A Thiourea-UV Assay for Total Glucosinolate Content in Rapeseed Meals[1]

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ABSTRACT

A rapid and sensitive method for the determination of the total glucosinolate content in rapeseed is described. The method is based on the specific UV absorbance of the thioureas and oxazolidine-2-thiones. Results obtained were confirmed by gas-liquid chromatography. Recoveries varying from 94 to 103% were obtained for samples containing mixtures of isothiocyanate and oxazolidine-2-thione (a total of 0.25 to 0.78 mg per assay). The relative standard deviation for rapeseed meals varied from 4 to 10% for the total glucosinolate content (expressed as 3-butenylisothiocyanate) and depended on the size of sample taken. The relative standard deviation for oxazolidine-2-thione varied from 5 to 35% for the same meal. The lower limit of detection for rapeseed meal is of the order of 0.25 mg of 3-butenylisothiocyanate per g.

INTRODUCTION

There is a need for a more rapid, accurate, and simple assay for the glucosinolates of rapeseed meal. The processing and feed industries would find such an assay helpful as they could then monitor their operations on a semicontinuous basis.

Two of the more popular methods for glucosinolate determinations are the gas-liquid chromatographic (GLC) technique developed by Youngs and Wetter (1) and the selective extraction procedure of Appelqvist and Josefsson (2). Both have their disadvantages: the GLC method requires the utilization of costly equipment while the extraction method is long and tedious. Other assays of varying degrees of complexity are available, most of them determining products released by enzymatic action. Isothiocyanates have been estimated by GLC (1,3) or after conversion to the thioureas by UV (4) or by chemical methods (5,6). Others estimate the amount of glucose released (7-9), or sulphate released (10). A comparison of the three analyses most frequently utilized for rapeseed and its meal are summarized by Prevot, Bloch, and Barbati (11). Recently a report (12) has appeared that describes the quantitative estimation of rapeseed glucosinolates without prior enzymatic hydrolysis.

This paper describes an analysis for glucosinolates based on the UV absorption of thioureas and oxazolidine-2-thiones. It is rapid, simple, and accurate, and one operator can do ca. 30 samples a day on a routine basis.

[1]NRCC No. 15090.

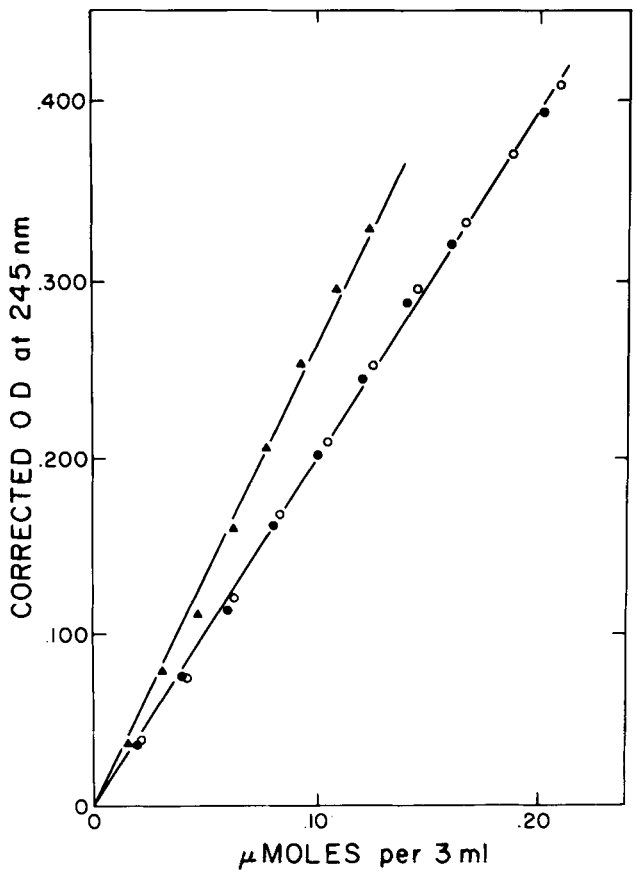

FIG. 1. Curves obtained by plotting the UV absorption of three compounds derived from the major glucosinolates found in rapeseed. 3-Butenylthiourea (●); 4-pentenylthiourea (○); 5-vinyloxazolidine-2-thione (▲). For the corrected optical density at 245 nm, see the text.

EXPERIMENTAL PROCEDURES

Materials

Commercial samples of rapeseed meal were obtained from various sources. These meals were assayed as received. Laboratory meals were prepared by extracting and grinding rapeseed in Swedish tubes (13) in Skellysolve F. Standard glucosinolate samples of 3-butenylgluosinolate and 2-hydroxy-3-butenylglucosinolate were made as described elsewhere (14). 3-Butenylthiourea, 4-pentenylthiourea, and 5-vinyloxazolidine-2-thione were synthesized in our laboratory.

