Kinetics of solanidine hydrolytic extraction from potato (*Solanum tuberosum* L.) haulm in solid-liquid systems

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Abstract: Dried and milled haulm of potato (*Solanum tuberosum* L.) was used as the solid phase. An ethanolic solution of hydrochloric acid mixed with chloroform in different volume ratios was the liquid phase. The aim of paper was to unite in a single step the processes of glycoalkaloids extraction from haulm, their hydrolysis to solanidine and the extraction of solanidine. This could make the procedure of obtaining solanidine faster and simpler. The best degree of solanidine hydrolytic extraction of 84.5 % was achieved using 10 % w/v hydrochloric acid in 96 % vol. ethanol mixed with chloroform in a volume ratio of 2:3, after 120 min of hydrolytic extraction.

Keywords: potato, haulm, glycoalkaloids, hydrolysis, solanidine, extraction.

INTRODUCTION

Plants of the Solanum genus contain glycoalkaloids (GA) which are present as secondary plant metabolites.[1] GA are class of nitrogen containing steroid glycosides.[2] They are synthesized under the conditions of bud activity and stress, such as exposure to light and mechanical injury.[3–4] In the haulm of potato (*Solanum tuberosum* L.), glycoalkaloids are normally present in the concentration range 0.25–0.62 %.[5] There are two major glycoalkaloids, α-solanine and α-chaconine[6–7] which represent more than 95 % of the total GA.[8] The carbohydrate moiety (solatriose in α-solanine and chacotriose in α-chaconine) is attached to the 3-OH position of the aglycon.[9] By hydrolysis of glycoalkaloids with mineral acid, the 3β-*O*-glycosidic bond is cleft yielding aglycon solanidine (Fig. 1).[8]

Solanidine is an important precursor for hormone synthesis.[5,9] Chemical transformations of solanidine for obtaining 16-dehydropregnenolone acetate, the key-intermediate in the industrial synthesis of progesterone and cortisone derivatives are already presented in the literature.[10]

In literature the procedures for solanidine isolation from potato haulm and sprouts are known, mainly by hydrolysis of GA with mineral acid solutions, for analytical purposes.[11–13] There are less data for solanidine isolation from fermented plant material after

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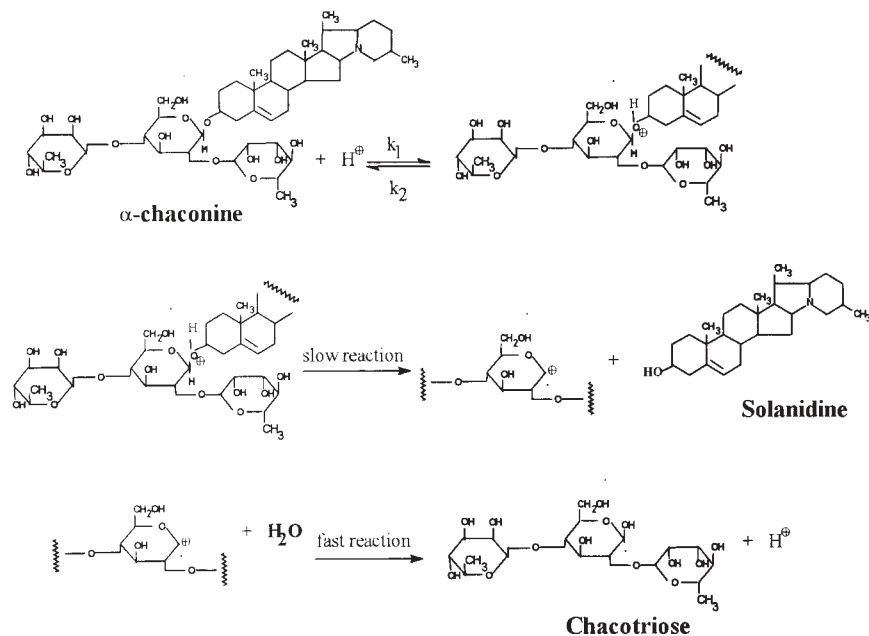
Fig. 1. The solanidine obtained by mineral acid hydrolysis of α-chaconine.

