Determination of Glucosinolates Using Their Alkaline Degradation and Reaction with Ferricyanide

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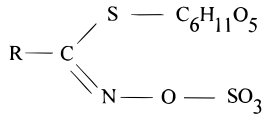
Glucosinolates, a group of naturally occurring thioglucosides, are significant factors impairing the nutritional quality of rapeseed and postextraction rapeseed meal, restricting its use as high-quality protein animal feed. Currently, the European Community standards and Canola definition are being brought in line recommending cultivation and marketing of rapeseed with a glucosinolate content below 18 μmol of total glucosinolates per gram of seeds. Furthermore, some glucosinolates are of increasing interest in *Brassica* vegetables due to their proven cancer-preventing activities. A novel approach to the analysis of total glucosinolates is reported in this paper based on their alkaline degradation and subsequent reaction of released 1-thioglucose with ferricyanide. The reaction was followed spectrophotometrically using sinigrin and glucotropeaolin as model glucosinolates. The applicability of the method was demonstrated using rapeseed extracts after reducing the interfering effect of phenolics by their adsorption onto polyvinylpolypyrrolidone. Good agreement with official ISO methods was shown.

Keywords: *Alkaline degradation; ferricyanide; glucotropaeolin; rapeseed; sinigrin; total glucosinolates; thioglucose*

INTRODUCTION

Glucosinolates are naturally occurring β-D-thioglucosides (I) found in 11 families of plants. Most of the cultivated plants that contain glucosinolates belong to the *Brassica* genus of the Cruciferae family. Myrosinase



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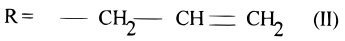

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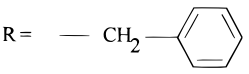

(III)

(thioglucoside glucohydrolase, EC 3.2.3.1), also found in these plants, acts on glucosinolates to produce glucose, sulfate, and a range of physiologically active products including isothiocyanates, thiocyanates, and nitriles depending on both the substrate and the reaction conditions (McGregor et al., 1983). These released compounds are responsible for the characteristic biting taste of important condiments (e.g., mustard, horseradish) and contribute to the desirable sensory properties and flavors of many vegetables, e.g., cabbage, Brussels sprouts, broccoli (McGregor et al., 1983). High levels of the hydrolysis products (especially in sprouts), however, may produce an unpleasant bitter taste. Glucosinolates and the hydrolysis products are beneficial for the growth of the *Brassica* plants as they are toxic to some insects and belong to the group of natural pesticides (Stoewsand, 1995).

When consumed by humans as part of the normal diet, some of the hydrolysis products exhibit anticarcinogenic properties (Jongen, 1996). However, excessive consumption of glucosinolate-containing vegetables can lead to increased risk of goiter (Stoewsand, 1995). When consumed in large amounts by animals as part of their feed, e.g., in rapeseed meal, the hydrolysis products reduce palatability and are toxic. Rapeseed varieties low in glucosinolates have been available as a result of breeding programs over nearly two decades, but even these glucosinolates levels are still too high for effective use of the high-protein rapeseed meal for animal feed or for its inclusion in human consumption (Shahidi and Gabon, 1990).

HPLC separation with UV detection (ISO 1995) or gas chromatography (Thies, 1984) are the most frequently used methods of analysis for individual glucosinolates, but it is often necessary, or sufficient, to determine the total quantity of glucosinolates in a sample. Two official ISO and European Community (EC) methods for determination of total glucosinolates in rapeseed and rapeseed meal involve HPLC of individual desulfoglucosinolates followed by their summation (ISO, 1995) and X-ray fluorescence (XRF) determination of total sulfur in the rapeseed sample, which can be correlated with total glucosinolates (ISO, 1994). Although the methods are satisfactory in terms of precision and reproducibility, they require sophisticated equipment and are either lengthy (HPLC) or need extensive calibration (XRF). Other currently adopted analytical procedures for total glucosinolates rely either on sample treatment by myrosinase followed by determination of released glucose (Koshy et al., 1988; Kershaw and Johnstone, 1990) or on formation of colored complexes of glucosinolates with thymolsulfuric acid (DeClercq and Daun, 1989) or tetrachloropalladate (Thies, 1982) after sample preparation and cleanup. The repeatability and reproducibility of most of the currently used methods have been

