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High-performance liquid chromatographic determination of glycoalkaloids in potatoes from conventional, integrated, and organic crop systems

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Abstract

During the last decade organic agro-food products, including potatoes, have been progressively introduced in the Portuguese consumer market. Nevertheless, the quality of these products with regards to chemical safety still requires the implementation of monitoring programmes. In this communication we compare the glycoalkaloid content in varieties Santé and Raja of marketed Portuguese potatoes from conventional, integrated, and organic crop systems, using HPLC. In Santé tubers, the corresponding total amount of α-solanine and α-chaconine was respectively 37.3, 44.2, and 38.4 mg/kg fresh weight, whereas for Raja a decreasing concentration on the total glycoalkaloid content was observed from conventional (79.5 mg/kg) to integrated (59.6 mg/kg) and organic tubers (44.6 mg/kg).

Keywords: Potatoes; *Solanum tuberosum*; Glycoalkaloids; α-solanine; α-chaconine; HPLC; Conventional agriculture; Integrated agriculture; Organic agriculture

1. Introduction

Integrated and organic produced foodstuffs have been progressively introduced in the Portuguese consumer market during the last 20 and 10 years, respectively. Although these two modes of production are subjected to general rules, in accordance with specific regulations (Council Regulation, 1991; Ministério da Agricultura, Portugal), more attention should be paid to chemical safety aspects related to the presence of toxicants (pesticides, nitrates, environmental, food processing, and natural toxicants) in the corresponding agro-food products. The chemical risks able to affect organic and conventional agro-food products, the differences between organic and conventional plant products from the view of possible effects on human health, and a comparison of the quality of organically and conventionally grown foods, and foods produced with the aid of different fertilization systems, have been discussed in previous reviews (Brandt & Mølgaard, 2001; Pussemier, Larondelle, Van Peteghem, & Huyghebaert, 2006; Woese, Lange, Boess, & Bögl, 1997).

With respect to the production of potatoes (*Solanum tuberosum* L) one major concern regards the content of glycoalkaloids (GA), that should be controlled so as not to exceed 200 mg/kg fresh weight of tissue. Glycoalkaloids are secondary metabolites that serve as natural defenses against insects and other pests but, in high doses they are also capable of producing various toxic effects in humans, and several cases of poisoning from consumption of potato tubers have been reported (Chen & Miller, 2001; Friedman, 2004; Friedman & Mc Donald, 1997; Jadhav, Lutz, Mazza, & Salunkhe, 1997; Jadhav, Sharma, & Salunkhe, 1981;

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Maga, 1994; Morris & Lee, 1984; Plhak & Sporns, 1997; Smith, Roddick, & Jones, 1996; Sporns, Abell, & Driedger, 2000). The two major glycoalkaloids present in tubers of *S. tuberosum* are α-solanine and α-chaconine (Fig. 1), which together account for 95% or more of GA content (Hellenäs, 1986). Reported chemical analysis of marketed potatoes revealed that the levels of these two substances often exceed the safety limits (Hellenäs, 1986; Hellenäs, Brandzell, Johnsson, & Slanina, 1999; Sotelo, Contreras, Sousa, & Hernandez, 1998; Sotelo & Serrano, 2000). A comparison of the levels of GA in one potato variety (Karin) cultivated in different regions of Czech Republic in conventional and ecological ways, indicated, in all cases, higher amounts of α-solanine and α-chaconine in the tubers growing from organic farming methods (Hamouz, Čepl, Vokál, & Lachman, 1999). In a six-year crop rotation trial carried out in Sweden, three varieties of potatoes growing in organic and integrated production were analyzed for their GA content, but no differences were observed (Fjelkner-Modig, Bengtsson, Stegmark, & Nyström, 2000). In a study reported (Hellenäs & Branzell, 1995), different varieties of conventional and organic potatoes from two different trials showed opposite results regarding their α-solanine and α-chaconine content.

