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High-performance liquid chromatographic determination of glycoalkaloids in potato products

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SUMMARY

A method for the determination of α-solanine and α-chaconine, two major potato glycoalkaloids, in commercial potato products by high-performance liquid chromatography (HPLC) was developed. The glycoalkaloids were extracted with methanol and then purified using Sep-Pak C_{18} or Sep-Pak NH_2 cartridges. A Nucleosil 5-NH_2 column was employed for HPLC with acetonitrile–20 m*M* potassium dihydrogenphosphate (75:25, v/v) as the mobile phase. The calibration graph was linear in the range 1–50 μ g/ml for both α-solanine and α-chaconine. The average recoveries were 82.4–92.6% for α-solanine and 86.5–97.4% for α-chaconine added to various commercial potato products at a level of 5 mg per 100 g. The proposed method is applicable to all commercially available potato products and potato starch.

INTRODUCTION

The two major glycoalkaloids present in potato tubers are the steroidal triglycosides α-solanine and α-chaconine, which possess the same aglycone, solanidine, but differ in their sugar moiety. They account for more than 95% of the total glycoalkaloid content found in the potato tubers[1,2]. They are haemolitically active like saponin, fungitoxic[3] and highly toxic to humans. The toxicity of potato glycoalkaloids (PGAs) generally involves gastrointestinal disturbances and neurological disorders[4,5]. Sprouted cull and green potatoes which contain over 20 mg of total PGAs per 100 g are considerd toxic to humans[6]. A few cases of PGA intoxication have occurred and have been reported[7].

Although increasing numbers of commercial potato products, such as potato chips, fried potatoes and croquettes, are now on the market, very little information for evaluating their quality is available. Therefore, there was a need to develop a simple method for determining their PGA content in order to control the quality and evaluate the safety of commercial potato products.

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A number of methods, such as spectrophotometry[8], titrimetry[9], thin-layer chromatography[10], gas chromatography[11], high-performance liquid chromatography (HPLC)[12–14] and enzyme-linked immunosorbent assay (ELISA)[15], have been used for the determination of individual or total PGAs in potato tissues. HPLC is now becoming the most widely used method because it is rapid, accurate and reproducible and can be used to determine both individual and total PGAs. However, most studies have involved the detection of PGAs in fresh potato tissues and not in commercial products, except for Bushway and Ponnampalam's LC method[12]. Their technique, although of great value, included an ammonium precipitation clean-up step that was complicated and very time consuming.

This paper proposes a more sophisticated HPLC method that is simple and precise for the determination of PGAs in commercial potato products using combined Sep-Pak C_{18} and NH_2 cartridges for the sample preparation procedure.

EXPERIMENTAL

Chemicals and reagents

A glycoalkaloid stock standard solution (1 mg/ml) was prepared by dissolving 10 mg of α-solanine and α-chaconine (Sigma, St. Louis, MO, U.S.A.) in 10 ml of methanol. Working standarad solutions were prepared by dilution with methanol.

The HPLC mobile phase was prepared by mixing acetonitrile and 20 m*M* potassium dihydrogenphosphate (KH_2PO_4) buffer solution (75:25, v/v).

The clean-up columns were Sep-Pak C_{18} and Sep-Pak NH_2 cartridges (Millipore, Milford, MA, U.S.A.). The Sep-Pak C_{18} cartridge was preconditioned by elution with 10 ml of methanol and then 10 ml of water prior to use, and the Sep-Pak NH_2 cartridge was conditioned with 10 ml of methanol and then 10 ml of acetonitrile.

Acetonitrile and methanol were of HPLC grade (Kanto Kagaku, Tokyo, Japan). Water was glass distilled and deionized. All other chemicals were of analytical-reagent grade and used without further purification.

Apparatus

HPLC was carried out using a Shimadzu LC-6A system (Shimadzu, Kyoto, Japan) equipped with a Model SPD-6A spectrophotometer. The analytical column was Nucleosil 5-NH_2 (250 mm × 4.6 mm I.D.) (Machery, Nagel & Co., Düren, F.R.G.). The mobile phase was run isocratically at 30°C at a flow-rate of 0.7 ml/min. The detection wavelength and the sensitivity were set at 208 nm and at 0.04 a.u.f.s., respectively.

