DETERMINATION OF α-SOLANINE CONTENT IN TWO VARIETIES OF POTATOES BY THE DENSITOMETRIC METHOD

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:

Solanine is a glycoalkaloid found in the *Solanaceae* family, such as the potato. It is very poisonous even in small quantities because it has pesticide and fungicide effects and represents a natural plant defense mechanism. Its concentration increases when the plant is exposed to the agents that can cause plant stress (fertilization, insecticide use, etc.). This paper aims to examine the influence of three cultivation systems (conventionally, organically and naturally) on the biosynthesis of α-solanine (αS) through his quantification in young potatoes using densitometry. Two varieties of potatoes were analyzed: *Aladdin* (*Ala*) and *Mona Lisa* (*MoL*). For statistical analysis, the Student's t-test was used.

The results showed that the use of artificial insecticides caused a very intense biosynthesis of αS in the conventionally grown *Ala* variety (1.19 mg/100 g of fresh tubers (f.t.)) in comparison to the average α-solanine content (AαSC) by the organically grown *Ala* (0.62 mg/100 $g_{f.t.}$) (it is close to the statistical significance, (p=0.08)). It is difficult to explain the very high AαSC of natural *Ala* cultivation (1.62 mg/100 $g_{f.t.}$).

Analysis of potatoes of the *MoL* variety showed that the AαSC of conventionally grown potatoes (1.35 mg/100 $g_{f.t.}$) was statistically higher than the AαSC of naturally grown potatoes (0.59 mg/100 g of f.t.) ($p^*<0.05$). Also, AαSC of the organically grown *MoL* (1.40 mg/100 $g_{f.t.}$) was higher than the AαSC of naturally grown *MoL*, but without statistical significance (p>0.05).

Concentrations of αS founded in the case of conventionally, organically and naturally grown potatoes are considered safe and such potatoes are suitable for consumption. However, because of a slight reduction in toxic αS, it is recommended to consume organically grown potatoes (*Ala* variety), and naturally grown potatoes (*MoL* variety).

KEYWORDS: glycoalkaloids; α-solanine; potatoes; densitometry.

INTRODUCTION

Most plants of the *Solanaceae* family are very significant to humans. These include tobacco (*Nicotiana spp*.), sweet pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), tomatoes (*Lycopersicon esculentum*) and potatoes (*Solanum tuberosum*). Of all these, the most important are potatoes.Although potatoes are perceived as a source of carbohydrates, they are equally good sources of high-quality proteins [1], [2]. Fresh potatoes contain 2% of protein, while dried potatoes have up to 10% protein. Markakis (1975) calculated, based on amino acid composition, that the proportion of high-quality proteins in potatoes was 70%, compared to proteins contained in whole eggs. It should be emphasized that the proteins in potatoes constitute an excellent source of lysine, but not of cysteine and methionine, which limited their nutritional value [3].

Historically, solanine is the first isolated alkaloid [4] that has been recognized as a glycoalkaloid [5]. It was not until 1955 that Kuhn and Low proved that solanine was a mixture of two components, α-solanine, and α-chaconine [6]. From 300 representatives of the genus Solanum, 90 structurally distinct steroidal alkaloids were isolated and characterized [7], [8]. In conventionally grown potato varieties the primary glycoalkaloids present are α-solanine and α-chaconine. These two components show similar toxic effects on humans [9], [10]. When crossing varieties, care should be taken that the glycoalkaloid content does not reach the limit at which it would not be safe

to use potatoes in the diet. However, many factors can affect the level of glycoalkaloids in potatoes. Climate change in the cultivation area can cause variations in glycoalkaloid concentrations among representatives of the same variety [11], [12]. The tuber does not cease to produce glycoalkaloids after harvesting, and improper storage can result in a dramatic increase in these components [13]. Sinden (1972) [14] indicated a greater increase in glycoalkaloids due to mechanical damage to the tuber, and Petersen (1993) emphasized that the damaged tuber should be discarded, as should green potatoes [15].

The glycoalkaloids are not evenly distributed within the tuber. The largest content is just below the crust. Potatoes naturally produce solanine and the related glycoalkaloid chaconine, which serve as a mechanism of defense against insects, diseases, and pests. Potato leaves, stems and shoots are particularly rich in glycoalkaloids. When the potato tuber is exposed to sunlight, it becomes green and the biosynthesis of glycoalkaloids is increased. This process reveals a tuber that "protects" from being eaten. The green color is derived from chlorophyll and as such is harmless. However, it is an important indicator that solanine and chaconine are present in elevated concentrations in the tuber.

