

## Glucosinolates content, an important quality parameter monitored at each stage of the French rapeseed production chain

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### INTRODUCTION

High level of Glucosinolates (GLS) in rape seeds and meals are considered to be undesirable due to their toxic effect on thyroid and to the growth disorders which are observed especially for monogastric animals. The European standard called "low GLS" has been fixed below 25 micromoles grams<sup>-1</sup> at 9% of water in the seed. Since the year 2000, cultivation of rapeseed has been expanding in Europe in relation to the biodiesel market development mainly based on rapeseed oil. The rapeseed meal volumes produced in Europe are therefore increasing and new outlets have to be found especially in the monogastric feeding industry. In France (1.45 million hectares of rapeseed in 2010) a great attention has been paid for several years to the GLS control the whole production chain along. Results obtained in France at the variety registration stage, at the seed production and at the meal manufacturing steps during a period of 6 years GLS monitoring are presented.

### MATERIALS AND METHODS

**At the variety registration step:** During the first year of experiment, managed by the French official organisation GEVES, the GLS content is measured on all the evaluated varieties on 8 to 10 locations of the national network (2 samples of 200 grams taken in 2 different replicates). During the second year the GLS content is checked only on the varieties which have a statistical risk to reach or overpass the threshold according to the first year analytical results. GLS analyses are performed by the BioGEVES lab. The GLS average over 1 or 2 years is calculated for each variety and compared to the maximum GLS content threshold fixed by the registration rules in France. From 2005 till 2010 (6 years), 152 registered winter type rapeseed varieties have been studied for their GLS content. The data analyse has been carried out for the overall collection and then separately according to the genetic structure of the cultivars (cv): inbred line (72 cv), dwarf restored hybrid (13 cv), 3 ways hybrid (2 cv), simple restored hybrids (58 cv) and varietal association (7 cv). Finally the three hybrid types have been gathered in one group and their performances regarding GLS have been compared to the inbred lines ones.

**At the rapeseed production step:** The national oilseed rapeseed organization (ONIDOL) and the oilseed technical institute (CETIOM) have been monitoring the quality of the rapeseed collected in France for 18 years. Samples coming from the harvest of the different cropping areas are yearly collected when farmers deliver the rapeseed harvest for storage according to a provisional sampling plan established by the CETIOM and the cooperatives. The varieties are not identified at the seeds delivering.

During the 6 years period 1 481 samples were evaluated for glucosinolates and also for other quality parameters by several certified French labs coordinated by the CETIOM. The CETIOM lab in Ardon (45) participated to the analytic works and carried out the data treatment. The mean value for GLS content of the domestic harvest was estimated taking into account the mean GLS content of each considered cultivated area and the French share of the area in terms of seeds production. Samples distribution histograms have been established.

**At the meal processing step:** ONIDOL and CETIOM started in 2003 a similar monitoring of the quality of the rapeseed meal manufactured in France. From 2005 to 2010, samples of delivered meals were monthly collected in the French crushing plants (7 in 2003, 8 in 2008, 9 in 2010). Glucosinolates contents were checked by the CETIOM lab. The average GLS content of the meal produced in France

has been calculated for every year. Data were also analysed separately for each plant and then compared each to other.

### Analytical methods

GLS content determination was based on high performance liquid chromatography. Standardised methods were applied: NF V03-908-3 or NF EN ISO 9167-1 for seeds and NF ISO10633-1 for meals. Results were expressed in micromoles gram<sup>-1</sup> of seeds at 9% of water and in  $\mu$ moles of gram of dry matter (DM) for meal.

### RESULTS

**At the variety registration step:** Since 1990, GLS content in the seeds has to be less than 18  $\mu$ moles g<sup>-1</sup> to allow the variety to be registered in France. The restored hybrids took advantage from an adjusted threshold of 20  $\mu$ moles g<sup>-1</sup> until the year 2002 (year of cv application).

The overall mean GLS content of the registered varieties slowly decreased by 1.2 points, from 14.1  $\mu$ moles g<sup>-1</sup> of seed in 2005 to 12.9  $\mu$ moles g<sup>-1</sup> in 2010 (table 1).