Sample preparation

Products with low lipid levels (potato tubers, mashed potato and potato starch). A 5-g sample was homogenized with 30 ml of methanol for 2 min, followed by filtration through a suction filter containing a Toyo Roshi No. 4 filter-paper. The residue was rinsed with *ca.* 10 ml of methanol and the rinsings were combind with the original filtrate. The filtrate was brought to a final volume of 50 ml with methanol. A 5-ml aliquot of the extract (when potato starch was treated, 50 ml of the extract were concentrated to *ca.* 5 ml using a rotary evaporator) was mixed with 8 ml of water. The mixture was applied to the Sep-Pak C_{18} cartridge, which was then washed with 5 ml of

40% methanol, and was subsequently eluted with 15 ml of methanol. The eluate was evaporated to dryness *in vacuo*, and the residue formed was dissolved in 1 ml of methanol. An aliquot (20 μl) of the solution was injected into the HPLC system.

Products with high lipid levels (potato chips, fried potatoes, croquettes and potato salad). A 5-g sample was treated in a manner similar to that already described. The final 1 ml of methanol solution obtained was mixed with 19 ml of acetonitrile. The mixture was applied to the Sep-Pak NH_2 cartridge, which was then washed with 5 ml of acetonitrile, and was subsequently eluted with 10 ml of methanol. The eluate was evaporated to dryness *in vacuo* and the residue was dissolved in 1 ml of methanol. An aliquot (20 μl) of the solution was injected into the HPLC system.

Calibration graph

A 20-μl volume of the PGA standard solution (1–50 μg/ml each of α-solanine and α-chaconine) was injected into the HPLC system. A calibration graph was prepared by measuring the peak heights of α-solanine and α-chaconine.

RESULTS AND DISCUSSION

Chromatographic conditions

As for the separation of PGAs by HPLC, the use of a reversed-phase column was not appropriate, because α-solanine and α-chaconine have the same aglycone, solanidine, in their structures. Although Carman *et al.*[14] separated PGAs by using an ion-pair technique with a C_{18} column, the resolution was poor.

On the other hand, Bushway and co-workers[12,13] employed an NH_2 column with a mixture of tetrahydrofuran, acetonitrile and phosphate buffer as the mobile phase. However, according to the method described by Carman *et al.*[14], because of the lack of inhibitors in HPLC-grade tetrahydrofuran, the formation of decomposition products such as peroxides caused the background absorbance to become so high that the solvent mixture was not usable.

Therefore, a mixture of acetonitrile and phosphate buffer without tetrahydrofuran was employed for the resolution of PGAs with a Nucleosil 5-NH_2 column. The parameters examined, which were varied in order to effect the required separation, were the concentration of the KH_2PO_4 and water in the mobile phase, its pH and the column temperature.

Fig. 1A shows the effect of the water concentration in the mobile phase on the capacity factor (k'). The k' values of both α-solanine and α-chaconine decreased with increase in the concentration of water (22–30%). This phenomenon suggested that the PGAs were chromatographed in the normal-phase mode. A 25% water concentration, approximately the middle of the range examined, was chosen for subsequent work.

The effect of the KH_2PO_4 concentration in the mobile phase on the k' values is shown in Fig. 1B, and revealed a convex shape for α-solanine and α-chaconine over the range 5–50 m*M* KH_2PO_4. Relatively constant k' values were obtained in the range 20–30 m*M*. A symmetrical shape peak and a good baseline separation of the PGAs were also observed in this range.

The k' values of the PGAs showed a dependence on the pH of the mobile phase, as shown in Fig. 1C. A good baseline separation was achieved in the pH range 2–7. As the pH of the acetonitrile–20 m*M* KH_2PO_4 (75:25, v/v) mobile phase was *ca.* 6, no additional pH adjustment was employed.

