Production of Glucosinolates During Winter Rape Seed Ripening and the Stability of Their Content in Glucosinolate-free Varieties in the Course of cultivation.

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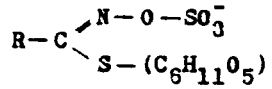
Introduction.

Oilseed rape meal contains 35-39% of protein of a well balanced aminoacid composition and is thus potentially an excellent source of protein for animals and man. However, the use of the meal is limited by its content of glucosinolates which yield toxic cleavage products: isothiocyanates and oxazolidinethiones are goitrogenic, the latter interfering with the synthesis of thyroxin.

The glucosinolates(Fig.1) are substances characteristically found in cruciferous plants. Approximately 90 naturally occuring glucosinolates have been identified and these 15-20 are supposed to occur within the genus Brassica(Fenwick and Heaney,1983).

Fig.1. General Formula for Glucosinolates.



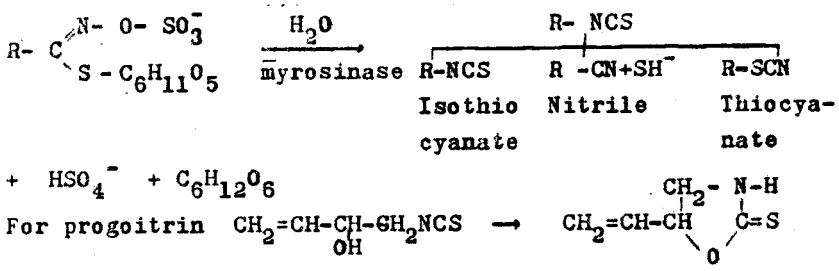
The four major compounds in Brassica napus are progoitrin , gluconapoleiferin,gluconapin, glucobrassicanapin(Fig.2). Indolyl glucosinolates have also been detected.(McGregor,1978)

Fig.2. The Major Glucosinolates Found in B. Napus

Trivial Name	Systematic Name	R Group
Progoitrin	2-hydroxy-3-butenyl-	CH_2= CH-CH(OH)-CH_2-
Gluconapoleiferin	2-hydroxy-4-pentenyl-	CH_2= CH-CH_2-CH(OH)-CH_2-
Gluconapin	3-butenyl -	CH_2= CH-CH_2-CH_2-
Glucobrassicanapin	4-Pentenyl-	CH_2= CH-CH_2-CH_2CH_2-

The breakdown products of glucosinolates (Fig.3)formed in the presence of moisture and enzyme myrosinase(Craig and Draper,1979)are responsible for deleterious effects when oilseed rape meal is used in animal fodder.

Fig.3. Enzymatic Hydrolysis of Glucosinolates.



The range of the glucosinolate content in the rapeseed meal of Brassica napusL. -Winter oilseed rape varieties is 120-150uMol.g^{-1}defatted meal.

In order to improve the quality of the rapeseed meal various methods. For the removal of the glucosinolates in industrial several technological methodshave been considered. Those methods have been too expensive and have resulted in protein losses or reduced protein quality or both.(Bowland et al.1965)For this occasion plant breeders studied the possibilities of reducing the glucosinolate content in the rape seed (Röbbelen, Thies ,1980).For the selection, the breeders must know as early as possible the material with the minimum glucosinolate content in meal,i.e.the so called "double zero" varieties.

From this reason the production of glucosinolates during winter rapeseed ripening was studied with the aim to learn thus the earliest time in which it is possible to determine significant differences in the production of glucosinolate and low glucosinolate varieties.

By introducing the low glucosinolates varieties in practice in Czechoslovakia changes in the glucosinolate content of winter oilseed rape varieties was reported. Thus a decrease of stability of the glucosinolates can lead to a decrease in quality of the "double zero " varieties;Therefore this economically significant problem was studied in detail.

Material and Method.

