OIL CROPS: BRASSICA SUBNETWORK

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A SIMPLE METHOD FOR IDENTIFYING LOW-ERUCIC ACID AND LOW-GLUCOSINOLATE RAPESEED-TURBIDITY TITRATION-COLORIMETRY

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A simple analytical method for rapeseed breeding, producing, purchasing and processing is reported. It is based on turbidity titration(1). Determination of erucic acid and glucosinolate for 5 samples can be carried out within a quarter. Absolute error for erucic acid estimation less than 0.5% when the content of erucic acid below 6%, and for glucosinolate less than 2 μmole/g when glucosinalate content below 30 μmole/g. Very similar results have been required by different operators.

The main studies presented in the paper are the condition of extracting polar glucosinalate and nonpolar fat from rapeseed at one time in room temperature, the curve about turbidity titration and the effects of various factors in glucosinolate colormetry.

EXPERIMENTAL CONDITION

I. **Instrument and reagents**:

1. Extract: dissolve 5 mL Triton X-100 in 500 mL absolute ethand.
2. Titration solution: 75-80% aqueous ethanol.
3. Oxide silicon dust (SiO_2)
4. $PdCl_2$ solution: put 177 mg dCl_2 in the mixture of 2 mL 2M HCl and 10 mL H_2O, than heat and solutize and make up to 1 litre with H_2O.
5. $CHCl_3$
6. Little spoon (0.5g) made by self.
7. Glass mortar
8. Test tube with plug (10m)
9. Graduated pupet (2mL, 10mL)
10. Sucking globe
11. Centrifuge (or hand set centrifuge)
12. Standard rapeseed (erucic acid: 2% and 5%, glucosinolate: 15 and 25 μmole/g)

II. **Extract fat and glucosinolate from rapeseed at one time**:

1. Choice of extract:
Effort was made to search for an extract which can extract polar glucosinolate and nonpolar fat at one time in room temperature so as to determine erucic acid and glucosinolate with same extracting solution. It proved that 1% Toriton X-100 (neutral surface active agent) absolute ethanol solution is efficient to extract erucic acid and glucosinolate from rapeseed at one time in room temperature. Data presented in Tables 1 and 2 were obtained from the determination of glucosinalate with colormetry and of fatty acid methyl ester by GC.

Table 1. Relation between glucosinolate content and absorbance.

Glucosinolate (μmole/g)	12.500	20.000	24.600	31.200	40.000
Absorbance (E)	0.030	0.048	0.060	0.192	0.200

Table 2. Percentage of fatty acid in extracting solution (%).

	Fatty Acid			
	C_{16}	C_{18}	C_{20}	C_{22}
avg. of 5 analyses	4.62	88.25	4.04	2.93
standard value	4.26	88.01	4.68	3.06

2. Other factors:
Fat can't disolve in water. The moisture content in extract had great effect on extracting of fat, especially with the fat containing erucic acid. So the extract-Triton X-100 solution must be prepared with absolute ethanol.

Disposing the extract in the air for 21 hours (no test for much long), different temperature and humidity have no effects on the result.

III. Effective factors in the determination of glucosinolate with colormetry:

1. Tentative plan and test for the concentration of color-developing agent: This method is used in identifying low-glucosinolate rapeseed. Distinguishing vaults are suggested that .30 μmole/g rape cake (about 15 μmole/g rapeseed) for original seed and 50 μmole/g rape cake (about 25 μmole/g rapesed) for commercial rapeseed(2). So we expected that the sudden change of colour is clear for visual photometry when glucosinolate content above 50 μmole/g rape cake, so as to identifying commercial rapeseed. And expected that there was Linear relate between the content and colour when glucosinolate content below 50 μmol/g rapecake, so as to know the valid content for screening original rapesed. Optimum content of color-developing agent and sample amount used were chosen and get ideal effect expected, Table 1.

2. Removal of the effects of fat and pigment: Glucosinolate and fat were extracted together. Adding color-developing agent into the extracting solution made fat colloidal flocculent precipitate. Pigment also interfered with visual photometry. An effect and simple method was adopted. That was adding $CHCl_3$ into the extracting solution before adding color-developing agent. By $CHCl_3$ extracting fat and pigment, the extracting solution was transparent, decoloured and to the benefit of visual photometry.

3. Effect of colour developing time:

Colour developing by glucosinolate and $PdCl_2$ need a long time-more than 10 hours. Color thickness is about 70% of the maximum in 5 minutes. Standard rapeseed and sample rapeseed parallel analysis can remove effect of color develop-ing time.

