GLYCOALKALOIDS AND PHENOLIC COMPOUNDS IN GAMMA IRRADIATED POTATOES

A food irradiation study on radiation-induced stress in vegetable products

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W.W.A. Bergers

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
Dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 4 juni 1980
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

Het onderzoek werd verricht op het Instituut voor Toepassing van Atoomenergie in de Landbouw (I.T.A.L.) te Wageningen.

STELLINGEN

1. In met hoge dosis (3 kGy) gammastraling behandelde aardappelen van het ras Eba vindt tijdens de bewaring opeenhoping van het β-glycoside van 6-methoxy - 7-hydroxycoumarin (scopolin) plaats.
   Dit proefschrift

2. De risicoschatting gebaseerd op het optreden van chemische veranderingen in bestraalde voedingsmiddelen verdient de voorkeur boven het gebruik van toxicologische onderzoeksmodellen m.b.v. proefdieren.

3. De kritiek van Coxon et al. (1979), dat kleurreacties van aardappel-glycoalkaloiden in sterke zuren onvoldoende specifiek zijn, is onjuist.
   Coxon, D.T., Price, K.R. & Jones, P.G. (1979). A simplified method for the determination of total glycoalkaloids in potato tubers. J. Sci. Food Agric. 30, 1043-1049.

4. Kloppende hartcel cultures zijn een geschikt *in vitro* systeem om de biologische activiteit van glycoalkaloiden te testen.
   Dit proefschrift

5. De bevinding van Lau-Cam (1978) dat "scopolin" niet door middel van fluorescentie op dunne laag platen gedetecteerd kon worden, doet vermoeden, dat er een verkeerde verbinding gebruikt is.
   Lau-Cam (1978). Thin-layer chromatography of coumarins of medical and phytochemical interest on buffered layers. J. of Chromatography 151, 391-395.

6. De visuele vergelijkingsmethode van McMillan & Thompson (1979) is niet geschikt voor bepaling van het solanine gehalte van aardappels.
   McMillan, J.D. & Thompson, P. (1979). An outbreak of solanine poisoning in schoolboys. Quarterly J. of Medicine 190, 227-243.

7. Het gebruik van enkelvoudige extractie vloeistoffen voor de primaire extractie van aardappel-glycoalkaloiden verdient de voorkeur boven toepassing van binaire mengsels.
   MacKenzie, J.D. & Gregory, P. (1979). Evaluation of a comprehensive method for total glycoalkaloid determination. Am. Potato J. 56, 27-33.

8. Toepassing van electrochemische detectie bij de hoge druk vloeistof chromatografie van glycoalkaloiden kan hun analyse bevorderen.

   Bushway, R.J., Barden, E.S., Bushway, A.W. & Bushway, A.A. High performance liquid chromatographic separation of potato glycoalkaloids. J. of Chromatography 178, 533-541.

9. De opvatting dat aardappelknollen enkel in de schil glycoalkaloiden zouden bevatten, is niet juist.

   Anonymus (1979). British Medical Journal, 8 December, 1458-1459.

10. Blootstelling aan 1-pentyne-3-ol als methode voor het isoleren van nieuwe ADH (Alcohol Dehydrogenase) deficiënte mutanten bij *Drosophila* (O'Donnell et al., 1975) is niet zonder meer te gebruiken voor het vinden van ADH deficiënte mutanten bij andere insekten.

    O'Donnell, J., Gerace, L., Leister, F. & Sofer, W. (1975). Chemical selection of mutants that affect Alcohol Dehydrogenase in Drosophila II. Use of 1-pentyne-3-ol. Genetics 79, 73-83.

11. De ogenschijnlijk eenvoudige techniek van vloeistofscintillatie kan tot grote fouten leiden.

12. Topschakers dienen hun mindere collega's te onderhouden en niet andersom.

W.W.A. Bergers

Glycoalkaloids and phenolic compounds in gamma-irradiated potatoes. A food irradiation study on radiation-induced stress in vegetable products.

Aan Ada, Xander en Lizet

VOORWOORD

Bij het verschijnen van dit proefschrift wil ik diegenen bedanken, die dit mede mogelijk hebben gemaakt.

Professor Pilnik ben ik erkentelijk voor het doorwerken en begeleiden van diverse manuscripten en Professor Koeman voor het ondersteunen van het initiatief tot het promotie onderzoek. De directie van het ITAL bedank ik voor het geven van de gelegenheid tot dit onderzoek. Mijn collega's van de groep voedselbestraling bedank ik voor hun medewerking en collegialiteit; in het bijzonder Ing. H. Leveling (Ames test) en Ir. J.G. van Kooij. De medewerkers van het IBVL: Ing. A. van Es, Mw. Drs. K. Hartmans en H. de Ruiter, ben ik erkentelijk voor hun spontane medewerking. Dr. G.F. Wilmink bedank ik voor zijn belangstelling als ISAC lid.

Mijn dank gaat voorts uit naar de dienstverlenende sector van het ITAL en de medewerkers van het proefbedrijf voor voedselbestraling. Mw. C. Mons-Enklaar ben ik erkentelijk voor het typen van het manuscript.

Voorts ben ik erkentelijk voor de medewerking van en contacten met de vakgroepen Botanie, Organische Chemie en Toxicologie van de LH en de instituten het Spelderholt en het CABO. Tenslotte, wil ik J. Davies, H. Bosman and D. Kennett bedanken voor hun aandeel in het analytisch gedeelte.

CURRICULUM VITAE

De auteur werd geboren op 22 april 1948 te Leiden. Hij behaalde in 1966 het einddiploma Gymnasium B aan het Bonaventura Lyceum te Leiden. In datzelfde jaar begon hij met de studie van Scheikunde met Natuurkunde, Biologie en Wiskunde als bijvakken aan de Rijksuniversiteit te Leiden. Het kandidaatsexamen werd in juli 1969 behaald. In februari 1972 werd het doctoraalexamen Scheikunde afgelegd met hoofdvak Biochemie, bijvak Organische Chemie en derde richting Bacteriegenetica.

Van juli 1972 tot oktober 1973 vervulde hij de militaire dienstplicht. Daarna was hij van maart 1974 tot december 1974 werkzaam als wetenschappelijk ambtenaar (TAP plaats) bij de werkgroep Plantevirussen van het Biochemisch Laboratorium van de Rijksuniversiteit te Leiden. In december 1974 trad hij, als wetenschappelijk ambtenaar, in tijdelijke dienst, bij het Instituut voor Toepassing van Atoomenergie in de Landbouw. Na een jaar voorafgaand literatuuronderzoek over straling in relatie tot bederf van plantaardige producten, werkte hij van februari 1976 tot november 1979 aan een promotie onderzoek met als onderwerp glycoalkaloiden en fenolen in bestraalde aardappelen.

CONTENTS

Parts of this study are published in detail in the following papers:

I W.W.A. Bergers, A rapid quantitative assay for solanidine glycoalkaloids in potatoes and industrial potato protein.
Accepted on June 4, 1979 by Potato Research.

II W.W.A. Bergers, On the colour reactions of potato glycoalkaloids in strong acids in the presence of (para)formaldehyde.
Accepted on September 6, 1979 by Food Chemistry.

III W.W.A. Bergers, Investigation of the contents of phenolic and alkaloidal compounds of gamma irradiated potatoes during storage.
Accepted on December 4, 1979 by Food Chemistry.

IV W.W.A. Bergers & G.M. Alink, Toxic effects of the glycoalkaloids solanine and tomatine on cultured neonatal rat heart cells.
Accepted on February 5, 1980 by Toxicology Letters.

1. GENERAL INTRODUCTION

1.1 Chemical changes in irradiated vegetable products

Irradiation is a recent food preservation method (Urbain, 1978). Ionizing radiation, usually gamma rays, can kill microorganisms in food and influence specific physiological processes in vegetable products. The main stumbling block for application is the legal prohibition of food irradiation. Wholesomeness of an irradiated food item has to be proved by long-term animal feeding tests.

This approach, according to the food additive principle, is not satisfactory, because a no-effect level cannot be established for irradiated food (Diehl, 1974). Therefore, in addition to limited animal feeding tests, evaluation of chemical changes in irradiated foodstuffs has been suggested in order to obtain general acceptance of irradiated food (Elias & Cohen, 1977).

In principle, chemical changes are estimated for metabolically inactive foodstuffs on the basis of radiolytical data (Diehl & Schertz, 1975). Chemical changes in irradiated, metabolically active foodstuffs, i.e. vegetable products, are more difficult to estimate, because metabolic stress compounds can be induced or radiolytic products can be metabolized.

Pronounced increases in metabolic stress compounds have been reported for irradiated citrus fruit, e.g. in grapefruit peel (Riov et al., 1972). Stress factors may enhance toxin formation in vegetable products (Liener, 1969; Anonymus, 1973; Coxon et al., 1973; Haard & Salunkhe, 1979). The study of radiation-induced increases in metabolic stress compounds is therefore a valuable tool for the wholesomeness evaluation of irradiated vegetable products. Furthermore, radiation may also induce chemical changes, which influence sensoric quality of irradiated vegetable products.

1.2 Potatoes as a model for irradiation experiments; choice of target compounds

Potatoes are an important food item in food irradiation technology (Urbain, 1978). Irradiation doses of 0.05 to 0.15 kGy of gamma irradiation completely inhibit sprouting.

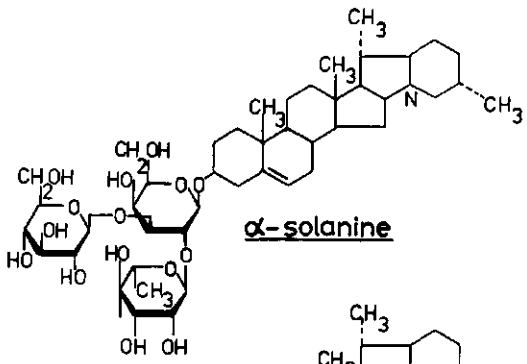
Potato tubers, stored at an appropriate temperature, e.g. 10 ^{o}C, maintain their metabolizing capacity for several months.

The effects of several post-harvest stresses, e.g. greening, damage and microbial infection of potato tubers, are known for several classes of compounds. The contents of glycoalkaloids, phenolic acids (mainly chlorogenic acid) and hydroxy coumarins, scopolin, of potato tubers are influenced. Thus, effects of radiation stress can be compared and evaluated on the basis of these three types of compounds, which have been chosen as main target compounds in this study.