10.1021/jf9906026 CCC: $18.00
Published on Web 10/28/1999

compared in several ring tests organized by ISO (Wathelet et al., 1995). Near-infrared reflectance spectroscopy has also recently been successfully tested for determination of total glucosinolate content in a range of *Brassica* seed samples (Velasco and Becker, 1998).

Sinigrin (II, allylglucosinolate) is the principle glucosinolate present in commercial crops of yellow mustard seed (Sang et al., 1984). Within the European Community, and across the world, it is the primary reference standard for HPLC analysis of glucosinolates, while glucotropaeolin (II, benzylglucosinolate) is an alternative standard (ISO, 1995). These compounds are known to be decomposed at high pH: sinigrin is degraded (>90%) to 1-thioglucose, vinylglycine, and sulfate by a first-order reaction with respect to both sinigrin and hydroxide ion (Friis et al., 1977):

$$\text{sinigrin} \xrightarrow{OH^-} C_6H_{11}O_5S^- + \text{vinylglycine} + SO_4^{2-} \quad (1)$$

Thiol compounds are known to be readily oxidized by ferricyanide (Dawson et al., 1989), which is both chromogenic and electroactive; therefore, the alkaline treatment of glucosinolates and subsequent reaction of the breakdown products with ferricyanide offers the possibility of spectrophotometric or electrochemical measurement of glucosinolates. The purpose of the work reported here was to investigate this new approach to the rapid determination of total glucosinolates.

Both the determination of isolated glucosinolates (singrin and glucotropaeolin) in model solutions and the determination of total glucosinolates in rapeseed samples are described. Spectrophotometric measurements were used to follow the reduction of ferricyanide ion, and strategies were developed for circumventing or reducing interfering effects when analyzing vegetable samples. To enable comparison, the rapeseed was independently analyzed using ISO methods.

EXPERIMENTAL PROCEDURES

Chemicals. Glucotropaeolin (potassium salt) was obtained from Merck; sinigrin monohydrate, 1-thioglucose, glucose, insoluble polyvinylpolypyrrolidone, and potassium ferricyanide was obtained from Sigma-Aldrich. Unless otherwise stated, fresh aqueous solutions were prepared shortly before use.

Spectrophotometry. A diode-array spectrophotometer (Hewlett-Packard, model 8452A) with HPUV software was used for measurements of UV−vis spectra. Measurements of reaction kinetics with the ferricyanide ion were carried out at 420 nm ($\epsilon = 1020$; Dawson et al., 1989). Unless indicated otherwise, a 1 cm cuvette and an integration time of 25 s was used.

Identification of Disulfide of Thioglucose. Ligand exchange HPLC was used to identify 1-thioglucose and its disulfide. An aqueous mobile phase was used with a column packed with the sodium form of a strongly acidic cation-exchange resin LGKS 0802 (Spolchemie, Czech Republic). Detection was by means of an infrared detector. A reference sample of the disulfide was prepared by oxidation of 1-thioglucose using a methanolic solution of iodine (Horton, 1963).

Extraction of Glucosinolates from Rapeseed. Rapeseed was obtained from the National Institute of Agricultural Botany (NIAB, Cambridge, U.K.). Ground whole-fat seeds (500 mg) were added to a solution of almost boiling acetate buffer (pH 4.2, 0.2 M, 7.5 mL). The mixture was kept in a boiling water bath for 15 min. After cooling (5 min), the whole extract was mixed with a solution containing 0.5 M barium and lead acetates (1.5 mL), and polyvinylpolypyrrolidone (0.4 g) was added. The mixture was incubated while being stirred (15 min) before adding sodium sulfate (2 M, 1.5 mL) and centrifuging (14 000 rpm, 5 min). In some cases, sinigrin (~5 mg) was added to duplicate samples of cooled extract before addition of the acetates. These samples were used to calculate the recovery of sinigrin in the subsequent reactions.

