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Isolation and chemoenzymatic treatment of glycoalkaloids from green, sprouting and rotting *Solanum tuberosum* potatoes for solanidine recovery

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ABSTRACT

The estimation of glycoalkaloids in the flesh of different types of decayed potatoes was evaluated. The results showed that turned green and also sprouting or rotting potato flesh contain high amounts of toxic solanine and chaconine, exceeding by 2–5-fold the recommended limit, and ranging from 2578 ± 86 mg/kg to 5063 ± 230 mg/kg of dry weight potato flesh. For safety consideration, these decayed potatoes should be systematically set aside. To avoid a net economic loss and encourage the removal of this hazardous food, a recycling process was investigated to generate added-value compounds from the toxic glycoalkaloids. A simple chemo-enzymatic protocol comprising a partial acidic hydrolysis followed by an enzymatic treatment with the β-glycosidase from *Periplaneta americana* allowed the efficient conversion of α-chaconine to solanidine.

1. Introduction

Toxic steroidal glycoalkaloids are naturally occurring compounds, which are commonly found in plants of the Solanaceae family (potatoes, tomatoes, sweet pepper, egg plants etc.). In potato tubers from *Solanum tuberosum* L., the most abundant glycoalkaloids are α-chaconine and α-solanine. They represent over 95% of the total glycoalkaloid content of the potato plant (Friedman & McDonald, 1997) and are known to be toxic molecules, as revealed by numerous animal tests (Crawford & Myhr, 1995; Friedman, Rayburn, & Bantle, 1991; Langkilde et al., 2012) and clinical investigations (Benilova et al., 2006; Hellenäs, Nyman, Slanina, Lööf, & Gabrielsson, 1992; McGehee et al., 2000; Mensinga et al., 2005). In humans, ingestion of 1–2.5 mg of potato glycoalkaloids per kg of body weight can induce poisoning and cause health related problems including gastroenteritis, gastrointestinal discomfort, diarrhea, vomiting, fever, low blood pressure, fast pulse rate along with neurological and occasional death in human and farm animals (Friedman & McDonald, 1997; Hellenäs et al., 1992; Slanina, 1990) and the fatal dose is between 3 and 6 mg/kg body weight (Slanina, 1990). Glycoalkaloid toxicity is attributed to their inhibiting effect on acetylcholinesterase activity in the central nervous system and their action on membrane integrity, adversely affecting the digestive system and general body metabolism (Benilova et al., 2006; Roddick, Weissenberg, & Leonard, 2001). *In vitro* experiments showed that both α-solanine and α-chaconine are potent cytotoxins that rapidly induce cell lysis. Moreover, their cytotoxic potency is considerably increased when both compounds are acting together, thus revealing a synergistic action of the two molecules in disrupting membranes (Rayburn, Friedman, & Bantle, 1995; Yamashoji & Matsuda, 2013). Notably, poisoning due to these glycoalkaloids is often underdiagnosed because the observed symptoms are close to those caused by other common gastrointestinal ailments. Given this toxicity, safety guidelines have been established. According to FAO/WHO (1999) recommendation, the safety limit for the level of total glycoalkaloids in potato tubers is 200 mg/kg fresh weight, a value corresponding to 1000 mg/kg dry weight (Koleva, van Beek, Soffers, Dusemund, & Rietjens, 2012). The glycoalkaloid content can vary greatly depending on potato cultivars and environmental factors, such as light, mechanical injury, storage temperature and/or post-harvest treatment (Dale, Griffiths, Bain, & Todd, 1993; Machado, Toledo, & Garcia, 2007; Percival & Baird, 2000; Percival & Dixon, 1996). Glycoalkaloids are also known to accumulate in

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potato peels and according to many authors, the losses of glycoalkaloids in peeled potatoes (in light-fleshed and colored-fleshed) can account for 20–80% depending on potato cultivar, tuber size and peeling methods (Eltayeb, Al-sinanp, & Khan, 2003; Lachman et al., 2013; Rytel, Tajner-Czopek, Kita, Miedzianka, & Bronkowska, 2015).

