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Formation and Control of Chlorophyll, Solanine Alkaloids, and Sprouts of Potato (Solanum tuberosum L.) Tubers and Carbonyl Compound of Tomato (Lycopersicon esculentum mill.) Fruits

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FORMATION AND CONTROL OF CHLOROPHYLL, SOLANINE ALKALOIDS, AND SPROUTS OF POTATO (*SOLANUM TUBEROSUM* L.) TUBERS AND CARBONYL COMPOUNDS OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) FRUITS

by

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S. J. Jadhav

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ABSTRACT

Formation and Control of Chlorophyll, Solanine Alkaloids, and Sprouts of Potato (Solanum tuberosum L.) Tubers and Carbonyl Compounds of Tomato (Lycopersicon esculentum Mill.) Fruits

by

S. J. Jadhav, Doctor of Philosophy

Utah State University, 1973

Major Professor: Dr. D. K. Salunkhe
Department: Nutrition and Food Sciences

Part I. Formation and control of chlorophyll, solanine alkaloids, and sprouts of potato (Solanum tuberosum L.) tubers

Incorporation of radioactive carbon from acetic acid-2-^{14}C (sodium salt), β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C, L-leucine-U-^{14}C, L-alanine-U-^{14}C, and D-glucose-U-^{14}C into the predominant glycosidic steroidal alkaloids, α-solanine and α-chaconine of potato sprouts was 4.88, 9.0, 15, 24, and 20 times less than that of mevalonic acid (MVA)-2-^{14}C (DBED salt), respectively. The efficiency ratio revealed that β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C was incorporated via acetate or acetoacetate. The distribution of radioactivity originated from D-glucose-U-^{14}C was nearly nine times higher in the glycoside moiety than that in the aglycone part of the glycoalkaloids. Apparently, Alar (succinic acid 2,2-dimethylhydrazide), Ethrel or Ethephon (2-chloroethylphosphonic acid), and Telone (1,3-dichloropropene and related

chlorinated hydrocarbons) significantly reduced the rate of incorporation of β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C into the alkaloids.

A catalytic conversion of solanidine and UDP-glucose-U-^{14}C to β-glucoside by the enzymatic system in a suspension of potato slices and the enzyme preparation from sprouts demonstrated the presence of β-glucosyltransferase in Solanum tuberosum L. Stepwise synthesis of α-solanine and α-chaconine from solanidine in potato tubers or sprouts seems possible.

Formation of solanine alkaloids in peeled potato slices was stimulated when stored at 15 and 24 C in dark or light (200 foot-candles). The slices held under light developed nearly three to four times more alkaloids than those held in the dark. Significantly higher concentrations of solanine alkaloids were formed in the late stage (after 24 hours) than in the early stage of the storage period. Hence, it can be concluded that when potatoes are sliced for chips or French fries, they should be processed immediately, before the glycoalkaloids are synthesized in higher concentrations.

Post-harvest application of chemicals, such as Phosfon (tributyl 2,4-dichlorobenzylphosphonium chloride), Phosfon-S (tributyl 2,4-dichlorobenzylammonium chloride), Amchem 72-A42 [2-(p-chlorophenylthio)-triethylamine], Amchem 70-334 or CPTA [2-(p-chlorophenylthio)-triethylamine hydrochloride], Nemagon (1,2-dibromo-3-chloropropane), and Telone (1,3-dichloropropene and related chlorinated hydrocarbons) at the concentrations of 250, 500, and 100 parts per million (ppm) in water; glycerin (10, 20, and 30 percent weight by volume [w/v] in water); and mineral oil (1.25, 2.5, 5, 10, 15, 20, and 100 percent [w/v] in ether or petroleum ether) significantly inhibited the formation of chlorophyll

and solanine alkaloids in the peripheral (periderm and outer parenchyma) zone of potato tubers exposed to a fluorescent light (200 foot-candles) for 6 or 7 days at 16 C and 60 percent relative humidity. The rates of inhibition increased with concentration of chemicals studied. A 10 percent solution of mineral oil was the minimum required concentration for effective control of chlorophyll and solanine alkaloids. The tubers dipped in 10 percent mineral did not develop chlorophyll on exposure to light (200 foot-candles) for 4 weeks, while the overall rate of inhibition of alkaloids was significantly high. In general, oil treatments were the most effective in controlling the formation of chlorophyll, solanine alkaloids, and sprout growth.

Part II. Formation and control of carbonyl compounds of tomato (Lycopersicon esculentum Mill.) fruits

Incubation of unsaturated fatty acids such as linoleic and linolenic acids with the crude soluble extract from tomato fruits produced carbonyl compounds. The enzyme preparations did not catalyze the conversion of saturated or monounsaturated fatty acids to carbonyls. Inability of potassium cyanide to inactivate the crude soluble extract proved that degradation of these fatty acids was mediated by lipoxidase and nonenzymatic oxidation by heme compounds was eliminated. These findings were supported by the fact that hydrogen peroxide, an inhibitor of lipoxidase enzyme, had inhibitory effects on the degradation of linoleic and linolenic acids by the tomato extract.

Hexanal was found to be one of the products of the enzyme reaction. The identity of hexanal was confirmed by comparing the physical properties such as retention time, infra-red and ultra-violet absorption bands,

and R_f value with those of an authentic sample. Biogenesis of hexanal from linoleic or linolenic acid was further substantiated by the use of uniformly labeled ^{14}C isotopes of these fatty acids with the crude soluble extract, filtered homogenate, and tissue slices.

Maximum activities (as evidenced by the production of carbonyls) were observed in the extract prepared with and incubated in a buffer medium of pH 7.5 (0.1 M, Tris-HCl). The degradation of linoleic and linolenic acids was maximum at 30 C when incubated for 4 hours with 1 ml of the crude soluble extract. The enzymatic activity was enhanced by metal ions and compounds containing free -SH groups. Increase in the production of carbonyls by addition of citric and L-ascorbic acid may result from their metabolism. In general, ripe fruits contained greater enzymatic activities but smaller amounts of linoleic and linolenic acids than green fruits. The activity of the crude extract was increased by dialysis and the ammonium sulfate fractionation between 30 and 70 percent saturation. The rates of degradation of linoleic and linolenic acids catalyzed by the insoluble fractions of tomato extracts were more than those by the corresponding soluble fractions.

Tomato fruits (green-wrap or large green) stored under hypobaric or sub-atmospheric pressures were analyzed for their volatiles after ripening. The concentrations of selected carbonyls (acetaldehyde, 2-methyl propanal, butanal, 3-methyl butanal, and hexanal) and some other volatiles decreased substantially with decrease in storage pressure.

(154 pages)

PART I

FORMATION AND CONTROL OF CHLOROPHYLL, SOLANINE ALKALOIDS, AND SPROUTS OF POTATO (*SOLANUM TUBEROSUM* L.) TUBERS

INTRODUCTION

The potato (Solanum tuberosum L.), with a total production of nearly 300 million metric tons, is one of the major food crops in the world (Food and Agriculture Organization of the United Nations, 1966). Because they yield heavily, are relatively inexpensive, and can be grown in a wide variety of soils and climates, potatoes are the mainstay in the diets of people in many parts of the world. The Union of Soviet Socialist Republics, Poland, Germany, France, and the United States of America are the leading producers of this crop. A total production of over 16 million metric tons in 1970 (United States Department of Agriculture, 1972) followed the general upward trend since 1920 in the USA (Wadleigh and Dyal, 1972).

Potatoes are an excellent source of carbohydrates and have a significant content of phosphorus, potassium, and vitamins, especially vitamin C. Their over 10 percent protein content on a dry-weight basis brings them relatively close to the 11 percent protein in wheat flour. Because of the high nutritive value and lysine content of potato protein, it is a valuable supplement to cereal proteins.

Considerable losses of potatoes occur between the field and the marketing place due to physiological and/or mechanical damage. When potatoes are exposed to light during post-harvest handling and marketing, a green pigmentation develops at the surface. This condition, known as "greening," indicates the formation of chlorophyll (Larsen, 1949). Although chlorophyll (Figure 1) is harmless and tasteless, green potatoes have less nutritive value and are considered unfit for human consumption.

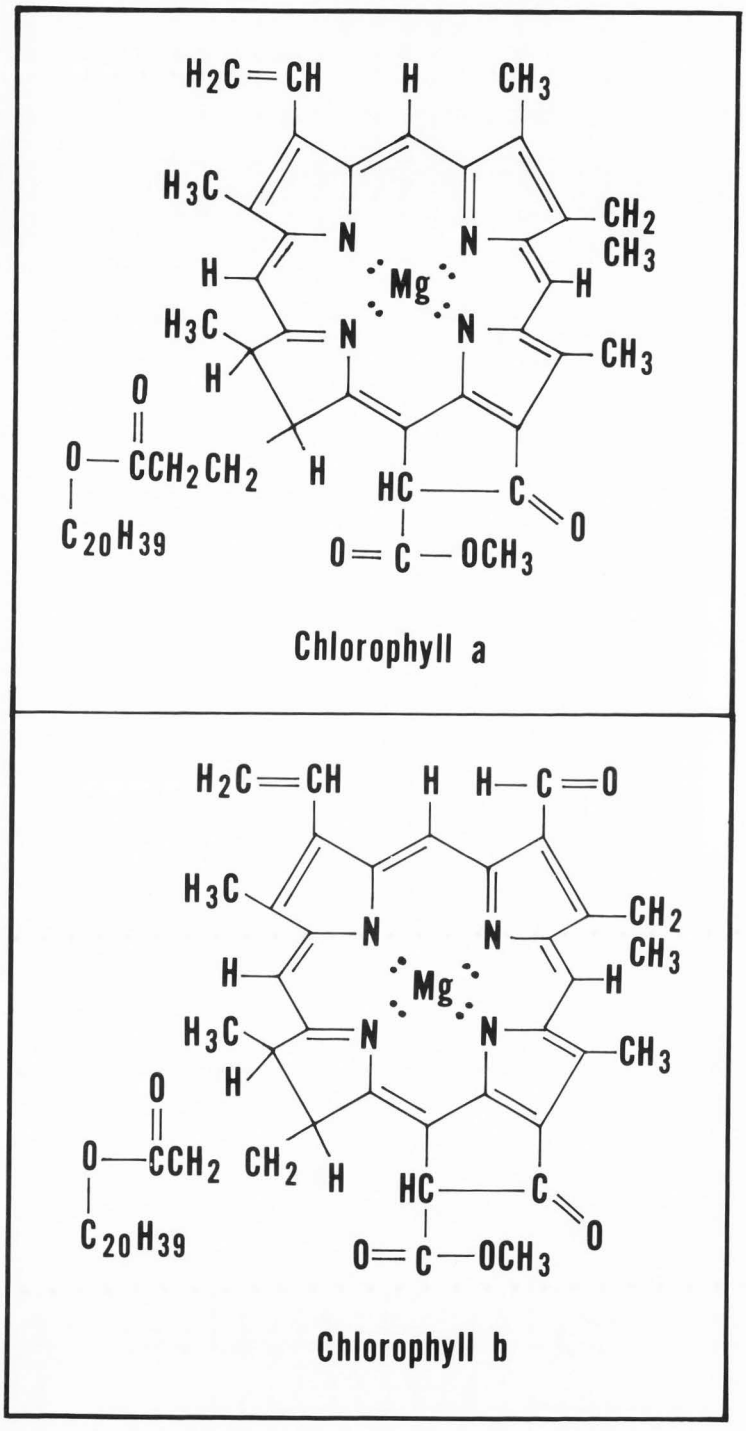

Figure 1. Structural formulas of chlorophyll a and b.

Thus additional losses of potatoes can occur in supermarkets and food stores where high intensity lights are used to attract customers. According to U.S. standards for potatoes issued on July 15, 1958, greening is defined as "damage" if more than 5 percent of the total weight of the potato must be removed to eliminate the greened tissue, and as "serious damage" if the loss is over 10 percent. The green potatoes are not marketable even though sold for a reduced price (Figure 2). Surveys conducted by Motts (1937) and DeLoach and Sitton (1941) indicated that severity of potato greening varied from 14 to 27 percent. Gull and Isenberg (1958) conducted a survey of 85 stores in various localities in the state of New York. They observed that the potatoes were exposed to artificial light up to 350 foot-candles (ft-c) intensity and the malady of potato greening was noticed in all stores. Although recent literature does not reveal reports on losses due to greening, the present-day merchandising practices do not eliminate the incidence of potato greening.

Certain environmental conditions stimulate a synthesis of solanine, a bitter-tasting glycoalkaloid possessing poisonous characteristics and normally present in all tubers in very small amounts. Green potatoes are usually associated with an increased level of this component. However, the processes of chlorophyll and solanine formation in potatoes are independent of each other (Conner, 1937). In the literature, solanine, solanine content, glycoalkaloid, or TGA (total glycoalkaloids) content(s) of potato are the terms used to represent a mixture of α-solanine and α-chaconine, the predominant glycoalkaloids (Figure 3).

Accidental consumption of potatoes containing high amounts of solanine has caused severe illness and, on some occasions, death. In

Figure 2. Severely green potatoes "on sale" for a reduced price in a supermarket.

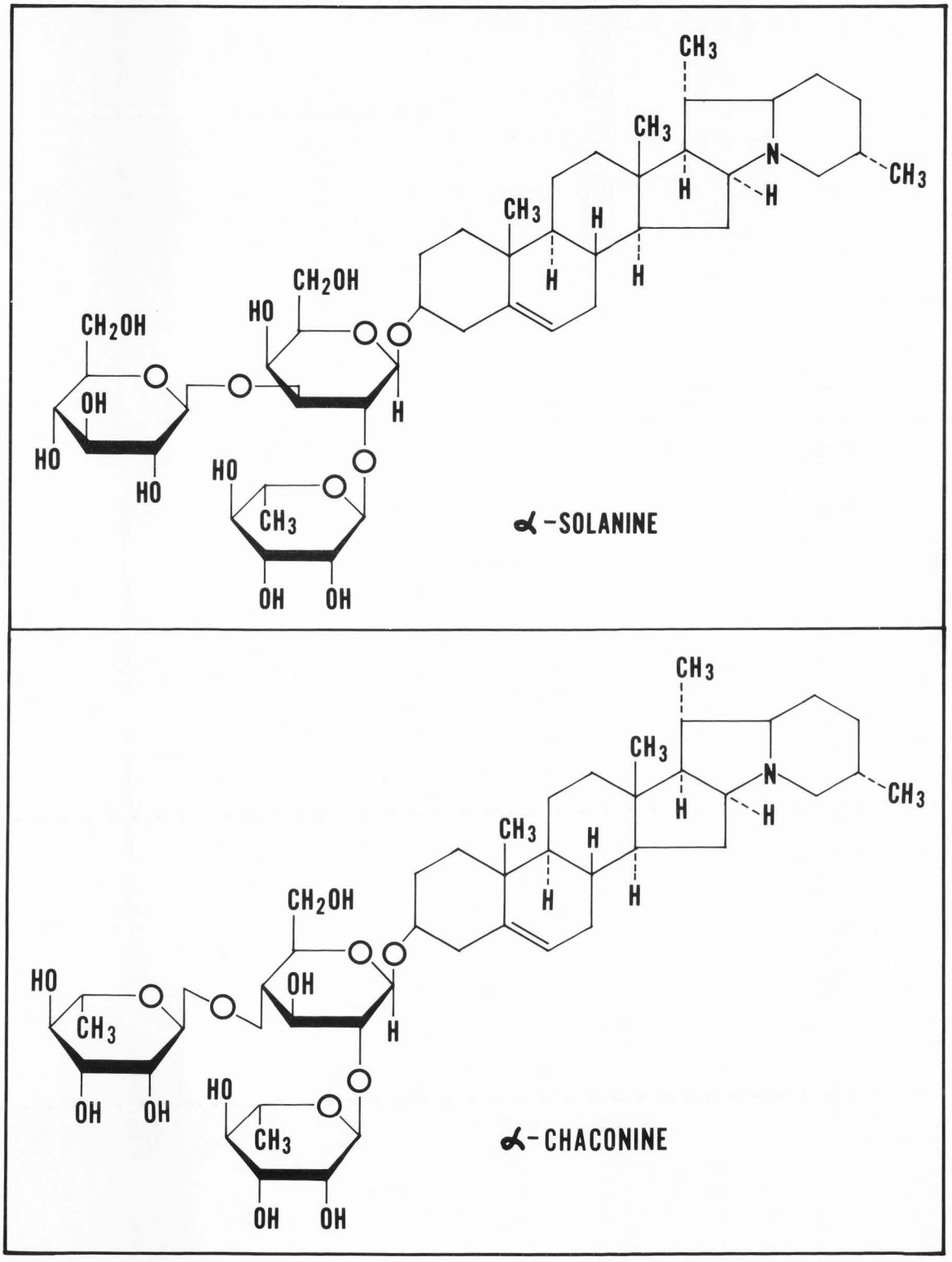

Figure 3. Structural formulas of α-solanine and α-chaconine.

Canada, several complaints from potato growers and consumers concerning unpalatable and bitter-tasting potatoes have been noticed and traced back to high solanine contents (Zitnak, 1961). Losses of livestock and poultry caused by ingestion of potato vines, sprouted potatoes, cull potatoes, and potato peels containing solanine have been reported (Hansen, 1925; Willimott, 1933). The symptoms of solanine poisoning are generally those of an acute gastrointestinal upset with abdominal pain, vomiting, and diarrhea.

The importance of the problem of potato greening and solanine production is two-fold--consumer acceptance and possible food poisoning hazards. It is significant that solanine is not destroyed by cooking, baking, or frying. Therefore, the only effective way to control this alkaloid is to inhibit its synthesis in the tubers. The United States Department of Agriculture and the Canadian Department of Agriculture in a joint action have withdrawn from commerce the potato cultivar Lenape because of its high glycoalkaloid content. This regulatory action has renewed interest in the pharmacological and toxicological aspects of potato glycoalkaloids (Zitnak, 1970), while the Renwick (1972) hypothesis on severe birth defects resulting from intake of potatoes containing certain unidentified toxicants has opened a new area of research.

To study formation and control of chlorophyll and solanine alkaloids, the following investigations were undertaken:

1. Light-induced chlorophyll development in potato tubers.

2. Biogenetic relationship of certain precursors of potato alkaloids.

3. Glucosylation of solanidine by the enzyme extracts from potato tubers and sprouts.

4. Temperature- and light-induced solanine alkaloids in tuber slices.

5. Control of light-induced synthesis of chlorophyll and solanine alkaloids by surface application of chemicals and mineral oil.

6. Control of sprout growth by surface application of mineral or vegetable oil.

REVIEW OF LITERATURE

Hardenburg (1964) reviewed earlier work on greening of potatoes. Literature pertaining to distribution, biosynthesis, factors affecting formation and control of chlorophyll and solanine alkaloids, and toxicological aspects and sprouting of potatoes is summarized.

Distribution of chlorophyll

A freshly harvested potato tuber contains very little chlorophyll; however, light will induce synthesis in its peripheral (periderm and outer parenchyma) zone. According to Larsen (1949), the synthesis is confined mainly to the first 3 mm of tissue, with the highest concentration in the first, where it seldom exceeds 1 mg per 100 cm^2 surface area. The formation of chlorophyll is especially vigorous in areas of high metabolic activity such as apical end, eyes, and meristematic region. Patil (1972) showed that slightly more chlorophyll a than b developed when White Rose potatoes were exposed to light (100 ft-c) for a period of 1-15 days. The amounts of other forms of chlorophyll are negligible in potato tubers.

Chemistry and distribution of solanine alkaloids

The chemistry of alkaloids present in Solanaceaeous plants has been extensively reviewed by Schreiber (1968). Until 1954 it had been considered that the cultivated form of potato contained only one alkaloid, solanine, discovered nearly 150 years ago. Kuhn and Löw (1954) reported the discovery of another glycoalkaloid, α-chaconine, in the leaves and

shoots of cultivated potato and in the leaves of the wild potato (Solanum chacoense, from which it was named. α-Solanine and α-chaconine have the same aglycone--solanidine--but differ with respect to the sugar chain (Figure 3). Besides these main alkaloids representing up to 95 percent of the total alkaloids β and γ forms of both solanine and chaconine possessing a shortened chain (Kuhn and Löw, 1955a, 1955b) were found in leaves of Solanum tuberosum and Solanum chacoense. The occurrence of leptinines and leptines (hydroxy- and acetoxy- derivative of solanine and chaconines) have been reported only in wild potato, Solanum chacoense (Schreiber, 1968). However, the several alkaloids existing in potatoes may have arisen through hybridization of such species with the cultivated potato plant. Zitnak (1961) detected free solanidine in concentrations up to 33 percent of the total glycoalkaloid level in bitter Netted Gem potatoes. Additionally, other alkaloids containing different aglycones such as tomatidenol, demissidine, and 5β-solanidan-3α-ol have been identified in Solanum tuberosum L. (Schreiber, 1968). Zitnak (1968) found several unknown alkaloids that could be obtained from flowers of potato plants by different extraction procedures. It is interesting to note that Kennebec potatoes, when sliced and aged at room temperature for 48 hours, synthesized two new alkaloids identified as α and β solamarine not previously present (Shih, 1972).

In the potato plant, most of the tissues contain the major glycoalkaloids. The alkaloid concentration is high in the tip shoots, and the flowers are particularly rich in solanine (Lampitt et al., 1943). As mentioned by Wolf and Duggar (1940), the solanine content is high in the meristematic regions such as leaf buds and young leaves down to

about the eighth node, beyond which there is a marked decrease. It is known that the maximum amount of alkaloid is found in sprouts.

According to Guseva, Borikhina, and Paseshnichenko (1960), α-solanine and α-chaconine represent about 40 percent and 60 percent of the total glycoalkaloids of sprouts, respectively. Results on the solanine contents of different potato cultivars in studies by Wolf and Duggar (1946) revealed that solanine accumulated continuously in the tubers of all cultivars studied. The alkaloid is formed in the parenchyma cells of the periderm and cortex of the tubers and in areas of high metabolic activity such as eye regions (Wolf and Duggar, 1940; Hilton, 1951; Reeve, Hautala, and Weaver, 1969), and the concentration is arranged in a descending gradient from the outside inward. Little or none is found in the pith and only small amounts are present in the intermediate region (Lampitt et al., 1943). Zitnak (1961) reported that potato peels of Netted Gem contained solanidine in amounts equal to those in peeled tubers, although the peels represented only one-seventh of the whole tuber weight. According to Zitnak and Johnston (1970), the glycoalkaloids diffuse through the entire tuber on reaching a high concentration.

Biosynthesis of chlorophyll and solanine alkaloids

A biosynthetic pathway to chlorophyll has been reviewed by Bogorad (1965, 1966) and Ellsworth (1972).

Biosynthetically all steroidal compounds such as sterols, certain sapogenins, terpenes, hormones, and alkaloids are interrelated and pathways leading to a synthesis of a structurally similar compound could be postulated on the basis of known ones. Thus, the regular

pathway starting from acetate via mevalonate, isopentenyl pyrophosphate, farnesyl pyrophosphate, squalene, and cholesterol is applicable to steroidal alkaloids (Figure 4). Reviews of biochemistry and possible biogenetic relationships of steroidal alkaloids of the Solanum group have been conducted by several authors (Heftmann and Mosettig, 1960; Heftmann, 1963; Clayton, 1965; Willuhn, 1965; Schreiber, 1966, 1968). The most significant findings in relation to the glycosidic steroidal alkaloids of potato are summarized here.

The first tracer work on the biogenesis of potato alkaloids was initiated by Guseva and Paseshnichenko (1958). They demonstrated the uptake and utilization of radioactive acetate by potato sprouts. The glycoalkaloids isolated from such sprouts grown under conditions of normal illumination had the labeled carbon chiefly in the aglycone, and from sprouts grown in the dark it was in the sugar portion of the glycoalkaloids. Radioactivity in the glycoalkaloids reached maximum when the feeding of labeled acetate was continued for two days. In a later experiment, Guseva, Borikhina, and Paseshnichenko (1960) found that α-chaconine contained nearly twice as much specific activity as α-solanine. Mevalonate was more effectively utilized in the biosynthesis of glycoalkaloids of potato seedlings than was acetate (Guseva, Paseshnichenko, and Borikhina, 1961). Cholesterol has been shown to be metabolized to solanidine when applied to leaf surfaces of potato plants (Tschesche and Hulpke, 1967).

The hypothesis that the distribution of labeled carbon atoms in all the steroidal rings of solasodine, synthesized from radioactive acetate or mevalonate by Solanum aviculare, agrees with that expected on the basis of the known biosynthetic and cyclization scheme of

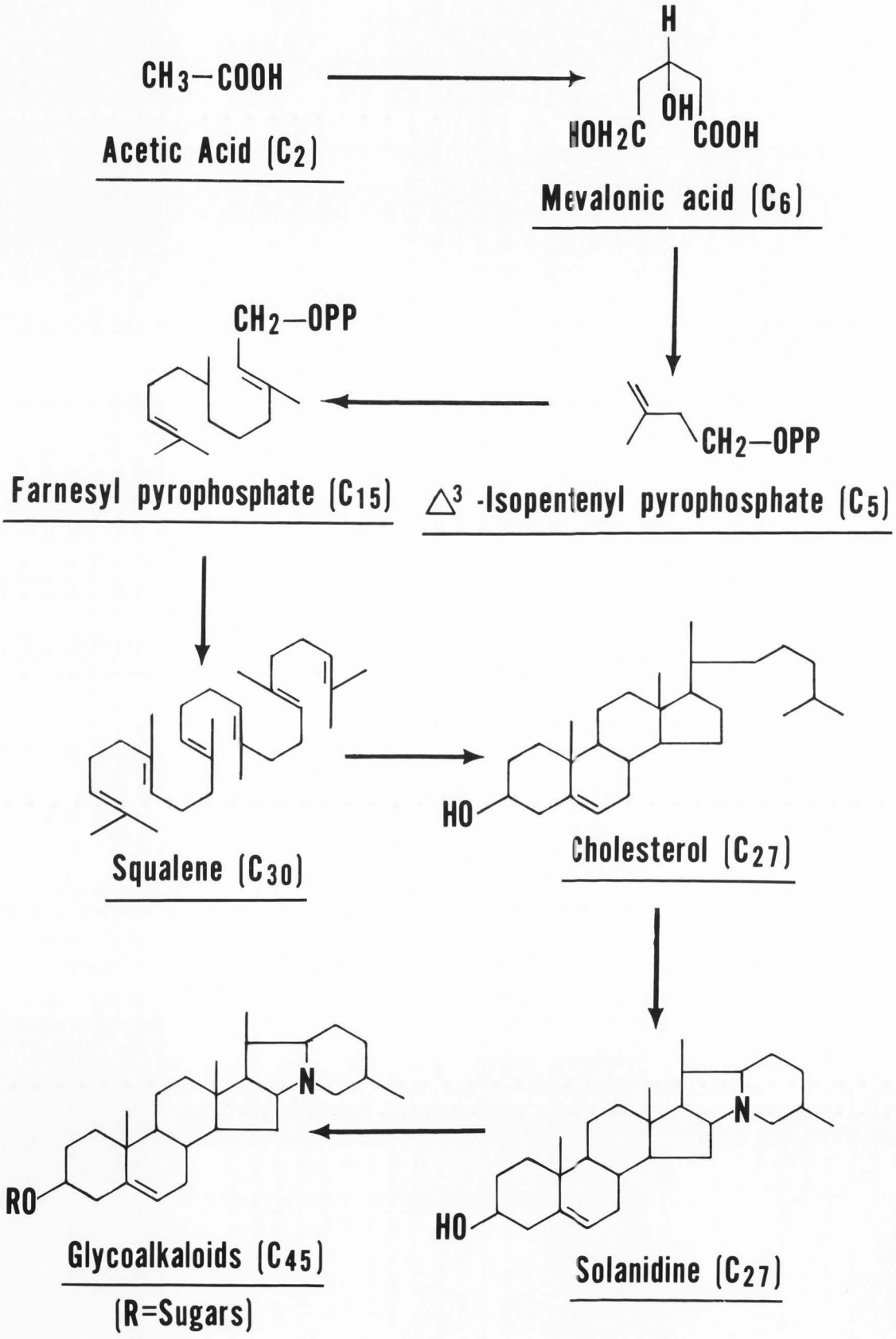

Figure 4. Outline of biogenesis of steroidal glycoalkaloids of potato.

squalene (Guseva and Paseshnichenko, 1962) and may be applied to α-solanine and α-chaconine because of their structural similarity with solasodine. The origin of the nitrogen atom in solanine alkaloids remains an unsolved biosynthetic problem. However, according to the hypothesis of Heftmann (1967), cholesterol may be undergoing cyclization in the side chain subsequent to the formation of 27-hydroxycholesterol followed by a direct replacement of the hydroxyl group by an amino function.