The other reagents were employed without further purification.

The phosphate-citrate buffer (pH 7.0) was prepared by mixing 3.5 ml of 0.1 M citric acid solution with 16.5 ml of 0.2 M dibasic sodium phosphate solution. Thioglucoside glucohydrolase (EC 2.3.2.1), commonly called myrosinase, was prepared according to the method of Schwimmer (15).

Methods

The assay procedure is based in part on the earlier GLC method (1). Rapeseed meal (100 mg) is weighed into a 4 ml screw-cap vial with a teflon cap liner. One milliliter of the phosphate-citrate buffer containing 3 mg of thioglucoside glucohydrolase and exactly 2.5 ml of methylene chloride is added to the vial. The methylene chloride did not contain an isothiocyanate marker. The capped vial containing one glass bead is shaken on an oscillating shaker for 2 hr at room temperature.

After completion of the enzyme incubation, the emulsion is broken by centrifuging at ca. 1000 x g. A clear methylene chloride layer is essential, as traces of aqueous extract will introduce large errors into the assay. The total

TABLE I

3-Butenyl Isothiocyanate (3-BITC) and 5-Vinyloxazolidine-2-thione (VOT) Content of a Standard Glucosinolate Solution as Determined by the Thiourea-UV Method

Amount added[a]		Total by	Total by		VOT by	
3-BITC (mg)	VOT (mg)	GLC (mg)	thiourea-UV assay (mg)	Recovery (%)	thiourea-UV assay (mg)	Recovery (%)
0.0151	0.0101	0.0252	0.0228	90	ND	---
0.0378	0.0253	0.0631	0.0540	86	ND	---
0.0604	0.0404	0.1008	0.1000	99	ND	---
0.0755	0.0505	0.1280	0.1142	89	ND	---
			0.1140	89	0.040	78
0.151	0.101	0.252	0.239	94	0.095	94
0.227	0.142	0.379	0.379	100	0.150	99
			0.377	100	ND	---
0.303	0.203	0.506	0.496	98	0.214	105
0.379	0.253	0.632	0.650	103	0.270	107
			0.631	100	ND	---
0.455	0.304	0.759	0.776	102	0.329	108

[a]The values in the first two columns are the mg of material in each assay as determined by the gas-liquid chromatographic method (1). The value in the third column was obtained by adding columns 1 and 2.

[b]ND = not determined.

isothiocyanate plus oxazolidine-2-thione content is determined by adding 50 μl of the methylene chloride extract to 3 ml of 20% ammoniacal ethanol (1 part concentrated NH_4OH to 4 parts anhydrous ethanol) and heating for 2 hr at 50 C in a water bath. The reaction is carried out in a culture tube (16 x 125 mm) equipped with a screw cap. After cooling, the optical density is determined at 235, 245, and 255 nm. The blank is made up of 50 μl of methylene chloride in 3 ml of 20% ammoniacal ethanol. The corrected optical density is obtained as follows:

$$OD_{245_{corr}} = OD_{245} - \tfrac{1}{2}(OD_{235} + OD_{255})$$

The total isothiocyanate content is expressed as mg 3-butenylisothiocyanate per g of meal (mg of 3-butenylisothiocyanate per g meal = $OD_{245_{corr}}$ x [28.55].

5-Vinyloxazolidine-2-thione content is determined by following the steps outlined above, except that 95% ethanol is substituted for the ammoniacal solution. The amount in the meal is expressed as mg of 5-vinyloxazolidine-2-thione per g of meal (mg of 5-vinyloxazolidine-2-thione per g meal = $OD_{245_{corr}}$ x [22.1]).

RESULTS AND DISCUSSION

Figure 1 shows that the molecular absorption for 3-butenyl- and 4-pentenylthiourea are identical, while that for 5-vinyloxazolidine-2-thione was higher. Since the molecular absorption for the thioureas and the oxazolidine-2-thione are similar, it is possible to obtain a reasonable estimate for glucosinolate by expressing the content as mg of 3-butenylisothiocyanate in the meal.

A standard solution of *n*-butyl isothiocycanate was employed to test the method described above. Twelve assays performed at different times resulted in an average recovery of 96.6% with a standard deviation of 1.9%. This indicates that the isothiocyanate was being converted quantitatively to the thiourea.

A comparison of the GLC method (1) and the present method is given in Table I. For this comparison, varying amounts (0.02 to 0.6 ml) of a standard glucosinolate solution containing 3-butenylglucosinolate and 2-hydroxy-3-butenylglucosinolate were employed. The recovery is good for samples that contain > 0.38 mg per assay; less than this amount results in somewhat lower recoveries, in the order of 90%. Samples of < 0.15 mg of 5-vinyloxazolidine-2-thione are difficult to assay, but above this value the recoveries are again good.