In some countries such as Ivory Coast that do not cultivate potatoes and are forced to import, potatoes often tend to turn green, to sprout or rot on the markets due to the delay between importation and consumption, the difficulties of storage and the climatic conditions. These deterioration processes are known to stimulate α-solanine and α-chaconine production. Moreover, as these molecules are fairly heat-stable with melting points in the range of 190–285 °C, they are not destroyed by home cooking, frying, baking, or microwaving (Bushway & Ponnampalam, 1981; Lachman et al., 2013; Tajner-Czopek, Rytel, Kita, Pęksa, & Hamouz, 2012). In consequence, they represent a real danger to consumers and also to the environment where they are discharged. Potato glycoalkaloids are derived from a solanidine aglycone and include carbohydrate side-chains. The side-chain of α-solanine is composed of the trisaccharide solatriose ((α-L-rhap(1-2)[β-D-Glcp (1-3)]β-D-galactopyranosyl) and that of α-chaconine comprised of chacotriose ((α-L-rhap(1-2)[α-L-rhapGlcp1-2)]β-D-glucopyranyl) (Friedman & McDonald, 1997). The biological and toxicological activity of these compounds is strongly influenced by the nature and the arrangement of the carbohydrate substituents that mediate interactions with the receptor sites of cell membranes. For example, α-chaconine is reported as a much more toxic compound than α-solanine (Rayburn, Bantle, & Friedman, 1994). Interestingly, solanidine, the aglycone part of these molecules, is a precursor for hormone synthesis, such as progesterone, testosterone and cortisone and some pharmacologically active compounds (Vronen, Koval, & Groot, 2003) and decayed potatoes could be used as a source of this valuable precursor. To that end, acidic hydrolysis of the sugar part of potato glycoalkaloids was proposed to isolate solanidine (Attoumbré, Giordanengo, & Baltora-Rosset, 2013; Friedman & McDonald, 1995; Nikolić & Stanković, 2003). Chemical hydrolysis was efficient but required strong conditions of hydrolysis using 10% hydrochloric acid for 2 h at 75 n°C and also led to the formation of solanthrene, an undesired secondary compound resulting from solanidine dehydration (Attoumbré et al., 2013). Enzymatic hydrolysis was also attempted but it required 72–96 h treatment, and often resulted in partial hydrolysis of the glycoalkaloids (Nikolic & Stankovic, 2005; Oda, Saito, Ohara-Takada, & Mori, 2002). To recycle potato wastes and avoid pollution, the challenge is thus to establish conditions that could result in total hydrolysis of glycoalkaloids while preventing solanidine dehydration. To date, chemo-enzymatic treatments of potato glycoalkaloids have never been envisioned, although they could certainly be helpful to obtain solanidine in higher yields. In this context, the objective of the present study was first to determine the glycoalkaloid content in the flesh of turned green, decaying and sprouting *Solanum tuberosum* potatoes, purchased in different market places of Abidjan (Ivory Coast). Secondly, a chemo-enzymatic treatment of α-chaconine and α-solanine was investigated to propose a simple recycling process of decayed potatoes and produce solanidine, a value-added and important precursor of pharmacologically active compounds.

2. Material and methods

2.1. Material

In this investigation three types of potatoes (*Solanum tuberosum*) were analyzed; the first was turned green potatoes, the second was sprouting potatoes, and the third was rotting (decayed) potatoes. These potatoes were purchased in different market places of Abidjan (Ivory Coast) washed and peeled manually with a knife, sliced, frozen at −80 °C, and freeze-dried The glycoalkaloids α-solanine (label purity 99% by HPLC), α-chaconine (label purity 99% by HPLC), and the aglycone solanidine, were obtained from LGC Standards (Chromadex, Molsheim, France), and used as standard compounds.

2.2. Sample preparation

Freeze-drying was performed with Alpha 1-2 LD freeze dryer (Bioblock Scientific, Illkirch, France) at a working pressure of 0.0090 mbar and temperature of −84 °C for 48 h. Then, the dry material was ground in an electric grinder and stored at −20 °C. For each variety and type of potato, 3 samples of 4 g were prepared. These powders were then tested for their α-solanine and α-chaconine content.

2.3. Extraction of glycoalkaloids

The extraction procedure was based on that of Percival and Dixon (1996). The potato powder (4 g) was weighed and dissolved in 80 mL of extraction buffer (water:acetic acid:$NaHSO_3$ 100:5:0.5 *v*/*v*/*w*). The mixture was homogenized using a polytron homogenizer for 2 min at high speed. Then, the solution was centrifuged at 7000*g* for 30 min. The supernatant was collected and passed through a Whatman No. 4 filter paper (Millipore Co., Bedford, MA,) and stored at 4 °C in dark vials. The extraction and analysis of glycoalkaloids was performed in triplicate starting with three independent freeze-dried samples.

2.4. Clean-up

This procedure was accomplished according to Hellenäs and Branzell (1997). A SepPak Plus C18 cartridge (Waters Corp., Milford, MA) was conditioned overnight in 5 mL of acetonitrile and then rinsed with 15 mL of extraction buffer (water:acetic acid:$NaHSO_3$ 100:5:0.5 *v*/*v*/*w*). Afterwards, the sample solution (10 mL) was passed through the cartridge that was subsequently washed with 4 mL of solid-phase extraction buffer (water/acetonitrile 85:15 *v*/*v*). The glycoalkaloids were then eluted with 4 mL of acetonitrile:0.022 M potassium phosphate buffer (pH 7.6) 60:40 *v*/*v*. The eluate was finally adjusted to 5 mL with the same solution. Eluted sample was then transferred into a dark amber vial and stored at 4 °C for further HPLC analysis. Before injection into the liquid chromatograph, all samples were filtered through Whatman No. 4 filter paper (Millipore Co., Bedford, MA).

2.5. HPLC analysis

The method of HPLC analysis was adapted from AOAC (2000). This analysis was performed using an HPLC system (Ultimate 3000; Dionex, Sunnyvale, CA) equipped with a Venusil C18 column (5 μm particle size, 100 Å, 150 × 4.6 mm) with UV detector. A 20-μL glycoalkaloid sample was loaded into an HPLC vial and placed in the autosampler. Isocratic elution with 50% acetonitrile in 0.01 M phosphate buffer (pH 7.6) was used at a flow rate of 1 mL/min for 20 min at 30 °C. Quantification was carried out with UV-detection at 202 nm using external standards of α-solanine and α-chaconine. All samples were analyzed in duplicate. The standard curve using 50, 100, 150, 200, 250 and 500 μg/mL of α-solanine and α-chaconine solutions were prepared in methanol. Chromeleon software was used for data acquisition and analysis. The glycoalkaloid content was expressed in mg per kg dry weight.