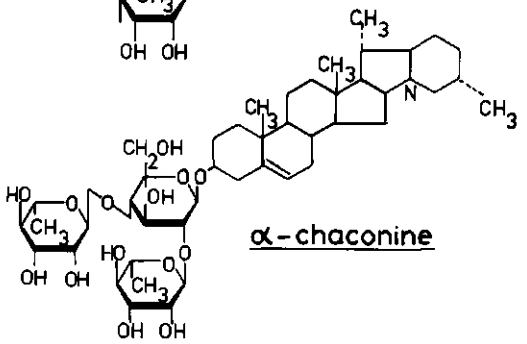
Potatoes contain 2 to 10 mg/100 g fresh weight glycoalkaloids, mainly α-solanine and α-chaconin, Figure 1. The glycoalkaloid content is primarily determined by genetic factors. However, glycoalkaloid contents can be influenced during post-harvest storage by light (greening) and damage. The distribution of glycoalkaloids in tubers is not homogeneous, but is concentrated near the peel and eyes of the tubers. Considerably higher contents of glycoalkaloids occur in other parts of potato plants and tuber sprouts, i.e. up to 3% fresh weight (Jadhav & Salunkhe, 1975).

The phenolic compound, chlorogenic acid, occurs in many plants. It is a bound form of caffeic acid and quinic acid, see Figure 1. In plants the trans isomer predominates, but during isolation the cis isomer may be formed (Harborne, 1973). Potato tubers contain 6 to 32 mg/100 g fresh weight chlorogenic acid with higher contents occurring near the peel than in the centre (Penner & Fromm, 1972). In slices of potatoes (Zucker, 1965) and wound tissue (Clarke, 1973) chlorogenic acid is synthesized.

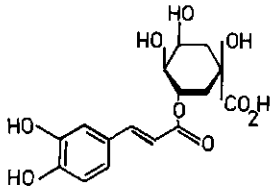
Scopoletin and its bound form scopolin, see Figure 1, have been found in several plants (Bate-Smith, 1954; Herrmann, 1976). Scopoletin (coumarin, 7-hydroxy, 6-methoxy) is usually found bound to glucose. A specific property of coumarins is their bright fluorescence (Goodwin & Kavanagh, 1950). In potato tubers a scopolin content of approx. 0.1 mg/100 dry weight was found by Hughes & Swain (1960). A 10 to 20 fold increase in the scopolin content, compared with control tubers, has been found in fungal infected potatoes (Hughes & Swain, 1960; Clarke, 1973), and scopoletin accumulation has been observed in virus infected potatoes (Andreae, 1944). Accumulation of scopolin appears to be variety specific, i.e. synthesis of hydroxy coumarins is depdendent on host control (Clarke & Baines, 1976).



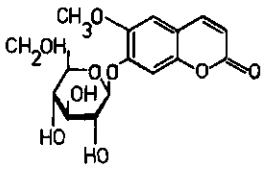
α-solanine



α-chaconine



chlorogenic acid



scopolin

Figure 1 - Chemical structures of primarily investigated alkaloidal and phenolic target compounds in alcoholic extracts of irradiated stored potatoes.

1.3 Toxicity of target compounds

The toxicity of potato glycoalkaloids is well known (Nishie, 1971; McMillan & Thompson, 1979). During this study, it was found that measuring beating cultured neonatal rat heart cells was a sensitive system to detect cellular toxicity of glycoalkaloids. This type of toxicity may be important in view of *in vivo* toxicity of glycoalkaloids (Bergers & Alink, IV).

For coumarins, genetic effects have been found in plant cells and hepatotoxicity of coumarin in rats (Grigg, 1978). Chlorogenic acid has a low toxicity (Chaube & Swineyard, 1976), but is important in view of sensoric quality of potatoes, i.e. after cooking darkening (Thomas et al., 1979).

Glycoalkaloids, chlorogenic acid and scopoletin were examined in several animal feeding tests, teratogenicity tests, after a remarkable hypothesis of the presence of teratogenic substances in blighted potatoes, based on epidemiological grounds (Renwick, 1972). Although this hypothesis was refuted later also on epidemiological grounds (Nevin & Merrett, 1975), many teratogenicity tests have been performed (Keeler et al., 1978). No evidence for teratogenic properties of chlorogenic acid (Chaube & Swineyard, 1975), scopoletin (Ruddick et al., 1974) and potato glycoalkaloids (Keeler et al., 1978) were found). 25 mg/kg/day of scopoletin fed to pregnant rats reduced mean litter size. Results of teratogenicity tests however, may vary greatly for different animal species (Khera, 1976) and have to be carefully evaluated. At present, except in a few cases, toxicology of stress metabolites associated with edible plant tissues is unknown (Wood, 1979).

1.4 Purpose of the study

Potatoes were used as a model in order to study the metabolic stress effects in irradiated vegetable products. The changes of the contents of specific target compounds, i.e. glycoalkaloids, phenolic acids and coumarins in alcoholic extracts of irradiated potatoes were studied for metabolic irradiation stress. Doses of up to 3 kGy were applied to potatoes of several varieties. The use of a direct method for detecting radiation stress by fluorescence was also explored. Analytical studies on qualitative and quantitative assays of the target compounds were done.

In view of conflicting literature data on mutagenic properties of alcoholic extracts of irradiated potatoes (Kopylov et al., 1974; Levinsky & Wilson, 1975; Hossain et al., 1976) glycoalkaloids and phenolic compounds as well as alcoholic extracts were tested with a bacterial mutagenicity test (Ames test).

2. EFFECTS OF GAMMA IRRADIATION ON METABOLIC ACTIVITIES IN VEGETABLE PRODUCTS

Doses of 0.05 to 3 kGy (5 to 300 krad) can be used for the irradiation of vegetable products. In the lower dose range, 0.05 to 0.15 kGy, sprouting of tuberous plant foods is inhibited. Slightly higher doses 0.2 to 0.5 kGy can be used to induce a ripening delay in climacteric fruits, e.g. mangoes and bananas. Higher doses, 0.5 to 3 kGy can be used to kill microorganisms, especially moulds (Urbain, 1978). Such doses of ionizing radiation do not inactivate enzymes in vegetable products very considerably. Enzymes in a cellular environment behave in a very radio resistant way compared with enzymes in aqueous solutions (Marples & Glew, 1958; Vas, 1966).

2.1 General post-harvest metabolic activities

Biochemical activities of plant tissues are closely connected with the physiological status and with the post-harvest environmental conditions, i.e. temperature and atmosphere. The kind of activities are determined by the plant-organ type: leaves and fruits, on ageing and during storage, exhibit a progressive physiological breakdown called senescence, which is associated with the breakdown of cellular membranes. Tubers sprout after a dormancy period.

The respiratory rate gives a rough indication of the longevity of a commodity (Duckworth, 1966; Haard, 1971a). Vegetables and fruits generally show a declining rate of respiration after harvest, indicating a progressive breakdown in function of the cellular membranes. Climacteric fruits behave somewhat differently after harvest and show a characteristic upsurge in respiratory rate, associated with ripening. After reaching the climacteric maximum, respiratory rate slows down, being associated with progressive senescence (Biale, 1960). The respiratory rate is proportional to the temperature of the storage environment within physiological limits. In addition, respiratory rate is controlled by the O_2 and CO_2 in the surrounding atmosphere (Salunkhe & Wu, 1974). Relative humidity may influence the respiratory rate in some cases (Haard, 1971b).

Ripening of fruits after harvest is limited to fruits of the climacteric type, e.g. bananas; these fruits are harvested in a mature, usually green stage and ripen during post-harvest storage, when they undergo pronounced biochemical changes in pigment, texture, flavouring compounds and soluble solids (Biale, 1960; Biale & Young, 1962).

Leaves, non-climacteric fruits and climacteric fruits, reaching the climacteric maximum exhibit a kind of regulated physiological breakdown during storage (senescence). This is associated with a progressive disorganisation of the cell's metabolic apparatus, i.e. membranes and cytological fine structures ultimately causing cell death (Varner, 1961; Bonner & Varner, 1965; Sacher, 1973).

In tubers after a certain period of dormancy, sprouting begins, accompanied by several biochemical activities (Wareing & Saunders, 1971).

During storage and handling of fruit and vegetables, physiological disorders, induced by environmental stresses, may occur. Low temperatures, between 0 °C and 10 °C, cause chilling injury particularly in tropical and subtropical commodities (Lyons, 1973). Damaged or cut tissues of tubers, e.g. sweet potatoes, exhibit a marked increase in metabolic activity some time after the original damage (Ogawa & Uritani, 1969). A marked increase in the respiratory rate is associated with an increase in several metabolic processes, e.g. protein synthesis and phenolics. In infected plant tissue, specific metabolic activities take place in response to the infection and phytoalexines are formed in the infected tissue (Kuc, 1972; Deverall, 1972). In addition, ethylene may induce specific stress metabolites (Coxon et al., 1973).

2.2 Effects of gamma radiation on post-harvest metabolism

Radiochemical lesions in nucleic acids, membranes and phytohormones can cause metabolic stress in post-harvest plant tissues (Romani, 1966b). Metabolic effects of gamma irradiation on fruit and vegetables largely depend on the physiological status of a commodity, with a declining effect in more senescent systems. In fact Romani (1972) interpreted the respiratory response of ripening climacteric fruits after irradiation with ionizing radiation as an index for senescence.

Effects of gamma irradiation on fruits are complicated. The effects of ionizing radiation are closely connected with the physiological status at the moment of irradiation and with the kind of fruit. Climacteric fruits, e.g. mangoes and bananas, show a ripening delay with 0.2 to 0.5 kGy doses of gamma irradiation; whilst in the case of irradiation during the climacteric phase no ripening delay is observed (Thomas & Screenivasan, 1970). Delay in ripening has been observed in several climacteric fruits, e.g. apples, plums, sapotas, papayas, pears and tomatoes. However, in peaches and nectarines a radiation-induced stimulation of ripening has been shown. Pears and tomatoes exhibit a ripening delay when irradiated with 1 to 3 kGy doses of gamma irradiation during the

climacteric respiratory rise; whilst ripening is not quite normal if the irradiation treatment takes place during the pre-climacteric stage (Romani, 1966a, b; Maxie & Abdel-Kader, 1966). Ripening-delaying doses cause a shift in the respiratory pattern and an increase in fructose content in irradiated bananas (Nair et al., 1973). The increase in fructose content was explained as a shift in carbohydrate metabolism from the glycolytic to the pentose phosphate pathway.