Factors affecting formation of chlorophyll and solanine alkaloids

Cultivar. Potato cultivars differ markedly in their rate of greening and solanine production when exposed to light. Several cultivars have been known for their differential solanine contents (Wintgen, 1906; Morgenstern, 1907; Bömer and Mattiis, 1923; Wolf and Duggar, 1946; Zitnak, 1955; Zitnak and Johnston, 1970; Sinden and Webb, 1972). Investigations conducted by Bömer and Mattiis (1924), Lepper (1949), Zitnak (1970), Sanford and Sinden (1972), and Sinden and Webb (1972) revealed that cultivars with high glycoalkaloid contents are more likely to produce excessive solanine than the cultivars with low glycoalkaloid contents when subjected to less than ideal environmental conditions or to improper handling. Patil, Salunkhe, and Singh (1971) reported that various cultivars differed significantly in chlorophyll and solanine formation and the results were in conformity with those of Wolf and Duggar (1946), Larsen (1949), Lepper (1949), Zitnak (1955), Gull and Isenberg (1958), Akeley, Houghland, and Schark (1962), and Sinden and Webb (1972). It appears, therefore, that the greening

potential and solanine content are genetically controlled characteristics which vary with the cultivar.

Location, climate, and environment. Sinden and Webb (1972) concluded that deviations of solanine contents above the average to excessive levels (above 20 mg/100 gm) in the five cultivars at certain locations were rare, and usually could be explained by abnormal growing conditions (environment) or improper handling. They (1972) contradicted the results of Zitnak (1955) that the influence of locations was insignificant. The toxic level of solanine in the bitter tubers from several locations in Alberta (Canada) was attributed to one or more unfavorable climatic conditions (Hutchinson and Hilton, 1955). However, these samples showed no greening. Yamaguchi, Hughes, and Howard (1960b) showed that White Rose potatoes were more susceptible to greening when harvested in winter than during summer.

Maturity and specific gravity. Immature and small potatoes show more tendency toward greening and solanine production as compared to mature and large ones (Bömer and Mattis, 1924; Sinden and Webb, 1972). In contrast, Buck and Akeley (1967) indicated less greening in tubers harvested at an early date than those harvested at a later date. Wolf and Duggar (1946) noticed an inverse relationship between the tuber size and solanine concentration. The results of Patil, Salunkhe, and Singh (1971) revealed that chlorophyll development in Kennebec tubers was inversely related to specific gravity; however, alkaloid synthesis was independent of specific gravity of the tubers.

Storage and temperature. Several workers studied the effect of storage temperature and/or temperature during light exposure on chlorophyll and solanine accumulation (Wolf and Duggar, 1946; Folsom,

1947; Larsen, 1949; Hilton, 1951; Zitnak, 1953; Gull and Isenberg, 1960; Yamaguchi, Hughes, and Howard, 1960b; Buck and Akeley, 1967). In general, the rate of greening was rapid at room temperature during light exposure and the greening decreased as the temperature was lowered. Storage temperatures were, however, inversely related to the production of solanine alkaloids whether illuminated or kept in darkness.

Relative humidity. According to Larsen (1949), variation in the humidity did not affect the development of greening. Nothing is known about possible effects of humidity on the solanine content of tubers.

Light intensity and quality. The probability of tubers being exposed to a certain quantity and duration of light, singly or in certain combinations including daylight, sunshine, UV light, fluorescent or incandescent light, varies with environmental factors and marketing conditions. Several workers (Larsen, 1949; Gull and Isenberg, 1958, 1960; Isenberg and Gull, 1959; Liljemark and Widoff, 1960; Yamaguchi, Hughes, and Howard, 1960a; Patil, Salunkhe, and Singh, 1971) have studied the effect of light on greening and solanine contents of tubers. The investigators showed that greening is dependent on the duration of exposure and the intensity and quality of light. Light intensity as low as 5 ft-c produced chlorophyll which increased with increases in light intensity (Yamaguchi, Hughes, and Howard, 1960a; Liljemark and Widoff, 1960). The findings of Patil, Salunkhe, and Singh (1971) indicated synthesis of chlorophyll with increased light intensity up to 100 ft-c, slow and gradual degradation up to 150 ft-c, and rapid degradation at 200 ft-c, but revealed insignificant differences in the high solanine contents.

By contrast, Gull and Isenberg (1958) found no significant increase in the amount of chlorophyll above 50 ft-c light intensity. Zitnak (1953) found greening and exceptionally rapid synthesis of solanine in tubers exposed to solar radiation.

Conner (1937) found that the blue end of the spectrum encouraged solanine formation the most, while the yellow-red end of the spectrum was most efficient for chlorophyll but did not increase solanine. According to the investigations of Zitnak (1953), infra-red light was effective on chlorophyll and alkaloid synthesis, while ultra-violet light was effective only on alkaloid synthesis. Pink, blue, and daylight fluorescent lights caused more greening; while green, gold, and warm fluorescent lights reduced greening (Isenberg and Gull, 1959; Yamaguchi, Hughes, and Howard, 1960a; Liljemark and Widoff, 1960). Solanine determinations of the tubers gave irregular results (Liljemark and Widoff, 1960). The effect of colored cellophane filters on chlorophyll content of tubers was due to the change in spectrum of light incident on the tubers from a cool fluorescent light (Yamaguchi, Hughes, and Howard, 1960a).

Duration of light exposure. The exposure time is an important factor in greening because of its cumulative effect. Gull and Isenberg (1958) reported a direct relation between greening and duration of light exposure. The length of exposure required to cause greening and an increase in solanine formation varies in the literature reports (Larsen, 1949; Gull and Isenberg, 1960; Patil, Salunkhe, and Singh, 1971; Yamaguchi, Hughes, and Howard, 1960a; Howard, Yamaguchi, and Timm, 1957).

Methods of controlling chlorophyll and solanine alkaloids

Several physiochemical methods for the control of greening and solanine have been studied. Practical use of these methods has certain limitations because of marketing trends and priority to health problems.

Genetics. The breeding of cultivars resistant to greening may be possible because genetic differences controlling this biochemical change are apparent among existing strains (Akeley, Houghland, and Schark, 1962). None of the commercially-acceptable cultivars of potatoes now on the market are immune to greening and solanine formation. Consumer protection, therefore, requires a development and growing of cultivars with low glycoalkaloid contents.

Packaging. One approach to the problem of greening is to protect tubers from light. Use of packaging material recommended for tubers was made through research findings of Larsen (1949); Lutz, Findlen, and Ramsey (1951); Hardenburg (1954); Howard, Yamaguchi, and Timm (1957); Liljemark and Widoff (1960); and Newman (1966).

Colored lights and colored-film filters. Although placing tubers under green light or green cellophane filters reduces greening, the appearance does not attract the customer (Larsen, 1949; Liljemark and Widoff, 1960). According to Yamaguchi, Hughes, and Howard (1960a), tango (amber) cellophane seemed the most promising. Present knowledge does not show any convincing evidence that packaging, colored lights, and colored film filters protect tubers from solanine developments.

Chemicals. Numerous chemicals are known to prevent greening and/or solanine synthesis in potato tubers. Foliar spraying of chelating

compounds and Ethrel or Ethephon (2-chloroethylphosphonic acid) and N6BA (N6-benzyladenine) was studied by Gull and Isenberg (1958) and Jeppsen, Salunkhe, and Jadhav (1973), respectively. Schwimmer and Weston (1958) found that potatoes dipped in a solution of 3-amino-1,2,4-triazole developed less chlorophyll when exposed to light. Tubers tested with nicotinic acid by a vacuum injection method depressed glycoalkaloid level during illumination (Parups and Hoffman, 1967). Inhibition of chlorophyll and solanine alkaloids by Ethrel or Ethephon (2-chloroethylphosphonic acid) and Alar (succinic acid 2,2-dimethylhydrazide) was first reported by Patil, Salunkhe, and Singh (1971). Immersion of tubers in a 2 or 3 percent household detergent solution followed by a tap water rinse was found effective in controlling light-induced chlorophyll and solanine alkaloids (Sinden, 1971). The most effective chemicals such as hot paraffin wax and oils were reported by Wu and Salunkhe (1972a, 1972b, 1972c).

Controlled atmosphere storage. Forsyth and Eaves (1968) and Patil, Singh, and Salunkhe (1971) explored controlled atmosphere storage of tubers. They found 15 percent and higher concentration of CO_2 will prevent greening; no significant effect on the formation of solanine glycoalkaloids was observed (Patil, Singh, and Salunkhe, 1971).

Hypobaric storage. Application of sub-atmospheric pressure (hypobaric) controls greening, but not the formation of solanine (Jadhav, Patil, and Salunkhe, 1973).

Ionizing radiation. Increasing doses of ionizing radiation (5-250 krad) inhibited but did not completely suppress chlorophyll formation (Schwimmer and Weston, 1958). Gull and Isenberg (1958) observed a nearly 50 percent reduction in the chlorophyll contents of tubers

subjected to a 40 krad dose followed by an illumination. A 10 krad dose, alone or in combination with 15 percent CO_2 reduced chlorophyll when held under light; however, it did not affect solanine synthesis (Patil, Singh, and Salunkhe, 1971). The results of Ziegler, Schanderl, and Markakis (1968) showed two apparent trends: decrease of greening with irradiation, irrespective of CO_2 treatment; and decrease of greening with increasing CO_2 in the atmosphere, irrespective of irradiation.

Toxicological aspects of solanine alkaloids

Cases of potato poisoning have been reported by several investigators (Harris and Cockburn, 1918; Rothe, 1918; Bömer and Mattis, 1923; Griebel, 1923; Hansen, 1925; Damon, 1928; Willimott, 1923; Wilson, 1959). The toxicity of α-solanine attributed to its inhibitory effect on cholinesterase was established through the results of Pokrovskiĭ (1956), Orgell, Vaidya, and Dahm (1958), Harris and Whittaker (1959, 1962), Orgell (1963), and Patil et al. (1972). Several animals have been tested for differences in sensitivity to a total potato alkaloid as well as to α-solanine. The data are presented in Table 1. Information on the pharmacology and toxicity of α-chaconine is meager. Except for the anticholinesterase activity of leptine I (Orgell, 1963), pharmacological and toxicological properties of trace alkaloids of potato (leptines and leptinines) are unknown.

Sprouting of potatoes

Freshly harvested tubers usually undergo a rest period (dormant state) of several months during which there is little or no sprout growth regardless of environmental conditions. Following the rest period sprout growth occurs at temperatures of 4.4 C or above.

Table 1. Evaluation of α-solanine toxicity

Experiment	Dose of α-solanine: Administration	Dose of α-solanine: Amount	Effect	Reference
Human[a]	Oral	≥ 2.8 mg/kg[b]	Toxic[c]	Rühl (1951)
	Oral	20-25 mg[b]	Toxic[c]	Wilson (1959)
Sheep	Oral	225 mg/kg	Toxic[c]	Konig (1953)
	Oral	500 mg/kg	Lethal	Konig (1953)
	Intravenous	17 mg/kg	Toxic[c]	Konig (1953)
	Intravenous	50 mg/kg	Lethal	Konig (1953)
Pregnant rat	Oral	10% of sprout diet	Death of all pups before weaning age	Kline et al. (1961)
Rat	Gastric intubation	590 mg/kg	50% death within 24 hours	Gull, Isenberg, and Bryan (1970)
	Intraperitoneal	75 mg/kg	50% death in a few hours	Gull, Isenberg, and Bryan (1970)
Mice	Oral	1000 mg/kg	Nontoxic	Nishie, Gumbmann, and Keyl (1971)
	Intraperitoneal	42 ± 1.8 mg/kg	50% death in 7 days	Nishie, Gumbmann, and Keyl (1971)
	Intraperitoneal	10 mg/kg	Toxic[c]	Patil et al. (1972)

Table 1. Continued

Experiment	Dose of α-solanine: Administration	Dose of α-solanine: Amount	Effect	Reference
	Intraperitoneal	32.3 mg/kg	50% death	Patil et al. (1972)
	Intraperitoneal	≥ 50 mg/kg	Lethal	Patil et al. (1972)
Chick embryo (4-day-old)	Injection into yolk sac	18.8 ± 1 mg/kg	50% mortality in 18 days	Nishie, Gumbmann, and Keyl (1971)
Rabbit	Intraperitoneal	20 mg/kg	Overnight death	Nishie, Gumbmann, and Keyl (1971)
			Death in 2.5-24 hours, recovery if survived for at least 24 hours	Patil et al. (1972)
	Intraperitoneal	30 mg/kg	Death in 6.25 hours	Nishie, Gumbmann, and Keyl (1971)
			Death in 50 minutes	Patil et al. (1972)
	Intravenous	10 mg/kg	Death in 2 minutes	Nishie, Gumbmann, and Keyl (1971)

[a]In a case of potato poisoning (total alkaloid).
[b]Determined from potatoes consumed (total alkaloid).
[c]General symptoms of food poisoning.

Sprouting of tubers is another problem that confronts the potato industry during long-term storage periods. Sprouting not only leads to considerable losses in weight and nutritive value of tubers, but makes them unfit for consumption. Initiation of sprout activity results in accumulation of solanine alkaloids in the eye regions of potato tubers and further growth develops highest concentrations of the alkaloids in sprouts. Inhibition of sprout growth by chemicals, ionizing radiation, and controlled atmosphere storage has been discussed by Sawyer (1959) and Smith (1968).

EXPERIMENTAL

Plant material

Tubers of Russet Burbank and Norgold Russet were purchased from a local grower. Russet Burbank tubers were selected on the basis of uniform size (U.S. No. 1), while Norgold Russet tubers were classified for uniformity of specific gravity (1.08-1.10) by the brine flotation method (Clark, Lombard, and Whiteman, 1940; Salunkhe et al., 1953). The selected tubers were washed and stored at 4.4 C until utilized. The tubers were held at 16 C for a day and then subjected to various treatments.

Chemicals

Chemicals used in this study were obtained as follows: Alar (succinic acid 2,2-dimethylhydrazide) from UniRoyal Chemical, Division of UniRoyal, Incorporated, Bethany, Connecticut; Amchem 72-A42 [2-(p-chlorophenylthio)-triethylamine], Amchem 70-334 or CPTA [2-(p-chlorophenylthio)-triethylamine hydrochloride], and Ethrel or Ethephon (2-chloroethylphosphonic acid) from Amchem Products, Incorporated, Ambler, Pennsylvania; Nemagon (1,2-dibromo-3-chloropropane) from Shell Chemical Company, Modesto, California; Phosfon (tributyl 2,4-dichlorobenzylphosphonium chloride) and Phosfon-S (tributyl 2,4-dichlorobenzylammomium chloride) from Mobil Chemical Company, New York, New York; Telone (1,3-dichloropropene and related chlorinated hydrocarbons) from Dow Chemical Company, Midland, Michigan; glycerin, mineral oil (Squibb), and vegetable oil (Wesson) from a local market; α-solanine from K and K Laboratories, Incorporated, Plainview, New York,

and Sigma Chemical Company, St. Louis, Missouri; solanidine from Schwarz/Mann, Division of Becton, Dickinson and Company (B.D.), Orangeburg, New York; PPO (2,5-diphenyloxazole) and Dimethyl POPOP-1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene from New England Nuclear, Boston, Massachusetts, and Packard Instrument Company, Incorporated, Downers Grove, Illinois, respectively.

Radioactive compounds

L-Alanine-U-^{14}C (100 mC/mM), L-leucine-U-^{14}C (248 mC/mM), β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C (7.1 mC/mM), D-glucose-U-^{14}C (4.92 mC/mM), and DL-mevalonic acid (MVA)-2-^{14}C (DBED salt, 12.66 mC/mM) were purchased from New England Nuclear, Boston, Massachusetts. Acetic acid-2-^{14}C (sodium salt,, 4.47 mC/mM) and UDP-glucose-U-^{14}C (50-150 mC/mM) were obtained from ICN Chemical and Radioisotope Division, Irvine, California.

Formation of chlorophyll and solanine alkaloids

Whole tubers. To demonstrate light-induced formation of chlorophyll, tubers were exposed to light for 7 days at 16 C and 60 percent relative humidity. Light was obtained from a bank of fluorescent tubes 8 feet long (ITT F96T 12/CW cool white). The light intensity was measured in foot-candles with a Weston illumination meter, Model 603, No. 610. Figure 5 shows the light arrangement with temperature control set up to simulate the conditions in grocery stores or supermarkets.

Administration of radioactive compounds

In vivo. Russet Burbank tubers were transferred to a dark room at 16 C and 60 percent relative humidity and stored to develop sprouts

Figure 5. An experimental arrangement in a temperature controlled set-up showing exposure of potato tubers to light.

void of chlorophyll. These sprouts, because of their high metabolic activity and ability to synthesize a maximum amount of alkaloids, were used for biosynthetic precursor incorporation studies according to the procedures outlined by Guseva and Paseshnichenko (1958) with suitable modifications. Fresh sprouts weighing 10 gm were supported on a perforated plastic disc in a glass container 3 inches in diameter. A 50 ml nutrient (Hoaglandi) solution (pH 6.7) containing 10 μC of the respective substrate was added to each container and the sprouts incubated (25 C) under 100 ft-c light intensity for 2 days with constant shaking. L-Alanine-U-^{14}C, L-leucine-U-^{14}C, β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C, D-glucose-U-^{14}C, sodium acetate-2-^{14}C, and DL-mevalonic acid (MVA)-2-^{14}C (DBED salt) were used as various substrates. 20 μC of DL-mevalonic acid (MVA)-2-^{14}C (DBED salt) were added to the incubation medium since plant cells utilize only L isomer. To minimize a loss of incubation medium due to evaporation, each glass container was covered with a thin sheet of plastic with a small hole (1 cm in diameter) at the center. In inhibition studies, Alar (succinic acid 2,2-dimethylhydrazide), Ethrel or Ethephon (2-chloroethylphosphonic acid), or Telone (1,3-dichloropropene and related chlorinated hydrocarbons) were separately added to each flask containing β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C. A flask without an inhibitor served as control. Incubations were similarly conducted. After 2 days, the sprouts were removed, washed with water, and the respective batch of labeled sprouts frozen and held at -20 C.

In vitro. Enzymatic glucosylation of solanidine was carried out in two stages.

In the early stage, cylinders (7 mm diameter) of storage tissue were removed from potato tubers (Norgold Russet cultivar) with a cork

borer and 1 mm thick discs were cut using a hand microtome. The tuber discs (10 gm) were placed in a 250-ml Erlenmeyer flask that contained 0.4 μmole ATP, 1 μmole solanidine, 0.5 μC UDP-glucose-U-^{14}C in a 25-ml aqueous medium. Incubations, in duplicate, were carried out at 25 C under light (100 ft-c) for 16 hours. At the end of the incubation period, the aqueous medium was separated from the discs and basified with 10 ml of ammonium hydroxide.

In the second stage, chlorophyll-void sprouts (3.5-4 inches in length) of Norgold Russet tubers were utilized as the source of enzymes. The sprouts (25 gm) were cut into small pieces and soaked in a 0.5 percent sodium sulphite solution to inhibit enzymatic browning. After 30 minutes, the tissues were washed thoroughly with distilled water, mixed with 12.5 gm polyvinylpolypyrrolidone (PVP), and homogenized in a mortar containing a phosphate buffer (pH 7, 0.05 M). The homogenate was passed through four layers of cheesecloth and the filtrate was centrifuged at 10,000 x g for 10 minutes. The supernatant was fractionated between 10 and 60 percent saturation by $(NH_4)_2SO_4$, the precipitate collected by centrifugation (10,000 x g, 30 minutes), dissolved in 20 ml phosphate buffer, centrifuged, and the soluble fraction was dialyzed against the same buffer for 16 hours at 4 C. This enzyme preparation was diluted fourfold and subsequently 2 ml were added to a reaction mixture of 0.1 μmole ATP, 0.25 μmole solanidine, and 0.2 μC UDP-glucose-U-^{14}C in 1 ml phosphate buffer (pH 7, 0.05 M). The reaction mixtures in polyethylene test tubes were incubated at 25 C for 0.25, 0.5, 1, 2, and 3 hours with constant shaking. After each incubation period, the test tubes were held in boiling water for 10

minutes to inactivate the enzymes. The product was precipitated with 2 ml of ammonium hydroxide.

Extraction of alkaloids

The frozen sprouts were cut into small pieces, blended with 70 ml of 95 percent ethyl alcohol, and the residue was extracted in a soxhlet with the same solvent for 2 days. The alcohol extract (150 ml) was evaporated to near dryness, dissolved in 25 ml of 5 percent sulfuric acid, filtered, and the filtrate was precipitated by 10 ml of ammonium hydroxide. The alkaloid fraction was acidified, reprecipitated, and washed with 1 percent ammonium hydroxide followed by ether. The precipitate was dissolved in 10 ml of 0.05 percent HCl for measurement of radioactivity. The reaction product in *in vitro* studies was similarly treated. However, the final alkaloid fraction was radioassayed in 1 percent sulfuric acid in methanol.

Hydrolysis of alkaloid fraction

The half quantity of the alkaloid fraction obtained from sprouts fed with D-glucose-U-^{14}C was dissolved in 1 N sulfuric acid in 50 percent ethyl alcohol and the solution (20 ml) was boiled under reflux for 16 hours. The aglycone, solanidine, liberated was precipitated with ammonium hydroxide (10 ml), collected by centrifugation, thoroughly washed with 1 percent ammonium hydroxide, and dissolved in 5 ml of 1 percent sulfuric acid in methanol. Radioactivity in methanolic solution of solanidine and the supernatant consisting of sugars was measured.

Radioassay

Aliquots of solutions of alkaloid preparations in *in vivo* and *in vitro* studies and hydrolyzed products (aglycone and sugars) were added to 15 ml of scintillation liquid (5 gm PPO, 0.3 gm Dimethyl POPOP, and 333 ml Triton X-100 in toluene made to 1000 ml). Radioactivity was measured with a Unilux II-A Scintillation Counting System, Nuclear-Chicago.

Radioautography

Samples were spotted on a silica gel thin layer (0.25 mm) plate, developed in butanol saturated with water, dried, covered with Saran wrap, and placed in contact with X-ray film for 60 days.

Tuber slices

Russet Burbank potato tubers were peeled with a hand peeler and then cut into 0.7 x 0.7 x 5 cm slices. Such slices were divided into two groups: one was stored in the dark and the other was exposed to a fluorescent light of 200 ft-c intensity. Each group of slices was subjected to four temperature treatments: 0, 8, 15, and 24 C at 90-95 percent relative humidity. A 400 gm sample was taken every 12 hours over a period of 48 hours and analyzed for solanine content.

Solanine analysis. The method for extraction and determination of solanine alkaloids was that of Gull and Isenberg (1960) with certain modifications. The slices were macerated in a Waring blendor containing 225 ml of 95 percent ethyl alcohol for 4 minutes. The extract was filtered, the residue was wrapped in a filter paper, and it was transferred to a soxhlet. The extraction was carried on for 2 days in a total volume of 250 ml of the solvent. The alcohol extract was

evaporated in a porcelain dish to near dryness. The dried material was dissolved in 15 ml of 5 percent sulfuric acid and filtered into a 50 ml pyrex centrifuge tube. The dish was rinsed with another 10 ml of sulfuric acid and filtered. The contents of the centrifuge tube held in an ice-water bath were neutralized by a dropwise addition of ammonium hydroxide. An excess of alkali (10 ml) was added to adjust the pH of the solution to 9.5. The solution was heated in a water-bath at 80 C to flocculate the alkaloids and stored overnight at 4 C. The suspension was centrifuged, residue washed with 1 percent ammonia solution, and then dissolved in 1 percent sulfuric acid and diluted to 100 ml in a volumetric flask.

The determination of solanine (total alkaloids) in the above solution was based on a color producing characteristic of steroidal compounds. An aliquot of 2.5 ml of the diluted solution was cooled in an ice-bath, 5 ml of concentrated sulfuric acid were added dropwise over a period of 3 minutes with vigorous shaking followed by 2.5 ml of 1 percent formaldehyde solution in the same manner over a period of 2 minutes. Special glass tubes, 3 inches in length, were fabricated to deliver the sulfuric acid and formaldehyde reagents in 3 and 2 minutes, respectively. The reaction mixture was held at room temperature (25 C) for 90 minutes to allow the development of color. Optical density of the color was measured on a "spectronic 20" at 565 mμ. A standard curve was constructed with known concentrations of pure α-solanine in 1 percent sulfuric acid and developing the color as outlined above. A regression equation, $\hat{Y} = 0.425 + 49.132\ \overline{X}$, calculated from the data obtained was used in the determinations of solanine contents.[1]

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[1] $\overline{X}$ = Optical density, $\hat{Y}$ = mg solanine/100 ml stock solution.