hydrolysis of GA by specific enzymes from the plant material. All of these procedures require several phases: extraction of GA from the plant material, separation of extracts, GA hydrolysis in the extracts and organic liquid phase re-extraction of solanidine and then its isolation. In case of acid hydrolysis, the process of GA acid hydrolysis can be carried out during GA extraction from the plant material (process of hydrolytic extraction). In case of enzymic hydrolysis, it takes several tens of hours for fermentation of plant material, and solanidine has to be extracted from the fermented plant material, re-extracted with an organic solution and then isolated. Typically the degree of enzymic GA hydrolysis is lower than the degree of GA hydrolysis by mineral acid.

A detailed procedure for the preparative isolation of solanidine from potato sprouts by the simultaneous extraction of GA from sprouts and their hydrolysis with 1.8 mol/dm^3 hydrochloric acid in methanol was described by Gaši *et al.* (1984).[14] After chloroform extraction, purification in a column with aluminum oxide, and recrystallization from ethanol, solanidine was obtained in a yield of 0.47 % based on dry potato sprouts (87.7 % of theoretical yield). Stanković *et al.* (1994)[15] isolated solanidine from potato haulm and sprouts after fermenting the plant material for 50 h, and by boiling the fermented plant material in carbon tetrachloride. A solanidine yield of approximately 55 and 65 % from haulm and tuber sprouts, respectively, was obtained (calculated as % of the amount of solanidine extracted from the plant material). However, a detailed procedure for the isolation of solanidine from potato haulm as the secondary product in the production of this culture, was not found in the available literature.

This research concerns the kinetics of solanidine hydrolytic extraction in two-phase,

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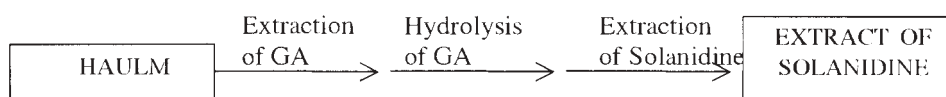of GA of GA of Solanidine SOLANIDINE

HAULM

Fig. 2. The model of solanidine hydrolytic extraction using solid-liquid systems.

solid-liquid systems, with the aim of determining the optimal system for solanidine extraction. The model of solanidine hydrolytic extraction in solid-liquid systems in shown in Fig. 2.

In these systems, the processes of glycoalkaloids extraction, their hydrolysis to solanidine and the extraction of solanidine were united in a single step, making the procedure of obtaining solanidine faster, simpler and more economic. The presence of chloroform in the liquid phase reduces the reaction of solanidine transformation into solanthrene,[16] and enables a more selective extraction of solanidine in comparison to a solution of hydrochloric acid in 96 vol. % ethanol without chloroform, where increased quantities of hydrophilic substances are extracted from the plant material. This procedure enables the easier purification of the extracts and the obtaining of a more pure solanidine.

A series of investigations was planned regarding the kinetics of GA hydrolysis from potato haulm and sprouts using different liquid and solid-liquid systems, the results of which would enable a comparison and choice of the optimum system of obtaining solanidine.

EXPERIMENTAL

Plant material

The haulm of potato cv. Désirée was harvested in mid July, dried at room temperature in trays for 21 days, and milled to an average particle size 0.14 mm. The moisture content was obout 10 %.

Solanidine hydrolytic extraction

The dried and milled haulm (40 g) was treated with 800 mL of a mixture consisting of 2, 5 and 10 % w/v hydrochloric acid in 96 vol. % ethanol and chloroform in separate flasks. The volume ratio of hydrochloric acid in 96 vol. % ethanol and chloroform in the mixture was 2:1, 1:1, 3:2 and 4:1. The flasks with reflux condenser were placed in a bath with boiling water. Aliquots of 1 mL of the liquid phase were taken at 10, 15, 30, 45, 60, 90 and 120 min intervals, from each flask and the content of solanidine was determined.

