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Gas Chromatographic Method for the Determination of Solanidine and Its Application to a Study of Feed–Milk Transfer in the Cow

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An extraction method based on alkaline saponification of milk, partitioned into toluene, and subsequent gas chromatography quantitation has been developed to estimate the glycoalkaloid metabolite solanidine in bovine milk. Spiking studies indicated approximately 93% recovery of added solanidine at levels of 1.12, 0.56, and 0.28 ppm with a detection limit of 0.14 ppm. A study to determine the presence of solanidine in milk from cows consuming diets containing 0, 10, and 20% tater meal showed no detectable amounts of solanidine in any of the milk samples at 60 and 150 days of lactation.

Today processed potatoes represent 57% of the total potato production in the United States and the perentage continues to rise (Thorton and Sieczka, 1980). Considerable waste is generated during processing, and one means of utilizing it is the production of animal feed like tater meal, which is a potato waste product comprised of cull potatoes and process byproducts such as peel, screen, drum, French fry, and filter cake wastes. Studies conducted by Bushway et al. (1980b) and Bushway (1982) have shown that these waste products contain high levels of naturally occurring toxicants, potato glycoalkaloids, which at elevated levels can impart a bitter flavor to tubers and are toxic to man and animals (Sinden and Deahl, 1976; Hansen, 1925; Willimott, 1933; Jadhav and Salunkhe, 1975; Maga, 1980).

In Maine and Canada much of the potato waste is fed to dairy cattle as a feed supplement. A recent investigation by King and McQueen (1981) has demonstrated that rumen microorganisms cleave glycoalkaloids, yielding solanidine, its dihydro analogue 5β-solanidan-3β-ol, and monosaccharides. Although very little research has been performed on the toxicological and physiological properties of solanidine, there is evidence that suggests that it is similar to the glycoalkaloids (α-chaconine and α-solanine) as far as bitterness (Zitnak, 1961). The toxicological data are conflicting. Some studies indicate solanidine is more toxic than the glycoalkaloids (Nair et al., 1981; Zitnak, 1961) while others have demonstrated it is less toxic (Nishie et al., 1971, 1975). Physiological and toxicological properties of the dihydro form of solanidine are unknown.

Because of the extensive use of potato waste (which contains approximately 400 ppm of solanidine in the form of glycoalkaloids) as animal feed for dairy cows, the possible detrimental effects of solanidine, and the large consumption of milk and milk products, an investigation was conducted to develop a gas–liquid chromatographic method to quantify solanidine in whole milk and to determine if a feed ration containing potato byproducts resulted in the passage of solanidine into the milk of lactating dairy cows.

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EXPERIMENTAL SECTION

Reagents. A mixture of α-chaconine and α-solanine extracted from Katahdin potato blossoms using the procedure of Bushway et al. (1980a) was hydrolyzed following the method of Coxon et al. (1979) to obtain solanidine standard. For extractions, certified-grade solvents were used with the exception of methanol, which was purified grade (Fisher Scientific Co., Medford, MA). HPLC-grade solvents were employed for HPLC analysis (Fisher Scientific Co.). Sodium hydroxide, potassium hydroxide, and sodium sulfate were obtained from Fisher Scientific Co. Dragendorff's reagent was purchased from Sigma Chemical Co. (St. Louis, MO). The column packing, 3% OV-17 on 80–100-mesh Chromosorb W HP, was obtained from Supelco, Inc. (Bellefonte, PA).

Gas Chromatography (GC). The GC conditions for the detection of solanidine were those of King (1980) with slight modifications. Equipment included a Perkin-Elmer Signa-2 gas chromatograph with a nitrogen–phosphorus detector (Perkin-Elmer Corp., Norwalk, CT) and a Hewlett-Packard 3390 A reporting integrator (Hewlett-Packard Avondale Division, Avondale, PA).

A 1.2 m × 2 mm i.d. glass column was packed with 3% OV-17 on 80–100-mesh Chromosorb W HP. Helium (25 mL/min) was employed as a carrier gas while hydrogen (1–2 mL/min) and compressed air (600 mL/min) were used for the nitrogen–phosphorus detector. Operating temperatures were as follows: injection port, 285 °C; oven, 265 °C; detector, 290 °C; bead, 550 °C.