For each treatment, two samples were taken and two determinations of each sample were made. The results of solanine (total alkaloids) contents were expressed as mg per 100 gm of fresh slices.

Control of chlorophyll and solanine alkaloids

To investigate control of chlorophyll and solanine alkaloids synthesized in the surface layer of tubers, chemical and mineral oil treatments were conducted in experiments I and II, respectively.

Experiment I (chemicals). Solutions (0, 250, 500, and 1000 ppm) of Phosfon (tributyl 2,4-dichlorobenzylphosphonium chloride), Phosfon-S (tributyl 2,4-dichlorobenzylammonium chloride), Amchem 70-A42 [2-(p-chlorophenylthio)-triethylamine], Amchem 70-334 or CPTA [2-(p-chlorophenylthio)-triethylamine hydrochloride], Nemagon (1,2-dibromo-3-chloropropane), and Telone (1,3-dichloropropene and related chlorinated hydrocarbons) were prepared in distilled water containing 0.01 percent Triton B-1956 as a wetting agent. The solutions were placed in desiccators and the tubers of Russet Burbank cultivar were dipped in the respective solutions. Air was drawn off by a suction pump (18 inches vacuum) for 5 minutes, followed by dips for 25 minutes. Glycerin treatments (0, 10, 20, and 30 percent w/v) were likewise conducted on the tubers of Norgold Russet cultivar. Control tubers were similarly treated with the 0.01 percent Triton B-1956 water solution.

Experiment II (mineral oil). Treatments with mineral oil (0, 1.25, 2.5, 5, 10, 15, 20, and 100 percent w/v in petroleum ether or ether) were accomplished by dipping individual potato tubers (Norgold Russet cultivar) for 0.5 second at an ambient temperature. The solvent was allowed to vaporize under fan-forced air. Tubers without any dips were

considered control. Excess of oil on tubers dipped in 100 percent mineral oil was removed with tissue paper.

Light arrangement for experiments I and II

Control and treated potatoes (8 tubers per treatment in duplicate) in experiments I and II were exposed to a fluorescent light (Figure 5). Unless otherwise mentioned, the tubers were exposed to light for 7 days at 16 C and 60 percent relative humidity.

Analytical methods for experiments I and II

Chlorophyll. Peels ranging in thickness from 1.5-2.0 mm were uniformly removed by a hand peeler from surfaces of tubers exposed to light. The peels were cut into small pieces, thoroughly mixed, and a sample of 10 gm was weighed for chlorophyll analysis according to the Association of Official Agricultural Chemists (1965) method with suitable modifications. The peels were placed in a Waring blendor (Waring Products Company, Winsted, Connecticut) containing 15 mg $MgCO_3$ and 100 ml of 85 percent cold acetone (4 C). The contents were then macerated at high speed for 4 minutes followed by filtration under suction. The pulp was reblended with 100 ml of cold acetone, filtered under suction, and finally washed with 50 ml of acetone. The acetone extract was diluted with cold water to 350 ml, and extracted twice with 100 and 40 ml of anhydrous ether. The ether layer was washed with water to remove the remaining acetone. The ether extract was dried over anhydrous sodium sulfate, adjusted to 100 ml, and subsequently used for optical density readings at 660 and 642.5 mμ with a Bausch and Lomb "spectronic 20" spectrophotometer. The total chlorophyll was

calculated according to the formula given in Association of Official Agricultural Chemists (1965) and the results were expressed as mg per 100 gm fresh peel.

Solanine alkaloids. A sample of 20 gm of cut peels was extracted with 150 ml of 95 percent ethyl alcohol and analyzed for solanine content according to the procedure described heretofore.

Control of sprouting

Experiment III (mineral or vegetable oil). To study the inhibition of sprouting, Norgold Russet potatoes (70 tubers per treatment in duplicate) were treated with 5, 10, and 100 percent mineral or vegetable oil and stored in the dark at 16 C and 60 percent relative humidity. Periodically, visual observations were made on the development of sprout growth.

Statistical analysis

All experiments were arranged in a completely randomized block design. The experiments with precursor incorporation studies, enzymatic glucosylation of solanidine, and treatment of tubers with chemicals were arranged in two replicates and two determinations were made on each replicate. Statistical analyses were computed and the means were compared according to the least significant difference (LSD) procedure (Steel and Torrie, 1960) wherever possible.

RESULTS AND DISCUSSION

Formation of chlorophyll

In vivo studies with tubers. Norgold Russet potatoes exposed to a fluorescent light (200 ft-c) for 7 days at 60 percent relative humidity were examined for chlorophyll development in the peripheral (periderm and outer parenchyma) zone. Green and unattractive appearance of such tubers in the surface layer could be revealed by removal of peels or scratching. Figure 6 shows a difference between tubers unexposed and exposed to light.

The peripheral cells of potato tubers contain large amyloplasts (leucoplasts), which are formed from proplastids. During the process of greening, these are converted into chloroplasts. Large vesicles appear at the edges of amyloplasts during early stages of chlorophyll formation. As chloroplast formation proceeds, the vesicles are replaced by vacuolated grana. The chloroplasts that are formed maintain large starch granules. These chloroplasts, the cell organelles, are the photosynthetic apparatus and synthesize chlorophyll pigments which are confined to the lamellae.

Formation of solanine alkaloids

In vivo studies with sprouts. It is evident from the results in Table 2 that sprouts utilize Acetate-2-^{14}C, β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C, L-leucine-U-^{14}C, L-alanine-U-^{14}C, D-glucose-U-^{14}C, and mevalonic acid (MVA)-2-^{14}C (DEBED salt) for the synthesis of solanum alkaloids under the experimental conditions described. Incorporation

1. Normal potatoes not exposed to light
2. Potatoes exposed to a fluorescent light (200 ft-c) for 7 days and 60 percent relative humidity

Figure 6. Light-induced formation of chlorophyll in the peripheral (periderm and outer parenchyma) zone of potato tubers. (The tubers were partially peeled.)

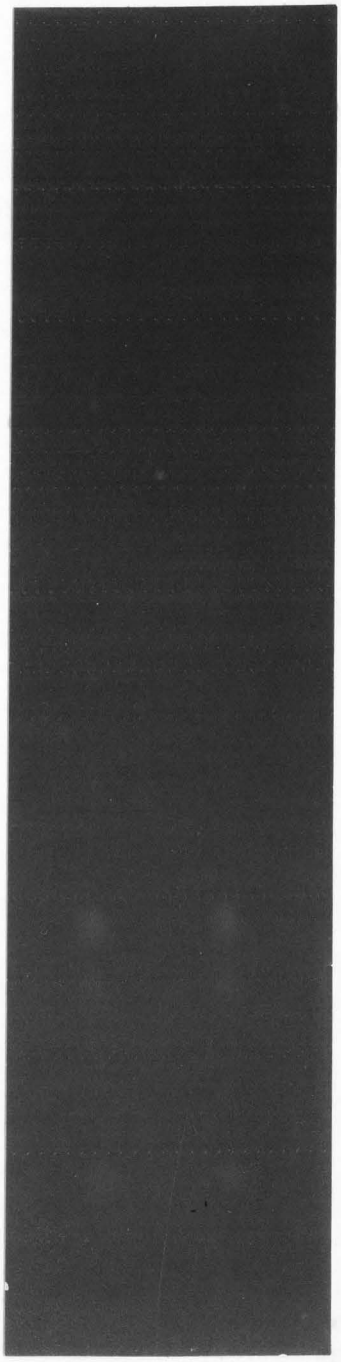
Table 2. Utilization of precursors by potato sprouts[a]

	Precursors					
	Acetate	HMG	L-Leucine	L-Alanine	D-Glucose	DL-MVA
Alcohol extract (dpm x 1000)	1270.4 (35.4)	719.525 (79.12)	967.300 (33.47)	575.575 (51.34)	842.200 (48.91)	8,259.625 (793.40)
Alkaloid fraction (dpm x 1000)	51.56 (5.17)	27.477 (5.60)	16.541 (3.13)	10.497 (1.08)	12.393 (1.18)	252.177 (8.15)
% Incorporation of added label into alkaloid fraction	0.2323	0.1238	0.0745	0.0473	0.0558	1.135
Efficiency ratio[b]	1/4.88	1/9	1/15	1/24	1/20	1/1

[a]Ten gm sprouts were incubated (25 C) in a 50 ml Hogland solution (pH 6.7) containing the respective ^{14}C precursor (10 μC) for 2 days under 100 ft-c light. 20 μC of DL-MVA were added.
[b]Incorporation of each precursor into the alkaloid fraction/that of MVA.
Abbreviations: HMG, β-hydroxy-β-methylglutaric acid; DL-MVA, DL-mevalonic acid (DBED salt).
Note: Values in parentheses represent standard deviation.

of a label from β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C into α-solanine and α-chaconine is indicated by the results of radioautography (Figure 7). The spot corresponding to α-chaconine was more intense than that for α-solanine. The concentration of α-chaconine and α-solanine in the proportion of 3:2 in potato sprouts has been reported by Guseva and Paseshnichenko (1960). The formation of solanine alkaloids from these substrates, expressed as percent incorporation of added label or efficiency ratio, was 4.88, 9.0, 15, 24, and 20 times less than that of mevalonic acid (MVA)-2-^{14}C (DBED salt) (Table 2).

In higher plants, the role of β-hydroxy-β-methylglutaric acid (HMG) as a precursor of isoprenoid compounds such as carotenoids, steroids and steroidal alkaloids, terpenes, or intermediate metabolites has not been clearly established. However, in the light of the evidence related to the biogenesis of isoprenoids, the mechanism of incorporation of β-hydroxy-β-methylglutaric acid (HMG) can be explained in two possible ways. First, plants are able to synthesize a β-hydroxy-β-methylglutaric acid (HMG)-activating enzyme or HMG-CoA reductase (EC.1.1.1.34) which is responsible for the utilization of β-hydroxy-β-methylglutaric acid or HMG (Hepper and Audley, 1969; Berry, 1971). Consequently, the rate of incorporation of β-hydroxy-β-methylglutaric acid (HMG) lies between those of acetate and mevalonic acid (MVA). The second hypothesis assumes that β-hydroxy-β-methylglutaric acid (HMG) is degraded to acetate or acetoacetate which in turn enters the isoprene units (Steele and Gurin, 1960; Potty, 1969). As a result, the incorporation of β-hydroxy-β-methylglutaric acid (HMG) becomes less efficient as compared to acetate or acetoacetate.



0 = Origin
1 = α-Solanine
2 = α-Chaconine

Figure 7. Radioautogram of the alkaloid fraction extracted from sprouts administered with β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C and separated on TLC plate.

The incorporation of acetate into alkaloids of potato seedlings (Guseva and Paseshnichenko, 1961) has been shown to be nearly one-fourth the active form of DL-mevalonic acid (MVA); while in the present experiment, β-hydroxy-β-methylglutaric acid (HMG) was found to incorporate at about one-ninth of the efficiency of mevalonic acid (MVA). Thus, a comparison of the rates of incorporation of β-hydroxy-β-methylglutaric acid (HMG), acetate, and mevalonic acid (MVA) indicates that the second pathway is applicable to the synthesis of alkaloids and the possibility of β-hydroxy-β-methylglutaric acid (HMG) going directly to mevalonic acid (MVA) is eliminated. The incorporation of β-hydroxy-β-methylglutaric acid (HMG) would be more than acetate if it followed the first pathway.

The amount of label from L-leucine which appeared in the alkaloid fraction indicates an incorporation pathway similar to that for β-hydroxy-β-methylglutaric acid (HMG). According to Davies, Giovanelli, and Ap Rees (1964), enzymatic degradation of leucine in plants occurs as follows: Leucine --> α-ketoisocaproate --> isovaleryl-CoA --> 3-dimethylacrylyl-CoA --> 3-methylglutaconyl-CoA --> HMG-CoA --> acetoacetate and acetyl-CoA. However, attempts to demonstrate the possibility of HMG-CoA --> mevalonic acid (MVA) were unsuccessful (Davies, Giovanelli, and Ap Rees, 1964).

Alanine is known to be metabolized through its corresponding keto acid. However, a very low rate of incorporation may be due to its transformation into various plant metabolites. The alkaloid fraction of the sprouts incubated with D-glucose-U-^{14}C was hydrolyzed and the radioactivity in the aglycones and the sugar moieties measured. The incorporation of the label into the sugar fraction was nearly nine times higher than that into the aglycone. If glucose were incorporated

after glycolytic breakdown via acetate, the efficiency would be smaller than 1/20 and more incorporation into aglycone would be expected. However, the results indicate predominant glycosylation. Schematically, the proposed role of various precursors in the alkaloid biogenesis is presented in Figure 8.

As shown in Table 3, Alar (succinic acid 2,2-dimethylhydrazide), Ethrel or Ethephon (2-chloroethylphosphonic acid), and Telone (1,3-dichloropropene and related chlorinated hydrocarbons) significantly inhibited the synthesis of alkaloids in comparison to the control by 49.4, 75.5, and 58.2 percent, respectively. However, neither Alar (succinic acid 2,2-dimethylhydrazide) nor Telone (1,3-dichloropropene and related chlorinated hydrocarbons) reduced the amount of ^{14}C found in the alcohol extract; whereas, the alcohol extract from Ethrel or Ethephon (2-chloroethylphosphonic acid)-treated sprouts contained nearly twice the amount of radioactivity as that of control sprouts. It appears from the inhibition studies that ethylene released from Ethrel or Ethephon (2-chloroethylphosphonic acid) affects plant metabolism and effectively reduces alkaloid synthesis in sprouts. Elmer (1932, 1936) discovered that germinating potatoes were inhibited in their sprout development if ripening apples and pears were stored in close proximity to them, an effect which he attributed to the action of ethylene produced with fruit volatiles. The effect of Alar (succinic acid 2,2-dimethylhydrazide) is based on the fact that Alar (succinic acid 2,2-dimethylhydrazide) and related substances are involved as inhibitors in the pathways leading to isoprenoids (Ryugo and Sachs, 1969). Telone (1,3-dichloropropene and related chlorinated hydrocarbons) reduced incorporation (dpm) into the alkaloid fraction more than Alar (succinic acid, 2,2-dimethylhydrazide).

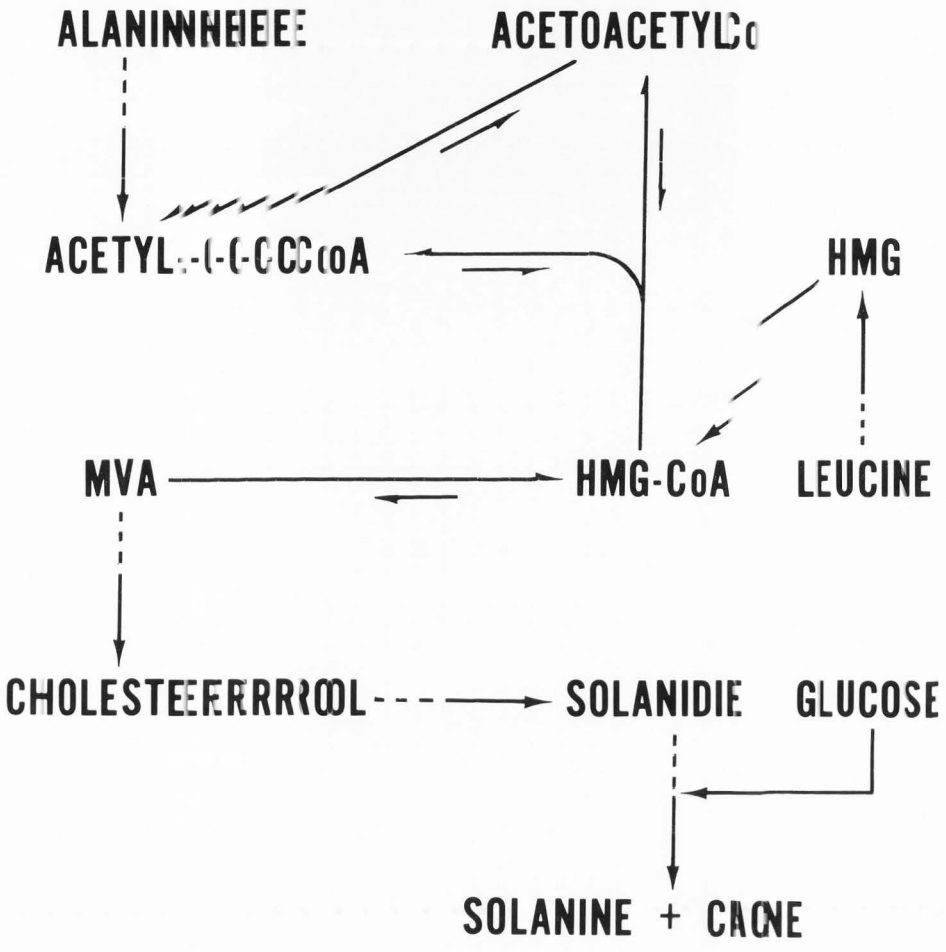


Figure 8. A proposed role of various precursors in the formation of potato glycoalkaloids.

Table 3. Effect of inhibitors on utilization of β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C by potato sprouts[a]

	Inhibitor, 10^{-3}M			
	Control	Alar	Ethrel	Telone
Alcohol extract (dpm x 1000)	719.525 (79.12)	732.825 (30.72)	1,602.275** (166.77)	840.875 (44.54)
Alkaloid fraction (dpm x 1000)	27.477 (5.60)	13.780** (2.70)	6.732** (0.90)	11.490** (1.98)
% Inhibition of alkaloids	0.0	49.4	75.5	58.2

[a]Ten gm sprouts were incubated (25 C) in a 50 ml Hoagland solution (zero or 10^{-3}M inhibitor) containing β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C (10 C) for 2 days under 100 ft-c light.

**Significantly different from control at 0.01 level.

Trade names: Alar (succinic acid 2,2-dimethylhydrazide), Ethrel or Ethephon (2-chloroethylphosphonic acid), and Telone (1,3-dichloropropene and related chlorinated hydrocarbons).

Note: Values in parentheses represent standard deviation.

Berry (1971) reported the effect of Telone (1,3-dichloropropene and related chlorinated hydrocarbons) on carotenogenesis and predicted that the inhibition of carotene synthesis could be attributed to the formation of a chlorine free radical from this chemical.

Although Alar (succinic acid 2,2-dimethylhydrazide) and Telone (1,3-dichloropropene and related chlorinated hydrocarbons) decreased the rate of incorporation of label into the alkaloids of potato sprouts, the uptake of the label in the alcohol soluble fraction of the tissue was not affected (Table 3). It may be possible, therefore, that these chemicals somehow alter or affect physiological responses and metabolic activities to counteract the physiological role of solanum alkaloids in growing sprouts. The apparent increase in total radioactivity in the alcohol extract of sprouts treated with Ethrel or Ethephon (2-chloroethylphosphonic acid) may result from changes in the shape, size, and permeability of cells in contact with the radioactive material. According to the reports of Amchem Products (1967-70), potato plants sprayed with Ethrel showed swelling of terminal buds.

In vitro studies with tuber or sprout extracts. The alkaloid fraction isolated from the suspension of potato slices contained nearly 0.56 percent (6224 ± 224 dpm) of the total radioactivity administered. The incorporation of label indicated that the enzymatic system of potato tubers was capable of glucosylating the 3β-hydroxyl group of solanidine when UDP-glucose-U-^{14}C served as a donor. Similar investigations by Procházka (1971) on the specificity of the enzymatic system of potato tubers led to the conclusion that a sterically unhindered 3β-hydroxyl group of steroids could be glucosylated if the steroids belong to the 5α-H or Δ^5-series.

The formation of β-glucoside also occurred in enzyme extracts from sprouts. The results are presented in Figure 9. The rate of synthesis was faster in the early period of incubation than in the later. Glucosylation of solasodine by enzyme extracts from Solanum laciniatum has been reported by Liljegren (1971). On the basis of his results and on sequential synthesis (Barber, 1962; Harborne, 1963; Miles and Hagen, 1968; Hahlbrock and Conn, 1970) and degradation (Guseva and Paseshnichenko, 1959) of secondary metabolites such as glycosides and flavonoids in certain plants, he supported the theory that glycosylation is the last step in the synthesis of solasonine and solamargine. This hypothesis may be similarly applied to the synthesis of α-solanine and α-chaconine because of genetical factors existing in the same Solanum species. Since γ, β, and α forms of solanine and chaconine (1, 2, and 3 sugars in the glycosidic part, respectively) occur in potato tubers as well as sprouts, stepwise synthesis of α-solanine and α-chaconine from solanidine seems possible. The formation of β-glucoside in both the cases indicated the presence of β-glucosyltransferase in Solanum tuberosum L.

In vitro studies with tuber slices. The effect of temperature on the level of solanine concentrations in potato slices is shown in Figure 10 (top). At low temperatures (0 and 8 C) there was a slow but significant increase in solanine content during a 48-hour period in the dark, while the storage temperatures of 15 and 24 C vigorously stimulated the formation of solanine alkaloids. After 48 hours at 24 C in the dark, the solanine content reached a concentration of 2.05 mg per 100 gm slices. This is seven times as much as that in the original (zero-time) sample. Another fact to be noted from Figure 10 (top) is

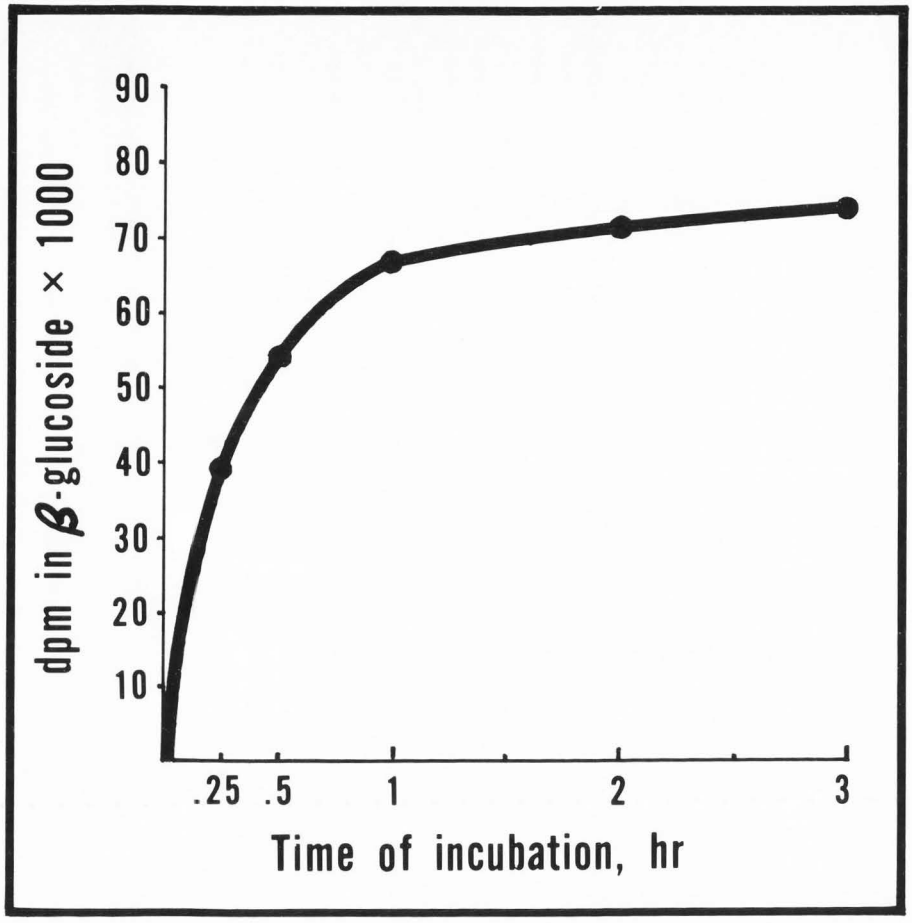


Figure 9. Conversion of solanidine and UDP-glucose-U-^{14}C to β-glucoside by the extracts from potato sprouts.

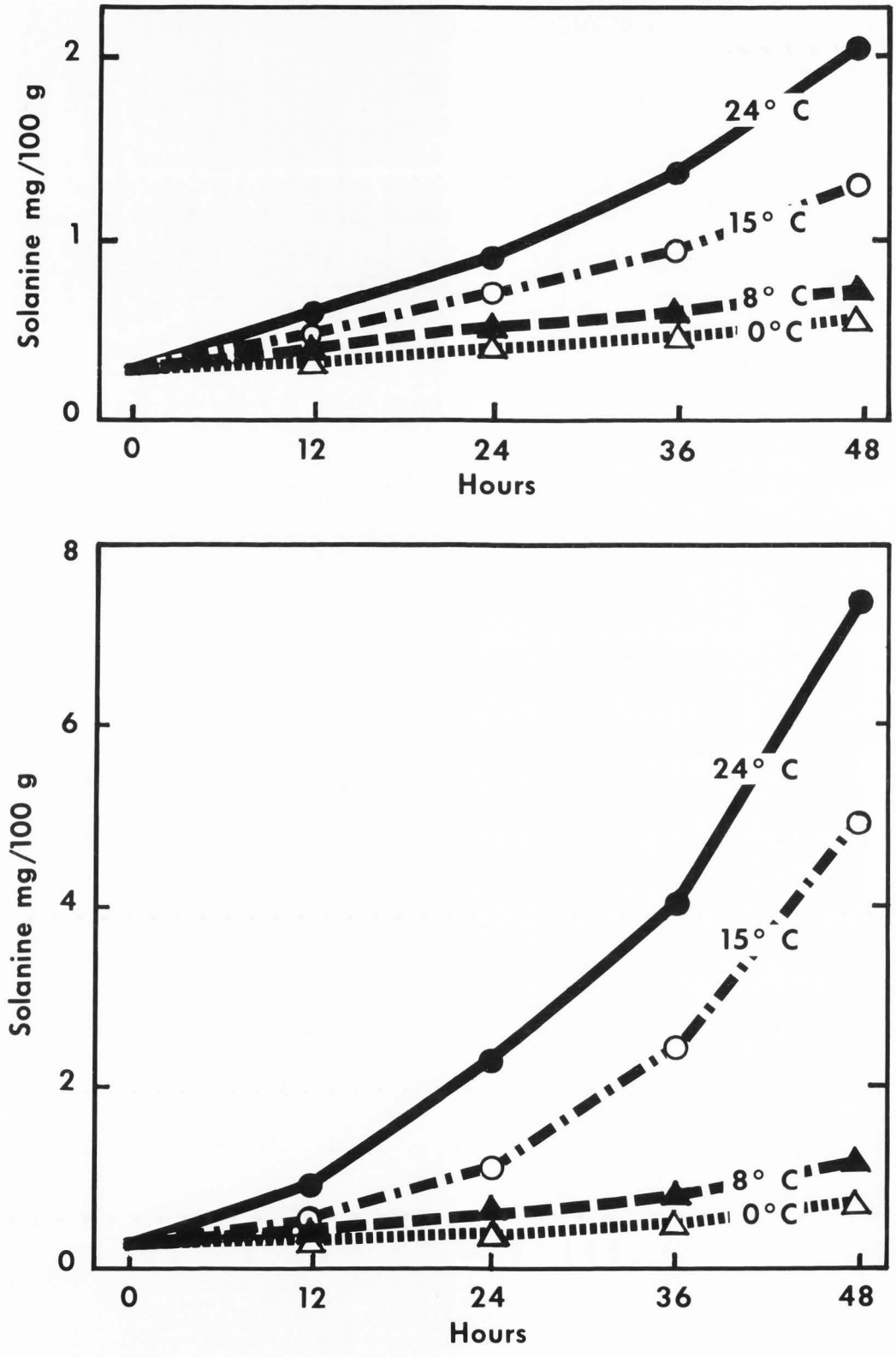


Figure 10. Solanine contents of tuber slices of Russet Burbank cultivar stored in the dark at different temperatures (top) and that of exposed to a fluorescent light (200 ft-c) at different temperatures (bottom).

the increasing rate of alkaloid synthesis in the later stage. It could, therefore, be assumed that potato slices require an induction period for alkaloid synthesis.

