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Analysis of the potato glycoalkaloids by using of enzyme biosensor based on pH-ISFETs☆

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Abstract

The applicability of an enzyme biosensor based on pH-ISFETs for direct determination of total glycoalkaloids content in real potato samples, without any pre-treatment, is shown. The results of determination of the total glycoalkaloids concentrations in potato samples from different experimental varieties obtained by the biosensor are well correlated with the analogous data obtained by the HPLC method with standard complex sample pre-treatment procedure. The detection of total glycoalkaloids content by biosensors is reproducible, the relative standard deviation was around 3%. The dependence of total glycoalkaloids content on various parts of the potato tuber and their size, different growing area has been shown using the biosensor developed.

The method based on biosensors is cheap, easy to operate and requires a shorter analysis time than the one needed using traditional methods for glycoalkaloids determination. The biosensor can operate directly on potato juice, or even directly on a suspension of potato or plant material. It can provide a way to save time and costs, with a possibility of taking rapid assessment of total glycoalkaloids content in a wide variety of potato cultivars. Furthermore the operational and storage stability of this biosensor are quite good with a drift lower than 1% per day and response being stable for more than 3 months.

Keywords: Biosensor; ISFET; Butyryl cholinesterase; Glycoalkaloids; Potatoes

1. Introduction

One of the world's major agricultural crops, the cultivated potato (*Solanum tuberosum* L.) is consumed daily by millions of people from diverse cultural background. Potatoes are grown in the almost 80% of all countries, and worldwide production stands in excess of 350 million tonnes per annum, a figure exceeded only by wheat, maize and rice [1]. Despite its status as a food of first importance, the potato tuber contains toxic glycoalkaloids that cause sporadic out-breaks of poisoning in humans [2]. Glycoalkaloid poisoning elicits a wide variety of symptoms – ranging from gastrointestinal disorders, through confusion, hallucination and partial paralysis to convulsion, coma and death – but is thought to stem from one or both of two quite distinct modes of action. The first is inhibition of the enzyme acetylcholinesterase, which allows hydrolysis of the neurotransmitter acetylcholine, a key process in nerve impulse conduction across cholinergic synapses [3]. Neurological symptoms such as weakness, confusion and depression, which have been noted in patients suffer-

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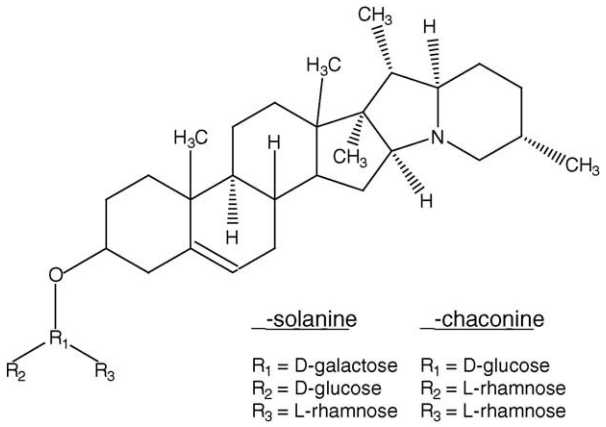


Fig. 1. Molecular structures of α-solanine and α-chaconine. Solanidine consists of a steroidal backbone without attached sugar moieties.

ing from glycoalkaloid poisoning, are likely manifestations of this anti-acetylcholinesterase activity [4]. The other major biological action of glycoalkaloids is their ability to disrupt sterol-containing membranes [5]. This action is thought to be responsible for damaging cells in the gastrointestinal tract and also in other tissues organs into which glycoalkaloids are transported (e.g. blood, liver) following absorption.

Glycoalkaloids were first identified in potatoes by Baup early in the 19th century [6], a wider information on their chemistry, biochemistry, distribution, physiology and toxicology became available, and reviewed later [7,8]. Main glycoalkaloids in potatoes are α-solanine and α-chaconine, both triglycosides of solanidine, a steroidal alkaloid derived from cholesterol. Their molecular structures are shown in Fig. 1. Solanidine is a steroidal backbone without attached sugar moieties.

