DETERMINATION OF 5-VINYL-2-OXAZOLIDINETHIONE IN MILK

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INTRODUCTION

Rapeseed meal is to a large extent used for feeding dairy cows. Although the enzyme system, myrosinase, which hydrolyses 2-hydroxy-3-butenylglucosinolate and other glucosinolates, is inactivated in commercial rapeseed meal, some of the glucosinolate may be hydrolysed *in vivo* yielding among other things 5-vinyl-2-oxazolidinethione (I). This substance causes goitre in animals (1). Virtanen *et al.* found that cows given feed containing 2-hydroxy-3-butenylglucosinolate excreted about 0.05% of I that was found in the minced feed (2). The content of I in the milk did not exceed 100 µg/l. After studying the influence of I on the uptake of radioactive iodide in the thyroid gland, Vilkki *et al.* (3) concluded that the content of I in the milk from cows fed with a high amount of green *Brassica* plants was not high enough to cause goitre in man. However, Peltola (4) found that a level of I, which might be found in milk, caused goitre in rats with long-term consumption. For this reason it was of interest to analyse the content of I in milk in Sweden.

Kreula and Kiesvaara (5) developed a method for the determination of I in milk. After extraction and clean-up the sample was chromatographed with two-dimensional paper chromatography. The spot of I was eluted and the oxazolidinethione content was determined by spectrophotometric measurements.

After Kreula and Kiesvaara published their method (5) the technique of high pressure liquid chromatography (HPLC) has been rapidly developed. This technique was considered to be more precise and less time-consuming than the one based on paper chromatography and spectrophotometry and was utilized for the development of the present method.

METHOD

Materials

Milk was obtained from retail shops at Uppsala and from the Department of Animal Husbandry, the Agricultural University of Sweden, Alnarp and Öjebyn.

5-vinyl-2-oxazolidinethione was prepared from rapeseed meal. According to spectrophotometric determination the purity was 99%. The compound was dissolved in chloroform to a concentration of 10 µg/ml.

Mobile solvent for HPLC - Chloroform-hexane (2+1), reagent grade.

Liquid chromatograph.-Spectra-Physics Model 3500 B with a 250x3 mm id stainless steel column packed with Spherisorb 5 µm Silica (Spectra-Physics 2905 Stender Way, Santa Clara, CA 95051).

UV detector - Shoeffel Model SF 770 spectroflow monitor (Schoeffel Instrument Corp., 24 Booker St, Westwood, NJ 07675) or SP 8200 (Spectra-Physics).

Extraction

Milk (300 ml) is heated to 85-90°C for ca 5 min and then rapidly cooled. The sample is extracted twice with 500 ml of dichloromethane in a separatory funnel.

Clean-up

The combined dichloromethane solutions are evaporated to dryness. The residue is extracted with 5 ml 1% ammonia solution by gentle shaking and warming in a water bath simultaneously. This procedure is repeated four times in all. The combined ammonia solutions are neutralized with 18% acetic acid to pH 6.5-7.5 (5). Milk fat is removed by extraction with 2x5 ml hexane in a separatory funnel. The water phase is extracted with 4x30 ml of dichloromethane. After evaporation of the combined dichloromethane solutions to dryness the residue is dissolved in 1.0 ml of dichloromethane.

Liquid chromatography

5-vinyl-2-oxazolidinethione is separated and detected with a high pressure liquid chromatograph with a flow rate of 1.2 ml /min. The UV detector is set at a wavelength of 249 or 254 nm. Usually the size of the injected sample has been 10 µl. The I-content has been calculated by comparison of the peak area of the sample with that of an injected standard solution.

RESULTS AND DISCUSSION

Kreula and Kiesvaara (5) found that I very rapidly disappeared from the milk if it was not heated. Even when the milk was analysed immediately after the addition of I heating to +85°C increased recovery.

Kreula and Kiesvaara (5) reported a recovery of about 75%. Using the extraction with ethylacetate and the clean-up according to their method we obtained recoveries of only about 50%. A better recovery was obtained when dichloromethane was utilized for the extraction.

As shown in Table 1 the recovery of I added to milk was 74% on the average. The limit of detection of the method is 1 µg/l. A typical chromatogram of a spiked sample is shown in Fig. 1.

Some milk samples from retail shops or dairies in various parts of Sweden have been analysed for I-content by using the present method. As shown in Table 2, I was found in all the samples. However, the contents were low and it does not seem probable that they may constitute a health problem.

Rapeseed varieties with a low glucosinolate content will be available in a not too distant future. As shown in Table 3 the I-content was very low in milk from cows fed with rapeseed with a low glucosinolate content even when the seed was not heat-treated.