The increase in respiration in climacteric fruits generally decreases if irradiation takes place during more advanced stages of the climacteric rise (Maxie & Abdel-Kader, 1966). In irradiated pears there is an overall declining capacity for repair of mitochondria and ribosomes if irradiated during the climacteric rise, indicating a loss of compensatory action by radiation stress in more senescent tissue (Romani et al., 1968).

Irradiation of tomatoes resulted in the inhibition of carotogenesis, especially β-carotene synthesis. This effect was more pronounced in less mature fruits (Villegas et al., 1972).

Terminal leaf senescence in excised wheat leaf tissue was found to be radio-resistant up to a dose of 5 kGy. Leaf senescence was measured by chlorophyll contents as an index for senescence (Haber & Walne, 1968).

2.2.1 Potato tubers

An immediate effect of irradiation in potatoes is an increase in respiratory rate, reaching a maximum 24 hours after irradiation and returning to the normal level in about a week (Kodencherry & Nair, 1972). The increase in the respiratory rate reflects an increase in metabolic activities, because it has been found that irradiation doses below 3 kGy do not uncouple oxidative phosphorylation. An increase in decarboxylation is excluded, because O_2 uptake studies show a similar pattern as for CO_2 evolution (Sussmann, 1959; Kodencherry & Nair, 1972). Further metabolic changes in irradiated potatoes are changes in sugars (Jaarma, 1968) and free amino acids, induction of phenyl ammonia lyase and asparagine synthetase (Herrmann & Raths, 1958; Pendharkar & Nair, 1975).
Early findings indicate an effect of ionizing radiation on plant hormones as a sensitive response of plant tissue (Gordon, 1957). In irradiated potatoes, a constantly suppressed activity of the IAA synthesizing system has been reported (Ananthaswamy et al., 1972; Nair et al., 1973; Ussuf & Nair, 1974). Inhibition of sprouting was reversed by the exogeneous addition of auxine within 6 hours of radiation treatment.

2.2.2 Polyphenoloxydase, phenyl ammonia lyase and phenolics

If pre-climacteric bananas are irradiated with doses above 0.5 kGy, they become entirely brown during storage; whereas bananas irradiated during progressive stages of the climacteric rise can withstand doses of up to 2 kGy without much radiation damage (Thomas & Sreenivasan, 1970; Thomas & Nair, 1971).

Several studies have been undertaken to correlate browning phenomena with changes in polyphenoloxidase activity. Moderate increases in polyphenoloxidase activity were noted. In bananas, a differential effect on creolase and catecholase activities of the polyphenoloxidase enzyme complex was found. Catecholase activity increased with increasing dose, whereas normally catecholase exhibits an increase with doses of up to 1 kGy and a decrease with higher doses (Thomas & Nair, 1971). Irradiation of pre-climacteric mango fruits with doses above 0.75 kGy causes browning during subsequent storage; in this case, a time dependent increase of polyphenoloxidase activity was found (Thomas & Janave, 1973). For potatoes, irradiation-induced browning was reported which was dependent on the storage period between harvest and irradiation treatment. A transient increase in polyphenoloxidase activity was found (Ogawa & Uritani, 1970). In another investigation an immediate increase in creolase activity (45%) and a decrease in catecholase activity (25%) was reported for gamma-irradiated potatoes (Pendharkar & Nair, 1974).

A 2 kGy dose of gamma irradiation caused a marked increase in the phenyl ammonia lyase (PAL) activity in the peel of irradiated grapefruits. The PAL activity reached a maximum 24 hours after irradiation, which parallels ethylene production in irradiated fruits (Riov et al., 1972). Peel damage was manifested mainly in those fruits which maintained a high level of PAL activity for 5 days after irradiation. Riov et al. (1972) also showed that there was an increased synthesis of phenolic compounds, mainly the coumarins: scopolin and scopoletin. Another coumarin compound: 6,7 dimethoxy-coumarin was also found. Evidence for the correlation bretween increased PAL activity and increase of coumarins was based on the incorporation of labelled phenylalanine into scopolin (Riov et al., 1971; Riov, 1971; Riov et al., 1972). Irradiation of potato tubers with 0.1 kGy also caused induction of PAL activity in cortex and bud tissue (Pendharkar & Nair, 1975). The induced activity declined within 3 hours of irradiation. Further transient increases of chlorogenic acid in irradiated potatoes have been reported (Penner & Fromm, 1972).

Studies examining the effect of sliced irradiated potatoes indicate a decrease in PAL activity in parenchymatous tissue proportional to the dose (Shirsat & Penner, 1973).

However, discs of irradiated sweet potato roots reveal an increase of PAL activity (dose 0.9 kGy) and induction of polyphenol synthesis (Ogawa & Uritani, 1969). In their study, the change in polyphenol synthesis after cutting varied with respect to the storage period between irradiation and cutting. Shortly after irradiation (1 day), a decrease was found and after 2 days a definite increase. Later studies suggested a relationship between gamma irradiation and effects of exogenous ethylene (Ogawa & Uritani, 1971).

3. ANALYTICAL STUDIES ON THE TARGET COMPOUNDS

Chemical analysis of glycoalkaloids and phenolic compounds of potato samples was done starting from freeze-dried potato material. The tubers were sliced and immediately frozen in liquid nitrogen (to prevent enzymic changes) and subsequently freeze-dried. Then, 10 g amounts of the powdered slices were extracted with 80% ethanol. Further preparative steps are shown in Figure 2.

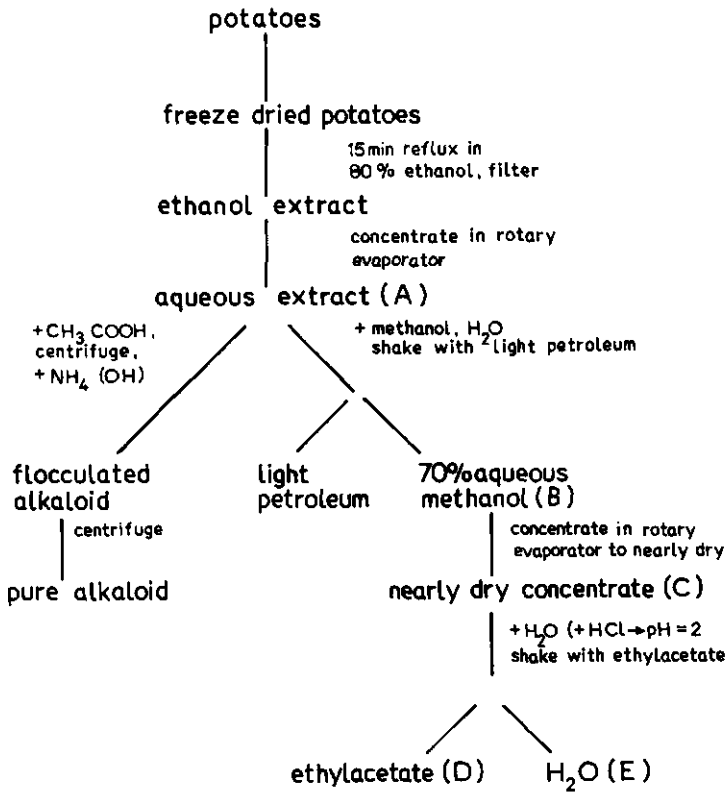


Figure 2 - Outline of chemical analysis.

3.1 Quantitative assay of solanidine glycoalkaloids

A quantitative assay for potato glycoalkaloids was worked out from an existing method by modifications in the work-up procedure. Acetic acid was added to the aqueous extract (A) and this solution was centrifuged. The glycoalkaloids

were precipitated by $NH_4(OH)$ and flocculated by heating. After cooling, the precipitated glycoalkaloids were centrifuged and the pellet containing the glycoalkaloids was dissolved in 7% (w/w) aqueous phosphoric acid. The glycoalkaloids were assayed colorimetrically by mixing with concentrated phosphoric acid in which paraformaldehyde was dissolved. This method could also be used for the quantitative assay of glycoalkaloids in fresh potatoes and industrial potato protein (Bergers, I).

3.1.1 Mechanism of quantitative colour reactions

Because of objections (Fitzpatrick & Osman, 1974; Coxon et al., 1979) to the 'Clarke' and 'Marquis' colour reactions of potato glycoalkaloids as quantitative assay (Bergers, I) these reactions were investigated.
The 'Clarke' reaction takes place after the addition of potato glycoalkaloids to a mixture of phosphoric acid and (para)formaldehyde and the 'Marquis' reaction takes place after the addition of (para)formaldehyde when the glycoalkaloids have previously reacted in 66% sulphuric acid. The spectral changes of these typical colour reactions are given in Figures 3 and 4. It was found that the influence of time of addition of paraformaldehyde was the major factor for the type of colour changes encountered making the Clarke reaction more suitable.

According to the structural similarity of the steroidal part of potato glycoalkaloids and cholesterol, colour reactions of both were compared indicating that both steroids react in strong acids in the presence of an oxidator, provided that the more lypophilic character of cholesterol was taken into account. A similar mechanism of a serial oxidation of carbonium ions as in the case of cholesterol, was suggested therefore as the basis for these specific colour reactions of potato glycoalkaloids (Bergers, II).

3.2 Qualitative and quantitative assay of phenolic compounds

Quantitative analysis of phenolic compounds in potato samples is outlined in Figure 2. UV spectra, 250-400 nm and fluorescence intensities (excitation 350 nm, emission 450 nm) were measured of fractions (B), (D) and (E). Qualitative results were obtained by TLC on cellulose plates, developed in 10% HAc or BEW (buthanol-ethanol-water, 4:1:2,2).

Both qualitative and quantitative data were obtained by HPLC chromatography of extracts (B) according to the reversed phase principle on an ion exchange column with an ionic and pH gradient. Through UV absorbance detection at 310 nm direct quantitative results, particularly for chlorogenic acid and scopolin, were obtained (Bergers, III).