In this communication we present the high-performance liquid chromatographic determination of glycoalkaloids in marketed potatoes from conventional, integrated, and organic crop systems. We have selected two common varieties, Raja and Santé, which are known to possess desirable traits such as high yields, tuber quality, and resistance to pests, diseases, and physiological disorders. The three crop systems were experimented in a field trial according to the conditions described in Section 2, and Tables 1 and 2.

2. Materials and methods

2.1. Materials

High-performance liquid chromatography (HPLC) and extraction solvents (acetonitrile, MeOH, water, and acetic acid) were purchased from Merck and Riedel-de-Haen. Glycoalkaloid standards (α-solanine, α-chaconine, and solanidine) and reagents (heptane sulfonic acid sodium salt, sodium bisulfite, triethylamine, phosphoric acid) were obtained from Sigma-Aldrich. C_{18} Sep Pak cartridges were procured from Waters Associates, Milford, MA.

2.2. Potato samples

The potato varieties Raja and Santé were experimented at the location of Marinhais—Núcleo de Ensaios e Controlo do Escaroupim, Portugal, with fertilization systems and plant protection products as described in Tables 1 and 2. Seeds were originary from Holland, category Base, diameter 35–45 mm, and planted in 2003/03/06. Before planting, sand soil was analysed to determine inherent fertility and fertilizer requirements for the organic and integrated production systems, on a base of 40 t/ha of potato production, whereas for conventional crop system the ap-

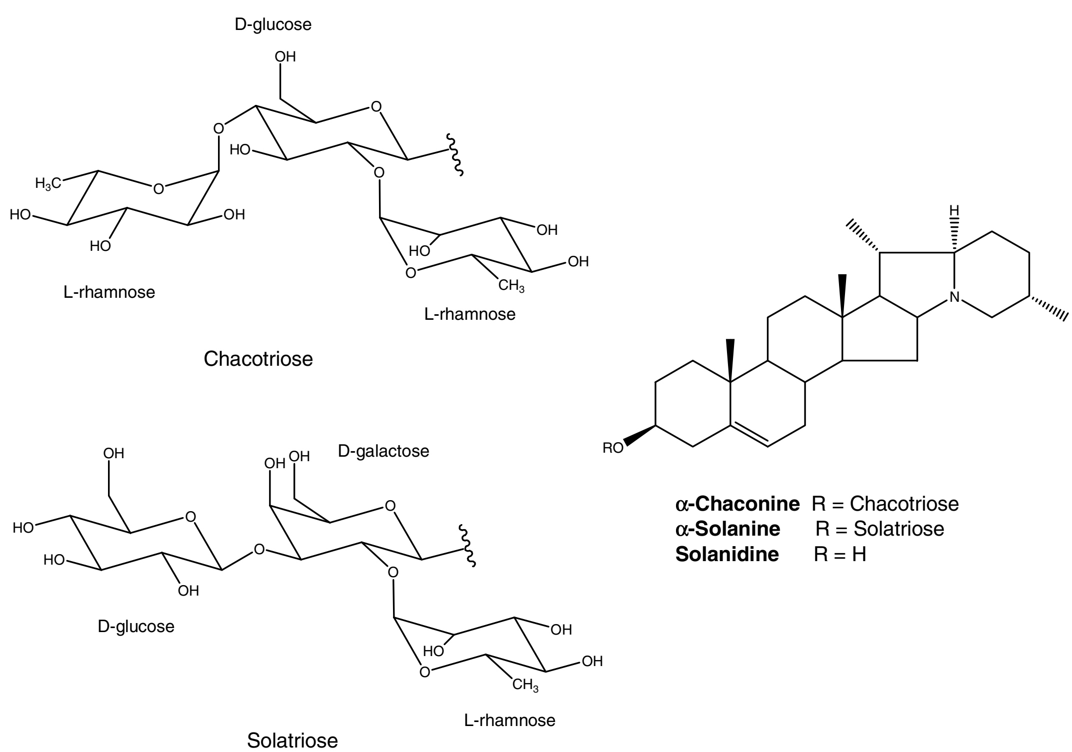

Fig. 1. Structures of α-chaconine, α-solanine, and solanidine.

