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SCIENTIFIC PAPER

633.491 + 66.094.941:547.421

## ACID AND ENZYMATIC HYDROLYSIS OF *Solanum tuberosum* L. GLYCOALKALOIDS – COMPARISON RESULTS

*A comparison of the results of the acid and enzymatic hydrolysis of GA from potato haulm and tuber sprouts is presented in this paper. Different systems were used for acid hydrolysis: monophasic liquid, two-phase liquid-liquid or solid-liquid and three-phase solid-liquid-liquid systems. Enzymatic hydrolysis was carried out by enzymes present in fresh haulm and fresh tuber sprouts and the fresh juice obtained from fresh plant material. The procedure of acid hydrolysis of GA from potato sprouts in a two-phase liquid-liquid system was selected as the optimal procedure for obtaining solanidine. The liquid-liquid system was composed of GA extract with hydrochloric acid as the first liquid phase and chloroform as the second liquid phase. A degree of GA hydrolysis of 96% and a solanidine yield of 1.50 g per 100 g of dried tuber sprouts after 90 minutes were then achieved.*

*Key words: Potato, Glycoalkaloids, Solanidine, Acid hydrolysis, Enzymatic hydrolysis.*

Potato (*Solanum tuberosum* L.) glycoalkaloids (GA) are potential toxic secondary metabolites of plants from the *Solanaceae* family [1–3]. In dried haulm the range of the GA content is 0.25–0.62%, while the content in dried sprouts is substantially higher: 0.56–5.03% [4,5]. By mineral acid [6–10] or enzymatic [11–13] hydrolysis, GA yield solanidine, an important precursor for the synthesis of hormones and some pharmacologically active compounds [14].

In this paper a comparison of the results of the acid and enzymatic hydrolysis of GA from potato haulm and tuber sprouts is presented. Different systems were used for acid hydrolysis: monophasic liquid, two-phase liquid-liquid [8,9] or solid-liquid [7] and three-phase solid-liquid-liquid systems, at boiling water and by a reflux condenser [10].

The monophasic system was composed of GA extract where the hydrochloric acid was added as a hydrolysis agent. The two-phase liquid-liquid system was composed of GA extract with hydrochloric acid as the first liquid system and organic solution as the second liquid phase, while the two-phase solid-liquid was composed of plant material as the solid phase and a mixture of solution hydrochloric acid in ethanol and chloroform as the liquid phase. The three-phase system had haulm or sprouts as the solid phase, a solution for glucoalkaloid extraction and hydrolysis as the first phase and organic solution for solanidine extraction as the second phase. By hydrolysis in two and three-phase systems, GA extraction from plant material, GA hydrolysis to solanidine and solanidine extraction by the organic phase were combined in a single step. This

approach can make the procedure for solanidine isolation simpler, faster and more economic. Enzymatic hydrolysis was carried out by enzymes present in the fresh plant material and the fresh juice obtained from fresh haulm and tuber sprouts. The purpose of this study was to define the optimal procedure for solanidine isolation, by comparing the results of the degree of GA hydrolysis (DH) and the yield of solanidine (YS).

### EXPERIMENTAL SECTION

**Plant material.** The potato (*Solanum tuberosum* L.) cv. Désirée haulms were harvested in mid-July, dried at room temperature in trays for 21 days, and milled to an average particle size of 0.14 mm. The content of GA was 0.53 g per 100 g and the ratio of  $\alpha$ -solanine to  $\alpha$ -chaconine was 1.27:1. The tuber sprouts were grown at 20–22°C for 60 days in the dark. They were dried at room temperature and milled to an average particle size of 0.21 mm. The content of GA was 3.38% and the ratio of  $\alpha$ -solanine to  $\alpha$ -chaconine was 1.12:1. The corresponding maximal yield of solanidine which could be obtained was 0.24 and 1.57 g per 100 g of dried haulm and potato sprouts, respectively (determined by the ratio of  $\alpha$ -solanine to  $\alpha$ -chaconine considering that 1 mole of  $\alpha$ -solanine or  $\alpha$ -chaconine yields 1 mole of solanidine).

