3-1966

Effects of gamma ray irradiation on pigment betanin (betacyanins and betaxanthins) and its influence on color of beta vulgaris

B. P. Poovaiah

To the Graduate Council:

I am submitting herewith a thesis written by B. P. Poovaiah entitled "Effects of gamma ray irradiation on pigment betanin (betacyanins and betaxanthins) and its influence on color of beta vulgaris." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Melvin R. Johnston, Major Professor

We have read this thesis and recommend its acceptance:

Ivon E. McCarty, M. C. Bell

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

March 9, 1966

To the Graduate Council:

I am submitting herewith a thesis written by B. P. Poovaiah entitled "Effects of Gamma Ray Irradiation on Pigment Betanin (Betacyanins and Betaxanthins) and Its Influence on Color of Beta vulgaris." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

Major Professor

We have read this thesis and recommend its acceptance:

J. E. McCarty

M. C. Bell

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

EFFECTS OF GAMMA RAY IRRADIATION ON PIGMENT BETANIN (BETACYANINS AND BETAXANTHINS) AND ITS INFLUENCE ON COLOR OF *BETA VULGARIS*

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

B. P. Poovaiah

March 1966

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. Melvin R. Johnston, Head of the Department of Food Technology, and Professor Ivon E. McCarty for their advice and criticism throughout this study; to Dr. M. C. Bell, Subject Leader of Animal Nutrition, UT-AEC Research Station, Oak Ridge, for kindly making available laboratory space, facilities and counsel throughout the project; to Mr. James E. Johnson, President, and Mr. B. J. Byrd, General Manager of Southern Drug Manufacturing Company, for their constant encouragement and cooperation throughout this study; to Mr. Carroll D. Shell, Mrs. E. L. Russell and their colleagues of UT-AEC Histology Laboratory for their suggestions in the preparation of slides; to Dr. B. M. Anderson, Department of Biochemistry, for rendering facilities during the process of preliminary studies; to Mrs. G. Reed for her valuable assistance in the preparation of photomicrographs; to Mr. Ron Helms and his colleagues for their technical assistance in the operation of the IBM 7040 computer and to the staff of the reference library for their kind and considerate help in finding reference materials.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

LIST OF PLATES

CHAPTER I

INTRODUCTION

Radiation processing of food materials has been a subject of interest in recent years because of its ability to inactivate enzymes and destroy microorganisms. Radiation may, however, change the color, flavor, and texture and therefore the acceptability of a food. Its exact effect depends upon the food in question, the processing conditions and the total radiation dose employed. Use of heavy particle radiation like alpha, neutrons, protons and deuterons in food products must be excluded since they have the further property of bringing about nuclear transformation. Also, with these heavy particles at their normal energy levels, penetration is small. The gamma ray is not a particle. It is a non-corpuscular electromagnetic radiation of extremely short wavelength. It is similar to X-ray and is a highly penetrating ray. Hence, it has a tremendous possibility in pasteurization; as well as, in sterilization of foods.

The potential value of radiation to food preservation is unlimited and hardly touched. A particular advantage of irradiation is that it does not appreciably raise the temperature of the food during the treatment. Thus perishable fruits and vegetables can be preserved in their fresh state.

Betanin is the conspicuous pigment of the root, Beta vulgaris, comprised of red-violet group of pigments called betacyanins and the

other yellow group of pigments known as betaxanthins (26). These two pigments occur in the cell sap (vacuoles) and thus belong to the chymochromes (113). When either of these two pigments occur alone, the pigmented part of the plant is either red-violet or yellow. They are also often found together and thus color of the pigmented part of the plant may vary from violet-red, red, fire red, orange-red, and orange to yellow, depending on the relative amounts of the two types of pigments (26).

Several investigators have shown that the high level of irradiation causes visible bleaching of fruits and vegetables. The intensity of the pigmentation in fruits and vegetables has an important relationship to the commercial value of irradiated products.

This investigation was undertaken to study the effect of gamma radiation on the pigment betanin at and above the pasteurization dose levels; and its relative influence on color, ascorbic acid, enzymatic activity and histological structure.

