HPLC-DAD METHOD FOR ANALYSIS OF GLYCOALKALOIDS ALPHA-SOLANINE AND ALPHA CHACONINE IN CONVENTIONAL POTATO

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Abstract

A simple and fast HPLC-DAD method has been developed for separation and quantification of two glycoalkaloids, alpha-solanine and alpha-chaconine, in potatoes. Separation of the analytes was performed on a Shimadzu Shim-pack GIST C18 column (250mm x 4mm I.D., 5µm particle size) applying isocratic elution of the mobile phase consisting of 30% (v/v) acetonitrile and 70 % KH_2PO_4 (20 mM and pH 6.57). The flow rate on the mobile phase was 1 mL/min and the detection of solanine and chaconine was performed on a wavelength of 204 nm. The retention time of α-solanine was 12.8 min and for α-chaconine 14.6 min. Liquid-liquid extraction of analytes was performed from dehydrated potatoes using methanol-acetic acid (95:5, v/v), followed by SPE with Sep-Pak C18 columns. Satisfactory results for the repeatability and accuracy of the method confirmed that the extraction method as well as HPLC method are appropriate for analysis of alpha-solanine and alpha-chaconine in potatoes samples.

Keywords: *alpha-solanine,alpha-chaconine, potatoes. HPLC-DAD*

1. INTRODUCTION

Potato is the fourth most important crop worldwide after maize, wheat and rice and is rich source of proteins, carbohydrates, vitamins, minerals and phytonutrients. However, potatoes are also known to contain nitrogenous steroidal glycosides, called glycoalkaloids. Due to the toxicity and bitter taste conferred by glycoalkaloids, they are unwanted potato constituents for consumers. In potato tubers α-solanine and α-chaconine account for 95% of glycoalkaloids, which are generally referred as total glycoalkaloids (Sorensen et al., 2008).

These alkaloids consist of a non-polar lipophilic steroid nucleus that is extended by two fused nitrogen-containing heterocyclic rings at one end and bound to a polar water-soluble trisaccharide at the other end. Both solanine as well as chaconine share the same aglycone, namely solanidine, but differ in their carbohydrate component. Small amounts of aglycone and partial glycosides are normally present in potato (Smith et al., 1996).

Naturally occurring in potato tubers, peels, sprouts and blossoms, potato glycoalkaloids when found in tubers at high concentrations are reported to cause poisoning and in a few instances death. Basically, glycoalkaloids are stress metabolites, arising in tubers, in response to excessive light, wounding, premature harvesting and other adverse conditions (Sinden and Webb, 1974).

They are thought to function in the chemical defense of the plant, as non-specific protectors or repellents against potential pests and predators (Roddick, 1989).

Glycoalkaloids are present throughout the potato plant with the highest levels observed in those parts of plant with high metabolic rates (Van Gelder, 1990). Tubers have much lower glycoalkaloids content with higher levels found in the periderm and cortex, decreasing markedly towards the pith but the distribution is not uniform (Dale et al., 1998).

Glycoalkaloids content of the foliage is also an important factor in protecting plants from fungi, insects and predators (Uppal, 1987).

In humans, glycoalkaloid poisoning elicits a wide variety of symptoms ranging from gastrointestinal disorders, through confusions, hallucination and partial paralysis to convulsions, coma and death.

Chaconine and solanine are equally potent inhibitors of acetylcholinesterase responsible for hydrolysing the neurotransmitter acetylcholine, a key process in nerve impulse conduction across cholinergic synapse thus resulting in neurological symptoms. Ability of glycoalkaloids to disrupt sterol containing membranes is responsible for damaging cells in the gastrointestinal tract and also in other tissues or organs into which glycoalkaloids are transported following absorption. Keeping these in view, new potato varieties are checked for glycoalkaloids levels since there is a guideline stating that new potato varieties should not contain more than 20 mg of glycoalkaloids per 100 g of fresh weight. However, some researchers feel that the safe upper limit is much lower i.e., 6 mg/100mg (Morris and Lee, 1984).