Thin-Layer Chromatography (TLC). Separation of solanidine was achieved on 10 × 10 cm, 200-μm thickness, Whatman high-performance thin-layer chromatographic plates (Whatman, Inc., Clifton, NJ). Plates were developed with the lower layer of a mixture of methanol–chloroform–1% ammonium hydroxide (100:100:50 v/v). Solan-

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idine was visualized with Dragendorff's reagent.

High-Performance Liquid Chromatography (HPLC). The HPLC equipment consisted of a Waters Model ALC/GPC 254 liquid chromatograph with a Model 6000A solvent delivery system, a Model U6K injector (Waters Associates, Milford, MA), a Schoeffel variable-wavelength detector (Westwood, NJ), and an Omni-Scribe recorder (Houston Instruments, Austin, TX).

The operating conditions employed were those of Bushway et al. (1979) and included a 25 cm × 4.5 mm 5-μm amino column (IBM Instruments, Inc., Danbury, CT) and a mobile phase of tetrahydrofuran–water–acetonitrile (55:15:30 v/v) pumped at a rate of 1.1 mL/min. Ultraviolet detection was accomplished at 215 nm (0.04 absorbance unit full scale).

Preparation of Solanidine Standard. Stock solutions of 0.28, 0.14, and 0.07 mg/mL were prepared by dissolving crystalline solanidine in methanol.

Preparation of Spiked Solanidine Samples. Homogenized milk was purchased from local retail outlets. Aliquots of 250 mL were spiked with standard solanidine at three different concentrations (1.12, 0.56, and 0.28 ppm) and freeze-dried to remove excess water. Each level of spiking was repeated 6 times. A blank received 1 mL of methanol.

Preparation of Tater Meal Samples. Bovine milk was obtained from the University of Maine Farm (Orono, ME). As a regular part of their dietary regime, the cows consumed feed containing 0%, 10%, and 20% tater meal depending on their stage of lactation. During the time of maximum milk production (31–40 kg of milk/day), cows received a feed ration containing 20% tater meal (4.5 kg of tater meal/day). Milk samples were taken approximately day 60 of lactation. As milk production decreased (13–18 kg of milk/day), the tater meal in the feed ration was reduced to 10% (2.25 kg of tater meal/day). These milk samples were taken approximately day 150 of lactation or 90 days after the high tater meal ration was stopped. No tater meal was given during the drying off period (4–7 kg of milk/day). These samples were used for no tater meal.

Samples containing 1.5 L from each diet treatment were centrifuged to separate the cream. Both milk and cream were stored at −15 °C. For analysis, 8.0 g of cream was added to 250 mL of milk and the mixture was freeze-dried. Each diet treatment was analyzed in duplicate.

Extraction of Solanidine from Milk. The freeze-dried milk solids were refluxed 10 min in a solution of 300 mL of methanol and 18 mL of 60% aqueous potassium hydroxide, then held in a 45°C water bath for 90 min. Sonication was used to aid the transfer of the refluxed mixture to a 1-L separatory funnel, rinsing with a total of 260 mL of distilled water, 150 mL of toluene, and 20 mL of methanol. The separatory funnel was shaken thoroughly and the contents were allowed to settle. The aqueous layer was transferred to a clean separatory funnel and reextracted with 100 mL of toluene. The two toluene fractions were combined, washed with 100 mL of distilled water, filtered through sodium sulfate into a 1-L round-bottom flask, and evaporated to dryness on a rotary evaporator. The residue was transferred with methanol to a 100-mL round-botton flask, evaporated to dryness, and then redissolved in 5 mL for analysis by GC and TLC.

Determination of Solanidine by Gas and Thin-Layer Chromatography. Solanidine standards for GC analysis were prepared by removing 1 mL of each of the three stock solutions (0.28, 0.14, and 0.07 mg/mL) and diluting to 5 mL with toluene. A linear response was observed. Therefore, when quantifying samples, a standard corresponding to the level of the sample was injected followed by the sample and then the standard. All injections were 5 μL.

Table I. Recovery of Solanidine Added to Homogenized Milk

	% recovery		
sample no.	0.28 ppm	0.56 ppm	1.12 ppm
1	94.7	94.3	105.4
2	87.5	101.7	110.2
3	84.7	99.4	95.7
4	112.8	83.7	80.8
5	95.3	91.6	89.3
6	89.5	103.0	
mean	94.5	92.5	96.3
CV, %	10.7	8.1	12.4

For the determinations of solanidine by TLC, plates were spotted with 10 μL of the GC standards and samples described above. Development required about 30 min. Dragendorff's reagent was sprayed to detect the presence of an orange spot on a yellow background.