Table 1
Fertilization used in conventional, integrated, and organic crop systems

Crop	Preplant fertilization		Cover fertilization
	Organic manure	Fertilizer	
Conventional	Thonumus (organic N 1.7%, P_2O_5 0.8%, K_2O 1.15%, MgO 3%, CaO 4%, total organic matter 65%)	N 60 kg/ha P_2O_5 120 kg/ha K_2O 240 kg/ha	60 kg/ha of $MgNO_3$
Integrated	Thonumus	N 50 kg/ha $P_2O_5$100 kg/ha K_2O 200 kg/ha	50 kg/ha of $MgNO_3$
Organic	Frayssinet (organic N 2.0%, P_2O_5 1.0%, K_2O 1.5%, C/N 15, total organic matter 70%)		Liquid organic matter (dried matter 52.5%, organic matter 37.5%, N 3.5%, P_2O_5 0.1%, K_2O 7.5%, CaO 0.7%, MgO 0.1%, SO_3 1.3%, density 1.22 g/mL)

Table 2
Plant protection products

Crop system	Date	*Phytophthora infestans* active ingredient	*Leptinotarsa decemlineata* active ingredient
Conventional	03/04/08	Mancozeb (dithiocarbamate)	Cypermethrin (pyrethroid)
	03/04/14	Mancozeb + metalaxyl (phenylamide)	
	03/04/23	Chlorothalonil (phtalic derivative)	Chlorpyrifos (organophosphate) + phosalone (organothiophosphate)
	03/05/05	Mancozeb + metalaxyl	
	03/05/15	Chlorothalonil	
	03/06/09		Deltamethrin (pyrethroid)
Integrated	03/04/14	Mancozeb	
	03/04/23	Mancozeb	
	03/05/05	Copper oxychloride	
	03/05/15	Copper oxychloride	
	03/06/09		*Bacillus thuringiensis*
Organic	03/04/14	Copper oxychloride	
	03/04/24	Copper oxychloride	
	03/05/05	Copper oxychloride	

plied fertilization was the one normally used by the local growers. The results for sand soil analysis were as follows: pH (H_2O) = 6.6, P_2O_5 = 73 ppm, K_2O = 69 ppm, Mg = 40 ppm, Fe = 43 ppm, Mn = 8 ppm, Zn = 0.6 ppm, Cu = 8.1 ppm, B = 0.45 ppm, $CaCO_3$ = 0%, organic matter = 0.29%. The analysis of the irrigation water showed the following results: pH = 8.6, conductivity = 0.45 mS/cm, Ca^{2+} = 6.62 mg/L, Mg^{2+} = 5.60 mg/L, Na^{+} = 92.00 mg/L, B^{-} = 0.09 mg/L, Cl^{-} = 63.83 mg/L, CO_3^{2-} = 3.00 mg/L, CO_3H^{-} = 122.00 mg/L, SO_4^{2-} = traces, solids in suspension = none, Fe < 0.50 mg/L, Mn < 0.20 mg/L, NO_3^{-} = 2.7 mg/L, saturation index = −0.07, adjusted Na adsorption ratio = 5.37. Harvest took place in 2003/07/02, after the plants have reached complete maturation. The tubers were kept in a cool chamber at a temperature of 6–8 °C, and a relative humidity of 90–95%.