CHAPTER II

REVIEW OF LITERATURE

I. THE PIGMENT BETANIN

In 1918 Schudel (111) coined the word "betanin" and its sugar-free moiety, betanidin. He also reported that the red pigment (betanin) of Beta vulgaris is associated with nitrogen and thus differs from common water soluble red pigment known as anthocyanins. The betanin is similar to anthocyanins in that it contains a saccharide (glucose) and displays different solubilities in various solvents (60). Nitrogen containing anthocyanins are reported to be present in plants other than Beta vulgaris (65, 88). The structure of betanin or other nitrogen containing anthocyanins are not known (4, 112). The anthocyanins are the only class of flavonoid groups credited with members containing nitrogen. According to Peterson and Joslyn (79) betanin is a pyrrole pigment and not a nitrogenous anthocyanin. This does not preclude the presence of nitrogenous anthocyanins of other plant species (102). Dreiding (26) has pointed out that many of the similarities of betanin with anthocyanins and flavones are superficial and it is no longer permissible to classify betanin as nitrogenous anthocyanins as was once believed.

Among the two groups of betanin, betacyanins have received much more attention than the betaxanthins. Very little is known about the

betaxanthins beyond their distribution and that they usually accompany the betacyanins (26). The formulas hitherto proposed for betanin, are all based on the assumption that betanin really is an anthocyanin derivative, so far there has been no proof of that assumption (41).

II. PIGMENTS IN FRUITS AND VEGETABLES

The color of fruits and vegetables is exceedingly important to our pleasure at the table. Most of the pigments occur in plastides, (specialized bodies lying in the protoplasm of the cell). Occasionally a pigment may be present in the protoplasm as a crystal. Sometimes the water-soluble pigments are dissolved in the vacuoles and not generally distributed throughout the cell.

The chief pigments of fruits and vegetables are:

1. The carotenoids, a group of yellow, orange and orange-red fat soluble pigments. These are a mixture of three isomers, α-β- and γ-carotine. Lycopine also comes under this category but differs from carotine isomers in the cyclization of the end carbons. Carotenoids which contain hydroxyl groups are called xanthophylls.

2. The chlorophylls are a group of green pigments. Chlorophyll has been isolated in two forms chlorophyll "a" and chlorophyll "b". Chemically, they are very similar. They belong to the group of important biological pigments porphyrins, which includes hemoglobin. These pigments are fat soluble.

3. The flavonoids are the group of compounds most widely distributed in the plant kingdom. They are water soluble and often

present in the juices of plants. The true flavonoids consist of the anthocyanins which constitute the red-blue purple pigments of plants; the anthoxanthins are yellow in color. The catechins and the leucoanthocyanins, the last two groups of compounds, are colorless but readily change to brownish pigments. They are the so-called food tanins.

4. The anthocyanins, most of the red, blue and violet pigments that occur in flowers, fruits and other parts, occur in plant cells as glycosides which are ethers of monosaccharides. Sometimes with one monosaccharide moiety and sometimes with two, the color results from the structure of the anthocyanidin which is combined with the monosaccharides. The carbohydrates commonly bonded to the anthocyanidins are glucose, galactose, rhamnose and occasionally a fructose. Most of the anthocyanins are soluble in water.

Only three types of anthocyanidins have been identified in plant tissues, although a number of methyl derivatives of these three have been isolated. The three types of anthocyanidins are pelargonidin, cyanidin, and delphidin.

Anthocyanin groups of pigments are sensitive to pH. At high pH' values, the anthocyanins pass through a violet and then blue color. Some turn green and then yellow at very high pH' values. At low pH' values these pigments are red but the hues may be different. Thus pelargonidin is orange-red in acid solution, while delphianidin is a bluish red (68, 61).

III. PHENOMENA OF COLOR

Relationship Between Molecular Structure and Color Theory

If radiant energy could be spread out in a spectrum-like fashion, the following classifications of radiation would be found, progressing in order from the longer wavelengths to the shorter wavelengths: radio-wave, heat wave, infrared light, visible light, ultraviolet light, X-rays, and gamma rays. The human eye is sensitive only to the small portion of energy between the wavelengths of 800 mμ and 400 mμ (100).