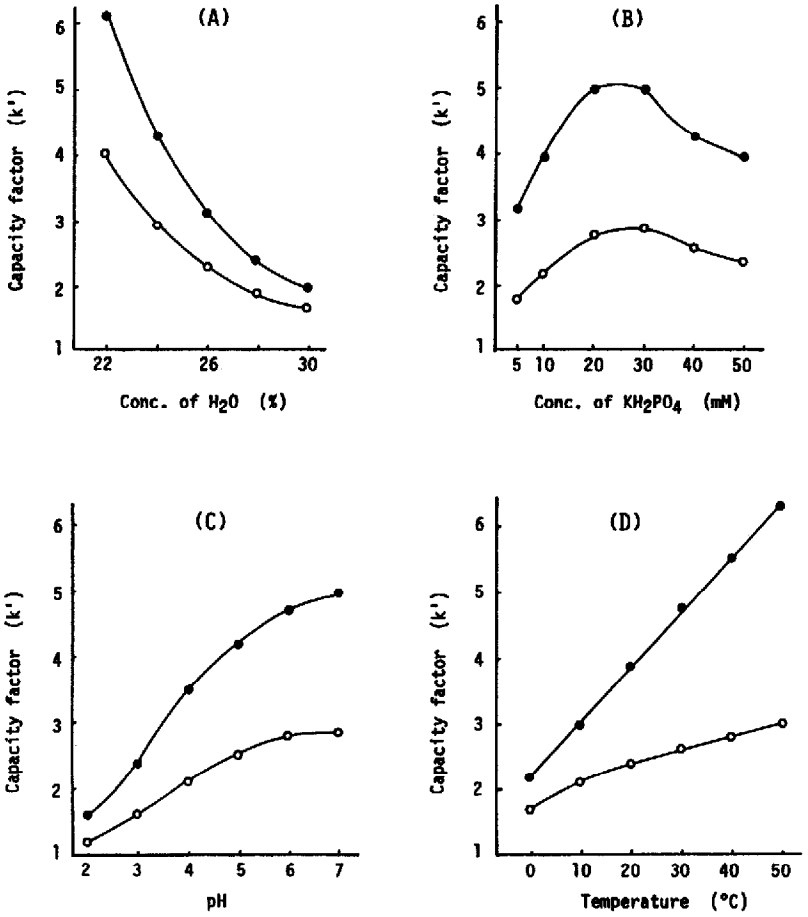

Fig. 1. Effect of (A) water concentration, (B) KH_2PO_4 concentration, (C) pH of the mobile phase and (D) column temperature on capacity factor of (●) α-solanine and (○) α-chaconine.

Preliminary experiments revealed that the column temperature affected the reproducibility of the retention times of the PGAs and the stability of the baseline. Therefore, the effect of the column temperature in the range 0–50°C on the k' values were examined. The dependence on column temperature is illustrated in Fig. 1D. The k' values, especially for α-solanine, increased with increase in temperature. Above 30°C, the elution of α-solanine was markedly slower than that of α-chaconine. Therefore, the column temperature was maintained at 30 ± 0.5°C in subsequent work.

The calibration graphs of peak height *versus* alkaloid concentration were linear in the range 1.0–50.0 μg/ml for both α-solanine and α-chaconine. The detection limit was 20 ng (signal-to-noise ratio = 3) for each alkaloid at a sensitivity of 0.04 a.u.f.s.

Clean-up

Carman *et al.*[14] used a Sep-Pak C_{18} cartridge for the sample preparation procedure. The Sep-Pak C_{18} cartridge was applied to potato tubers[16] and to the

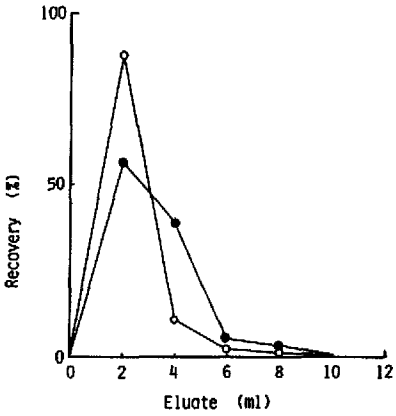

Fig. 2. Elution pattern of (●) α-solanine and (○) α-chaconine from the Sep-Pak NH_2 cartridge. Amounts of 200 μg of α-solanine and α-chaconine were applied to Sep-Pak NH_2 cartridge. Eluent: methanol.

various potato products, such as potato chips and fried potatoes. However, the clean-up of the potato products was insufficient because of their high lipid contents. The HPLC traces showed interferences from impurities, which may lead to a decrease in the ability of the HPLC column to separate the compounds of interest.

Attempts were made to clean up samples using Sep-Pak NH_2 cartridge, which revealed an interaction in the normal-phase mode similar to that with the Nucleosil 5-NH_2 column used for the HPLC analysis. When a methanolic solution of the PGAs was placed on the Sep-Pak NH_2 cartridge, the PGAs were not retained because of the high polarity of the solvent. Addition of acetonitrile to the methanolic solution of the PGAs in order to lower the methanol concentration below 10% resulted in good retention of the PGAs. Fig. 2 shows the elution patterns of α-solanine and α-chaconine

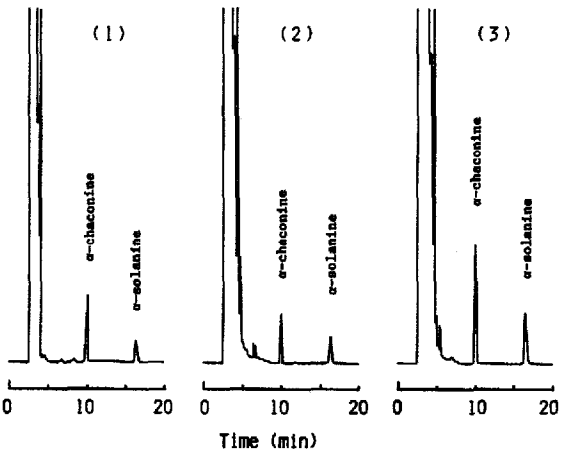

Fig. 3. Typical chromatograms of α-solanine and α-chaconine from various potato products. Volumes of 20 μl were injected into the chromatograph. (1) Potato starch (3.0 μg/ml of α-solanine, 6.0 μg/ml of α-chaconine); (2) mashed potato (4.3 μg/ml of α-solanine, 5.3 μg/ml of α-chaconine); (3) potato chips (7.4 μg/ml of α-solanine, 13.1 μg/ml of α-chaconine).