Content of solanidine

The corresponding liquid phase was evaporated to dryness under vacuum. The dry residue was dissolved in 10 mL of 2 % w/v aqueous acetic acid. The pH of the solutions was adjusted to 4.0 by adding aqueous sodium hydroxide (first 50 and then 1 % w/v). The solutions were transferred to a separatory funnel for the formation of the complex with methyl-orange, as described by Tuckalo and Tsarik.[13] The coloured complex was extracted with chloroform (5 times by 5 mL), dried with anhydrous sodium sulphate and its volume adjusted to 25 mL. The absorbance of the extract was measured at 420 nm (UV-Vis Spectrophotometers, Lambda V Perkin Elmer). The content of solanidine was determined from the standard curve.

TLC analysis

A 0.03 mL aliquot of the liquid phase obtained after 10, 15, 30, 45, 60, 90 and 120 min was applied to 2×20 cm plates, 120 μm thick Silica gel G 60 (Merck). The plates were developed to a height of 16 cm with a

mixture of methanol-chloroform-1 % ammonium hydroxide (50:50:25 v/v). The spots were visualized by treatment with 50 % aqueous sulphuric acid and heating at 110 ºC for 30 min.

RESULTS AND DISSCUSION

The maximal achieved degree of solanidine hydrolytic extraction (DHE), the hydrolytic extraction time, yield of solanidine, yield of total extracted matter and the content of solanidine in the total extracted matter, using different solid-liquid systems, are given in Table I.

TABLE I. Maximal achieved DHE of solanidine[1] (%), hydrolytic extraction time[2] (minutes), yield of solanidine[3] (g per 100 g of dried and milled haulm), yield of total extractive matter[4] (%) and content of solanidine in the total extractive matter[5] (mg/g) in solid-liquid systems

Concentration of HCl in ethanol % (w/v)	Ratio of HCl ethanol solution and chloroform in the liquid phase (v/v)			
	2:3	1:1	3:2	4:1
2	52.2[1]	60.5	52.0	32.8
	40[2]	120	60	120
	0.13[3]	0.15	0.13	0.08
	0.18[4]	0.48	0.52	0.63
	35.28[5]	15.42	12.21	6.35
5	57.8	62.0	57.4	63.1
	120	30	30	120
	0.14	0.15	0.14	0.15
	0.26	0.57	0.62	0.69
	27.11	13.25	11.29	11.16
10	84.5	35.0	27.5	24.1
	120	45	30	30
	0.23	0.09	0.06	0.06
	0.28	0.63	0.67	0.73
	40.18	6.75	4.78	4.04

The DHE is expresed as the ratio of the solanidine content in the liquid phase after a certain hydrolysis time to the maximal yield of solanidine which could be achieved from the used plant material. The maximal yield of solanidine which could be achieved was calculated according to the glycoalkaloids content and the ratio of α-solanine and α-chaconine in the haulm, considering that one mol of α-solanine or α-chaconine yields one mol of solanidine. The yield of solanidine was calculated according to the DHE and the maximal possible yield of solanidine.