A considerable difference in solanine content between potato slices in cold (0 and 8 C) and warm (15 and 24 C) storage under light is illustrated in Figure 10 (bottom). The latter showed a relatively high amount compared to the former. In the 48-hour exposure to 200 ft-c light at 24 C, the solanine concentration increased up to 7.4 mg per 100 gm slices. In general, light increased the rate of synthesis of solanine alkaloids nearly three to four times more than dark. However, the amount of solanine alkaloids in the early and late stages followed a similar correlation that was observed in the dark storage.

The experimental evidence presented in this work establishes the fact that light and relatively high storage temperatures stimulate solanine biosynthesis in potato slices, a basically wounded tissue. The production of solanine has been reported by McKee (1955) in wounded potatoes. Injury of tubers caused by either bruising or mechanical grading after harvesting induced alkaloid synthesis in tubers (Sinden, 1972). Kuć (1964) reported that fresh potato slices increased in concentration of glycoalkaloids from an undetectable amount to 20 mg/100 gm after storage at room temperature in the dark for 3 days. However, the alkaloid contents were far more than those found in our studies. Perhaps the different rates of alkaloid synthesis in tuber slices may be an inherent genetical characteristic variable with the cultivar.

The phenomenon discussed above may be a form of a physiological defense mechanism in tubers or in slices when exposed to stress such as high light intensity in grocery stores or wounding as in the case

of slices or strips prepared for chips and French fries. In many instances, in potato processing plants slices, cubes, mash, strings, strips, shreds, and others are stored or held at relatively high light intensity and temperature for some time before cooking or dehydration. This may cause synthesis and subsequent accumulation of solanine alkaloids which cannot be destroyed during cooking, baking, or frying (190 C). Therefore, the only effective means of avoiding high concentrations of alkaloids in potato products is to process slices, strips, or other products as soon as they are prepared.

Control of chlorophyll and solanine alkaloids

Experiment I (chemicals). The results on the inhibitory effects of chemicals on chlorophyll and alkaloid synthesis in potato tubers are presented in Figures 11 and 12, respectively. Amchem 72-A42 [2-(p-chloropenylthio)-triethylamine] was the most effective to inhibit both chlorophyll and solanine alkaloids. The rates of inhibition by Nemagon (1,2-dibromo-3-chloropropane) and Telone (1,3-dichloropropene and related chlorinated hydrocarbons) were considerably less at lower concentrations. In general, the higher the concentration, the greater was the inhibition--regardless of the chemicals studied.

The effectiveness of Phosfon (tributyl 2,4-dichlorobenzylphosphonium chloride) and Phosfon-S (tributyl 2,4-dichlorobenzylammonium chloride) may be accounted for by the growth-retarding properties of these substances. Moreover, these compounds are known to decrease the biosynthesis of a natural gibberellin, an isoprenoid resulting from mevalonate (Dennis, Upper, and West, 1965). Amchem 72-A42 [2-(p-chlorophenylthio)-triethylamine], Amchem 70-334 or CPTA [2-(p-chlorophenylthio)-triethylamine

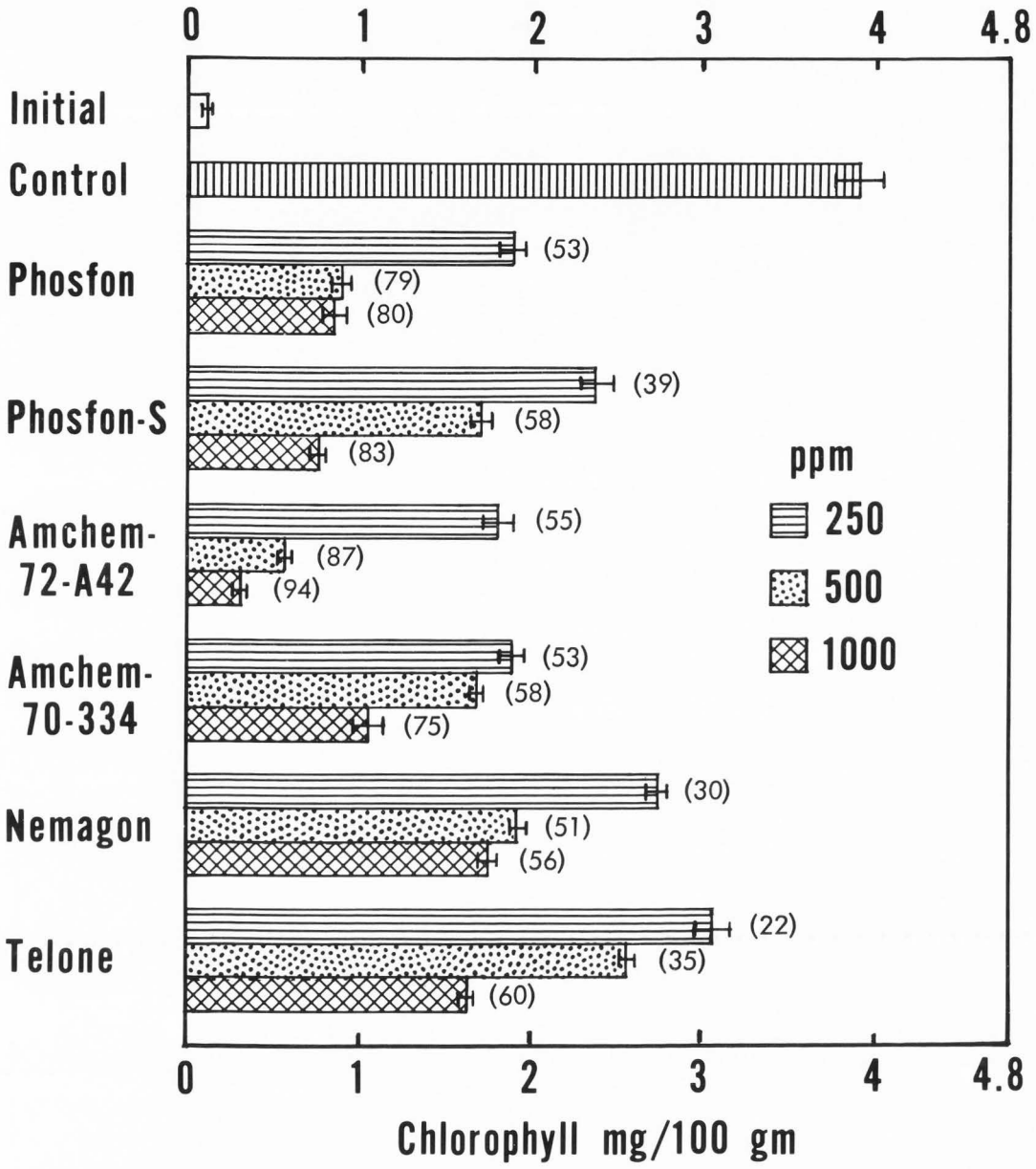


Figure 11. Effect of surface application of chemicals (0, 250, 500, and 1000 ppm in water) on the formation of chlorophyll in the peripheral (periderm and outer parenchyma) zone of potato tubers (Russet Burbank cultivar) exposed to a fluorescent light (200 ft-c) for 6 days at 16 C and 60 percent relative humidity. (Means are significantly different from control mean at 0.01 level. Values in parentheses represent percent inhibition of control compared to initial chlorophyll content. Data expressed per 100 gm of fresh peels.)

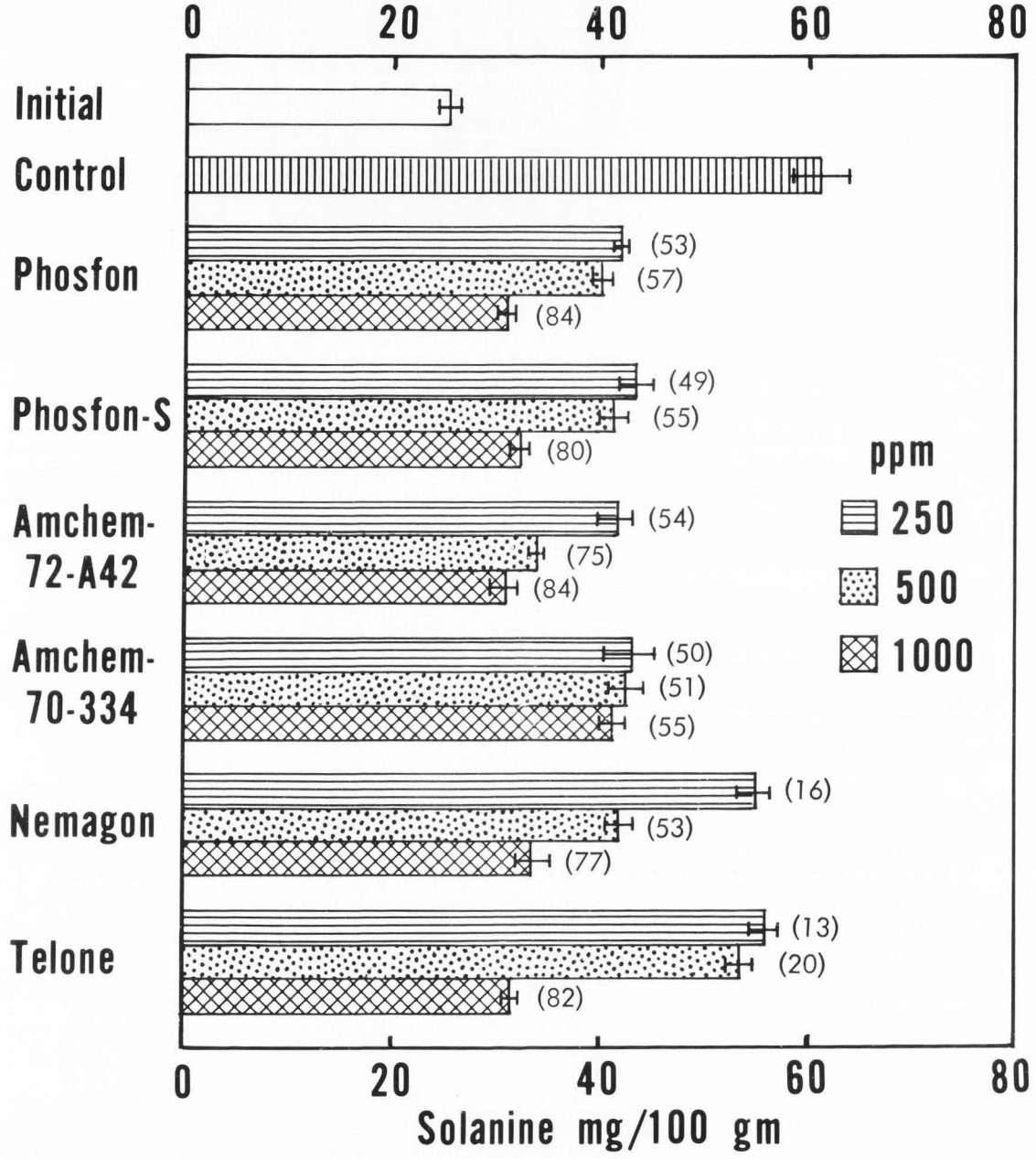


Figure 12. Effect of surface application of chemicals (0, 250, 500, and 1000 ppm in water) on the formation of solanine alkaloids in the peripheral (periderm and outer parenchyma) zone of potato tubers (Russet Burbank cultivar) exposed to a fluorescent light (200 ft-c) for 6 days at 16 C and 60 percent relative humidity. (Means are significantly different from control mean at 0.01 level. Values in parentheses indicate percent inhibition of control compared to initial solanine content. Data expressed per 100 gm of fresh peels.)

hydrochloride], and Ethrel or Ethephon (2-chloroethylphosphonic acid) contain a $-CH_2CH_2-$ functional unit in their chemical structures and also behave as ripening agents (Jadhav and Salunkhe, 1972; Rabinowitch and Rudich, 1972) of fruits. Ethylene, a plant hormone, released from Ethrel or Ethephon (2-chloroethylphosphonic acid) directly to plant tissues is known to accelerate degradation of chlorophyll in fruits (Amchem Products, 1967-1970; Salunkhe, Anderson, and Patil, 1971). Since Ethrel or Ethephon (2-chloroethylphosphonic acid) has been reported (Patil, Salunkhe, and Singh, 1971) as an inhibitor in the synthesis of chlorophyll and solanine alkaloids in potato tubers, Amchem 72-A42 [2-(p-chlorophenylthio)-triethylamine] and Amchem 70-334 or CPTA [2-(p-chlorophenylthio)-triethylamine hydrochloride] may be following a similar mode of action. The effects of Telone (1,3-dichloropropene and related chlorinated hydrocarbons) and Nemagon (1,2-dibromo-3-chloropropane) could be attributed to the release of halogen free radicals (Berry, 1971) from these two compounds.

As compared to untreated control, development of chlorophyll and solanine alkaloids was also significantly less when tubers were treated with glycerin followed by an exposure to light (Figure 13). The mode of action of glycerin, however, is not known. The structures of effective chemicals are presented in Figure 14.

Experiment II (mineral oil). Mineral oil treatments of tubers at various concentrations in the range of 0-100 percent are shown in Figure 15. In general, the tubers treated with mineral oil up to 10 percent concentration exhibited an attractive appearance as compared to untreated and excessively treated ones. Above 10 percent concentration, the consumption of oil and oily appearance of the tubers increased.

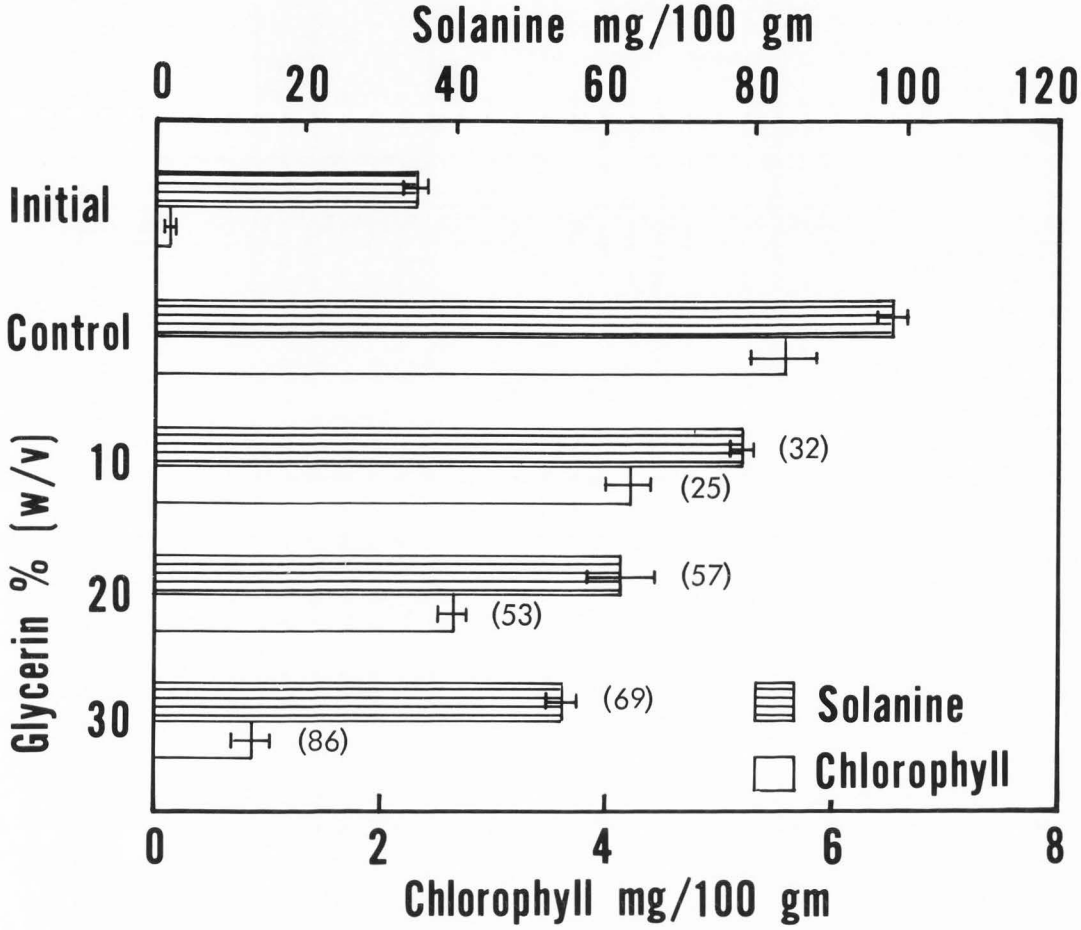


Figure 13. Effect of surface application of glycerin (0, 10, 20, and 30 percent w/v in water) on the formation of chlorophyll and solanine alkaloids in the peripheral (periderm and outer parenchyma) zone of potato tubers (Norgold Russet cultivar) exposed to a fluorescent light (200 ft-c) for 7 days at 16 C and 60 percent relative humidity. (Means are significantly different from the respective control mean at 0.01 level. Values in parentheses represent percent inhibition of control compared to initial chlorophyll or solanine content. Data expressed per 100 gm of fresh peels.)

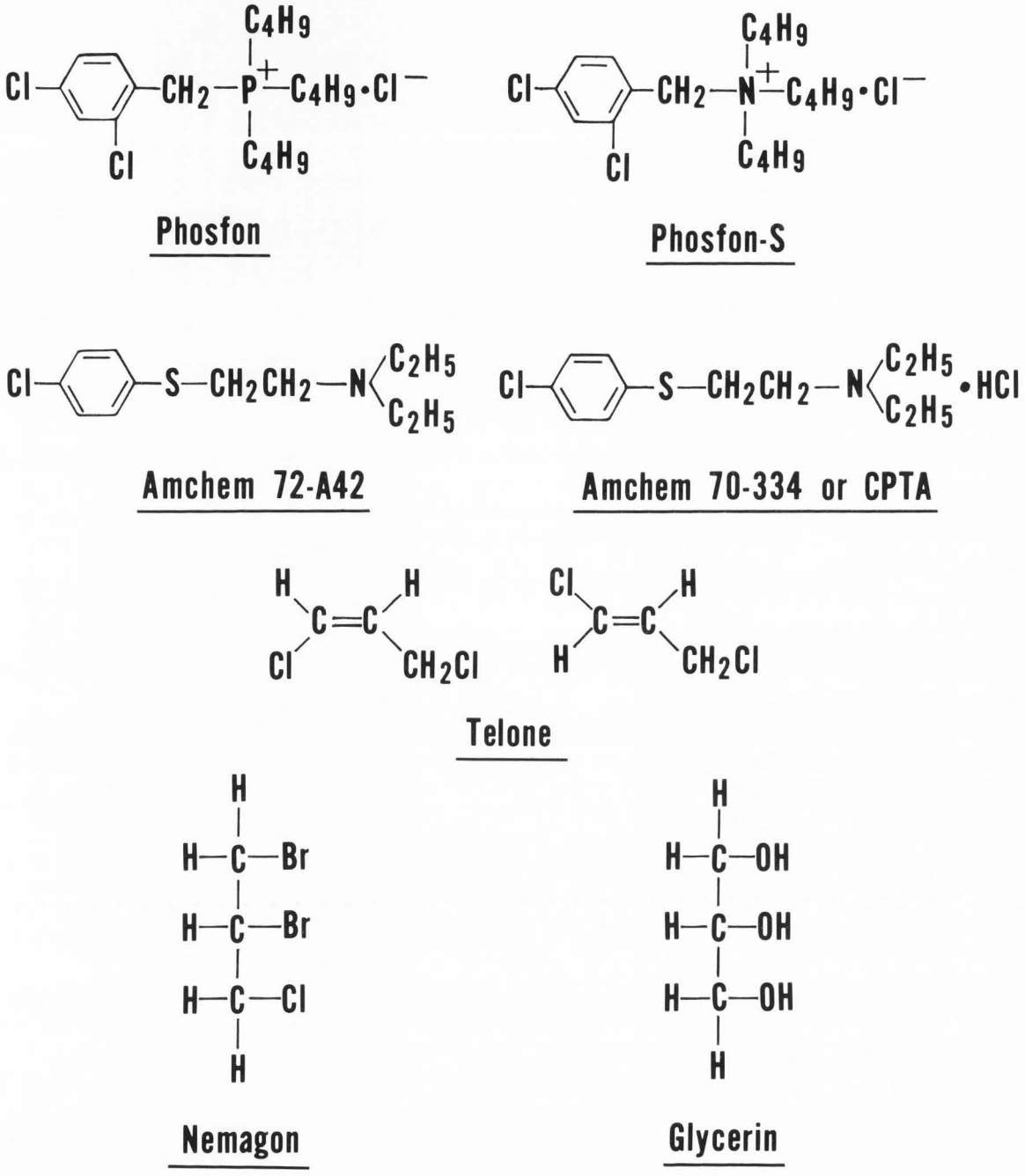


Figure 14. Structural formulas of chemicals effective on controlling light-induced formation of chlorophyll and solanine alkaloids.

1. Control
2. Petroleum ether
3-9. Mineral oil treatments at the concentrations of 1.25, 2.5, 5, 10, 15, 20, and 100 percent (w/v), respectively

Figure 15. Surface application of mineral oil in petroleum ether at various concentrations.

These tubers showed differential rates of greening when exposed to light for 7 days. A visual comparison of the pigmentation which resulted from the light effect on the tubers treated with mineral oil could be made in Figure 16 (top). Actual greening in the surface layer is revealed by peeling the skin (Figure 16, bottom). It is clear that the development of chlorophyll decreases as concentration of mineral oil increases.

Chlorophyll and solanine contents of such tubers were determined and the results are presented in Figure 17. The efficiency of the treatment increased with increasing concentration up to 10 percent of mineral oil and then remained almost constant and maximum up to 100 percent. A concentration of 10 percent mineral oil was a minimum requirement for effective inhibition of both chlorophyll and solanine alkaloids. There were no significant differences between the chlorophyll and solanine contents of tubers displayed under light with or without petroleum ether dips. The effect of ether as a solvent in all treatments was almost similar to that of petroleum ether (Figure 18). Since tubers were dipped in solutions for 0.5 second and the solvents used were highly volatile at room temperature, rupture of cell structure in the peripheral (periderm and outer parenchyma) zone usually caused by these solvents was eliminated.

The tubers dipped in 10 percent mineral oil did not turn green when continuously exposed to light for 28 days (4 weeks). A comparison of such tubers was made in Figure 19 with the tubers exposed to light for 0 or 7 days. It was interesting to note that there was no difference between the treated and the normal tubers in relation to greening. The analytical results on chlorophyll and solanine contents are

1. Control
2. Petroleum ether
3-9. Mineral oil treatments at the concentrations of 1.25, 2.5, 5, 10, 15, 20, and 100 percent (w/v), respectively

Figure 16. Differential greening of tubers (Norgold Russet cultivar) treated with mineral oil at various concentrations followed by an exposure to a fluorescent light (200 ft-c) for 7 days at 16 C and 60 percent relative humidity.


1 2 3 4 5 6 7 8 9


1 2 3 4 5 6 7 8 9

Figure 17. Effect of surface application of mineral oil (0, 1.25, 2.5, 5, 10, 15, 20, and 100 percent w/v in petroleum ether) on the formation of chlorophyll and solanine alkaloids in the peripheral (periderm and outer parenchyma) zone of potato tubers (Norgold Russet cultivar) exposed to a fluorescent light (200 ft-c) for 7 days at 16 C and 60 percent relative humidity. (Means are significantly different from the respective control mean at 0.01 level. Values in parentheses represent percent inhibition of control compared to initial chlorophyll or solanine content. Data expressed per 100 gm of fresh peels.)

