)	9.8 (BCD)	38.5 (BCD)
DKF3333	46.8 (DEF)	51.8 (BC)	38.3 (AB)	7.0 (D)	37.8 (BCD)
LG5528	46.8 (DEF)	51.5 (BC)	38.4 (AB)	8.7 (BCD)	39.3 (ABCD)
SY EDENIS	46.6 (DEF)	50.8 (BC)	33.2 (C)	10.0 (BCD)	34.7 (D)
EXTRASOL	46.5 (DEF)	57.9 (AB)	38.5 (AB)	9.7 (BCD)	40.2 (ABC)
ES AKUSTIC	46.2 (DEF)	57.0 (AB)	39.2 (AB)	10.0 (BCD)	41.1 (ABC)
LG5625	45.7 (EF)	55.4 (ABC)	35.0 (BC)	14.3 (A)	39.9 (ABCD)
ES TEKTONIC CL	45.6 (F)	54.6 (ABC)	37.0 (ABC)	13.5 (A)	41.5 (ABC)
LG5656HO	44.4 (G)	50.8 (BC)	38.8 (AB)	14.7 (A)	44.4 (A)
Cultivar effect	***	***	***	***	***
Location effect	***	***	***	***	***

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means per cultivar within a column followed by the same letter are not significantly different (P < 0.05) with Student-Newman-Keuls comparison test

Location and cultivar effects were significant for all parameters in Table 5: oil content, seed size (TSW), percentage protein content of DDM, hullability and the calculated protein content of the dehulled meal. We were able, therefore, to distinguish varieties with contrasting characteristics, not only for oil content, but also concerning parameters that affect the possibility of obtaining meal with high protein content.

Cultivars LG5656HO and Dougllas had significantly higher calculated protein content in their dehulled meal (44.4% and 42.5%) than cultivars LG5687HA and SY EDENIS (34.8% and 34.9%). However, for all other varieties, we could not draw a firm conclusion, as the differences concerning this parameter were not significant. Thus, with this wider number of varieties, various combinations of seed characteristics were identified:

- Varieties with low or medium oil content, but high protein content (as a percentage of DDM) and good or medium hullability, giving a high protein meal (LG5656HO, ES TEKTONIC, Extrasol, ES Akustic)
- Some varieties with high oil content, high protein content (as a percentage of DDM) and medium hullability, giving a high protein meal (Dougllas, Kapllan)
- Some varieties with medium oil content, poor protein content (as a percentage of DDM) and medium hullability, giving a lower protein meal compared to other varieties (SY Edenis, LG5687HO)
- Some varieties with very high oil content, high protein content (as a percentage of DDM) but low or very low hullability, giving a medium protein meal (Clloser, Meddia CS).

Turning to the economic aspect, some varieties would be more profitable than others. The outlines of an economic approach can be suggested, but would require further development if sunflower ideotypes are to be determined. Using 2015 market data (oil price of 750€/t, 36% protein meal at 260€/t and hulls at 80€/t), and by calculating the rate of hull removal necessary to produce a 36% protein-content meal (based on the formula presented in section 2.5), we calculated the likely achievable income of some cultivars. Oil content was the main factor affecting income, with percentage protein content of DDM as the second factor (using high protein content seeds, a lower percentage of hulls can be removed to produce 36% protein meal, the quantity of which is therefore greater). The cultivars that would produce the highest expected incomes belonged to the varietal groups combining high or very high oil contents and high protein contents: Meddia CS, Clloser, Kapplan, Dougllas (494 to 505 €/ton of processed seeds). The lowest incomes were obtained for cultivars displaying low/medium oil contents: LG5625, SY Edenis, LG5656HO and ES Tektonic (472 to 477 €/ton of processed seeds).

Crude fibre content analysis could replace hullability tests?

In 2013, hullability (percentage of extracted hulls) was significantly correlated with: oil content, Thousand Seeds Weight, percentage protein content of DDM, percentage crude fibre content of DDM and percentage crude fibre content of DM (Table 6). Only percentage protein content of DM was not correlated. The closest correlation was with crude fibre content in DM (see Figure 4). The results of the 2012 correlation matrix gave the same conclusions.

