ABSTRACT

GLYCOALKALOID AND DEMETHYL STEROL CONTENT IN SOME POTATO VARIETIES AND CLONES

By

Samuel Lo-Tung Wang

A bisolvent mixture, MeOH-$CHCl_3$ (2:1), was developed for the extraction of glycoalkaloid from potato tubers. Blending the sample and the solvent mixture at a ratio of 1 to 20 completely dehydrated the sample, denatured the soluble protein and eliminated the adhesiveness of starch. A higher yield of glycoalkaloid was obtained by the bisolvent extraction method than by the Soxhlet extraction method. A complete recovery of added glycoalkaloid was also obtained.

The glycoalkaloid isolated from the surface of potato tubers was shown to contain both α-solanine and α-chaconine by thin-layer chromatography. Both glycoalkaloids had identical absorption spectra after reacting with the Marquis reagent, the Clarke reagent or 85% H_3PO_4.

Demethyl sterols were obtained from the chloroform fraction of the bisolvent mixture. After saponification, free sterols and steryl glycosides were separated by thin-layer chromatography, and both fractions were found to contain cholesterol, stigmasterol and β-sitosterol. The quantity of above sterols was determined by thin-layer and gas-liquid chromatography. β-sitosterol was the most abundant one.

The glycoalkaloid levels of Russet Burbank, Kennebec, 709 and 321-65 potato tubers during control storage at 40 and 50F generally decreased. Most of the decrease occurred during the first two months of storage and it was most pronounced in tubers with initially high glycoalkaloid content. A rise in storage temperature (45F to 60F) resulted in an increase in glycoalkaloid content with the greatest increase occurring in the Russet Burbank variety. The glycoalkaloid content of tubers with an initially high glycoalkaloid level was generally reduced by sprouting, while in those with lower glycoalkaloid levels, there was slight increase of glycoalkaloid. None of the potato tubers had a glycoalkaloid level that could be considered undesirable.

Glycoalkaloid level was found to increase rapidly when potatoes were treated with white light, but no increase was obtained from the maleic hydrazide treated 709 after 2 months storage at 45F.

Red and UV lights also induced synthesis of glycoalkaloid but to a less extent, while far-red light caused less increase of glycoalkaloid than did darkness.

Total sterol of potato tubers stored at 40F increased with time, and an initial increase with a subsequent decrease of total sterol was found in the MH-30 treated potatoes stored at 50F. The increase of total sterol was primarily due to the increase of β-sitosterol, although cholesterol decreased. The glycoalkaloid level of the untreated potatoes stored at 50F did not have a consistent pattern of change.

Potatoes with more than 15 mg/100 g w.b. of glycoalkaloid were considered as unsafe for human consumption, but this level would not be detected by the consumers according to the results of this study. The glycoalkaloid level higher than 25 mg/100 g w.b. was detected and rejected significantly. The taste of the glycoalkaloid appeared either as an astringent or burning sensation.

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Samuel Lo-Tung Wang

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INTRODUCTION

Due to degeneration of the old potato varieties, breeding of new clones is carried on continuously. New clones are evaluated mainly by their total solids, yield per unit, disease resistance, processing characteristics and flavor. At times, the presence of glycoalkaloid is overlooked. The glycoalkaloid in potatoes is not only a hazard to human health, but also causes off-flavor. With the increasing number of clones and species hybrid seedlings being bred each year, the possibility of introducing a potato variety with a high level of glycoalkaloid is also increasing. Therefore, an efficient and rapid method of glycoalkaloid determination is needed for examination of the glycoalkaloid content of the new potatoes.

Studies were undertaken to determine the glycoalkaloid content of two established commercial varieties, Russet Burbank and Kennebec, which have excellent processing characteristics, and are ranked number 1 and 3 respectively in total acreage in the States (Talburt and Smith, 1967), and two clones, 709 and 321-65, which are to be released by the Crop and Soil Science Department, Michigan State University, during storage.

The effect of white, far-red, red and ultraviolet light on the synthesis of glycoalkaloid was studied. It has been established that potatoes exposed to sunlight prior to harvest generally contain a high level of glycoalkaloid.

Representative plots of the potatoes were also sprayed with maleic hydrazide, a preharvest sprout inhibitor, to evaluate its effect on the synthesis of glycoalkaloid during storage.

LITERATURE REVIEW

The Occurrence and Distribution of Glycoalkaloid in Potatoes

Solanum alkaloids, a group of steroidal glycoalkaloids occurring in the Solaneceae genus, has been a subject of interest to scientists for many years. The study of steroidal glycoalkaloids in potatoes was initiated when Desfosses (1820) reported the existence of a new substance, which he designated as "Solanin," in berries of S. nigrum and S. dulcamara potatoes. Solanine was also found in sprouts of S. tuberosum potatoes by Baup (1826), and in tubers by Wackenroder (1843).

For many years, the steroidal glycoalkaloid isolated from potato plant was thought to be a single compound, until Kuhn and Löw (1954), using paper chromatography, resolved the seemingly single compound into six compounds, namely α-, β-, γ-solanine and α-, β-, γ-chaconine. β-, γ-solanine and β-, γ-chaconine are found only in potato plants, and can only be isolated during the active synthesis of α-solanine and α-chaconine. In potato tubers, both α-solanine and α-chaconine are present (Allen and Kûc, 1968).

Therefore, solanine, defined as "the steroidal alkaloidal fraction of potatoes soluble in acidified alcohol and insoluble in slightly alkaline aqueous solution" by Talburt and Smith (1967), is a mixture of solanine and chaconine. However, it should be mentioned that the word "solanine" appearing in literature published since 1954 frequently refers only to α-solanine. Many researchers now use the term "glycoalkaloid" to avoid possible confusion.

Morgenstern (1907) investigated the solanine level in potatoes of 39 varieties and reported that the average content of solanine was higher in reddish varieties than in yellowish varieties, and higher in food-potatoes than in fodder-potatoes. A number of studies carried out in different countries (Lampitt et al., 1943; Wolf and Duggar, 1946; Arnold, 1950; Gull and Isenberg, 1960; Zitnak, 1961; Simek, 1962; Gawecki and Ilecki, 1966; Vecher, 1967) confirmed that solanine level of different potato varieties varied widely.

Solanine level of potato is a function of many factors in addition to variety. Potatoes subjected to different climates, soils and fertilizer treatments are likely to have different levels of solanine (Simek, 1962). Potatoes from different crop years, if not grown under identical environmental conditions, may be expected to have different solanine content (Gawecki and Ilecki, 1966).

Solanine is present in all parts of the potato plant in different concentration. Early analysis by Gmelin (1859) showed that different parts of potato contained different amounts of solanine. Potato sprouts had the highest content of solanine, and potato tubers had the lowest, the ratio being 68 to 5 in the sample he used. Meyer (1895) reported that the solanine content was higher in unpeeled potatoes than in peeled ones, indicating that the solanine content was higher in the potato cortex. He also found that the average level of solanine decreased as the sprouts grew longer. Several studies on the distribution of solanine in different parts of potato plant (Lampitt et al., 1943; Street et al., 1946; Wolf and Duggar, 1946; Paseschnitschenko, 1957) agreed that sprouts, leaves, stolons, roots, flowers and buds had very high concentration of solanine, whereas the solanine concentration of the tubers was low, normally ranging from 10 to 50 mg per 100 g (dry weight basis), and was predominantly present in cortex of tuber (traditionally considered as potato peel). Relatively higher concentration of solanine was detected in the eyes of tuber (Lampitt et al., 1943; Turapin, 1951). Griebel (1924) reported that unripe tubers had a higher solanine content than mature ones and small tubers more than large ones.

Change of solanine level in various parts of potato plant during the growing season was studied by Morgenstern (1907), Street et al. (1946), and Wolf and Duggar (1946). The solanine level in each part of the potato plant seemed to increase in the early period of emerging and then gradually decreased until maturity. Solanine content in tubers did not change in a consistent pattern. Wolf and Duggar (1946) reported that a higher solanine content was found between 50 and 70 days after emerging in tubers of White Rural and Irish Cobbler, but solanine content in Russet Burbank tuber decreased continuously with the growing period.

Factors Affecting Level of Glycoalkaloid in Stored Potato Tubers

Potato tuber is metabolically active. Various biochemical changes take place during storage. Without outside interference, the tuber undergoes a resting period before change occurs. Upon exposure to light, synthesis of chlorophyll (Larsen, 1949), anthocyanin (Stanko, 1962), chlorogenic acid (Zucker, 1963) as well as glycoalkaloid in tuber is induced. Sunburned potato tubers have a high level of glycoalkaloid (Morgenstern, 1907; Bömer and Mattis, 1924).

Conner (1937) reported that flourescent light with wavelength of 300 nm stimulated the synthesis of solanine rapidly, and light capable of promoting chlorophyll synthesis did not necessarily increase solanine synthesis. Using freshly harvested potato tubers as samples, Barug (1962) found that solanine content of potato increased with the intensity and duration of light, and also pointed out that solanine concentration in potato peels was highly correlated with the intensity of light. Gull and Isenberg (1960) reported that both solanine and chlorophyll increased with time of exposure up to 96 hours. Whether these two biochemical processes are closely related was not certain.

Potato tubers exposed to different lights appeared to differ in the amount of solanine and chlorophyll synthesized. Blue or green cellophane papers were more effective than red ones in reducing tuber greening under light (Larsen, 1949). Liljemark (1960) reported that daylight induced more solanine and chlorophyll than did red light, and green light induced little chlorophyll.

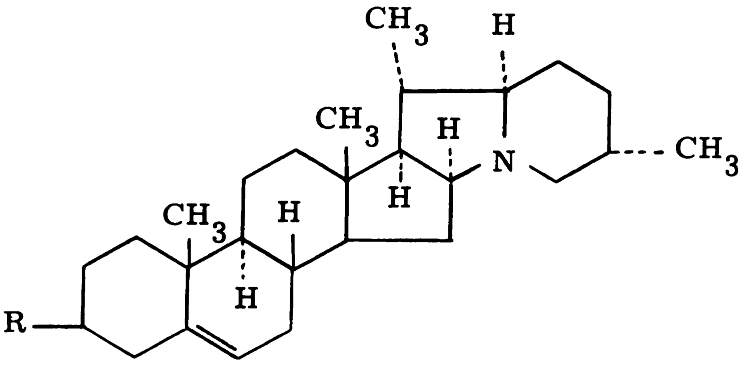
Solanine level of tubers in storage was not studied to any extent. Arutyunyan (1940) reported that solanine level did not change after 45 days of storage at -6C to -8C. Solanine was actively synthesized when potato was sprouting, and most, if not all, of the synthesized solanine was located in sprouts (Lampitt _et al._, 1943). Moskaleva and Pogorelova (1969), using fluorescent microscopy and histochemical analysis, found that distribution of both solanine and chaconine in sprouts was not uniform; maximum content was in meristematic tissues of top and side buds. Synthesis of solanine during sprouting was not light-dependent.

Varietal differences of potatoes in the response to light irradiation was also found. Genetic difference in the greening of potato tubers was reported by Gull and Isenberg (1958), and Akeley et al. (1962). Barug (1962) compared the potential of solanine synthesis in 9 varieties exposed to daylight for 72 hours, and suggested that varietal difference with respect to solanine increase in potatoes upon exposure to light also existed.

Chemical and Physical Properties of Solanine and Chaconine

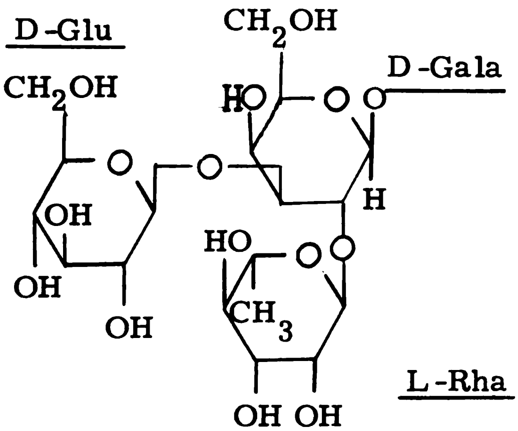
Both solanine and chaconine are steroidal glycoalkaloids; this group of compounds is characterized by consisting of three moieties: sugar, steroidal structure and alkaloid. Their aglycone is solanidine. The only difference between solanine and chaconine is in the sugar moiety.

The structure of solanine was studied soon after its discovery (Blanchet, 1838; Zwenger, 1861). The correct empirical formula was not worked out until Shörpf and Hermann (1933) proposed a formula $C_{27}H_{43}ON$ for solanidine; and later, Clemo (1936) proposed a formula $C_{47}H_{73}O_{15}N$ for solanine with one secondary alcohol group and one tertiary amino group. Structural formula of solanidine, Δ^5-solaniden-3β-ol (Figure 1) was proposed by Prelog and Szpilfozel (1942) and confirmed by Uhle and Jacob (1945) by



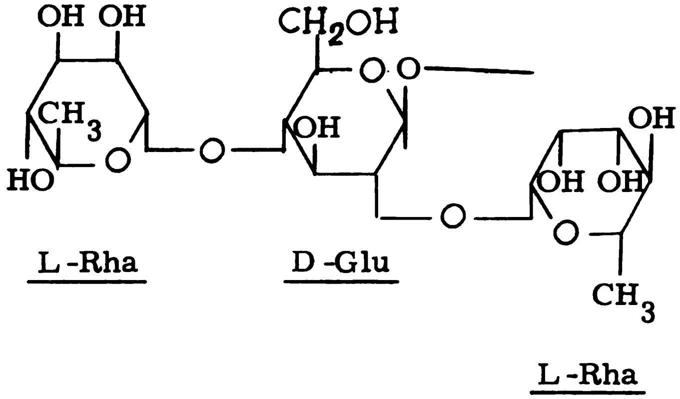
1. R = HO- SOLANIDINE

2. R =



α-SOLANINE

3. R =



α-CHACONINE

Figure 1. --Structures of solanidine, α-solanine and α-chaconine

converting a known compound, sarsasapogenin, to solanidine derivative.

The sugar moiety of solanine, solanose, consists of one mole each of glucose, galactose and rhamnose. Brigg and Vining (1953) studied the mode of linkage of sugar moiety of solanine by means of periodic oxidation, and reported that it was α-L-rhamnopyranosyl-(1→2 galact)-β-D-glucopyranosyl-(1→3 galact)-β-D-galactopyranosyl-Δ^5-solaniden-3β-ol (Figure 1). β- and γ-solanine are compounds formed by depleting one and two sugars from α-solanine respectively (Kuhn and Löw, 1954).

Chaconine was first separated from a solanine preparation using paper chromatography and the solvent system, pyridine-HOAc-H_2O (3:1:3) (Kuhn and Löw, 1954). Its sugar moiety, chaconose, was found to be α-L-rhamnopyranosyl-(1→2 gluc)-α-L-rhamnopyranosyl (1→4 gluc)-β-D-glucopyranosyl-Δ^5-solaniden-3β-ol. β- and γ-chaconine are isomers lacking either one or two rhamnose molecules.

A summary of the physical properties of these compounds is given in Table 1. It should be noticed that the different melting point of α-solanine and α-chaconine reported by investigators is probably due to low purity of sample. The highest value is regarded as authentic.

Table 1. --Summary of some physical properties of solanine and chaconine

Compound	Empirical Formula	Mol. Wt.	Sugar[a]	M. P.	$[\alpha]_D$	Reference
α-solanine	$C_{45}H_{73}O_{15}N$	868.09	Gal+Glu+Rha	286° 285° 276-278° 248-249° 228-229°	-59° (Py)[b]	Kuhn & Low, 1954 Soltys & Wallenfels, 1936 Prelog & Jeger, 1960 Paquin & Lepage, 1963 Allen & Kúc, 1968
β-solanine	$C_{39}H_{63}O_{11}N$	721.94	Gal+Glu	290°	-31° (Me)[c]	Kuhn & Löw, 1954
γ-solanine	$C_{33}H_{53}O_{6}N$	559.79	Gal	250° 252-254°	-26° (Me)[c]	
α-chaconine	$C_{45}H_{73}O_{14}N$	852.09	Glu+Rha+Rha	243° 230-236° 216-217°	-85° (Py)[b]	Kuhn & Löw, 1954 Allen & Kúc, 1968 Paseshnichenko, 1956
β-chaconine	$C_{39}H_{63}O_{10}N$	705.94	Glu+Rha	255°	-61° (Py)[b]	Kuhn & Löw, 1954
γ-chaconine	$C_{33}H_{53}O_{6}N$	559.79	Glu	244° 240-242°	-40° (Py)[b]	

[a] Gal = D-galactose, Glu = D-glucose, Rha = L-rhamnose.

[b] Py = Pyridine

[c] Me = Methanol

Solanine and chaconine are identical in the following aspects. Both are very soluble in dilute acid or acidified alcohol, and insoluble in alkaline aqueous solution (Reuling, 1839; Swenger, 1859).

According to Autenrieth (1928), solanine is slightly soluble in water, about 1 part in 8000 parts of water, soluble in 500 parts of cold and 125 parts of boiling ethanol and 4000 parts of ether. Precipitation of both components from solution is completed as the pH value is raised to 9.4, where, according to Wolf and Duggar (1946), the solubility of solanine is in the order of 0.1 mg per 100 ml.

Both compounds give identical colors with the following color tests: $SbCl_3$ reagent (Wierzchowski and Wierzchowska, 1961), red; Marquis reagent (Alberti, 1932), purple; Clarke reagent (Clarke, 1958), blue; H_3PO_4, orange.

Solanine and chaconine can be recrystallized from aqueous alcohol. Solanine precipitates after repeated recrystallization in needle form. Chaconine does not crystallize readily, and crystallization is induced by lowering the temperature slowly to avoid gel formation (Allen and Kúc, 1968).

The major difference rests on the chromatographic behavior of these two compounds. They have been separated on paper chromatography, column chromatography, and thin-layer chromatography. With paper chromatography using ethyl acetate-HOAc-H_2O (3:1:3) as solvent system, relative R_f value for α-solanine and α-chaconine was found to be 1.00 and 1.61 respectively (Kuhn and Löw, 1954). Paquin and Lepage (1963) obtained R_f values 0.22 and 0.54 for α-solanine and α-chaconine respectively with 95% EtOH-HOAc (3:1) and silica gel plate. Using H_2O saturated n-butanol as solvent system, Allen and Kúc (1968) reported that R_f for α-solanine and α-chaconine on Al_2O_3 plate was 0.16 and 0.28 respectively, and R_f on silica gel plate, 0.36 and 0.52 respectively. Column chromatography was first employed by Paseschnichenko and Guseva (1956) for separating chaconine from solanine. They eluted the mixture with H_2O saturated n-butanol on dry neutral Al_2O_3 column and obtained pure chaconine and solanine successfully. Patt and Winkler (1960) purified solanine with an ion-exchange column.

Infrared absorption spectra of solanine and chaconine only differ slightly at 8 and 12.2 μ according to Allen and Kúc (1968).