In general, analysis of the potato glycoalkaloids is not a simple task. It needs three complex steps: (1) extraction of all the compounds of interest, (2) elimination, if necessary, of all interfering species, and finally (3) determination of the specific analyte concentration [8,9]. Further methods were proposed including more steps, such as derivatisation or hydrolysis.

Extraction solvents can be non-aqueous, acidic or combined. The literature describes over 20 different types of the solvents used for the glycoalkaloids extraction, for example, ethanol [10], 5% acetic acid [11], methanol–acetic acid–water (94:1:6) [12], methanol–chloroform (2:1) [13], etc.

Glycoalkaloids are commonly purified through one of the following paths: (1) precipitation with ammonium hydroxide [14]; (2) extraction with either aqueous Na_2SO_4 solutions [13] or water-saturated butanol [15]; (3) use of a C_{18} ion-pair chromatography cartridge [16]. A combination of these techniques can also be employed.

Current methodologies for the analysis of the potato glycoalkaloids and related compounds include: (1) colorimetry [17]; (2) thin layer chromatography [18]; (3) gas chromatography [19]; (4) high-performance liquid chromatography (HPLC) [12,15,16,27]; (5) immunoassays (ELISA) [20]. All these methods are complex. They need an expensive and bulky instrumentation with high power consumption and well-trained operators.

Recently biosensors based on pH-sensitive field effect transistor as a transducer and enzyme butyrylcholinesterase as a recognition element have been developed, characterised and optimised for sensitive detection of the glycoalkaloids in model solutions [21,22]. This paper describes the analysis of glycoalkaloids in real potatoes samples by using such an enzyme-based system.

2. Materials and methods

2.1. Materials

Butyrylcholinesterase (BuChE) (EC 3.1.1.8, from Horse Serum) with a specific activity of 13 U/mg solid; bovine albumin (fraction V, 98% purity), butyryl choline chloride (BuChCl) (98% purity), α-chaconine (95% purity), α-solanine (95% purity) from potato sprouts, and glutaraldehyde (grade II, 25% aqueous solution) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All other reagents were of analytical grade and were used without any further treatment.

2.2. Sensor design and measurements

Ion sensitive field effect transistors (ISFETs) were fabricated at the Research Institute of Microdevices (Kiev, Ukraine). The potentiometric sensor chip contains two identical Si_3N_4-ISFETs, their design and operation mode have been previously described [23,24]. ISFETs were operated at a constant source current and drain-source voltage mode (I_s = 200 μA, V_{ds} = 1 V). Their pH-sensitivity was linear for pH values ranging from 2 to 12 with a slope of about 40 mV/pH.

All measurements were performed in daylight at room temperature in an open glass vessel filled with a vigorously stirred 5 mM phosphate buffer solution, pH 7.2. A 200 mM stock solution of BuChCl in deionised H_2O, and 2 mM stock solution of the glycoalkaloids in 5 mM acetic acid were prepared. The concentrations of substrates and inhibitors were adjusted by adding defined volumes of the stock solution of proper concentration. The differential output signal between the measuring and reference ISFETs was registered using laboratory ISFET-meter from Institute of Microtechnology (Neuchatel, Switzerland). After the estimation of the level of the enzyme inhibition, the initial enzyme activity was restored by washing out the biosensor enzymatic membrane in the working buffer solution for 5 min.