Additionally, potato tubers typically contain about 4 to 12mg of GAs per 100g in total weight of fresh potato and 20 to 60mg of GAs per 100g of total weight of freeze or dried potato (Sotelo and Serrano, 2000). However, in case of increase GAs more than the normal value (4 to 12mg fresh potato and 20 to 60mg for freeze potato) could be considered as a toxic and gives a bitter taste. The toxicity GAs can cause diarrhea, vomiting, nausea, stomach and abdominal pains, fever, headache, rapid and weak pulse, hurried breathing, delirium, hallucination and in extreme cases, coma (Mondy and Seetharaman 1990; Friedman and McDonald, 1997).

Generally, the concentration of GAs accumulates to high levels in response to a number of factors, like exposure to light, mechanical injury, poor conditions of growth, fungal attack, environmental factors and storage conditions (Edwards et al.,1996; Dao and Friedman, 1994; Machado et al., 2007).

Furthermore, α-chaconine and α-solanine molecules are heat stable and the structure of these chemicals do not change by frying, home cooking, baking and microwaving, thus, they are fairly heat-stable with melting points in the range of 190-285^{0}C (Tajner-Czopek et al., 2012; Lachman et al., 2013).

For these reasons, the concentration of GAs is critical and should be monitor before consumption of potatoes by human (Bushway et al., 1981).

However, in pharmaceutical industry, α-solanine and α-chaconine are suitable for utilization. The aglycone-solanidine is a precursor for the synthesis of hormones like progesterone, cortisone and testosterone (Nikolic & Stankovic, 2003).

According to the literature, concentration of glycoalkaloids are typically 3 to 10 times greater in the potato peel than flesh. Variation in potato glycoalkaloid quantity is dependent on crop variety, geographic location of crop growth, post-harvest storage conditions and type of fertilizer applied to soil (Gosselin and Mondy, 1989; Mondy and Munshi, 1990).

Various methods have been developed for the determination of GAs, such as ultraviolet–visible (UV–vis) spectrophotometry (Li, Long, Wan, Ding, Zhang, & Wan, 2015), high-performance thin layer chromatography (HPTLC) (Mäder, Rawel, & Kroh, 2009), immunoassays (Driedger, LeBlanc, LeBlanc, & Sporns, 2000), high-performance liquid chromatography coupled with ultraviolet detector (HPLC-UV) (Backleh et al., 2004), liquid chromatography coupled with mass spectrometry (LC-MS) (Nie, & Guo, 2017) and matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Ha et al., 2012). Among these methods, HPLC and HPLC-MS were the most commonly used, whereas their accuracy and sensitivity were often severely influenced by the coexisting complicated sample matrices. To reduce the interference of complicated matrices, an efficient sample pretreatment method is needed for selective extraction and isolation of the target GAs prior to the instrumental analysis.

Some *in vitro* studies indicate certain beneficial effects for instance anti-allergic, anti-diabetic, antipyretic, antibiotic properties and anti-inflammatory (Friedman, 2006; Kenny et al., 2013).

GAs has some inhibitory effects similar to natural pesticide and insecticide, as well as GAs can be used against fungi, bacteria and insect pests (Jadhav et al., 1991; Boulogne et al., 2012).

Moreover, α-chaconine and α-solanine, both are functioning as antifungal activities (Fewell and Roddick, 1997). As indicated by these beneficial and harmful properties of GAs, it is necessary for enforcing the regulation of GAs in potatoes and their products.

In a general manner, alkaloids extraction is carried out by extracting them using non-polar solvents followed by polar solvents, and finally purified and separated using chromatographic methods such as TLC (thin layer chromatography) combined to column chromatography. The separation and purification could also be achieved by using the latest separation and purification techniques such HPLC (preparative high-performances liquid chromatography), and HPTLC (high-performance thin-layer chromatography) (Huie, 2002; Kaufmann and Christen; 2002).