2.3. Extraction of alkaloids for HPLC analysis

Fresh potato tubers (50 g) were cleaned, and ground to a smooth consistency in a commercial warning blender with 60 mL of extracting solution (4.0 g of 1-heptane sulfonic acid sodium salt in 1 L of distilled water containing 10 mL of acetic acid) and filtered to obtain the crude extract. Sodium bisulfite (0.5 g)/100 g of sample was added to retard the oxidation during sample preparation and analysis. For weight/volume calculations, all samples were assumed to have a water content of 80% (Watt & Merril, 1963). This resulted in a sample/solvent ratio of 50 g/100 mL. Solid phase extraction technique was adopted for concentrating glycoalkaloid content from potato samples (Carman, Kuan, Ware, Francis, & Kirshenheuter, 1986). Ten milliliter each of the sample extracts were applied on to a preconditioned C_{18} Sep Pak cartridge. After removing interfering compounds by elution with 5 mL of acetonitrile:water (2:8), glycoalkaloids were eluted using 3 mL of acetonitrile:water (8:2) at a rate of 1 drop/s. The samples were stored at 4 °C before being injected to HPLC. All the experiments were repeated in triplicate.

2.4. HPLC analysis

HPLC analysis of glycoalkaloids was performed using a Waters 600 instrument (Milford, MA, USA) with UV detector (Waters 2487), and Chromeleon 4.3 software (Dionex, Germany), on a Zorbax Rx-C18 column

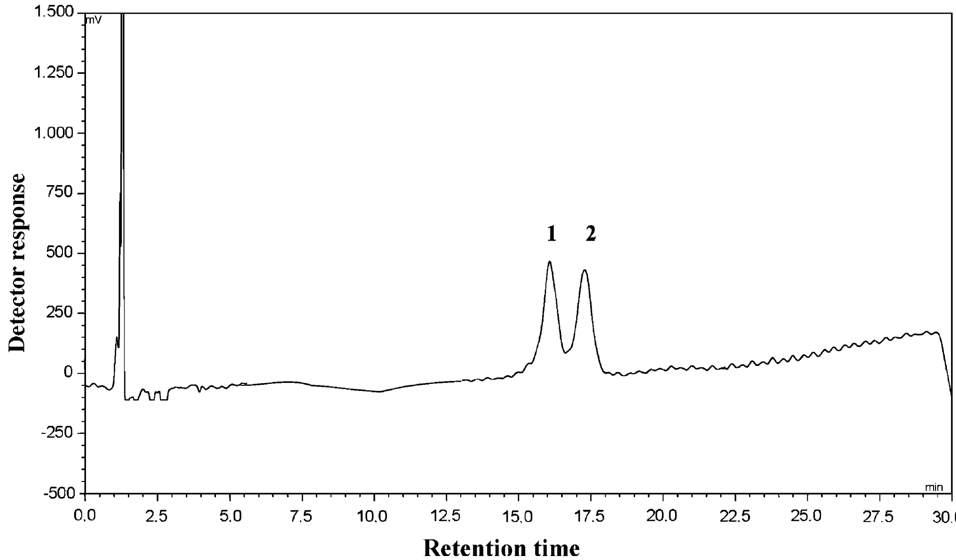

Fig. 2. HPLC separation of α-solanine and α-chaconine from an extract of organic Santé tubers, on a Zorbax Rx-C18 column (4.6 × 250 mm, 5 μm), eluent TEAP (pH 3.0):MeCN (80%:20% mixture from 0 to 20 min, linear gradient elution from 20 to 30 min till 50%:50% mixture, isocratic elution from 30 to 35 min, and linear gradient return to the initial conditions), flow rate 2.0 mL/min, column temperature 50 °C, UV absorbance detector 202 nm, sensitivity of 0.025 AUFS from 0 to 20 mn, and 1.0 AUFS from 20 mn. Peaks: α-solanine (**1**), retention time 16.0 mn, and α-chaconine (**2**), retention time 17.5 mn.