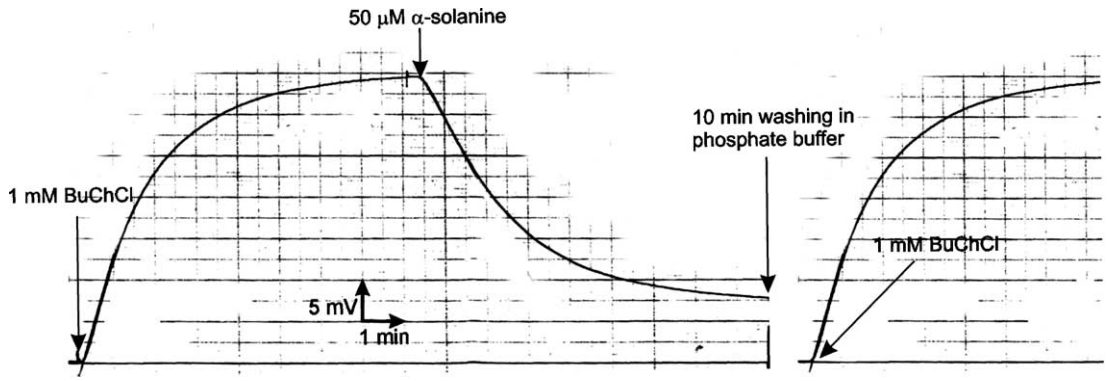


Fig. 2. Typical response of an enzyme biosensor based on pH-ISFETs. Measurements conditions: base line obtained in a 5 mM phosphate buffer at pH 7.2, the arrows indicate the BuChCl and α-solanine adding and beginning of the washing step.

2.3. Enzyme immobilisation

Biologically active membranes were formed by cross-linking butyrylcholinesterase with bovine albumin on the transducer surface in a saturated glutaraldehyde vapour [25,26]. The mixture containing 5% (w/v) enzyme, 5% (w/v) bovine albumin, 10% (w/v) glycerol in 20 mM phosphate buffer (pH 7.2) was deposited on the sensitive surface of one transducer by the drop method, while the mixture of 10% (w/v) bovine albumin and 10% (w/v) glycerol in 20 mM phosphate buffer (pH 7.2) was placed on the surface of a reference transducer. The sensor chip was then placed in a saturated glutaraldehyde vapour. After a 30 min exposure in glutaraldehyde, the membranes were dried at room temperature for 15 min.

2.4. Glycoalkaloids quantification in potato samples

The 17 different varieties of potatoes studied were grown in Experimental station of ARVALIS Institut du Végétal. Furthermore eight experimental varieties from five different sites were analysed. After selection of 5–6 samples from each variety tubers were cleaned manually by gentle washing under running water, dried and then crushed. The solid particles were removed by filtration through an inert carbon tissue. Prepared in such a way the samples of juice (without any additions and additional treatments) were analysed directly using the biosensor.

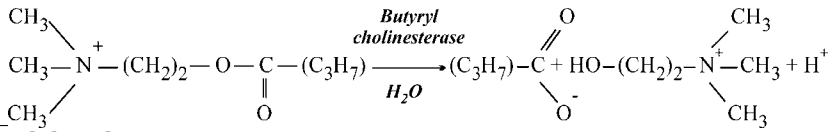
The level of enzyme inhibition by glycoalkaloids was evaluated by the biosensor via the following steps (Fig. 2):

- The sensor was placed into the buffer solution and the output signal (base line), was registered.
- BuChCl solution was added in the measuring cell (volume of 5 ml) in order to reach a 1 mM concentration and the steady-state output signal was registered.
- An appropriate volume of standard glycoalkaloids or potato juice sample was added (for example 5 μL of pure potato juice, in this case, after injection, dilution factor was 1/1000), and the inhibition effect was estimated.
- The sensor was placed into the buffer solution for washing, and after 10 min base line signal was fully recovered, the sensor was then ready for a new set of experiments.

Reference measurements of the glycoalkaloids in potatoes by HPLC technique were entrusted to ARVALIS-Institut du Végétal. The methodology used is adapted from [16]. The glycoalkaloids were extracted from tuber with a solution of acetic acid/Na-heptanesulfonate in water and purified using solid phase extraction. Separation and quantification of α-chaconine, β-chaconine and α-solanine were realised on a Zorbax Rx-C_{18} column, with 60% acetonitrile/water as eluent, at a wavelength of 202 nm.

3. Results and discussion

The feasibility of enzyme biosensors based on pH-ISFETs and butyryl cholinesterase for sensitive detection of α-solanine and α-chaconine was shown earlier [21,22]. The principle of operation of biosensor for glycoalkaloids determination is based on following basic reaction.



This reaction results in proton generation, inducing a pH change in the sensor membrane, which allows to use a potentiometric method of measurements. Solanaceous glycoalkaloids inhibit cholinesterases as it has been shown in previous in vitro and in vivo studies [3,28–30]. The level of BuChE inhibition due to the action of glycoalkaloids can be evaluated by a comparison of the biosensor responses before and after contact with a glycoalkaloids solution.