### Acid hydrolysis

**Monophasic system.** The extracts of GA were obtained by extraction from 20 g of haulm or tuber sprouts with 50% volume methanol and 96% volume ethanol, applying the plant material to a solution ratio of 1:20 and 1:10 (w/v), respectively. The extractions were carried out by using a reflux condenser, at the water bath boiling temperature. The reaction temperature was 80±2°C in ethanolic and 83±2°C in methanolic solution. Samples of the extract were taken after 120 minutes from each flask and filtered. The filtered extract (3 mL)

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Paper received: September 29, 2005  
Paper accepted: July 10, 2006

was hydrolyzed by adding 36% (w/v) hydrochloric acid using a reflux condenser to concentrations of 10 and 2% w/v, at the water bath boiling temperature, for 10, 15, 30, 45, 60, 90 and 120 minutes. The content of solanidine was then determined.

**Content of solanidine.** The value of the pH of the appropriate solution was adjusted to 4.0 by adding sodium hydroxide solution (at first by 50 and then 1% w/v). The solutions were transferred to a separatory funnel for the complex formation with methyl-orange. The coloured complex was extracted by chloroform, 5 times by 5 mL, dried with anhydrous sodium-sulfate and its volume was adjusted to 25 mL. The absorbance of the extract was measured at 420 nm (UV-Vis Spectrophotometers, Lambda V Perkin Elmer) and the content of solanidine was determined on the standard curve [16]. In the organic liquid phase (chloroform, trichloroethylene or carbon tetrachloride), for solanidine content determination, aliquots of 5 mL were evaporated to dry under vacuum and the dry residue dissolved in 10 mL of 2% w/v acetic acid by using a reflux condenser, at the water bath boiling temperature. The pH of the solutions was then adjusted to 4.0 and the content of solanidine determined as before.

### Two-phase system

**Solid-liquid system.** Dried and milled haulm and tuber sprouts were treated by a mixture consisting of 10% w/v hydrochloric acid in 96% ethanol and chloroform in a volume ratio of 2:3 and 1:1, respectively. The flasks were placed in a bath with boiling water and connected to a reflux condenser. Aliquots of 1 mL of filtered liquid phase were taken at 10, 15, 30, 45, 60, 90 and 120 minutes and filtered in order to determine the solanidine content.

**Liquid-liquid system.** The extracts of GA from haulm and tuber sprouts, obtained by using a monophasic system were evaporated under vacuum to half of their volume and diluted by distilled water. Hydrochloric acid was then added to the concentration of 10 and 2% w/v and chloroform was also added in a volume ratio of 1:1. The flasks were placed in a water bath at the boiling temperature and connected with a reflux condenser. Aliquots of 5 mL of the organic liquid phase were taken after 10, 15, 30, 45, 60, 90 and 120 minutes, from each flask and the content of solanidine was determined.

**Three-phase solid-liquid-liquid system.** Dried and milled potato haulm and tuber sprouts were treated with 10% w/v hydrochloric acid in 50% vol. aqueous methanol and chloroform, in a volume ratio to the acid phase of 1:1, was added. The flasks were placed in the bath with boiling water and connected with a reflux condenser. The temperature of the lower layer was approx. 65°C and the temperature of the upper layer was approx. 95°C. Aliquots of 1 mL of the organic liquid phase were taken at 10, 15, 30, 45, 60, 90 and 120

minute intervals, and from each flask the content of solanidine was determined.

### Enzymatic hydrolysis

**Fresh haulm.** Fresh and milled haulm and sprouts were treated at 35 and 37°C, respectively. After a certain incubation time (12–96 h), 1 g of fresh incubated plant material was taken. Acetic acid aqueous solution (10 mL, concentration of 2% w/v) was added and the obtained solanidine extracted at room temperature by stirring at 200 min<sup>-1</sup>, for 60 minutes. The pH of the extract was adjusted to 4.0 and the content of solanidine was determined as before.