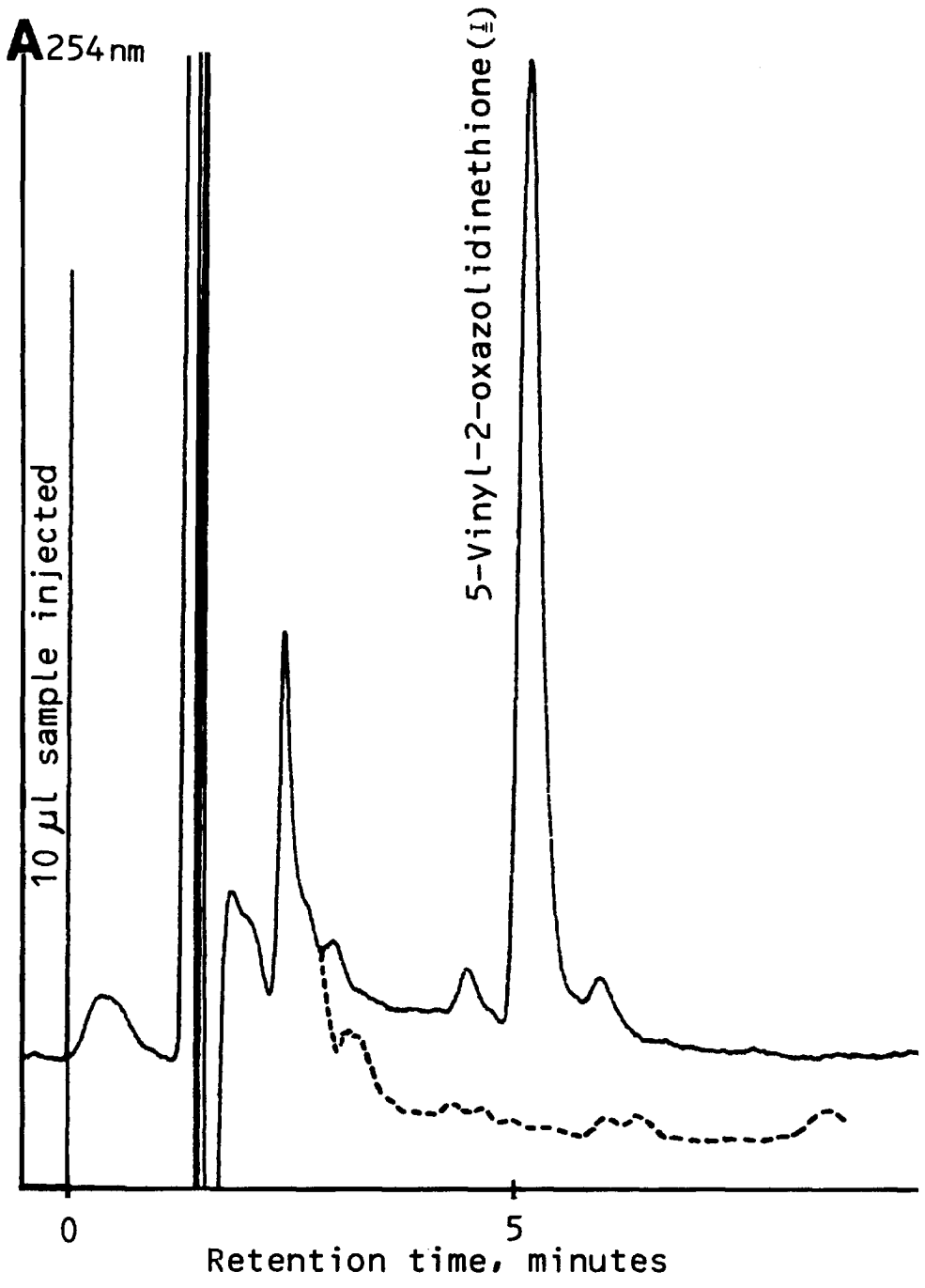

Fig. 1. High pressure liquid chromatogram of a milk sample without I (-----), and chromatogram of a milk sample after addition of 67 µg I/l (———)

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TABLE 1

RECOVERY OF 5-VINYL-2-OXAZOLIDINETHIONE ADDED TO MILK CONTAINING 3% FAT

Addition µg/l	Recovery %
143	82
100	70
67	71
67	70
67	70
67	90
33	70
33	69
M_8	74

TABLE 2

CONTENT OF 5-VINYL-2-OXAZOLIDINTHIONE (I) IN MILK FROM RETAIL SHOPS AND DAIRIES SEPT. 1977 - MARCH 1978

Fat content of milk %	Obtained from	Number of samples	Content of I µg/l	
			range	mean
3	Retail shops	8	7-30	16
0.5	"	6	7-36	24
3	Dairies	6	8-34	24
0.5	"	1	54	54

TABLE 3

CONTENT OF 5-VINYL-2-OXAZOLIDINETHIONE IN MILK FROM COWS FED COMMERCIAL RAPESEED MEAL OR CRUSHED RAPESEED WITH A LOW GLUCOSINOLATE CONTENT, µg/l. THE FEEDING EXPERIMENT WAS PERFORMED BY THE DEPARTMENT OF ANIMAL HUSBANDRY, THE AGRICULTURAL UNIVERSITY OF SWEDEN, ALNARP

Sampling No.	Composition of concentrate mixture		
	Control without rapeseed	With 5.7% commercial rapeseed meal	With 8.4% of crushed rapeseed, low glucosinolate content
1	<1	3	2
2	<1	16	2
3	<1	7	4

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