Different methods have been described to extract glycoalkaloids from potato. The most used methods consist of polar solvent, usually methanol or ethanol (Maldonado *et al*., 2014), acidic solvent, usually acetic acid, tricholo-acetic acid (TCA) or sulfuric acid (Matsuda *et al*., 2004), or combined alcohol-acidic solutions (Nikolic and Stankovic, 2003; Distl *et al*., 2009). However, the recovery yield of the extracted glycoalkaloids from potato samples varied widely for the major PGAs, α-solanine and α-chaconine. The different solvents used have shown that acetic acid is the most appropriate for dried samples (Speroni and Pell, 1980; Bushway *et al*., 1985) compared to nonaqueous ones, while acidic solutions in alcohols have shown to have pool recovery yield of α-solanine (Friedman *et al*., 1997a)

Therefore, the main objective of this study was to develop a rapid, sensitive and accurate method for determination of α-solanine and α-chaconine (Fig.1) in the flesh and skin of conventional, Solanum tuberosum potatoes.

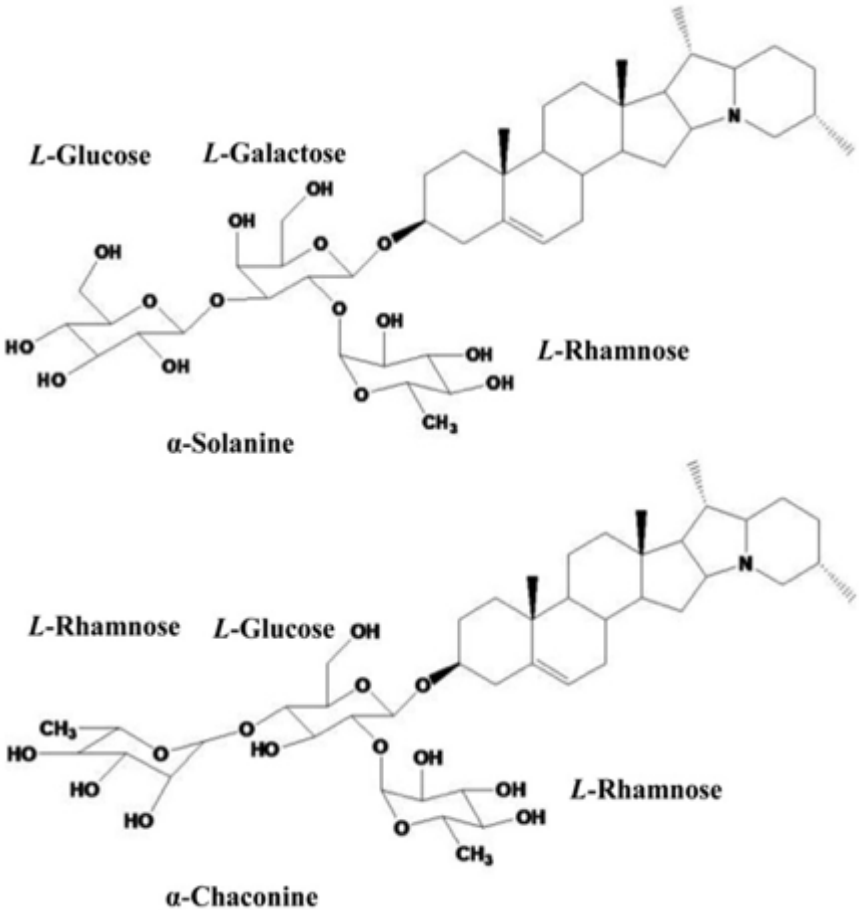

Fig. 1. Chemical structures of α-solanine and α-chaconine

Source: Wang, Huiying & Liu, Mingyue & Hu, Xinxi & Li, Mei & Xiong, Xingyao. (2013). Electrochemical Determination of Glycoalkaloid

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Standards of α-solanine purity (HPLC) ≥98% and α-chaconine purity (HPLC) ≥98% were bought from Extrasynthesis – France. All other chemicals used in this study were as follows: methanol (HPLC grade), potassium dihydrogen phosphate (≥99%), acetic acid (≥99%), acetonitrile (gradient grade ≥99.9%). Sep-Pak C18 cartridges (silica-based octadecyl bonded phase with strong hydrophobicity, for solid-phase extraction (Waters).