**Juice of fresh haulm.** The juice was obtained after pressing fresh and frozen haulm or tuber sprouts through linen (200 mL) and treated at 35 and 37°C, respectively. After a period of incubation (12 to 96 h), 1 mL of juice was taken. The 2% w/v aqueous acetic acid solution (9 mL) was added. The pH of the solution was adjusted to 4.0 and the content of solanidine was determined.

**TLC analysis.** Aliquots of the appropriate liquid phase (0.03 mL) were applied to the 20x20 cm plates, 120 µm thick Silica gel G 60 (Merck reagents). The plates were developed to the height of 16 cm, with the lower layer of mixture of methanol-chloroform-1% ammonium hydroxide (50:50:25 v/v). The spots were visualized by treating with the 50% aqueous sulfuric acid and heating at 110°C for 30 minutes [17].

### RESULTS

The comparison results of GA hydrolysis and solanidine extraction are presented in Table 1. The degree of GA hydrolysis (DH) or the degree of GA enzymatic hydrolysis (DEH) were expressed as the ratio of the solanidine content in a certain extract to the maximal yield of solanidine which could be achieved from the plant material. The maximal yield of solanidine which could be achieved from the plant material was calculated according to the GA content and the ratio of  $\alpha$ -solanine and  $\alpha$ -chaconine in the plant material, considering that 1 mol of  $\alpha$ -solanine or  $\alpha$ -chaconine yields 1 mol of solanidine.

The results of the kinetics of extraction of solanidine in a two-phase liquid-liquid system by the optimal first phase and chloroform, trichloroethylene or carbon tetrachloride as second liquid phase were shown in Figure 1.

GA in fresh haulm and in juice from fresh haulm were fermented at 35°C, while fresh sprouts and juice from the sprouts were fermented at 37°C. The results of the kinetics of enzymatic hydrolysis are shown in Figure 2 and Table 2.

During GA hydrolysis different bonds in the molecule of GA can be cleft and different intermediate products such as  $\beta_2$ -chaconine,  $\gamma$ -solanine and  $\gamma$ -chaconine are obtained. The results of the TLC

Table 1. Maximally achieved DH of GA, time of hydrolysis, YS and S/TEM in different systems for GA acid hydrolysis

System	DH (%)	Time (min)	YS	S/TEM (g x 10 <sup>-3</sup> /g)
MONO-PHASE: Haulm: 10% w/v HCl in 96% vol. ethanol Sprouts: 2% w/v HCl in 50% vol. methanol	93.0 96.0	30 60	0.23 <sup>1</sup> 1.50 <sup>1</sup>	0.89 7.18
TWO-PHASE (solid-liquid): Haulm: 10% w/v HCl in mixture of 96% vol. ethanol and chloroform (2:3 v/v) Sprouts: 10% w/v HCl in mixture of 96% vol. ethanol and chloroform (1:1)	v/v)	84.8 82.5	120 120	0.21 <sup>1</sup> 1.29 <sup>1</sup>
TWO-PHASE (liquid-liquid): Haulm: 10% w/v HCl in 96% vol. ethanol-chloroform Sprouts: 2% w/v HCl in 50% vol. methanol-chloroform	93.1 96.0	120 90	0.19 <sup>1</sup> 1.50 <sup>1</sup>	53.03 458.18
THREE-PHASE (solid-liquid-liquid): Haulm: 10% w/v HCl in 50% vol. methanol-chloroform Sprouts: 10% w/v HCl in 50% vol. methanol-chloroform	98.6 65.0	90 60	0.24 <sup>1</sup> 1.02 <sup>2</sup>	42.5 364.64

<sup>1</sup> – g/100 g of dried plant material, <sup>2</sup> – g/g of dried plant material