Quantitative Determination of Glycoalkaloid of Potatoes

The gravimetric method of solanine determination involved isolation and purification of solanine from potato tissue and direct weighing after it was dried in an oven to a constant weight. Morgenstern (1907) heated the ground potato samples with dilute HOAc for one hour, filtered and rinsed the residues with water. The residues were then re-extracted with 96% alcohol for 12 hours, filtered and the filtrate condensed. Solanine was precipitated by NH_4OH from the combined filtrates. Bömer and Mattis (1924) proposed a method which called for extraction of ground potato samples with HOAc 4 times, and subsequent extraction of the dried crude precipitates with 95% ethanol in Soxhlet extractors for 5 hours. Gmelin (1859) estimated the solanine content by measuring the reducing power of sugar after hydrolysis of solanine with H_2SO_4. Conner (1937) in his study of light effect on solanine synthesis hydrolysed solanine with HCl and measured the reducing power. These methods, however, were laborious and their precision depended entirely upon the technique in isolating and purifying the compound.

Alberti (1932) reported the first colorimetric method using conc. H_2SO_4 and 1% HCHO, known as Marquis reagent. Solanine was allowed to react with conc. H_2SO_4 for a short period, and a purple color developed upon addition of 1% HCHO. This method was modified by Pfankuch (1937), Rooke et al. (1943), Wolf and Duggar (1946), Dabbs and Hilton (1953), Baker et al. (1955). The last method called for Soxhlet extraction with 3% HOAc in EtOH for 18 hours, evaporation of ethanolic solution to near dryness, coagulation of soluble protein with 5% H_2SO_4, and filtration. The filtrate was diluted with 1% H_2SO_4 to volume. Three ml of sample solution was added dropwise to 3 ml of conc. H_2SO_4 in a 3-minute period, and kept cool with constant swirling in an ice bath. Three ml of 1% HCHO was then added and purple color was measured at 570 nm after 90 minutes.

Clarke (1958) discovered that a mixture of conc. H_3PO_4 and dilute para-HCHO could produce a color reaction for determination of solanine; he also noted that chaconine underwent the same reaction. This reaction was adopted by Patt and Winkler (1960) for solanine determination, and was modified by Schwarz (1962). Solanine was extracted with 5% HOAc, precipitated by NH_4OH, centrifuged, and the precipitate dissolved in 3 ml of conc. H_3PO_4 plus 3 drops of para-HCHO saturated aqueous solution. The blue color was measured at 595 nm after 60 minutes. Vecher (1967) employed the above method to determine the solanine content of the important and new potato varieties in Russia.

Sachse and Bachmann (1969) made a comprehensive comparison of the two methods described above and concluded that the Clarke reagent was superior to the Marquis reagent. They found that refluxing of the potato sample in 80% ethanol or 90% methanol for 30 minutes yielded more solanine than did the Soxhlet extraction with 3% HOAc in 95% EtOH and proposed the following procedure for solanine determination: Reflux the potato sample in 80% EtOH for 30 minutes, filter, precipitate the glycoalkaloid from filtrate with NH_4 OH, filter again, and dissolve the residue into 1% H_3PO_4. Mix 1 ml aliquot of sample solution with 10 ml of reagent solution made of 0.03 g of para-HCHO and 100 ml of 85% H_3PO_4. Measure the blue color after 30 minutes at 600 nm.

Wierzchowski and Wierzchowska (1961) determined the solanine concentration with a mixture of 30 g of $SnCl_3$ and 20 ml of conc. HCl successfully. This method did not gain popularity due to the toxic nature of the reagent. A polarographic method of glycoalkaloid determination was reported by Pierchalski and Mrozowska (1968).

Mechanism of Glycoalkaloid Synthesis in Potatoes

Guseva and Paseschnichenko (1957) found that an enzyme preparation from potato sprouts was capable of cleaving the sugars from solanine. Studying with C^{14}-acetate, Guseva and Paseschnichenko (1958) found that, if the sprout was grown in light, most C^{14} was incorporated into aglycone, and if in darkness, C^{14} was incorporated into both sugars and aglycone. If radioactive mevalonic acid was used, the rate of incorporation was higher. However, more radioactivity was incorporated into the non-saponifiable fraction than the glycoalkaloid.

Not much progress was made in regard to the elucidation of biosynthetic mechanism of glycoalkaloid until newer chromatographic techniques were introduced in the 1960's. Successful isolation of many intermediate triterpenes and sterols from plants such as soybeans and potatoes made it possible to postulate the metabolic pathways toward glycoalkaloid synthesis.

Phytosterols of potatoes were first isolated by Schwarz et al. (1955). Using conventional methods, they identified these phytosterols as β-sitosterol and stigmasterol. Schreiber et al. (1961) reported that the major sterol in both potato and potato beetle was β-sitosterol. Johnson et al. (1963) discovered cholesterol from extracts of a growing potato sprout with gas-liquid chromatography. This was the first time that cholesterol was found to be present in plant tissue.

Subsequent isolation of triterpenes and phytosterols from leaves by a group of workers led by Dr. K. Schreiber in Germany confirmed the presence of cycloartenol (Schreiber and Osske, 1962), 24-ethylidene lophenol (Schreiber and Osske, 1963), 24-methylene cycloartanol (Osske and Schreiber, 1964), 24-methylene lophenol (Osske and Schreiber, 1965) in potatoes. Based on these findings, Schreiber and co-workers (Ardenne et al., 1965) proposed a scheme of the possible routes leading to the syntheses of cholesterol, campesterol, β-sitosterol, stigmasterol and solanidine (Figure 2).

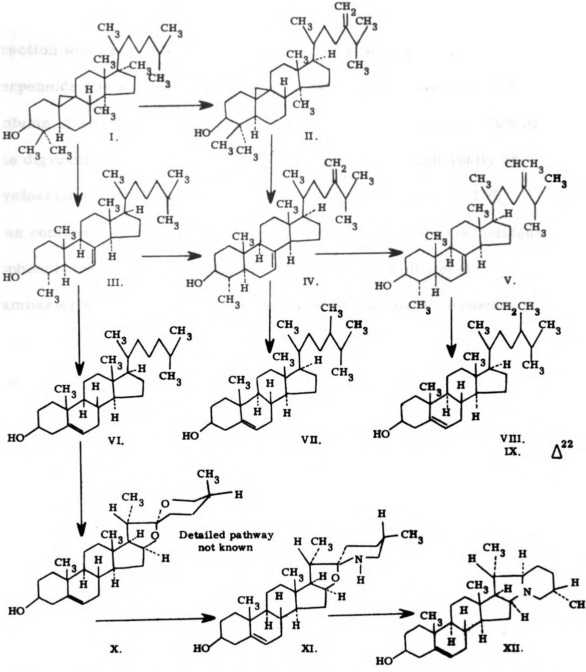


Figure 2. --Biosynthetic routes of β-sitosterol and stigmasterol; campesterol; cholesterol and solanidine

Note: I. Cycloatenol; II. 24-methylene cycloartenol; III. Lophenol; IV. 24-methylene lophenol; V. 24-ethylidene lophenol; VI. Cholesterol; VII. Campesterol; VIII. β-sitosterol; IX. Stigmasterol; X. Yamogenin; XI. Tomatid-5-en-3β-ol; XII. Solanidine.

According to Schreiber and Osske (1962), the raw fat extract of potato leaves was first saponified, and the unsaponifiable fraction was precipitated with digitonin. The digitonide of triterpenoids and sterols could be fractionated into 3 fractions on a column of silica gel or florisil. Fraction A, comprising 60-70% of the digitonin-precipitable material, was composed principally of cycloartenol and 24-methylene cycloartanol; fraction B, 7-13%, was composed of lophenol, 24-methylene lophenol and 24-ethylidene lophenol; fraction C, 18-33%, was composed of cholesterol, campesterol, β-sitosterol. An analysis of fraction C (Ardenne et al., 1965) showed that it contained 49% β-sitosterol, 36% stigmasterol, 12% cholesterol and 3% campesterol.

The biosynthetic route from cholesterol to yamogenin, tomatid-5-en-3β-ol and solanidine was based on the finding that both yamogenin and tomatid-5-en-3β-ol could be obtained from the hydrolysis product of solanine (Schreiber, 1957), that tomatid-5-en-3β-ol could be synthesized from yamogenin-like compound (Schreiber and Rönsch, 1965a), and that solanidine could be synthesized from tomatid-5-en-3β-ol (Schreiber and Rönsch, 1965b).

Rees et al. (1968) reported the isolation of a phytosterol, 4α, 14α-dimethylcholesta-8,24-dien-3β-ol, from potatoes. They hypothesized that cycloartenol, like lanosterol, an intermediate for animal sterol biosynthesis, might be the most important intermediate for phytosterol biosynthesis. The scheme of biosynthetic pathway for cholesterol formation was suggested to be cycloartenol → 31-norcycloartenol → 4α, 14α-dimethylcholesta-8, 24-dien-3β-ol → lophenol → cholesterol, although 31-norcycloartenol has not been isolated from any plant source yet. This scheme can be added to that of solanidine biosynthesis shown in Figure 2. Further details in the solanidine biosynthesis are unavailable.

Since relatively significant amount of cholesterol exists in potato leaves along with β-sitosterol, stigmasterol and campesterol, it is very likely that cholesterol is an important intermediate for solanidine biosynthesis (Bennett and Heftmann, 1965).

Toxicity of Solanine

A number of outbreaks of potato poisoning were reported. Harris and Cockburn (1918a, 1918b) cited that 56 Berlin soldiers were ill after a meal of greened potatoes in 1899, and at the end of 1917, quite a few poisoning cases were reported in Glasgow, England, where 61 individuals were involved with one death. The symptoms included gastro-enteritis, diarrhea, colicky pain, headache and depression. The suspected potatoes were examined and it was found that the uncooked potatoes contained 38 mg solanine per 100 g fresh potato and the cooked potatoes contained 24 mg solanine per 100 g. Poisoning by potatoes was attributed to the unusual high content of solanine. The hazard of eating sprouted or sunburned potatoes was emphasized.

Potato varieties with a high level of solanine were reported frequently during the 1920's. One variety examined by Alfa and Heyl (1923) contained 68.7 mg, 40.0 mg and 29.8 mg of solanine per 100 g of potatoes respectively for three different lots. Similar results were also reported by Griebel (1923), Bömer and Mattis (1923, 1924).

According to a USDA report cited by Hansen (1925), 64 cows were poisoned after being fed with some potato leaves; 6 pigs died after eating sprouted raw potatoes. The solanine concentration in stomach content of the poisoned animals was found to be 60 to 85 mg per 100 g of fluid (Lowe, 1929).

Rühl (1951) reported that ingestion of berries of a potato plant caused death of a 2-year-old girl after 13 days, and found that solanine was the primary factor causing physiological lesions. He noted that large doses of solanine irritated the gastro-intestinal mucosa, and caused vomiting and diarrhea. Kingsbury (1964) recorded the symptoms of solanine poisoning as being apathy, drowsiness, salivation, dyspnea, trembling, progressive weakness or paralysis, unconsciousness, gastro-enteritis, anorexia, abdominal pain, constipation, diarrhea, and headache.

Sprouted potatoes fed to rats caused marked weight loss and death, but the similar potatoes had no toxic effect on rats when peeled (Smerha and Českoslow, 1948). König and Staffe (1953) reported that the injection of 2 g of solanine into 40 kg sheep caused a change in hemoglobin, erythrocyte and leucocyte, and shock in 30 minutes. Abbott _et al._ (1960) found that solanine had a high anti-cholinesterase activity _in vitro_. Similar results were also obtained by Orgell and Hibbs (1963a, 1963b). Kline _et al._ (1961) studied the toxic effects of potato sprouts or crystalline solanine on pregnant rats, and he found that rats fed with either one had a lactation deficiency, which was probably due to anti-hormonal action of solanine.

Toxicity level of solanine on human beings has not been established. However, it will be regarded as a dangerous level if the potato contains higher than 100 mg per 100 g (dry basis) (Talburt and Smith, 1967).

The resistance of certain potato plants to potato beetles was attributed to the presence of glycoalkaloids in potato leaves. Sturkow and Löw (1961) found that crystallized solanine and chaconine when infiltrated into potato leaves in concentration of 0.02 to 0.8% had repellent effect against both normal and DDT-resistant potato beetles. Allen and Kúc (1968), using a paper bioassay technique, found that solanine and chaconine were the principal fungitoxic compounds in addition to caffeic acid in extracts of Irish potato tubers.

Relationship Between Glycoalkaloid and Off-Flavor of Potatoes

Glycoalkaloid is a bitter substance, which in small amount is not noticeable, and is probably regarded as a flavor constituent of potato tubers. If the level of glycoalkaloid in potato tubers increases, a bitter taste arises (Zitnak, 1961). Higher concentration of glycoalkaloid is found in cortex of potato tubers, particularly those exposed to light. Thus off-flavor of potato is likely to be enhanced by consuming the unpeeled tubers.

From the rather scarce information available, that off-flavor of potatoes is entirely due to the high level of glycoalkaloid has not been confirmed. An organoleptic test on flavor of light-treated or sunburned potatoes, and controls did not show any significant difference in score (Gull and Isenberg, 1958). Barug (1962) also failed to correlate the flavor change with change of glycoalkaloid concentration. Instead, he found that the flavor score was highly correlated with surface discoloration of potato tubers.

MATERIALS AND METHODS

Materials

The potatoes used in this study were grown at the Montcalm County Experiment Station Farm, Michigan, by the Department of Crop and Soil Science, Michigan State University. The crops were harvested at the end of September and in early October, 1969.

Two commercial varieties, Russet Burbank and Kennebec, and two clones, designated as 709 and 321-65, were chosen. Russet Burbank and Kennebec were used mainly as controls. 709, a clone of S. tuberosum, was chosen because it was one of the new clones found to have marketing potential. 321-65, a S. tuberosum and S. stoloniferum species hybrid, was selected on the basis of its high solids content (28.3%) and commercial value as a yellowish potato product (Pope, 1969). In addition, B5141-6, a potato reported to have off-flavor (Thompson, 1969), was used in the flavor study.

Treatment of Potatoes with Maleic Hydrazide (MH-30)[1]

Potatoes (all except B5141-6) were sprayed with a 4000 ppm water solution of MH-30,[1] a sprout inhibitor, four weeks before harvest, and the vines were killed by sodium arsenate solution 10 days before harvest.

Storage of Potatoes

Potatoes were stored at 40F and 50F to provide for long-term storage and also to permit different physiological activities.

The MH-30 treated potatoes were harvested on September 25, 1969, stored immediately at 45F in the Experiment Station Farm, and brought to the Food Science Department on October 14, 1969. They were divided into two lots, and stored at 40F and 50F respectively. During the first 10 days, the temperatures rose to 44F and 58F due to the respiratory heat generated in the close chamber. Small lots were stored at 45F for light treatment studies.

Untreated potatoes were harvested on October 10, 1969, stored at 60F, which gradually dropped to 45F in 35 days, and brought to the Food Science Department on December 1, 1969. They were similarly divided and stored at 40F and 50F respectively. Small

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[1] A product of Uni-Royal Chemical Co. containing 58% diethanol amine salt of 6-hydroxy-3-(2H) pyridazinone as active ingredient.

batches of untreated potatoes were brought in earlier (November 1, 1969) and stored at 45F for light treatment.

B5141-6 potatoes were harvested in September, 1969, and stored at 45F and 65F respectively. They were brought to the Food Science Department on January 13, 1970, for flavor evaluation and glycoalkaloid determination.

Experimental

The chemical analyses were initiated about 1 month after harvesting and were continued at monthly intervals until vigorous sprouting of the potatoes occurred. The storage and analysis scheme is presented in Figure 3.

I. Analytical procedure

A. Sample preparation

Three types of sample were employed:

1. Surface sample

Three tubers from each sample lot are blanched in live steam for 20 minutes, 10 minutes on each side, cooled with cold water spray immediately and hand-peeled. The 2 to 3 mm thick surface of the entire tuber is scraped into a small plastic cup with a stainless steel spatula and mixed thoroughly.

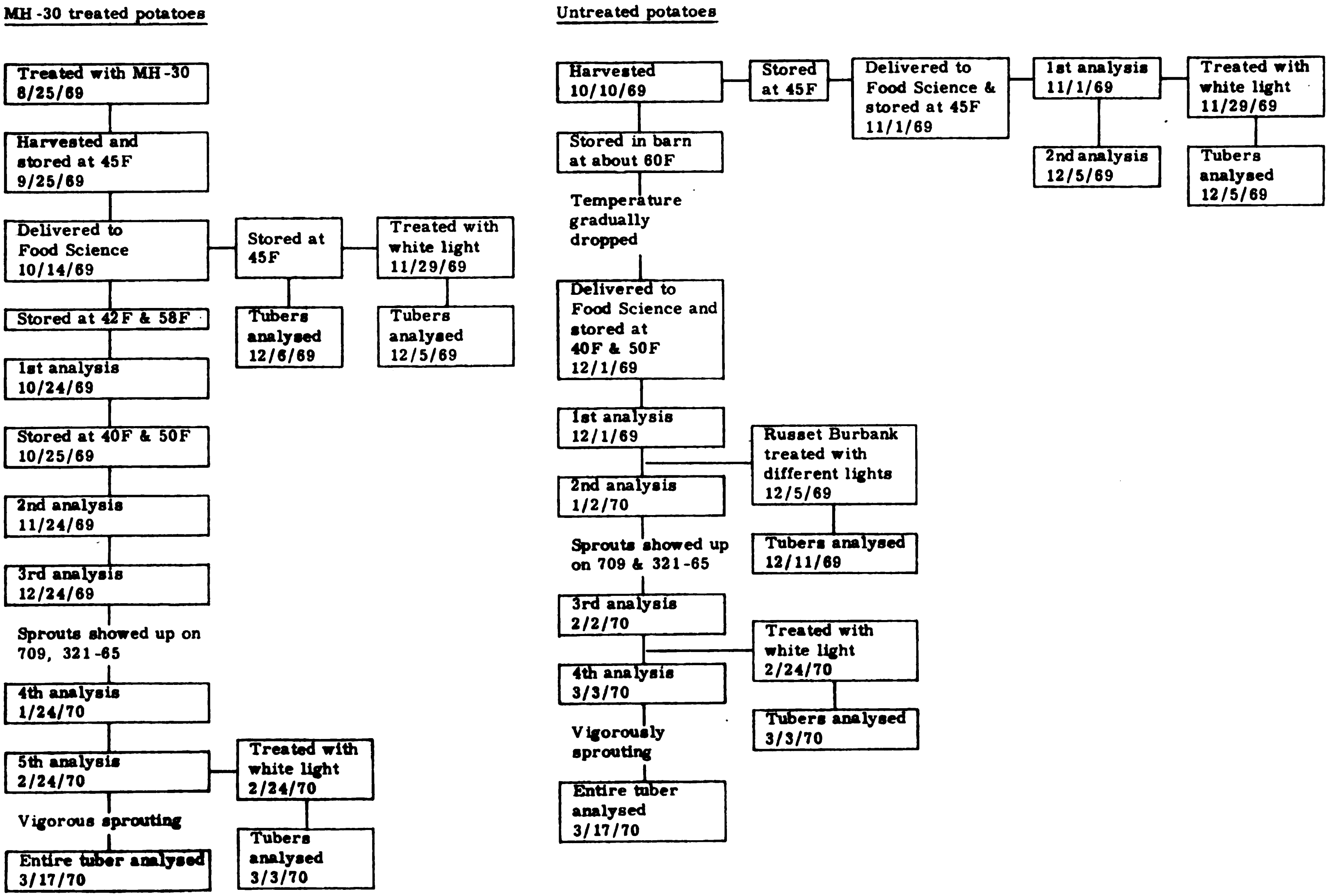

Figure 3. --Storage and analysis scheme of the MH-30 treated and untreated potatoes

2. Boiled flesh sample

Three tubers from each sample lot are boiled in a stainless steel kettle for about 30 minutes, drained, cooled with cold water and hand-peeled immediately. The peeled tubers are then mashed and mixed with mortar and pestle.