Table 6: Pearson correlation matrix concerning 2013 trials (first line Pearson correlation coefficient (R), second line number of samples)

	TSW	**Protein content (%DDM)**	**Protein content (%DM)**	**Crude fibre (%DDM)**	**Crude fibre (%DM)**	**% extracted hulls**	**Calculated protein content of dehulled meal (% RM)**
Oil content	-0.38452*** 157	-0.20423* 157	-0.59629*** 157	-0.17711 *NS* 32	-0.78159*** 32	-0.59706*** 107	-0.51507 *** 107
TSW		0.08842 *NS* 157	0.23748** 157	0.33684 *NS* 32	0.31760 *NS* 32	0.40733*** 107	0.39198 *** 107
Protein content (%DDM)			0.90667*** 157	0.08418 *NS* 32	0.43585* 32	-0.28675** 107	0.76529 *** 107
Protein content (%DM)				0.13256 *NS* 32	0.63598*** 32	-0.00416 *NS* 107	0.85291 *** 107
Crude fibre (%DDM)					0.72126*** 32	0.35681* 31	0.25643 *NS* 31
Crude fibre (%DM)						0.70678*** 31	0.64457 *** 31
% extracted hulls							0.38716 *** 107

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

This could be explained by the fact that the percentage cellulose content of dry matter was both highly correlated with the percentage cellulose content of DDM (the richer is the whole seed in fibre, the richer also in fibre is the defatted fraction of the seed) and oil content (the richer is the seed in oil, the greater is the reduction of the defatted fraction, which lowers the proportion of cellulose). Previous studies had shown on the one hand that hullability is significantly and negatively correlated with the seed oil content (Denis *et al.*, 1994; Dauguet *et al.*, 2015); and on the other, since the crude fibre is concentrated mainly in the hulls, that hullability is strongly and positively correlated with the seed hull content (not assessed in this present study, but demonstrated by Denis *et al.*, 1994; Baldini *et al.*, 1994; Nel, 2001). Thus, the crude fibre content as a percentage of DM incorporates both the effect of fibre content as a percentage of DDM, and the effect of oil content on hullability.

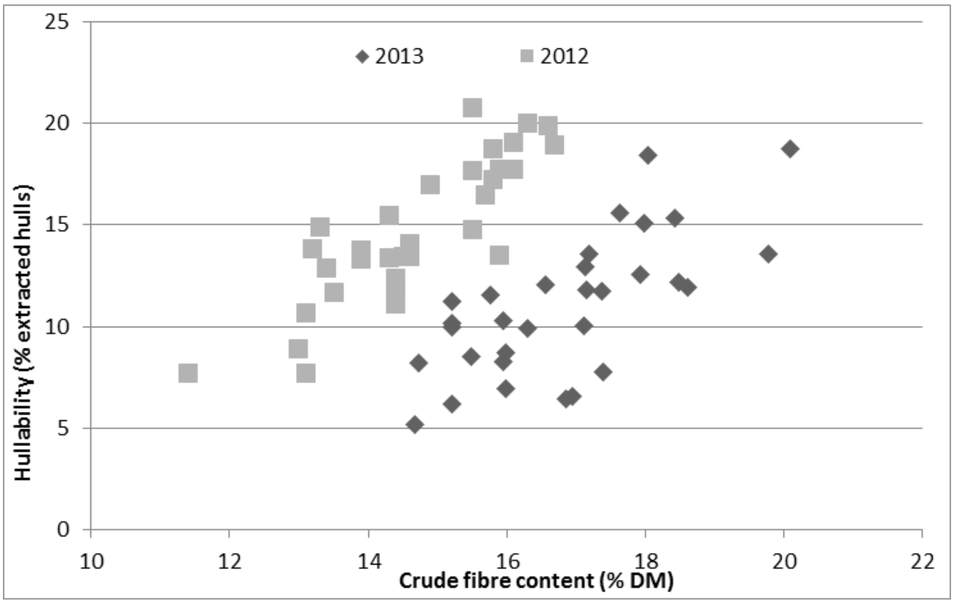


Figure 4. Relationship between on hullability (% of extracted hulls) and crude fibre content of sunflower seeds

An analysis of covariance was conducted, testing the effects of crude fibre content expressed as a percentage of DM, year and the interaction "Crude fibre DM * Year" on the extracted hull rates (Table 7). It showed a year effect, i.e. a different intercept but, no interaction effect (similar slopes).