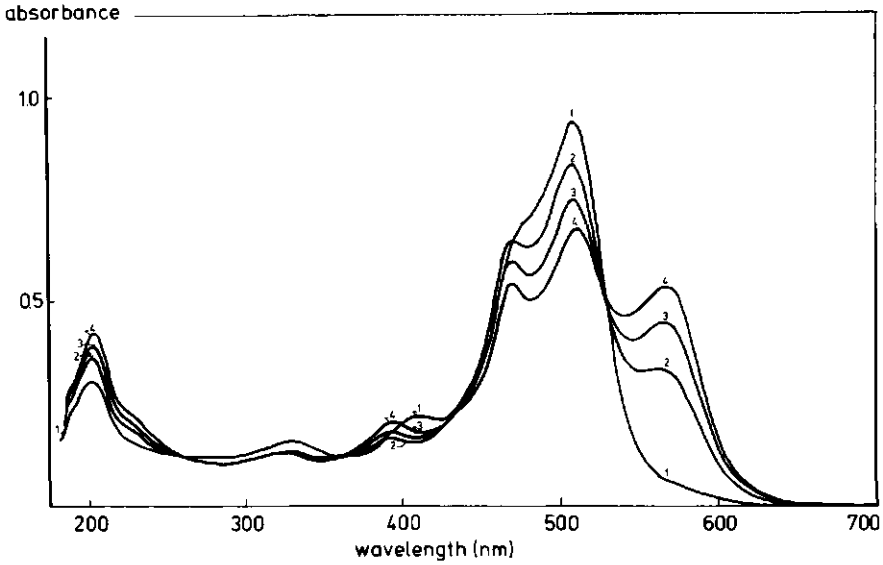


Fig. 3 - Marquis colour reaction. Spectra of glycoalkaloids in sulphuric acid/ paraformaldehyde taken at 1-5, 15-20, 40-45 and 80-85 minutes, respectively. The numbers 1, 2, 3 and 4 refer to the spectra recorded at 1-5, 20-25, 40-45 and 80-85 minutes, respectively (Bergers, II).

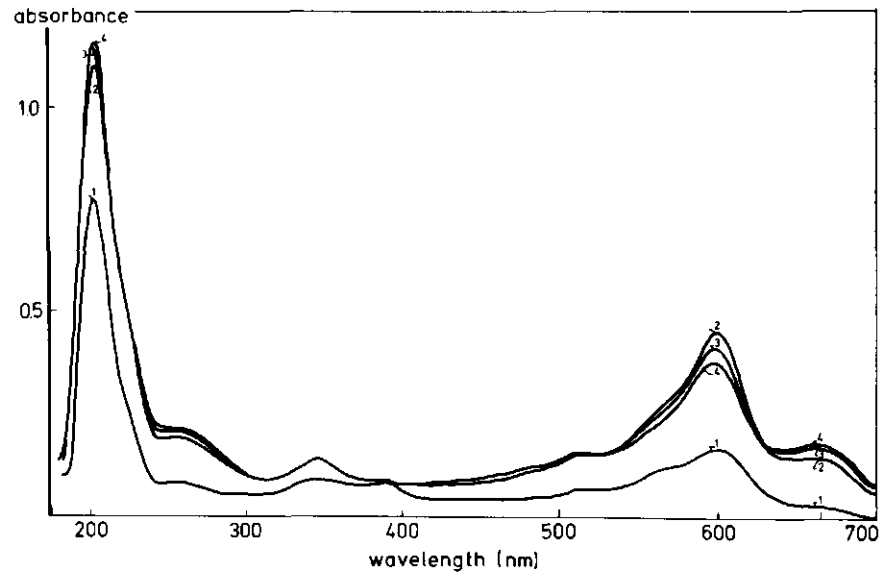


Fig. 4 - Clarke colour reaction. Spectra of glycoalkaloids in phosphoric acid/ paraformaldehyde taken at 1-5, 20-25, 40-45, 60-65 minutes. The numbers 1, 2, 3 and 4 refer to the spectra recorded at 1-5, 20-25, 40-45 and 60-65 minutes, respectively (Bergers, II).

4. POTATO IRRADIATION EXPERIMENTS

4.1 Glycoalkaloids and phenolic compounds in irradiated stored potatoes

Potato tubers were irradiated with gamma rays from a ^{60}Co source of approx. 190 kCi at the Pilot Plant for Food Irradiation in Wageningen.
Dose levels of 0.1, 0.5 and 3 kGy were given. Dosimetry was done by the Fricke ($FeSO_4$) and the perspex dosimeter. After irradiation, potatoes were stored at 10 °C, 90% RH. The target compounds were analyzed, as described in Chapter 3, after various periods of storage.

The results of the target compounds analyzed in the potato samples are represented in Tables 1, 2 and 3. Glycoalkaloid contents of stored irradiated (up to 3 kGy) potatoes, analyzed by two-way analysis of variance, showed no significant effects according to dose or storage time after the radiation treatment, Table 1.

Phenolic compounds, particularly scopolin show pronounced increases during storage in the case of high dose (3 kGy) irradiated potatoes of the Eba variety, Table 2; chlorogenic acid levels decline in 3 kGy irradiated Bintje and Eba potatoes, Table 3. Increases of fluorescent phenolic compounds of irradiated and control potatoes, analyzed during storage, show a time-dependent increase in fluorescent compounds in the high dose treated Eba and Bintje potatoes, Figure 5.

UV absorbance patterns obtained by HPLC chromatography of extracts (type (B), Figure 2) of 3 kGy irradiated potatoes of the Bintje and Eba varieties are shown in Figure 6. They show a change of the phenolic compounds in the irradiated potatoes dependent on the storage time after the radiation treatment. The patterns of the control and lower dose treated potatoes, analyzed during the 3 months storage period, do not change seriously and are similar to the patterns shown in Figure 6 of the 3 kGy dose treated potatoes, analyzed 1 day after irradiation.

Identification of the accumulated main fluorescent compound in 3 kGy irradiated Eba potatoes was achieved by hydrolysis with β-glycosidase of an aqueous extract (type (A), Figure 2) and isolation of the hydrolyzed aglycone. The mass spectra of scopoletin and the isolated product were identical, Figure 7 (Bergers, III).

Table 1 - Solanine contents of control and irradiated Bintje and Alpha potatoes during subsequent storage at 10°C (Bergers, III).

	Storage days after gamma irradiation													
	t = 1 d		t = 4 d		t = 7 d		t = 14 d		t = 32 d		t = 74 d		t = 125 d	
Bintje														
control	14.7[a]	(1.73)[b]	12.0	(1.44)	19.2	(0.71)	13.4	(0.61)	14.7	(1.31)	10.0	(1.61)	[c]	
0.1 kGy	13.9	(0.53)	16.3	(1.87)	18.2	(1.17)	12.9	(1.68)	11.2	(1.90)	12.3	(1.89)	17.0	(2.19)
0.5 kGy	14.6	(1.19)	8.4	(2.20)	15.4	(1.87)	12.6	(0.61)	15.6	(3.5)	10.4	(1.03)	16.5	(2.41)
3 kGy	16.8	(0.72)	10.1	(3.52)	14.3	(0.40)	13.3	(0.46)	11.2	(3.2)	9.0	(2.87)	17.7	(3.31)
	Storage days after gamma irradiation													
	t = 1 d		t = 4 d		t = 7 d		t = 14 d		t = 32 d		t = 74 d		t = 125 d	
Alpha														
control	8.0[a]	(0.68)[b]	5.7	(0.62)	7.8	(1.14)	6.9	(1.23)	8.9	(0.14)	7.2	(0.82)	[c]	
0.1 kGy	10.2	(1.04)	6.1	(1.18)	11.0	(2.25)	7.2	(1.47)	8.9	(0.21)	8.4	(1.31)	8.5	(0.23)
0.5 kGy	13.5	(0.80)	11.5	(0.40)	14.8	(0.91)	3.8	(0.75)	9.8	(0.29)	8.6	(1.05)	8.0	(1.80)
3 kGy	5.7	(0.26)	9.8	(2.18)	16.2	(0.64)	9.6	(1.94)	6.9	(0.47)	8.0	(2.03)	7.3	(1.46)

a) solanine contents (measured as total glycoalkaloid) from 10 symmetrical quarters of potatoes in mg/100 g freeze-dried tissue
b) standard deviation of triplicate chemical analysis
c) discarded because of severe sprouting

Table 2 - Chlorogenic acid contents of irradiated and control potatoes of BINTJE and EBA variety (mg/100 g freeze dried potato tissue) during storage at 10 °C (Bergers, III).

	t = 1 d	t = 4 d	t = 14 d	t = 33 d	t = 61 d	t = 90 d
Bintje						
control	26	23	24	26	26	17
0.1 kGy	42	35	30	57	39	30
0.5 kGy	30	27	41	41	37	24
3 kGy	45	34	26	24	21	18
Eba						
control	35	38	33	28	32	30
0.1 kGy	44	40	42	40	44	51
0.5 kGy	46	54	48	62	40	56
3 kGy	46	55	27	12	10	13

Table 3 - Scopoletin-β-glycoside contents of irradiated and control EBA potato tubers during storage (mg/100 g freeze dried potato tissue) at 10 °C (Bergers, III).