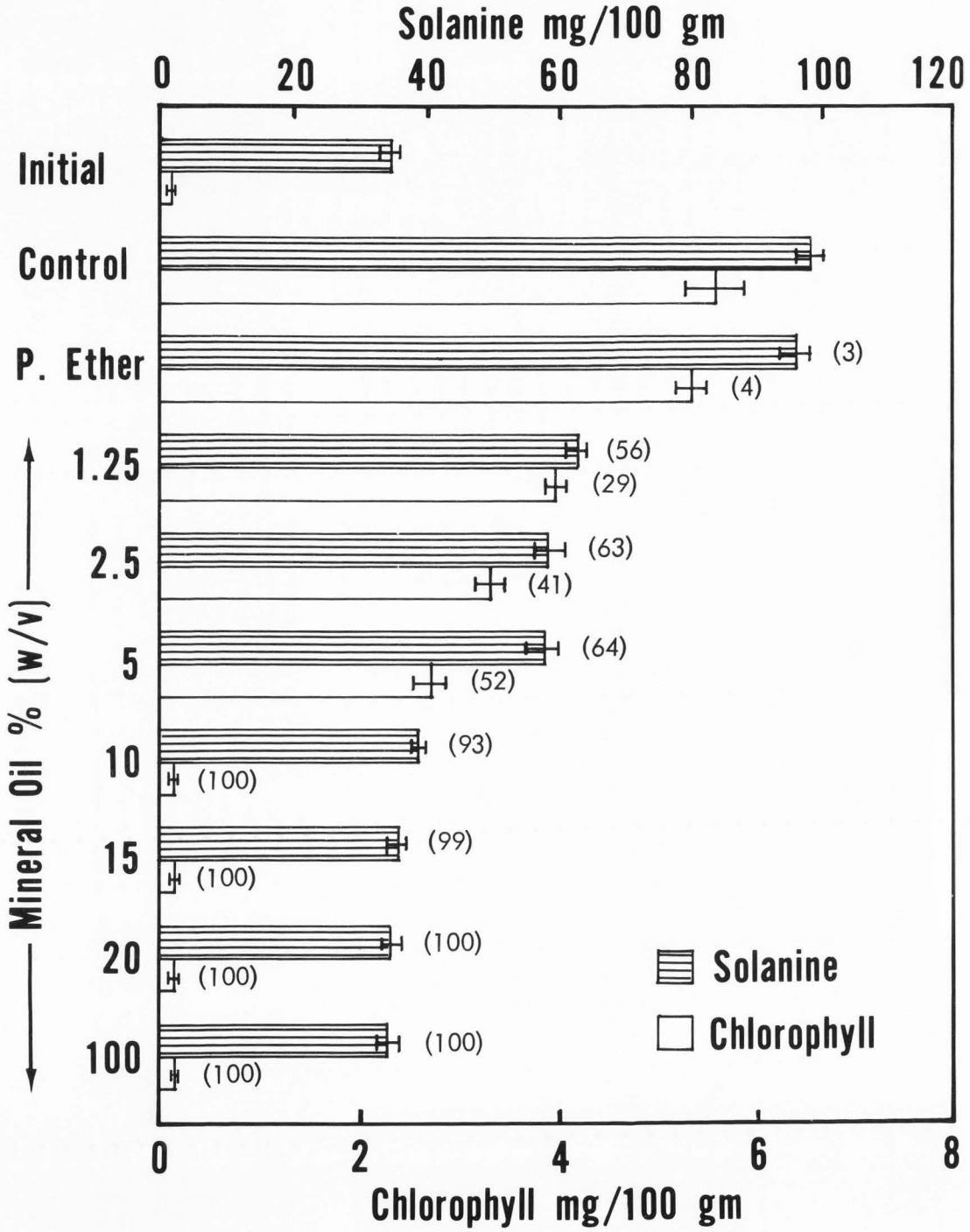
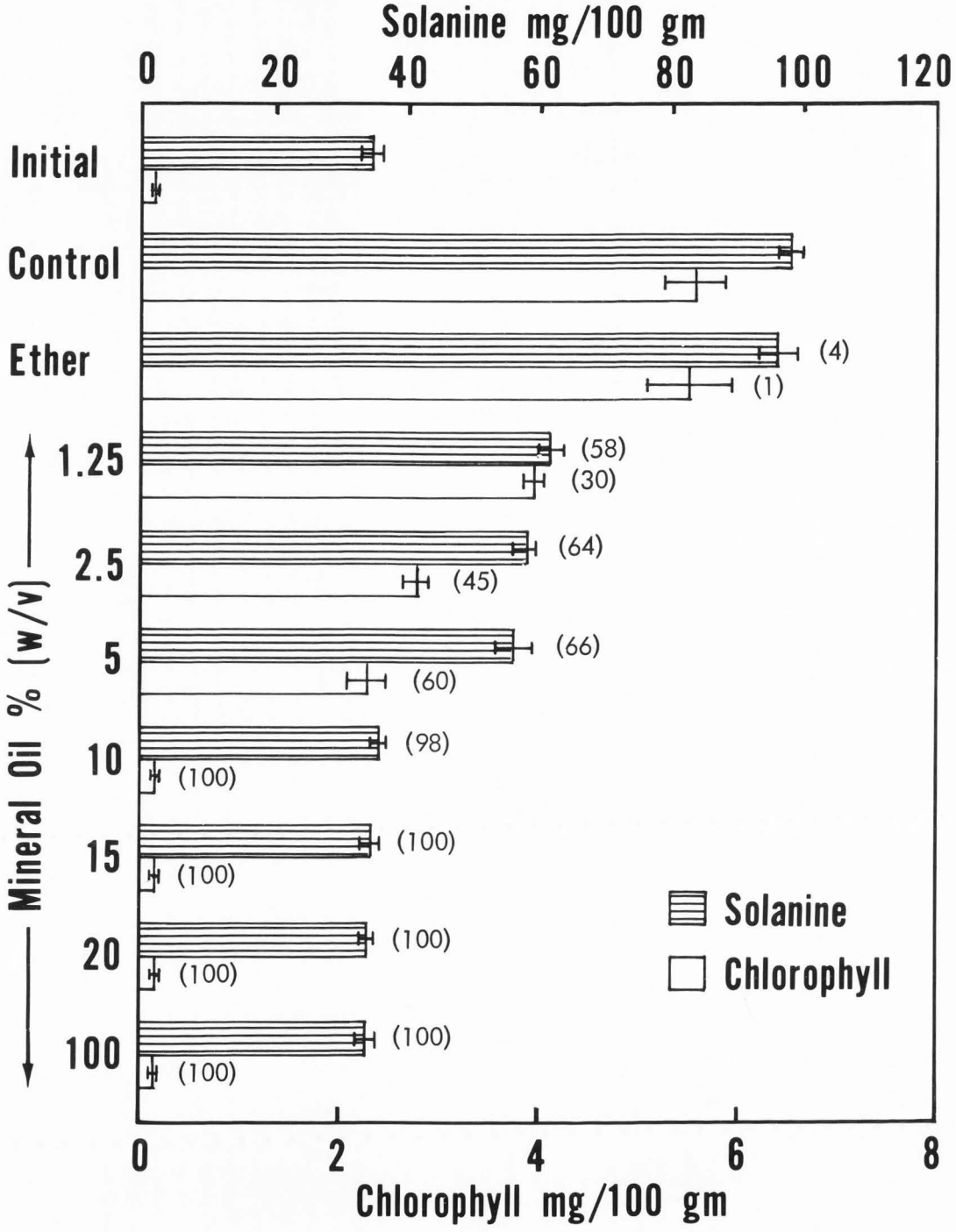

Solanine mg/100 gm
0
20
40
60
80
100
120
Initial
Control
P. Ether
1.25
2.5
5
10
15
20
100
Mineral Oil % (w/v)
(3)
(4)
(56)
(29)
(63)
(41)
(64)
(52)
(93)
(100)
(99)
(100)
(100)
(100)
(100)
(100)
Solanine
Chlorophyll
0
2
4
6
8
Chlorophyll mg/100 gm

Figure 18. Effect of surface application of mineral oil (0, 1.25, 2.5, 5, 10, 15, 20, and 100 percent w/v in ether) on the formation of chlorophyll and solanine alkaloids in the peripheral (periderm and outer parenchyma) zone of potato tubers (Norgold Russet cultivar) exposed to a fluorescent light (200 ft-c) for 7 days and 16 C and 60 percent relative humidity. (Means are significantly different from the respective control mean at 0.01 level. Values in parentheses represent percent inhibition of control compared to initial chlorophyll or solanine content. Data expressed per 100 gm of fresh peels.)


Solanine mg/100 gm
0
20
40
60
80
100
120
Initial
Control
Ether
1.25
2.5
5
10
15
20
100
Mineral Oil % (w/v)
(4)
(1)
(58)
(30)
(64)
(45)
(66)
(60)
(98)
(100)
(100)
(100)
(100)
(100)
(100)
(100)
Solanine
Chlorophyll
0
2
4
6
8
Chlorophyll mg/100 gm

1. Normal tubers not exposed to light
2. Control tubers exposed to light for 7 days
3. Mineral oil-treated tubers exposed to light for 28 days

Figure 19. Differential greening of tubers (Norgold Russet cultivar) treated with 10 percent mineral oil (w/v in petroleum ether) followed by an exposure to a fluorescent light (200 ft-c) for 28 days (4 weeks) at 16 C and 60 percent relative humidity.

presented in Figure 20. The rate of solanine inhibition decreased in the second and third weeks and then increased in the fourth week to the level at the end of the second week. However, the overall rate of inhibition was significantly high over a period of 4 weeks. The results in this experiment agreed with the findings of Conner (1937) that the processes of chlorophyll and alkaloid synthesis in potato tubers are independent of each other.

The importance of this method of controlling greening and alkaloid synthesis lies in the fact that it is simple, effective, and inexpensive. Because mineral oil is used as a laxative and could be removed during peeling off the peripheral layer of the tuber, residual problems are not as serious as in any other chemical inhibitors which are not a part of the biological system. However, the exact mode of its action is not known. This method may be useful in grocery stores and supermarkets where the sellout number of potato tubers is relatively high.

Control of sprouting

Experiment III (mineral or vegetable oil). Complete inhibition of sprouts occurred when potatoes were applied with 10 percent as well as 100 percent mineral or vegetable (Wesson) oil and stored for 30 days (Figure 21). A concentration of 5 percent of both oils delayed the initiation of sprout activity by 5-6 days. After this period, the rate of sprout growth was also less than that for control tubers. The tubers treated with petroleum ether did not show significant differences from control tubers in relation to sprout development.

Although oil treatments were effective in controlling sprout growth, the tubers developed rancidity during the second week followed

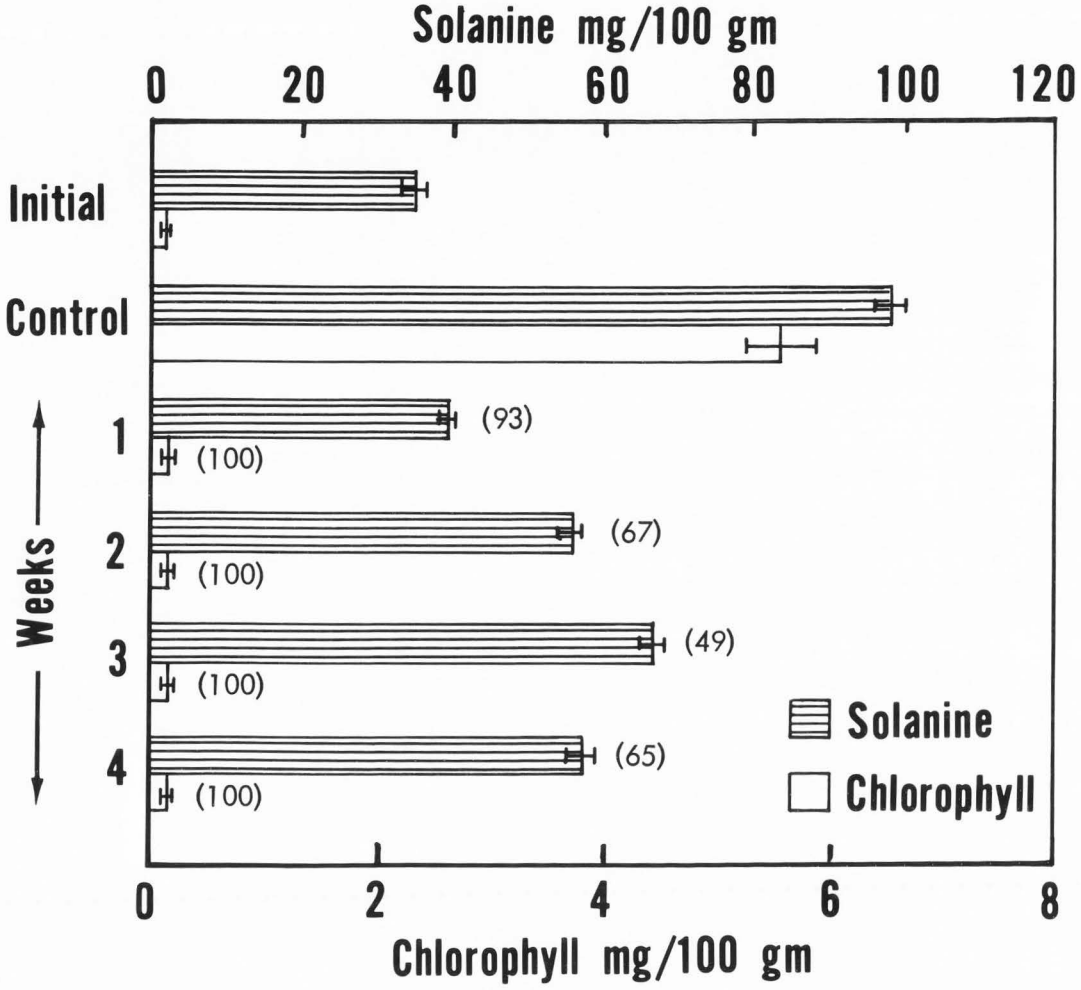


Figure 20. Effect of duration of light (200 ft-c) exposure on the formation of chlorophyll and solanine alkaloids in the peripheral (periderm and outer parenchyma) zone of potato tubers (Norgold Russet cultivar) treated with 10 percent mineral oil (w/v in petroleum ether) and displayed at 16 C and 60 percent relative humidity. (Means are significantly different from the respective control mean at 0.01 level. Values in parentheses represent percent inhibition of control (7 days light exposure) compared with initial solanine or chlorophyll content. Data expressed per 100 gm of fresh peels.)

1. Control
2. Petroleum ether
3. 5 percent mineral oil
4. 5 percent vegetable oil
5. 10 percent mineral oil
6. 10 percent vegetable oil
7. 100 percent mineral oil
8. 100 percent vegetable oil

Figure 21. Effect of surface application of mineral or vegetable oil on sprouting of Norgold Russet tubers after 30 days storage at 16 C and 60 percent relative humidity.

by dark spots in the peripheral zone after nearly 3 weeks of storage. When cut open, such potatoes had interior tissue that appeared normal at first, but, upon exposure to air pink discoloration occurred which gradually became dark brown or black. The pink discoloration was intense in the peripheral zone. Bartholomew (1915) demonstrated such discoloration in tubers developing a disease (blackheart) because of oxygen lack. Mann and Joshi (1920) produced blackheart in tubers coated with paraffin or collodion. Thus, oils may be involved in a process of blocking lenticles responsible for gas exchange, causing anaerobic fermentation and putrefaction and consequently accelerating disintegration of the tubers.

A bacterial soft rot, a characteristic disease of fleshy vegetables, was followed by blackening of tubers. The extent of spoilage of tubers increased with concentration of oils--less than 5-6 percent at the lowest, 14-18 percent at moderate, and 20-26 percent at the highest concentration. The oil treatments at low concentration in combination with certain edible waxes could be used to eliminate decay of potato tubers.

SUMMARY AND CONCLUSIONS

A fluorescent light induced the formation of a green pigment, chlorophyll, and highly toxic solanine alkaloids in the peripheral zone of potato tubers.

Tracer studies indicated incorporation of a ^{14}C label from acetate, β-hydroxy-β-methylglutaric acid (HMG), leucine, alanine, glucose, and mevalonic acid (MVA). The efficiency ratio revealed that β-hydroxy-β-methylglutaric acid (HMG) was incorporated via acetate or acetoacetate. The results with glucose showed predominant glycosylation. Alar (succinic acid 2,2-dimethylhydrazide), Ethrel or Ethephon (2-chloroethylphosphonic acid), and Telone (1,3-dichloropropene and related chlorinated hydrocarbons) inhibited the incorporation of the ^{14}C label into the alkaloids. Enzymatic glucosylation of solanidine by the extracts from potato tubers and sprouts supported the hypothesis on stepwise synthesis of solanine alkaloids from solanidine.

The tuber slices induced the synthesis of solanine alkaloids when subjected to relatively high temperature or high intensity light. Therefore, immediate utilization of tuber slices for chips or French fries is recommended.

Investigations on control of greening showed that Phosfon (tributyl 2,4-dichlorobenzylphosphonium chloride), Phosfon-S (tributyl 2,4-dichlorobenzylammonium chloride), Amchem 72-A42 [2-(p-chlorophenylthio)-triethylamine], Amchem 70-334 or CPTA [2-(p-chlorophenylthio)-triethylamine hydrochloride], Nemagon (1,2-dibromo-3-chloropropane), Telone (1,3-dichloropropene and related chlorinated hydrocarbons),

glycerin, and mineral oil were effective in inhibiting the formation of chlorophyll and solanine alkaloids. Mineral oil was the most effective. A 10 percent concentration of mineral oil in ether or petroleum ether was the minimum requirement for complete inhibition of chlorophyll and the alkaloids. Mineral or vegetable oils were also effective in controlling sprouting to tubers. Further modifications in oil treatment will be useful for its commercial application.

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PART II

FORMATION AND CONTROL OF CARBONYL COMPOUNDS OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) FRUITS

INTRODUCTION

The tomato (Lycopersicon esculentum Mill.) fruit is one of the most popular, as well as important, commodities in the world. Over 20 million metric tons of tomatoes are produced each year on a world basis. The United States, Italy, and Spain are the leading producers of this crop. In the United States alone, it ranks second only to potatoes in production among vegetable crops (Figure 22) and contributes approximately 400 million dollars to the economy. As a processing crop, it takes first rank among the vegetables.

Though the tomato was not recognized as a valuable food until about a century ago, its merit is now universally accepted. It is often referred to as "the poor man's orange" because of its high vitamin, malic acid, and citric acid contents, and the fact that it serves as a fine appetizer. The tomato is also popular because it is a most rewarding crop for the home garden since it grows well practically everywhere and it provides nutrients in many forms such as raw in salads; cooked in soups, preserves, catsups, and sauces; pickled; and in other forms.

Demand for and acceptance of fresh tomato fruit are based largely on the flavor. Flavor is a composite of taste and odor (aroma), which are entirely different from physiological and chemical points of view. Taste is a function of the taste buds in the mouth, which constitute a selective mechanism. A relation exists between the kind of taste that a substance has and its chemical constitution. On the basis of psychological studies, the four primary sensations of taste are: sourness

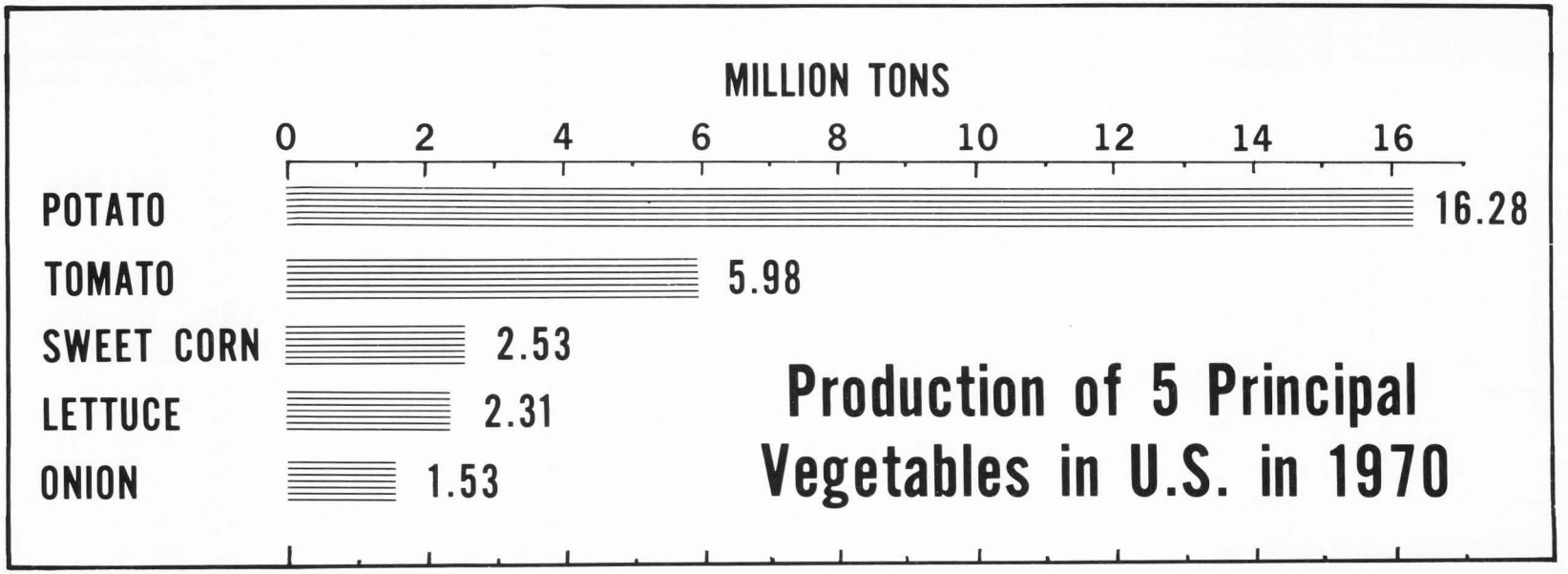


Figure 22. Yield of the five most prevalent vegetables in the United States of America in 1970.

of acids; saltiness of ionized salts; sweetness of sugars, glycols, alcohols, aldehydes, ketones, amides, esters, amino acids, sulfonic acids, and halogenated acids; and bitterness of long chain organic substances and alkaloids. Salty and sour tastes show a much better correlation with structure than do sweet and bitter tastes.

From the consumer's point of view, odor or aroma excites sensation in the brain when the aroma compounds contact the nasal cavity. The sensation depends upon the aroma substance fitting the olfactory cells in the nose. Fruit aroma is generally considered to consist of various volatile substances such as esters, aldehydes, ketones, alcohols, lactones, hydrocarbons, acids, etc., which exist in minute quantities in the fruit.

Tomato fruit quality is determined mainly by color, texture, and flavor. Among these, color and flavor are probably the most useful criteria for estimating maturity of tomato fruit. High quality is associated with redness of color and prominence of flavor. The flavor of a fruit becomes pronounced when the sugar content is at its maximum, at which time the skin acquires its richest color.

Although aroma of tomato fruit is due to total volatiles, contribution of carbonyl compounds (aldehyde and ketones) to the typical aroma of tomato is quite significant. The aliphatic nature of the carbonyls of tomato volatiles led Shah, Salunkhe, and Olson (1969) to hypothesize that their formation may be from an oxidative degradation of fatty acids and/or transamination of certain amino acids. Oleic, linoleic, and linolenic acids have been found to be the major unsaturated fatty acids in tomato fruit (Kapp, 1966). The unsaturated fatty acids have been implicated as the precursors in the formation of

a number of volatile compounds (Nye and Spoehr, 1943; Hultin and Proctor, 1962; Drawert et al., 1965; Anjou and von Sydow ,1967). Volatiles of tomato vary in concentration in tomato fruit with the stage of maturity, light conditions, nutrient availability, and enzymatic activity during ripening (Shah, Salunkhe, and Olson, 1969).

In view of the above considerations, studies were initiated to investigate the formation and control of carbonyls of tomato fruits from fatty acids. The following objectives were undertaken:

1. Enzymatic production of carbonyls from fatty acids.

2. Biogenetic relationship of linoleic and linolenic acids with hexanal of tomato fruit.

3. Factors affecting production of carbonyls from linoleic and linolenic acids.

4. Production of carbonyls of tomatoes stored under hypobaric or sub-atmospheric pressures.

REVIEW OF LITERATURE

A review pertaining to the chemistry of tomato fruit is available in the literature (Yamada, 1959). Studies on biochemistry of living cells have revealed that many volatile (aroma) compounds which contribute to the individual flavors of foods are derived from nonvolatile compounds such as carbohydrates, proteins and amino acids, fats, minerals, and vitamins. Therefore, it is imperative to discuss compositional changes of tomato fruit during growth and maturation. Additionally, a survey is conducted dealing with the chemistry of tomato volatiles and factors affecting their production in the fruit.

Compositional changes during growth and maturation

The relative concentrations of the chemical constituents of tomato fruit are important in assessing the quality in respect to color, texture, appearance, nutrient value, taste, and aroma.

Sugars. The soluble solids of tomatoes are predominantly sugars, which in turn are important contributors to flavor. In general, the flavor of a fruit becomes pronounced when its sugar content peaks. The free sugars, representing more than 60 percent of the solids in tomatoes, are mainly D-glucose and D-fructose. The sugar content of tomato fruit is a function of the stage of maturity. It increases uniformly from small and green mature to large and red-ripe tomatoes (Rosa, 1925; Winsor, Davies, and Massey, 1962; Lambeth, Fields, and Huecker, 1964; Dalal et al., 1965).

Starch. The starch content of tomato fruit depends upon maturity, cultivar, and ripening conditions, and varies from 1-1.22 percent in immature fruit to 0.1-0.15 percent in red-ripe fruit. Yu, Olson, and Salunkhe (1967) studied the composition of tomato fruit at nine different stages of maturity and observed that starch accumulated until nearly the large-green stage and then rapidly decreased. Relatively low starch contents in the last stage of fruit maturation were noticed by Sando (1920), Rosa (1925), Saywell and Cruess (1932), and Davies and Cocking (1965). Yu, Olson, and Salunkhe (1967) made the important observation that the increases and decreases in free reducing sugar and starch contents were not parallel as the maturation progressed.

Pectins. The texture of the fruit is satisfactory only when pectase, calcium, and pectin are in sufficient quantities. Changes in pectic substances or firmness during maturation have been studied by several workers (Appleman and Conrad, 1927; Kattan, 1957; Woodmansee, McClendon, and Somers, 1959; Luh et al., 1960; Dalal et al., 1965). The pectic enzymes, if not inactivated by heat, cause loss of viscosity in the processed products. Production of methanol in tomato is due to pectin esterase activity during maturation and ripening (Jacquin and Tavernier, 1955).

Ascorbic acid. Tomato fruit is a rich source of ascorbic acid (vitamin C). On the basis of fresh weight, vitamin C content averages about 25 mg/100 gm (Olliver, 1967); however, the values vary with the cultivars. The ascorbic acid content changes little during fruit maturation and ripening according to several reports (MacLinn and Fellers, 1938; Wokes and Organ, 1943; Kaski, Webster, and Kirch, 1944).

Organic acids. Citric and malic acids are organic acids that contribute most to the typical taste of tomato fruit. As whole fruit ripens from mature green to red, acidity increases to a maximum value and then decreases (Janes, 1941; Rosa, 1925; Winsor, Davies, and Massey, 1962; Dalal et al., 1965). Maximum acidity was found at breaker (Winsor, Davies, and Massey, 1962) and at pink stages (Janes, 1941; Rosa, 1925; Dalal et al., 1965).

Acidity of tomato fruit is important for flavor. It is also important to the processor because high acidity reduces the processing time and temperature necessary to kill spoilage microorganisms. Low acidity of fruits is responsible for the increased incidence of spoilage of canned tomatoes (Lambeth, Fields, and Huecker, 1964).

Amino acids. Several authors reported the presence of 20 amino acids in ripe tomatoes (Carangal et al., 1954; West, 1959; Burroughs, 1960; Freeman and Woodbridge, 1960; Wong and Carson, 1966; Davies, 1966; Yu, Olson, and Salunkhe, 1967). Concentrations of these materials are higher in the pulp than in the walls of the fruit. Freeman and Woodbridge (1960) observed that glutamic and aspartic acids increased markedly; while alanine, arginine, leucine, and valine decreased with ripening. Davies (1966) reported that glutamic acid increased approximately 10-fold and aspartic acid more than doubled as tomatoes passed from the mature green to the red stage of ripeness. In their studies of eight varieties of tomatoes, Hamdy and Gould (1962) noted that glutamic acid peaked at the ripe stage. Yu, Olson, and Salunkhe (1967) found that glutamic acid doubled as the fruit passed from breaker to pink stages of development. Total amino acid content was shown to be

constant throughout the ripening process (Freeman and Woodbridge, 1960; Yu, Olson, and Salunkhe, 1967).