Table 7: Analysis of covariance results for the percentage of extracted hulls variable (2012 and 2013 data)

Parameter	**Estimated value**	**Standard error**	**Pr > |t|**
Intercept	-22.85	3.57	<0.0001
Crude fibre content	2.01	0.21	<0.0001
Year 2012	8.04	0.72	<0.0001
Year 2013 (reference)	0		
R^2 model = 0.69			

The crude fibre content analyses were performed on: DKF3333, Vellox and Extrasol in 2012 and NK Kondi, ES Tektonic, Fydgi and SY Valeo in 2013. However, the climatic context was probably the most impacting parameter since hullability was lower overall in 2013 compared to 2012 (see Tables 2 and 3), which may be linked to a higher oil content in 2013 compared to 2012 (see Table 4).

Thus, if significant advances are made in the near future in the development of rapid non-destructive analysis methods, determining the crude fibre content as a percentage of DM would be an appropriate way to assess the hullability of varieties in sunflower breeding programmes. An annual calibration does, however, appear necessary.

Predicting the potential of a variety for producing a meal with high protein content?

It appears that protein content of dehulled sunflower meal (calculated data for each sample from the seed protein content and rate of extracted hulls by the method outlined in section 2.5) was most closely correlated with seed protein content as a percentage of DDM ($p < 0.0001$ and $R^2 = 0.59$, see Table 6 for 2013 data) and much less related to the rate of hulls extracted ($p<0.0001$, $R^2 = 0.15$, see Table 6 for 2013 data). From this, it may be concluded that the initial seed protein content is of paramount importance for obtaining high protein meals.

Taking all the data for 2012 and 2013 together, we obtained Equation 1.

Equation 1. Relationship between protein content of dehulled meal and protein content as a % of DM (2012 and 2013 data, Figure 5)

Prot_dehulled_meal = 18,198 + 0,6391 * (protein content DDM) [$R^2 = 0.43$]

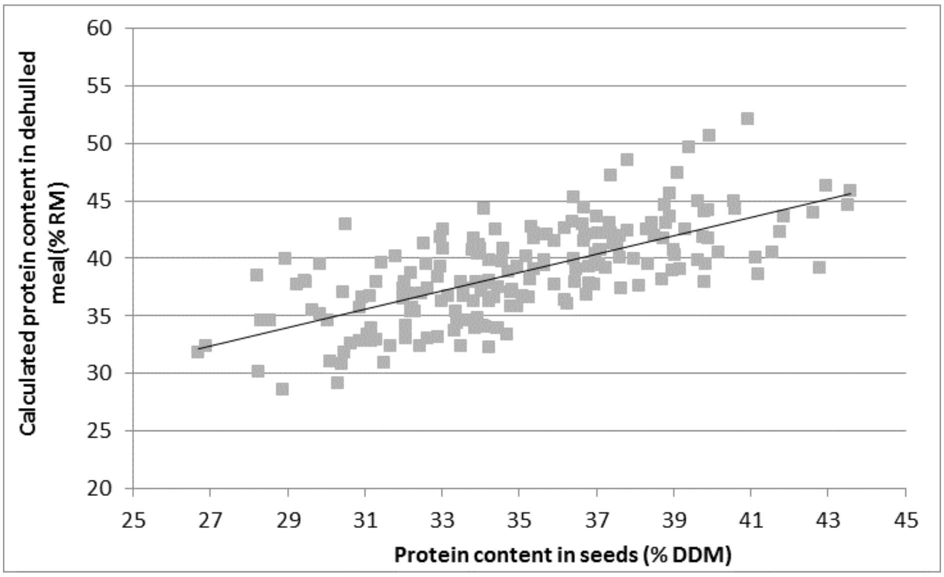


Figure 5. Relationship between calculated protein content in dehulled meal (% RM) and protein content of sunflower seeds (% DDM)

Adding crude fibre content to the model could improve the equation, as this parameter was correlated with hullability. Taking into account the year effect, as shown in section 3.4, could further improve the predictive model.

Equation 2. Protein content of dehulled meal (% RM) as a function of protein content DM and crude fibre content DM, and including year effect (2012 and 2013 data)

2013: Prot_dehulled_meal = -26.22 + 1.12*(protein content DDM) + 1.48*(Crude Fibre content DM)

2012: Prot_dehulled_meal = -20.73 + 1.12*(protein content DDM) + 1.48*(Crude Fibre content DM) Significance: General model $p<0.0001$; Intercept $p<0.0001$; protein content DDM $p<0,0001$; Crude Fibre content DM $p<0.0001$; Year effect $p<0.0001$

R^2 (model)=0.86 ; partial R^2 (protein content DDM)=0.66 ; partial R^2 (Crude Fibre content DM)=0.16 ; partial R^2 (year effect)=0.05

A calibration of this Equation 2 according year results in a more accurate estimate, and enables classification of varieties according to their capacity to produce meal with improved protein content after dehulling.