Certain diseases such as potato decay can dramatically increase the concentration of glycoalkaloids. It is believed to be a natural reaction of the plant, response to infection or mechanical damage. If green color is observed beneath the bark, this strongly suggests that solanine is rewarded, although the two processes can occur independently of one another. Second and more reliable indicator of potato toxicity is its bitter taste. Because of this appearance and the bitter taste of potatoes, solanine poisoning is rare if storage is taken into account. The most common symptoms are vomiting and diarrhea, so poisoning can be misdiagnosed as gastroenteritis. Most people who are poisoned by solanine completely recover. However, fatal cases were reported due to poor and inadequate treatment. The deaths were also due to the consumption of other plants of the willow family, such as *Solanum dulcamara* (woody willow) [10], [16], [17]. Recently, the relevance of α-solanine has increased because of its possible role as an inhibitor of breast, pancreatic, and esophageal cancers and melanoma [18].

This study aimed to examine the influence of three cultivation systems (conventionally, organically and naturally) on the biosynthesis of α-solanine (αS) through his quantification in young potatoes using densitometry.

EXPERIMENTAL

Thin-layer chromatography with densitometry detection (HPTLC) (Desaga, CD60 TLC scanner with appropriate software, and Desaga AS 30 TLC applicator, Heidelberg, Germany) was used to determine α-solanine in potatoes of the Mona Lisa and Aladdin variety.

The extraction and precipitation were done according to the protocol based on a quantitative method for solanidine glycoalkaloids [19]-[21]. The washed, unpeeled potato tubers are cut into small pieces or rendered. 30 g of ground potatoes was weighted into a 300 mL flask and 200 mL of 96% ethanol was added. The flask with the alcoholic potato pulp was placed in a water bath at 90 °C and incubated for 10 minutes. The resulting extract was filtered through Whatman filter paper no. 1 via the Bühner funnel. The filtered extract was evaporated to a volume of 20 mL on a rotavapor, at 60 °C.After evaporation, 50 mL of 10% acetic acid was added to the resulting concentrate, mixed and centrifuged at 8000 rpm for 30 minutes at 10 °C. After centrifugation, the solution is poured into an Erlenmeyer flask with a glass stopper and brought to pH 10 with ammonia. The flask is immediately sealed (to prevent evaporation of the ammonia) and heated in a water bath at 70 °C for 20 min. After heating, the solution was poured into 15 mL centrifugal tubes and cooled at 4 ºC for at least 3 h. The solution was centrifuged at 8000 rpm for 30 minutes at 10 °C.

After centrifugation, the liquid portion is carefully decanted and the glycoalkaloid precipitate remains at the bottom of the cuvette. The precipitate was dissolved in 200 μl of the mobile phase.

CHROMATOGRAPHIC PLATE PREPARATION AND CHROMATOGRAPHY

The glass is well cleaned with methanol before developing the chromatographic plates. Silica gel was applied to the plate and allowed to dry overnight. Before use, the TLC plate was activated at 110 °C. Twenty-one samples of 5 μl volume were applied at 8 mm intervals to the chromatographic plate. This sample plate was heated to 90 °C for 30 minutes and then placed in a mobile phase bath for 1 hour.

MOBILE PHASE SELECTION

Acid and neutral mobile phases did not show the good separation of α-solanine and α-chaconine. Best distributed was achieved in the alkaline mobile phase containing methanol, chloroform and ammonium hydroxide. The selected mobile phase was the di-

chloromethane-methanol-water-ammonium hydroxide system (70 + 30 + 4 + 0.4 v / v), in which the stain is well separated and the R_f values are in the range between 0.2 and 0.98.

SELECTION OF DETECTION REAGENTS

The Carr-Price reagent was chosen because of its high sensitivity. Antimony(III) builds a double bond with the steroid component, forming a red colour. The reagent must be anhydrous as water interferes with the reaction of antimony chloride with glycoalkaloids. Therefore, it is very important to heat the plates to 90 °C (between the analysis steps) and preferably keep them in a desiccator. The Carr-Price reagent was modified because the addition of acetic acid increased the sensitivity and colour remained stable for a longer period.

DETECTION AND READING

After developing in the mobile phase, the plates are air-dried for 15 minutes and then placed in the oven for 60 minutes at a temperature of 90 °C. Direct visualization is achieved when the plate is sprayed with a modified Carr-Price reagent and then dried for 5 minutes at 105 °C. Glycoalkaloids will appear as red spots. Densitometry reading is performed by reflection scanning at 507 nm. The determination is best done 20 minutes after heating the plates, as the red chromatographic zones of the glycoalkaloids turn purple over time. For all these analyses, standards of α-solanine were prepared in ethanol, with the following concentration: 0.91 μg/ml, 9.01 μg/ml, 90.1 μg/ml, and 1000 μg/ml.

RESULTS AND DISCUSSION

Three samples of conventional, organic and natural cultivation were used to determine α-solanine in the potato of Aladdin and Mona Lisa varieties. The selected samples (tubers) were of approximate size so that the samples had a close-ratio of peel to pulp as possible. The amounts of α-solanine found in potato samples of the Aladdin variety of conventional cultivation ranged from 0.47 to 1.73 mg per 100 g of the fresh tuber (f.t.), with a mean of 1.19±0.64 mg/100 $g_{f.t.}$.