3. Diced fresh sample

The tuber chosen for glycoalkaloid determination is peeled, trimmed and diced into small pieces.

All samples used for glycoalkaloid determination were dried at 60C in a vacuum oven for 18 hours, weighed and percent dry material computed.

B. Extraction

1. Reflux extraction method

To evaluate the reflux extraction method, 200 g of potato sprouts were refluxed with 1000 ml of acidified 95% ethanol (3 ml HOAc per 100 ml) for 24 hours. The ethanolic solution was cooled, filtered and sufficient saturated lead acetate solution was added to precipitate colloidal materials. The mixture was centrifuged, the supernatant removed and evaporated to a small volume, and diluted with ca. 200 ml of 2% HOAc. The pH was 3.8.

Differential precipitation was induced by continuous addition of dilute NH_4OH, and the precipitates were collected by centrifugation at pH 4.5, 7.0, 8.0 and above 9.0 as recorded by a glass electrode pH meter. The nature of the precipitation was evaluated in terms of purity of the precipitates.

2. Bisolvent extraction method

Five gram samples in triplicate were blended with 100 ml of a mixture of MeOH-$CHCl_3$ (2:1) in a semi-micro stainless steel blender assembly for 4 to 5 minutes. The suspension was filtered through a sintered glass Büchner funnel (medium porosity), and the residue rinsed twice with a 25 ml portion of the same solvent mixture. The filtrate was transferred into a 500 ml separatory funnel, 60 ml of 0.8% Na_2SO_4 added, and the mixture vigorously shaken. The layers were allowed to separate and the lower layer ($CHCl_3$) was discharged into a 150 ml beaker. The methanolic layer was re-extracted with 10 ml more of 0.8% Na_2SO_4, and the bottom layer ($CHCl_3$) again collected in the 150 ml beaker. The combined $CHCl_3$ fractions, which contained various pigments and steroids, were air-dried, and kept for chromatographic analysis of sterols.

The methanolic fraction was collected into a 250 ml beaker, and separatory funnel rinsed with 1% HOAc. This solution was concentrated on a steam bath to less than 10 ml and made to volume with 1% HOAc (10 to 50 ml depending on the concentration of glycoalkaloid). It was used as the sample solution for glycoalkaloid determination.

3. Percent recovery by the bisolvent extraction method

To 5 g surface samples with either low or high level of glycoalkaloid, 1 mg of pure α-solanine (K & K Laboratory, Plainview, N. Y.) was added, and glycoalkaloid determined with or without addition of the pure solanine.

4. Comparison between the bisolvent and Soxhlet extraction method

Diced flesh samples of raw potatoes as well as boiled flesh samples were extracted with 150 ml of

3% HOAc in EtOH for 18 hours with Soxhlet extractors. The ethanolic solution was collected into a 250 ml beaker, and evaporated to near dryness on a steam bath. Five ml of 5% H_2SO_4 was added to coagulate the colloidal substance. The solution was filtered, and rinsed with 5 ml of 5% H_2SO_4. The glycoalkaloid was precipitated from the filtrate by raising the pH to 9.4 with NH_4OH, centrifuged and redissolved in 10 ml of 1% HOAc. Identical samples were also extracted with the bisolvent extraction method. The yields of glycoalkaloid from both methods were compared.

C. Colorimetric determination of glycoalkaloid

A modification of the colorimetric method described by Schwarze (1962) was used. Five or 10 ml of the sample solution were pipetted into a 40 ml centrifuge tube (heavy duty, bottom tapered). The pH value was adjusted to 9.4 with 5M NH_4OH, the tube heated at 80C for 1 minute and left to stand for 10 minutes. It was centrifuged at 2,000 rpm for 15 minutes, the supernatant siphoned off, the precipitate washed with 0.5 to 1 ml of water, recentrifuged and the water decanted off.

To the precipitate, 5 ml of 85% H_3PO_4 was added, followed immediately by 5 drops of saturated para-HCHO solution. It was stirred well and left to stand for 60 minutes at room temperature. The absorbance was read at 600 nm on a Beckman DB Spectrophotometer. The concentration of glycoalkaloid was determined from a standard curve obtained for a series of α-solanine concentrations from 0.06 mg to 0.6 mg prepared in the same manner as the sample. Higher concentration of glycoalkaloid was determined with a standard curve ranging from 0.1 mg to 1.2 mg constructed by replacing 85% H_3PO_4 with 70% H_3PO_4 and developing for 120 minutes at room temperature.

D. Chromatographic analysis of glycoalkaloid and sterol of potatoes

1. Thin-layer chromatography of glycoalkaloids

The glycoalkaloid precipitate obtained in the same manner as described for its colorimetric determination was dissolved in a 1% HOAc solution in MeOH. It was spotted on a thin-layer plate (E-merck Silica Gel F-254, abrasion resistant), and developed in one of the following solvent systems until the solvent front travelled 12 to 15 cm:

1) MeOH -EtOAc -HOAc -H_2O (20:30:10:1)
2) 95% MeOH -EtOAc -HOAc (10:15:2)
3) BuOH -HOAc -H_2O (40:8:20)
4) 3 -Heptanone -HOAc -H_2O (8:5:1)
5) EtOAc -Pyridine -H_2O (3:1:3)

The plate was then air-dried, and sprayed with one of the following visualizing reagents:

1) Lowry reagent: A mixture of 50 mg of $FeCl_3 . 6H_2O$, 90 ml of H_2O, 5 ml of glacial acetic acid and 5 ml of conc. H_2SO_4.
2) $SbCL_3$ reagent: A mixture of 30 g of $SbCl_3$ and 40 ml of conc. HCl.
3) Dragendorf reagent: Made by mixing 5 ml of solution A (0.8 g of Bismuth subnitrate, 10 ml of glacial acetic acid and 40 ml of H_2O), 5 ml of solution B (40% aqueous KI), with 20 ml of glacial acetic acid and diluted to 100 ml with H_2O.

After spraying, the plate was heated at 90C in an oven for 5 to 10 minutes for visualization. A long-wave UV lamp (Ultra-Violet Products, Inc., San Gabriel, California) was used to aid the visualization of faint spots.

Preparative thin-layer chromatography was also employed for the preparation of a large amount of pure α-solanine and α-chaconine. The glycoalkaloid isolate was redissolved in 1% HOAc, treated with active carbon, reprecipitated, and then dissolved in 1% HOAc (in MeOH) after water washing. It was applied to a 0.5 mm thick preparative plate (E-merck Silica Gel PF-254), and developed in the no. 1 solvent system for 90 minutes. Small quantities of the isolates were spotted on both sides of the plate as markers, and were sprayed with the no. 1 visualizing reagent after development. The bands corresponding to α-solanine and α-chaconine were collected and eluted with warm methanol. The methanolic solution was condensed and made aqueous prior to addition of 5M NH_4OH for precipitating solanine and chaconine. The respective precipitates were washed with water, and the α-solanine and α-chaconine obtained were tested with the following reagents:

1) Marquis reagent: Six ml of conc. H_2SO_4 were added to 0.2 mg of each glycoalkaloid, with cooling in an ice bath, and followed by 3 ml of 1% HCHO.

2) Clarke reagent: Five ml of conc. H_3PO_4 were added to about 0.2 mg of each glycoalkaloid with shaking, followed by 5 drops of saturate para-HCHO solution.

3) Conc. H_3PO_4: Five ml of conc. H_3PO_4 were added to about 0.2 mg of each glycoalkaloid.

Their absorption spectra were determined with a Beckman DB Spectrophotometer.

2. Thin-layer chromatography of sterols

The air-dried $CHCl_3$ fraction from the bisolvent extraction procedure was redissolved in 20 ml of dry ethyl ether and saponified with an equal amount of KOH saturated MeOH. The solution was shaken vigorously for 2 minutes and allowed to stand for 20 minutes in a 250 ml separatory funnel. The mixture was separated into 2 layers with the addition of 80 ml of distilled water and moderate swirling. The lower layer was discarded, and the ethereal layer was washed with additional water until the washing showed neutral reaction to litmus paper.

The washed ethereal fraction was filtered through a layer of anhydrous Na_2SO_4 into a small beaker, and the separatory funnel rinsed with dry ethyl ether. This ethereal solution was spotted on a thin-layer plate (E-merck Silica Gel F-254, abrasion resistant), and developed in one of the following solvent systems for 45 to 60 minutes:

1) Benzene-EtOAc (1:1)
2) Hexane-EtOEt-HOAc (80:20:1)
3) 3-Heptanone-HOAc-H_2O (8:5:1)

The plate was dried and sprayed with one of the following visualizing reagents:

1) Lowry reagent
2) Liebermann-Burchard reagent: A mixture of chilled glacial acetic acid and conc. H_2SO_4 (19:1).
3) Iodine vapour
4) Benzidine-$NaIO_4$ reagent: A mixture of solution A (0.1% $NaIO_4$) and solution B (2.8 g of benzidine in 80 ml of ethanol, 70 ml of H_2O, 30 ml of acetone and 1.5 ml of 1N HCl).

The sprayed plate was heated at 90C in an oven for 5 to 10 minutes, and the spots examined with the aid of a short- or long-wave UV lamp (Ultra-Violet Products, Inc., San Gabriel, California).

Two-dimensional thin-layer chromatography of sterols was also carried out using a combination of solvent system no. 1 and 2, other conditions being the same.

3. Thin-layer and gas-liquid chromatographic analysis of sterols

The entire ethereal sample solutions obtained from three 5 g potato samples were combined and streaked onto a 0.5 mm thick plate (E-merck Silica Gel PF-254) with small quantities as markers on both sides, and developed in the no. 1 solvent system for 45 minutes. The plate was air-dried, and the markers sprayed with the no. 1 visualizing reagent and heated at 90C in an oven for 5 to 10 minutes. The bands of steryl glycosides and free sterols were collected and eluted with $CHCl_3$-MeOH (2:1). To the eluent, 40μg of authentic 5α-cholestane was added as internal standard (The Anspec Co., Inc., Ann Arbor, Michigan), and the mixture dried in a warm (about 60C) water bath under a flow of nitrogen gas.

Quantitative determination of sterols with GLC has been carried out by the use of trimethyl silyl ethers of cholesterol (Wells and Makita, 1962), campesterol, stigmasterol and β-sitosterol (Rozanski, 1966). Therefore, trimethyl silyl derivatives of the sterol fraction were made in this study for quantitative analysis. The silylating reagent was made of 3 parts of hexamethyl disilazane, 1 part of trimethyl chlorosilane and 9 parts of water-free pyridine. The steryl glycoside fraction (spot 1) was first hydrolysed with 1N HCl in MeOH at 75C over night, and the sterols were extracted with chloroform, dried and silylated with 40 ul of the silylating reagent. The free sterols from spot 4 were silylated directly. After 10 minutes, the mixture was injected directly into a F & M 5075 Gas Chromatograph (Hewlett Packard Co., Southfield, Michigan).

The gas-liquid chromatography was performed on an 8-foot long glass column (O.D. $\frac{1}{4}$ in) packed with 1% OV-101[1] on 100-120 mesh Gas-Chrom Q (The Anspec Co., Inc., Ann Arbor, Michigan) under the following conditions: Column temperature, 240C; Injection port temperature, 290C; Detector block temperature, 295C; Flow rate of Helium, 30 ml/min; Hydrogen, 20 ml/min; Air, 350 ml/min. Each injection was allowed to run

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[1] OV-101 is a methyl derivatove of silicone oil.

for 40 minutes, and duplicate injections were made for each sample. The relative retention time for each component was computed with reference to 5α-cholestane, the internal standard.

II. Studies of the effect of storage

Monthly analyses were made on the untreated and MH-30 treated potatoes (Russet Burbank, Kennebec, 709 and 321-65) stored at 40F and 50F for the determination of the glycoalkaloid and sterol levels using surface samples. Six months after harvest (4 months controlled storage) at 40F and 50F, average levels of glycoalkaloid in these potato tubers were determined using boiled flesh samples.

III. Studies of the effect of white light on glycoalkaloid and sterol formation

The MH-30 treated and untreated potatoes (Russet Burbank, Kennebec, 709 and 321-65) after storage at 45F for about 60 days were randomly sampled and exposed to white light (both fluorescent and incandescent) for 6 days, 3 days on each side, at 52F. To retain the moisture, the tubers were sacked in punched polyethylene bags. Glycoalkaloid and sterol were determined using surface samples.

IV. Studies of the effect of different lights on glycoalkaloid and sterol formation

Untreated Russet Burbank potatoes after storage at 45F for 60 days were exposed to white, far-red, red, ultra-violet and no light for 6 days, 3 days on each side, under the conditions listed in Table 2. The levels of glycoalkaloid and sterol were determined using surface samples.

Table 2. --Conditions of various lighting systems

Light	Range of Wavelength nm	Energy erg/sec/cm^2	Temperature ° C
Dark	---	---	19
UV	<400	---	25
Red	600-700	3770	24
Far-Red	615-775	11426	28
White	400-700	---	25

V. Studies of the effect of glycoalkaloid on the potato flavor

A. Flavor evaluation of the B5141-6 potatoes

The mashed potatoes were prepared for flavor evaluation of the B5141-6 potatoes (45F and 65F storage) against the control, Russet Burbank (40F storage).

Potatoes were peeled, boiled for 30 minutes in a stainless steel kettle, and blended with 0.1% salt, 2-3% margarine and 15-17% milk on wet weight basis. Using a flavor difference procedure, the samples and the control were presented to a 24-member taste panel for comparison. Panel members were instructed to judge whether the samples were similar, better or worse than the control. Glycoalkaloid level of these potatoes was determined without addition of ingredients.

B. Determination of the threshold level of glycoalkaloid affecting the potato flavor

A dilution procedure was employed to determine at what level glycoalkaloid would affect the potato flavor. Surface material of the light-treated potatoes, or juice of the albino sprouts, were used as sources of glycoalkaloid. The surface material was collected in the same manner as was the surface sample, and was blended to homogeneity in a Waring blendor. The juice was expressed from the sprouts, which were steam-blanched for 10 minutes, cooled, chopped and blended. The glycoalkaloid and moisture content of both materials were determined before use. Increasing amounts of glycoalkaloid were added to several aliquots of the plain mashed potatoes which contain very little glycoalkaloid. The samples were presented to the taste panel using either the paired comparison procedure, or the single sample procedure.

In the paired comparison procedure, the simulated samples were paired with the control samples, and were presented to the taste panel in an increasing order. In the single sample procedure, the simulated samples were presented alone. The panelists were asked to detect the off-flavor of the samples and to judge the acceptability of the samples.

RESULTS AND DISCUSSIONS

Sprouting

Potatoes stored at 40F up to nine months were still free of sprouts. At 50F storage, sprouts began to appear on the MH-30 treated 709 and 321-65, untreated 709 and 321-65 three months after harvesting. Vigorous sprouting took place 1 month later. Russet Burbank and Kennebec seemed to respond to the sprout inhibitor more favorably and were not vigorously sprouting until the fifth month of storage. Therefore, monthly chemical analyses were carried on until the fifth month for the MH-30 treated potatoes, and until the fourth month for the untreated potatoes.

Methodology of Glycoalkaloid Determination

Since it is well-known that the peelings of raw potatoes contain a high level of glycoalkaloid (Lampitt et al., 1943), a change in glycoalkaloid level should be pronounced in the surface area of the tubers. Therefore, in this study, a surface sample composed primarily of the cortical tissue rather than the composite sample of the whole tuber was used to determine the change in glycoalkaloid during storage. Steam-blanching provided the most satisfactory method for removing the skin and separating the cortical tissue, and it also inhibited the enzymatic browning reaction, which takes place rapidly in the fresh tissue of potato.

Boiled flesh samples were used for evaluating the average level of glycoalkaloid in potato tubers, and diced fresh samples were used for comparison purpose.

Since the glycoalkaloids, solanine and chaconine, have high melting point and low solubility in water, it is expected that there would be little or no loss of the glycoalkaloids during steam-blanching. The loss of glycoalkaloid from the whole tuber after boiling for 30 minutes was not determined, but it was offset by the identical experimental condition of each sample.

The total solids content of the potato samples, Russet Burbank, Kennebec, 709 and 321-65, at the beginning of storage were 24.1, 19.5, 19.0 and 28.3% respectively, and 21.3, 22.1, 19.9 and 26.5% respectively at the end of 4 months of storage (Pope, 1969). The total solids content of the surface samples after steam-blanching varied from 15 to 23.5%, and hence the levels of glycoalkaloid in different lots of potatoes were compared on dry weight basis.

In the study of the reflux extraction method, it was observed that, as the time of refluxing increased, the solution became more brown in color. This was probably due to polymerization of the phenolic compounds. It was impossible to remove the brown polymerized pigments to any significant extent by saturated lead acetate precipitation in the purification procedure. The extract after being diluted with 2% acetic acid had a tea-brown color.

The precipitate collected at pH 4.5 was a dark brown, clay-like substance, which had a high affinity for glass and could not be redissolved by 2% acetic acid. The bulk of the glycoalkaloid started precipitating at pH 6.3, and completed precipitation at pH 9.3. The mother liquor, highly buffered, was light brown in color. Precipitates collected at pH 7.0, 8.0, and 9.0 were suspended in 95% ethanol, and heated in 70C water bath. Recrystallization failed to remove the polymerized pigments. It was thought that the reflux method with aqueous ethanol was infeasible for quantitative determination of glycoalkaloid because of the polymerization of phenolic compounds, and also, the presence of a large quantity of starch and protein in the extract further lowered the purity of the precipitates.

The bisolvent system, MeOH-$CHCl_3$ (2:1), modified from MeOH-$CHCl_3$ (1:2) employed by Folch (1957) for lipid extraction, was found to be an excellent mixture for glycoalkaloid extraction, which is polar in nature. Blending this solvent mixture with the sample at a ratio of 20 to 1 (100 ml of solvent to 5 g of sample) completely dehydrated the sample, denatured the protein and eliminated the adhesiveness of the starch. This permitted rapid filtration of the solution through a sintered glass Büchner funnel (medium porosity).