A mixture of all wavelengths in the visible range having equal relative intensities produced by a body at white heat temperature is known as "white light." If the light striking the retina of the eye does not contain all of the wavelengths in the visible spectrum, or if the intensity of some of them is reduced considerably, the sensation of color results. Light striking the retina of the eye may produce a sensation of color because (1) only a limited region of the visible spectrum is emitted by light source, or because (2) source of the wavelengths are absorbed either by passage through a transparent medium or by reflection from an opaque substance. The visual color of a material is complimentary to the color absorbed and in the sensation of the wavelengths striking the object minus the wavelengths absorbed (77).

Pigments owe their color to the absorption of visible light. The absorption of light acts to excite molecules to discrete energy levels. However, light is quantized in units of energy called "light quanta" or "photons," and the amount of energy per quantum varies

inversely with the wavelength of the light. The wavelengths which will be absorbed by a molecule are determined by the energy differences between the excited and unexcited levels of molecules (42).

Excitation of a molecule by visible light involves principally an increase in the electron energy of the molecule. The ease of excitation of a molecule is dependent upon the relative electron mobility within the structure. Electrons bonding the atoms of saturated molecules are strongly stabilized and only excited by high-energy radiation well within the ultraviolet range. However, the more mobile electrons as the non-bonding electrons of sulfur, oxygen, and nitrogen, the unpaired electrons of free radicals, and the pi electrons associated with double bonds as in carbon to carbon, are excited more easily. All colored organic molecules contain one or more doubly bonded groups such as -N=N >C=N >C=O, and -N=O which are called "chromophores." The presence of "chromophores" in molecules is necessary for color but is not always sufficient to lower the energy requirement for excitation to a level within the visible spectrum (42).

In the discussion of color the term "deepening of color" is synonymous with "bathochromic" change and means the progressive migration of absorption bands (increase in wavelength) from the violet region through the red end of the spectrum or a change in color from green-yellow to yellow to orange to red to purple to violet to indigo to blue to blue-green to green. Shifts in the opposite direction are called "hypsochromic" (42).

The effect of increased conjugation is generally bathochromic. The condensation of aromatic rings is usually bathochromic; however, when aromatic rings are added to fuleven or quinone units the shift is hypochromic. The effect of methyl substitution is an alternate system and is usually bathochromic. (An "alternate" system is a conjugated system in which it is possible to "start" every other carbon atom in a way that every starred carbon atom is completely surrounded by unstarred carbon atoms and vice versa). Any distortion in the spatial relationships of a color molecule which impairs the conjugation, as may result from a substitution, serves to insulate portions of the molecule and may shift the color in either direction but almost always causes a loss in strength (42).

The effect of any condition which concentrates the charge in a molecule is usually bathochromic. "Auxochromes" are groups which have no chromophoric properties, but produce bathochromic effects when substituted in a molecule, by increasing its polarizability. The auxochrome groups include (1) the electron donating (basic, ortho- and para-directing) groups: $CH_3 < Cl < Br < OH < OCH_3 < NH_2 < O^-$, and (2) the electron acceptor (acidic, meta-directing) groups: $NH_3^+ < SO_2 < NH_2 < CO_2{=}CN < COOH < COCH_3 < CHO < NO_2$. The groups which partake of the resonance of the conjugated system into which they are substituted are most effective. Two auxochromes so placed that they interact through a conjugated system may produce an effect greater than the sum of their individual effects. If an auxochrome is forced out of

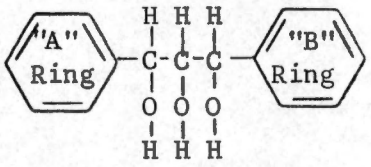
coplanarity with the molecule by ortho substitution, its effect is decreased (42).

An organic molecule can show its complete, unperturbed spectrum only when isolated as in the vapor state at low pressure. The spectrum of a color molecule in situ is subject to alteration due to interactions of the molecule with other molecules with which it is closely associated. Aggregation, adsorption, solvent effects, or metalization (especially if the metal is bound to the dye in a covalent chelate) may have strong effect on the color of the molecule (42, 53).

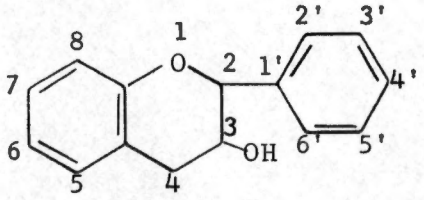
Unfortunately very little recent literature dealing specifically with the anthocyanins in terms of molecular structure-color theory is available. However, the manifestation of some of the above principles will be readily apparent when the chemistry of anthocyanin is considered later.