On the basis of the patterns of changes noted for the volatile or aroma components (Dalal et al., 1965) and amino acids during maturation, Yu, Olson, and Salunkhe (1967) hypothesized that certain amino acids could serve as precursors of volatile compounds in tomato fruit. This was further verified by using crude preparations from tomato fruits (Yu, Olson, and Salunkhe, 1968a, 1968b; Yu, Salunkhe, and Olson, 1968; Yu and Spencer, 1969, 1970). Nitrogen fertilization (Saravacos, Luh, and Leonard, 1958), the α-amino acid-citric acid ratio (Hamdy and Gould, 1962), and the dicarboxylic amino acid-soluble carbohydrate ratio (Taverna, 1965) all seem to affect the flavor of raw or processed tomatoes. Oxidative breakdown of sulfur compounds leads to the generation of sulfurous volatiles.

Proteins and enzymes. Tomato proteins represent 3 percent of total solids. Yu, Olson, and Salunkhe (1967) noted protein changes during ripening which correlated with aroma production.

The effective amount of enzymes present at any given stage of maturity of tomato is generally determined by the relative rates of synthesis and degradation, and by environmental conditions. Yu, Olson, and Salunkhe (1968a) demonstrated that the degradation of amino acids to carbonyls was higher when catalyzed by enzyme extracts from field-grown tomatoes than by those from greenhouse-grown tomatoes. This degradation increased with the maturity of the fruit. The nature and amounts of volatile compounds produced also depended on the maturity stage of the tomato fruit (Yu, Salunkhe, and Olson, 1968; Yu, Olson and Salunkhe, 1968b). It is generally agreed that several compositional

changes accompanied by the development of the typical aroma upon ripening due to catalytic actions of various enzymes ultimately reflect on the quality of the fruit.

Pigments. Consumers buy tomatoes by "eye" judgment. Color is perhaps the most important and reliable index of tomato maturity. Consequently it contributes significantly to the grade of both raw and processed products. As destruction of chlorophyll progresses during ripening, different shades of color such as green-yellow, yellow-orange with some trace of green, orange-yellow, orange-red, and red develop in sequence. According to Ferrari and Benson (1961), β-carotene (yellow) and lycopene (red) contribute 7 and 87 percent of the carotenoids in a normal red tomato. It is known that β-carotene decreases as lycopene increases with ripening (Dalal et al., 1965; Dalal, Salunkhe, and Olson, 1966; Meredith and Purcell, 1966). Other forms of carotenes such as xanthophyll (yellow) pigments (Curl, 1961; Edwards and Reuter, 1967) and phytoene and phytofluene (Porter and Zscheile, 1946; Rabourn and Quackenbush, 1953; Tomes, 1963) in tomato have been reported.

On the basis of structural similarity and high correlation between the high boiling volatiles (terpenoids) of tomato, Stevens (1970b) hypothesized the production of such volatiles from oxidation of the polyene-carotenes.

Lipids. The lipid fraction of tomatoes is composed of triglycerides, diglycerides, sterols, sterol esters, free fatty acids, and hydrocarbons. Kapp (1966) initiated investigations on the total lipids in the pericarp of tomatoes, but found no definite relationship with color development. He reported that total lipids varied with cultivar, fruit maturity at harvest, and storage treatment. A total of 33

saturated and unsaturated fatty acids were found in the pericarp of all tomato varieties tested. Linoleic, linolenic, oleic, stearic, palmitic, and myristic acids comprised the major portion of the fatty acid fraction and increased during the period of greatest color development. During the same period, linoleic and palmitic acids decreased in percent of total fatty acids.

Ueda et al. (1970) found considerable amounts of total lipids in green tomato fruits on plants and lesser amounts in fruits harvested at the breaker stage. A slight increase in neutral lipids occurred at the full ripe stage. The fatty acid composition of triglyceride showed a decrease in linoleic and oleic acids at the stage of color development.

Minerals. Mineral content increases during growth and maturation of the tomato fruits because of increased cell organization and permeability, acid-base balance, and control or activation of enzyme systems. Although mineral elements represent a small fraction (0.55 percent ash) of the dry matter of tomato fruits, they all play an important role in the nutritional composition and final quality of the product.

Chemistry of tomato volatiles

The volatile constituents of tomatoes have been the subject of several studies. According to the investigations of Pyne and Wick (1965), the volatiles constitute only 2-5 ppm in total, but comprise at least 50 compounds, among which hexanal, 2- and 3-methyl butanol, cis-3-hexenol, and some other unidentified carbonyls are the most important in amount. Acetaldehyde and ethanol were first reported in

tomato volatiles by Gustafson (1934). Spencer and Stanley (1954) reported that typical tomato odor fraction contained alcohols, carbonyls, and unsaturates, and these were modified by many other odor fractions, some terpene in nature. The change in ethanol and acetaldehyde content during ripening was reported by Rakitin (1945). Chromatograms of a tomato concentrate showing numerous components have been published by Bidmead and Welti (1960). However, no identifications were made. Matthews (1961) tentatively identified furfural, acetaldehyde, and acetone in ripe tomatoes. Schormüller and Grosch (1962, 1964, 1965) found hexanal, 2-hexenal, glyoxal, methyl glyoxal, cinnamaldehyde, hydrocinnamaldehyde, 2-butanone, 2-pentanone, methyl heptenone, diacetyl, and a 5-carbon dicarbonyl in a tomato extract. Hein and Fuller (1963) presented evidence for the occurrence of diacetyl, α-pinene, citronellal, limonene, and citral. Miers (1966) detected volatile sulfur compounds in canned tomatoes and canned tomato juice and suggested that these compounds contributed to "cooked" tomato flavor.

Extensive investigations conducted by several workers revealed the occurrence of a number of new aroma compounds in tomato fruit (Ryder, 1966; Giannone and Baldrati, 1967; Katayama, Tubata, and Yamato, 1967; Dalal et al., 1968; Shah, Salunkhe, and Olson, 1969; Buttery et al., 1971; Kazeniac and Hall, 1970). In all these investigations on tomato volatiles, carbonyl compounds (aldehydes and ketones) and alcohols occurred consistently. However, large variations in quantitative data were reported. According to the studies conducted by Shah, Salunkhe, and Olson (1969), typical aroma of tomato is predominantly due to volatiles of which aldehydes and ketones represent 32 percent; short-chain alcohols 10 percent; and hydrocarbons, long-chain alcohols, and

esters 58 percent. Recently, Johnson, Nursten, and Williams (1971) surveyed the volatile compounds in tomato fruit that have been identified by several workers. Altogether 65 carbonyls (aldehydes and ketones), 34 hydroxy compounds (alcohols), 19 esters, 18 acids, 14 hydrocarbons, 6 nitrogen compounds, 5 lactones, 4 acetals and ketals, 4 sulfur compounds, 3 ethers, and 3 chlorine compounds have been reported.

The chemistry of volatile (aroma) compounds was made successful with the remarkable developments in analytical instrumentation. A complete analysis of volatiles occurring in trace amounts requires a three-step procedure: extraction, fractionation, and identification. A simple method for the extraction of tomato volatiles is by use of a proper solvent. Inclusion of non-volatile compounds such as pigments, however, makes the analysis complex. Steam distillation often causes chemical changes in the aroma. Distillation under reduced pressure coupled with low temperature condensation appears to be the most desirable means of obtaining the volatile fraction. The effectiveness of different techniques used in the isolation of aroma fraction from food material has been discussed by Chang (1973). The concentrated essence obtained by one of the preceding methods can be separated into its components by various types of chromatography. However, gas-liquid chromatography seems the most appropriate for the separation of very small quantities of mixtures. Identification of individual components of a tomato essence can be accomplished by physical properties (boiling point, chromatographic R_f values, enrichment retention techniques) and by spectroscopic data obtained from infra-red, ultra-violet, nuclear magnetic resonance, and mass spectra. The problems encountered in

gas-liquid chromatography of volatiles and subsequent identification have been thoroughly discussed by Jennings (1972) and Chang (1973).

Factors affecting the formation of tomato volatiles

Cultivar and harvest time. Quantitative differences in volatile compounds between three different cultivars of unprocessed tomatoes were studied by Nelson and Hoff (1969). They found acetaldehyde, methyl sulfide, methanol, ethanol, and isopentanal in greatest quantities in processed tomatoes of Rutgers cultivar. The cultivar, H 1350, on the other hand, contained the smallest amounts of methyl sulfide, acetone, and methanol. Acetone and methanol occurred in Roma at higher concentrations. The effect of cultivar on the amount of volatiles was also reported by Johnson et al. (1968). Rutgers and KC 146 cultivars, which were related genetically, showed similar relationships between harvests in the three volatiles measured in fresh tomato juice. Both cultivars tended to have a smaller amount of hexenol present compared with the amount of isoamylol detected. These authors (1968) also reported that fruits harvested one week apart differed considerably in the volatile contents. This may be due to variation in weather and soil conditions. Fertility, moisture, heat, and sunlight would affect the composition of volatiles. Stevens (1970a) examined the volatiles from Campbell 146 and 1327 tomato cultivars and found heritable concentration differences for 2-isobutylthiazole, methyl salicylate, and eugenol. The various genes controlling the concentration of these compounds were also investigated. Compositional variations in tomato volatiles seem to be inherent genetical characteristics which vary with the cultivar.

Growth, maturation, and ripening process. Dalal (1965) analyzed tomato fruits harvested at different stages of maturation for volatile compounds. The gas chromatograms were rather similar in both the field- and the greenhouse-grown tomatoes. The concentration of components, with the exception of isopentanal and hexanal, increased during the ripening process. Isopentanal and hexanal, presumed to give the "green leafy" aroma of tomatoes, were found in their highest concentrations at the breaker and the large-green stages, respectively. Dalal et al. (1967) found that concentrations of volatiles, except for isobutanol, hexanol, and hexanal, were higher in the field-grown than the greenhouse-grown tomatoes. The artificially ripened and greenhouse-grown tomatoes contained less volatiles than the field-grown and naturally ripened.

Shah, Salunkhe, and Olson (1969) evaluated volatiles of artificially ripened, field ripened, and overripened tomato fruits and indicated quantitative differences in certain volatiles. The differences were attributed to the environmental factors such as light, nutritional balance, and temperature.

Hypobaric or sub-atmospheric pressure storage. This is a recent approach of increasing the storage life of horticultural produce (Burg and Burg, 1966; Tolle, 1969; Dilley, 1973). This type of controlled atmosphere (CA) storage includes a reduction of atmospheric pressure, and has the same sort of effects as standard CA storage.

EXPERIMENTAL

Material

Tomatoes (experimental cultivar VF 7) were grown in a greenhouse. To study the effect of growth and maturation on enzyme activity the sample tomatoes were classified into nine stages of maturity in the following way (Dalal et al., 1965): 1 = 0.5 inch (in diameter) or below; 2 = 0.75 to 1.0; 3 = 1.00 to 1.25; 4 = 1.25 to 1.75; 5 = 2 to 3 (large green); 6 = 2 to 3 (breaker); 7 = 2 to 3 (pink); 8 = 2 to 3 (red); 9 = 2 to 3 (red ripe). The selected fruits were frozen and subsequently stored at -20 C. For the studies involving radiochemicals and manometric determination of lipoxidase activity, fresh fruits at the breaker and red stage of maturity were used, respectively. The green-wrap or large green tomatoes of uniform maturity which sank in 35 percent ethyl alcohol and floated in 25 percent ethyl alcohol were utilized for a hypobaric storage experiment. These tomatoes (cultivar DX 54) were grown in field conditions.

Chemicals

Chemicals were obtained as follows: fatty acids such as caproic, caprylic, capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids from Sigma Chemical Company, St. Louis, Missouri; ATP, NADH, and PVP (Polyvinylpyrrolidone K-30) from N. B. C. Research Biochemicals, Cleveland, Ohio; Coenzyme A (acid free) from Calbiochem, Los Angeles, California; PPO (2,5-diphenyloxazole) and Dimethyl POPOP-1,4 bis 2-(4-methyl-5-phenyloxazolyl)-benzene from New England Nuclear, Boston, Massachusetts, and

Packard Instrument Company, Incorporated, Downers Grove, Illinois, respectively.

Solvents and reagents

All solvents and reagents were purchased from the standard commercial sources.

Chromatographic adsorbents

Silica Gel G. with gypsum binder for thin-layer chromatography (TLC) was supplied by Warner-Chilcott Laboratories Instrument Division, Richmond, California. Basic alumina (pH 7.4) and magnesia were obtained from J. T. Baker, Los Angeles, California.

Radioactive compounds

Linoleic acid-U-^{14}C (1,000 mC/mM) and linolenic acid-U-^{14}C (1,000 mC/mM) were purchased from Amersham/Searle, Des Plaines, Illinois.

Preparation of enzyme extracts

A hundred grams of sample fruit were blended in a Sorvall Omni-Mixer with 100 ml Tris-HCl buffer (0.1 M, pH 7.5) for 2 minutes at 4 C. The homogenate was filtered through four layers of cheesecloth and defined as the filtered homogenate. It was then centrifuged at 10,000 X g for 10 minutes in a Servall RC-2 refrigerated centrifuge to remove the debris and cell fragments. The supernatant, a crude soluble extract, was dialyzed against the buffer at 4 C for 24 hours with changes of buffer every 6 hours. Ammonium sulfate was added to a part of the supernatant, a precipitate was collected between 30 and 70 percent of saturation, and subsequently dissolved in 15 ml of the buffer.

The various enzyme preparations were tested for enzymatic activities in terms of carbonyl compounds produced.

Protein determination

Protein concentration of various enzyme preparations was determined by the method of Wadell (1956). For this purpose, a 1 ml extract was diluted to 100 ml with 0.9 percent NaCl and the diluted mixture was read in a Beckman DB-G spectrophotometer at 215 and 225 mμ. The protein content of the 1 ml extract was calculated.

Production and determination of carbonyls

A direct spectrophotometric assay (Kachmar and Boyer, 1953) was employed to indicate an increase in carbonyls produced as a result of enzyme reaction. Solutions of various fatty acids were prepared separately in a buffer of pH 7.5 (0.1 M Tris-HCl) and were stored under N_2 to protect them from oxidation until they were used for an enzyme assay. The reaction mixture contained 3 μmoles of corresponding fatty acid and 0.1 μmole of each ATP, $MgCl_2$, CoA (acid free), and NADH in a test tube. The reaction was initiated by an addition of 1 ml of the enzyme extract. The final volume of the assay mixture was 2 ml. Unless otherwise stated, the assay system was incubated at 30 C for 3 hours with frequent agitation.

After the incubation period, enzymes were inactivated by 1 ml of 0.013 percent 2,4-dinitrophenylhydrazine in 2 M HCl. After 10 more minutes incubation at 37 C, the test tubes were removed from the water bath, 2 ml of water and 5 ml of 0.6 M NaOH were added to each test tube and were allowed to stand for 10 minutes for the color development. The intensity of the color was measured at 510 mμ by "Spectronic 20."

In the blank experiment, the reaction mixture and enzyme extract were incubated separately, mixed, and inactivated. The net increase in the absorbance was attributed to the enzymatic production of carbonyls. Experiments were conducted in duplicate and the average values were recorded.

Enzyme activity. A unit of enzyme activity was defined as a net increase in absorbance by a 0.001 unit at 510 mμ per mg protein under the specified incubation period.

Lipoxidase activity

The crude soluble extract from red fruits and the buffer, 1 ml each, were added to the manometric flask and 1 ml solution of linoleic or linolenic acid (3 μmoles) was placed in the side arm. After equilibration at 25 C in O_2 for 10 minutes, the contents were mixed. A reaction mixture replacing enzyme extract by 1 ml buffer served as a blank. Consumption of O_2 per 60 minutes for duplicate samples was recorded and averaged.

Reaction product

Enzyme reaction. To characterize the product of the enzyme reaction, the contents of the reaction mixture were increased 10 times. The reaction was carried out in a 50 ml test tube containing 10 ml of the dialyzed enzyme extract, 30 μmoles substrate, 5 μmoles $MgCl_2$, 6 μmoles ATP, 1 μmole CoA, and 3 μmoles NADH. At the end of 3 hours, the enzymes were inactivated with 5 ml of 10 percent trichloroacetic acid (TCA). The mixture was centrifuged and the supernatant was utilized further for extraction of volatiles.

Solvent extraction. The above supernatant was extracted two times with 20 ml absolute ether. The total extract was washed with 10 ml of 5 percent NaOH followed by 10 ml distilled water. The extract was treated with anhydrous Na_2SO_4 and animal charcoal to remove moisture and coloring matter, respectively. The filtered extract was concentrated to a volume of about 2 ml at 35 C.

2,4-Dinitrophenylhydrazone (DNPH-one) derivatives. Fifty mg of 2,4-dinitrophenylhydrazine (DNPH-ine) were dissolved in 5 ml ethyl alcohol (95 percent) containing 1 ml concentrated HCl. The reagent was added to the above ether concentrate. The resulting mixture was evaporated to dryness after an hour. The mixture of the derivatives was dissolved in methylene chloride and purified by passing through a column of activated (1 hour at 120 C) alumina.

Resolution of 2,4-DNPH-one of hexanal. A sample of the hydrazone derivative of hexanal for the spectral studies was obtained by a preparative TLC on Silica Gel G developed by a benzene-ethyl acetate (20:5) mixture. A band corresponding to the authentic sample (2,4-DNPH-one of hexanal) was removed from the plate and rechromatographed. The same procedure was repeated two more times for a desired separation and purification.

Absorption spectra of 2,4-DNPH-one of hexanal. Ultra-violet spectra were obtained in absolute methanol with a Beckman DB-G spectrophotometer. Infra-red spectra were recorded in chloroform with a Beckman IR 20 A.

Gas-liquid chromatography. A Micro-Tek 2500 R with dual flame ionization detector (block temperature, 210 C) connected to a Westronics recorder with a chart speed of 15 inches per hour was used for

the separation and identification of the volatiles. The ether extract of the enzyme reaction was concentrated to about 0.25 ml and 4 µl of this sample was injected into the stainless steel, 0.25 inch (outer diameter) x 18 feet column packed with 10 percent Carbowax 20 M on Chromosorb P acid-washed, 60-80 mesh. The column temperature was programmed from 75 C (10 minutes initial hold) to 150 C at the rate of 4 C/minute with a final hold at 150 C for 45 minutes. Helium was used as a carrier gas with a flow rate of 60 ml/minute. Input and output attenuation were 10 and 4 x, respectively. Hexanal peak was identified by means of an enrichment technique and retention time data.

Administration of radioactive compounds

Tissue slices (1.5 x 10 mm), the filtered homogenate, and a crude soluble extract from fruits (breaker) were incubated at room temperature with linoleic or linolenic acid (0.5 µC) for 4 hours. Reaction products were extracted with 50 ml absolute ether, concentrated, and an aliquot was used for TLC. After developing plates in benzene, a band corresponding to hexanal was scraped off and collected in a scintillation vial containing 15 ml scintillation liquid (5 gm PPO, 0.3 gm Dimethyl POPOP, and 333 ml Triton X-100 in toluene made to 1000 ml). Radioactivity was measured with a Unilux II-A Scintillation Counting System, Nuclear-Chicago.

Analysis of total linoleic acid and total linolenic acid

Preparation of samples. Fruit slices were freeze dehydrated in a freeze dryer (Hull Corporation, Hatbord, Pennsylvania). A homogenous

powder was prepared from the Wiley mill using a 20 mesh sieve. Samples were stored in plastic bags in a desiccator under vacuum.

Extraction of lipids. A 100 mg sample was blended for 5 minutes at 0 C with 20 ml chloroform and methanol mixture (2:1). The homogenate was filtered through a cintered glass funnel and the residue was washed in the filtrate with 15 ml of the same solvent two times. The total solvent extract was evaporated under vacuum at 40 C. The residue in petroleum ether (50-60 C boiling point range) was adsorbed on a column of activated magnesia (100 C) covered with 2 gm PVP and 2 gm anhydrous Na_2SO_4 and eluted with 100 ml petroleum ether. The eluate was evaporated to about 5 ml under the stream of N_2 gas and transferred quantitatively to a test tube. Further, petroleum ether was completely evaporated.

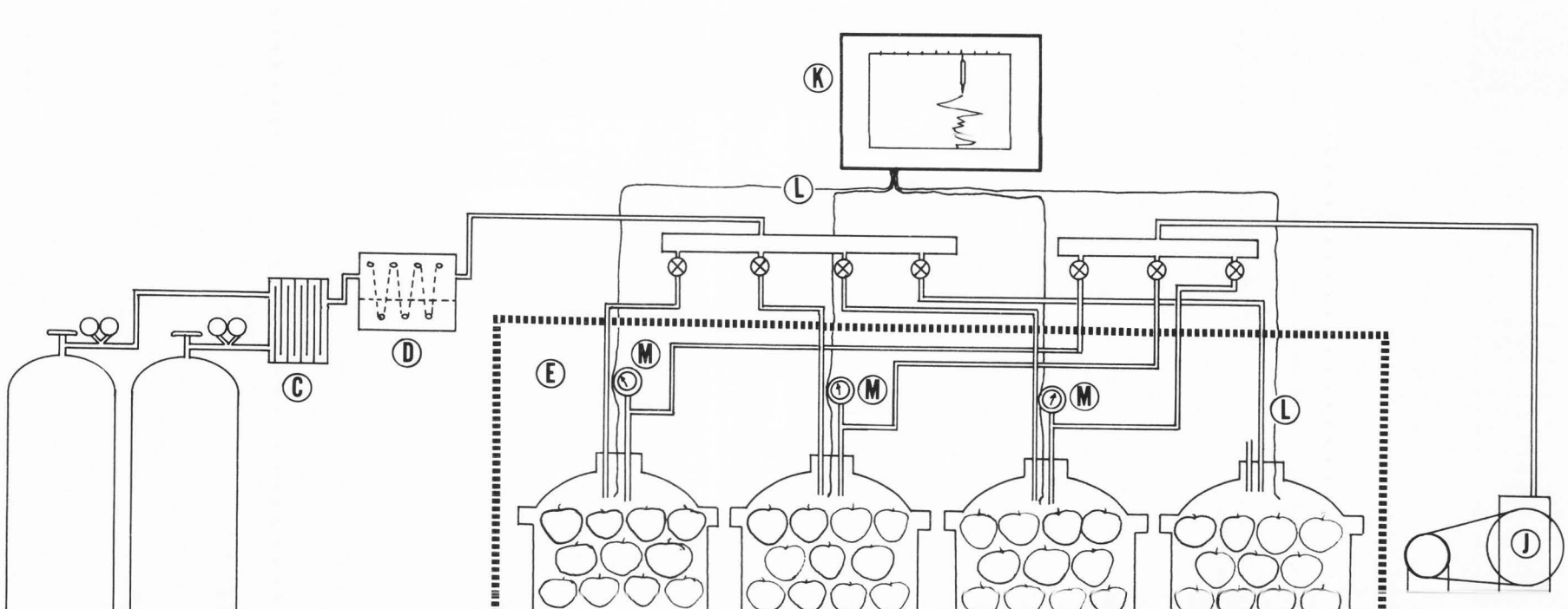
Analysis. Analysis of total linoleic and total linolenic acids was accomplished by the method of Herb and Riemenschneider (1953). The reaction test tube containing the lipid fraction was blanketed with oxygen-free nitrogen, 5 grams of 21 percent KOH-glycol reagent were added, and it was heated in an oil bath at 180 C with constant shaking. After 15 minutes, the test tube was removed from the bath, cooled rapidly in cold water, and the contents were diluted to known volume with absolute methanol until suitable spectral optical density was reached. Appropriate readings were made in a Beckman DB-G spectrophotometer at 233 and 268 mμ. The total linoleic and linolenic acid contents were calculated according to the formula. The average value of duplicate determinations was recorded and the results were expressed as mg fatty acid/100 gm wet weight of fruit.

Hypobaric or sub-atmospheric pressure storage

Pressure treatments. The fruits were subjected to three sub-atmospheric pressure treatments (471 mm Hg, 278 mm Hg, and 102 mm Hg) and one control (646 mm Hg, the atmospheric pressure at Utah State University, Logan, Utah). Both control and treated tomatoes were stored in 19-liter fruit chambers maintained at 12.8 ± 1.1 C and continuously evacuated by means of a vented-exhaust, oil-seal pump. Constant ventilation was achieved by allowing air to each chamber through a vacuum regulator (Matheson Model 49) which maintained the selected vacuum (none in the control) by flowing air to be bled into the system at a proper rate. Incoming air was saturated with moisture (containing a volatile fungicide-sec. butylamine) by passing through a humidifier. The air flow through the apparatus (Figure 23) was regulated with a valve at the rate of 30 ml per minute. By so doing, relative humidity was maintained at 90-95 percent.

The fruits were ripened (red ripe) in 35, 65, and 87 days stored at 646, 471, and 278 mm Hg pressures, respectively. The tomatoes stored at 102 mm Hg for 100 days were transferred to a 646 mm Hg pressure chamber and allowed to ripen for 7 days at 12.8 ± 1.1 C and 90-95 percent relative humidity. The red ripe tomatoes were then frozen and stored at -20 C until used for analysis of volatile compounds.

Extraction of volatiles. Tomato volatiles were extracted according to the method of Shah, Salunkhe, and Olson (1969). A 500 gm sample of tomato pulp was put into a 2-liter distilling flask. A glass tube extension from a boiling water flask was inserted to a level of 1 inch above the bottom of the distilling flask. The distillates were trapped



A, Nitrogen; B, Oxygen; C, Mixer; D, Humidifier; E, Constant temperature chamber; F, Storage container with 471 mm Hg pressure; G, Storage container with 278 mm Hg pressure; H, Storage container with 102 mm Hg pressure; I, Storage container with 646 mm Hg (atmospheric pressure at USU); J, Vacuum pump; K, Temperature recorder; L, Thermocouple; M, Vacuum gauge

Figure 23. Apparatus for storage of fruits at the sub-atmospheric pressures.

in a receiver cooled with an ice-salt mixture. Seven hundred ml of the condensate were collected, saturated with NaCl, and extracted with 350 ml purified ethyl ether. The ether extract was concentrated to 200-225 µl at 35 C.

