The Situation in Rapeseed Cultivation in Lithuania

Variation of Glucosinolate Contents and Analytical Problems.

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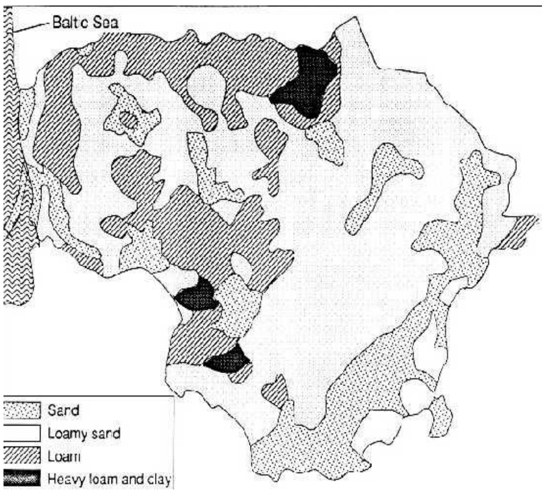
General overview. Lithuania is situated on the south eastern shore of the Baltic Sea. Country occupies the western fringe of the East European Plain, covering an area of 6.5 million ha. It lies between longitudes 21°and 27° east and latitudes 54° and 57° north, and borders Latvia in the north, Belarus in the south east and Poland and Kaliningrad (Figure 1a). The utilised agricultural area (UAA) is 3.5 million ha (i.e. 53.4% of the total); arable land accounts for 2.95 million ha (84.1% of UAA) followed by meadows and natural pastures (0.496m ha, or 14.2% of UAA). A high proportion of the arable land is in forage cropping, including temporary grassland (44.4% of crop area); approximately 1.8 million ha (1/3 of territory) are covered with forest while more than 1 million ha of hilly and other land are not used for agriculture. In 2000 about 28.1% of the UAA land was planted with cereals, rapeseed accounted only for 1.6%
(by http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPC/doc/Counprof/lith.htm and http://www.europa.eu.int/comm/agriculture/external/enlarge/publi/countryrep/lithuania.pdf).




Latvia
Riga
Lithuania
Vilnius
Belarus
Minsk
Poland

rea in Lithuania (a). Soil characteristics (b). By ULT/AGP/AGPC/doc/Counprof/lith.htm

regions in terms of soil types (Figure 1b): the most ntry; the western part has wetter acid soils; sandy hills untry; in northern areas there is a region of calcareous

ween maritime and continental; in the 12-15 km-wide e eastern part of the country it is continental by moderate heat in summer as well as by adequate days. The negative features include long autumns and od (169 – 202 days). The average annual temperature is approximately 6.2° C with average January temperature of -4.8° C, and average July temperature of +17.2° C. The difference between the mean monthly temperatures of July, which is the hottest month, and January, which is the coldest month is as high as 22º C. Winters tend to be overcast and variable in temperature, and snow cover is by no means continuous because cold periods can be followed by sudden thaws. The average annual precipitation is 662 mm. However, its distribution within Lithuania's territory is uneven and varies from 500 to 900 mm. Such great differences result from the great variation in the country's relief. The weather is generally very changeable and there is a significant variation in the rainfall patterns and minimum temperatures between the years.

Rapeseed cultivation. Winter and spring rape are long-day plants. They are quite demanding in terms of climate and soil and perform well in wet and mild climatic conditions. Winter rape is susceptible to wintering conditions, therefore the cultivation of rapeseed, especially of winter crop, is rather complex in Lithuania. Nevertheless rape is the most suitable oil crop for cultivation in Lithuania. Rapeseed cultivation for industrial processing in Lithuania was started during 1994-1995, and since then the area sown with rapeseed has been steadily increasing (Fig. 2). In 1999, a sharp decline in oilseed rape price resulted in a significant reduction in the cultivation area.