	Storage days after irradiation					
	t = 1 d	t = 4 d	t = 14 d	t = 33 d	t = 61 d	t = 90 d
control	-*	0.81	0.79	1.62	0.43	0.63
0.1 kGy	0.67	0.53	1.52	1.09	3.51	0.54
0.5 kGy	0.70	0.61	-	0.15	1.48	0.25
3 kGy	0.81	-	9.07	28.4	23.3	35.0

* no peak detected on HPLC chromatogram

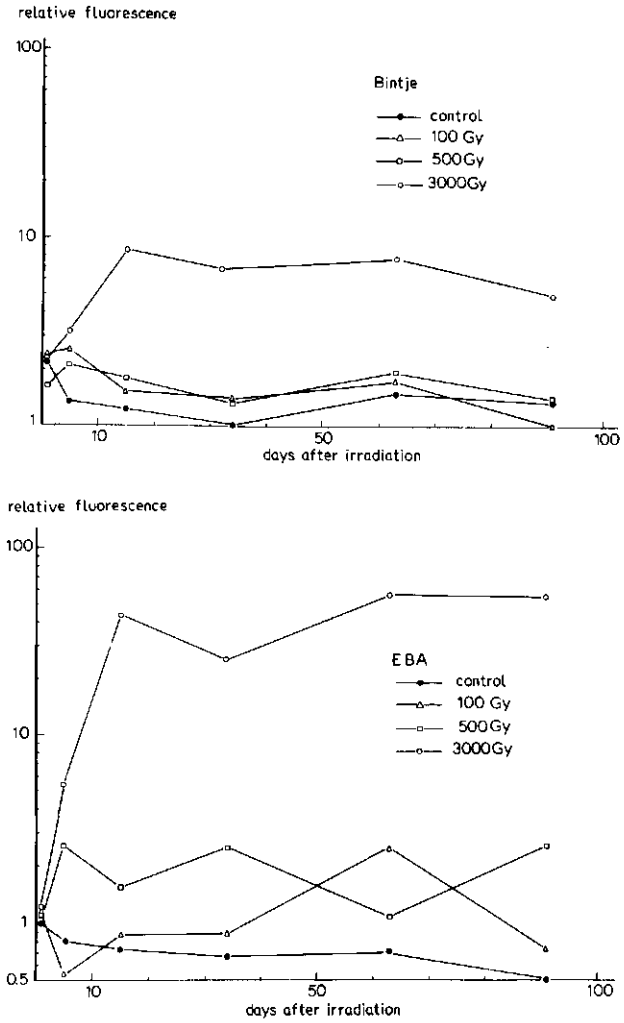


Fig. 5 - Relative fluorescence values of diluted alcoholic extracts (8 mg*/ml) in Phosphate buffer pH = 7 for irradiated and control potatoes of Eba and Bintje varieties during storage. Excitation wavelength 350 nm, Emission 450 nm (Bergers, III).

* original freeze-dried potato material

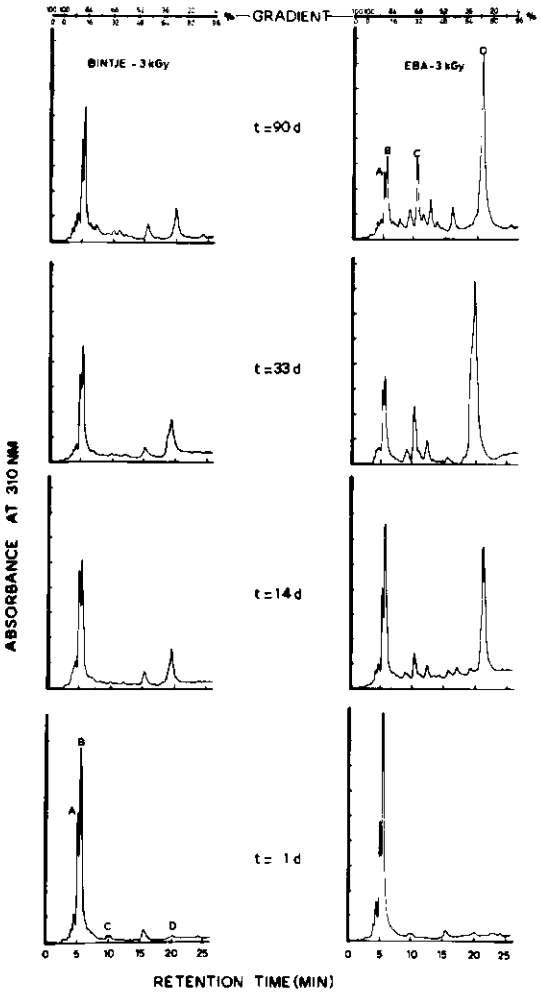


Fig. 6 - HPLC chromatograms of alcoholic extracts of Eba and Bintje potatoes irradiated at 3 kGy and sampled resp. 1, 13, 33 and 90 days after irradiation. Peak A, B and C represent respectively caffeic acid, chlorogenic acid and scopoletin-ß-glycoside. Peak D represents a major unidentified component. As mobile phase a gradient of critic acid/phosphate buffer, pH = 2.85 and 8.2 was used (Bergers, III).

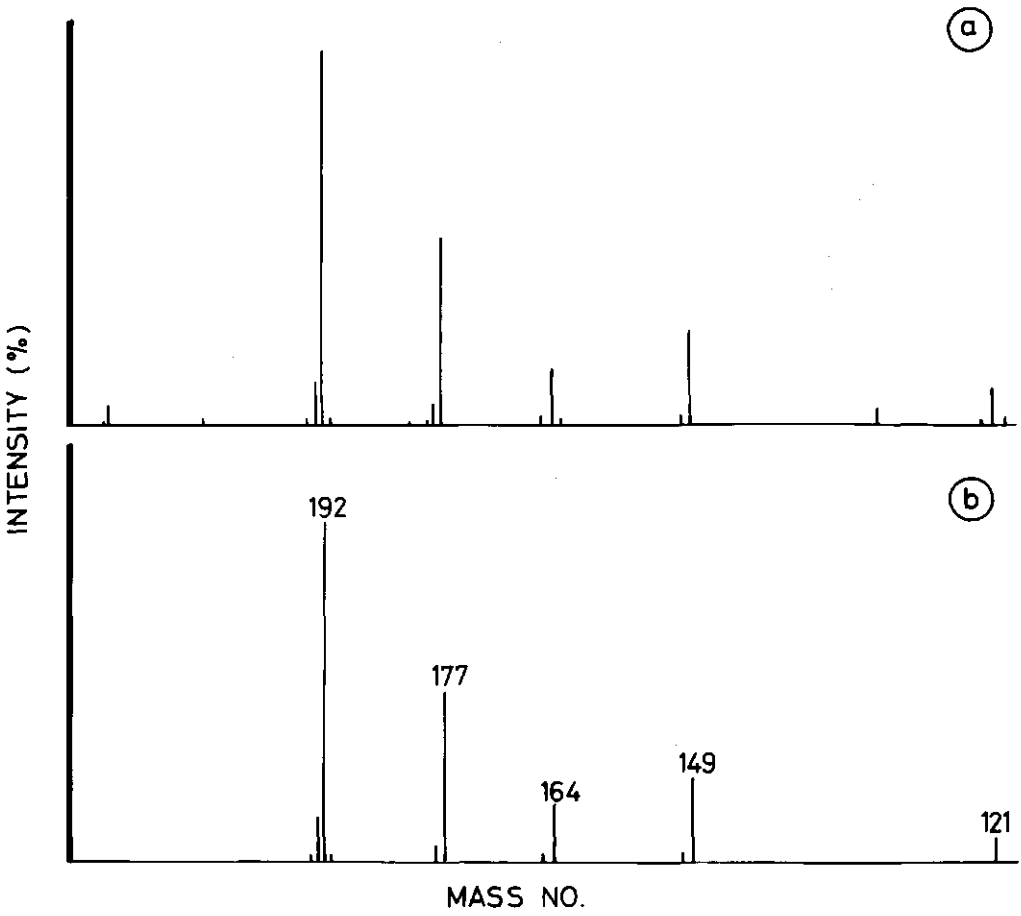


Figure 7 - Mass spectra of isolated product of 3 kGy irradiated potatoes of the Eba variety (a) and scopoletin (b).

4.2 Direct examination of fluorescent compounds using UV

4.2.1 Introduction

It has been shown in 4.1 that potatoes irradiated at high dose (3 kGy) accumulate fluorescent compounds which can be obtained from alcoholic extracts. The main accumulated fluorescent compound was identified as scopolin. Koeppe et al. (1970) also observed an increase in scopolin in tobacco after X-radiation, and Riov et al. (1972) noted the same effect after gamma irradiation.

By examining potato halves under UV light it was found that accumulation of fluorescent compounds could easily be observed. In this way, the response of tubers of several varieties could be studied after high dose (3 kGy) irradiation for induced fluorescence. Also, the effect of a dose range (0.1 to 3 kGy) on radiation-induced fluorescence was examined for a sensitive potato variety (Eba).

Cellular localization of fluorescent compounds was studied by fluorescence and light microscopy of sections of heavily fluorescent tissue.

4.2.2 Materials and methods

Potato tubers were irradiated with a gamma ^{60}Co source at several dose levels and stored after the irradiation treatment at 10 ^{o}C, 90% RH. Tubers of several potato varieties from clay soils were obtained from the Institute for Storage and Processing of Agricultural Produce (IBVL) in Wageningen.

Tubers were sliced in halves, from stem to bud end, and examined under long wave UV light (366 nm) of a chromatography lamp in the dark.

For the microscopy examinations, fresh sections of fluorescent tissues were cut on a sliding microtome (angle 10^{o}) at thicknesses of approx. 150 µm. The sections were examined under a fluorescence microscope (Wild) in a dark field. Excitation source Hg; excitation filter UG 1; emission cut-off filter 460 nm. Microphotographs were taken on a 50 ASA Agfa daylight colour-film and exposed for 2 to 4 min. Plasmolysis was studied by placing the sections in saturated KNO_3 solutions.

4.2.3 Results and Discussion

Direct visible accumulation of a fluorescent compound under UV was first seen in high dose (3 kGy) irradiated Eba potatoes. About a week after irradiation, strongly fluorescent tissue was seen in the cortex of sliced potato halves of the Eba variety. During subsequent storage, the fluorescent tissue increased inwards and after a month's storage, the small potatoes became totally light blue fluorescent. After prolonged storage, the vascular ring became brownish in some potatoes, whilst fluorescence remained, Figure 8.

Several varieties were examined for irradiation-induced (3 kGy dose) fluorescence. Accumulated fluorescence, yellow and light blue, was seen scattered throughout the potato tissue or around the cortex. Accumulation of fluorescence was determined two weeks after the radiation treatment and divided into three classes of 9 varieties.

Eba, Surprise, Pimpernel	strong fluorescence
Bintje, Alpha	moderate fluorescence
Marijke, Lekkerlander, Doré, Provita	little or no fluorescence

A dose range of gamma-irradiated Eba potatoes (0.1 to 3 kGy) showed accumulation for doses ranging from 1 to 3 kGy. Lower doses are comparable with unirradiated controls, see Figure 9.