The production and content of glucosinolates during seed ripening was studied in the French variety"Jet neuf" having a normal glucosinolate content(154.4 μMol.g^{-1}defatted meal)and in the low glucosinolate variety"Librador"(33.9 μMol. g^{-1}defatted meal).The experiment was performed at the University experimental station near Praque in a sugar beet growing region with fertile brown soils in the season1982/83. Threesowing terms were used.

The samples of the seed were taking weakly till the full maturation. In these samples the content of individual glucosinolates were determined by gaschromatography after derivatization to trimethylsilylderivates. (Zukalová,Vašák,1978)

The changes in the glucosinolate content of the winter oilseed rape variety "double zero"-Tandem in succesive generation were studied in practice in the Northern Bohemia in yeer 1985/86.

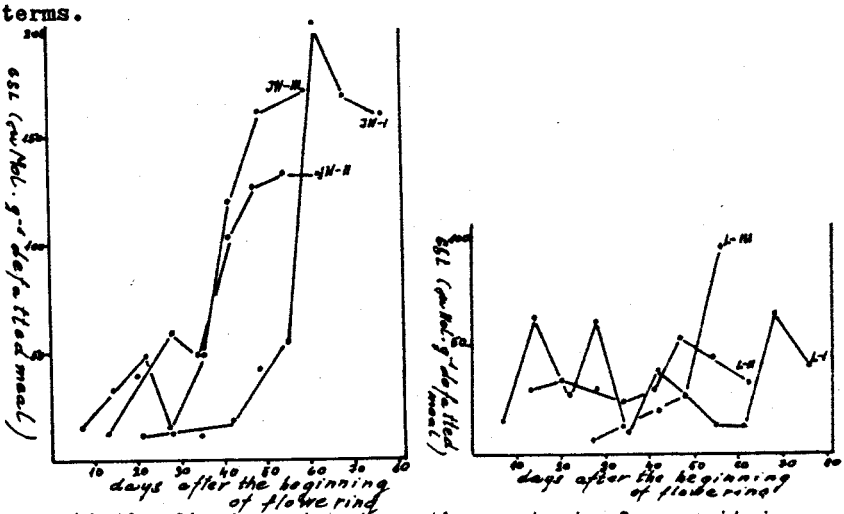
Results and Discussion.

The alkenylglucosinolates represent the major compounds from the group of glucosinolates in rapeseed. In the "Jet neuf" the content of these compounds starts to increase during the ripening from the 30th to the 40th day after the beginning of flowering in the agronomic date of sowing and late sowing.For early sowing the content of alkenylglucosinolates increases between the 40thto the 50thday after the beginning of the flowering.The increase of the content in the alkenylglucosinolates exhibits a slight drop at beginning of the maturation with the second and third sowing terms, followed between the 30th and 40th day by an increase to the level of comparable glucosinolate containing varieties i.e.to 160 μMol.g^{-1}defatted meal. In the final stages of the ripening in crops from the first sowing term a new drop is observed. The significantly lowest glucosinolate content was recorded in the crops grown from the second sowing term.(Fig.4.)No significant glucosinolate production trend during ripening was observed in the "Librador" variety whose glucosinolate content is 25% of that in the variety "Jet neuf", though at the beginning of the proces from the

end of flowering to the 30th day, the content is about the same , ranging between 30-60 μMol.g^{-1} defatted meal. The second sowing term leads to by far the lowest value. The third sowing term is definitely unsuitable. (Fig.5.)