Table 1. Summary of Calibration Results from Spectrophotometric Determinations of Sinigrin and Glucotrapaeolin after Their Alkaline Degradation and Subsequent Reaction with Ferricyanide in pH 7

glucosinolate	time[a] (min)	slope ± s.e.[b] (mM^{-1})	intercept ± s.e.[b]	R^2 [c]
sinigrin	5	0.822 ± 0.005	0.003 ± 0.004	0.999
	5[d]	0.804 ± 0.005	0.007 ± 0.004	0.999
glucotropaeolin	5	0.312 ± 0.005	−0.008 ± 0.006	0.996
	30	0.791 ± 0.009	0.011 ± 0.003	0.998

[a] Incubation time in mixture with sodium hydroxide (1 M). [b] s.e. is the standard error. [c] Coefficient of determination. [d] Second calibration carried out 5 months after the first one using the same batch of sinigrin.

Alkaline Treatment and Reaction with Ferricyanide. The clear supernatant was mixed with an equal volume of sodium hydroxide (2 M). HCl was added after 30 min incubation (1 part of concentrated HCl to 13 parts of the alkaline solution) to neutralize the solution. The resulting mixture was centrifuged (14 000 rpm, 3 min), and the supernatant was mixed with an equal volume of ferricyanide (2 mM) prepared in phosphate buffer (pH 7, 0.2 M). The absorbance of the solution was measured within 15 s against phosphate buffer (pH 7, 0.2 M) at 420 nm.

Quantification of Total Glucosinolates in Rapeseed Sample. The content of total glucosinolates in the rapeseed sample was calculated from the absorbance reading using a standard sinigrin calibration curve, Table 1

$$c = \frac{\Delta OD - \text{intercept}}{\text{slope}} \times \frac{Vd}{m} \quad (2)$$

where c is the concentration of glucosinolates in the seeds ($mmol\ kg^{-1}$), ΔOD is the change in optical density (420 nm) caused by the solution, the slope and intercept are those of the sinigrin calibration curve (Table 1), V is the volume of acetate buffer used for extraction of glucosinolates (L), d is the dilution factor of the extract during the alkaline treatment and reaction with ferricyanide (6.02 for the described procedure), and m is the mass of seeds used in the sample (kg).

Due to the background absorbance of the extracts, however, it was necessary to measure the absorbance of blank samples, which were prepared by mixing the neutralized alkaline-treated extract with phosphate buffer (pH 7, 0.2 M). In those cases when duplicate samples were spiked with sinigrin, the concentration of glucosinolates in the original samples could be calculated from the absorbance difference readings:

$$c = \frac{n_{\text{sinigrin}}}{m_{\text{seeds}}} \times \frac{\Delta OD_1}{\Delta OD_2 - \Delta OD_1} \quad (3)$$

where c is the concentration of glucosinolates in the seeds ($mmol\ kg^{-1}$), n is the number of millimoles of sinigrin spiked into the sample, m is the mass of seeds used in the sample (kg), ΔOD_1 is the change in optical density (420 nm) caused by the unspiked solution, and ΔOD_2 is the change in optical density (420 nm) caused by the spiked solution.

Safety Considerations. The available material safety data for glucotropaeolin was sparse; therefore, it was handled in a similarly cautious manner to sinigrin, which is corrosive, causes burns, and is a lachrymator. Target organs include the blood and liver.