Fluorescence microscopy of sections of fluorescent 3 kGy irradiated Eba tissue revealed specific fluorescent cells scattered throughout the tissue, especially near the vascular ring. The presence of non fluorescent plasma strings indicates a vacuolar localization of the fluorescent compounds (Figures 10, 11).

Similar observations of fluorescent cells have been demonstrated in fungal infected potato tubers (Clarke, 1973; Clarke & Baines, 1976). In addition, certain potato varieties, infected with virus, exhibit bright blue fluorescence (Andreae, 1944). These findings have in common the specific response of different varieties and accumulation of fluorescent compounds by a certain stress factor. Specific plant cells accumulate fluorescent compounds in stressed tubers and this finding may be related to the finding of specific phenol-storing cells in plant tissue by Beckmann (1972). Other phenolics, e.g. chlorogenic acid have been shown by Reeve (1969) to be unevenly distributed in potato tissue. An important

difference with irradiation-induced phenolics in citrus fruits, which is accompanied by peel pitting (Riov, 1975), is that radiation-induced fluorescence in potatoes is not directly accompanied by visible tissue damage.

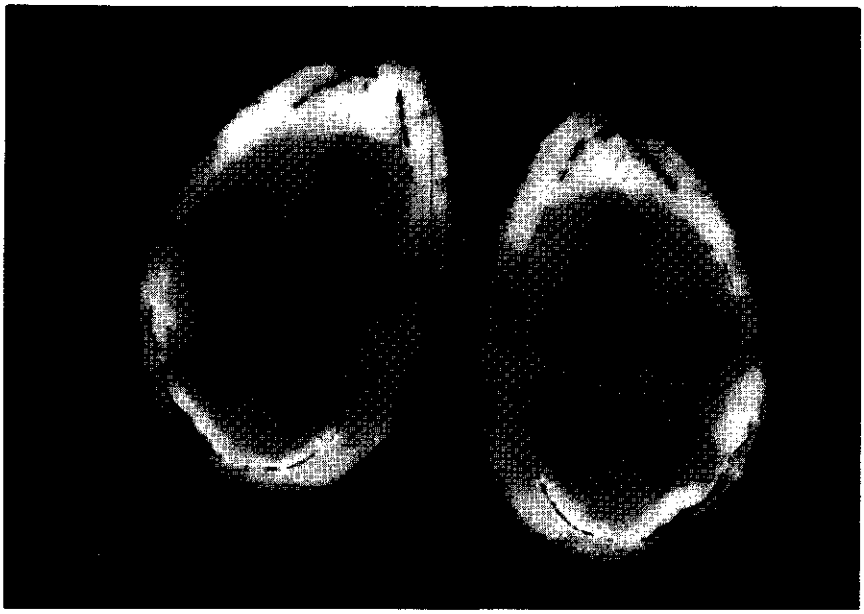


Figure 8 - Bright fluorescence visible under long-wave UV in halves of 3 kGy irradiated potato of the Eba variety after prolonged storage at 10 °C (circa 3 months). Necrotic tissue is visible near the vascular ring.

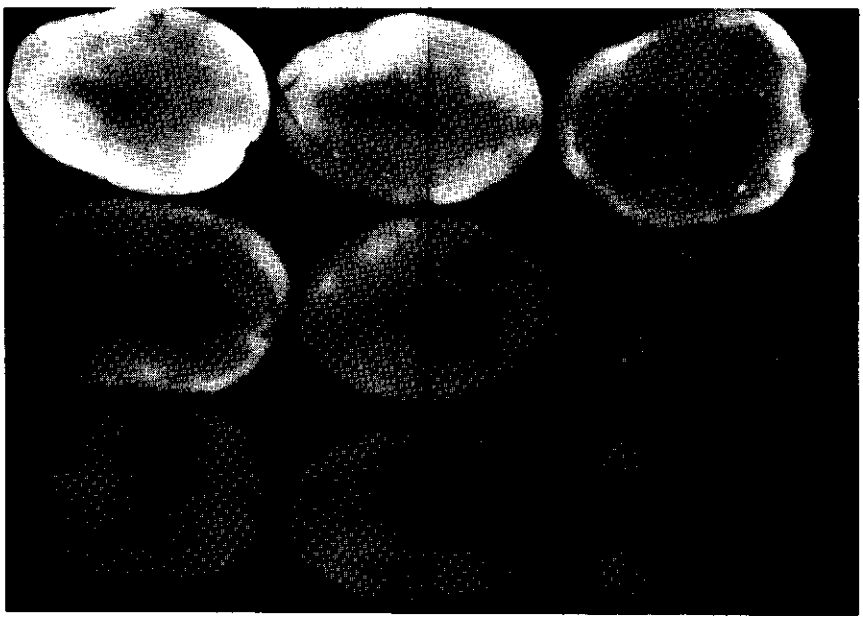


Figure 9 - Halves of potatoes of the Eba variety treated with several doses of gamma irradiation after a month's storage at 10 °C. Upper row, from left to right: 3.0, 2.5 and 2 kGy; middle row: 1.5, 1.0 and 0.5 kGy; lower row: 0.25, 0.1 and 0 kGy. Bright fluorescence is visible under long-wave UV in high dose (> 0.5 kGy) irradiated potatoes.

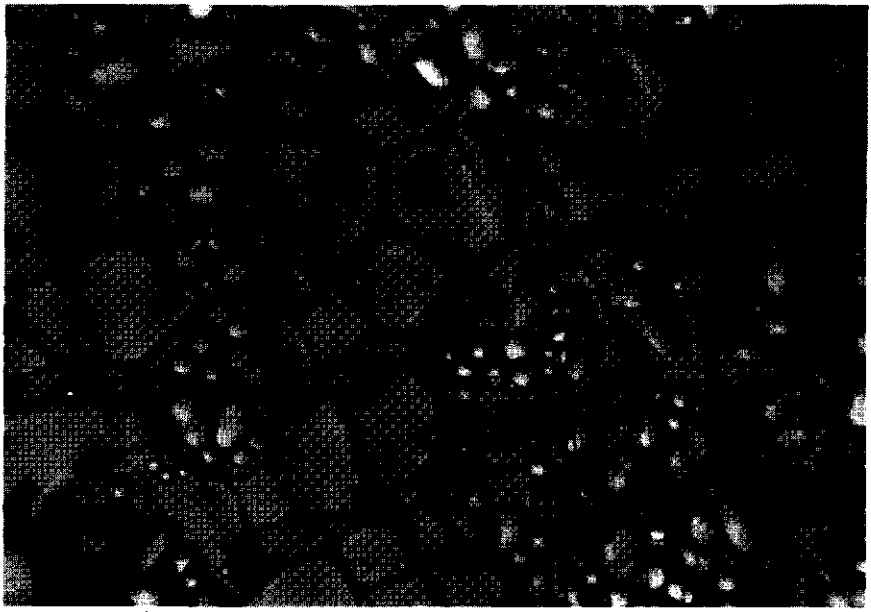


Figure 10 - Section of cortex tissue of 3 kGy irradiated potato tuber of the Eba variety seen by light microscopy (approx. 300 x).

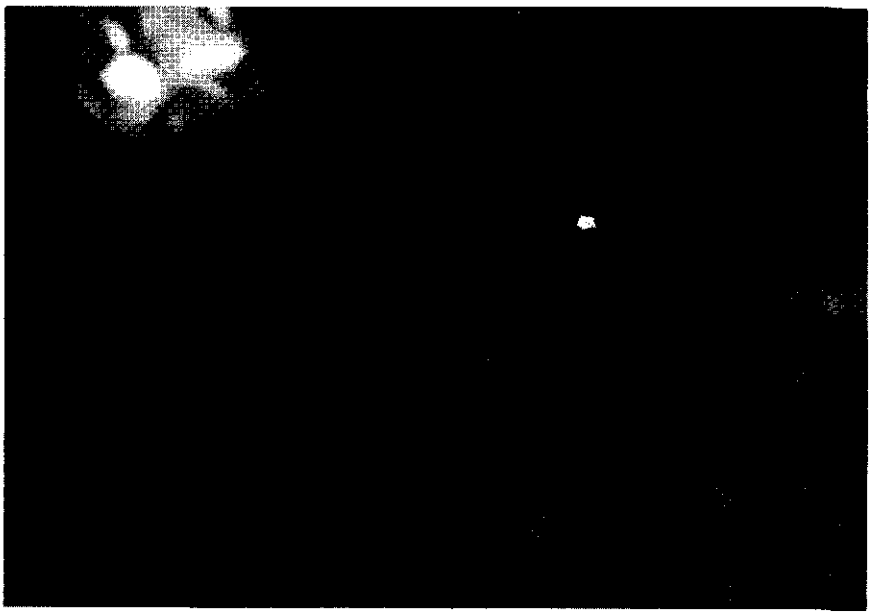


Figure 11 - The same section as in Figure 10, seen by dark field microscopy with UV light. Now brightly fluorescent cells become visible (approx. 300 x).

5. MUTAGENICITY TESTS OF TARGET COMPOUNDS AND ALCOHOLIC EXTRACTS

5.1 Introduction

Mutagenicity tests are frequently included in wholesomeness studies of gamma-irradiated foods (Münzner & Renner, 1975; Van Kooy et al., 1977; Hattori et al., 1979). Increased mutagenic activity was reported for alcoholic extracts of 0.1 kGy irradiated potato tubers (Kopylov et al., 1974). However, in two separate mutagenicity studies of alcoholic extracts no increase was found (Levinski & Wilson, 1975; Hossain et al., 1976).

In order to evaluate mutagenic effects of compounds from metabolically stressed potatoes, alcoholic extracts were examined in bacterial mutagenicity tests. In addition, reference glycoalkaloids and phenolic compounds were tested for mutagenicity.

5.2 Material and Methods

Strains and tests

The Ames *Salmonella typhimurium* strains TA 98, 100, 1535, 1537 and 1538 were used. Mutagenicity tests were performed by the plate assay as described by Ames et al. (1975). Microsomal liver enzymes (S9) were obtained from rats, induced with Arochlor 1254. Mutagenicity is measured by counting His^+ revertants. Positive controls were included in each test.