IV. CHEMICAL STRUCTURE OF CLASSES OF THE FLAVONOID GROUP

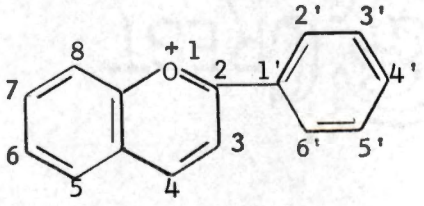
Geissman and Hinriener (35) state "the flavonoid compounds are characterized by their possession of a C_6-C_3-C_6 carbon skeleton consisting of two aromatic rings linked by an aliphatic three carbon chain. Chiefly, on the basis of the oxidation state of aliphatic fragment, the very large number of compounds included in the flavonoid classification is subdivided into such well known types as anthocyanins, flavones, chalcones, etc. The carbon skeleton (Figure 1, formula I)



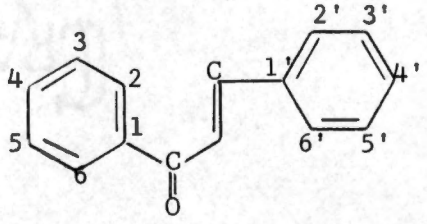
I. Flavonoid Group



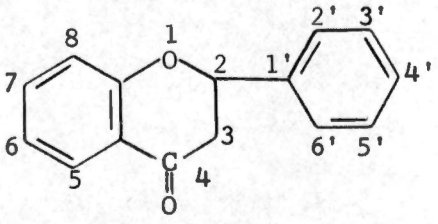
II. Catechins



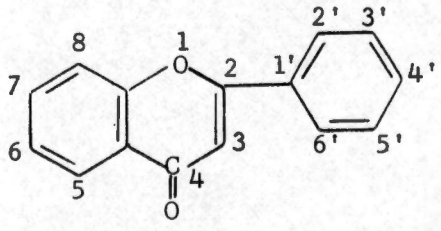
III. Anthocyanins



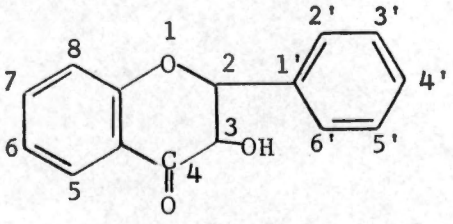
IV. Chalcones



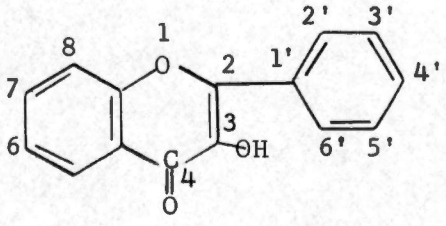
V. Flavanones



VI. Flavones



VII. Flavanonols



VIII. Flavonols

Figure 1. Carbon skeletons of the flavonoid group series.

can be visualized as an "A" benzene ring connected to a "B" benzene ring by a three carbon chain which can be in various states of oxidation.

Bate-Smith (4) has arranged the classes of the flavonoid group into two series on the basis of the level of oxidation of the $-C_3-$ fragment. In the "flavan" series the carbon adjacent to the "A" ring is unoxidized while in the flavanone series, the carbon adjacent to the "A" ring is oxidized to a carbanyl. In the flavan series no higher state of oxidation is possible than that of the anthocyanins, while in the flavanone series, no higher state of oxidation is possible than that of the flavonols.

Flavan series:		
Catechins	A-CH_2·CHOH·CHOH-B	Formula II
Leuco-anthocyanins	Structure uncertain	
Anthocyanin	A-CH_2·CO·CO-B	
	A-CHOH·CHOH·CO-B	Formula III
Flavanone series:		
Dihydrochalcones	A-CO·CH_2·CH_2-B	
Chalcones, flavanones	A-CO·CH_2·CHOH-B	Formula IV, V
Flavones	A-CO·CH_2·CO-B	Formula VI
Benzalcoumaranones	A-CO·CO·CH_2-B	
Flavanonols	A-CO·CHOH·CHOH-B	Formula VII
Flavonols	A-CO·CO·CHOH-B	Formula VIII

In most of the classes of the flavonoids the "A" benzene ring is believed to be linked to the oxygen of the carbon adjacent to the "B"

benzene ring, thus forming a heterocyclic structure. The carbon skeletons for the various classes of flavonoids and their carbon numbering schemes, as presented by Seshadri (112) are shown in formulas II through VIII.

