A rapid quantitative assay for solanidine glycoalkaloids in potatoes and industrial potato protein

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Summary

A rapid quantitative assay for potato glycoalkaloids with solanidine (solanid-5-en-3β-ol) as steroidal aglycone is described. The method of Sachse & Bachmann has been modified with respect to the extraction and working up procedures to increase the speed and ease of analysis. A considerable simplification of analysis was achieved by replacing the original ether extraction and celite filtration by centrifugation. Fresh potato and industrial potato protein were analysed with this method. Industrial potato protein from several sources contained widely divergent glycoalkaloid contents (5-165 mg/100 g).

Introduction

Determination of potato glycoalkaloids became important in view of their toxic properties (Nishie et al., 1971) and has been the subject of continuing attention (Nishie et al., 1976; Brown & Keeler, 1978).

Potato tubers normally contain small amounts (10-50 mg/100 g dry weight) of the glycoalkaloids α-solanine and α-chaconine, which share the same alkaloidal aglycone solanidine and differ only with respect to the sugar moiety. These glycoalkaloids are concentrated near the peel and may vary according to environmental conditions during growth, wounding and light exposure as well as genetic factors (Jadhav & Salunkhe, 1975).

Methods for quantitative assay of potato glycoalkaloids have been intensively explored with respect to the method of primary extraction, the work-up procedure and the ultimate quantitative assay. For the primary extraction, hot alcoholic extraction, extraction with a bisolvent mixture (chloroform, methanol) and extraction with aqueous acidic alcohol have been used. The work-up procedures vary from the classical precipitation in alkaline solution to absorption on an ion exchange resin (Patt & Winkler, 1960) and hydrolysis and extraction of the aglycones (Fitzpatrick & Osman, 1974). The ultimate quantitative assay has been shifted from a gravimetric assay (Lepper, 1949) to colorimetric (Baker et al., 1955; Sachse & Bachmann, 1969; Bretzloff, 1971), titrimetric (Fitzpatrick et al., 1978) and polarographic assays Pierzchalski & Mrozowska, 1968). In addition several chromatographic methods are known (Herb et al., 1975; Hunter et al., 1976; Roosen-Runge & Schneider, 1977; Cadle et al., 1978; Osman et al., 1978).
The main problem of most of these quantitative methods is the many steps involved and therefore the rather time-consuming nature of the analysis. Starting from the rather versatile quantitative assay of Sachse & Bachmann (1969), based on hot alcoholic extraction, precipitation in alkaline solution and the chromogenic reaction of Clarke (1958), a more rapid assay was developed by modifications of the extraction and working up procedures. This method was applied to fresh and freezedried potatoes and also to industrial potato protein.

Material and methods

Chemicals
Phosphoric acid 85 % PA, paraformaldehyde reinst from Merck; α-solanine, from USDA ARS Eastern Regional Research Center; diethylether PA; ethanol 96 % technical quality; acetic acid PA; ammonia (25 %) PA.

Sample preparation
Potatoes, including peel, are sliced (3–5 mm) and immediately frozen in liquid nitrogen, freezedried and thereafter powdered. Fresh potatoes are mashed mechanically for a few minutes and 50-g portions directly transferred to 200 ml 96 % ethanol to prevent enzymic browning. Industrial potato protein is extracted without prior handling.

Primary extraction of potato glycoalkaloids
The first coarse extraction is done by a 15-min period refluxing of 10 g freeze-dried potato or protein with 100 ml 80 % aqueous ethanol (for fresh potatoes 50 g/200 ml 96 % ethanol) on a temperature-controlled water-bath (about 90 °C).

Work-up procedure
The hot extract is filtered by suction over a Büchner funnel and this extract (becoming cloudy on standing) is concentrated to about a tenth of the original volume with the aid of a rotary evaporator (water-bath, 60 °C). The concentrated extract is mixed with 50 ml 10 % (v/v) aqueous acetic acid; then centrifuged in a cooled centrifuge at 10 °C for 30 min at 10 000 g, and carefully poured off. To this supernatant concentrated ammonia is added to a final pH = 10, accompanied by some clouding and an intense yellow colour shift, which is caused by the presence of phenolic compounds (mainly chlorogenic acid). The glycoalkaloids are then precipitated by heating for 20 min at 70 °C. The flasks are cooled to 4 °C for at least 3 hours and the flocculated glycoalkaloids are centrifuged at 10 °C for 30 min at 10 000 g. After centrifuging, the supernatant is carefully poured off and the pellet is dissolved in a small volume of 7 % (w/w) aqueous phosphoric acid (1–10 ml), depending on the glycoalkaloid concentration.

Quantitative colorimetric determination
0.4 ml of the solution of potato glycoalkaloids is mixed with 4 ml 85 % H₃PO₄ (w/w) containing 1.2 mg paraformaldehyde (0.03 % w/v) and this mixture is thoroughly mixed. Colour development: a blue colour develops, reaching a maximum after 30–40 min and then slowly fades. The absorbance at 600 nm is read after 40 min and