IV. Turbidity Titration Curve:

Water content in titration solution had great effect. In order to identify the rapeseed in which erucic acid content below 5%, 75-80% aqueous ethanol was adopted to have great difference in solution volume. Titration curve is shown in Fig. 1.

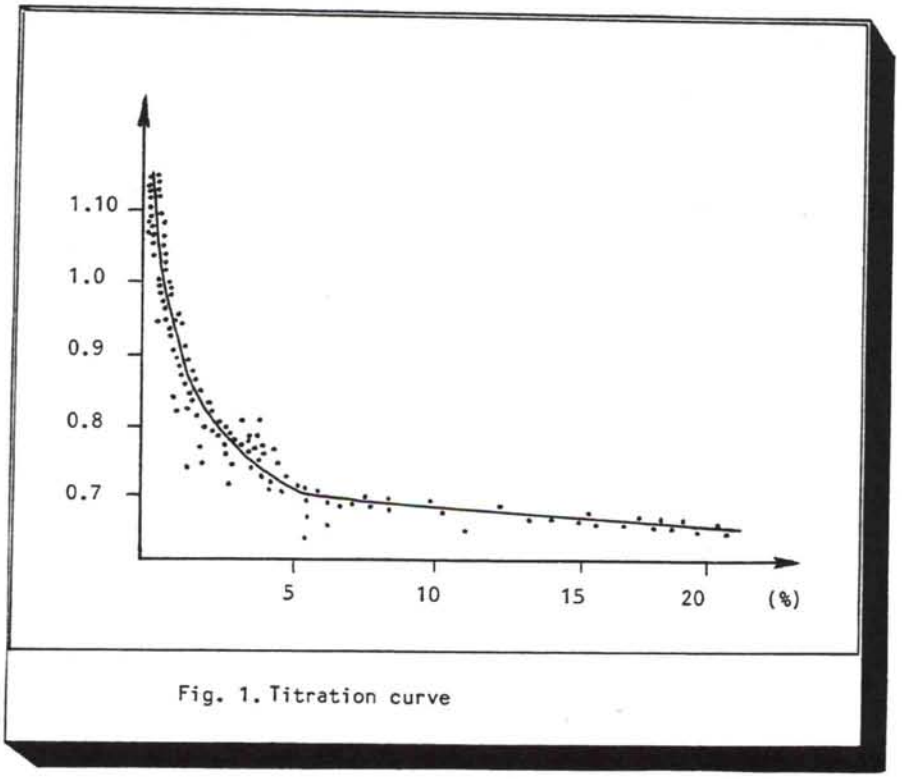

Fig. 1. Titration curve

SAMPLE ANALYSIS

Turbidity titration-colormetry procedure:

1. Representative rapeseed of different position is about 20g.
2. Put the sample put into a little clean bottle and mix.
3. Then abandon half of the rapeseed, shake and abandon another half.
4. Repent the operation till about 2.5 g rapeseed remains.
5. Take 0.5g sample with little spoon and put into glass mortar.
6. Add 0.5 g drug oxide silicon dust.
7. Grind.
8. Put into a test tube (10mL, with plug).
9. Add 8 mL extract solution.
10. Accurately extract 3 mins.
11. Centrifug.
12. Take 1 mLx2 of the supernatant liquid into test tube 1 and 2.

Determination of erucic acid:

1. Add 2mL of extract into tube 1.
2. Mixing with 75-80% aqueous ethanol titrate till turbidity.
3. Recording volume of titrating solution.
4. Erucic acid content in sample was estimated by comparing with titration volume of standard rapeseed (2% of 5% erucic acid) of same operating procedure.

Determination of glucosinolate:

1. Add 1 mL $CHCl_3$ into tube 2.
2. Mix.
3. Add 1 mL $PdCl_2$ solution.
4. Mix.
5. Draw out $CHCl_3$ below.
6. 5 mins.
7. Compare the color thickness between sample and standard rapeseed (15 or 25 umol/g rapeseed) and estimate the glucosinolate content of sample.

RESULT

Turbidity titration-colormetry have been tested and verified for three years. Table 3 is comparing the result with GC.

Table 3. Comparison between the method and GC.

erucic acid	>5%	5-2 %	<2 %
coincidence*	99%	98.7%	99.2%

* For glucosinolate, coincidence was 100%.

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