RESULTS AND DISCUSSION

Reduction of Ferricyanide. At neutral pH, neither sinigrin nor glucotropaeolin was able to reduce ferricyanide but its reduction by 1-thioglucose was completed in a matter of seconds. Interestingly, the reaction of

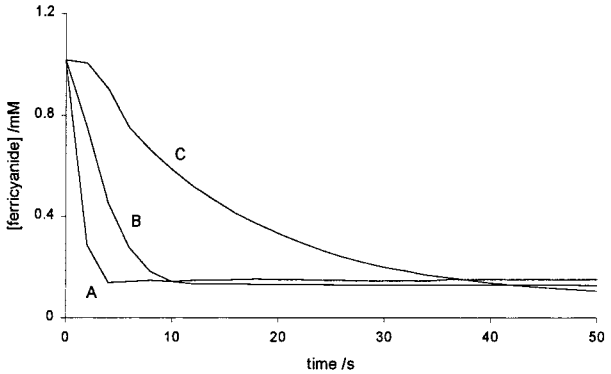

Figure 1. Reactions of 1-thioglucose (1 mM) with ferricyanide (1 mM). (A) In phosphate buffer, pH 7, 0.2 M. (B) In phosphate buffer, pH 12, 0.2 M. (C) In sodium hydroxide, 1 M. (Signal integration time, 0.5 s.)

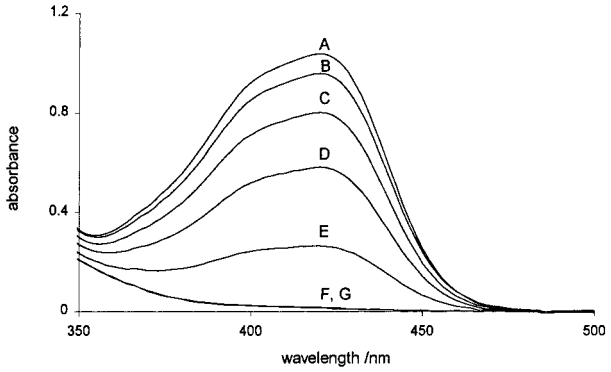

Figure 2. Changes in absorbance resulting from addition of sinigrin (final concentration 1 mM) into a solution of ferricyanide (1 mM; in phosphate buffer, pH 12, 0.2 M). Spectrum A is of the background electrolyte. Time (min) after addition of sinigrin: (B) 10, (C) 30, (D) 60, (E) 120, (F) 180, and (G) 240.

1-thioglucose with ferricyanide in sodium hydroxide (1 M) was less rapid than in neutral phosphate buffer, Figure 1. HPLC showed that the disulfide of thioglucose was the product of the reaction with ferricyanide:

$$2C_6H_{11}O_5S^- + 2Fe(CN)_6^{3-} \rightarrow C_6H_{11}O_5SSO_5H_{11}C_6 + 2Fe(CN)_6^{4-} \quad (4)$$

Sinigrin (1 mM) added to a solution of ferricyanide (1 mM) in phosphate buffer (pH 12, 0.2 M) caused a slow decrease in absorbance at 420 nm (Figure 2), which was caused by reduction of ferricyanide by 1-thioglucose slowly released from sinigrin by the action of hydroxide anion. Incubation of sinigrin in concentrated base brought about rapid degradation, as shown by subsequent reaction of the breakdown products with ferricyanide present in the reaction mixture, Figure 3. The rate of the reaction was in accord with published rate data (Friis et al., 1977): rate/M^{-1} s^{-1} = 8×10^8 exp $\{-63$ kJ $mol^{-1}/RT\}$. Glucotropaeolin was similarly hydrolyzed, the reaction showing a lower rate compared with that of sinigrin, Figure 3.