Extraction of Tater Meal Glycoalkaloids. Three samples of tater meal, which was incorporated in the bovine feed ration and obtained from the University farm, were analyzed for glycoalkaloids. Glycoalkaloids were extracted from the tater meal by the method of Bushway et al. (1983).

Determination of Tater Meal Glycoalkaloids by HPLC. The HPLC was calibrated by injecting 0.208, 0.416, 0.624, and 0.832 μg/5 μL α-chaconine standard and 0.222, 0.444, 0.666, and 0.888 μg/5 μL α-solanine standard. Standards were injected in duplicate. Tater meal samples, 4 μL, were injected in duplicate. Peak height was employed for quantification.

RESULTS AND DISCUSSION

Difficulties were encountered with the development of a method for the extraction of residue levels of solanidine from milk. Because of the chemical structure of solanidine and the amount of fat in milk, it was concluded that a saponification procedure would be needed. A dry column saponification method developed by Maxwell et al. (1981) was first tried. However, recovery was poor 30–40% and the method was very time consuming.

A wet saponification technique with methanol and aqueous potassium hydroxide, followed by extraction with toluene and water, proved successful. Refluxing in the methanolic potassium hydroxide accomplished both the saponification of milk lipids and the digestion of milk solids. Solanidine was readily extracted into toluene due to the nonpolar nature of the aglycon and its low solubility in alkaline medium. In order to avoid troublesome emulsions, it is necessary to use a critical volume of water to ensure separation of the toluene and methanol layers. A water volume of 260 mL was found most suitable.

The GC method for solanidine developed by King (1980) was first employed, but a retention time of 21 min was obtained instead of the reported 9 min. This discrepancy could not be explained. However, by changing the GC column from 1.8 m × 4 mm i.d. to 1.2 m × 2 mm i.d. and decreasing the helium flow rate from 60 to 25 mL/min, we observed a retention time of 7 min for solanidine.

A recovery study was performed (Table I). Percent recoveries for all three spiking levels of solanidine ranged from 80.8 to 110.2% with CV varying from 8.1 to 12.4%. Although there was some variability in percent recoveries from the different concentrations, the CV for each spiking level and the overall average CV was good for a residue

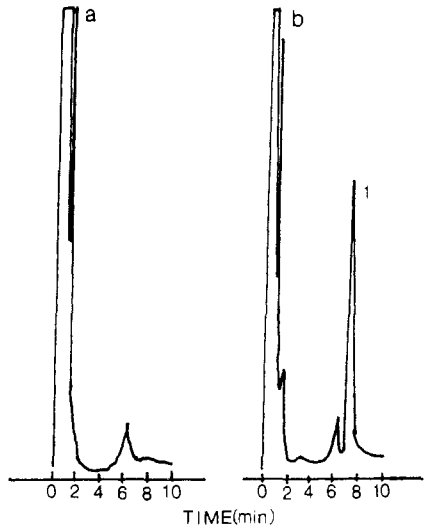

Figure 1. Gas chromatograms of (a) 20% tater meal milk sample and (b) milk spiked with 1.12 ppm of solanidine. Conditions: column, 1.2 m × 0.2 mm i.d., packed with 3% OV-17 on 80–100-mesh Chromosorb W HP; carrier gas, helium, 25 mL/min; hydrogen, 1–2 mL/min; compressed air, 600 mL/min; oven temperature, 265 °C; injector temperature, 285 °C; detector temperature, 290 °C; bead temperature, 550 °C; detector, nitrogen–phosphorus; chart speed, 0.2 cm/min. (1) Solanidine.

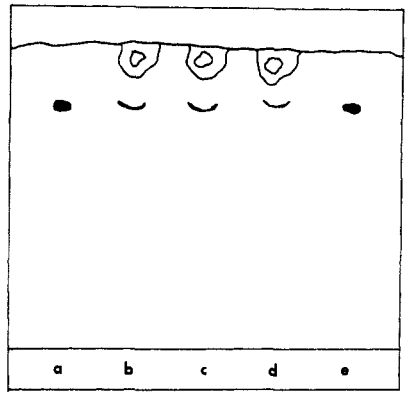

Figure 2. Thin-layer chromatogram of milk spiked at three levels with standard solanidine. (a, e) Standard solanidine; (b) 1.12 ppm of solanidine spike; (c) 0.56 ppm of solanidine spike; (d) 0.28 ppm of solanidine spike.

method. Thus, the results indicate the method of extraction is adequate for obtaining low levels of solanidine from cows milk.