Gas-liquid chromatography. A 1 µl sample of the ether concentrate was injected into a glass column (180 x 0.6 cm, length x outer diameter) packed with 10 percent SE-30 on Chromosorb W. acid washed 60/80 fixed to a Packard 7400 gas chromatograph with a single hydrogen flame ionization detector. The column temperature was programmed from 80 C (10 minutes initial hold) to 160 C at the rate of 4 C/minute for 20 minutes with a final hold at 160 C for 25 minutes. The selected peaks were identified on the basis of enrichment retention techniques. The concentrations of the volatiles were determined by measuring the area of individual peak.

Statistical analysis

Several tomatoes (4 to 10, depending on sizes) of the same maturity were cut into halves. Only one half from each fruit was selected for experimental use. The halves were cut into small pieces, from which samples were randomly taken. Each experiment was carried out twice, and for each experiment duplicate samples were used; the values thus obtained were averaged. Statistical analyses were computed and the means were compared according to the least significant difference (LSD) procedure (Steel and Torrie, 1960) wherever possible.

RESULTS AND DISCUSSION

Formation of carbonyl compounds

In the early stage of the present work, a preliminary experiment was carried out to investigate whether or not the enzyme preparation had activity in converting fatty acids to carbonyl compounds. For this purpose, a mixture containing 1 ml of crude soluble extract, 1 ml of Tris-HCl buffer (0.1 M, pH 7), and 1 ml of the respective fatty acid (0.001 M) was incubated at 37 C for 4 hours. The products (carbonyls) were measured. The experimental results revealed that the preparation appeared to have that activity when linoleic or linolenic acid was used as a substrate (Tables 4 and 5). The enzyme extract did not catalyze the conversion of saturated fatty acids (C_6 to C_{18}) or even monounsaturated fatty acids (C_{16}, C_{18}).

The lipoxidase enzyme is known to catalyze oxidation of unsaturated fatty acids. The presence of this enzyme in tomato fruits has been reported by Shishiyama, Araki, and Akai (1970). It specifically requires cis,cis-1,4-diolefinic unsaturated acids such as linoleic, linolenic, and arachidonic acids (Tappel, 1964). Whitfield and Shipton (1966) predicted that production of certain carbonyls (alk-2-enals, alka-2,4-dienals, and some alkanals) in stored unblanched frozen peas was due to enzymatic oxidation of linoleic and linolenic acids. The evidence for the enzymatic formation of carbonyls from unsaturated fatty acids in apple fruits has been presented by Drawert et al. (1965). In view of these evidences, it is apparent that oxidation of linoleic and linolenic acids in the presence of tomato extract could be due to

Table 4. Effect of linoleic and linolenic acids on absorbance when added to the enzyme extract from red tomatoes (maturity 8)

Substrate	Absorbance at 510 mμ[a]		Difference
	With substrate[b]	No substrate[c]	
Linoleic acid	0.19	0.11	0.08
Linolenic acid	0.20	0.10	0.10

[a]Absorbance after addition of 2,4-dinitrophenylhydrazine and alkali.
[b]The reaction mixture containing 1 ml of each substrate (0.001 M), crude soluble extract, and Tris-HCl buffer (0.1 M, pH 7.5) was incubated at 37 C for 4 hours.
[c]In control or blank experiment, the respective substrate was replaced by 1 ml of the buffer.

Table 5. Response of fatty acids to enzyme preparations

Response of fatty acids[a]	
Positive response[b]	No response[c]
Linoleic acid	Caproic acid
Linolenic acid	Caprylic acid
	Capric acid
	Lauric acid
	Myristic acid
	Palmitic acid
	Palmitoleic acid
	Stearic acid
	Oleic acid

[a]Fatty acids were tested with enzyme preparations from red tomato fruits (maturity 8).
[b]Fatty acids which indicated increase in absorbance compared to blank (formation of carbonyls).
[c]Fatty acids which indicated no change in absorbance compared to blank (no formation of carbonyls).

lipoxidase enzyme. However, enzymatic degradation of unsaturated fatty acid (linoleic or linolenic) has been questioned because of catalysis by heme compounds.

To establish the oxidation of linoleic and linolenic acids to carbonyls by either lipoxidase or heme compounds, the effect of some inhibitors on lipoxidase activity in tomato extract was studied (Table 6). It was observed that potassium cyanide had no inhibitory effect on the enzyme extract even at high concentrations. Holman (1947) and Süllman (1941) noted that potassium cyanide does not inhibit lipoxidase; on the contrary, it is an inhibitor of heme compounds (Barron and Lyman, 1938). Owing to this distinguishing property, it was confirmed that oxidation of linoleic and linolenic acids was catalyzed by lipoxidase and nonenzymatic degradation by heme compounds was eliminated. Furthermore, EDTA and p-chloromercurybenzoats were found to be ineffective inhibitors. Propyl gallate served as an antioxidant. The degree of inhibition of lipoxidase by certain polyphenols such as phloridzin, pyrocatechol, and pyrogallol is pointed out in Table 6.

Additionally, the enzymatic formation of carbonyls was demonstrated when the boiled crude soluble extract could not catalyze degradation of linoleic and linolenic acids. Hydrogen peroxide, when added at the concentration of 100 M, inhibited the enzyme system in the tomato extract (Table 10). Since this chemical is known for oxidation and inactivation of lipoxidase (Mitsuda, Masumoto, and Yamamoto, 1967), degradation of linoleic and linolenic acids to carbonyls was attributed to the lipoxidase enzyme.

In view of the current knowledge on volatiles in tomato and in some other plants, the formation of volatiles in general and carbonyls

Table 6. Effect of inhibitors on lipoxidase activity[a] in tomato fruits (red, maturity 8)

Inhibitor	Concentration x 10^{-3} M	Lipoxidase activity: Linoleic acid	Lipoxidase activity: Linolenic acid	% Inhibition of lipoxidase activity: Linoleic acid	% Inhibition of lipoxidase activity: Linolenic acid
		μl O_2 Uptake/60 min./ml enzyme extract			
1 Control		88	91	0	0
2 KCN	1.10	88	91	0	0
	0.11	88	90	0	1
	11.00	86	88	2	3
3 EDTA(disodium)	1.10	87	89	1	2
4 p-Chloromercury benzoate	1.10	88	91	0	0
5 Propyl gallate	1.10	53	46	40	49
	4.40	40	39	55	57
6 Phloridzin	1.10	67	74	24	19
7 Pyrocatechol	1.10	53	56	40	38
8 Pyrogallol	1.10	0	0	100	100
9 H_2O_2	5.50	4	6	95	93

[a]Determined manometrically using a crude soluble extract.

(aldehydes or ketones) in particular are schematically presented in Figure 24. The possibility that some of the carbonyls may be formed through α-oxidation of saturated fatty acids and subsequent oxidative decarboxylation has been suspected (Hitchcock and James, 1965). However, the inability of oxidative degradation of saturated fatty acids to carbonyls by the tomato enzyme extract in the present investigations could be due to improper experimental conditions. The hypothesis may be demonstrated by the use of labeled saturated fatty acids on a whole tomato fruit.

Reaction products

To study the reaction products of linoleic and linolenic acids, experiments were designed in which the amount of ingredients in the reaction mixture was increased to 10-fold. Hexanal, a C_6 aldehyde (carbonyl), was found to be one of the products synthesized from both linoleic and linolenic acids. It was purified as its 2,4-dinitrophenylhydrazone (DNPH-one) derivative on a preparative TLC by repeated chromatography. The identity of hexanal was confirmed by co-chromatography on a thin-layer silica gel plate, infra-red, and ultra-violet spectra of 2,4-DNPH-one derivative, and finally by gas-liquid chromatography of the ether extract of the reaction products. The physical properties of hexanal or its derivative coinciding with those of an authentic sample are presented in Table 7.

The findings in the previous experiment that linoleic and linolenic acids are precursors of hexanal were confirmed by tracer studies. A labeled carbon (^{14}C) was incorporated into hexanal when uniformly labeled linoleic or linolenic acid was incubated with crude soluble

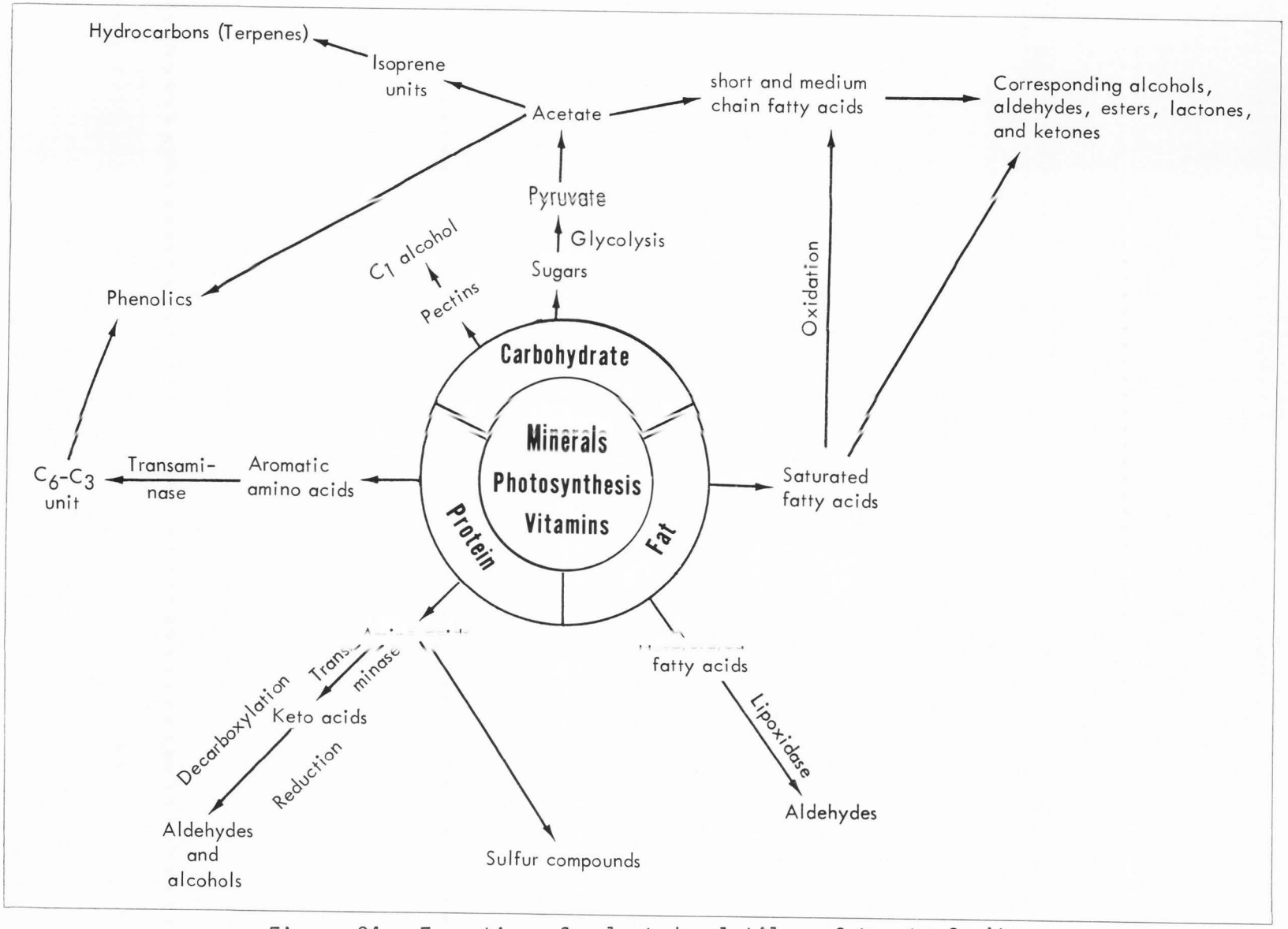


Figure 24. Formation of selected volatiles of tomato fruit.

Table 7. Physical properties of hexanal and its 2,4-dinitrophenylhydrazone (DNPH-one)

Method	Properties
GLC	Retention time,[a] 29.8 minutes
IR	Absorption bands,[b] cm^{-1}, 920, 1200, 1305, 1330, 1620, 2990, 3290
UV	Absorption bands,[b] nm, 225, 358
TLC	R_f value,[b] 0.7

[a]Physical property of hexanal.
[b]Physical property of 2,4-DNPH-one derivative.

Table 8. Incorporation of radioactivity from fatty acids into hexanal

Enzyme preparation	Linoleic acid[a]	Linolenic acid[a]
	Radioactivity in hexanal (dpm)	
1 Crude soluble extract[b]	1346 (72) [0.1213]	1372 (63) [0.1355]
2 Filtered homogenate[b]	920 (35) [0.0829]	616 (28) [0.0555]
3 Tissue slices (10 gm)	758 (40) [0.0683]	580 (31) [0.0522]

[a]Amount of linoleic or linolenic acid (1,000 mC/mM) administered to the respective enzyme preparation was 0.5 μC.
[b]Corresponds to 10 gm fresh weight of tissue slices.
Note: Values in parentheses represent standard deviation, while those in brackets represent percent of fatty acid incorporated into hexanal.

extract, filtered homogenate, and fruit slices (Table 8). Apparently, the crude soluble extract prepared from breaker tomatoes showed more activity compared with filtered homogenate and fruit slices. Since hexanal is one of the products of the enzyme reaction, a scheme of the enzymatic breakdown of linoleic and linolenic acids is presented in Figure 25. The reaction mechanism is considered to be similar to that of autoxidation (Bergström and Holman, 1948) of unsaturated oils where a chain reaction initiated by the removal of a hydrogen atom from the hydrocarbon chain leads to a conjugation of the double bond system and subsequently hydroperoxide is further degraded to various components, mainly carbonyls.

Control of carbonyl compounds

pH effect. An experiment was conducted in which the activities of the crude soluble extract (as evidenced by the production of carbonyls) prepared in two different buffer systems were compared. The substrates dissolved in the corresponding buffer were incubated at 30 C for 3 hours. As shown in Table 9, pH 7.5 was more suitable than pH 6.5 and 8.5 when the buffers were used for extraction of enzymes and for incubation medium.

Experimental conditions. Conversion of linoleic and linolenic acids to carbonyls was studied under different sets of conditions such as substrate concentration, temperature, time of incubation, and enzyme concentration (Figure 26). All these characteristics discussed here are those of the crude soluble extract of red tomatoes (maturity 8). The effect of the parameters mentioned heretofore on enzymatic activity differed with fatty acids used as substrates. In general, the production of carbonyls was more with linolenic acid rather than linoleic

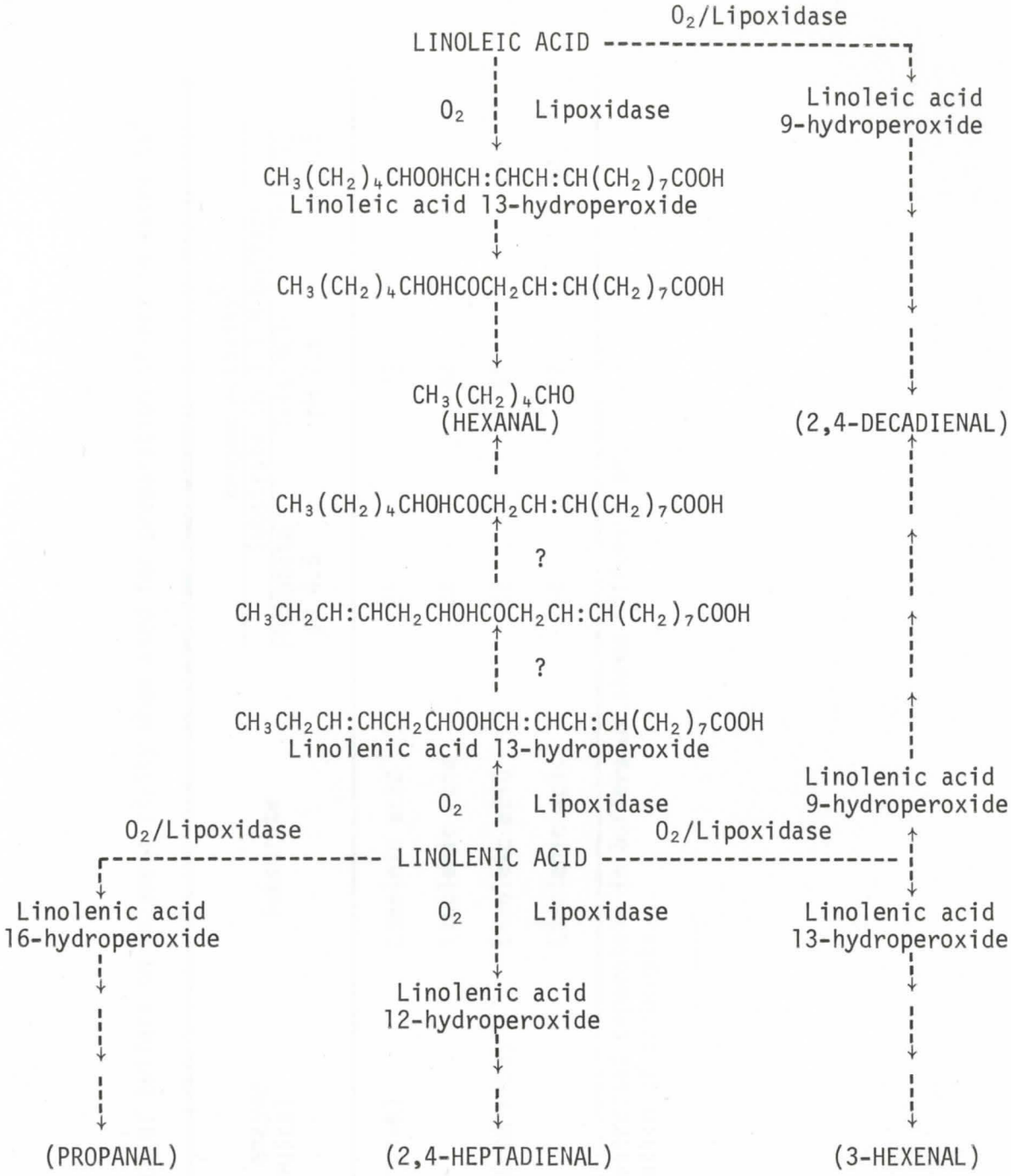


Figure 25. A possible scheme for the enzymatic degradation of linoleic and linolenic acid. (Volatile compounds in the above scheme represented in parentheses occur in tomato fruit.)

Table 9. Effect of buffers on enzyme activity when used for preparation of enzyme extracts[a]

Source of enzyme (tomato fruits)	Substrate	Enzyme activity[b] Substrate in 0.1 M buffers		
		Phosphate pH 6.5	Tris-HCl pH 7.5	Tris-HCl pH 8.5
Maturity 7 (pink)	Linoleic acid	21	75	30
	Linolenic acid	22	82	43
Maturity 9 (red ripe)	Linoleic acid	12	61	23
	Linolenic acid	14	77	42

[a]Enzymes were extracted separately in buffers of three different pH.
[b]Based on formation of carbonyls.

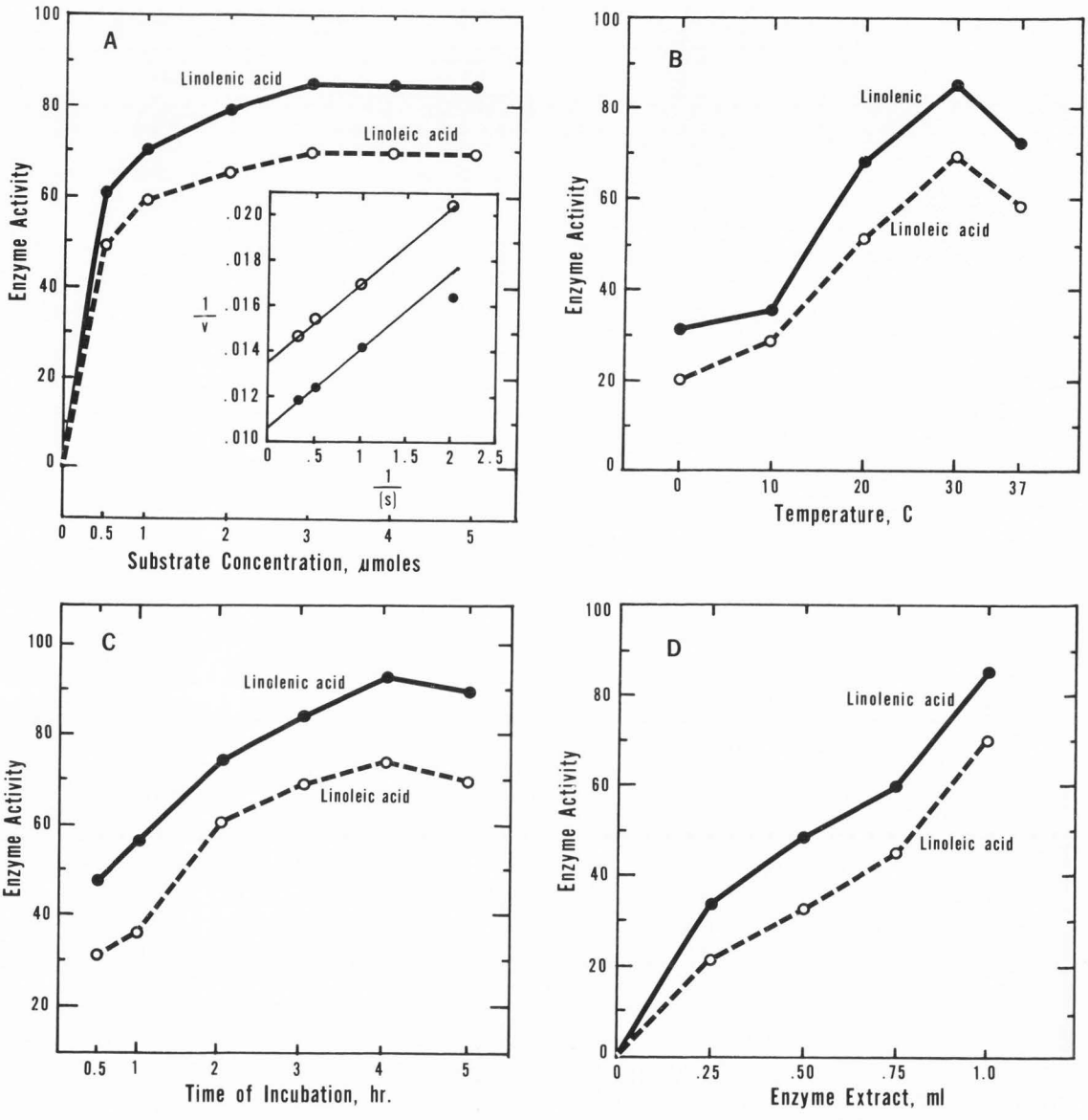


Figure 26. Effect of (A) substrate concentration, (B) temperature, (C) time of incubation, and (D) enzyme concentration on enzyme activity. (A crude soluble extract prepared from tomato fruits [red, maturity 8] with a 0.1 M Tris-HCl buffer of pH 7.5. Incubations were conducted at 30 C for 3 hours with linoleic and linolenic acid as substrates. Enzyme activity is based on formation of carbonyls.)

acid. Unless otherwise stated, incubations were conducted at 30 C for 3 hours using 3 μmoles of the substrates in a final volume of 2 ml of the reaction mixture containing 1 ml of the crude soluble extract and 0.1 μmole of each co-factor (ATP, $MgCl_2$, CoA, and NADH).

As seen from Figure 26, the enzyme activities responsible for degradation of both the unsaturated fatty acids increased with substrate concentration. The soluble fraction from tomato fruit (maturity 8) had its maximum activity at a concentration of 3 μmoles for both the substrates. At above this concentration, substrate saturation was observed. The nature of the plot of concentration vs enzyme activity is hyperbolic. The Km values for linoleic and linolenic acids are 2.53×10^{-5} M and 2.98×10^{-5} M, respectively.

The effect of temperature of incubation was studied at 0, 10, 20, 30, and 37 C. Enzymatic activities were low for incubation temperatures below 10 C. Above this temperature, activities increased very rapidly and reached their optimum at 30 C, followed by a slight decrease at 37 C. Inactivation of the crude soluble extract due to boiling was observed.

The reaction mixture containing 1 ml of the crude soluble extract, 1 ml of the respective substrate, and co-factors was incubated for different time intervals (0.5, 1, 2, 3, 4, and 5 hours) at 30 C. At the end of the incubation period, the production of carbonyls was measured colorimetrically. The plot of incubation time vs enzyme activity showed 4 hours as the optimum time for incubation of both linoleic and linolenic acids to obtain a maximum activity (Figure 26).

While selecting a proper concentration of the crude soluble extract, the volume of the reaction mixture was maintained at 2 ml by an

addition of the buffer. It is evident from Figure 26 that the rate of reaction increased almost linearly when the concentration of the enzyme extract was increased from 0.25 ml to 1 ml.

Effect of reagents. Experiments were conducted to investigate whether or not the crude soluble extract would be activated or inhibited by some reagents. The results are presented in Table 10. Metal salts such as $MgCl_2$, $MnCl_2$, $FeSO_4$, and $CuCl_2$ enhanced the rate of degradation of both the fatty acids to carbonyls by the enzyme preparations. $FeSO_4$ and $CuCl_2$ were more effective in the catalytic oxidation and the results support the fact that enzymatic degradation of linoleic and linolenic acids takes place via free radical intermediates. The requirement of free -SH groups for the enzyme action was indicated by the effects of glutathione, mercaptoethanol, and L-ascorbic acid. Moreover, the stimulation by citric and L-ascorbic acids may result from their metabolism to certain carbonyls. Although ATP, CoA, and NADH are suggested to be the cofactors in the degradation of unsaturated fatty acids to CO_2 (Davies, Giovanelli, and Ap Rees, 1964), no significant effects of those compounds were noted in this experiment.

Maturity of tomato fruits. Enzyme preparations from tomatoes harvested at various stages of maturity showed differential activities in converting linoleic and linolenic acids to carbonyl compounds. As shown in Figure 27 (top), activities were low for extracts from young fruits. As the fruit developed, enzymatic activities appeared to increase. In general, activities were greater in the ripe fruits (maturities 6, 7, 8, and 9) than in the green fruits (maturities 2, 3, 4, and 5). This was augmented by the fact that the amounts of unsaturated fatty acids decreased with ripening (Figure 27, bottom).

Table 10. Effect of various reagents on enzymes from tomato fruits (red, maturity 8)[a]

Reagent	Concentration (μmoles)	Substrate	
		Linoleic acid	Linolenic acid
		Absorbance increase at 510 mμ[b]	
$MgCl_2$	5.0	0.044	0.021
$MnCl_2$	5.0	0.064	0.021
$FeSO_4$	1.0	0.135	0.190
$CuCl_2$	1.0	0.140	0.175
GSH	3.0	0.022	0.020
EtSH	3.0	0.024	0.026
L-Ascorbic acid	3.0	0.122	0.104
Citric acid	3.0	0.040	0.036
H_2O_2	100	-0.096	-0.096

[a]Each assay mixture contained 0.5 ml of crude soluble extract, 0.5 ml of reagent, and 1 ml substrate (3 μmoles).
[b]Increase in absorbance after addition of 2,4-DNPH-ine and alkali.
Abbreviations: GSH, glutathione; EtSH, mercaptoethanol.