2.2. Preparation of Standards

Stock solutions of α-solanine (3,8 mg/mL) and α-chaconine (2,8 mg/mL) were prepared in methanol-acetic acid (99+1, v/v).

2.3. Sample preparation

Fresh potatoes bought from local market were washed, sliced and then dried at 35-40^{0}C in an air-circulation drying oven for 48h. The water loss was assessed at 70-80%. Dried sliced unpeeled tubers were then ground. After dried potatoes were ground, 10g was weighed into a glass and shaken with 40 mL extraction solution (methanol acid and acetic acid, 95:5, v/v) for 15 min on magnetic stir. After stirring, samples were put for 15 min on ultrasonic bath and after that 10 min on centrifugation at 8000 rpt. After centrifugation the supernatant was collected and cleaned through SPE column (Waters Sep-Pak C18, 500mg) which were conditioned with 10 mL acetonitrile, 10 mL extraction solution (methanol acid and acetic acid , 95:5, v/v). Then, a volume of 10 mL sample extract was passed through the column, washed with 10mL 15% acetonitrile, eluted with 5mL mobile phase (acetonitrile and KH_2PO_4, 30:70, v/v) and the volume adjusted to 10 mL with mobile phase.

2.4. HPLC analysis

The instrumentation used for identification of glycoalkaloids in potato samples was an Shimadzu HPLC Nexera XR system (model CBM-20A) consisted of a binary pump (LC-20ADXR), degassing unit (DGU-20A5R), autosampler (SIL-20ACXR), column oven (CTO-20 AC), di ode array detector (SPD-M20A), and communication bus module (CB-20A) (Shimadzu, Oregon, USA). The data processing was performed on the Shimadzu LabSolutions soft ware (version 5.86). Separation of the analytes was performed with isocratic elution at room temperature on a Shimadzu Shim-pack GIST C18 column (250 mm×4 mm I.D., 5 μm particle size). The mobile phase was 30 % (*v/v*) acetonitrile and 70 % KH_2PO_4 with concentration of 20 mM and pH 6.57. Detection of solanine and chaconine was performed on a wavelength of 204 nm at a flow rate of 1 mL/min.

3. RESULTS

Figure 2 illustrates chromatogram of standards of the analysed glycoalkaloids α-solanine and α-chaconine**.** Separation of α-chaconine and α-solanine peaks was with mobile phase consisted of 30 % (*v/v*) acetonitrile and 70 % KH_2PO_4 with concentration of 20 mM and pH 6.57.