**Table 1: Glucosinolates content (GLS) of the varieties registered in France between 2005 and 2010- GLS in micromoles g<sup>-1</sup> of seeds at 9 % humidity**

	2005	2006	2007	2008	2009	2010	Total over years
number of registered varieties	12	18	35	40	34	13	152
mean GLS content	14.1	13.5	14.1	12.9	12.6	12.9	13.4
minimum GLS content	11.6 (IL)	9.5 (IL)	10.1 (IL)	8.1 (IL)	9.7 (RH)	10.8 (IL)	8.1
maximum GLS content	17.7 (L)	16.5 (RH)	16.9 (RH)	16.9 (L)	16.6 (RH)	15 (RH)	17.7

#### (IL): inbred line - (RH) : restored hybrid

At the beginning of the studied period, hybrids expressed in general higher GLS concentration than inbred lines (table 2). According to the breeders, some male sterility sources (OGU-INRA source for example) involved in the hybrid process should be linked with GLS higher content.

During the 6 years period, the GLS content of inbred lines remained quite stable with a slight GLS decrease of around 0.5/1 point meanwhile the GLS mean value of the hybrid types was significantly lowered of more than 2 points (table 2). Since 2008, the GLS mean value of inbred line and hybrids have been joining 12.5 to 13  $\mu$ moles g<sup>-1</sup> of seeds. The proportion of varieties displaying GLS rates bellow 15  $\mu$ moles/g increased during the period and reached 100 % in 2009 for inbred lines and in 2010 for both types (table 2). The 10  $\mu$ moles g<sup>-1</sup> seems however a difficult barrier to overcome for the rapeseed winter types but some cultivars succeeded in it since 2008 showing that genetic progress goes on with this trait (table 2).

**Table 2: Comparison for glucosinolates content (GLS) between inbred line and hybrid type cultivars registered in France between 2005 and 2010, GLS expressed in  $\mu\text{moles g}^{-1}$  of seed at 9 % water**

		2005	2006	2007	2008	2009	2010
mean GLS value	Inbred lines (a)	13.8	13.1	13.6	12.7	12.5	13.2
	Hybrid types (b)	15.1	13.8	14.3	13.0	12.6	13.1
	gap between hybrids and inbred lines (b-a)	1.3	0.76	0.7	0.30	0.11	-0.07
% of varieties with GLS content $\leq 15$	inbred lines	78	67	71	77	100	100
	hybrid types	33	60	59	88	86	100
% of varieties with GLS content $\leq 10$	inbred lines	0	8	0	23	8	0
	hybrid types	0	0	0	0	9	0

**At the rapeseed production stage:** the average GLS value of rapeseed harvests over the period was good ( $14.9 \mu\text{moles g}^{-1}$ ) but the standard deviation remained quite high between 3 and 4, excepted in 2010 (2.5). In addition, huge differences between the lowest samples ( $5$  to  $8 \mu\text{moles g}^{-1}$ ) and the highest one's ( $22$  to  $30 \mu\text{moles g}^{-1}$ ) were observed each year (table 3). These indicators suggest that the variability of GLS content remains important and that extreme values could be reached while cumulating effects of the variety and of pedoclimatic conditions.

**Table 3: Glucosinolates content (GLS) of seeds samples collected in France at the seeds delivering between 2005 and 2010, GLS expressed in  $\mu\text{moles g}^{-1}$  of seeds at 9 % water**

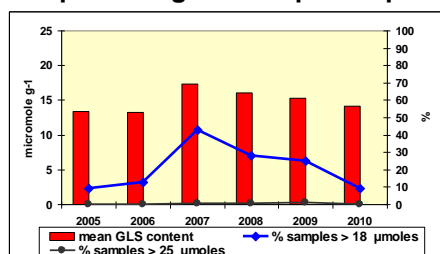
	2005	2006	2007	2008	2009	2010	mean value over years
Number of checked samples	471	104	260	187	214	245	246
mean value	13.4	13.3	17.3	16.1	15.3	14.2	14.9
standard deviation	3.1	3.7	3.9	4.1	3.7	2.5	3.5
minimum value	6.2	7.3	7.1	7.1	5.1	8.1	6.8
maximum value	24	24.6	30.4	30	25.4	22.7	26.2

In spite of this variability, the French seeds production met largely the current European standard requirement of maximum glucosinolates content of  $25 \mu\text{moles g}^{-1}$  each year (figure 1). If we simulate a maximum target value of  $18 \mu\text{moles/g}$ , we observe that a great proportion of the delivered seeds lots would have over passed this limit in 2007 (43%), 2008 (28%) and in 2009 (25%). In 2010, the 2005 level was recovered with 9 % of the French samples above  $18 \mu\text{moles g}^{-1}$  which can be considered as a good result to be stabilised in the next years.