Considering the kinetic aspects of the alkaline degradation of glucosinolates, sodium hydroxide (1 M) was used in subsequent experiments for degradation of glucosinolates to determine their content by allowing the released 1-thioglucose to reduce ferricyanide. Thus, sinigrin and glucotropaeolin were prepared in the hydroxide (1 M) while aqueous glucosinolate-containing plant extracts were mixed with equal volumes of sodium

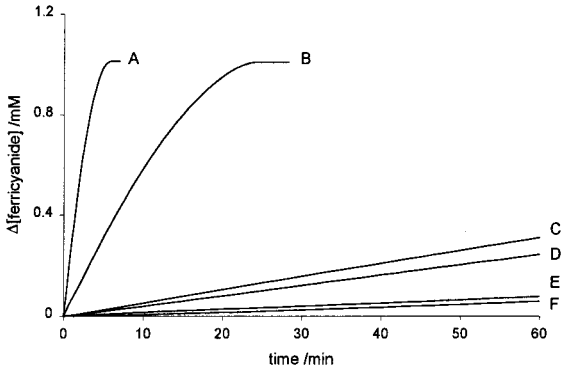

Figure 3. Reactions of sinigrin, glucotropaeolin, and glucose with ferricyanide (1 mM) in phosphate-buffered solution and in aqueous sodium hydroxide. (A) Sinigrin, (B) glucotropaeolin, and (C) glucose in sodium hydroxide (1 M) solution. (D) Sinigrin, (E) glucotropaeolin, and (F) glucose in phosphate buffer solution (pH 12, 0.2 M). In each case the initial concentration of the compound was 1 mM. (Signal integration time, 2 s.)

hydroxide (2 M). The determination of isolated glucosinolates can be carried out in one step in a matter of minutes by incubation in alkaline solution (1 M NaOH) of ferricyanide and reading the decrease in absorbance at 420 nm. While this may be of some limited use, for more complex solutions there are practical reasons to suggest that the alkaline glucosinolate degradation and ferricyanide detection of 1-thioglucose produced should be carried out separately: (1) ferricyanide reduction by hydroxide has been described (Bhattacharyya and Roy, 1986). Despite being a slow process, at high pH it could interfere with the measurement of 1-thioglucose. (2) More importantly, there would be substantial interference from other compounds present in real samples, e.g., reducing sugars are split into smaller molecules at high pH (Yang and Montgomery, 1996) and their alkaline reaction with ferricyanide is well-known (Myklestad et al., 1997; Avigad, 1968). In particular, the breakdown products of glucose are known to react rapidly with ferricyanide in alkaline solutions, Figure 3. (3) It is also of concern that 1-thioglucose, produced by the alkaline breakdown of glucosinolates, eq 1, will undergo further degradation to give other products that will react with ferricyanide.

To circumvent these problems, the decomposed glucosinolates were reacted with ferricyanide at neutral pH. Reaction of the glucosinolates in sodium hydroxide followed by injection of the alkaline solution into a buffered neutral solution of ferricyanide resulted in rapid and quantitative reduction of ferricyanide, whereas there was no reduction when glucose was used in place of the glucosinolates (Figure 4). Unless stated otherwise, subsequent results were obtained using this pH switch procedure.

Temporal Effects of the Treatment of Glucosinolates in 1 M NaOH. Complete degradation of glucotropaeolin was achieved in about 25 min, whereas the complete degradation of sinigrin took only 5 min. In excess of these minimum degradation times, the alkaline incubation of sinigrin and glucotropaeolin had no effect on the subsequent reactions with ferricyanide. Glucose treated with sodium hydroxide for up to 16 h failed to exhibit any reaction with ferricyanide after the pH switch, Figure 5.

Quantitative measurements of isolated glucosinolates using the pH switch technique could be made with good