Organic-grown potatoes of Aladdin cultivar had an α-solanine content of 0.07 to 1.72 mg/100 g of the fresh tuber, with a mean of 0.62±0.95 mg/100 $g_{f.t.}$. According to these results, the amount of α-solanine found in organically grown samples is almost two times smaller than the amount of α-solanine determined from samples of conventionally grown potatoes (Table 1).

Table 1. Amount of α–solanine in analyzed potato samples Aladdin and Mona Lisa varieties

Variety of potato	System of cultivation	α-solanine content (mg in 100 g of fresh tuber)	The average α-solanine±S.D. (mg/100 $g_{f.t.}$)
Aladdin	Conventional	1.71	1.19±0.64
		0.47	
		1.38	
	Organic	0.08	0.62±0.95*
		1.72	
		0.07	
	Natural	1.76	1.62±0.33**
		1.25	
		1.86	
Mona Lisa	Conventional	1.45	1.35±0.19***
		1.12	
		1.45	
	Organic	2.96	1.40±1.35
		0.57	
		0.68	
	Natural	0.98	0.59±0.56
		0.20	
		0	

* the average α-solanine content (AαSC) of Aladdin variety was statistically smaller than the AαSC of Mona Lisa variety in organic cultivation ($p^*<0.05$);
** the AαSC of Aladdin variety was statistically higher than the AαSC of Mona Lisa variety in natural cultivation ($p^*<0.05$);
*** the AαSC was statistically higher than the AαSC of natural cultivation ($p^*<0.05$).

The content of α-solanine in samples of naturally grown potatoes ranged from 1.25 to 1.86 mg/100 $g_{f.t.}$. The mean of the measured concentrations of 1.62±0.33 mg/100 g $_{f.t.}$ is 1.36 times higher than that found in conventionally grown potatoes of the same variety.

Analysis of potato samples of the conventionally grown Mona Lisa variety found α-solanine content from 1.12 to 1.45 mg/100 $g_{f.t.}$ with an average content of 1.35±0.19 mg/100 $g_{f.t.}$ (Table 1). If this content is compared to an average amount of up to 1.40±1.35 mg/100 g of fresh organic-grown tubers, it may be observed that they do not differ significantly, while the average amount of α-solanine in naturally grown potatoes 0.59±0.56 mg/100 $g_{f.t.}$ was 2.3 times smaller.

The results obtained for α-solanine in samples of different varieties but with the same cultivation system were compared. The amount of α-solanine in Aladdin and Mona Lisa varieties of conventional cultivation does not differ significantly, while in organic farming the amount of α-solanine found in Aladdin variety is 2.3 times less than that found in the sam-

ples of Mona Lisa variety. Regarding natural cultivation, the amount of α-solanine in Aladdin samples was 2.7 times higher than that of Mona Lisa potatoes.

The results showed that the use of artificial insecticides caused a very intense biosynthesis of αS in the *Ala* variety by the conventionally cultivation system [AαSC 1.19 mg/100 $g_{f.t.}$] in comparison to the AαSC by the organically grown *Ala* (0.62 mg/100 $g_{f.t.}$) [it is close to the statistical significance, (p=0.08)]. It is difficult to explain the very high AαSC of naturally grown *Ala* (1.62 mg/100 $g_{f.t.}$).

Analysis of potatoes of the *MoL* variety showed that the AαSC of conventionally cultivation system (1.35 mg/100 $g_{f.t.}$) was statistically higher than the AαSC of naturally cultivation system (0.59 mg/100 $g_{f.t.}$) ($p^{*}<0.05$). Also, the AαSC of organically grown *MoL* (1.40 mg/100 $g_{f.t.}$) was higher than the AαSC of naturally grown *MoL*, but without statistical significance (p>0.05).

CONCLUSION

From the results obtained in this study, it could be observed that the use of artificial insecticides caused a rather intense biosynthesis of α-solanine in the conventionally grown Aladdin variety, compared to those organically grown. The average content of α-solanine was two times higher in conventionally vs organically grown young samples of potatoes of Aladdin variety.

The analysis of the potatoes of Mona Lisa variety showed that the average content of α-solanine in conventionally and organically grown potatoes did not differ. However, the average content of α-solanine in naturally grown potatoes is 2.3 times lower than that of conventionally grown potatoes.

Comparing the results obtained for α-solanine in samples of different varieties, but in the same cultivation system, we can see that the average content of α-solanine in both conventional varieties does not differ significantly. In a sample of organically grown potatoes, the average content of α-solanine found in the Aladdin variety is 2.3 times less than that found in the samples of the Mona Lisa variety. For naturally grown potatoes, the average content of α-solanine in Aladdin samples was 2.7 times higher than that of Mona Lisa potatoes.

The concentrations of α-solanine that were found in the case of conventional, organic and natural cultivation of potatoes are considered safe and such potatoes are suitable for consumption. However, because of a slight reduction in toxic α-solanine, it is recommended to consume organically grown potatoes (*Aladdin* variety) and naturally grown potatoes (*Mona Lisa* variety).

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