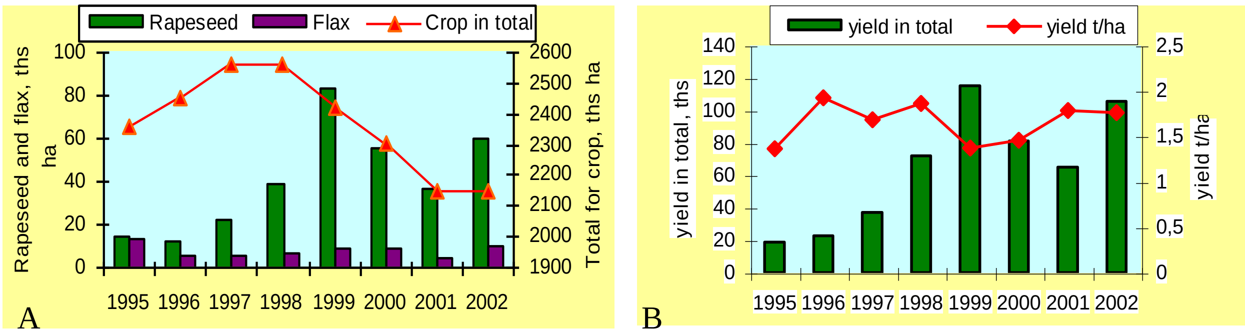


Figure 2. Are of oil crops grown in Lithuania (A) oilseed rape yield (B)
By http://www.std.lt/web/main.php?parent=234

In 2000 winter rape accounted for only a small part of the total area sown with rape. In 2001winter rape accounted for 25 per cent, in 2002 over 30 per cent of the total rapeseed production area. Winter rape is higher yielding than spring rape. It yields on average 2 t/ha. Individual growers and researchers achieve a yield of 3-4 t/ha.
In Baltic countries it is expected that with an increase in oilseed rape production area the yield will increase, and the total production will amount to 230 thousand tons in 2003/04. Currently 70-80 per cent of rapeseed grown in Lithuania is exported. According to Oil World analysts' predictions the exports from Baltic countries will increase by 40 thousand tons during 2003/04. This ought to compensate the declined rapeseed production in Northern and Eastern Europe. Oilseed rape is the first crop in today's Lithuanian agriculture that has found its way to the western market. The popularity of rapeseed cultivation is increasing. According to exports volumes (in 2001 69,3 thousand hectares for 51,7 million Lt) rapseed surrenders only to bread wheat.

Prospects for biofuel production from rapeseed are being currently discussed. The next year's production plans are 10 thousand tons of rapeseed methyl ester (RME) and 10 thousand tons of bioethanol. After accession to the EU, it is planned that 25 thousand tons of biofuel will be used in Lithuania in 2005. Lithuanian agricultural producers could easily grow about 350 thousand tons of rapeseed, therefore it is vital to develop processing industry, too. Rapeseed processing into biodiesel is a waste-free production. Lithuanian livestock producers value rapeseed cake as a forage rich in protein. Rapeseed growers enjoy rather favourable economic conditions: the state

pays 80 Lt direct payment per declared hectare of rapeseed crop. An extra 150 Lt direct payment per hectare (used to be 80 Lt) is paid if the yield is sold or transferred for storage to vegetable oil or biofuel producers on the domestic market. http://www.zpasaulis.lt/archyvas/43/ubik.html
Only high-yielding, low in erucic acid and glucosinolate content oilseed rape varieties are grown in Lithuania. The largest rapeseed cultivation area is sown with the varieties bred in Germany, Sweden, and Denmark. The chief rapeseed importer in Lithuania is the company Dotnuvos projektai (www.dotpro.lt) whose current range of varieties include the following spring rape varieties: Sponsor, Maskot (Sweden); Star (Denmark); Lisora, Ural, Terra F1, Heros, Liaison (Germany), and winter rape varieties: Accord, Valesca, Kasimir, Silva, Kronos (Germany) and Casino (Sweden).

