THE GLUCO-TEST AND THE PALLADIUM TEST - SUGGESTED PROCEDURES SUITABLE FOR THE SELECTION OF BREEDING MATERIAL OF WINTER RAPESEED FOR LOW GLUCOSINOLATE CONTENT

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1. The Gluco-test

A work procedure for the analysis is suggested, which is divided into single work sections in such a way, that the practical execution of the gluco-test gained a character of the continuous analysis with the production of: 1 result per 1 min., where time of the sample processing (with the regard to the materials used) is 23 min. The mechanical way of pulverizing the seed sample with quartz sand (Fig. 1) enables to work with the performance of pulverizing: 1 sample per 1 min. including simple and quick cleaning of the device after each sample, but also with keeping the required degree of fineness of pulverizing product. The procedure suggested is based on the results of published works (1 - 4). The optimum condictions were chosen with the regard to the properties of materials made in Czechoslovakia.

Work aids and appliances

1. Diagnostic strips "Glukophan", producer Lachema - Brno. They were adapted by removing the little indication area for reducing substances. This area was cut off closely to the little indication area for glucose. It is also possible to use for the analysis one half of the strip made by cutting it lengthwise;
2. Activated charcoal "Carbosorb", producer Imuna - Šarišské Michaľany, pulverized tablets;
3. Quartz sand washed by distilled water and dried;
4. China mortar (external diameter 10 cm, height 5.5 cm, depth 4.5 cm) with the device for mechanical pulverization of samples

(see below);

5. Low beaker, volume 50 ml, diameter 4 cm;

6. Sample batcher (vessel with the volume of 1.3 ml, which corresponds to the rapeseed weight about 0.8 g);

7. Quartz sand batcher (vessel with the volume of 2.2 ml, the corresponding dosage of sand is about 3.5 g);

8. "Carbosorb" batcher (vessel with the volume of 1.3 ml, the dosage quantity is about 0.8 g;

9. Water batcher (e.g. automatic burette);

10. Filter paper, circle with diameter of 8.5 cm and minor pieces quickly or medium filtering, for example "Filtrak 388 or 389";

11. Minor brush;

12. Rod;

13. Glass plate with a white base;

14. Electric stopwatch.

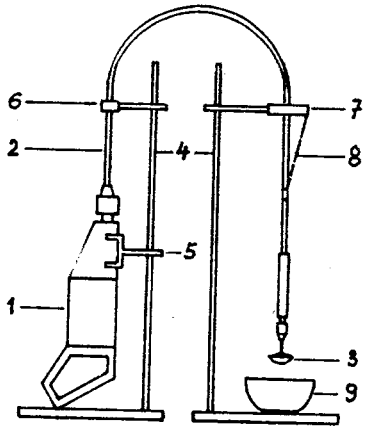
Work procedure

Time (min.)	work operations
0 - 1	Dose rape seeds and quartz sand into china mortar.
1 - 2	Pulverize the seed sample.
2 - 3	Transfer the sample quantitatively into 50 ml beaker in which "Carbosorb" was dosed in advance. Add 15 ml of distilled water and stir the mixture with a rod. Leave it to extract for 15 minutes.
16	Stir the mixture again.
17	Put the folded circle of filter paper to the bottom of the beaker.
18	Dip the adapted diagnostic strip into the filtered clear solution (for about 1 sec). After wetting the little indication area put the strip off immediately. Suck up the surplus solution on the strip (in front of the little indication area and from the back side of the strip) by putting a piece of quick-

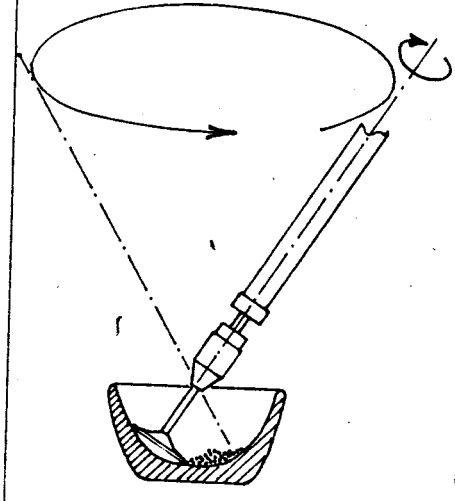
ly sucking filter paper to it. Don´t touch the little indication area! Put the strip on the glass plate with a white base.

23 Compare the intensity of colouring with the colour scale for the evaluation of the glucose content.

Fig. 1
The device for mechanical pulverization of the rapeseed sample.



1. Drilling machine
2. Flexible shaft
3. Abrasive disk
4. Laboratory stand
5. Holder for a drilling machine
6. Holder for a flexible shaft
7. Holder with a circle
8. Rubber suspension
9. Mortar



We pulverize the rapeseed sample with quartz sand by circle movements at mild pressure (at vertical direction of abrasive disk to the mortar wall). The optimum speed of turning we regulate by the help of a turning regulator (it is not depictured). We can clean the abrasive disk easily and quickly by putting the brush to its surface.