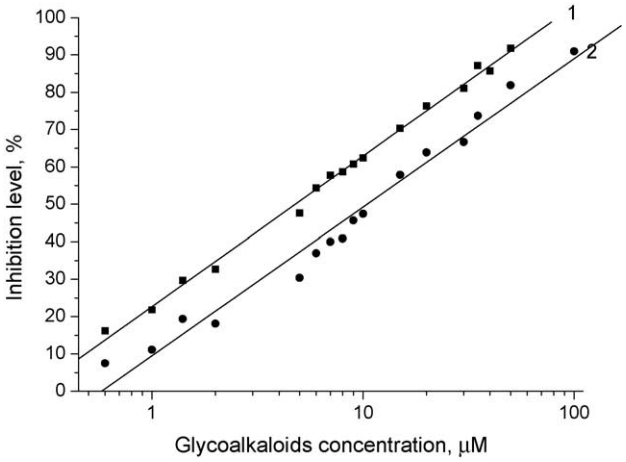


Fig. 3. Calibration curves for the detection of α-chaconine (1) and α-solanine (2) in semi-logarithmic plot. (Measurements were conducted in a 5 mM phosphate buffer at pH 7.2, and a 1 mM BuChCl concentration.)

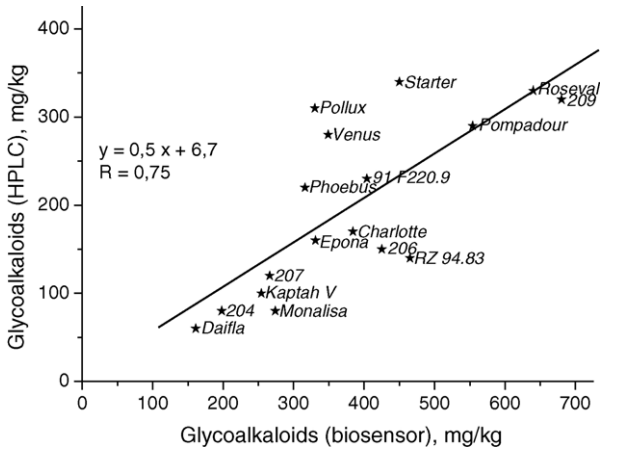


Fig. 4. Comparative results for total glycoalkaloids content in different potato varieties.

Fig. 3 presents the calibration curves for detection of α-solanine and α-chaconine. As it can be seen, the potato glycoalkaloids could be detected within the range of 0.2–100 μM depending on the type of alkaloid, with detection limits of 0.2 μM for α-chaconine and 0.5 μM for α-solanine. The dynamic ranges for the compounds examined show that such biosensors are suitable for a quantitative detection glycoalkaloids in potato samples.

α-Solanine and α-chaconine are known to represent around 95% of the total glycoalkaloids content in potatoes, other glycoalkaloid species being present only at trace levels. On the other hand, there is no specific reason to consider the toxicity of separate glycoalkaloids, since being present in potatoes in combinations they affect human altogether and therefore the total glycoalkaloids concentration in tuber has to be estimated.

Elaboration of a measurement protocol for real potato samples requires a preliminary biosensor calibration, i.e. detecting the kind and proportion of glycoalkaloids to be used in the sensor under development. Mostly, the ratio between main potato glycoalkaloids (α-solanine and α-chaconine) is known to be around 6:4, however, other ratios were quoted as well [14,31]. A mixture of α-chaconine/α-solanine with ratio 1/1 as model solution was used in further experiments for the sensor calibration and detection of the total glycoalkaloids concentrations in the potato juices For such a calibration, only concentrations lower than an equivalent concentration of 2 μg/ml for the mixture 1/1 are used and in this range the response is quasi linear with an inhibition level reaching 48% at 2 μg/ml.

Experiments for the determination of total glycoalkaloids concentrations were conducted on potato samples grown from 17 different experimental varieties. Results obtained using the biosensor method, compared with the analogous data obtained by HPLC are presented in Fig. 4. As it can be seen, a significant correlation between the two methods exists. Some discrepancies could be explained by slight differences between the procedures of samples preparation, as they were prepared in different places not simultaneously. Furthermore results obtained with biosensors show a glycoalkaloid level higher than the one obtained through the standard procedure. Such a behaviour can be partly attributed to the complexity of the HPLC sample pre treatment procedure involving extraction steps which can be not entirely quantitative.