The non-polar chloroform fraction was readily separated from the polar methanolic fraction in the filtrate by the use of 0.8% Na_2SO_4. After the two-step separation, less than 1 ml of chloroform remained in the methanolic fraction. It was noted that in the presence of either excessive water or excessive salt, separation could not be achieved because hydration of the chloroform fraction took place.

The methanolic fraction which contained glycoalkaloid was normally colorless, or occasionally pinkish if the content of anthocyanin was high. It is important that the methanol be evaporated completely from the methanolic fraction before the glycoalkaloid is redissolved in 1% acetic acid. The glycoalkaloid sample solution turned brownish upon addition of 5M NH_4OH due to the destruction of anthocyanin by alkali. However, the precipitated glycoalkaloid had a light gray appearance and could be redissolved in 1% acetic acid readily. There was no evidence of contamination with polymerized compounds.

Two sets of surface sample with different levels of glycoalkaloid were used in the recovery study, one with 1.37 mg/ 5 g w.b.[1] and another with 4.20 mg/5 g w.b.[1] The added solanine, which is equivalent to 73% of the glycoalkaloid in the first sample and 24% in the second sample, was completely recovered in both cases (Table 3).

Table 3.--Recovery of added solanine using the bisolvent extraction method

Sample	Glycoalkaloid mg/5 g w.b.[b]	Glycoalkaloid After Addition of 1 mg of solanine mg/5 g w.b.[b]	Recovered %
L_1[a]	1.37	2.45	
L_2	1.37	2.40	Ave. 105
L_3	1.37	2.40	
H_1[a]	4.25	5.10	
H_2	4.10	5.30	Ave. 105
H_3	4.20	5.30	

[a] L and H designate potato samples with low and high level of glycoalkaloid respectively.

[b] mg/5 g w.b. is mg per 5.0 g of potato sample on wet basis.

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[1] mg/5 g w.b. is mg per 5.0 g of potato sample on wet basis.

The glycoalkaloid yield obtained from the surface samples and from the diced fresh samples using the bisolvent extraction method was higher than that obtained using the Soxhlet extraction method (Tables 4 and 5).

Table 4. --Glycoalkaloid content of potato samples extracted with the bisolvent extraction and the Soxhlet extraction methods

Sample	Bisolvent Method mg/5 g w.b.[b]	Soxhlet Method mg/5 g w.b.[b]	Yield %
L_1[a]	1.37	1.05	
L_2	1.37	1.00	Ave. 79
L_3	1.37	1.20	
H_1[a]	4.25	3.10	
H_2	4.10	3.05	Ave. 74
H_3	4.20	3.15	

[a] L and H designate potato samples with low and high level of glycoalkaloid respectively.

[b] mg/5 g w.b. is mg per 5.0 g of potato sample on wet basis.

With the surface samples the yield with the Soxhlet method was about 75% of that obtained by the bisolvent method. With the diced samples, the yield varied. Analysis of diced fresh samples gave poor reproducibility of glycoalkaloid content for replicate samples, whereas the results obtained using peeled, cooked and mashed potatoes as samples were reproducible (Table 5). Since different areas of the tuber have different levels of glycoalkaloid (Lampitt et al., 1943), it is obvious that the diced fresh samples will not be as homogeneous as the boiled flesh samples.

Table 5. --Glycoalkaloid content of diced fresh sample and/or boiled flesh sample with the bisolvent extraction and Soxhlet extraction methods

Sample	Bisolvent Method		Soxhlet Method
	Diced Fresh Sample mg/5 g w.b.[b]	Boiled Flesh Sample mg/5 g w.b.[b]	Diced Fresh Sample mg/5 g w.b.[b]
L-45[a]	0.34	0.41	0.31
L-45	0.39	0.41	0.30
L-45	0.46	----	0.31
RB-40[a]	Nil	0.04	0.10
RB-40	Nil	0.04	0.07
RB-40	0.03	----	0.12
L-65[a]	0.81	0.41	0.37
L-65	1.04	0.41	0.58
L-65	1.24	----	0.39

[a] L-45 and L-65 are B5141-6 potatoes stored at 45F and 65F respectively, and RB-40 designates Russet Burbank potatoes stored at 40F.

[b] mg/5 g w.b. is mg per 5.0 g of potato sample on wet basis.

The standard curves for the determination of glycoalkaloid using 70% and 85% H_3PO_4 are given in Figure 4. With 85% H_3PO_4, the color reaction was more rapid and sensitive than with 70% H_3PO_4. The use of 70% H_3PO_4 for color development gave a less viscous solution and was more suitable for determining higher levels of glycoalkaloid such as that in potato sprouts.

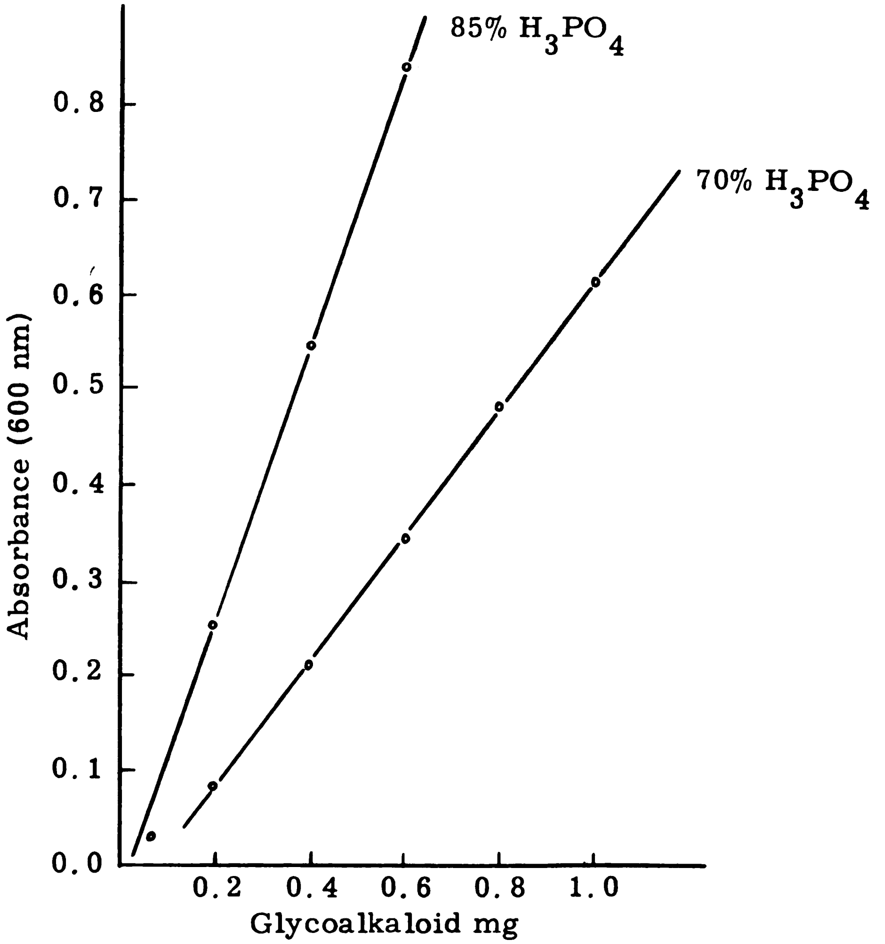


Figure 4. --Standard curves for glycoalkaloid determination

Thin-Layer Chromatography of Glycoalkaloids

The solvent systems which had been reported in the literature, such as BuOH-HOAc-H_2O (40:8:20) (Szendey, 1957), 3-heptanone-HOAc-H_2O (8:5:1) (Lepage, 1964), and EtOAc-pyridine-H_2O (3:1:3) (Kuhn and Löw, 1954) were first employed. The results obtained with all three solvent systems were not satisfactory. It took 390 and 200 minutes respectively for the solvent front of BuOH-HOAc-H_2O and 3-heptanone-HOAc-H_2O to travel the necessary distance, and yet the separation was very poor. Spots were not clearly detected after the glycoalkaloid precipitate was developed in EtOAc-pyridine-H_2O for 330 minutes. The low mobility and poor separation of the glycoalkaloids in these solvent systems necessitated the search of a new solvent system, and hence 95% MeOH-EtOAc-HOAc (10:15:2) was used. Early TLC results with this solvent system showed that small quantities of solanine from 0.5 to 4 μg could be chromatographed and visualized as distinct spots after being sprayed with the $SbCl_3$ reagent. This solvent system was then modified to MeOH-EtOAc-HOAc-H_2O (20:30:10:1). This mixture was very efficient as to the time consumed and the separation achieved. Roughly 70 minutes were required for the solvent to travel 12 cm.

A typical result (Figure 5) showed that all potato tubers contained both solanine and chaconine with R_f values 0.18 and 0.35

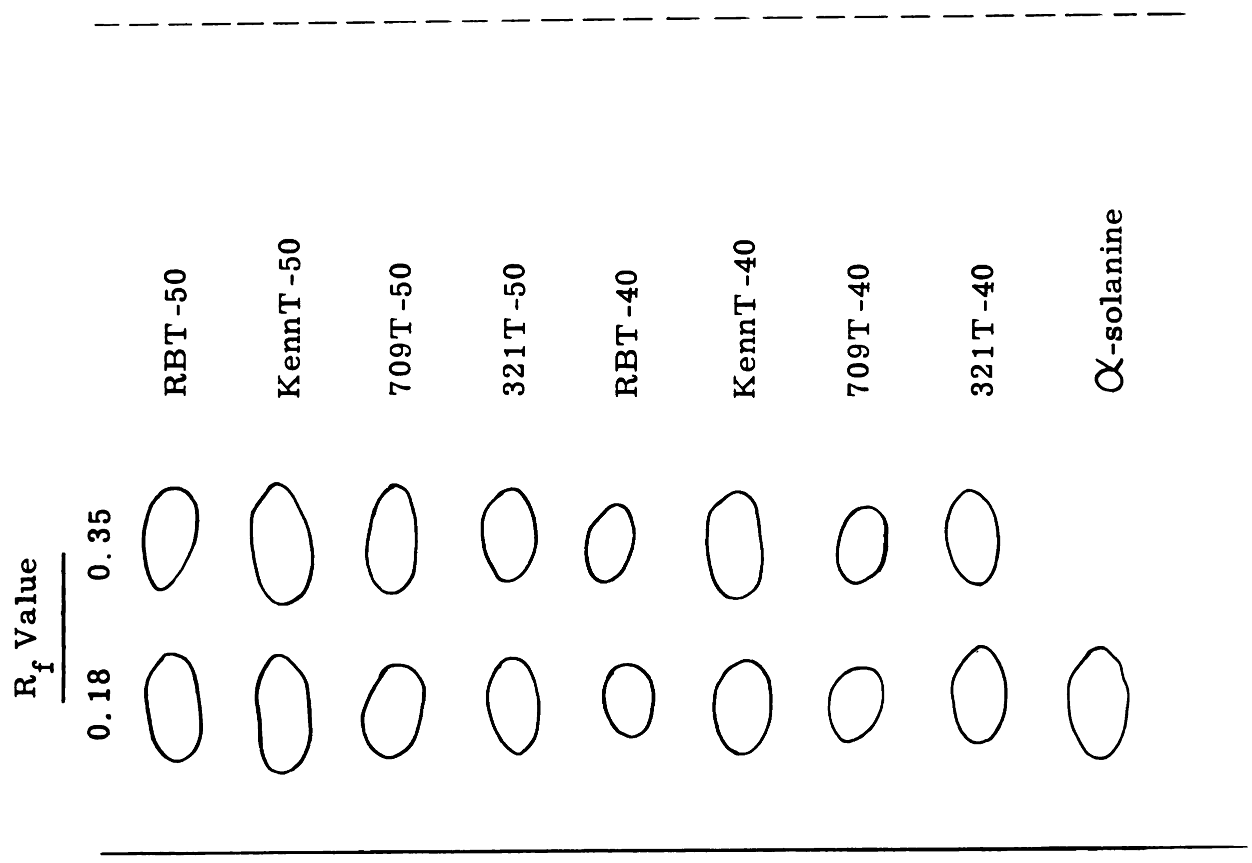


Figure 5. --Thin-layer chromatogram of glycoalkaloids from the MH-30 treated potatoes (Russet Burbank, Kennebec, 709 and 321-65) stored at 40F and 50F

Solvent system: MeOH-EtOAc-HOAc-H_2O (20:30:10:1)
Front and time: 10.9 cm and 70 min
Spray reagent: The Lowry reagent

respectively. The density of color seemed to correspond to the concentration of the glycoalkaloid, and hence a densitometer might be used to quantify the two compounds separately. However, the ratio of solanine to chaconine was estimated as 1 to 1 without using any instrument.

Using the same solvent system, the glycoalkaloid precipitate obtained from the Soxhlet extraction method was compared with that obtained from the bisolvent extraction method, and identical components were resolved (Figure 6). These results therefore showed the differences in glycoalkaloid content obtained by the bisolvent and Soxhlet method were only due to the difference in extraction efficiency.

Among the three reagents, the Lowry reagent, first used by Lowry (1967) for detection of cholesterol and its esters, was most satisfactory. A purple red color which fluoresces under long UV light developed after heating for 5 to 10 minutes at 90C. Although the $SnCl_3$ reagent was very sensitive in detecting the glycoalkaloid, it was not desirable because of its toxic nature and short shelf life. Dragendorf reagent was not satisfactory for detection of solanine and chaconine.

Successful separation of solanine and chaconine with TLC permitted preparative TLC of the two glycoalkaloids. In the preparation of a large amount of glycoalkaloid, preparative TLC is preferred

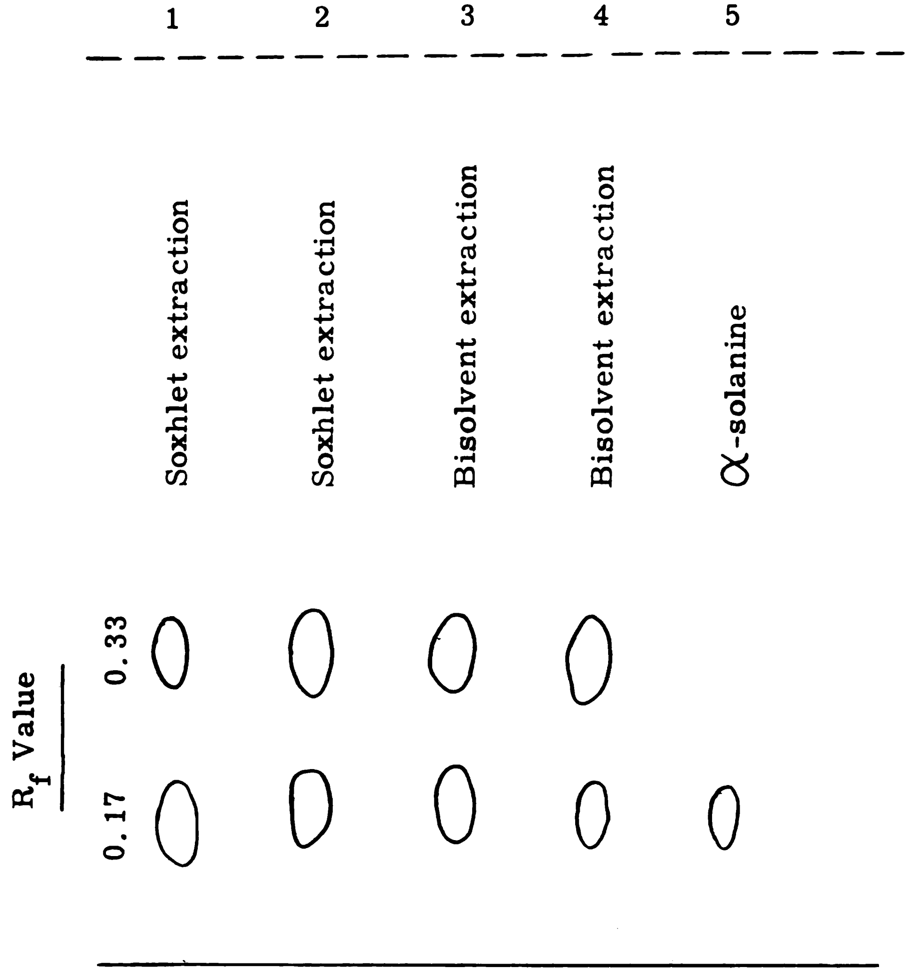


Figure 6. --Thin-layer chromatogram of the glycoalkaloids extracted by the bisolvent method and the Soxhlet method (Russet Burbank tubers)

Solvent system: MeOH-EtOH HOAc-H_20 (20:30:10:1)
Front and time: 12 cm and 70 minutes
Spray reagent: The Lowry reagent

to column chromatography because TLC requires less time and achieves better separation. After elution of the glycoalkaloids with MeOH, it is necessary to displace MeOH with 1% HOAc before the glycoalkaloids are precipitated since the glycoalkalids can only be precipitated by alkali from aqueous solution.

Absorption spectra of solanine were identical to those of chaconine with all three reagents tested (Figure 7). With the Clarke reagent, both glycoalkaloids showed the maximum absorption at 660, 600 and 512 nm. Use of 600 nm for quantitative determination of the glycoalkaloids was quite appropriate. This also confirmed that earlier data of the colorimetric determination of total solanine, which was the sum of solanine and chaconine, were acceptable.

Thin-Layer Chromatography of Sterols

In the chloroform fraction of potato sample, three components including sterols, carotenoids and chlorophylls were shown by thin-layer chromatography. After saponification, the majority of chlorophyll was lost to the aqueous fraction in the form of chlorophyllide, and the esterified sterols were freed from fatty acid.