2. The Palladium Test

a) Spectrophotometric evaluation

Our work procedure takes advantage of published findings (5). Differences mentioned in Tab. 1 result from using other types of photometers and also from our intention to measure by this way only the samples with lowered glucosinolate content. The ammount of the added reagent to the sample is in our case relatively increased, bacause with the volume ratio of the sample and the reagent 1:20 we can observe (at certain level of GSL in the sample) certain precipitation of the reaction product. The resulting solid phase can gradually soil the walls of the cell in case of measuring bigger series of samples. The effort to substitute the imported sodiumtetrachloropalladate (Na_2PdCl_4) (Aldrich, no. 20 581-8) by a reagent prepared from $PdCl_2$ (40 % solution, Safina - Vestec) and NaCl had further advantages - substantially increased stability of freshly prepared reagent solution and the possibility to measure the samples against the blank.

Table 1

The difference in our procedure in comparison with the procedure made by Thiese:

no.	parameters	work procedure Thiese	our one
1.	the ammount of extract for analysis	15 µl (< 15 µmol GSL/ /g of seed)	20 µl
2.	reagent	Na_2PdCl_4 (Aldrich, no. 20 581-8)	$PdCl_2$ + NaCl
	concentration of reagent	2 mmol/l of water	2 mmol/l of water
	added ammount of reagent	300 µl	1.5 ml
3.	ratio of the volumes of sample extract : reagent (for < content of GSL in seed sample)	1 : 20	1 : 75

4.	measuring instrument	8-channel photometer Titertek Multiscan (Flow Laboratories)	spectral photometer Spekol (VEB Carl Zeiss Jena)

b) Visual evaluation

The possibility of comparing the colouring intensity of the complex and the colouring of the sample complex, the GSL content of which is known (5), has several disadvantages. The reagent itself is yellow and the change to brown-red is not the best change of colouring for the visual evaluation, especially in the range of low GSL content.

Our method of quick, visual and reproducible evaluation consists in the following principle. If there is a surplus of the reagent in the reaction of GSL, the brown-red reaction product in the form of a solid phase appears. It is possible to bind this reaction product equably in the structure of a suitable type of the chromatographic paper, so that the equably coloured spot is created.

The colouring tone of the created spot is not only caused by the reaction product, but also by the surplus of the reagent. It is possible to remove it very easily by washing it with diluted hydrochloric acid, which does not dissolve the reaction product. After another washing by distilled water we get a spot the colouring intensity of which is proportional to the concentration of GSL in the sample. The colouring does not change, so it is possible to keep the analyzed sample as an evidence. The quantitative evaluation can be done by the help of prepared colour scale of samples with the known GSL content.

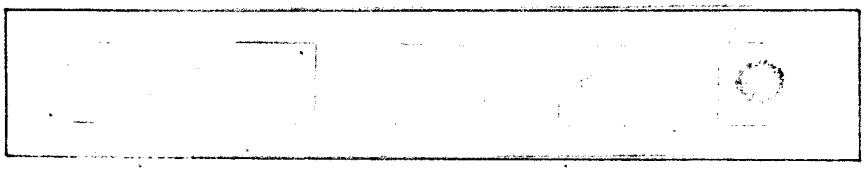
Work procedure

The preparation of the extract is the same as in the published work (5). We measure 10 µl of clear solution by a suitable batcher, e.g. by micropipette with the changeable tips. We apply this volume (1 drop) of a sample to a piece of chromatographic paper Schleicher-Schüll AG-591 in such a way, that we first push the sample out of the space of the tip (the drop "hangs") and we touch the surface of the chromatographic paper by this drop,

the paper sucks it up quickly. Only in the third minute after application of the sample (in the meantime we can apply further samples) we add in the same way 10 µl of the reagent with the concentration of 20 mmol Na_2PdCl_4/1 l of water (we can also use the reagent prepared from $PdCl_2$ and NaCl). We let the reaction continue for 30 minutes. It is necessary for the chromatographic paper to be laid on non-sucking base (e.g. glass).

After this reaction time we throw a piece of chromatographic paper with the spot to Erlenmayer flask with 1.5 % solution of HCl and stir vividly by circling. For the control of good washing we add to flask the same piece of chromatographic paper with the spot created after dropping the reagent itself (blank). Washing is finished when the spot of the blank disappears completely. Than we pour off the acid, we add distilled water and wash the samples by circling. After that we put the sample on the filter paper, where we let them dry. In the end we evaluate them. We carry out the evaluation from the side of application of the sample and the reagent by putting the paper to colour scale, which we can prepare from the samples of a known GSL concentration. We can glue the samples into a notebook and store them.

Fig. 2
The examples of the results of samples with the different content of GSL to 100 µmol/g of a sample, including the blank (without a spot).



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