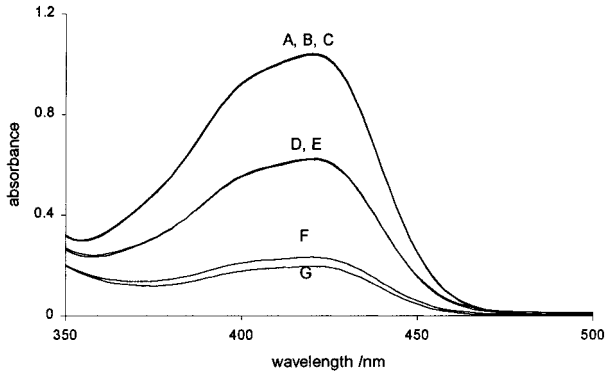

Figure 4. Optical spectra of ferricyanide solutions (1 mM in phosphate buffer, pH 7, 0.2 M) before and after addition of alkaline-treated sinigrin, glucotropaeolin, or glucose. Alkaline treatment was incubation with sodium hydroxide (1 M, 30 min). After addition to ferricyanide solution, final concentration (mM): (A) no addition (i.e., water only); (B, C) glucose, 0.5 and 1; (D) sinigrin, 0.5; (E) glucotropaeolin, 0.5; (F) glucotropaeolin, 1; (G) sinigrin, 1.

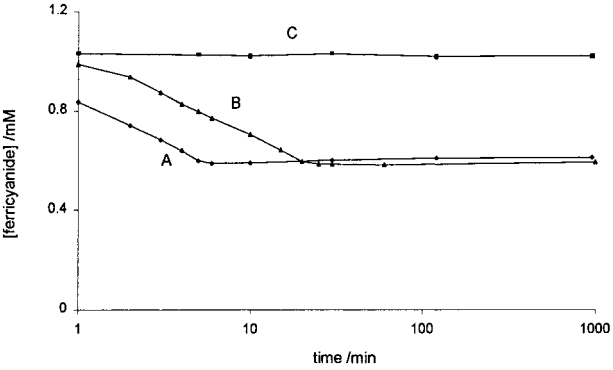

Figure 5. Reaction with ferricyanide (1 mM) of alkaline degradation products of (A) sinigrin, (B) glucotropaeolin, and (C) glucose. The indicated compounds (25 mM) were allowed to react in sodium hydroxide (1 M) for the times shown. Samples of the reaction mixture were taken and diluted in a ratio of 1 part to 49 parts of ferricyanide (1 mM in phosphate buffer, pH 7, 0.2 M).

precision (Table 1). It is noteworthy that when the base-catalyzed decomposition of glucotropaeolin was allowed to proceed to completion (30 min), the subsequent reaction with ferricyanide was quantitatively very similar to that obtained when sinigrin was used. This is despite both quantitative and qualitative differences in the degradation products of sinigrin and of glucotropaeolin (Friis et al., 1977). Furthermore, it should be noted that precise linear results were obtained even when alkaline degradation of glucotropaeolin was not allowed to proceed to completion, Table 1. This observation may be important in those applications where the speed of analysis is of more importance than measurement of an absolute glucosinolate concentration, e.g., in field determinations for estimation of crop development (Rosa et al., 1996).

Interference from Other Biochemicals. Ferricyanide reacts with a wide range of plant products in addition to glucose and glucosinolates. Reactions with plant phenolic compounds (Dawson et al., 1989; Côté and Nadeau, 1993; Carmona et al., 1991), vitamins (Dawson et al., 1989; Iyangi et al., 1985), thiols (Dawson et al., 1989), and carbohydrates (Myklestad et al., 1997; Avigad, 1968) are well documented. Although not exhaustive, a range of compounds that occur in either rapeseed (Simbaya et al., 1995) or other vegetable

Table 2. Ability of Chosen Biologically Important Chemicals To Reduce Ferricyanide in Neutral, Alkaline Solutions and pH-Switched Solutions. The Extent of Each Reaction Is Expressed as the Percentage Reduction of Ferricyanide Originally Present. Final Concentrations of Ferricyanide and of Test Compounds Were each 1 mM