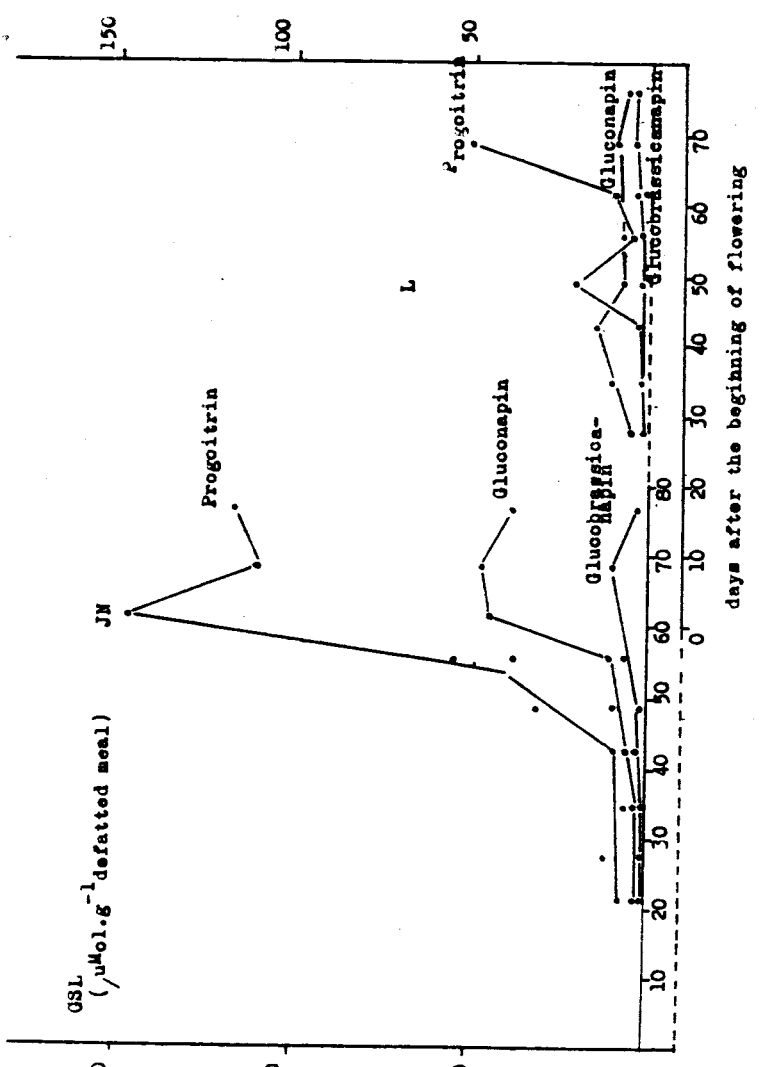
Fig.4. The formation of the total glucosinolate content during winter rape seed ripening; cultivar "Jet neuf" (JN); three sowing terms

Fig. 5. The formation of the total glucosinolate content during winter rape seed ripening; cultivar "Librador" (L); three sowing terms



At the first sowing term the content of progoitrine increases after 40days from the beginning of flowering from 9 μMol.g^{-1} defatted meal till 145 μMol.g^{-1} defatted meal and during the last 10days before maturation the content of this glucosinolate decreases. The content of gluconapin and glucobrassicanapin during seed ripening exhibits a drop similar to that one observed in the final stages of ripening. The variety "Librador" has a similar production of individual alkenylglucosinolates during ripening, though the content of glucosinolates in the first sowing date of the variety "Librador" is 34.4% of the content in the variety "Jet neuf". In the second and third sowing date the production of the glucosinolates during ripening is similar to the increase observed after about 35days after the beginning of the flowering. (Fig.6.)

Fig.6 Formation of the Glucosinolates During rthe Ripening of Winter Rapeseed: Cultivars "Jet Neuf"(JN) and "Librador" at the First Sowing Term.