Looking now at the GLS content trend during the past 6 years period, we observe that at the beginning of the period, in 2005 and 2006, the GLS average value of the French harvest was good, close to  $13 \mu\text{moles g}^{-1}$ , similar to the mean value of the inbred lines registered at that time. After that, a peak of

17.3 micromoles was reached in 2007 and between 2007 and 2010 the average value decreased of 1 point per year (figure 1). The annual variation could explain only a small part of the unusual value of 2007, probably not more than 1 point compared to 2006 if we refer to the variation of GLS content observed on the inbred lines during registration process between 2006 and 2007 (see table 2). The main part of the GLS variation in the French rapeseed harvest could be explained by GLS characteristics of the most cultivated varieties. During the years 2007 and 2008, two factors cumulated their effects in France: i) the expansion of hybrids acreage which displayed in that time higher GLS contents ii) the presence in top sales of some varieties expressing high GLS contents, between 16 and 25  $\mu\text{moles g}^{-1}$  (inbred line Grizzly and the hybrids Exagone, NK Aviator, Excel..) and coming for the highest GLS rates from the European catalogue of varieties (ex : Excel registered in the UK). In the following years 2009 and 2010, these varieties were less cultivated and their impact was progressively diluted in the global harvest. In addition, the new varieties coming in the market expressed less GLS content as shown before and contributed to decrease the mean GLS content of the French harvest.

**Figure 1: mean glucosinolates content of the samples collected in France from 2005 until 2010 and percentage of samples expressing glucosinolates content above 18  $\mu\text{moles g}^{-1}$  of seeds**



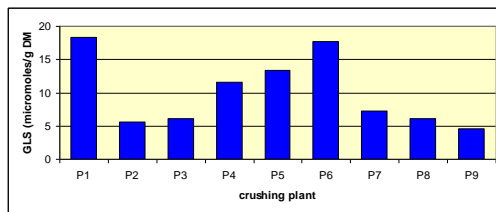
**At the meal processing step :** First of all, the mean annual value of residual GLS content measured in the rapeseed meal delivered by the main French crushing plants easily met the requirements of European standards (less than 30  $\mu\text{moles gram}^{-1}$  of DM) and can be then considered of good quality (table 4). During the period, the meal average GLS content remained quite stable in spite of a 1 point increase in 2007 and 2008 to be related to the GLS content peak observed in seeds for the 2007 and 2008 harvests. This relative stability of the French average GLS content in meal hid a great variability of unitary values which were found between 1.4 and 27.7  $\mu\text{moles g}^{-1}$  DM.

**Table 4: Glucosinolates content (GLS) of the rapeseed meal delivered by the French surveyed crushing plants between 2005 and 2010, GLS expressed in  $\mu\text{moles g}^{-1}$  of dry matter**

	2005	2006	2007	2008	2009	2010	over years
number of analysed samples	54	76	73	74	81	86	74.0
yearly mean value	11.1	10.7	11.7	11.3	10.3	9.5	10.8
minimum value	2.8	3.8	3.4	1.4	2.3	1.6	2.5
maximum value	16.8	18.0	24.1	25.4	21.6	27.7	22.3

This variability appeared to be related to the crushing plants as shown in the figure 2 and probably to the hydrothermal treatment during the desolventisation process, high temperatures leading to lower residual GLS contents. However, as it is shown in the communication entitled "A multi-year survey on chemical composition of rapeseed meal produced in France" and presented by the CETIOM, the process conditions could also affect the protein solubility and the digestibility of the meal. So a good balance has to be found between GLS degradation during process and protein digestibility of the meal in relation to the targeted uses.

**Figure 2: Yearly mean glucosinolates content of the rapeseed meal delivered by the 9 surveyed crushing plants in 2010**



### Conclusion

The rapeseed market trends led the European crushers to ask their suppliers to use only varieties registered with a GLS content below  $18 \mu\text{moles g}^{-1}$  in order to deliver more stable meal quality to the feeding industry and to enlarge the potential market for the rapeseed meals produced in Europe. Our study showed that registration process including GLS maximum threshold like in France ( $18 \mu\text{moles g}^{-1}$ ) could contribute to improve the quality of the varieties introduced in the market and as a consequence could have a clear impact on seeds production and on meal quality. Therefore it is crucial to monitor the GLS content at each step of the supply chain in order to have a good knowledge of the varieties, seeds and meal quality at large scale, to share the information between the rapeseed production chain actors and to point where improvements are still needed and feasible without affecting other traits.

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