Extracts and chemicals

Alcoholic extracts were obtained by hot alcoholic extraction of freeze-dried potato tubers, concentrated by rotary evaporator, diluted with methanol and shaken with light petroleum (the extract (B) shown in Figure 2). NaN_3, o-coumaric acid, caffeic acid, ferulic acid, coumarin, umbelliferon and aesculin were obtained from Merck; chlorogenic acid, scopoletin, tomatine, α-solanine, ethidium bromide and 9-amino acridine from Sigma; solanidine from Roth and solasodine from Pfaltz & Bauer, AF_2 (2-(2-furyl)-3-(5-nitro-2 furyl) acrylamide from Sigumura (National Cancer Center Research Institute, Tokyo).

5.3 Results and Discussion

The results of mutagenicity tests of several alcoholic potato extracts using the tester strains TA 100 and TA 98 with and without the addition of microsomal liver enzymes are given in Table 4. No significant increase in the mutagenicity was found in the extracts of the irradiated potatoes (Bintje and Eba varieties) with respect to dose or storage time after the radiation treatment.

Reference compounds, e.g. glycoalkaloids and phenolic compounds were tested on strains TA 98 and TA 100, Table 5. No mutagenic effects for the compounds tested were observed (up to 200 µg/plate) and tests with the additional strains TA 1535, 1537 and 1538 were also negative (Table 6). At 200 µg/plate the alkaloids solanidine and solasodine were very toxic to the bacterial strains. However, lower non-toxic concentrations were not mutagenic.

Plant extracts contain certain levels of free histidine, which may influence spontaneous His^+ revertants (Van Kooy et al., 1977). Histidine levels from the alcoholic potato extracts were approx. 10 µg/plate and did not seriously interfere with the test. However, small insignificant increases can be explained in this way. In bacterial mutagenicity tests described here, no mutagenicity for simple coumarins was seen, although there are some reports of genetic effects of coumarins on plant tissues (Grigg, 1978). However, some precaution in the interpretation of results is necessary because of the discovery of antimutagenic factors in vegetable extracts (Van Kooy et al., 1977; Kada et al., 1978). It may be supposed that by using hot alcoholic extraction such factors were destroyed, as found by Kada et al. (1978). Further no mutagenicity was found for the glycoalkaloids, the well-known naturally occurring toxic constituents of potatoes; nor was any found for the aglycone. The *Salmonella*/microsome system is, however, in principle suited for detecting alkaloidal mutagens (Wehner et al., 1979).

This study shows that compounds in alcoholic extracts of metabolic stressed potatoes are non-mutagenic in the Ames test.

Table 4 - Results of AMES mutagenicity tests of extracts from irradiated and non-irradiated potatoes of the Eba and Bintje varieties. Means of triplicate assay, standard deviation between brackets. Extracts were prepared 1 and 15 days after the irradiation treatment.

*Alcohol extract 0.2 ml/pl	His$^+$ revertants/plate			
	TA 100		TA 98	
	-S9	+S9	-S9	+S9
BINTJE				
0 kGy t = 1 d	128 (5)	117 (14)	17 (5)	21 (5)
0 kGy t = 15 d	138 (11)	168 (29)	21 (2)	30 (5)
0.1 kGy t = 1 d	133 (16)	147 (10)	29 (4)	31 (8)
0.1 kGy t = 15 d	130 (18)	151 (14)	22 (7)	30 (3)
0.5 kGy t = 1 d	146 (22)	124 (7)	19 (4)	28 (1)
0.5 kGy t = 15 d	150 (8)	152 (6)	17 (5)	31 (7)
3 kGy t = 1 d	127 (9)	146 (12)	24 (5)	28 (10)
3 kGy t = 15 d	151 (19)	152 (6)	18 (7)	29 (5)
control	96 (3)	86 (4)	14 (2)	24 (11)
EBA				
0 kGy t = 1 d	147 (24)	159 (15)	24 (5)	39 (9)
0 kGy t = 15 d	163 (18)	167 (18)	27 (4)	34 (5)
0.1 kGy t = 1 d	150 (7)	153 (8)	22 (4)	37 (5)
0.1 kGy t = 15 d	173 (7)	180 (9)	26 (11)	32 (3)
0.5 kGy t = 1 d	167 (15)	160 (34)	22 (4)	36 (6)
0.5 kGy t = 15 d	149 (4)	174 (9)	27 (2)	33 (5)
3 kGy t = 1 d	140 (40)	155 (18)	22 (4)	41 (3)
3 kGy t = 15 d	166 (12)	186 (14)	31 (6)	42 (4)
control	144 (23)	127 (9)	21 (10)	31 (9)
**NaN_3 (2 µg/pl)	498 (80)	-	-	-
**Eth.Br. (1 µg/pl)	-	-	-	185 (81)
**AF_2 (0.1 µg/pl)	-	-	291 (95)	-

* 0.2 g/ml original freeze-dried potato
** positive controls

Table 5 - Results of AMES mutagenicity tests with bacterial strains of *Salmonella typhimurium* TA 100 and TA 98. Means of triplicate assay, standard deviation between brackets.

Compounds tested at 200 µg/pl in 0.2 ml DMSO	His$^+$ revertants/plate				Survival %
	TA 100		TA 98		
	-S9	+S9	-S9	+S9	
p-coumaric acid	64 (11)	70 (14)	13 (2)	17 (3)	100
o-coumaric acid	73 (3)	77 (5)	15 (2)	22 (4)	100
caffeic acid	95 (10)	103 (5)	34 (2)	35 (6)	100
ferulic acid	67 (4)	74 (6)	9 (3)	21 (2)	100
chlorogenic acid	90 (12)	94 (15)	12 (2)	22 (6)	100
coumarin	76 (10)	92 (15)	12 (2)	19 (1)	100
aesculetin	89 (6)	88 (9)	20 (1)	31 (6)	100
scopoletin	96 (6)	108 (3)	14 (3)	29 (7)	100
umbelliferon	91 (5)	104 (17)	13 (1)	28 (2)	100
α-solanine	97 (6)	94 (6)	18 (6)	24 (1)	100
solanidine	83 (10)	124 (12)	17 (2)	24 (4)	toxic
tomatine	99 (18)	103 (12)	18 (4)	33 (8)	100
solasodine	87 (15)	96 (2)	18 (1)	21 (2)	toxic
control	101 (14)	101 (13)	14 (4)	24 (6)	100
*NaN_3 (2 µg/pl)	498 (80)	-	-	-	100
*Eth.Br. (1 µg/pl)	-	-	-	185 (81)	100
*AF_2 (0,1 µg/pl)	-	-	291 (95)	-	100

* positive controls

Table 6 - Results of AMES mutagenicity tests with bacterial strains of *Salmonella typhimurium* TA 1535, TA 1537 and TA 1538. Means of triplicate assay, standard deviation between brackets.

Compounds tested (200 µg/pl) in 0.2 ml DMSO	His$^+$ revertants/plate						Survival %
	TA 1535		TA 1537		TA 1538		
	-Sg	+Sg	-Sg	+Sg	-Sg	+Sg	
caffeic acid	14 (7)	12 (3)	5 (3)	6 (1)	5 (1)	12 (2)	100
chlorogenic acid	19 (5)	22 (3)	9 (3)	7 (2)	9 (1)	17 (6)	100
coumarin	23 (9)	10 (1)	7 (2)	9 (2)	7 (2)	16 (4)	100
umbelliferon	20 (5)	25 (2)	7 (1)	10 (3)	5 (2)	19 (3)	100
scopoletine	17 (6)	17 (4)	6 (3)	5 (1)	5 (2)	12 (3)	100
α-solanine	24 (8)	10 (6)	7 (2)	12 (3)	6 (1)	14 (3)	100
solanidine	16 (6)	11 (3)	6 (6)	6 (4)	11 (3)	9 (2)	toxic
solasodine	12 (3)	12 (5)	7 (3)	10 (2)	9 (2)	14 (6)	toxic
control	46 (8)	15 (2)	7 (1)	7 (4)	9 (2)	12 (3)	100
* NaN_3 (1 µg/pl)	223 (76)	-	-	-	-	-	100
* Eth.Br. (1 µg/pl)	-	-	-	-	-	149 (82)	100
* 9-Amino acridine (100 µg/pl)	-	-	412 (154)	-	-	-	100

* positive controls

6. GENERAL DISCUSSION

Chemical changes for evaluation of the wholesomeness of gamma-irradiated foodstuffs have been proposed as an additional approach to animal feeding tests (Elias & Cohen, 1977). For irradiated fresh vegetable products these changes are twofold, i.e. by radiochemical reactions or by metabolic stress. The latter is the subject of this study using irradiated potatoes. The observed increase of phenolic compounds, i.e. scopolin, does not seem important for potato irradiation, because in order to inhibit sprouting a dose of 0.1 kGy is sufficient. Irradiated potatoes (maximum 0.15 kGy) were also evaluated by WHO in 1976 as being safe for human consumption, based on several animal feeding tests (Urbain, 1978). However, in principle, vegetable products may be irradiated to approx. 3 kGy and therefore increases in phenolics may occur in products irradiated with high doses. Similar increases in phenolics in citrus fruit peel confirm this (Riov et al., 1972). The results obtained during this study indicate the importance of dose, potato variety and the duration of storage after the radiation treatment. Further literature data for metabolic stress effects of irradiation (Chapter 2) point to a decreasing response in more senescent vegetable products, e.g. non-climacteric fruits.

Both potatoes and citrus fruits contain small amounts of hydroxy-coumarins before irradiation and therefore a radiation-induced synthesis of coumarins should be expected primarily in those products having a natural coumarin content. Investigations of other vegetable products may be indicated.

Chemical changes resulting from decomposition products can be estimated from model foods (Diehl & Schertz, 1975). They estimated, for a theoretical foodstuff composed of 80% water and 6.6% fat, carbohydrate and protein, a maximum yield of approx. 55 mg/kg decomposition products for an irradiation dose of 5 kGy and stated that in actual measurements this would be at least 5 times smaller. In comparison, this investigation showed an increase in the scopolin content of 3 kGy irradiated Eba potatoes from 2 to approx. 60 mg/kg fresh weight (a water content of 80% is assumed). It follows that metabolic stress compounds can be formed in much higher yields than radiolytic products and must be carefully evaluated in relation to the wholesomeness of irradiated food.

SUMMARY

Irradiation is a recent preservation method. With the aid of ionizing radiation microorganisms in food can be killed or specific physiological processes in vegetable products can be influenced.