Glucosinolate content variation and analytical problems. Although 00 varieties are currently grown in Lithuania, very changeable climatic and soil conditions determine sowing and ripening time. All this can produce fluctuations from year to year in oil, protein and glucosinolate content (Butkutė et al., 2000a, b; Mendham, Shipway, Scott, 1981; Montvilas, 1998; Robertson et al., 1999; Šidlauskas, 2000; Walton, Bowden, 1999; Velička, 2002). In general, rapeseed grown in Lithuania differs little in quality from that grown in other countries (Butkute, 2003a). Glucosinolate content still remains one of the key rapeseed quality control criteria, since rapeseed quality depends not only on varietal peculiarities but also on growing location and technology. The recently raised issue of biofuel production and other uses of rapseed for technical purposes can result in the imports and cultivation of rape varieties with a high erucic acid or glucosinolate content, which is restricted for rapeseed used for human consumption. In this case an effective system for seed control must be created, cultivation regions must be foreseen and growers must be strictly controlled.
Determination of glucosinolate content, controlled compounds in rapeseed, in Lithuania's laboratories is very problematic. The methods accepted and approved in the EU countries and the rest of the world require costly facilities and equipment, and the standard method approved in the country (LST 1399: Determination of glucosinolate content in rapeseed by fermentation method) does not meet the contemporary requirements in terms of accuracy and data repeatability. The international standard HPLC and X-ray fluorescence methods are still not used in Lithuania for the determination of glucosinolate content. In the situation when only 00 rape varieties are cultivated, the growers are not interested and are not encouraged to have the glucosinolate content tested. The glucosinolate content usually does not exceed 20 µmol/g, i.e. the content allowed by the Lithuanian standard LST 1323:1993. Therefore this analysis is interesting only for the scanty number of researchers investigating seed yield and quality formation peculiarities in relation to the growing technology, or seed dealers (UAB "Linas ir viza" http://www.lv.lt/), or organisation inspected cargoes (UAB "Sekargas & Co" http://www1.omnitel.net/sekargas/), if the seed of unknown origin is purchased.
The Lithuanian Institute of Agriculture was involved in PHARE-funded project "Rapeseed growing and vegetable oil production in Lithuania" in 1995 – 1996. From the funds received from the project, the Institute purchased the instrument *NIRS-6500* with software and SPINNING module for analysing fine grain and ground grain samples. The role of NIRS in the routine analysis of several seed quality traits of intact rapeseed, including GSL content, is becoming increasingly more important (Biston et al., 1988; Daun, 1995; Tillmann et al., 2000; Velasco and Becker, 1998 and others). The development of robust and accurate NIRS prediction systems depends upon availability of a large calibration database, accuracy of reference methods used for calibration etc. Calibration equations developed on the basis of inaccurate reference methods cannot provide analysing accuracy. In order to achieve reliable performance of NIRS and the data of analyses close to those obtained by the recognised and EU-approved methods, we searched for ways to compose data base for the development of equation for the prediction of glucosinolates, based on the data of corresponding accuracy. For this purpose, with the benevolent assistance of dr. K.Michalski, the samples were analysed in small batches in the

laboratories of other countries by GC method of silyl derivatives (Michalski et al., 1995) or HPLC in other EU countries' laboratories. The accuracy of an NIRS prediction depends on the successful completion of several factors of calibration. Development of an accurate equation for GSL determination was affected by more factors compared with the equations intended for other rapeseed quality parameters. The first reason why calibration sensitivity of the equations for GSL was very high could be that GSL content in rapeseed is very low in comparison with the contents of the other quality components. The highest content of GSL (e.g. 85 μmol/g) accounts for only about 3.3 percent of the total seed mass. The absolute majority of the samples in our database were the seed of double low varieties, i.e. seeds with 3 - 20 μmol/g GSL, or 0.12- 0.8 per cent of the seed mass. The optimal spectral range for GSL calibration was 1500-1800 nm (Figure 3).

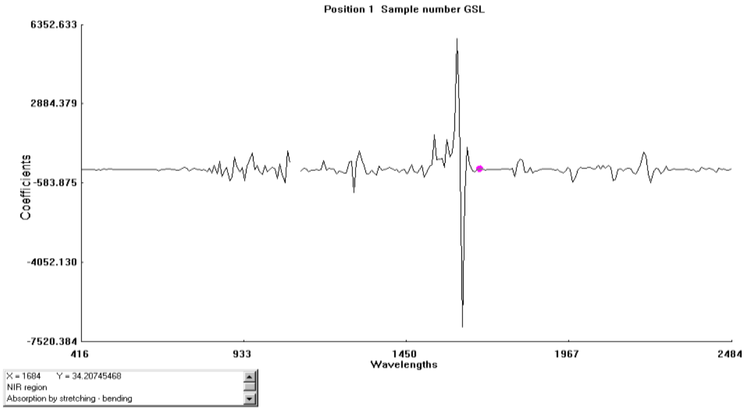


Figure 2. Plot of wavelength correlation with glucosinolate contents (Butkute, 2003b).