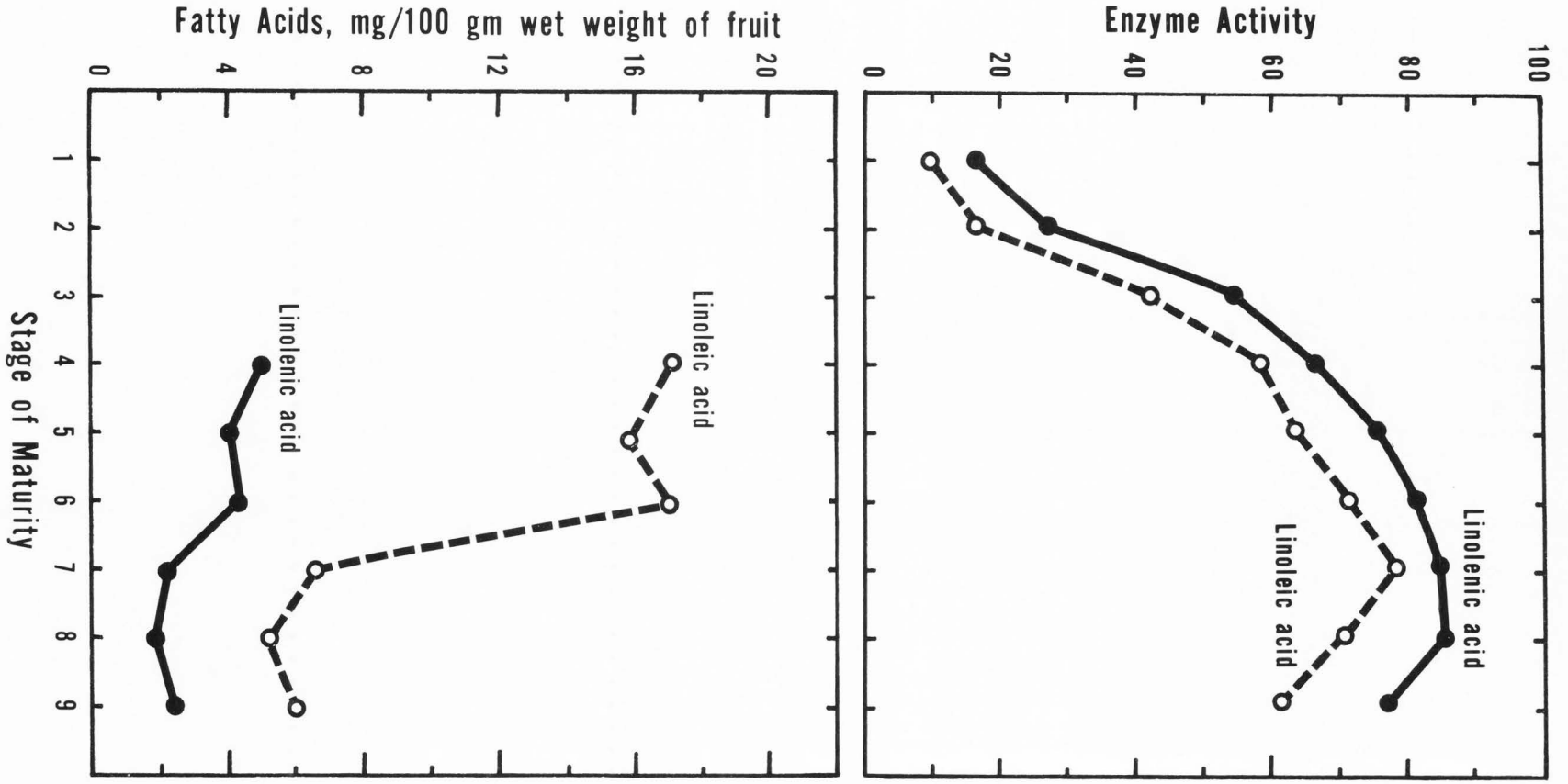


Figure 27. Effect of various stages of tomato fruits on enzyme activity (top) and the amounts of linoleic and linolenic acids (bottom). (Enzyme activity is based on formation of carbonyls.)

These findings are consistent with the findings of Dalal et al. (1968) that the rates of biosyntheses of the major volatile compounds of tomato fruit increase with the growth of the fruit. Apparently, synthesis of the enzyme system responsible for metabolism of unsaturated fatty acids seems dependent on the stage of maturity. It is also apparent that as the fruit ripens, more intricate enzyme systems become operative and utilize several kinds of substrates in the process of synthesizing volatile compounds (Shah, Salunkhe, and Olson, 1969).

Enzyme activity in different preparations. A comparative study was made on the capability of different enzyme preparations to catalyze the oxidation of unsaturated fatty acids. The enzyme fractions, such as filtered homogenate, crude soluble extract, dialyzed extract, ammonium sulfate fraction and its supernatant were obtained as described previously in the experimental section. The dialyzed extract and the ammonium sulfate fraction showed higher specific activities than the filtered homogenate, crude soluble extract, and the supernatant in the salt fractionation (Table 11). Evidently, certain low molecular weight inhibitors present in the crude extract were removed by dialysis.

The homogenate of tomato fruits (red ripe, maturity 9) was prepared as usual and the soluble fraction was obtained by centrifuging the filtered homogenate at different levels of gravitational forces for 10 minutes. The rates of degradation of linoleic and linolenic acids to carbonyls by the soluble fractions were compared with those of corresponding insoluble fractions. The results indicated more activities in the insoluble fractions than those in the soluble fractions (Figure 28).

Hypobaric or sub-atmospheric pressure storage. The patterns of the development of aroma components under the conditions of low pressures

Table 11. Steps in purification of enzymes from tomato fruits (red, maturity 8)

Steps	Substrate	
	Linoleic acid	Linolenic acid
	Enzyme activity[a]	
Filtered homogenate[b]	47	52
Crude soluble extract[c]	69	87
$(NH_4)_2SO_4$ fraction[d]	91	120
Supernatant[e]	19	24
Dialyzed extract[f]	150	162

[a]Based on formation of carbonyls.
[b,c,d,f]As defined in the experimental part.
[e]The supernatant in step d.

(102, 278, and 471 mm Hg) and atmospheric pressure (control, 646 mm Hg) are presented in Figure 29 and the analytical data of selected carbonyls (acetaldehyde, 2-methyl propanal, butanal, 3-methyl butanal, and hexanal) and some other volatiles are expressed in Table 12. An inverse relationship between the amount of volatiles and vacuum was observed. Compared to control, the fruits under the lowest pressure (102 mm Hg) for 100 days and subsequent ripening at atmospheric pressure had the least amount of aroma components.

According to Dalton's law the partial pressure of each gas in the storage chamber was reduced because of reduction in the total pressure. Consequently, ethylene, other gases, and volatile compounds produced by the fruits were removed by a continuous evacuation of air.

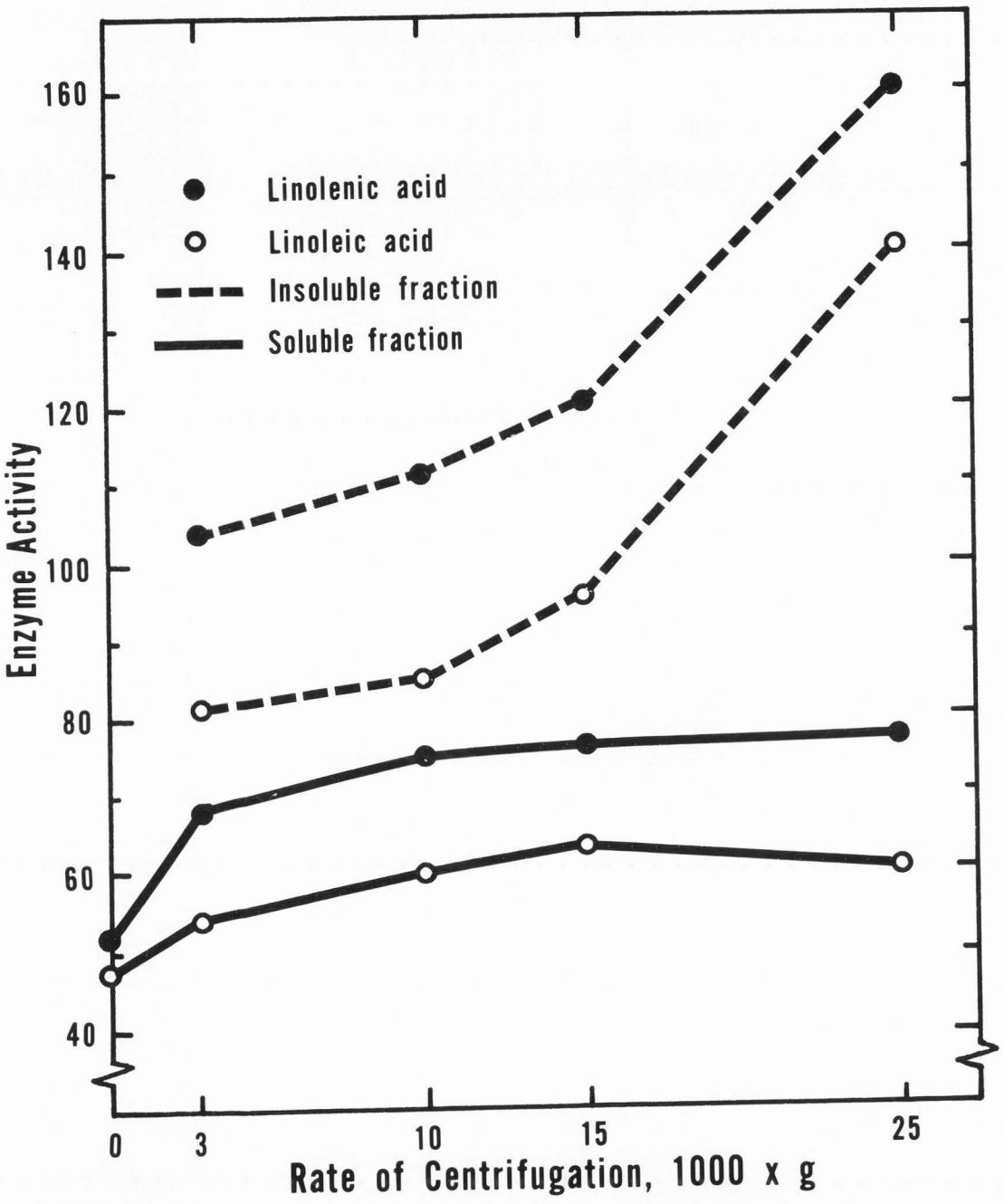


Figure 28. Comparison of enzyme activities between soluble and insoluble fractions of enzyme extracts obtained by a differential centrifugation. (The enzyme extracts were prepared from tomato fruits [red ripe, maturity 9]. Centrifugation was conducted at the stated rate for 10 minutes. Enzyme activity is based on formation of carbonyls.)

Figure 29. Gas chromatograms (SE-30 column) of the volatiles from tomatoes subjected to different levels of pressures.

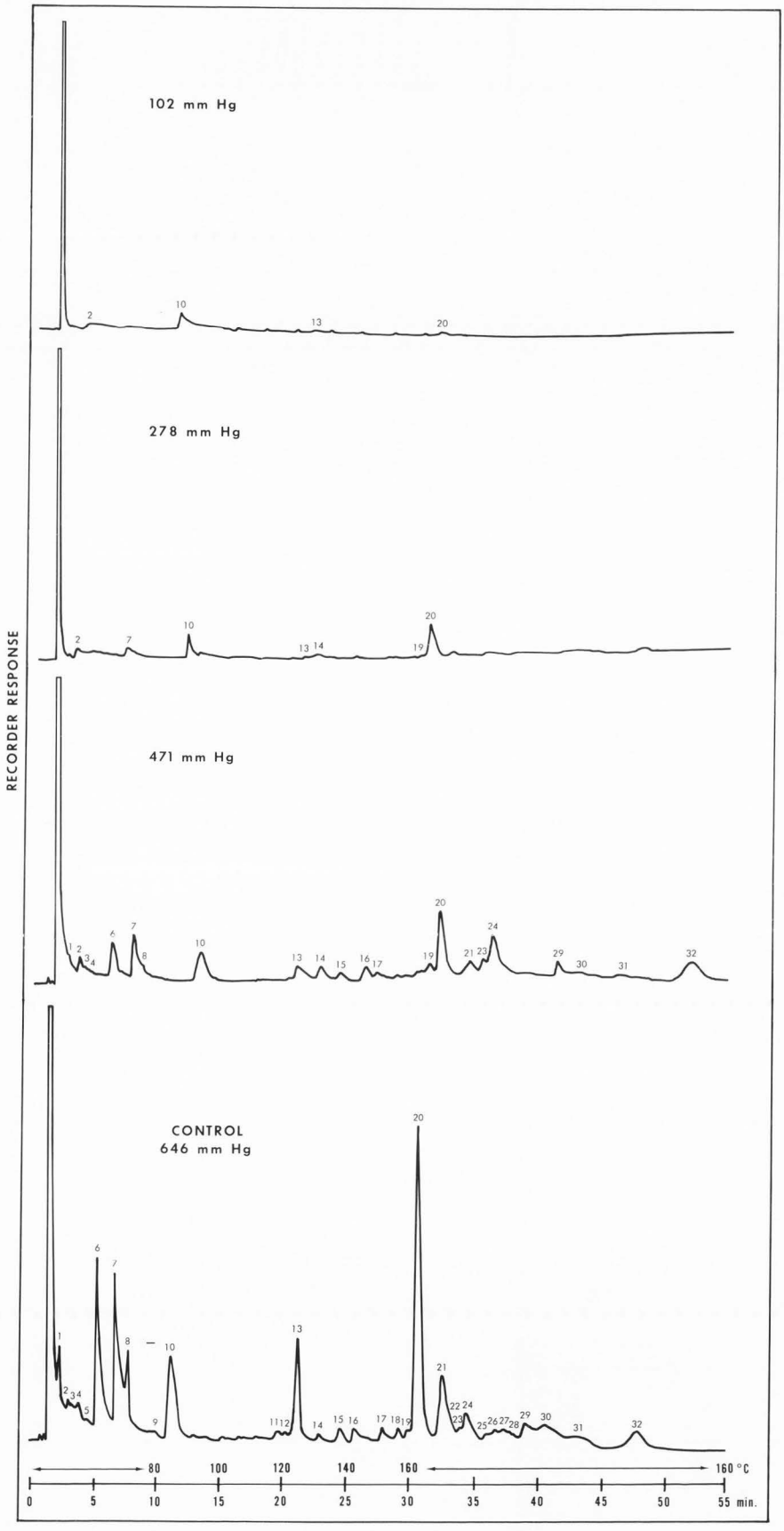

102 mm Hg
278 mm Hg
471 mm Hg
CONTROL
646 mm Hg
RECORDER RESPONSE
80
100
120
140
160
160 °C
0
5
10
15
20
25
30
35
40
45
50
55 min.

Table 12. Effect of sub-atmospheric pressure storage on the concentration of tomato volatiles

Peak no.	Compound[b]	Peak areas mm^2[a]			
		646 mm Hg	471 mm Hg	278 mm Hg	102 mm Hg
1	Acetaldehyde	32	14	6	1
6	2-Methyl propanal	99	29	2	1
7	Methanol	60	34	11	2
8	Butanal	28	6	2	-
10	3-Methyl butanal	99	42	9	3.6
13	Hexanal	60	19	2	1
14	Butanol	3	2	1	-
15	Isopentyl acetate	9	6	2.4	1
17	2-Methyl butanol	5	3	1.6	1
20	2-Methyl-3-hexanol	201	84	26	6
26	Hexanol	6	4	1.8	-
32	Linalool	72	42	18	2

[a]Average of duplicate results.
[b]Identified on the basis of enrichment retention techniques.

SUMMARY AND CONCLUSIONS

Enzyme preparations from tomato fruits catalyzed the conversion of linoleic and linolenic acids to carbonyl compounds. The formation of carbonyls was attributed to lipoxidase enzyme. Hexanal was found to be one of the products of the enzyme reaction. The biogenetic relation was confirmed by the use of labeled fatty acids.

The production of carbonyl compounds as affected by pH, temperature, time of incubation, concentration, activators and inhibitors, maturity of fruits, and partial purification of the enzyme extract was investigated. Additionally, effect of storage of tomato fruits under reduced pressures on the concentration of certain carbonyl compounds is discussed.

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APPENDIX

Table 13. Analysis of variance (ANOVA) of alcohol fraction (dpm x 1000) and alkaloid fraction[a] (dpm x 1000) corresponding to Table 2

Source	df	SS	MS	F	0.01
Treatment	3 (3)	2141700 (951.12)	713900 (317.04)	76.01 (29.19)	5.95 (5.95)
Error	12 (12)	112700 (130.33)	9392 (10.86)		
Total	15 (15)	2254400 (1081.45)			

LSD: 19.14 at 0.01 level
(7.12) at 0.01 level

[a]ANOVA of alkaloid fraction presented in parentheses.

Table 14. Analysis of variance (ANOVA) of chlorophyll and solanine contents[a] corresponding to Figures 11 and 12, respectively

Source	df	SS	MS	F	0.01
Treatment	19 (19)	290.05 (6924)	15.26 (364.42)	669.29 (109.43)	2.23 (2.23)
Error	60 (60)	1.37 (200)	0.0228 (3.3)		
Total	79 (79)	291.42 (7124)			

LSD: 0.28 at 0.01 level
(3.216) at 0.01 level

[a]ANOVA of solanine contents presented in parentheses.

Table 15. Analysis of variance (ANOVA) of chlorophyll and solanine contents[a] corresponding to Figure 13

Source	df	SS	MS	F	0.01
Treatment	4 (4)	82.52 (9203.39)	20.63 (2300.08)	5157.5 (85.6)	4.89 (4.89)
Error	15 (15)	0.06 (403.1)	0.004 (26.87)		
Total	19 (19)	82.58 (9606.4)			

LSD: 0.131 at 0.01 level
(10.8) at 0.01 level

[a]ANOVA of solanine contents presented in parentheses.

Table 16. Analysis of variance (ANOVA) of chlorophyll and solanine contents[a] corresponding to Figure 17

Source	df	SS	MS	F	0.01
Treatment	9 (9)	190.001 (22037)	21.11 (2448.5)	942.41 (695.59)	3.07 (3.07)
Error	30 (30)	0.672 (105.63)	0.0224 (3.52)		
Total	39 (39)	190.673 (22142.63)			

LSD: 0.29 at 0.01 level
(3.65) at 0.01 level

[a]ANOVA of solanine contents presented in parentheses.

Table 17. Analysis of variance (ANOVA) of chlorophyll and solanine contents[a] corresponding to Figure 18

Source	df	SS	MS	F	0.01
Treatment	9 (9)	184.998 (22656.10)	20.55 (2517.34)	987.98 (293.05)	3.07 (3.07)
Error	30 (30)	0.625 (257.62)	0.0208 (8.59)		
Total	39 (39)	185.623 (22913.72)			

LSD: 0.286 at 0.01 level
(5.70) at 0.01 level

[a]ANOVA of solanine contents presented in parentheses.

Table 18. Analysis of variance (ANOVA) of chlorophyll and solanine contents[a] corresponding to Figure 20

Source	df	SS	MS	F	0.01
Treatment	5 (5)	99.73 (10195.17)	19.94 (2039.03)	11729 (268.54)	4.29 (4.29)
Error	18 (18)	0.03 (136.69)	0.0017 (7.59)		
Total	23 (23)	99.76 (10331.86)			

LSD: 0.084 at 0.01 level
(5.61) at 0.01 level

[a]ANOVA of solanine contents presented in parentheses.

Table 19. Analysis[a] of variance (ANOVA) of linoleic and linolenic acid contents[a] corresponding to Figure 27 (bottom)

Source	df	SS	MS	F	0.01
Treatment	5 (5)	686.94 (7.93)	137.388 (1.586)	262.2 26.43	4.28 (4.28)
Error	18 (18)	9.93 (1.08)	0.524 (0.06)		
Total	23 (23)	696.37 (9.01)			

LSD: (1.473) at 0.01 level
(0.498) at 0.01 level

[a]ANOVA of linolenic acid content presented in parentheses.

VITA

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Candidate for the Degree of

Doctor of Philosophy

Dissertation: Formation and Control of Chlorophyll, Solanine Alkaloids, and Sprouts of Potato (Solanum tuberosum L.) Tubers and Carbonyl Compounds of Tomato (Lycopersicon esculentum Mill.) Fruits

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ABSTRACT

Formation and Control of Chlorophyll, Solanine Alkaloids, and Sprouts of Potato (*Solanum tuberosum* L.) Tubers and Carbonyl Compounds of Tomato (*Lycopersicon esculentum* Mill.) Fruits

by

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Part I. Formation and control of chlorophyll, solanine alkaloids, and sprouts of potato (*Solanum tuberosum* L.) tubers

Incorporation of radioactive carbon from acetic acid-2-^{14}C (sodium salt), β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C, L-leucine-U-^{14}C, L-alanine-U-^{14}C, and D-glucose-U-^{14}C into the predominant glycosidic steroidal alkaloids, α-solanine and α-chaconine of potato sprouts was 4.88, 9.0, 15, 24, and 20 times less than that of mevalonic acid (MVA)-2-^{14}C (DBED salt), respectively. The efficiency ratio revealed that β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C was incorporated via acetate or acetoacetate. The distribution of radioactivity originated from D-glucose-U-^{14}C was nearly nine times higher in the glycoside moiety than that in the aglycone part of the glycoalkaloids. Apparently, Alar (succinic acid 2,2-dimethylhydrazide), Ethrel or Ethephon (2-chloroethylphosphonic acid), and Telone (1,3-dichloropropene and related

chlorinated hydrocarbons) significantly reduced the rate of incorporation of β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C into the alkaloids.

A catalytic conversion of solanidine and UDP-glucose-U-^{14}C to β-glucoside by the enzymatic system in a suspension of potato slices and the enzyme preparation from sprouts demonstrated the presence of β-glucosyltransferase in Solanum tuberosum L. Stepwise synthesis of α-solanine and α-chaconine from solanidine in potato tubers or sprouts seems possible.

Formation of solanine alkaloids in peeled potato slices was stimulated when stored at 15 and 24 C in dark or light (200 foot-candles). The slices held under light developed nearly three to four times more alkaloids than those held in the dark. Significantly higher concentrations of solanine alkaloids were formed in the late stage (after 24 hours) than in the early stage of the storage period. Hence, it can be concluded that when potatoes are sliced for chips or French fries, they should be processed immediately, before the glycoalkaloids are synthesized in higher concentrations.

Post-harvest application of chemicals, such as Phosfon (tributyl 2,4-dichlorobenzylphosphonium chloride), Phosfon-S (tributyl 2,4-dichlorobenzylammonium chloride), Amchem 72-A42 [2-(p-chlorophenylthio)-triethylamine], Amchem 70-334 or CPTA [2-(p-chlorophenylthio)-triethylamine hydrochloride], Nemagon (1,2-dibromo-3-chloropropane), and Telone (1,3-dichloropropene and related chlorinated hydrocarbons) at the concentrations of 250, 500, and 100 parts per million (ppm) in water; glycerin (10, 20, and 30 percent weight by volume [w/v] in water); and mineral oil (1.25, 2.5, 5, 10, 15, 20, and 100 percent [w/v] in ether or petroleum ether) significantly inhibited the formation of chlorophyll

and solanine alkaloids in the peripheral (periderm and outer parenchyma) zone of potato tubers exposed to a fluorescent light (200 foot-candles) for 6 or 7 days at 16 C and 60 percent relative humidity. The rates of inhibition increased with concentration of chemicals studied. A 10 percent solution of mineral oil was the minimum required concentration for effective control of chlorophyll and solanine alkaloids. The tubers dipped in 10 percent mineral did not develop chlorophyll on exposure to light (200 foot-candles) for 4 weeks, while the overall rate of inhibition of alkaloids was significantly high. In general, oil treatments were the most effective in controlling the formation of chlorophyll, solanine alkaloids, and sprout growth.

Part II. Formation and control of carbonyl compounds of tomato (Lycopersicon esculentum Mill.) fruits

Incubation of unsaturated fatty acids such as linoleic and linolenic acids with the crude soluble extract from tomato fruits produced carbonyl compounds. The enzyme preparations did not catalyze the conversion of saturated or monounsaturated fatty acids to carbonyls. Inability of potassium cyanide to inactivate the crude soluble extract proved that degradation of these fatty acids was mediated by lipoxidase and nonenzymatic oxidation by heme compounds was eliminated. These findings were supported by the fact that hydrogen peroxide, an inhibitor of lipoxidase enzyme, had inhibitory effects on the degradation of linoleic and linolenic acids by the tomato extract.

Hexanal was found to be one of the products of the enzyme reaction. The identity of hexanal was confirmed by comparing the physical properties such as retention time, infra-red and ultra-violet absorption bands,

and R_f value with those of an authentic sample. Biogenesis of hexanal from linoleic or linolenic acid was further substantiated by the use of uniformly labeled ^{14}C isotopes of these fatty acids with the crude soluble extract, filtered homogenate, and tissue slices.

Maximum activities (as evidenced by the production of carbonyls) were observed in the extract prepared with and incubated in a buffer medium of pH 7.5 (0.1 M, Tris-HCl). The degradation of linoleic and linolenic acids was maximum at 30 C when incubated for 4 hours with 1 ml of the crude soluble extract. The enzymatic activity was enhanced by metal ions and compounds containing free -SH groups. Increase in the production of carbonyls by addition of citric and L-ascorbic acid may result from their metabolism. In general, ripe fruits contained greater enzymatic activities but smaller amounts of linoleic and linolenic acids than green fruits. The activity of the crude extract was increased by dialysis and the ammonium sulfate fractionation between 30 and 70 percent saturation. The rates of degradation of linoleic and linolenic acids catalyzed by the insoluble fractions of tomato extracts were more than those by the corresponding soluble fractions.

Tomato fruits (green-wrap or large green) stored under hypobaric or sub-atmospheric pressures were analyzed for their volatiles after ripening. The concentrations of selected carbonyls (acetaldehyde, 2-methyl propanal, butanal, 3-methyl butanal, and hexanal) and some other volatiles decreased substantially with decrease in storage pressure.

(154 pages)