	pH 7 (buffered)[a]		1 M NaOH[b]		pH switch[c]	
compound	5 min[d]	30 min[d]	5 min[d]	30 min[d]	5 min[d]	30 min[d]
Glucosinolates						
sinigrin	0	0	100	100	100	100
glucotropaeolin	0	0	39	100	39	100
Carbohydrates						
glucose	0	0	2	15	0	0
sucrose	0	0	0	2	0	0
Amino Acids						
isoleucine	0	0	1	3	0	0
lysine	0	0	1	3	0	0
phenylalanine	0	0	16	24	0	0
tryptophane	0	2	74	92	0	0
histidine	0	2	35	68	0	0
serine	0	0	1	3	0	0
aspartic acid	0	0	1	3	0	0
Proteins						
albumin[e]	0	0	19	38	0	0
Thiols and Disulfides						
cysteine	100	100	100	100	89 (41)[f]	84 (13)[f]
cystine	0	0	2	5	2	3
Phenols						
phenol	0	0	12	23	0	0
catechol	*g*	*g*	*g*	*g*	*g*	*g*
tannic acid	*g*	*g*	*g*	*g*	*g*	*g*
Vitamins and Coenzymes						
L-ascorbic acid	100	100	100	100	88 (76)[f]	75 (28)[f]
biotin	0	0	0	2	0	0
folic acid	2	5	71	95	0	0
NADH	26	100	100	100	0	0
thiamine	100	100	100	100	100	100

[a] Compound incubated in phosphate buffer (0.2 M, pH 7) containing ferricyanide (1 mM). [b] Compound incubated in sodium hydroxide (1 M) containing ferricyanide (1 mM). [c] Compound incubated in sodium hydroxide (1 M) before reducing pH to 7, by dilution, for reaction with ferricyanide. [d] Duration of exposure of indicated compound in sodium hydroxide (1 M) before measurement of ferricyanide at 420 nm. (pH switch solutions were quenched in neutral buffered ferricyanide for the measurements.) [e] Final concentration of albumin in the reaction mixtures was 0.1 mg/ml. [f] The value in brackets corresponds to alkaline incubation performed in the presence of traces of copper(II) cations. [g] Reaction with ferricyanide was difficult to quantify due to formation of strongly colored solutions even in the absence of ferricyanide.

samples were tested to evaluate the potential magnitude of their interference effect in the proposed procedure for glucosinolate determination, Table 2. Proteins and carbohydrates are concentrated in rapeseed meal, but neither the selected range of amino acids and proteins nor the selected saccharides produced any interference after the pH switch procedure. Of the compounds that showed any reaction within the first 30 min of their incubation with ferricyanide in either neutral or alkaline solution, only ascorbic acid, cysteine (representing naturally occurring thiols), oxidizable phenols, and thiamine showed a reaction when mixed with a neutral solution of ferricyanide after their alkaline degradation (1 M NaOH) for 5 or 30 min. The interfering effect of ascorbic acid and cysteine was reduced when the alkaline incubation preceding the neutral reaction with ferricyanide was performed in the presence of traces of copper. This was due to a catalytic effect on the

Table 3. Analysis of Rapeseed Using the Ferricyanide Method and the Official HPLC and XRF Methods (*n* Is the Number of Repeat Measurements). Sample 1 Was a 'Quality Control' Rapeseed Sample Analyzed in NIAB. Samples 2, 3, and 4 Were Routine Service Rapeseed Samples Analyzed in NIAB

	total glucosinolates/mmol ± s.d. in 1 kg of whole seeds[b]		
sample	$Fe(CN)_6^{3-}$ method	HPLC[a]	XRF[a]
1	20.8 ± 1.21 ($n = 7$)[c]	19.2 ± 2.44 ($n = 28$)	21.9 ± 1.06 ($n = 117$)
2	14.6 ± 0.55 ($n = 7$)[c]		13 ($n = 1$)
3	26.8 ± 1.54 ($n = 7$)[c]		26 ($n = 1$)
4	46.2 ± 1.20 ($n = 7$)[c]	48 ($n = 1$)	

[a] Data from NIAB, Cambridge. [b] s.d. is the standard deviation. [c] Calculated using standard curve (Table 1).

oxidation by oxygen (autoxidation) of these compounds (Dawson et al., 1989). The interference of oxidizable phenolics remained the most important practical problem.