A reproducibility test for the detection of total glycoalkaloids content in potatoes was done more specifically on the variety *Producent* Rubempré. Measurements done in a 5 mM phosphate buffer at pH 7.2, with a dilution level of 1/1000 for the potato juice are reproducible the relative standard deviation was about 3% for 20 measurements. Furthermore a test of operational stability demonstrated that biosensor responses remain stable with a drift of about 1% per day, this test being performed under operational conditions [22]. Furthermore, the storage stability in a 5 mM phosphate buffer, pH 7.2 at 4 °C was quite good, the biosensor responses remaining stable for more than three months.

Glycoalkaloids have been found in almost all parts of the potato but the highest concentrations are usually associated with tissues that are undergoing high metabolic activity [8,32,33]. These include potato flowers, unripe berries, young leaves, sprouts, peels, and area around potato eyes. The existence of a glycoalkaloid gradient of concentration in the potato has been evidenced, with higher concentration in the outer part of the tuber using the ISFETs based biosensor. For example, the total glycoalkaloids content in the peel of *Agata* variety was about 234 mg/kg of fresh weight, in contrast to 105 mg/kg found in flesh. Fig. 5 shows results for distribution of total glycoalkaloids contents in potato tuber of *Producent* Rubempré variety obtained with biosensor. As it can be seen, potato glycoalkaloids appear to be concentrated in a small layer immediately under the skin. Also it was shown that the concentration of glycoalkaloids in peel area around potato eyes is more than two times higher than in case of peel without eyes. This result is in a good agreement with results obtained by other authors [34]. With normal tu-

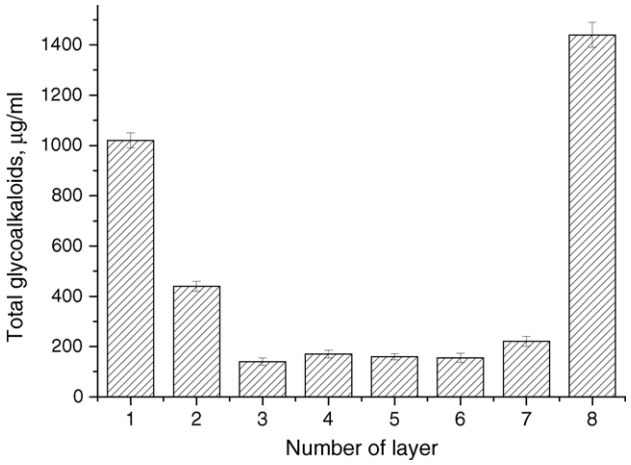


Fig. 5. The gradient of total glycoalkaloids content in potato tuber of *Producent* Rubempré variety obtained with biosensor. Measurements were done in 5 mM phosphate buffer, pH 7.2, 5 μl of pure potato juice was added to 5 ml of working buffer (dilution factor 1/1000). The 3 mm thickness layers was prepared by cutting of potato in direction from peel to peel of potato tuber (number from 1 to 8).

bers, the peeling could removed from 60 up to 96% of the glycoalkaloids content.

The fact that higher glycoalkaloids contents are present in the peel probably confort the observation that early harvest and small potatoes are richer in glycoalkaloids than larger tubers [34,35]. Fig. 6 shows results of total glycoalkaloids content in potato tuber with different sizes obtained with biosensor. As it can be seen, small immature tubers normally are high in glycoalkaloids since they are still metabolically active. Older, larger tubers, which have lower total glycoalkaloids level generally have a much lower skin to flesh ratio than the small tubers. It is possible that glycoalkaloids production may slow down as tubers mature. This is still an open question because many other factors can have an influence on the glycoalkaloids production.