Milk samples obtained from lactating dairy cows fed rations containing 20%, 10% and 0% tater meal were analyzed for solanidine. The alkaloid was not detected in the control or in tater meal milk samples at a detection limit of 0.14 ppm. A chromatogram depicting a typical separation is shown in Figure 1. No solanidine peak appeared in the 20% tater meal milk sample while the spiked sample had the alkaloid peak. The small peak at 6 min appeared in all milk samples including controls.

All GC results were confirmed by thin-layer chromatography. Figure 2 represents a thin-layer chromatogram of the three different spiked (0.28, 0.56, and 1.12 ppm) milk samples, all of which had a spot with the same R_f value as solanidine. No solanidine was observed in the extracts from the tater meal milk samples along with the control.

Even though the dihydro analogue of solanidine was not specifically determined in this study, the extraction, qualitative (TLC), and quantitative (GC) procedures used for solanidine should have been adequate to detect 5β-solanidan-3β-ol. However, there were no indications from the results that any solanidine alkaloids were present at the lower detection limit of 0.14 ppm.

Table II. Determination of Glycoalkaloid Content of Tater Meal by HPLC

	mg/100 g of dried product		
sample[a]	α-chaconine	α-solanine	total glycoalkaloid
1	54.8	28.9	83.7
2	52.7	29.8	82.5
3	47.0	26.9	73.9
mean	51.5 ± 4.0[b]	28.5 ± 1.5[b]	80.0 ± 5.3[b]

[a] Each analysis was done in duplicate. [b] Standard deviation.

Analysis of tater meal for the glycoalkaloids (Bushway et al., 1983) α-chaconine and α-solanine was performed to determine the maximum amount of solanidine one could expect to find in milk if 100% absorption occurred from the rumen. Quantification of α-chaconine and α-solanine should give an accurate value for the amount of solanidine since these two glycoalkaloids comprise 95–99% of the total glycoalkaloids (TGA) in potatoes and both compounds contain solanidine as the aglycon. Furthermore, one would expect to find very little free solanidine in this product. The glycoalkaloid composition of the three samples of tater meal is given in Table II. Average values for α-chaconine, α-solanine, and TGA content of tater meal were 51.5, 28.5, and 80.0 mg/100g, respectively. At this TGA level, cows given a feed ration containing 20% tater meal (4.5 kg/day) comsumed approximately 3.6 g of TGA/day while those on a 10% tater meal feed ration (2.25 kg/day) consumed about 1.8 g of TGA/day. On the basis of these calculations and the fact that approximately 83% of the glycoalkaloids are converted to the alkaloids solanidine (40–50% of this 83%) and the dihydro form by rumen microorganisms, the maximum amount that one could expect to observe in milk would be 20–30 ppm of solanidine if 100% of the dietary solanidine was transferred to the milk. With this much solanidine in the diet, one would expect to find detectable amounts of solanidine in milk if this was a primary elimination pathway in dairy cows.

The results of no detectable solanidine in milk are encouraging but do not entirely eliminate the possibility of alkaloid and/or glycoalkaloid transfer. For example, higher dietary levels of glycoalkaloids might be needed for detectable amounts of solanidine to be absorped into the milk because of poor absorption, or it is possible that glycoalkaloid metabolism in the rumen does not follow the proposed pathway of King and McQueen (1981). Clearly, continued research is needed to determine the fate of glycoalkaloids in dairy cows fed tater meal.

Registry No. Solanidine, 80-78-4.

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Mass Spectral Analysis of Some Naturally Occurring Polymethoxyflavones

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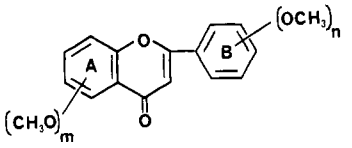
Ten polymethoxyflavones (PMFs) representative of a large class of naturally occurring compounds were prepared for mass spectral analysis. Intense peaks were observed at M^+ and $(M - CH_3)^+$, which could be used to identify and/or quantitate these or related compounds in foodstuffs or biological fluids. Characteristic peaks at m/z 167 and 139 were observed for certain 5,7,8-trimethoxy-PMFs, which may be of diagnostic value for structural recognition.