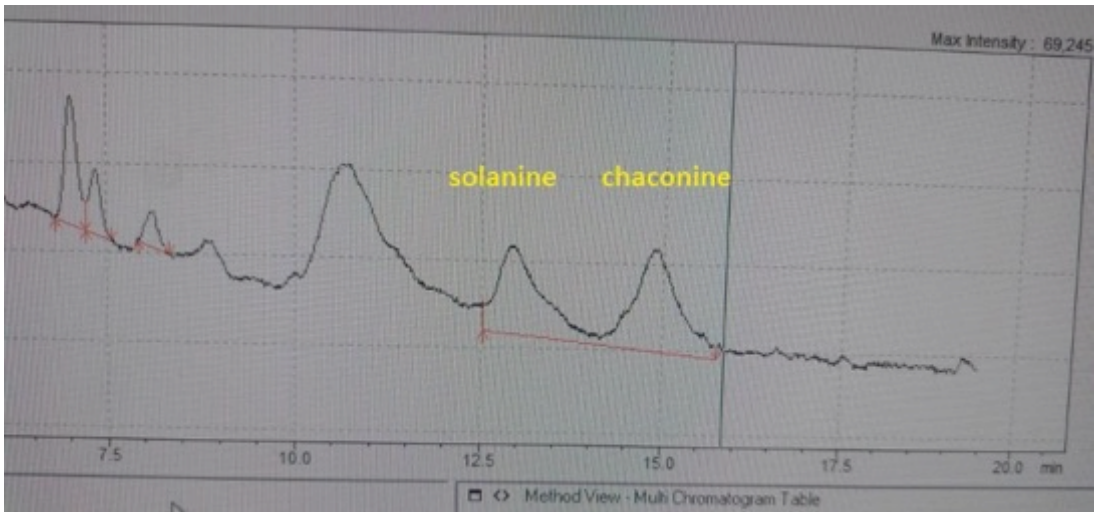

Fig. 2. HPLC chromatogram of standard glycoalkaloids. Peak 1: α-solanine; Peak 2: α-chaconine

Figure 3 presents a chromatograms of glycoalkaloids α-solanine and α-chaconine in extracts of skin and flesh of conventional potato. Separation of the analytes was performed on a Shimadzu Shim-pack GIST C18 column (250mm x 4mm I.D., 5µm particle size) applying isocratic elution of the mobile phase consisting of 30% (v/v) acetonitrile and 70 % KH_2PO_4 (20 mM and pH 6,57).The flow rate on the mobile phase was 1 mL/min.

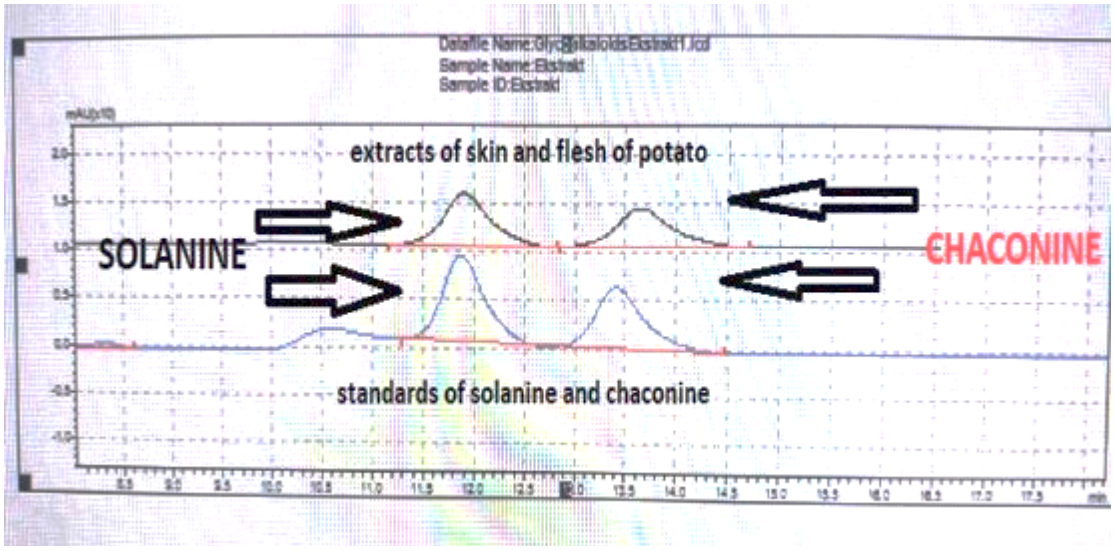

Fig. 3. HPLC chromatogram of extracts of skin and flesh of conventional potato

4. DISCUSSION

Since potato tubers contain approximately 77-79% water, the air-dried samples proved more than 10 times concentrations of glycoalkaloids. Air-dried samples can be easily grinded and stored for longer periods of time prior to measurements. Considering these advantages, air-dried tubers were used in this study.