TABLE I

RECOVERIES OF α-SOLANINE AND α-CHACONINE ADDED TO VARIOUS POTATO PRODUCTS

Sample[a]	*Recovery (%)*[b]	
	α-Solanine	*α-Chaconine*
Potato tuber	90.8 ± 3.5	93.8 ± 3.6
Potato starch	91.9 ± 3.5	97.4 ± 7.7
Mashed potatoes	89.1 ± 3.1	94.2 ± 2.0
Potato chips	92.6 ± 9.1	90.0 ± 8.3
Fried potatoes	82.7 ± 3.2	86.5 ± 5.2
Croquettes	89.2 ± 3.0	95.0 ± 2.2
Potato salad	82.4 ± 1.8	90.0 ± 2.1

[a] α-Solanine and α-chaconine were added to each sample at the level of 5 mg per 100 g.
[b] Mean ± S.D. (n = 5).

from the Sep-Pak NH_2 cartridge with methanol as the eluent. All of the α-solanine and α-chaconine were found in the first 10 ml of the eluate. Typical chromatograms are shown in Fig. 3 for potato starch, mashed potatoes and potato chips after removal of impurities.

TABLE II

CONTENTS OF α-SOLANINE AND α-CHACONINE IN VARIOUS POTATO PRODUCTS

Sample	*Content (mg per 100 g)*		
	α-Solanine	*α-Chaconine*	*Total PGAs*[a]
Potato starch A	0.12	0.23	0.35
Potato starch B	0.06	0.12	0.18
Potato starch C	0.03	0.08	0.11
Sweet potato starch	ND[b]	ND	ND
Wheat starch	ND	ND	ND
Corn starch	ND	ND	ND
Arrowroot starch	ND	ND	ND
Potato chips D	1.5	2.6	4.1
Potato chips E	0.6	1.1	1.7
Potato chips F	1.6	3.8	5.4
Potato chips G	1.3	2.4	3.7
Potato chips H	0.5	0.6	1.1
Potato chips I	0.5	0.9	1.4
Fried potatoes	1.7	1.6	3.3
Frozen fried potatoes	1.5	1.6	3.1
Croquettes	0.9	1.5	2.4
Frozen croquettes	2.7	4.0	6.7
Mashed potatoes	0.9	1.1	2.0
Potato tuber J	1.9	2.1	4.0
Potato tuber K	0.9	1.3	2.2
Potato tuber L	0.7	1.0	1.7

[a] Total PGAs = α-solanine + α-chaconine.
[b] ND = not detected.

Recovery study and analysis of commercial samples

Various commercial potato products fortified at a level of 5 mg per 100 g each of α-solanine and α-chaconine were used for the recovery study. Table I demonstrates that the mean recoveries were 82.4–92.6% for α-solanine and 86.5–97.4% for α-chaconine. The detection limits of both PGAs by the proposed method were 0.02 mg per 100 g for potato starch and 0.2 mg per 100 g for other potato products.

Table II shows that the total PGAs found in all commercial potato products and three brands of potato starch varied from 0.11 to 6.7 mg per 100 g, all below the critical level of 20 mg per 100 g which is used as the upper limit when screening new potato varieties for human consumption. On the other hand, PGAs were found not to be present in other kinds of starches such as wheat, sweet potato, corn and arrowroot. The reason why PGAs were found only in the potato starch is thought to be due to their imperfect purification during production of the starch from potato tubers. Therefore, it is considered that the PGAs were contaminants in almost all potato starches commercially available as foodstuffs.

CONCLUSIONS

The clean-up using Sep-Pak C_{18} and NH_2 cartridges gave excellent recovery, sensitivity and reproducibility. The chromatograms were significantly cleaner than those obtained by other HPLC methods. The determination of α-solanine and α-chaconine can be evaluated in terms of quality control and safety assessment of various potato products. Further, the proposed method can be applied as a confirmation method for a particular potato starch among the various kinds of starches by monitoring the PGAs.

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