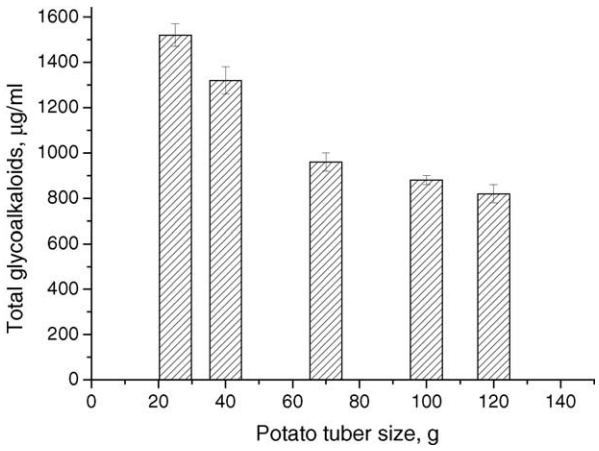


Fig. 6. Dependence of total glycoalkaloids content on size of potato tubers of *Producent* Rubempré variety obtained with biosensor. Measurements were done in 5 mM phosphate buffer, pH 7.2, 5 μl of pure potato juice was added to 5 ml of working buffer (dilution factor 1/1000).

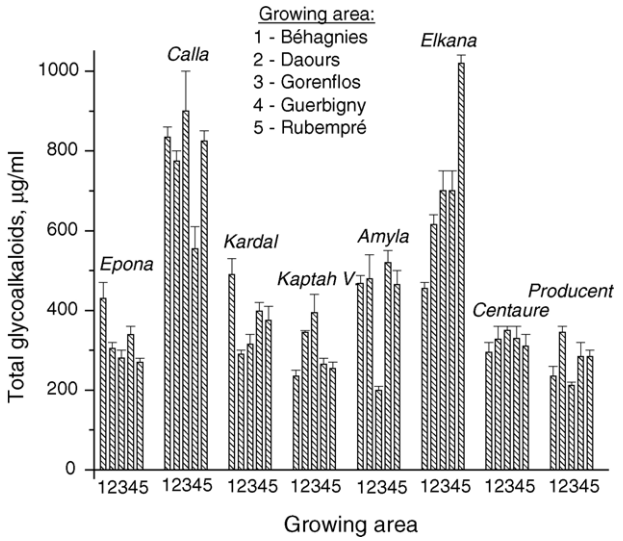


Fig. 7. Dependence of total glycoalkaloids content on different potato varieties grown in different areas obtained with biosensor. Measurements were done in 5 mM phosphate buffer, pH 7.2, 5 μl of pure potato juice was added to 5 ml of working buffer (dilution factor 1/1000).

Although the nature and relative concentrations of glycoalkaloids are genetically determined, the total concentrations are certainly influenced by environmental factors during the growing period. Seasonal, climatic and area variations seem to markedly influence glycoalkaloids biogenesis [8]. Fig. 7 shows the effect of differences in growing area on total glycoalkaloids contents obtained by biosensor. From these experiments we can confirm that although growing area may have an effect, sometimes even a dramatic impact (for example, variety with high concentration of glycoalkaloids), glycoalkaloids production is primary genetically controlled.

4. Conclusions

The results presented in this work demonstrate the applicability of butyrylcholinesterase biosensors based on pH-ISFETs for a sensitive detection of potato glycoalkaloids. A reproducible and sensitive biosensor for such detection will certainly provide a simple and quick method for screening a great number of samples, directly on fresh potato juices without any sample pre-treatment. A quite good correlation with results obtained with routinely used method has been shown. The biosensor can work after dilution on a potato extract, or even directly on a suspension of potato or plant material. It can provide a way to save time and costs, with a possibility of taking rapid assessment of total glycoalkaloids content on a wide variety of potato cultivars. If necessary, the selected samples can be then analysed more selectively by the standard methods for individual glycoalkaloids determination, which are more complex and time consuming.

Other choline esterase sensors based on inhibition phenomena, for the detection of toxic compounds as pesticides have been already proposed and studied (24 and works quoted

in this publication). Compared to such biosensors the main advantages of glycoalcaloïds detection is that no complex reactivation step is needed as inhibition by glycoalcaloïds is quite reversible. A simple washing in the measuring buffer allows a complete regeneration of the enzymatic activity.

If glycoalkaloids and pesticides are present together in a potatoes samples, it is then possible to discriminate both types of inhibition; a total inhibition level is first measured (glycoalkaloid + pesticides content) and after washing in buffer solution only irreversible effect remains (pesticides content). The biosensor developed could be used in different fields such as those needing an assessment of the food quality for human or animals.

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