Analysis of Rapeseed. The applicability of the pH switch method with ferricyanide was tested using glucosinolates extracted from rapeseeds. The predominant glucosinolates in these samples were expected to be (Sang et al., 1984) progoitrin (2-hydroxybut-3-enyl-glucosinolate) and hydroxyglucobrassicin (4-hydroxy-3-indolylmethylglucosinolate). Rapeseed varieties also contain high levels of oxidizable phenolics (Simbaya et al., 1995), e.g., soluble tannins, sinapine, phenolic acids, which represent a serious interference in the procedure, competing with 1-thioglucose in reduction of ferricyanide and obscuring the ferricyanide peak at 420 nm by a large background absorbance of the oxidation products. Moreover, the presence of proteins in rapeseed makes the extracts opaque, which complicates further the spectrophotometric analyses. However, the proteins could be removed by precipitation with lead and barium acetates, while the interfering phenolics could be removed simultaneously by adsorption with polyvinylpolypyrrolidone (Murdiati et al., 1991; Makkar et al., 1993). Samples prepared in this way gave absorption spectra, when used in the ferricyanide procedure, that enabled quantitative estimation of the total glucosinolate concentrations, Table 3. Quantification of total glucosinolates in the original samples was similar using either the simple calibration method (eq 2) or the sample spiking procedure (eq 3). It should be noted, however, that the initial step change in ferricyanide concentration (due to glucosinolates) was followed by a slow, continuous reduction. This effect was not observed with model solutions and was ascribed to residual polyphenols that were not removed by precipitation and adsorption since, over time, a characteristic peak at ~500 nm was observed. Repeatable results could be obtained only if the time between mixing with ferricyanide and the subsequent absorbance measurement was short (less than 30 s and constant). There was a linear correlation with standard HPLC and XRF data when this procedure was followed, Table 3.

CONCLUSIONS

The methods described in this paper may serve as the basis for the development of a convenient analytical assay for total glucosinolates in rapeseed and other plant materials. With the caveat that proteins and polyphenols should be removed from samples beforehand, the recommended procedure is the degradation of glucosinolates (1 M NaOH, 30 min) followed by adjustment to neutral pH and subsequent oxidation with ferricyanide (1 mM). The change in absorbance at 420 nm can be attributed to the ferricyanide consumed by reaction with the glucosinolates.

By comparison with other methods of total glucosinolate analysis, the ferricyanide method has several positive attributes. (1) The method of analysis is a technically straightforward, inexpensive procedure which does not require any special equipment. (2) There is no enzymic step, so concern over enzyme activity and purity is eliminated. (3) The method does not involve any long incubation periods (e.g., desulfation of glucosinolates) which makes the analysis much quicker than the official HPLC methods. (4) Unlike the XRF method, the ferricyanide procedure has application in samples other than rapeseed. Furthermore, it does not require extensive calibration using certified seeds. (5) In comparison with glucose release methods, the thymol method, and the palladium method, the ferricyanide procedure is not affected by glucose in the samples, which is of considerable importance when vegetable samples are to be analyzed. (6) In comparison with the ferricyanide procedure reported here, the reagents used in the other quick, simple, nonenzymatic methods (i.e., palladium and thymol methods) are less stable ($PdCl_4$) and more difficult to handle (thymol−H_2SO_4 at 100 °C). (7) The preliminary data presented here suggest that the ferricyanide method is nearly stoichiometric for all glucosinolates (this is not the case for the palladium reaction).

The described methodology is still open to improvement. Nevertheless, it provides an opportunity to analyze quickly the glucosinolate content in rapeseed or other samples using relatively simple procedures and inexpensive equipment. The possibility of adapting the procedures to a thick-film single-use sensor format, with immobilized reagents and electrochemical detection, is being investigated.

ACKNOWLEDGMENT

We thank Dr. Huw Jones from the National Institute of Agricultural Botany for providing rapeseed samples of known total glucosinolate content analyzed by HPLC and XRF methods.

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Received for review June 3, 1999. Revised manuscript received August 25, 1999. Accepted September 2, 1999.

JF9906026