Flavones 1 containing one or more methoxy groups in



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rings A and B occur throughout nature (Venkataraman, 1975) and most frequently in a wide variety of citrus species (Horowitz and Gentili, 1977). The prominence of these compounds in citrus has resulted in several reports describing their separation and quantitation by high-performance liquid chromatography (HPLC) (Bianchini and Gaydou, 1983; Ting et al., 1979) and by HPLC coupled with combined UV and fluorescence detection (Rouseff and Ting, 1979). The analytical profile of polymethoxyflavones (PMFs) is sufficiently characteristic to allow the determination of one type of citrus juice in the presence of another (Ting et al., 1979).

The biochemical role of PMFs in plants is largely unknown; but in the case of nobiletin, the material appeared to function as a natural fungicide (Ben-Aziz, 1967). Citrus PMFs also produce physiological activities in animals (Robbins, 1980), namely, by reducing blood viscosity (Robbins, 1976) and by their ability to indirectly detoxify certain polycyclic carcinogens (Wattenberg et al., 1968). The reported biological effects suggested that dietary flavones may play an important role toward alleviating cancer and/or degenerative diseases in man.

In this report we describe the preparation and mass spectral (MS) analysis of 10 representative PMFs, several of which occur naturally in citrus and other plants. The purpose of the work was to provide more detailed MS data for future identification and/or quantitation of PMFs and to test the generality of the currently accepted MS fragmentation mechanism of flavones, summarized earlier by Kingston (1971).

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45247.

Table I. Physical Properties of 2'-Hydroxychalcones

compound[a]	methoxy substituents	% yield	mp, °C	lit. mp, °C	TLC R_f
2a	3,3',4,4',5',6'	100	oil	116-117[b]	0.41[f]
2b	3,3',4,4'5,6'	34	170.5-172	169-170[c]	0.17
2c	2,3',4,4',6,6'	83	152.5-153.5	154[d]	0.14
2d	2,3,3',4,4',6'	68	164-166		0.21
2f	2,3,4,4',6'	34	134-135.5		0.39
2g	3,3',4,4',6'	84	141-142.5	146-148[c]	0.13
2h	3,4,4',6'	49	154-156	151[e]	0.33
2i	3',4,4',6'	89	138-140	144-145[c]	0.26

[a] Letters correspond to chalcones that were converted to flavones, 3a-i. Chalcone 2e corresponding to tangeretin was not synthesized. [b] Sastry and Row (1961). [c] Sherif et al. (1981). [d] Cummins et al. (1963). [e] Reidel et al. (1942). [f] Silica gel G plates; solvent was methyl *tert*-butyl ether.

Table II. Physical Properties of Polymethoxyflavones

structure no.	methoxy substituents	% yield	mp, °C	lit. mp, °C	TLC R_f
3a	5,6,7,8,3',4'	47	135-136	134[a]	0.78[e]
3b	5,7,8,3',4',5'	79	184.5-186.5	–[b]	0.82
3c	5,7,8,2',4',6'	32	246-247	–	0.82
3d	5,7,8,2',3',4'	46	178.5-181.5	180-182[c]	0.80
3e	5,6,7,8,4'		147-149.5	154[a]	0.82
3f	5,7,2',3',4'	32	156-159.5	–	0.84
3g	5,7,8,3',4'	51	194-195	197-198[a]	0.72
3h	5,7,3',4'	50	191-193	190-191[d]	0.84
3i	5,7,8,4'	65	206-210	211-213[a]	0.67
3j	7,3',4',5'	38	190-192	190-191[d]	0.87

[a] Horowitz and Gentili (1977). [b] mp not cited (DeSilva et al., 1980). [c] Govindachari et al. (1968). [d] Banerji and Goomer (1980). [e] Silica gel G plates; solvent was chloroform-methanol-water, 65:25:4.

EXPERIMENTAL SECTION

Materials. Tangeretin was purchased from the George F. Uhe Co. and recrystallized from ethanol before use; 3,4,5-trimethoxybenzoyl chloride, phloroacetophenone,

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