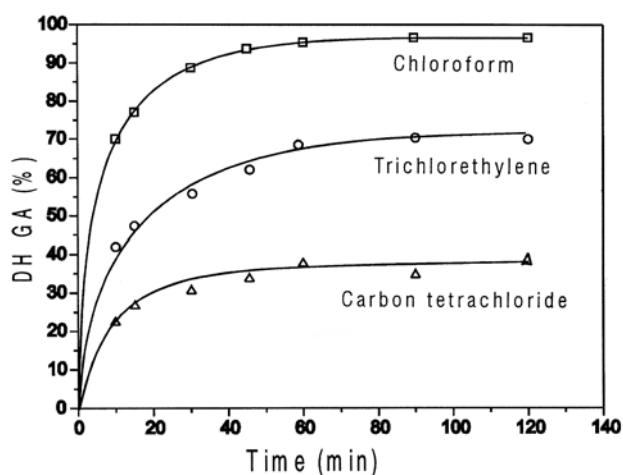


Figure 1. Kinetics of solanidine extraction in a two-phase liquid-liquid system by 2% w/v HCl in 50% vol. methanolic extract of GA from sprouts as the first phase and chloroform, trichloroethylene or carbon tetrachloride as the second liquid

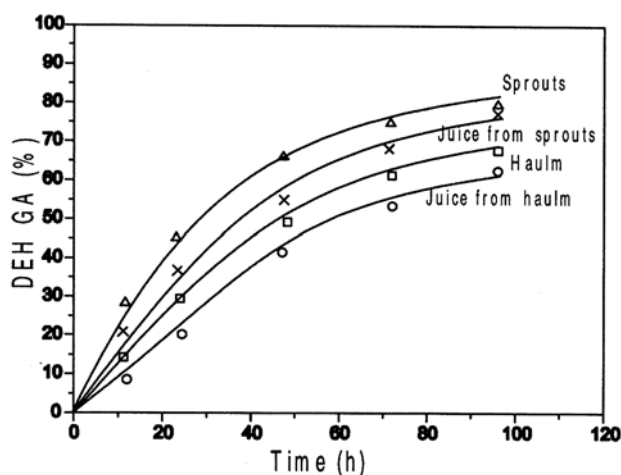


Figure 2. Kinetics of GA enzymatic hydrolysis during fermentation at 35°C (fresh haulm and juice from fresh haulm) and at 37°C (fresh sprouts and juice from fresh sprouts).

Table 2. Maximally achieved DEH and YS achieved during fermentation of GA in fresh plant material and juice from the fresh plant material

Plant material	DEH (%)	Time (h)	YS <sup>(1)</sup>	YS <sup>(2)</sup>
Haulm	70.0	96	0.02	0.17
Juice from haulm	61.5	96	0.02	0.15
Sprouts	81.5	96	0.29	1.28
Juice from sprouts	76.5	96	0.64	1.20

YS<sup>(1)</sup> – g per 100 g of fresh plant material or 100 mL of juice

YS<sup>(2)</sup> – g per 100 g of dried plant material

analysis of the pathway of GA acid and enzymatic hydrolysis are shown in Figure 3.

## DISCUSSION

**Acid hydrolysis.** Based on the results of the acid GA hydrolysis in different systems (Table 1) the maximal achieved DH of GA and DEH of GA was higher than 90%, except in the two-phase solid-liquid system when the DH was 84.8% and 82.5%, for GA hydrolysis from haulm and sprouts, respectively and in three-phase system when the DH of hydrolysis GA from the sprouts was 65%.

The maximal yield of solanidine was achieved in a three-phase system for hydrolysis GA from haulm (0.24 g per 100 g of dried plant material) and in two-phase liquid-liquid system for GA hydrolysis from sprouts (1.50 g per 100 g of dried plant material). Maximal contents of solanidine of 53 x 10<sup>-3</sup> and 458 x 10<sup>-3</sup> g per g of total extractive matters of the hydrolysate from haulm and sprouts, respectively, were achieved by using a two-phase liquid-liquid system.

