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Note

High-performance liquid chromatographic method for the determination of 5-vinyl-2-oxazolidinethione in milk

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Rapeseed meal is a valuable source of protein for dairy cows. However, it contains glucosinolates, of which 2-hydroxy-3-butenylglucosinolate is the most important[1]. The glucosinolates are hydrolysed by an enzyme system, myrosinase, which always yields glucose and sulphate. Further, thiocyanates, isothiocyanates, cyano compounds or oxazolidinethiones are formed[2]. Although the myrosinase is inactivated in commercial rapeseed meal, some of the glucosinolates may be hydrolysed *in vivo*[3], in which case 5-vinyl-2-oxazolidinethione (I) may be formed from 2-hydroxy-3-butenylglucosinolate. The oxazolidinethione causes goitre in animals[4]. Virtanen *et al.*[5] found that cows given feed containing 2-hydroxy-3-butenylglucosinolate excreted about 0.05% of the amount of I found in the minced feed. 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.*[6] concluded that the content of I in milk from cows fed large amounts of green *Brassica* plants was not high enough to cause goitre in man. However, Peltola and Krusius[7] found evidence that a daily dose of 100 μg of I given to man decreased the amount of thyroxine in the blood. In view of this, it is of interest to analyse I in milk.

Kreula and Kiesvaara[8] 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 I content determined spectrophotometrically.

McLeod *et al.*[9] showed that in milk I may be detected as its heptafluorobutyl derivative by gas–liquid chromatography using an electron-capture detector, after clean-up by high-performance liquid chromatography (HPLC). However, this seems to be a time-consuming method and experiments with milk were performed only at the very high level of 2 mg/kg of I.

Since Kreula and Kiesvaara[8] published their method, the technique of HPLC has developed rapidly. This technique is regarded as being 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.

EXPERIMENTAL

Materials

Milk from cows that had not been fed on rapeseed meal or *Brassica* plants was obtained from the Department of Animal Husbandry, Swedish University of Agricultural Sciences, Öjebyn, Sweden.

5-Vinyl-2-oxazolidinethione was prepared from rapeseed meal. The purity was 99% as determined by spectrophotometry. The compound was dissolved in chloroform to give a concentration of 10 μg/ml.

Extraction and clean-up

The extraction and clean-up were carried out essentially according to the method of Kreula and Kiesvaara[8], with the exception that dichloromethane was used instead of ethyl acetate for the extraction step. Milk (300 ml) was heated at 85–90° for *ca.* 5 min and then cooled rapidly. The sample was extracted twice with 500 ml of dichloromethane in a separating funnel and the dichloromethane solutions were evaporated to dryness. The residue was extracted with 5 ml of 1% ammonia solution by gentle shaking whilst warming in a water-bath. This procedure was repeated three times. The combined ammonia extracts were adjusted to pH 6.5–7.5 with 18% acetic acid. Fat was removed by extraction with two 5-ml volumes of *n*-hexane in a separating funnel. The aqueous phase was extracted with four 30-ml volumes of dichloromethane. After evaporation to dryness of the combined dichloromethane extracts, the residue was dissolved in 1.0 ml of dichloromethane.

Liquid chromatography

The liquid chromatograph used was a Spectra-Physics Model 3500B equipped with a 250 × 3 mm I.D. stainless-steel column packed with Spherisorb 5-μm silica (Spectra-Physics, Santa Clara, Calif., U.S.A.). I was detected by its UV absorption using a Schoeffel Model SF 770 spectroflow monitor (Schoeffel, Westwood, N.J., U.S.A.) or an SP 8200 (Spectra-Physics). Chloroform–*n*-hexane (2:1) (analytical-reagent grade) was used as the mobile phase with a flow-rate of 1.2 ml/min. The UV detector was set at a wavelength of 249 nm (SF 770) or 254 nm (SP 8200). Usually 10 μl of sample were injected into the chromatograph. The injections were made with a loop valve injector, which provides reproducible injections. Peak areas were measured by multiplying the peak height by the width at half-height. The content of I was calculated by comparing the peak area obtained with the sample with that obtained with standard solutions. The detector response was linear for the range tested (3-100 ng).

RESULTS AND DISCUSSION

Recoveries were studied by adding I dissolved in 100–200 μl of chloroform, which was evaporated before the heat treatment of the milk.

Kreula and Kiesvaara[8] found that I disappeared very rapidly from milk if it was not heated. Even when the milk was analysed immediately after the addition of I, heating to 85° increased the recovery.

TABLE I

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

Amount added (μg/l)	*Recovery (%)*
143	82
100	70
67	71
67	70
67	70
67	90
33	70
33	69
Mean:	74

Kreula and Kiesvaara[8] reported a recovery of about 75%. Using ethyl acetate extraction 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 I, the average recovery of I added to milk was 74%. The limit of detection of the method is 1 μg/l. A typical chromatogram of a spiked sample is shown in Fig. 1.

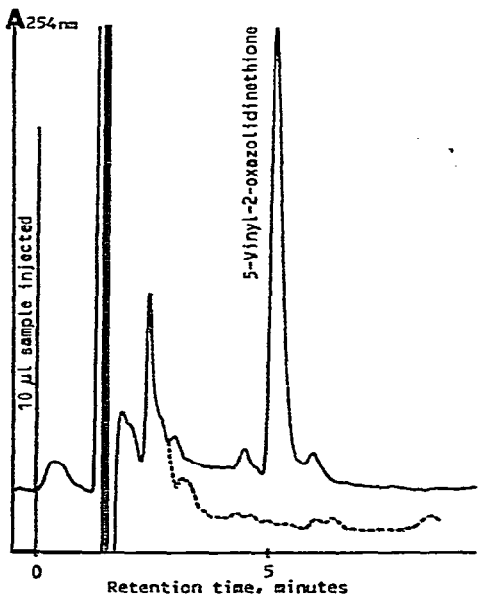

Fig. 1. High-performance liquid chromatograms of milk samples without 5-vinyl-2-oxazolidinethione (broken line) and after the addition of 67 μg/l of 5-vinyl-2-oxazolidinethione (solid line). Column, Spherisorb 5-μm silica; mobile phase, chloroform–*n*-hexane (2:1); flow-rate, 1.2 ml/min; Spectra-Physics SP 8200 UV detector, 0.04 a.u.f.s.

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