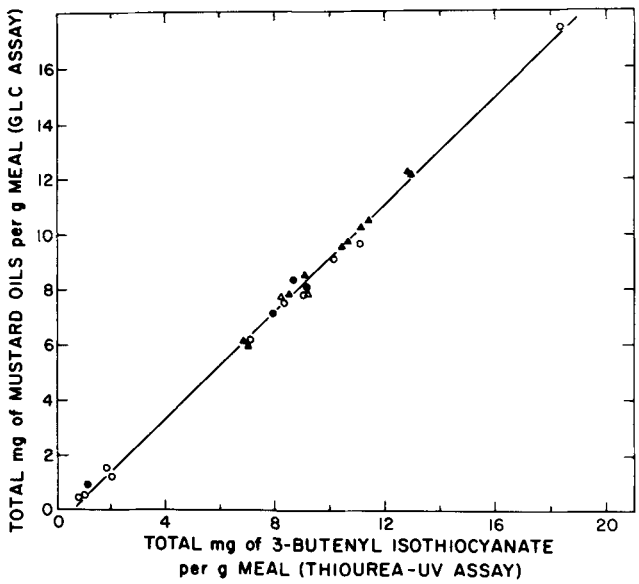

FIG. 2. The curve obtained by plotting values derived from the gas-liquid chromatographic (GLC) and the thiourea-UV assay. The mustard oil value was obtained by the summation of 3-butenyl and 4-pentenyl isothiocyanates and 5-vinyloxazolidine-2-thione from the GLC assay (1). Samples (Set I) prepared by us and Canada Agriculture (○); commercial samples (Set II) acquired from Dr. Bell, University of Saskatchewan, Saskatoon (▲); and commercial samples (Set III) secured from Canada Agriculture (●).

The thiourea-UV assay was tested on a number of commercial samples and on defatted meals prepared in the laboratory. The GLC method was also done on these samples and the total glucosinolate content calculated. These data are shown in Figure 2 and demonstrate the effectiveness of the present method in estimating the total glucosinolate content of a meal. A summary of the correlation coefficient and the linear regression is given in Table II. The correlation coefficient is high and significant beyond the 1% level.

The values given in Table III show the effect of sample size on the glucosinolate determination. Some of the variations undoubtedly are due to the low corrected optical density that one obtains when small amounts of material are employed. The standard deviation is considerably higher

TABLE II

Linear Regressions and Correlation Coefficients for the Three Sets of Samples

Set[a]	Linear regression	Correlation	Significance
I	Y = 0.63 + 1.034 X	0.998	beyond the 1% level
II	Y = 1.00 + 0.980 X	0.992	beyond the 1% level
III	Y = 0.18 + 1.064 X	0.996	beyond the 1% level
Combined	Y = 0.57 + 1.030 X	0.997	beyond the 1% level

where Y values for the thiourea-UV assay
X values for the gas-liquid chromatographic assay

[a]See Figure 2 for source of samples.

TABLE III

Glucosonilate Content Obtained for Various Samples at Different Levels of Material

Sample	Size (mg)	Total content as 3-butenyl isothiocyanate			5-Vinyloxazolidine-2-thione		
		OD[a]	mg/mg	SD[b]	OD[a]	mg/mg	SD[b]
Standard glucosinolate	2.0	0.175	0.250	0.013 (17)	0.098	0.108	0.004 (19)
	0.4	0.038	0.272	0.022 (10)	0.022	0.123	0.021 (10)
	0.2	0.017	0.238	0.026 (5)	0.011	0.124	0.022 (5)
			mg/g			mg/g	
Span rapeseed	100.0	0.293	8.37	0.30 (14)	0.133	2.92	0.15 (14)
	10.0	0.032	8.68	0.88 (12)	0.014	2.69	1.00 (12)
Bronowski rapeseed	100.0	0.027	0.78	0.07 (16)	0.014	0.30	0.10 (16)
	50.0	0.016	0.90	0.09 (8)	0.011	0.48	0.13 (8)
CDA-4 rapeseed	100.0	0.043	1.24	0.11 (16)	0.028	0.62	0.07 (16)

[a]This represents the corrected optical density at 245 nm.

[b]The standard deviation with the number of assays in brackets.

for the smaller samples, again suggesting that the low optical density is responsible. The lower limits of detection appear to be at an optical density of 0.01, which is equivalent to 25-30 μg of either 5-vinyloxazolidine-2-thione or 3-butenylisothiocyanate, which is equal to 0.25-0.30 mg per g of meal. The method appears, therefore, to be a good substitute for the GLC method, particularly when applied to the levels of glucosinolate present in rapeseed meal.

ACKNOWLEDGMENT

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