**Enzymatic hydrolysis.** The enzymatic kinetic curves show that by increasing the incubation time up to 96 h, the DEH also increases. The highest DEH of 81.5% was achieved with fresh sprouts, after 96 hours of incubation. The corresponding solanidine yields are

Table 3. Results of TLC analysis

Product	Time period of appearance (h)	Acid hydrolysis	Enzymatic hydrolysis			
			Fresh haulm	Juice from haulm	Fresh sprouts	Juice from sprouts
$\beta_2$ -chaconine	0-48	-	+	+	-	-
	48-120	-	+	-	+	+
$\gamma$ -solanine	0-48	-	-	-	-	-
	48-120	-	-	+	-	+
$\gamma$ -chaconine	0-48	-	-	-	-	-
	72-120	-	-	+	-	+
Solanidine	0-3	+	+	+	+	+
	3-120	-	+	+	+	+

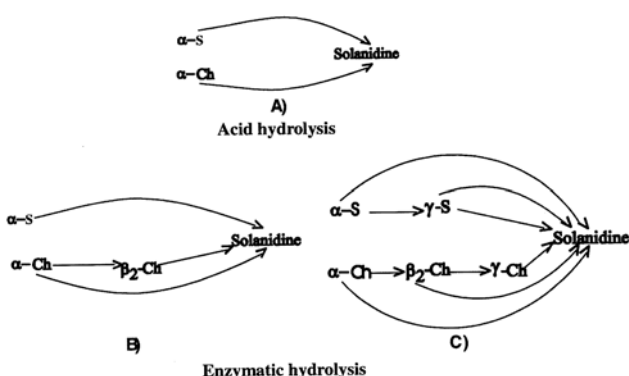


Figure 3. Products of acid (A) and enzymatic GA hydrolysis (B – fresh plant material; C – juice from fresh plant material)

presented in Table 2. The yields were calculated considering that 100 g of fresh haulm and sprouts yield 11 g and 23 g of dried haulm and tuber sprouts and 80 and 44 mL of juice, respectively.

**Products of hydrolysis.** GA hydrolysis by mineral acid or by enzymes, depends on the hydrolysis condition. Different glycosidic bond in molecule of glycoalkaloid can be cleaved and different intermediate products are obtained. By TLC analysis, the  $R_f$  value for  $\beta_2$ -chaconine was 0.55, for  $\gamma$ -solanine 0.67 and for  $\gamma$ -chaconine 0.71, while solanidine had a  $R_f$  value of 0.96. The results of TLC analysis, the products and retention times of the products, are shown in Table 3. During GA acid hydrolysis in all the applied systems,  $\alpha$ -solanine and  $\alpha$ -chaconine are directly hydrolyzed to solanidine, without the forming of intermediate products and without the degradation of solanidine to solanthrene (Figure 3A). During enzymatic GA hydrolysis some  $\beta$ - and  $\gamma$ -forms of intermediate products are identified showing that GA hydrolysis is gradual. Thus, during GA hydrolysis in fresh haulm and fresh sprouts (Figure 3B),  $\alpha$ -solanine is directly hydrolyzed to solanidine, while chaconine is hydrolyzed via  $\beta_2$ -chaconine. During enzymatic GA hydrolysis in the juice of haulm and sprouts  $\alpha$ -solanine is hydrolyzed via  $\gamma$ -solanine, while chaconine is degraded via the  $\beta_2$ - and  $\gamma$ -form and without the formation of solanthrene (Figure 3C). Solanthrene was not detected as a degradation product

of enzymatic GA hydrolysis in fresh haulm and fresh sprouts and in the juice from fresh plant material.

Solanidine isolated from the organic liquid phase of the two-phase system, by GC/MS analysis, shows a parent peak at 397 supporting the solanidine molecular formula and molecular mass of 397.3345. Fragments at  $m/z$  204 and  $m/z$  150 are also characteristic for the fragment formulas of  $C_{14}H_{22}N$  and  $C_{10}H_{16}N$ , respectively [18,19]. The IR spectrum had for major functional groups at  $3435\text{ cm}^{-1}$  for  $\nu$  (OH),  $2917\text{ cm}^{-1}$  and  $2849\text{ cm}^{-1}$  for  $\nu$  (CH),  $1632\text{ cm}^{-1}$  for  $\nu$  (C=C),  $1463\text{ cm}^{-1}$  for  $\delta$  (CH),  $1378\text{ cm}^{-1}$  for  $\nu$  (CN) vibrations, bands at  $1261\text{ cm}^{-1}$ ,  $1164\text{ cm}^{-1}$ ,  $1032\text{ cm}^{-1}$  of  $\gamma$  (C-O) and  $\gamma$  (C-C).