(4.6 × 250 mm, 5 μm) from Agilent (USA), calibrated with standard samples, and using the conditions indicated in Fig. 2. The GA content was calculated from the peak areas, which were plotted against a standard calibration curve for each glycoalkaloid analysed. The resolution factor (α) of α-solanine versus α-chaconine was calculated to be 1.06. The limit of quantification (LOQ) in the column was 3.8 μg for α-solanine and 3.3 μg for α-chaconine, whereas the corresponding limits of detection (LOD) were 1.2 and 1.0 μg. LOQ and LOD were determined as being respectively 10 and 3 times the standard deviation in the *y*-intercept of the standard regression lines. The mean recoveries obtained from triplicate samples were 93.6% for α-solanine and 82.7% for α-chaconine. These were determined by adding 1 mL of a 25 μg/mL standard solution of α-solanine and α-chaconine, to 10 mL of aliquots of extracted potato samples prior to ion-pair chromatography. The samples were then treated and analysed as described above.

3. Results and discussion

The content of α-solanine and α-chaconine in potato tubers from the two varieties growing under conventional, integrated, and organic conditions, is shown in Table 3. The total amount of these glycoalkaloids was nearly identical in Santé tubers from the three crop systems, whereas for the Raja variety we have observed a decreasing concentration on total GA from conventional (79.5 mg/kg) to integrated (59.6 mg/kg) and organic tubers (44.6 mg/kg).

The results obtained for the Raja variety suggest that the biosynthesis of glycoalkaloids could have been influenced by the different fertilization conditions. Conventional and integrated systems use large amounts of N fertilizer in addition to organic manure, which is not the case for organic production (Table 1), and this fact can affect the C/N balance, and carbon and nitrogen pools of secondary metabolism. Previous studies on cultivated plants demonstrated that N fertilization resulted in higher contents on N-containing compounds (Brandt & Mølgaard, 2001), including alkaloids, and free amino-acids that are the source of incorporated nitrogen in the glycoalkaloid nucleus (Friedman & Mc Donald, 1997; Kaneko, Tanaka, & Mitsuhashi, 1976). Nevertheless, from a conventional field experiment carried out in Poland with two varieties of potatoes (Beryl and Mila), it was observed that after six months of storage, cv. Beryl accumulated more glycoalkaloids with increasing nitrogen fertilization rates, while the opposite was observed for cv. Mila (Rogozinska & Wojdyla, 1999). When treated with fungicides against *Phytophthora infestans*, an increase on the GA content was observed for both varieties after six

Table 3
Glycoalkaloid content (mg/kg fresh weight) of Santé and Raja potato varieties from conventional, integrated and organic crop systems

Variety	Conventional crop system			Integrated crop system			Organic crop system		
	α-Solanine	α-Chaconine	Total	α-Solanine	α-Chaconine	Total	α-Solanine	α-Chaconine	Total
Santé	17.7	19.6	37.3	19.8	19.6	44.2	13.6	24.8	38.4
Raja	43.9	35.5	79.5	25.6	34.0	59.6	23.3	21.3	44.6

months of storage. In what concerns a possible effect of the plant protection products (Table 2) on the content of glycoalkaloids found in the tubers, we cannot draw a conclusive explanation, since it is expected that resistant cultivars in organic agriculture will cause higher contents of defence-related secondary metabolites (Brandt & Mølgaard, 2001), as α-solanine and α-chaconine, and this effect balance the one caused by the fertilization conditions. Moreover, data from greenhouse experiments revealed that the use of systemic pesticides appeared to affect the tested varieties differently (Wilson & Frank, 1975). The fact that the content of α-solanine and α-chaconine in Santé tubers did not vary in the three crop systems, could be possibly explained on a genotype basis.

As stated by Brandt and Mölgaard (Brandt & Mølgaard, 2001), it is difficult or impossible to compare the levels of secondary metabolites between organic and conventional products, since it has repeatedly been shown that the differences between cultivars are greater than those between plants of the same cultivar under different conditions. Aiming a better understanding of the influence of conventional, integrated, and organic agriculture methods on the content of glycoalkaloids in potato tubers, this research should be pursued for several years and extensive to other potato varieties.

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