Benzene-EtOAc (1:1) was the most satisfactory solvent system for the resolution of sterols in this study. Six spots (excluding carotenoids) were resolved from each of the potato tubers or

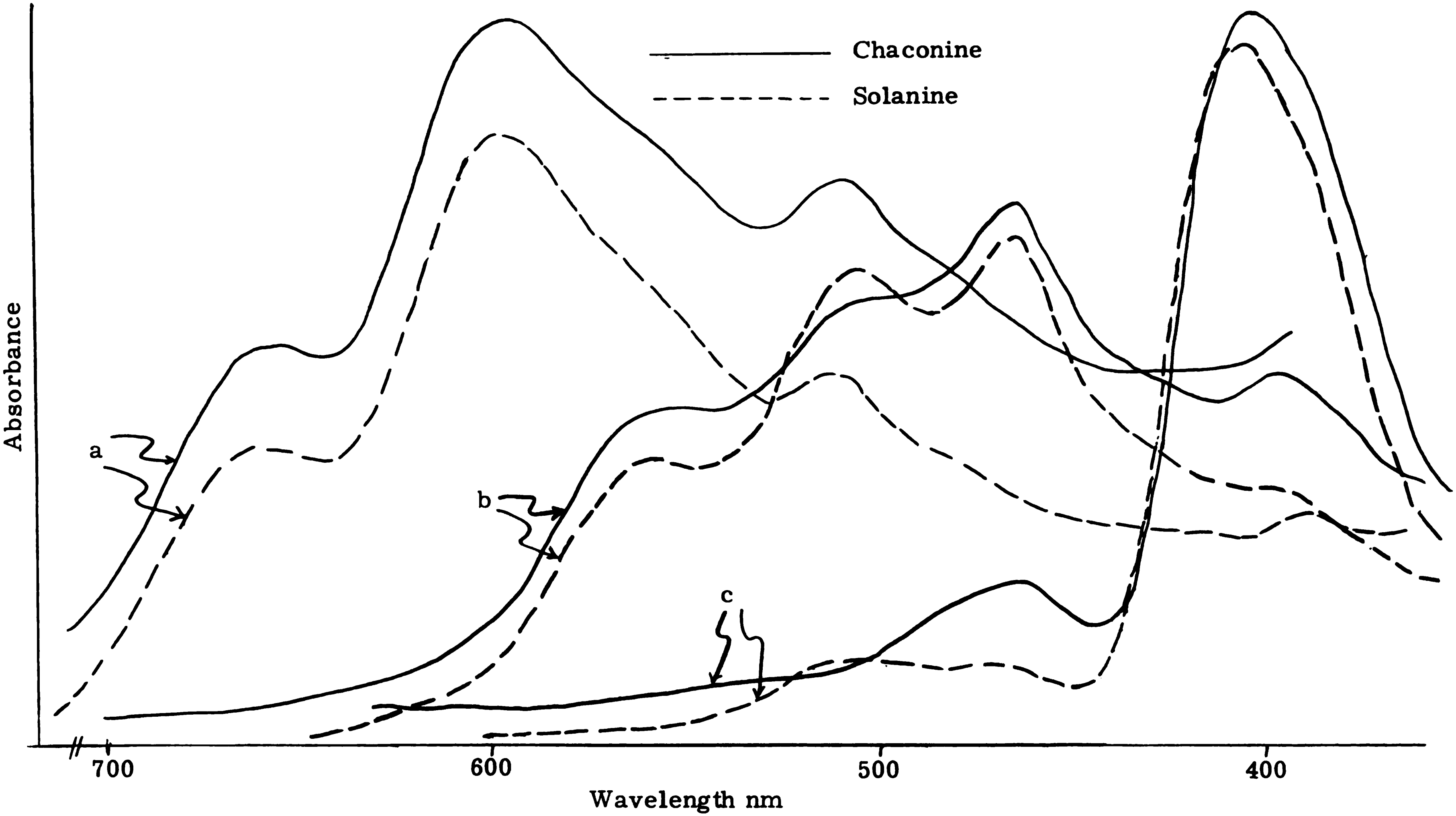


Figure 7. --Absorption spectra of solanine and chaconine after reacting with (a) the Clarke reagent, (b) the Marquis reagent, (c) 85% H_3PO_4

sprout extracts. Spot 3 was found only in potato sprouts, and spot 2 was found only in the potato tuber (Figure 8). Spot 6 absorbed short UV light without spraying. After spraying with the Lowry reagent, spots 1 to 5, and 7 fluoresced under long UV light, and spots 1 and 4 showed specific purple-red color of sterols. The R_f values of these spots were 0.00, 0.21, 0.36, 0.60, 0.67 and 0.72 respectively. Co-chromatography with the standard sterols indicated that spot 4 had similar R_f value as cholesterol, stigmasterol and β-sitosterol.

With hexane-EtOEt-HOAc (80:20:1), the R_f values changed considerably. Spots 2, 3 and 5 had similar R_f values, and spot 4 was next to spot 1. Spot 7 was resolved into 3 spots. The R_f values of these spots in numerical order are 0.00, 0.10, 0.10, 0.05, 0.10, 0.13, 0.47, 0.62 and 0.66 (Figure 9). With the more polar solvent system, 3-heptanone-HOAc-H_2O (8:5:1), spot 1 was moved with a R_f value 0.68, while the others were poorly resolved. The R_f value of spot 1 indicated that it was a steryl glycoside (Lepage, 1964). It was once suspected that spot 1 might contain solanine due to poor partitioning procedure between the chloroform fraction and the methanolic fraction. However, results with TLC showed that spot 1 did not contain any substance with a R_f value corresponding to solanine (Figure 10).

Two dimensional TLC of non-saponifiable from potato tubers or sprouts was carried out using a combination of

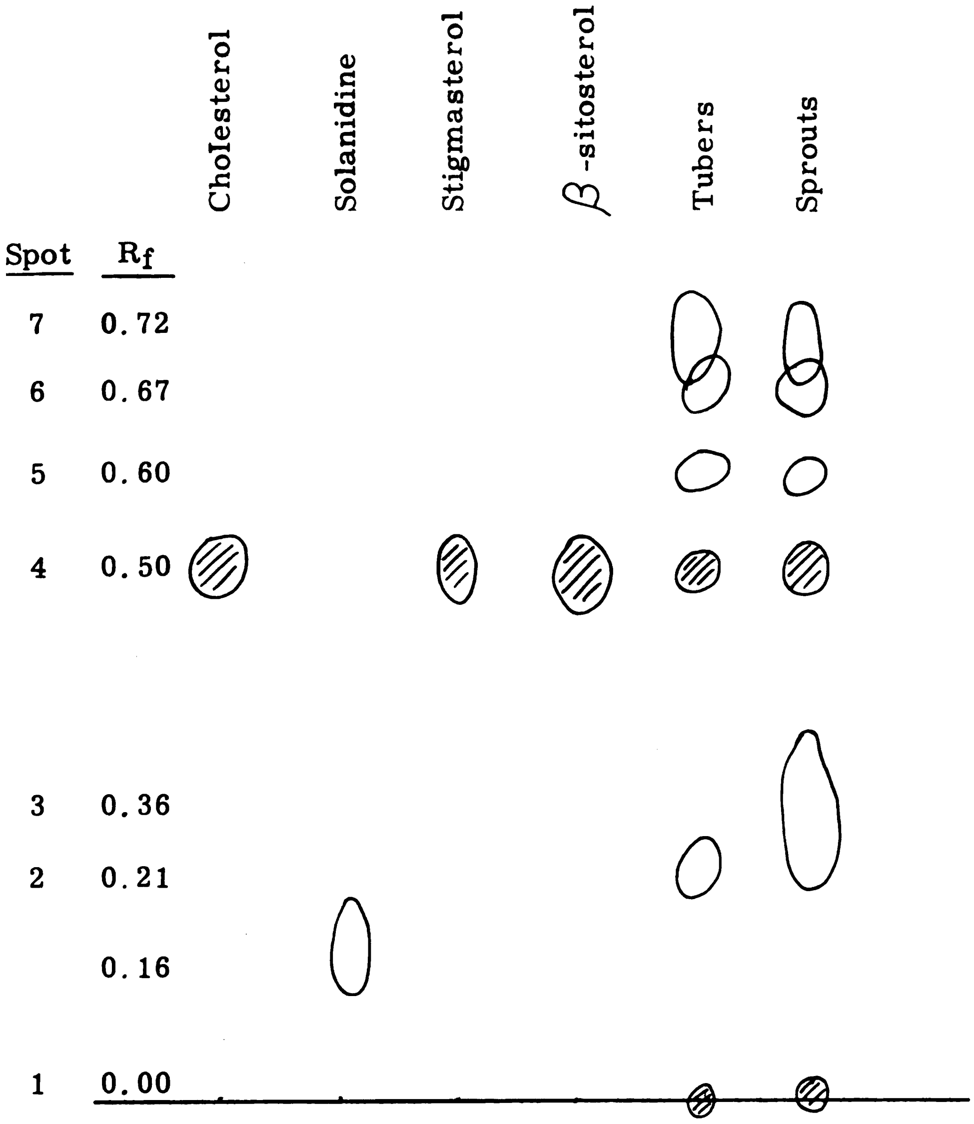


Figure 8. --Thin-layer chromatogram of the unsaponifiable compounds in the chloroform extract of the potato tubers, sprouts and authentic sterols (solanidine, cholesterol, stigmasterol and β-sitosterol)

Solvent system: Benzene-EtOAc (1:1)
Front and time: 12.8 cm and 45 minutes
Spray reagent: The Lowry reagent

▨ -- Purple-red color

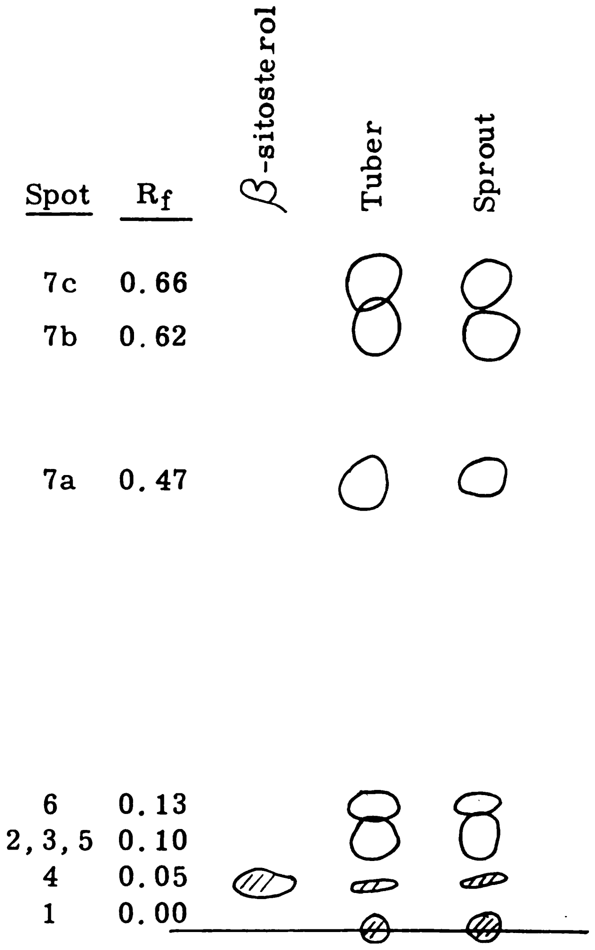


Figure 9. -- Thin-layer chromatogram of the unsaponifiable compounds in the chloroform extract of the potato tubers and sprouts

Solvent system: Hexane-EtOEt-HOAc (80:20:1)
Front and time: 13.0 cm and 31 minutes
Spraying reagent: The Lowry reagent

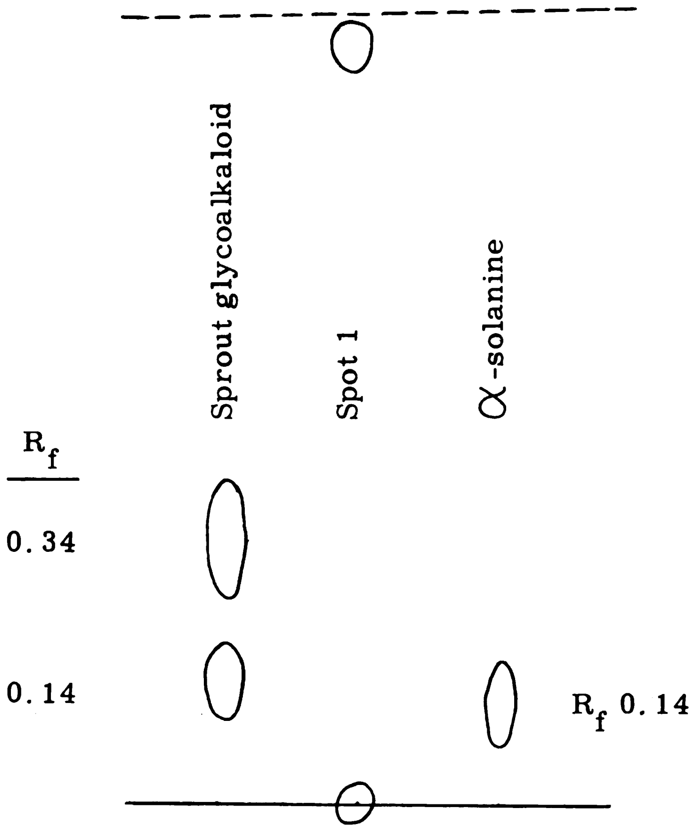


Figure 10. -- Thin-layer chromatogram of (a) sprout glycoalkaloid, (b) spot 1, (c) authentic solanine

Solvent system: MeOH-EtOEt-HOAc-H_2O (20:30:10:1)
Front and time: 10.6 cm and 60 minutes
Spraying reagent: The Lowry reagent

benzene-EtOAc (1:1) and hexane-EtOEt-HOAc (80:20:1). In tuber sample, all spots were clearly separated, but spot 1 and 7 were split into 2 spots in each case (Figure 11). Chromatogram for the sprout sample (Figure 12) was obtained by developing the plate in the same solvent systems, and spraying with the benzidine-$NaIO_4$ reagent and the Lowry reagent successively. After spraying with the benzidine-$NaIO_4$ reagent which reacts with sugars and glycosides, 2 spots, G1 and G2, were detected, and after spraying with the Lowry reagent on top of the first reagent, spot 1 showed same color as G1 and G2. This proved that spot 1 was a glycoside. Spot 3, the unique substance in sprouts, appeared to be a major substance accumulated during sprouting (Figure 12), but it was not identified.

Among the reagents for sterol visualization, the Lowry reagent was preferred although the Liebermann-Burchard reagent had similar visualizing function. Iodine vapor, on the other hand, was used only for temporary location of the spots, and plate was re-sprayed with other reagents after iodine sublimes.

Thin-Layer and Gas-Liquid Chromatographic Analysis of Sterols

The 8-foot long glass column packed with 1% OV-101 was very satisfactory in separating the standard sterols. Cholesterol, campesterol, sigmasterol and β-sitosterol had relative retention times of 2.072, 2.665, 2.880 and 3.263 respectively.

Sample: White light treated tuber

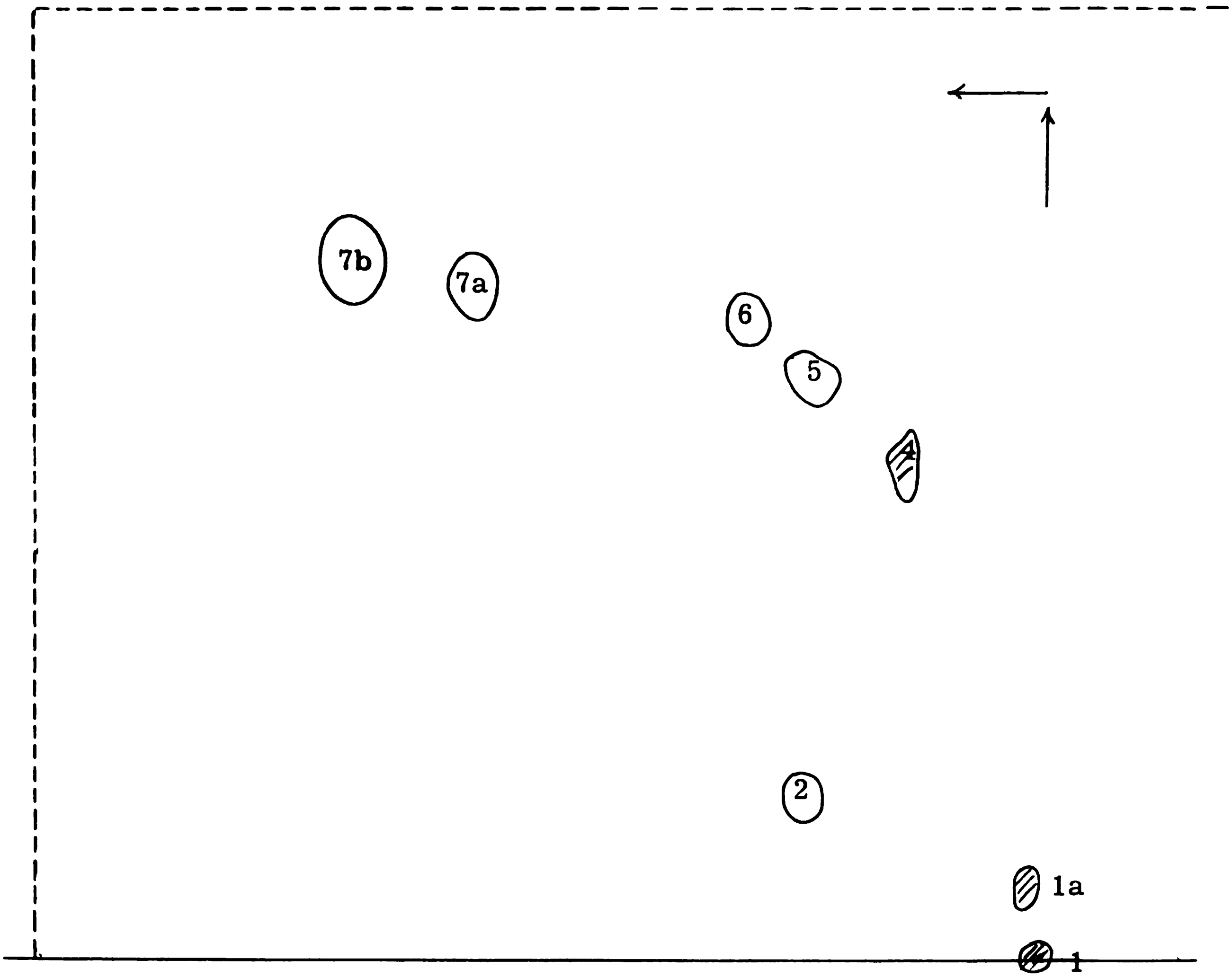


Figure 11. --Two-dimensional thin-layer chromatogram of the unsaponifiable compounds in the chloroform extract of the light treated potato tubers

Solvent system: 1. Benzene-EtOAc (1:1)
2. Hexane-EtOEt-HOAc (80:20:1)
Front and time: 1. 13 cm and 45 minutes
2. 13.9 cm and 45 minutes
Spray reagent: The Lowry reagent