Separation and retention of the α-solanine and α-chaconine was increased by raising the buffer pH. The optimum pH was found to be 6.50-6.66.

Reducing the amount of acetonitrile in the mobile phase also improved separation. Using 80% acetonitrile and 20% of potassium dihydrogen phosphate buffer with concentration of 20 mM and pH 6.57. The flow rate on the mobile phase was 1 mL/min., peaks of standards of α-solanine and α-chaconine were overlapping. Also 50 % (v/v) acetonitrile and 50 % KH_2PO_4 with concentration of 20 mM and pH 6.57. The flow rate on the mobile phase was 1 mL/min, peaks of standards of α-solanine and α-chaconine were not good with incorrect form (Fig.4).

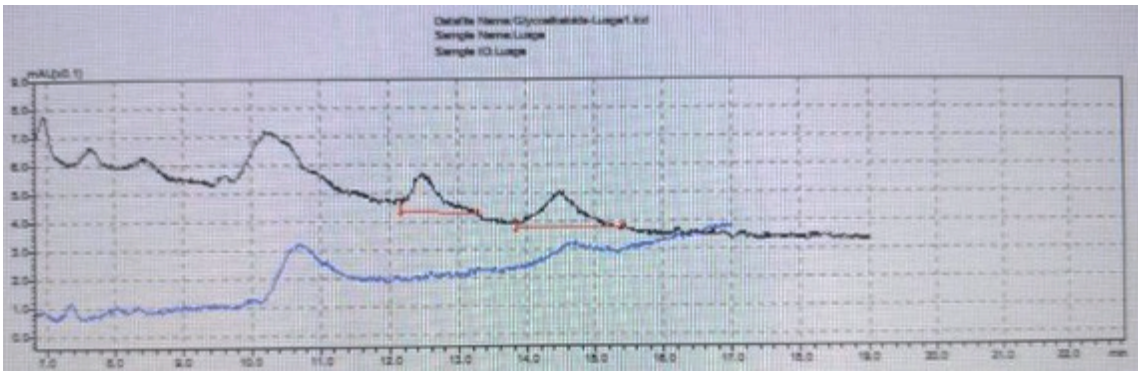

Fig. 4. HPLC chromatogram with mobile phase 50 % (v/v) acetonitrile and 50 % KH_2PO_4 with concentration of 20 mM and pH 6,57. The flow rate on the mobile phase was 1 mL/min

Using 30 % (v/v) acetonitrile and 70 % KH_2PO_4 with concentration of 20 mM and pH 6.57, gave maximum separation (Fig.2). Detection of solanine and chaconine was performed on a wavelength of 204 nm.

Common methods for the determination of glycoalkaloids in potatoes involve three steps: 1. Extraction with a suitable polar solvent like water or methanol, 2. Purification by precipitation or chromatography, and 3. Quantification by spectrophotometry. The solvent systems most commonly employed for glycoalkaloid extraction consist of aqueous acetic acid or combinations of acetic acid with methanol/chloroform, tetrahydrofuran or acetonitrile-methanol; some literature reports specify only methanol (Driedger and Sporns 2001; Nikolic et al., 2005; Jensen et al., 2007).

Separation of α-chaconine and α-solanine on extracts of skin and flesh of conventional potato (Fig.3) with mobile phase 30% (v/v) acetonitrile and 70% KH_2PO_4 with concentration of 20 mM and pH 6.57. Detection of solanine was on retention time 12.8 min and chaconine was on 14.6 min.

5. CONCLUSIONS

The method presented in this study is both rapid and specific for the direct quantification of solanine and chaconine in conventional potato tubers. We utilized dehydrated conventional potato tubers for analysis and obtained results that identified the predominant glycoalkaloids in potatoes, α-solanine and α-chaconine. This technique is applicable to research in potato breeding, production, and medical care. Moreover, laboratories could employ this method to explore how the concentrations of potato alkaloids react to environmental conditions during cultivation, as well as to investigate the biological activities of potatoes.

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