This spectral area seems very specific and highly sensitive (Biston et al., 1988; Butkute, 2000; Butkute 2001; Lila and Furstoss, 1986) and could be associated to a well defined structure of GSL as β-thioglucoside attached to carbon atom in N-hydroximine sulfate esters. The equation selected for practical work had standard error in calibration SEC 1.53 $\mu mol \cdot g^{-1}$ and the coefficient of correlation in calibration RSQ was 0.96.

Having analysed glucosinolate content by different methods (NIRS-6500 in the Analytical laboratory of the Lithuanian Institute of Agriculture, GC of silyl derivatives in the Institute of Plant Breeding and Acclimatisation, Poland, and HPLC ISO/AWI 9167-3 in the Laboratory of Analyses of CETIOM) and having mathematically estimated the variation of the parameter in relation to the analysing method, it was determined that the range of variation of the total glucosinolate content is similar (Table 1). A slightly higher variation was characteristic of the data obtained by the chromatography method. The accuracy of the total glucosinolate content determination by NIRS–6500 instrument is close to that obtained by GC and HPLC methods. The data obtained by NIRS closely correlate with those obtained by GC: R^2=0,92, regression equation $Y_{NIRS}= 1,00X_{GC} + 0,14$, for 00 rapeseed samples and R^2=0,96 for a set of various types of rapeseed. The correlation between the data obtained by NIRS and by HPLC method was also close: R^2=0,92, regression equation $Y_{NIRS}= 1,00X_{HPLC} – 0,20$.

Table 1. Variation of the total glucosinolate contents in the seed of 00 spring and winter rape varieties determined by different methods

Method / n of samples in the set	Standard deviation	Content μmol/g			Correlation between methods R^2
		Mean	Min	Max	
NIRS-6500/ 128	5,11	11,26	2,70	22,93	NIRS/GC
GC / 128	5,20	11,26	3,60	23,80	0.92
NIRS-6500*/ 74	6,19	11,19	2,70	24,94	NIRS/HPLC
HPLC* / 74	6,41	11,08	2,68	25,53	0.91

So the accuracy of the equation for prediction of GSL content by NIRS is sufficient and we used this method for the needs both of rapeseed researchers of LIA and rapeseed growers and dealers. Lithuanian Institute of Agriculture's Analytical Laboratory has accumulated a large collection of NIR spectra of spring and winter rapeseed. The quality of rapeseeds grown on the experimental plots of the Lithuanian Institute of Agriculture, Lithuanian University of Agriculture, variety testing stations, individual farms or agricultural partnerships was predicted by NIRS-6500, using the equation developed at LIA. Despite some year to year variation, maximum levels of the total content of glucosinolates only in exceptional cases approached the permissible limit for rapeseeds in Lithuania (Figure 4, Table 2).

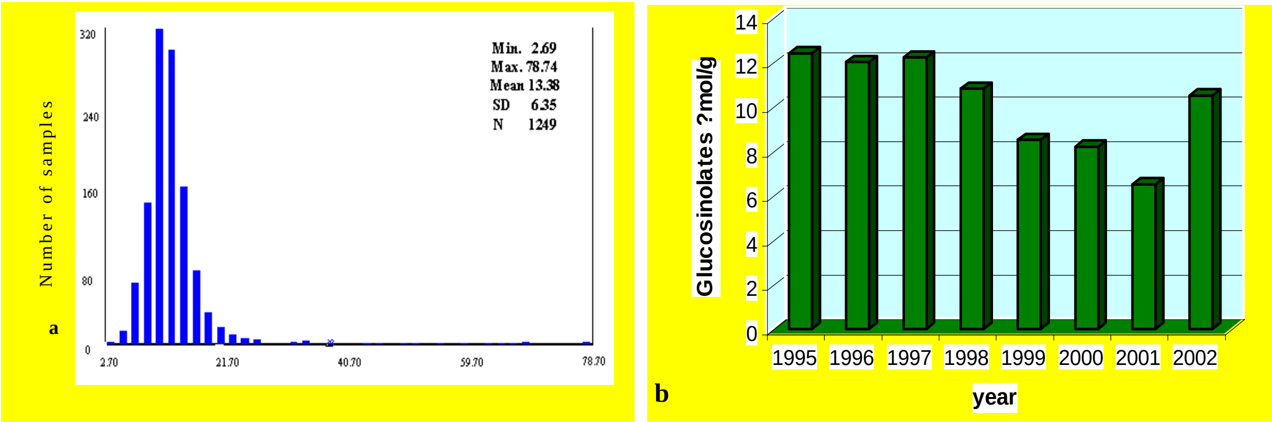


Figure 4. Histogram of the distribution of the total glucosinolate content (µmol/g seed) in the rapeseed samples, of 1994-2003 (a) and 1995-2002 yield, averaged data of rapeseed quality (b)