Sample: Sprout

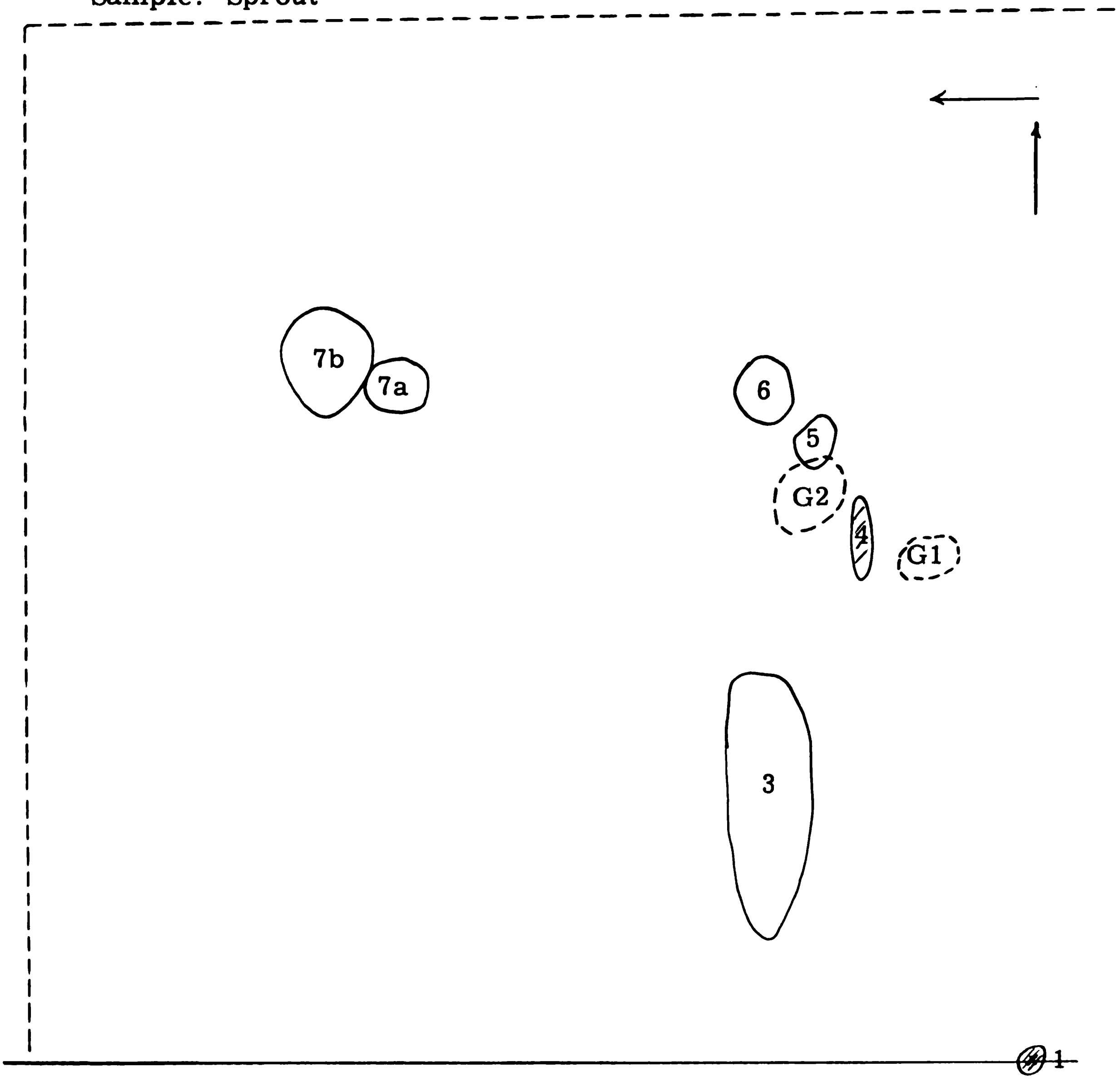


Figure 12. -- Two-dimensional thin-layer chromatogram of the unsaponifiable compounds in the chloroform extract of the potato sprouts

Solvent system:	1.	Benzene-EtOAc (1:1)
	2.	Hexane-EtOEt-HOAc (80:20:1)
Front and time:	1.	15.5 cm and 52 minutes
	2.	14.6 cm and 44.5 minutes
Spray reagent:	1.	Benzidine-$NaIO_4$ reagent
	2.	The Lowry reagent

Eight peaks were resolved from spot 1 (Figure 13). Peak 4, 6 and 7 were cholesterol, stigmasterol and β-sitosterol respectively. Proof of the presence of the above sterols was obtained by the mass spectrometry (Figure 14). Peak 3, which has a mass number 397, was present in larger amount than was cholesterol. Campesterol was not present.

Five peaks were resolved from spot 4 (Figure 13). Peak 1 was cholesterol; peak 2, unknown; peak 3, unknown; peak 4, stigmasterol; and peak 5, β-sitosterol. The chromatogram indicated that campesterol was present in a very small quantity in the potato tuber while 3% of campesterol was found in the potato leaves (Ardenne et al., 1965). Mass spectra of peak 1, 4 and 5 confirmed the presence of the above sterols. The mass spectrum of peak 2 (Figure 14) is given because of its possible significant role in the biosynthesis of glycoalkaloid.

Change of Glycoalkaloid Level During Storage and Light Treatment

Glycoalkaloid level in the surface of potato tubers generally tended to decline with the storage time. If the glycoalkaloid level was high at the beginning of storage, an obvious decrease would be seen, but if the tuber started at a low level of glycoalkaloid, change during storage was normally insignificant (Figure 15, 16).

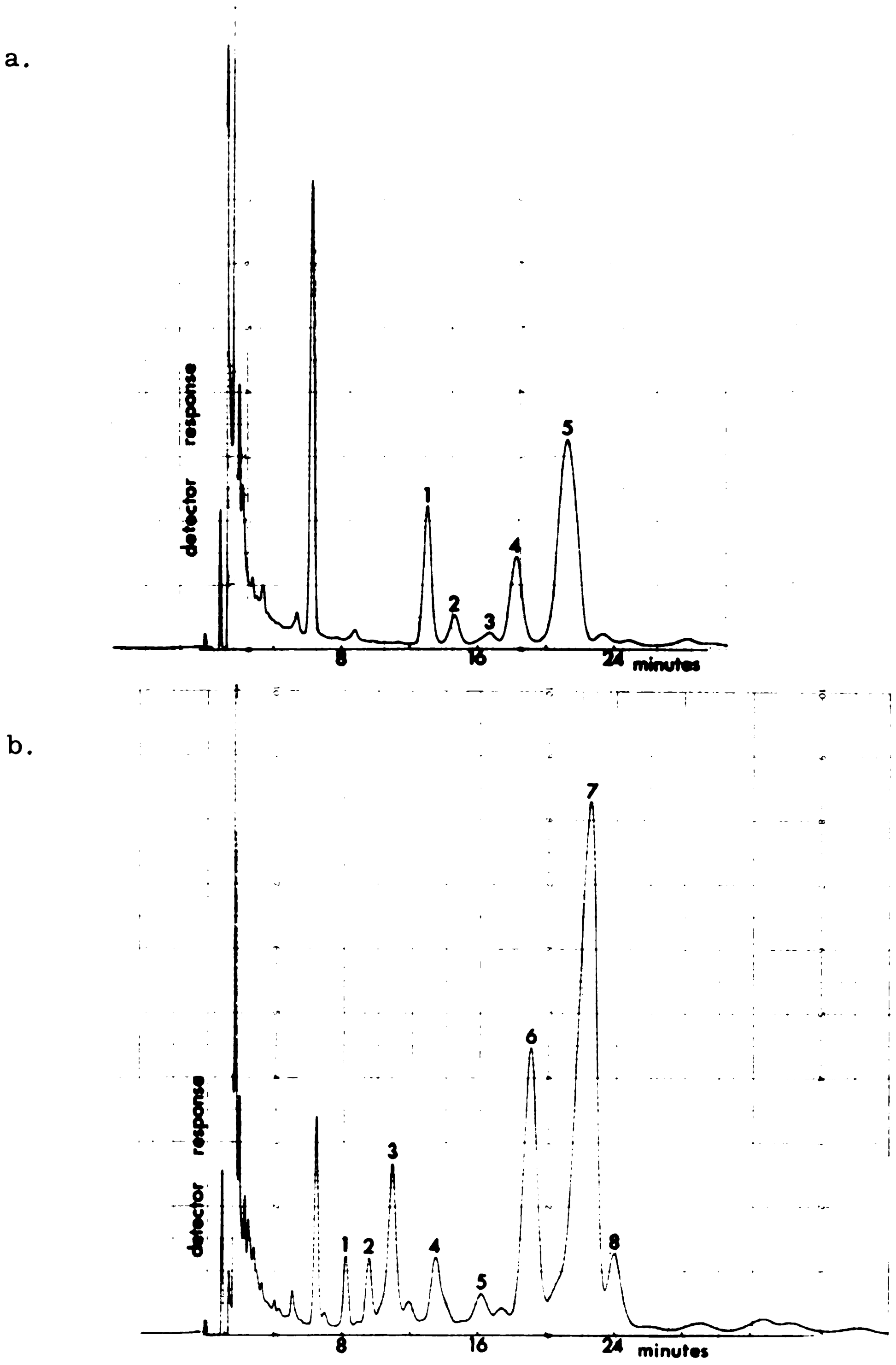
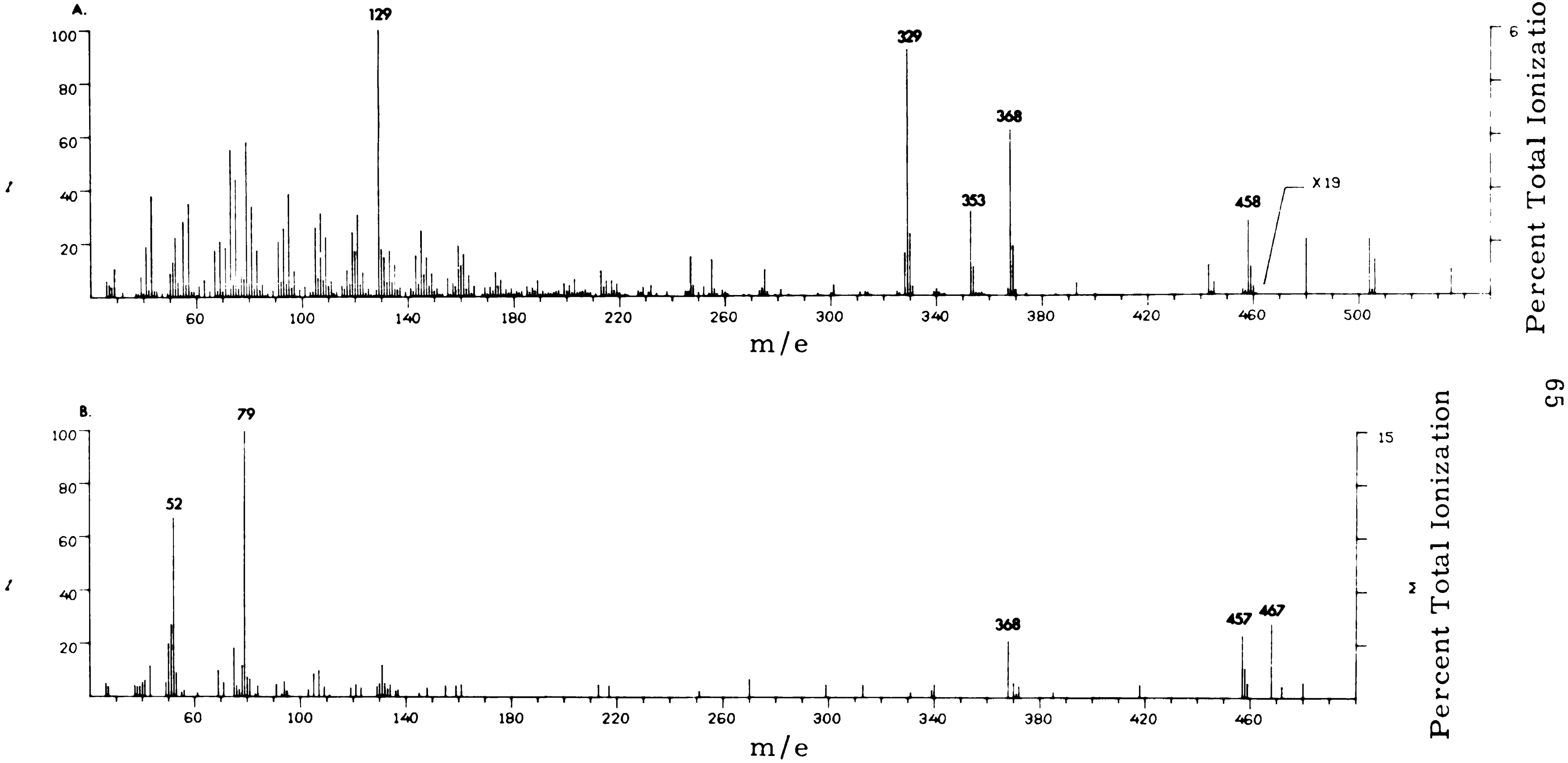


Figure 13. --Gas-liquid chromatogram of (a) spot 4, the free sterol fraction, (b) spot 1, the steryl glycoside fraction after acid hydrolysis


A.
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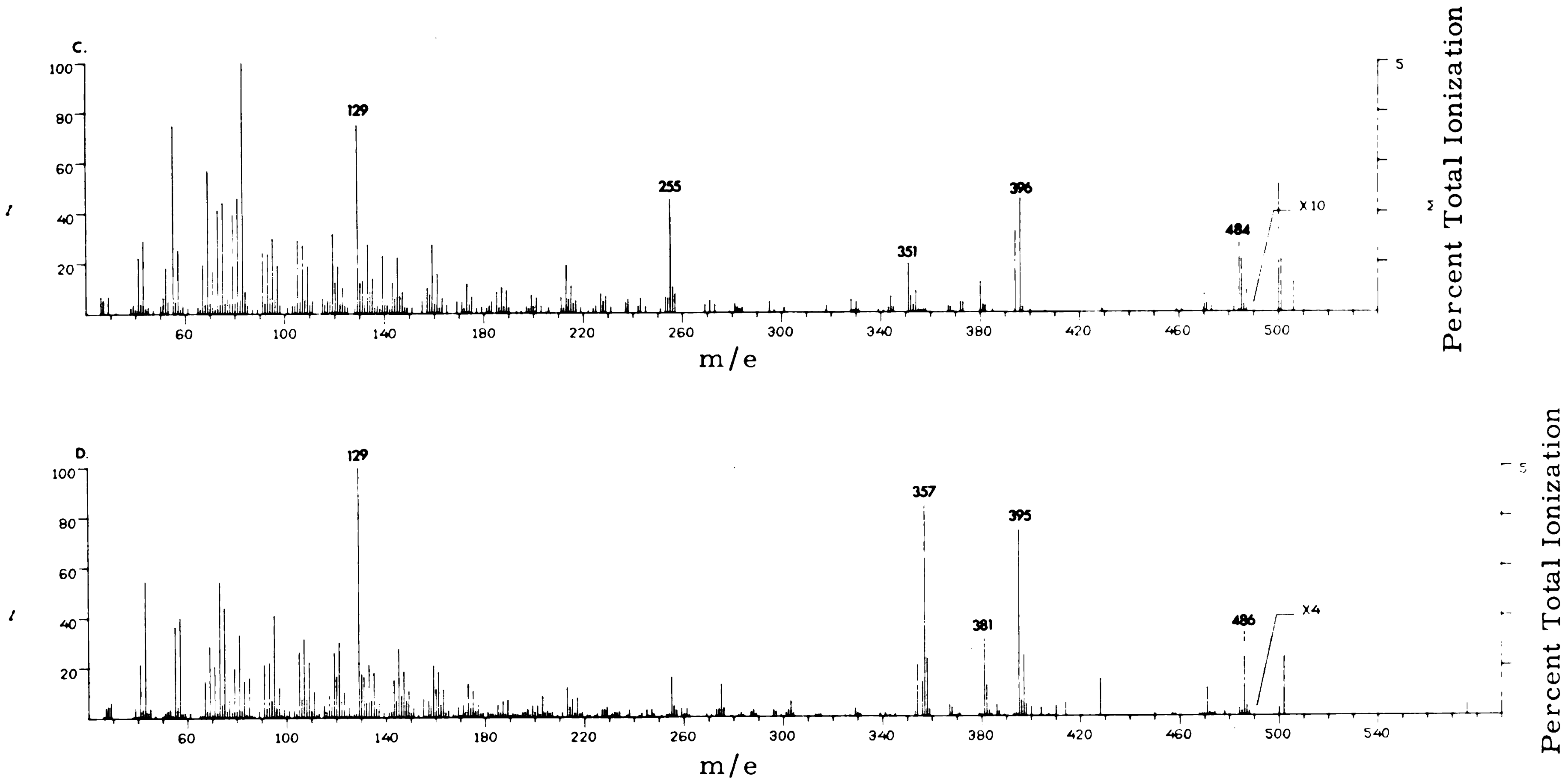

Figure 14. --Mass spectra of (a) cholesterol, (b) peak 2, (c) stigmasterol, (d) β-sitosterol

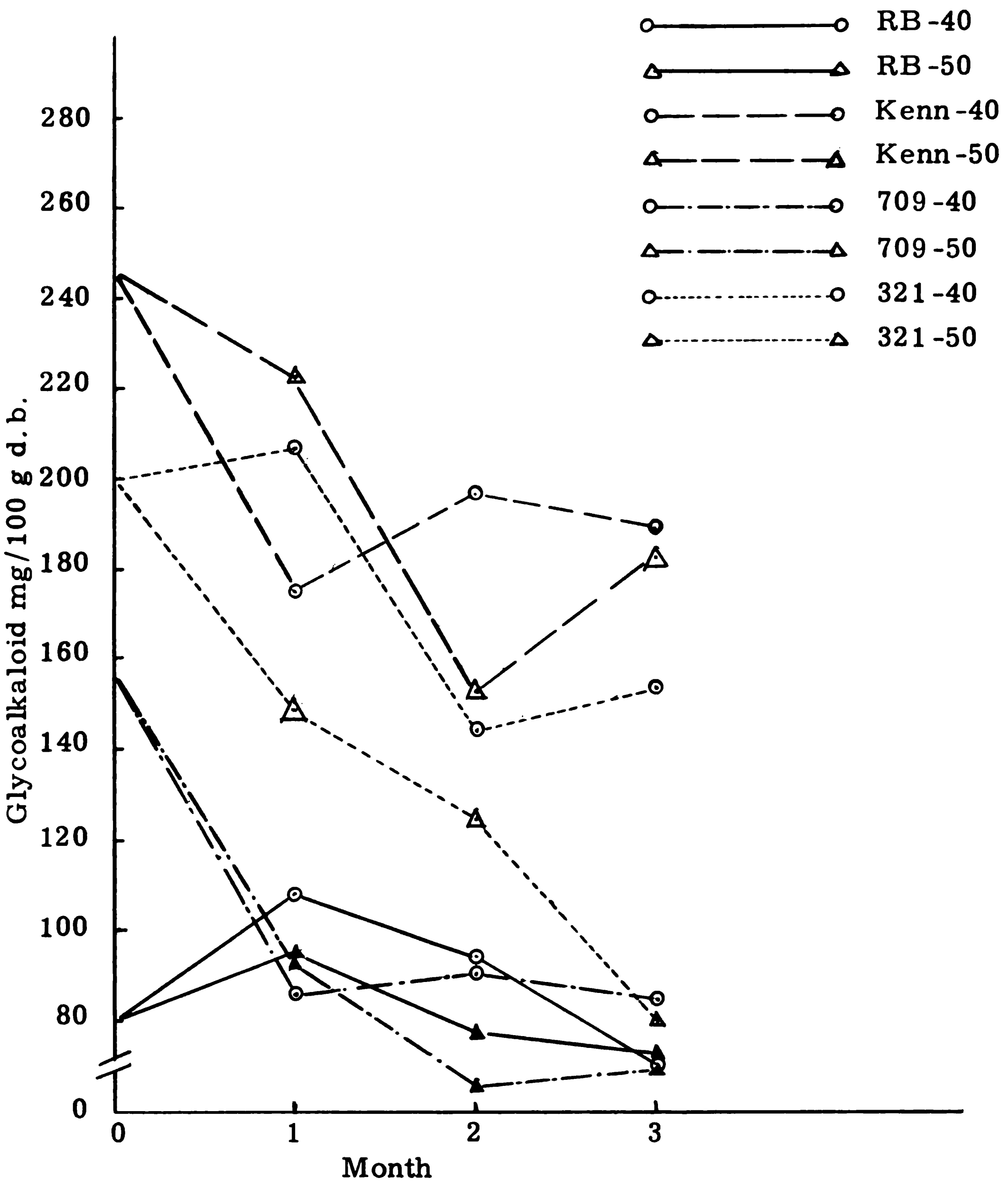

Figure 15. --Change of glycoalkaloid levels in the surface samples of potatoes (Russet Burbank, Kennebec, 709 and 321-65) stored at 40F and 50F