Biosynthesis of alkenylglucosinolates is according to the scheme:(Underhill et al. 1973)

Methionin →(1) Homomethionin →(2) 2Amino-6-methylthiohexan acid →(3) 2Amino-7-methylthioheptan acid

Sinigrin ↓(4)

Gluconapin ↓(5) Glucobrassicanapin ↓(6)

Progoitrin ↓(7) Gluconapoleiferin ↓(8)

Gluconapin represents a significant part of the total glucosinolate content (24%),while progoitrine represents the largest part(71%). These two main glucosinolates are produced by a highly active enzymatic hydroxyl system5and7 - which is competitive with the system 3. The biosyntesis of the individual alkenylglucosinolates is similar both with the glucosinolates and low glucosinolates varieties of rapeseed. In the glucosinolate-free varieties the highly active enzymatic hydroxyl system is probably strongly suppressed and the degree of this suppression determines the decrease of the content of glucosinolates.From these results it is apparent that the third date of sowing is atypical. The agrotechnical date of sowing represents clearly an optimum both for yield and for other fundamental qualitative characteristics as it follows from this and previous papers published by us.(Zukalová,Vašák,1985)

Besides the narrow correlation between the glucosinalate content in the seed and that in the green matter,the dynamics of the production of the glucosinolates gives the breeders a larger space of time for selection. It gives the possibility of determining the low-glucosinolate content already between 35-40days after flowering,i.e.six weeks before harvest.

In succesive generation there is a lack of the stabi - lity of the glucosinolates especially of low-glucosinolate varieties. The reasons for the decrease in the glucosinolate content of harvested seed are:

1. The genetic lack of stability.

From our results it is clear that the effect of the genetic lack of stability is small and could not account for the increase of the glucosinolate content.

2. Carry-over of high glucosinolate seed from preceding harvest.

The practice trial in the Northern Bohemia has shown the glucosinolate content tended to change from one generation to the next.(table1) Carry-over of high glucosinolate seed from the preceding harvest is the limiting factor of quality "double zero" rapeseed. These results show that the glucosinolate content in the succesive generation is twice as high. It was caused by the "memory-effect"of the fields . The sowing on the field with three years time -gap between two cultures of winter rapeseed causes the four times higher glucosinolate content. Asix years time-gap leads to a double content. In fields rapeseed was not grown the content of glucosinolate in the harvest rapeseed was the same as in the original seed.

In further work it must be established whether different sites, husbandry practices, diseases, cross-pollination or some other factors are responsible for the changes in the glucosinolate content.

Conclusion.

1. From the study of the production of the glucosinolates during winter rapeseed ripening it follows that already 35-40 days after the beginning of flowering,i.e. six weeks before harvest, it is possible to determine the low -glucosinolate breeding material.
2. The correlation between the glucosinolate content of the seed and that of the green matter gives the possibility of selecting parent genotypes for the breeding of fodder rapeseed.
3. The limiting factor for the increase of glucosinolate content is the"memory -effect",i.e. the carry-over of high glucosinolate seed.
4. A total change to the breeding of double-zero vrieties only will need the establishment of the effect of further factors,as husbandry practices, diseases, cross-pollination, weather, different sites, especially sulphur exhalation.

Table 1. Glucosinolate Content in Varieties Grown at Five Centre in The Northern Bohemia. - 1985/86.
(Variety "Tandem"-33,99 /uMol.g^{-1} defatted meal) original seed.

place	Gluco-napin	Progo-itrin	Gluco-brassica-napin		time-gap between two culture of winter rapeseed
St.st	33,90	85,34	3,29	122,53	1983
Dubá	34,39	63,47	3,53	101,39	1982
ø6places	22,94	50,35	1,76	75,10	1980
ø	25,22	55,16	2,09	82,47	
V.V.	28,00	81,32	5,41	114,73	1983
Zákupy	29,97	76,98	5,64	112,54	1983
	31,44	65,88	1,88	99,20	1984
ø5places	17,46	42,69	1,83	61,38	1980
ø	22,09	54,70	2,76	79,17	
St.st. Cvikov	33,41	78,19	8,23	119,83	1984
ø7places	20,72	49,65	1,95	72,32	1980
	22,31	53,22	2,65	78,26	
JZD Kravaře ø 3places	14,10	30,31	1,33	45,75	no breeding
JZD Brniště ø 2places	14,59	40,26	1,88	56,72	1981
ø in The Northern Bohemia	19,66	46,73	2,14	68,47	

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