During the process of hydrolytic extraction of solanidine by 2 % w/v HCl in ethanol mixed with chloroform in the volume ratio of 1:4 and 2:3, and by 5 % w/v HCl in ethanol mixed with chloroform in the volume ratio of 1:1 and 3:2, the presence of solanthrene, a product of dehydratation reaction of solanidine was detected by TLC after the periods of hydrolytic extraction given in Table I. Then, the content of solanidine in the extract, consequently, decreased and the DHE of GA was reduced.
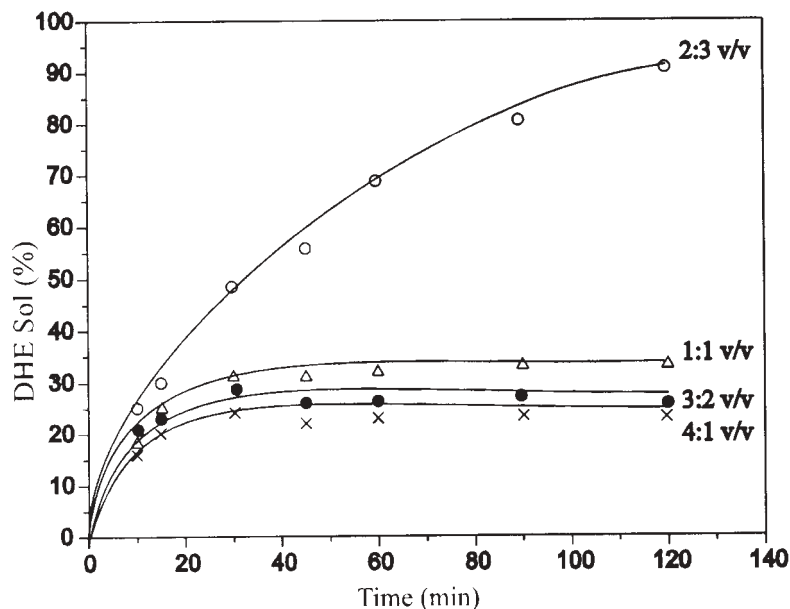
Fig. 3. The variation of the degree of solanidine hydrolytic extraction (DHE Sol, %) of haulm using solid-liqud systems with 10 % w/v HCl in 96 vol. % ethanol mixed with chloroform in different volume ratios, versus the hydrolytic extraction time.

The variations of the DHE with time for extraction by 10 % w/v HCl in 96 % vol. ethanol mixed with chloroform in different volume ratios, are shown in Fig. 3.

The best DHE of 84.5 % was achieved by 10 % w/v hydrochloric acid in 96 % vol. ethanol mixed with chloroform in the volume ratio of 2:3, after 120 min of hydrolytic extraction. During this process, solanthrene was not detected by TLC. The chloroform in the liquid phase of the optimal solid-liquid system protects solanidine from dehydration to solanthrene.[16]

The yield of solanidine depends on the DHE and the best yield of 0.23 g per 100 g of dried and milled haulm is achieved using the same system that gave best DHE achieved. The content of solanidine in the total extracted matters was also the best when this system was used. Its value is 40.18 mg per g of total extracted matter. This system was chosen as the optimal solid-liquid system.

By using a program for symbolic mathematical calculations, Maple V Release 4.00a (Waterloo Maple Inc.), the following equation for calculating the DHE (%), depending on the hydrolytic extraction time, t (min), according to kinetic curve for optimal system, was obtained:

$$DHE = 40 + 37.1 \times 10^{-2}\, t$$

According to this equation, the equations for calculating the yield of solanidine, q_S (g solanidine per 100 g dried and milled haulm), and the concentration of solanidine in the extract, C_S (mg/cm^3), in dependence on the hydrolytic extraction time are:

$$q_S = 9.76 \times 10^{-2} + 1.07 \times 10^{-3}\, t$$

$$C_S = 4.8 \times 10^{-2} + 5.26 \times 10^{-4}\,t$$

The variation of the rate of solanidine hydrolytic extraction calculated as mol solanidine per dm³ in second, in the optimal solid-liquid system with chloroform in liquid phase and the rate of solanidine hydrolytic extraction in a solid-liquid system without mixing the ethanolic solution of HCl with chloroform, versus the hydrolytic extraction time, are presented in Fig. 4. The variation of the corresponding yield of solanidine versus the hydrolytic extracton time are also presented in this Figure. The acid hydrolysis of GA in solid-liquid systems without chloroform in liquid phase depends on the hydrolysis conditions such as temperature, the concentration of mineral acid, the solvent, the hydrolytic time *etc.* and different intermediate products of α-chaconine are possible. Very often, aglycon solanidine was lost due to its conversion into solanthrene.[16]
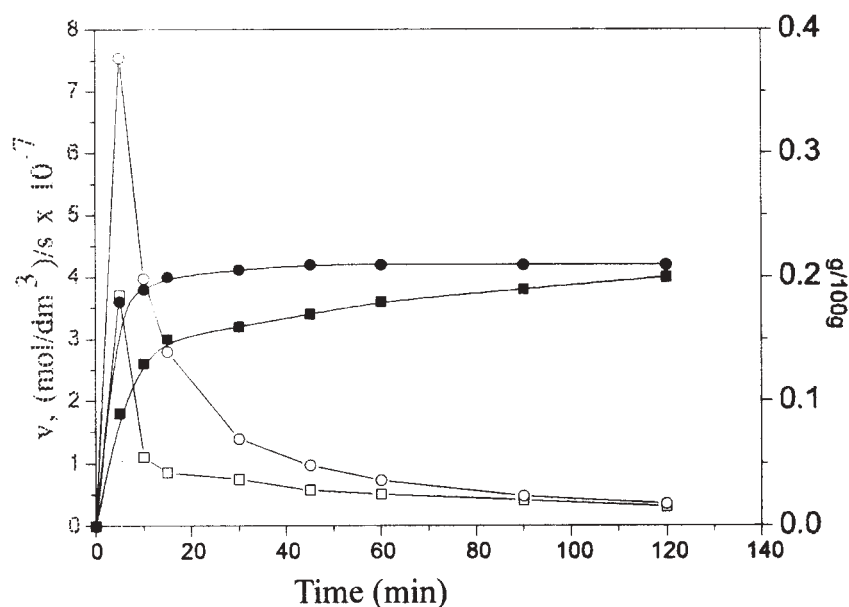

Fig. 4. The variation of the rate of solanidine hydrolytic extraction using the optimal solid-liquid system with chloroform in the liquid phase (-□-) and without chloroform in the liquid phase (-o-) versus the hydrolytic extraction time and the corresponding yield of solanidine (-■-,-●-).

From Fig. 4, it can be seen that the highest rate of solanidine hydrolytic extraction was achieved after 5 min of hydrolytic extraction. Its value was 7.5×10^{-7} mol solanidine per dm³ in second in the solid-liquid system without chloroform in the liquid phase and 3.7×10^{-7} mol solanidine per dm³ in second in the system with chloroform in the liquid phase.

The rate of solanidine hydrolytic extraction in the solid-liquid system after 5 min of hydrolytic extraction is nearly two times less when chloroform is present than without. After 90 min of hydrolytic extraction, the rates of solanidine hydrolytic extraction in these systems are equal, as is the case after 120 min extraction.

CONCLUSION

The best degree (84.5 %) of solanidine hydrolytic extraction in solid-liquid systems was achieved using 10 % w/v hydrochloric acid in 96 vol % ethanol mixed with chloroform in the volume ratio of 2:3, after 120 min of hydrolytic extraction. During this process, solanthrene was not detected, and the entire content of the obtained solanidine was extracted by the liquid phase. Chloroform in the liquid phase of the optimal solid-liquid system protects solanidine from dehydration to solanthrene. The yield of solanidine depends on the DHE and the best yield was achieved using the system which produced the best DHE. The content of solanidine in the total extracted matters was also the best when this system was used.

ИЗВОД

КИНЕТИКА ХИДРОЛИТИЧКЕ ЕКСТРАКЦИЈЕ СОЛАНИДИНА ИЗ НАДЗЕМНОГ ДЕЛА КРОМПИРА (*Solanum tuberosum* L.) У ЧВРСТО-ТЕЧНИМ СИСТЕМИМА

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У раду је испитана кинетика хидролитичке екстракције соланидина у двофазним, чврсто-течним системима. Суви, самлевени надземни део кромпира (*Solanum tuberosum* L.) коришћен је као чврста фаза, а етанолни раствор хлороводоничне киселине помешан са хлороформом у различитим запреминским односима као течна фаза. Циљ рада је био да се процес екстракције гликоалкалоида из биљног материјала, њихова хидролиза до соланидина и екстракција соланидина течном фазом обједини у један процес. На овај начин поступак добијања соланидина је једноставији, бржи и економичнији. Најбољи степен хидролитичке екстракције соланидина од 84,5 %, остврен је применом раствора 10 % m/v хлороводоничне киселине у 96 вол.% етанолу помешаног са хлороформом у запреминском односу 2:3 после 120 min.

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