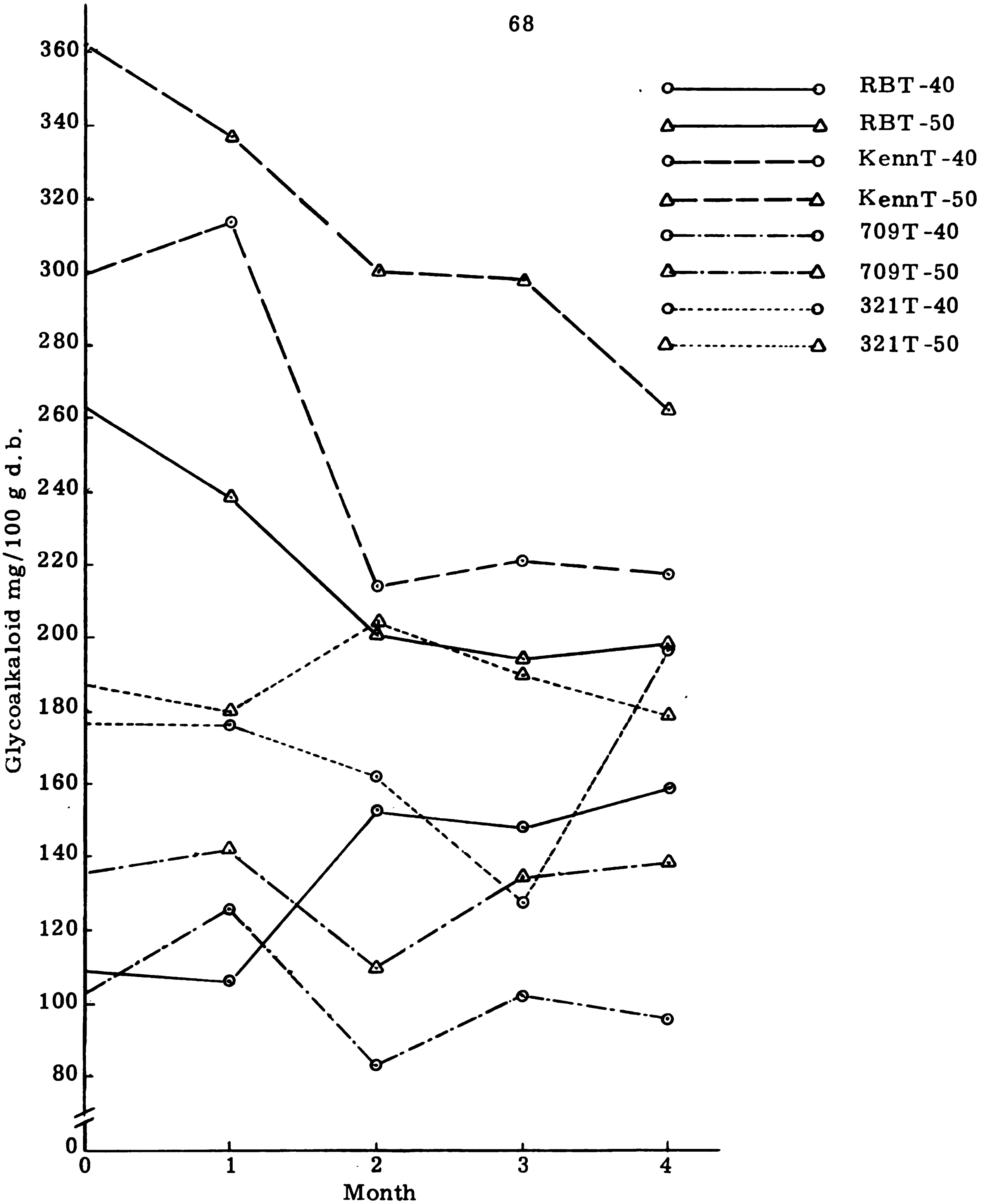

Figure 16. --Change of glycoalkaloid levels in the surface samples of the MH-30 treated potatoes (Russet Burbank, Kennebec, 709 and 321-65) stored at 40F and 50F

It was noticed that the greatest change took place in the untreated potatoes between the first and second month of storage, and in the MH-30 treated potatoes between the second and third month of storage. This seemed to correspond to the sprouting activities, which started in this period. Decrease of glycoalkaloid was probably due to the vigorous sprouting activities. However, glycoalkaloids of tubers in both 40F and 50F storage tended to reach an equilibrium level after a few months with only one exception; the untreated 321-65 in 50F storage continued to decrease.

Holding at 45F for 20 days after harvest, the MH-30 treated potatoes were stored at 58F for 10 days, and the glycoalkaloid content was increased markedly over that present in replicate lots stored at 42F (Figure 16). It would appear that during the dormant period the glycoalkaloid synthesis was greatly stimulated by a sudden rise in temperature.

After six months of storage (four months of controlled storage), the average glycoalkaloid level of the entire tuber was generally low (Figure 17). The MH-30 treated tubers of Russet Burbank and 709 had higher levels of glycoalkaloid than the untreated tubers. The difference of glycoalkaloid level was more distinct between the MH-30 treated and untreated Russet Burbank. The storage temperatures made large differences in the untreated

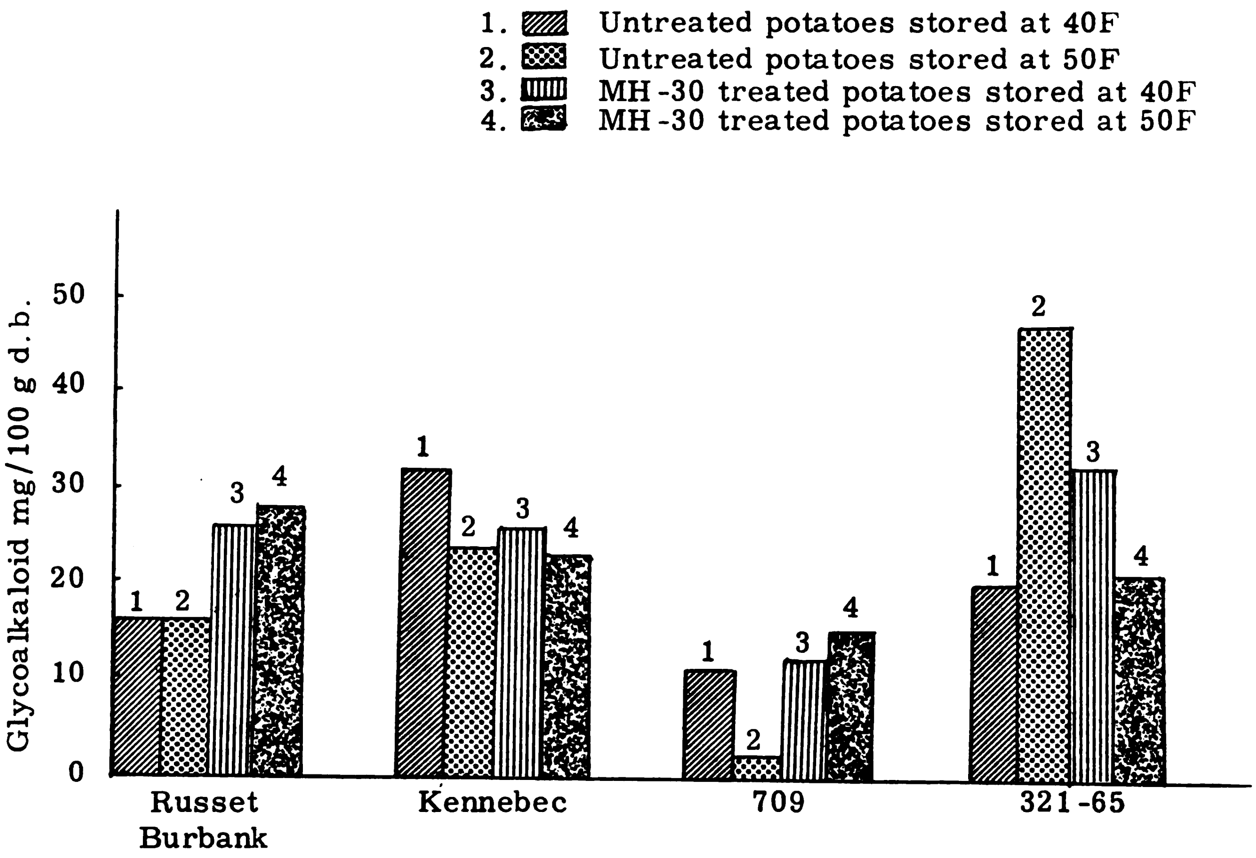

Figure 17. --Average glycoalkaloid levels of the MH-30 treated and untreated potatoes (Russet Burbank, Kennebec, 709 and 321-65) 6 months after harvest (4 months controlled storage at 40F and 50F)

Kennebec, 709 and 321-65. The untreated 321-65 in 50F storage had the highest glycoalkaloid level. However, among four different potatoes, 709 had the lowest level of glycoalkaloid under all conditions (Figure 17). This seemed to support the finding that 709 performs uniquely with the MH-30 and light treatment.

A comparison of the glycoalkaloid content of the surface layer and of the entire potato indicated that the ratio ranged from 5-10 to 1. The variation indicated that glycoalkaloid determinations of the surface layers could not be used as a means of predicting glycoalkaloid content of the entire tuber.

With the exception of MH-30 treated 709, the glycoalkaloid content of the tubers exposed to white light for six days, after 2 months storage at 45F, increased from 110 to 330 mg/100 g d.b.[1] over that of the controls (Figure 18). When a similar experiment was carried out with potatoes stored for five months at 40F, all light-treated potatoes had higher levels of glycoalkaloid. After 2 months storage, glycoalkaloid content in the MH-30 treated 709 after light treatment was 114 mg/100 g d.b. as contrasted to 137 mg/100 g d.b. in the control. After 5 months storage, the glycoalkaloid content of the light treated tubers was double that of the control tubers (194 and 96 mg/100 g d.b. respectively). The

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[1] mg/100 g d.b. = mg per 100 g of potato sample on dry basis.

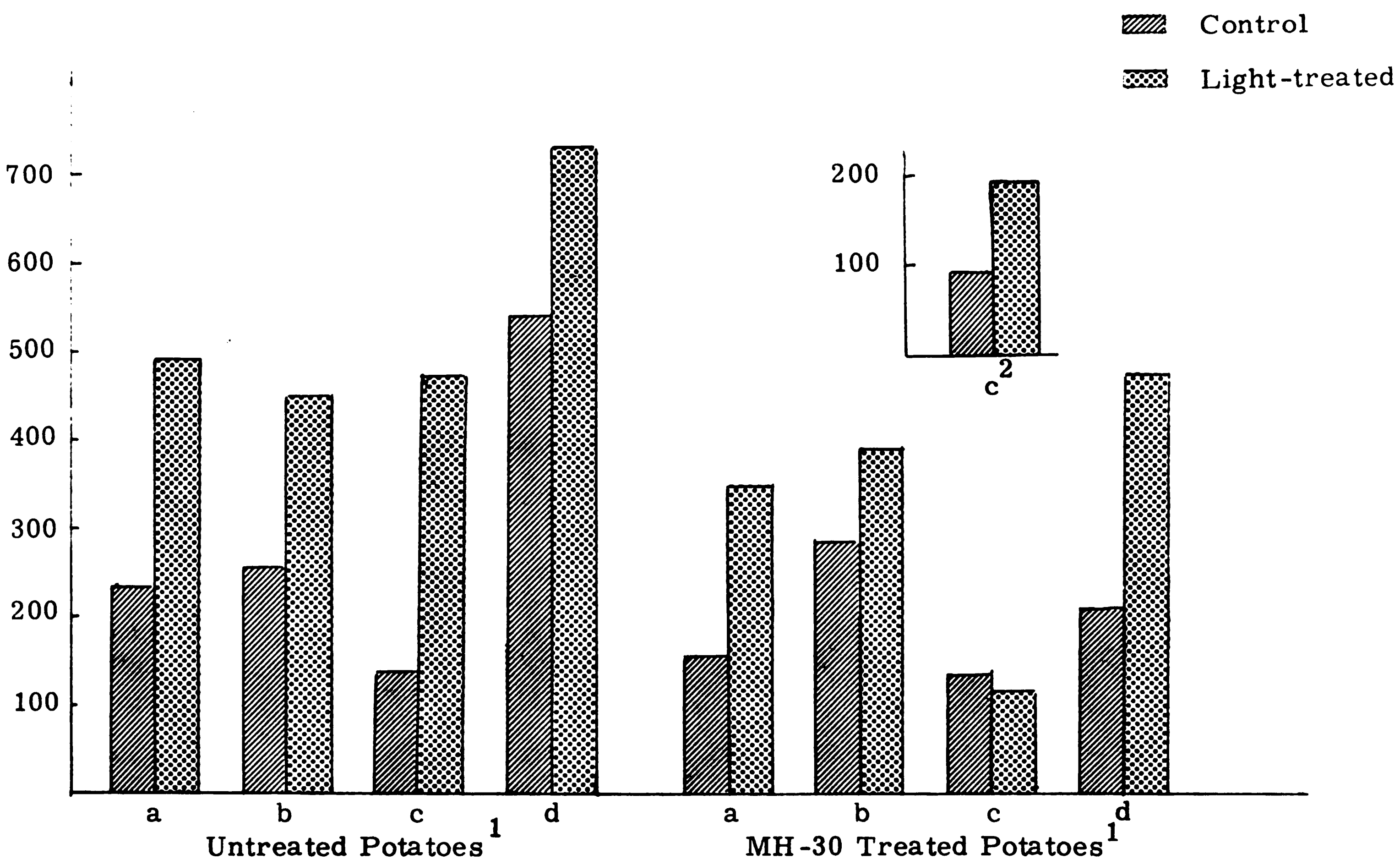

Figure 18. --Glycoalkaloid levels of untreated and MH-30 treated potatoes (a--Russet Burbank, b--Kennebec, c--709; d--321-65) with and without light treatment for 6 days

[1] Potatoes stored for 2 months

[2] Potatoes stored for 5 months

inhibitory effect of MH-30 on the production of glycoalkaloid, therefore, disappeared during the longer storage. This result agreed with an early report that the length of inhibition period of MH-30 was proportional to the MH-30 concentration used (Schoene and Hoffmann, 1949). The MH-30 treated potatoes after the light treatment tended to synthesize less glycoalkaloid than did the untreated potatoes (Figure 18).

The glycoalkaloid produced in Russet Burbank potatoes under white light in 100% CO_2 atmosphere increased less than under normal storage conditions (Figure 19a). After exposure to white light, anthocyanin of Russet Burbank also increased significantly. The sample solution, from which the glycoalkaloid is precipitated, became pinkish. The absorption spectra of the solution were taken with a Bausch-Lomb 505 spectrophotometer after concentration. The maximum absorption occurred at around 520 to 530 nm. The anthocyanin was not identified, but the absorbance of each sample solution was recorded on a quantitative basis. Increase of anthocyanin was not affected by CO_2 as much as by MH-30 (Figure 19b). Similar observations were made on the Kennebec variety.

Russet Burbank potatoes exposed to red and UV light for 6 days showed a slight increase in glycoalkaloid, but in the tubers exposed to far-red light it was slightly below the control (Figure 20a).

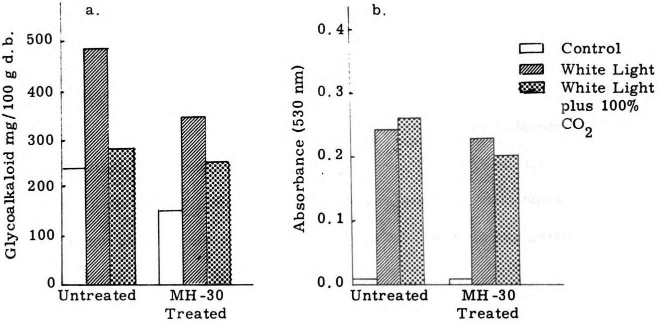

Figure 19. --Glycoalkaloid and anthocyanin synthesized by potatoes (Russet Burbank) after treatment with white light or white light plus 100% CO_2 for 6 days

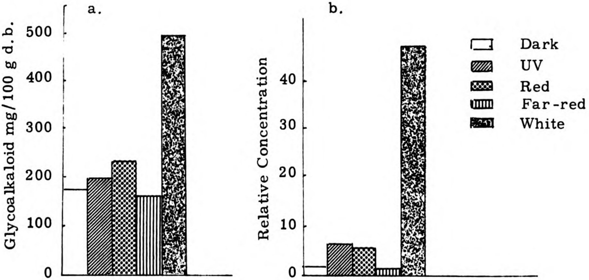

Figure 20. --Glycoalkaloid and chlorophyll synthesized by potatoes (Russet Burbank) after treatment with different lights for 6 days

Similar trends were obtained for the amount of chlorophyll produced (Figure 20b). The chlorophyll was determined using the chloroform fraction of these samples. The absorbance of the samples was taken with a Beckman DB Spectrophotometer at 660 nm after chloroform was displaced by ethyl ether. This suggests that far-red light may have a specific effect on the metabolic activities of potato tubers. It is not clear whether the high energy of far-red light has anything to do with this phenomenon.

Change of Sterols During Storage and Light Treatment

During dark storage, total sterol[1] in potatoes stored at 40F tended to increase during storage (Tables 6, 7). This increase was primarily due to an increase in β-sitosterol. At 50F there was a marked increase between the second and third month of storage and a slight decrease between the third and fourth month of storage with the MH-30 treated potatoes (Table 7). No consistent pattern of change was observed with the untreated potatoes, but the amount of cholesterol decreased while that of β-sitosterol and stigmasterol tended to increase during storage.