This parameter varied from 2.7 to 78.7 µmol/g seed (on average 13.9 µmol/g), standard deviation – as much as 6.34 (Figure 4a). However, as we can see from the figure, only individual samples had glucosinolate content exceeding 20 µmol/g. Most of Lithuania-grown double low oilseed rape varieties accumulate 6 -18 µmol/g glucosinolates in seed. A few samples of the collection accumulated over 50 µmol/g of glucosinolates, which can be explained by the genetic peculiarities of the varieties. However, there also were exceptions in our earlier experiments: depending on the branch of the plant on which the seed matured, glucosinolate content differed several times and exceeded the limits permissible for a variety or even for the 00 type of varieties under investigation (Butkutė et al., 2000b; Butkute et al., 2001).

Table 2. Variation of the total glucosinolate content in rapseed samples from 1995-2002.

	Samples from Lithuania's growers and researchers								Seed dealers	
	1995	1996	1997	1998	1999	2000	2001	2002	2000-2002	2002
N	132	376	351	137	139	125	298	100	190	98
SD	6,10	3,68	8,03	1,82	1,85	3,06	2,89	3,36	15,4	16,1
Mean	12,4	12,0	13,2	10,8	8,5	8,2	6,5	10,5	24,4	24,4
Max	30,2	20,4	78,7* /25,3	15,4	12,6	15,5	13,4	17,5	78,7	67,7
Min	7,2	5,3	6,2	7,3	2,7	2,7	2,0	3,8	3,3	3,5

* Samples with a glucosinolate content exceeding 30 µmol/g were provided from LUA's breeding material

The quality of the rapeseed samples provided for analysing, grown in the fields of the Lithuanian Institute of Agriculture, the Lithuanian University of Agriculture, the trial plots of the variety testing stations, individual farms or agricultural partnerships, was different in different years (Fig. 3b, Table 2). The weather conditions of these years differed not only in air temperatures but also in humidity. The ratio of oilseed rape varieties was different in individual years of investigation. During the period 1995-1998 *Star* variety accounted for the largest cultivation and investigation area, and consequently the largest share in the data base among spring rape varieties, while *Accord* accounted for the largest share in winter rape group. Since 2000 *Accord* variety has been superseded by *Kasimir*, which is characterised by a low glucosinolate content (about 3-6 μmol/g). Among spring rape varieties *Maskot* seed samples have accounted for the larger part in the described data base during the last 2 years. Each genotype is characterised by a distinctive plasticity and tolerance to extreme soil and cultivation conditions, therefore each of the investigated parameters describing rapeseed quality and chemical composition varied in relation to many factors. Comparison of the data of analyses of rapeseed samples from growers and dealers provided in Table 2 suggests that the content of glucosinolates in the rapeseed samples provided by the dealers (UAB "Linas ir viza" and UAB "Sekargas & Co") was more varied. The value describing the diversity of these samples, standard deviation, of individual parameters (especially of glucosinolates) is higher than that of the samples provided by the growers. A large part of the samples containing over 30 μmol/g glucosinolates, belong to a different type than 00 varieties, i.e. such high glucosinolate contents are determined by a genotype. Such rapeseed was grown not in Lithuania, it was imported from Belarus and the Ukraine and re-exported to West European countries.

Hence we may conclude that Lithuania has a good potential for the development of rapeseed production. Lithuanian rapeseed growers have a vast experience in seed cleaning, drying, storage, and procurement issues. Lithuania has a well-developed seed production system (UAB "Linas ir viza", UAB "Dotnuvos projektai"). Furthermore, the Lithuanian Institute of Agriculture and the Lithuanian University of Agriculture are the seats of oilseed rape researchers' potential. These research institutions provide know-how to growers on how to grow a high and disease-free seed yield. However, the quality issues still lack attention. The funds provided for more comprehensive quality analyses and purchasing of costly facilities are not available yet. On the one hand, this situation is determined by the current insufficient funding of research and science in Lithuania, on the other hand the too low and unstable rapeseed yields place focus on the solution of these problems.

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