4. CONCLUSION

At present, sunflower breeding programmes do not take into account the characters of protein content and hullability. So if the crushing industry wishes to produce a high protein meal it would have to review the dehulling process. In this study, we identified sunflower varieties that combine both high oil and high protein content. The protein content of sunflower seeds proved to be the key characteristic determining the quality of sunflower meal; improvement by breeding would help to improve both meal quality and the profitability of the crushing process. Selection of varieties with particularly high oil contents could have a negative impact on hullability; it may be worthwhile checking this in order to avoid difficulties at crushing plants.

Results observed in this study proved that for selection of cultivars producing sunflower meal with more than 40% protein content is perfectly feasible without having to remove more than 13% of seed mass in hulls. This study also highlighted important environmental effects (year and location) on protein content and hullability; this indicates that cultivar selection alone is not sufficient to ensure the production of a precise quality target for the seeds, although it should reduce the risk of failing to deliver meal of a commercial standard and/or losing too much oil in the hulls extracted. The question remains open as to whether the stakeholders in sunflower oil mills would benefit from negotiating specifications with their suppliers to segregate crops that have strong potential for producing high protein meal. A framework for sharing the earnings attributable to seed protein content could be set up for farmers. Further technical and economic assessments are needed to comprehensively address these possibilities. Action on them could lead to the adoption of new breeding strategies and significant improvements in the quality of sunflower meal.

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LITERATURE

Baldini M, Vannozzi GP, Cecco F, Macchia M, Bonari E, Benvenuti A. 1994. Genetic analysis of hullability in sunflower. *Industrial crops Production* 3, 29-35.

Dauguet S, Fine F, Guillemain C, Carré P, Merrien A, Krouti M, Champolivier. 2015. Impact of pedoclimatic and agricultural conditions on sunflower sedes characteristics in relation to the dehulling process. *OCL 2015*, 22(4) D402.

Denis L, Dominguez J, Baldini M, Vear F. 1994. Genetical studies of hullability in comparison with other sunflower seed characteristics. *Euphytica* 79: 29-38.

Denis L, Vear F. 1996. Variation of hullability and other seed characteristics among sunflower lines and hybrids. *Euphytica* 87: 177-187.

Evrard J, Burghart P, Carre P, Lemarie J, Messean A, Champolivier L, Merrien A, Vear F. 1996. Improvement of sunflower dehulling ability an interdisciplinary approach. In: Proc. 14th Int. Sunfl. Conf., Beijing/Shenyang, China. Int. Sunfl. Assoc., Paris, France.

Feedipedia, open access information system on animal feed resources, by INRA, CIRAD and FAO. www.feedipedia.org (checked on 3 December 2015).

Nel AA. 2001. Determinants of sunflower seed quality for processing. Ph. D. diss. Univ. of Pretoria, Pretoria, Republic of South Africa. Available from (verified 21 Aug. 2014).

Oraki H, Alahdadi I, Parhizkar khajani F. 2011. Influence of water deficit and genotype on protein, oil contents and some physical characteristics of sunflower seeds. *African Journal of Agricultural Research* 6(5)/1246-1250.

Peyronnet C, Pressenda F, Quinsac A, Carré P. 2012. Impact du décorticage du tournesol sur la valeur nutritionnelle et l’intérêt économique des tourteaux en fabrication d’aliments composés. *OCL* 2012; 19(6): 341-346. doi: 10.1684/ocl.2012.0486

Sharma R, Sogi DS, Saxena DC. 2009. Dehulling performance and textural characteristics of unshelled and shelled sunflower (*Helianthus annuus L.*) seeds. J Food Eng 92: 1-7.

Tostain S, Chervier P, Laulan A, Kermorgant T. 2012. Amélioration de l’autonomie énergétique et de l’impact environnemental d’une unité de trituration de tournesol par l’implantation conjointe d’un atelier de décorticage et d’une chaudière à coques. *OCL* 2012; 19(6) : 332-340. doi: 10.1684/ocl.2012.0485