Under white light cholesterol and stigmasterol content increased while β-sitosterol decreased (Table 8). It appears that

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[1] Total sterol is the sum of cholesterol, stigmasterol and β-sitosterol.

Table 6. --Sterol contents of potatoes (Russet Burbank, Kennebec, 709, and 321-65) at different stages of storage at 40F and 50F

Sample	Sterol	Month 1	Month 2	Month 3	Month 4
		mg/100 g w.b.			
Russet Burbank 40F	Cholesterol	.27	.17	.20	.33
	Stigmasterol	.43	.38	.40	.37
	β-sitosterol	.65	.66	.74	.94
	TOTAL	1.35	1.21	1.34	1.64
Kennebec 40F	Cholesterol	.18	.15	.16	.33
	Stigmasterol	.35	.34	.38	.36
	β-sitosterol	.24	.37	.43	.87
	TOTAL	.77	.86	.97	1.55
709 40F	Cholesterol	.18	.13	.15	.30
	Stigmasterol	.35	.38	.43	.93
	β-sitosterol	.25	.37	.43	1.00
	TOTAL	.78	.88	1.01	2.23
321-65 40F	Cholesterol	.20	.18	.17	.27
	Stigmasterol	.39	.38	.37	.32
	β-sitosterol	.48	.60	.50	.87
	TOTAL	1.07	1.16	1.04	1.46
Russet Burbank 50F	Cholesterol	.27	.20	.18	
	Stigmasterol	.43	.23	.36	
	β-sitosterol	.65	.73	.72	
	TOTAL	1.35	1.16	1.26	
Kennebec 50F	Cholesterol	.18	.18	.18	
	Stigmasterol	.35	.35	.39	
	β-sitosterol	.24	.40	.72	
	TOTAL	.77	.93	1.29	
709 50F	Cholesterol	.18	.17	.15	
	Stigmasterol	.35	.44	.42	
	β-sitosterol	.25	.52	.47	
	TOTAL	.78	1.13	1.04	
321-65 50F	Cholesterol	.20	.20	.15	
	Stigmasterol	.39	.37	.33	
	β-sitosterol	.48	.54	.39	
	TOTAL	1.07	1.11	.87	

Table 7. --Sterol contents of the MH-30 treated potatoes (Russet Burbank, Kennebec, 709 and 321-65) at different stages of storage at 40F and 50F

Sample	Sterol	Month 2	Month 3	Month 4
		mg/100 g w.b.		
Russet Burbank 40F	Cholesterol	.17	.22	.33
	Stigmasterol	.23	.40	.71
	β-sitosterol	.29	.56	.80
	TOTAL	.69	1.18	1.84
Kennebec 40F	Cholesterol	.28	.21	.27
	Stigmasterol	.38	.45	.61
	β-sitosterol	.40	.60	.64
	TOTAL	1.06	1.26	1.52
709 40F	Cholesterol	.19	.16	.29
	Stigmasterol	.39	.47	.69
	β-sitosterol	.38	.43	.60
	TOTAL	.96	1.06	1.58
321-65 40F	Cholesterol	.15	.19	.28
	Stigmasterol	.18	.44	.68
	β-sitosterol	.22	.60	.64
	TOTAL	.55	1.23	1.60
Russet Burbank 50F	Cholesterol	.13	.27	.27
	Stigmasterol	.19	.45	.43
	β-sitosterol	.21	.77	.45
	TOTAL	.53	1.49	1.29
Kennebec 50F	Cholesterol	.17	.35	.27
	Stigmasterol	.30	.48	.53
	β-sitosterol	.27	.66	.49
	TOTAL	.74	1.49	1.29
709 50F	Cholesterol	.20	.24	.17
	Stigmasterol	.45	.64	.51
	β-sitosterol	.33	.64	.51
	TOTAL	.98	1.52	1.19
321-65 50F	Cholesterol	.16	.41	.12
	Stigmasterol	.28	.47	.27
	β-sitosterol	.21	.60	.23
	TOTAL	.65	1.48	.62

under the influence of light, the system was mobilized towards increasing cholesterol and the unidentified compound of peak 2, although these compounds did not accumulate to any great extent. These results seem to be in agreement with the proposed biosynthetic pathway where cholesterol is an intermediate for solanidine biosynthesis. The decrease in β-sitosterol as well as total sterol during light treatment is probably due to a shift in kinetics in favor of synthesis of glycoalkaloid.

Table 8. --Sterol contents of potatoes (Russet Burbank, Kennebec, 709 and 321-65) with and without white light treatment for 6 days

Variety	Treatment	Cholesterol		Stigmasterol		β-sitosterol	
		Concn.[a]	%[b]	Concn.[a]	%[b]	Concn.[a]	%[b]
Russet Burbank	Control	.33	20	.37	23	.94	57
	Light	.37	25	.35	24	.74	51
Kennebec	Control	.32	21	.36	23	.87	56
	Light	.33	21	.49	32	.73	47
709	Control	.30	13	.93	40	1.10	47
	Light	.32	20	.67	42	.60	38
321-65	Control	.27	18	.32	22	.87	60
	Light	.27	18	.41	28	.79	54

[a] Concentration of sterol is expressed in mg/100 g w.b.

[b] % cholesterol + % stigmasterol + % β-sitosterol = 100

Table 9. --Sterol contents of Russet Burbank potatoes treated with white, far-red, red, UV light and in darkness

Light	Sterol	Content	
		Concentration mg/100 g w.b.	%[a]
White	Cholesterol	.35	37
	Stigmasterol	.31	33
	β-sitosterol	.28	30
	TOTAL	.94	
Far-red	Cholesterol	.22	30
	Stigmasterol	.25	35
	β-sitosterol	.25	35
	TOTAL	.72	
Red	Cholesterol	.37	37
	Stigmasterol	.34	34
	β-sitosterol	.29	29
	TOTAL	1.00	
UV	Cholesterol	.27	29
	Stigmasterol	.34	36
	β-sitosterol	.33	35
	TOTAL	.94	
Darkness	Cholesterol	.23	21
	Stigmasterol	.41	37
	β-sitosterol	.47	42
	TOTAL	1.11	

[a] % cholesterol + % stigmasterol + % β-sitosterol = 100

After the Russet Burbank potatoes were exposed to different lights, the result showed that the total sterol of potatoes stored in darkness was the highest and that stored in far-red light was the lowest among all samples (Table 9). As the tubers were treated with white, red or UV light, the level of cholesterol increased, while the level of β-sitosterol markedly decreased. Also, the percent of individual sterols varied in the same manner as obtained from the white light treatment for all varieties (Table 8).

Flavor of the B5141-6 Potatoes

The B5141-6 potatoes of both 45F and 65F storage have a high glycoalkaloid content with no greening (Table 10). The surface sample, as anticipated, had a much higher level of glycoalkaloid than did the flesh sample. The greatest difference between B5141-6 and Russet Burbank was in the flesh samples, with B5141-6 having 11 times as much glycoalkaloid as Russet Burbank. In the surface samples, the difference was only 3 times (Table 10).

In the flavor difference test between B5141-6 and Russet Burbank, the surface of both varieties was excluded. The test showed no significant flavor difference between the B5141-6 potatoes and the control. The majority of the panelists could not distinguish the control from the B5141-6. The B5141-6 samples were described as fresh as the control sample, even though the gummy texture of the B5141-6 potatoes stored at 65F was noticeable. It appears that potatoes with a glycoalkaloid level of 8.3 mg/100 g w.b. were acceptable to the taste panel under the described experimental condition.

Table 10. --Glycoalkaloid content in surface and flesh of some potato tubers (B5141-6 stored at 45F and 65F, Russet Burbank stored at 40F)

Sample	Area	Glycoalkaloid Content	
		mg/100 g w.b.	mg/100 g d.b.
L-45[a]	Surface	71	399
L-65[a]		69	329
RBT-40[b]		23	140
L-45[a]	Flesh	8.3	57
L-65[a]		8.3	51
RBT-40[b]		0.8	6

[a] L-45 and L-65 are B5141-6 potatoes stored at 45F and 65F respectively.

[b] MH-30 treated Russet Burbank stored at 40F is used as control.

Threshold Level of Glycoalkaloid Affecting the Potato Flavor

To facilitate the control of glycoalkaloid concentration, moisture content and homogeneity of the added glycoalkaloid in the simulated samples, drum-dried potato flakes without any additives were used as base material. Its moisture content was 8% as determined by a Cenco moisture balance, and its glycoalkaloid content was negligible as determined by the bisolvent extraction method.

In the first study, surface material from the light-treated potatoes was used as the source of glycoalkaloid. This material was green in appearance. Four levels of glycoalkaloid, 3.8, 6.8, 11.4 and 17.0 mg/100 g w.b. with 85% moisture were used, and a paired comparison test was run under red light. The result (Table 11a) showed that glycoalkaloid at a level higher than 6.8 mg/100 g w.b. could be detected by the panelists, and that all 4 levels were not acceptable to the panelists. This high rate of rejection gave rise to a thought that color difference between the samples and control was not masked by the red light used, and the color difference probably influenced the panelist's decision.

In the second series, glycoalkaloid concentrations of 3.9, 5.8, 7.0 and 8.7 mg/100 g w.b. respectively were used, and the reference sample dyed green. The panelists indicated a significant difference in flavor at the 8.7 mg/100 g level but did not reject any of the samples (Table 11b). In the third series of glycoalkaloid concentrations, 7.7, 9.2, 11.5 and 15.4 mg/100 g w.b., the panelists were able to detect the off-flavor of two high concentrations significantly, and both were considered as being unacceptable (Table 11c).

Table 11.--Paired comparison tests on potato samples using green potatoes as glycoalkaloid source

Sample	Glycoalkaloid Concentration mg/100 g w.b.	Total Trials	Number Detection	Number Rejection
		a. Reference Not Colored		
A	3.8	30	16	20*
B	6.8	30	23**	23**
C	11.4	30	24**	24**
D	17.0	30	26***	26***
		b. Reference Dyed Green		
A	3.9	30	18	11
B	5.8	30	14	11
C	7.0	30	19	15
D	8.7	30	22**	19
		c. Reference Dyed Green		
A	7.7	15	11	7
B	9.2	15	11	6
C	11.5	15	13**	13**
D	15.4	15	13**	12*

* 5% significance level
** 1% significance level
*** .1% significance level

Since the presence of green color in the glycoalkaloid donor seemed to influence the panelists even after the masking technique was used, the use of surface material from light treated potatoes as a glycoalkaloid source was discontinued.

Albino sprouts, having an extremely high level of glycoalkaloid, were used in subsequent flavor studies. In the first series, with levels of 5.0, 10.0, 15.0, 20.0 and 25.0 mg/100 g w.b. glycoalkaloid, significant detection of off-flavor occurred at the 10, 20 and 25 mg/100 g w.b. levels but not at the 15 mg/100 g w.b. level. No explanation can be given for the detection at the 10 mg/100 g w.b. level. Glycoalkaloid at 25 mg/100 g w.b. was rejected (Table 12). In the second series 20 and 25 mg/100 g w.b. glycoalkaloid were tasted with single sample procedure. Similar results were obtained, although the level of rejection was not as high (Table 13a). In the third series with levels of 10 and 15 mg/100 g w.b. glycoalkaloid, and the addition of 0.2% salt, significant detection of an off-flavor occurred at the 15 mg/100 g w.b. level, but it was not rejected (Table 13b).

An informal tasting with only 3 panelists indicated that a level of 35 mg/100 g w.b. glycoalkaloid was completely unacceptable and had a very strong off-flavor. The taste panelists reported that the off-flavor due to glycoalkaloid was astringent or gave a burning sensation and had a pronounced aftertaste. Zitnak (1961) reported the taste of glycoalkaloid as bitter.

Table 12. --Paired comparison tests on potato samples using albino sprouts as glycoalkaloid source

Sample	Glycoalkaloid Concentration mg/100 g w.b.	Total Trials	Number Detection	Number Rejection
A	5.0	15	4	3
B	10.0	30	21*	8
C	15.0	30	16	12
D	20.0	30	21*	18
E	25.0	15	15***	13***

Table 13. --Single sample procedure tests on potato samples using albino sprouts as glycoalkaloid source

Sample	Glycoalkaloid Concentration mg/100 g w.b.	Total Trials	Number Detection	Number Rejection
a. Without Salt				
A	20.0	40	26*	21
B	25.0	40	35***	27*
b. With 0.2% Salt				
A	10.0	30	15	3
B	15.0	30	24*	14

* 5% significance level
** 1% significance level
*** .1% significance level

Talburt and Smith (1967) suggested that potatoes with more than 100 mg/100 g d.b. (15 mg/100 g w.b.) of glycoalkaloid were not suitable for human consumption. In this study, none of the varieties under any of the storage conditions synthesized sufficient glycoalkaloid in the surface to bring the glycoalkaloid level in the total potato up to this level. However, this level would not be rejected by the consumers on the basis of flavor. Therefore, chemical determination of glycoalkaloid before a new lot of potatoes is marketed should be adopted as a safety measure against off-flavor or poisoning of potatoes due to glycoalkaloid.

SUMMARY AND CONCLUSIONS

An effective extraction method for glycoalkaloid determination using a bisolvent mixture, MeOH-$CHCl_3$ (2:1) was developed in this study. By blending the sample and the solvent mixture at a ratio of 1 to 20, the sample was dehydrated, and the glycoalkaloid extracted into the methanolic fraction. The glycoalkaloid so obtained did not have any polymerized pigment, and a complete recovery of added glycoalkaloid was obtained.

The glycoalkaloid isolated from the surface sample of potato tubers was resolved into α-solanine and α-chaconine by a solvent system MeOH-EtOAc-HOAc-H_2O (20:30:10:1) on a silica gel plate. Both glycoalkaloids had identical absorption spectra after reacting with the Marquis reagent, the Clarke reagent or 85% H_3PO_4, and were determined together quantitatively.

The chloroform fraction of the bisolvent extract contained sterols of potatoes. After saponification, 6 spots were resolved by the solvent system benzene-EtOAc (1:1) on a silica gel plate, but only 2 spots had the specific color reaction as the demethyl sterols such as cholesterol, stigmasterol, β-sitosterol or campesterol after spraying with the Lowry reagent. They were found to be steryl glycoside and free sterols.

The steryl glycoside and free sterols were analysed with gas-liquid chromatography. The steryl glycoside had 8 different steryl moieties including cholesterol, stigmasterol and β-sitosterol, and the free sterols included the above sterols plus two unknowns. Campesterol was not found in any significant quantity. Presence of cholesterol, stigmasterol and β-sitosterol was proved by mass spectrometry.

Potatoes including two commercial varieties, Russet Burbank and Kennebec, and two clones, 709 and 321-65, were used in studying the change of glycoalkaloid level during storage and light treatment. Glycoalkaloid level increased rapidly when potatoes were subjected to a higher storage temperature at the early stage of storage. No consistent change in glycoalkaloid level of the potatoes occurred during sprouting, but there was a tendency for it to decrease. Potatoes with low glycoalkaloid level at the beginning of storage maintained the low level throughout the storage period.

The potatoes were also treated with white, far-red, red and UV lights. Those stored under white light showed the greatest increase in glycoalkaloid content, while those held under far-red light had the lowest glycoalkaloid level. The MH-30 treatment generally decreased the rate of light-induced synthesis of glycoalkaloid, particularly during the early months of storage. The MH-30 treated 709 after 2 months storage had a lower glycoalkaloid level after white light treatment than those held in darkness.

Monthly analyses of sterols showed that total sterol increased in 40F storage, and an increase with a subsequent decrease of total sterol was observed in the MH-30 treated potatoes stored at 50F. The change of total sterol was primarily due to β-sitosterol. In the untreated potatoes stored at 50F, no consistent pattern of sterol change could be found.

During light treatment, β-sitosterol, as well as total sterol, tended to decrease. Stigmasterol increased at the expense of β-sitosterol. Cholesterol along with an unidentified compound increased slightly. There was no accumulation of any sterol in the same magnitude as the accumulation of glycoalkaloid during light treatment.

Based on the above data, it is suggested that these sterols, cholesterol in particular, are involved in the glycoalkaloid biosynthesis although they are not directly related.

Using a flavor difference procedure, B5141-6 potatoes (45F and 65F storage) which contained 8.3 mg/100 g w.b. glycoalkaloid were compared with a control potato (Russet Burbank at

40F storage) which contained 0.8 mg/100 g w.b. glycoalkaloid. No difference of flavor was detected by the 24-member taste panel.

A dilution procedure was employed to determine at what level glycoalkaloid would affect the potato flavor. Both the paired comparison procedure and the single sample procedure were used. When the green surface material of the light treated potatoes was used as the source of glycoalkaloid, the detection threshold was around 10 mg/100 g w.b., but when the juice of albino sprouts was used, the glycoalkaloid was not detected until the level was higher than 20 mg/100 g w.b. Since potatoes with more than 15 mg/100 g w.b. of glycoalkaloid were thought to be health hazards, it was concluded that potatoes with a glycoalkaloid level high enough to be a health hazard might not be rejected by the consumers on the basis of flavor.

PROPOSALS FOR FURTHER RESEARCH

1. In this study, cholesterol among other sterols was found to increase during the glycoalkaloid biosynthesis. According to the proposed biosynthetic pathway (Figure 2), cholesterol instead of stigmasterol and β-sitosterol is the intermediate of glycoalkaloid biosynthesis. The result of this study has shown that stigmasterol increases at the expense of β-sitosterol. There is no proof that stigmasterol and β-sitosterol are not the intermediates of glycoalkaloid synthesis. In addition, there seems to be an interrelationship among cholesterol, stigmasterol and β-sitosterol.

It is therefore suggested that C^{14}-labelled cholesterol be incorporated into the potato sprout or a piece of sprouting tuber, and the glycoalkaloid and sterols extracted with the bisolvent extraction method a few days later. The radioactivity in the glycoalkaloid precipitate can be measured, and the radioactivities in cholesterol, stigmasterol and β-sitosterol can also be measured if their intensities do not change after separation with preparative gas-liquid chromatography.

2. Higher concentration of glycoalkaloid is found in cortical tissue of potato tubers and sprouts. It is of interest that a histo-chemical procedure can be designed to locate the glycoalkaloid. An attempt has been made to stain the thin slices of tissue samples with the Clarke reagent in the hope that the color reaction between glycoalkaloid and the reagent can indicate the location of the glycoalkaloid. The color reaction, however, took place, but it was not exclusively blue. Presence of anthocyanin in this sample was an interfering factor. In pursuit of such a histo-chemical procedure, effort should be directed toward a better technique in stabilizing the glycoalkaloid, fixing the tissue and elminating the interference of anthocyanin.

3. Since far-red light has caused a lower level of glycoalkaloid and chlorophyll in potato tubers in this study, it may be useful in the conditioning of potatoes. Potatoes stored at low temperature are high in reducing sugar, which will cause darkening of potato chips. Consequently, it becomes common practice that potato tubers are brought to 65 to 70F for a few days in order to attain brighter final products. It is possible that treatment with far-red light may diminish the reducing sugar more rapidly and completely than the high temperature treatment.

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