## CONCLUSION

The procedure of the acid hydrolysis of GA from potato sprouts in a two-phase liquid-liquid system was chosen as the optimal. One the liquid-liquid system was composed of GA extract with hydrochloric acid as the first liquid phase and chloroform as the second liquid phase. Then, a degree of GA hydrolysis of 96% after 90 minutes and a solanidine yield of 1.50 g per 100 g of dried tuber sprouts were achieved. During acid hydrolysis,  $\alpha$ -solanine and  $\alpha$ -chaconine are directly hydrolyzed to solanidine, without intermediate products and without the degradation of solanidine to solanthrene. By enzymatic GA hydrolysis a lower DHE was obtained (81.5%) and the procedure is long (96 h). During enzymatic GA hydrolysis  $\beta_2$ -chaconine and the  $\gamma$ -form of solanine and chaconine are identified showing gradual GA hydrolysis.

## ACKNOWLEDGEMENTS

This work was supported under the project TR-6708 B by the Ministry of Science and Environmental Protection of the Republic of Serbia, Serbia and Montenegro.

## NOTATION

- GA – glycoalkaloids
- DH – degree of GA acid hydrolysis (%)
- DEH – degree of GA enzymatic hydrolysis (%)
- YS – yield of solanidine (g/100 g, g/g, g/100 mL)
- S/TEM – solanidine content in the total extractive matter ( $\text{gx}10^{-3}/\text{g}$ )

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## IZVOD

KISELA I ENZIMSKA HIDROLIZA GLIKOALKALOIDA *Solanum tuberosum* L.  
– UPOREDNI PREGLED REZULTATA

(Naučni rad)

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Glikoalkaloidi krompira (*Solanum tuberosum* L.) su potencijalno toksični sekundarni metaboliti biljaka familije *Solanaceae* koji sadrže azot. U suvom nadzemnom delu sadržaj glikoalkaloida je u granicama 0,25–0,62%, dok je sadržaj u suvim klicama krompira znatno veći i iznosi 0,56–5,03%. Hidrolizom glikoalkaloida dejstvom rastvora mineralnih kiselina ili enzimskom hidrolizom dobija se solanidin, važan prekursor u sintezi hormona i farmakološki aktivnih jedinjenja. U radu je dat uporedni pregled rezultata kisele i enzimske hidrolize glikoalkaloida iz nadzemnog dela i klica krompira. Kisele hidroliza je realizovana u različitim sistemima: jednofaznom tečnom, dvofaznom tečno–tečnom i čvrsto–tečnom i trofaznom čvrsto–tečno–tečnom sistemu. Enzimska hidroliza je ispitivana dejstvom enzima u svežem nadzemnom delu i svežim klicama i soku dobijenom ceđenjem svežeg nadzemnog dela i klica. Kao optimalan postupak za dobijanje solanidina izdvojen je postupak kisele hidrolize glikoalkaloida iz klica krompira u dvofaznom tečno–tečno sistemu (ekstrakt glikoalkaloida sa hlorovodoničnom kiselinom/hloroform). Primenom ovog sistema ostvaren je stepen hidrolize glikoalkaloida od 96% za 90 minuta i prinos od 1,50 g solanidina iz 100 g suvih klica krompira. Kisele hidroliza  $\alpha$ -solanina i  $\alpha$ -čakonina do solanidina odvija se direktno bez stvaranja intermedijernih produkata i bez procesa degradacije solanidina u solantren. Enzimskom hidrolizom ostvaren je niži stepen hidrolize (81,5%), a proces inkubacije je dugotrajan (96 sati). Enzimska hidroliza  $\alpha$ -solanina i  $\gamma$ -čakonina je postepena uz stvaranje  $\gamma$ -solanina i  $\beta_2$ - i  $\gamma$ -čakonina kao intermedijernih proizvoda.

Ključne reči: Krompir, Glikoalkaloidi, Solanidin, Kisele hidroliza, Enzimska hidroliza.