In order to study the effects of metabolic radiation stress on quantitative chemical changes in vegetable products, specific target compounds were investigated in stored irradiated potatoes. These target compounds, i.e. glycoalkaloids and phenolic compounds were choosen with a view to food toxicology and food sensoric quality.

Much attention has been spent on quantitative analyses of the pre-selected target compounds in the potato samples. Enzymic changes of the polyphenolic compounds were kept to a minimum by the direct freezing of potato slices in liquid nitrogen, freeze-drying and extraction by boiling in 80% ethanol.

A quantitative assay for solanidine potato glycoalkaloids was developed from a pre-existing method with minor changes, by which the rapidity of the assay is improved, without affecting its sensitivity. As a specific application, this method was used for the analysis of solanidine glycoalkaloids in industrial potato protein. Because of objections to the colour assays for glycoalkaloids, concerning their specificity (Fitzpatrick & Osman, 1974; Coxon et al., 1979), these quantitative reactions were investigated.

Quantitative analyses of phenolics and coumarins were done, starting from the alcoholic extracts. Qualitative data were obtained by analysis of UV spectra and fluorescence of diluted extracts and TLC chromatography on cellulose plates. For quantitative analyses a method was developed by HPLC chromatography of alcoholic extracts. Evidence with respect to identification of scopolin and scopoletin in the alcoholic extracts was obtained by comparison of extracts with and without enzymic hydrolysis with β-glycosidase. Scopoletin was also directly identified by UV, IR and Mass Spectra.

Results of glycoalkaloids, analyzed over several seasons, show no significant changes with regard to irradiation dose or storage time. On the other hand a change in phenolic compounds and coumarins was observed.
A 10 to 30 fold accumulation of scopolin was found in irradiated (3 kGy) potatoes of the Eba variety after approx. one month's storage at 10 ^{o}C, 90% RH and

also a decrease in the chlorogenic acid content. For irradiated (3 kGy) potatoes of the Bintje variety, the increase in fluorescent compounds was smaller. Several unidentified phenolic compounds increase in 3 kGy irradiated Eba potatoes, which were detected by UV absorbance at 310 nm.
Chemical analyses of samples of irradiated Eba potatoes indicate a dose *threshold* for accumulation of fluorescent compounds. Below 0.5 kGy no increase is observed.

By using the high fluorescence of the accumulated coumarins it was possible to detect accumulation of fluorescent compounds by simply examining potato halves under long wave UV light. The results agree with the chemical analyses of extracts of irradiated potatoes of the Eba and Bintje varieties. In this way potatoes of several varieties irradiated with a dose of 3 kGy and stored for at least 2 weeks at 10 ^{o}C could be examined. The results indicate pronounced differences between varieties. Further, a *threshold* for the accumulation of fluorescent compounds in irradiated Eba potatoes could be determined. In Eba potatoes irradiated above 0.5 kGy accumulation of fluorescent compounds could be seen. By fluorescence microscopy of sections of tubers it was observed that specific cells accumulate fluorescent coumarins. Examination of the fluorescent cells after plasmolysis, indicates a vacuolar origin of these compounds. Similar results have been reported for fungal infected potatoes (Clarke, 1973; Clarke & Baines, 1976). The increase in scopoletin can be explained by the increase in phenyl ammonia lyase, which has been shown to increase in irradiated citrus fruits (Riov et al., 1972) and irradiated potatoes (Pendharkar & Nair, 1975).

In view of conflicting earlier reports of increases in mutagenic compounds in alcoholic extracts of irradited potatoes, glycoalkaloids and phenolic compounds as well as the alcoholic extracts of irradiated and control potatoes were examined using a bacterial mutagenicity test system. No increased mutagenicity of extracts or reference compounds were found. These results agree with the negative findings of Levinsky & Wilson (1975) for mutagenic evaluation of extracts of irradiated potatoes and mutagenicity studies on irradiated potatoes and chlorogenic acid by Hossain et al. (1976).

Radiation-induced increase in coumarins is to be expected primarily in vegetable products having a natural coumarin content. Chemical changes as a result of radiolytic processes in a theoretical foodstuff can be estimated. Diehl & Schertz (1975) calculated 55 mg/kg radiolytic decomposition products for a 5 kGy dose. In comparison, in this study an increase in scopolin content from 2 to 60 mg/kg was found in 3 kGy irradiated Eba potatoes after a month's storage at 10 ^{o}C caused by metabolic stress.

SAMENVATTING

Gamma bestraling is een vrij recente techniek om de houdbaarheid van voedingsmiddelen te verlengen. Dit effect komt tot stand enerzijds door het afdoden of reduceren van bederf veroorzakende of pathogene microorganismen en anderzijds door gewenste biologische effecten tot stand te brengen, zoals spruitremming bij aardappelen en uien of rijpingsvertraging bij bepaalde vruchten of een combinatie van beide.

Plantaardige producten onderscheiden zich van "dode" voedselsystemen (b.v. vetten, suikers of producten, waarin de endogene enzymen geïnactiveerd zijn) en voedselsystemen met een beperkt metabolisme (vlees en vis) door een actief metabolisme tijdens de bewaring. Hierdoor kunnen naast de vorming van radiolyse producten door gamma bestraling mogelijk ook veranderingen optreden in van nature voorkomende toxische componenten in plantaardige producten.

Deze chemische veranderingen zijn van belang bij de beoordeling van de toxicologische risico's van bestraald voedsel en van belang bij de beoordeling van de sensorische kwaliteit van bestraald voedsel.

De aardappelknol werd gekozen om de effecten van metabole veranderingen na gamma bestraling, veroorzaakt door "stress", te bestuderen. De gehaltes aan fenolen en glycoalkaloiden, met name α-solanine en chaconine, chlorogeenzuur en scopoline (β-glycoside van coumarine, 7-hydroxy, -6-methoxy). De keuze van deze stoffen is gedaan vanwege hun toxicologisch belang. Het in geringe concentratie in aardappels natuurlijk voorkomende glycoalkaloid solanine is een bekende toxische verbinding; terwijl van coumarines genetische effecten bekend zijn. Chlorogeenzuur heeft een betrekkelijk lage toxiciteit, maar is meer van belang i.v.m. verkleuringen.

Veel aandacht werd besteed aan de analytische methoden ter bepaling van glycoalkaloiden en fenolen in bestraalde aardappelen. Ter vermijding van enzymatische omzettingen werden aardappelen in plakjes gesneden en meteen bevroren met vloeibare stikstof en later gevriesdroogd. Vervolgens werd het gevriesdroogde materiaal geextraheerd met 80% alcohol onder reflux. De alkaloiden werden bepaald met een gewijzigde bestaande methode, welke vooral de analysesnelheid ten goede kwam. Bovendien werden de kwantitatieve kleurreacties voor glycoalkaloiden uitvoerig onderzocht naar specificiteit en werkingsmechanisme. Voor de analyse van de fenolen werd gebruik gemaakt van een com-

binatie van UV spectra, fluorescentie, dunne laag chromatografie en hoge druk vloeistof chromatografie.

De resultaten voor glycoalkaloiden in bestraalde en niet bestraalde aardappelen toonden geen significante veranderingen t.a.v. de stralingsdosis en de bewaarduur na bestraling. Verschillende aardappelrassen vertoonden hetzelfde beeld.

Daarentegen werden voor fenolen en coumarines wel veranderingen waargenomen. Een 20-30-voudige toename van scopoline werd gevonden in bestraalde (3 kGy) aardappelen van de Eba variëteit ongeveer een maand na bestraling en tegelijkertijd een afname van chlorogeenzuur. Voor bestraalde (3 kGy) aardappelen van het ras Bintje was het effect van straling op de fenolen kleiner. Verschillende niet geïdentificeerde verbindingen namen toe in 3 kGy bestraalde Eba aardappelen, welke met UV absorptie detectie (310 nm) werden waargenomen. Bij de lagere doses (0.5 kGy en 0.1 kGy) werden echter geen veranderingen van de fenolen gevonden. Het bij hogere doses accumulerende scopoline werd geïsoleerd uit Eba aardappels en geïdentificeerd met UV-, Massa- en IR-spectra.

Accumulatie van fluorescerende verbindingen kon ook direct worden waargenomen onder langgolvig UV. De resultaten voor de bestraalde aardappelen van Eba en Bintje liepen parallel met de chemische analyses. Op deze wijze konden verschillende rassen met elkaar vergeleken worden. Voor de accumulatie van fluorescerende verbindingen in bestraalde Eba aardappelen werd een dosis grenswaarde van ongeveer 0.5 kGy vastgesteld. In fluorescerend aardappelweefsel werden d.m.v. fluorescentie microscopie specifieke fluorescerende cellen gevonden. Bestudering van plasmolyse duidde op lokalisatie van de fluorescerende verbindingen in de vacuole. Dergelijke bevindingen zijn ook gevonden voor accumulatie in met schimmel geïnfecteerde aardappelen (Clarke & Bainer, 1976).

Met het oog op tegenstrijdige literatuurgegevens omtrent een mutagene werking van alcohol extracten van bestraalde aardappelen, werden glycoalkaloiden en fenolen als ook alcohol extracten getest op mutageniteit. Geen verhoging van mutageniteit van extracten of referentie stoffen werd waargenomen in de Salmonella/microsoom test (Ames test).

Extrapolatie van het gevonden effect van toename van coumarines naar andere plantaardige producten lijkt niet zonder meer mogelijk. Hoewel in de schil van bestraalde grapefruits bij hogere doses een dergelijk effect werd waargenomen door Riov et al. (1972), lijkt het van belang, dat deze coumarines in ieder geval ook in niet bestraalde producten voorkomen.

De veranderingen ten gevolge van radiochemische processen in bestraald voedsel kunnen geschat worden op grond van model voedingsmiddelen (Diehl &

Schertz, 1975). Zij bedragen voor een theoretisch voedingsmiddel, bestaande uit 80% water en 6,6% vetten, suikers en eiwitten ten hoogste 55 mg/kg omzettingsproducten voor een dosis van 5 kGy, terwijl in werkelijkheid lagere waarden werden gevonden. Ter vergelijking: in deze studie werd voor scopoline in 3 kGy bestraalde Eba aardappels ten gevolge van "stress" een toename van 2 naar 60 mg/kg vers gewicht gevonden ongeveer een maand na bestraling.

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