With the exception of the catechins and possibly the leucoanthocyanins, the flavonoid compounds occur in the plant as glycosides in which certain of the phenolic hydroxyl groups are combined with sugar. The sugar-free molecules are called "aglycones." In anthocyanins, the glycones are called "anthocyanidins." Sugars which commonly occur in glycosidic combination with flavonoid substances include galactose, arabianose, xylose, and especially glucose and rhamonose. Sugars may be attached as mono-, di-, and trisaccharides. Glycosidation may occur at several positions on the same molecule. In addition, among the anthocyanins it is not uncommon for one or more of the sugar or anthocyanidin hydroxyls to be esterified with an organic acid such as p-hydroxybenzoic, malonic, p-hydroxycinnamic, or 3,5-dimethoxy-4-hydroxycinnamic acid (35).

In summary it can be seen that because of the many dimensions involved, the potential number of flavonoid group compounds are vast. The basic structure is the C_6-C_3-C_6 carbon skeleton. The "A" benzene ring may or may not be fused into a heterocyclic structure through an oxygen on the carbon adjacent to the "B" benzene ring. The aliphatic -C_3- fragment exists in different oxidation states, each state giving rise to a class of compounds. Usually, within each class, about eleven positions are available for different patterns of hydroxylation,

methoxylation, or for a combination of the two, to give rise to different types of compounds. Each type may be glycosidated with a number of sugars attached as mono-, di-, and/or trisaccharides in several positions on the same molecule. Esterification between the sugar or aglycone portion hydroxyl groups may exist. Finally, a number of classes are known to have isomers in which the "B" benzene ring is attached to the middle carbon of the aliphatic chain (C_6-$C_2(C_6)$-C) (35, 112, 36).

V. STRUCTURE OF ANTHOCYANINS

The glycone portion of the anthocyanin molecule is called "anthocyanidin." It consists of (A) C_6-C_3-C_6 (B) flavonoid carbon skeleton linked in a heterocyclic structure so that the "A" benzene ring is connected to an oxygen on the carbon adjacent to the "B" benzene ring (112). The resulting structure is a 2-phenylbenzopyrylium (or "flavylium") nucleus (12). The synthesized and isolated anthocyanins have been obtained as chlorides, thus the usual procedure in the literature is to depict the anthocyanidin as a positively charged moiety and to designate arbitrarily the point at the heterocyclic oxygen as the location of the charge (34). However, it must be kept in mind that the flavylium structure is a resonance hybrid (34). The anthocyanidin carbon skeleton structure is shown in formula III. The structure of the more common anthocyanidins are shown below.

The flavylium nucleus is usually hydroxylated in the 3, 5, 7 and 4' positions (112). In argument with the general structure of the flavonoids, hydroxyl substitution on the substitution of the substituted benzene ("B") ring usually consists of one, two, or three hydroxyl functions in the para (4'-), para and one meta (3', 4'-) and para and both meta (3', 4', 5'-) positions respectively (35). The other source of structural variation between anthocyanidin types is the methoxylation pattern. The hydroxylation and methoxylation patterns of the known types of anthocyanidins from Seshadri (112) are shown below.

Anthocyanidin	Position of the Hydroxyl Groups	Position of the Methoxyl Groups
Pelargonidin	3, 5, 7, 4'	--
Cyanidin	3, 5, 7, 3', 4'	--
Delphinidin	3, 5, 7, 3', 4', 5'	--
Peonidin	3, 5, 7, 4'	3'
Petunidin	3, 5, 7, 4', 5'	3'
Malvidin	3, 5, 7, 4'	3', 5'
Hirsutidin	3, 5, 4'	7, 3', 5'
Gesneridin	5, 7, 4'	--
Carajuridin	6, 7	5, 4'
Betanidin-type	(structures unknown)	

The structures shown in Figure 2 are a modification of that given by Bonner (13); it shows the anthocyanidins are arranged so as to demonstrate the effect of hydroxyl and methoxyl substitution upon the color. It can be seen that the effect of hydroxyl and methoxyl

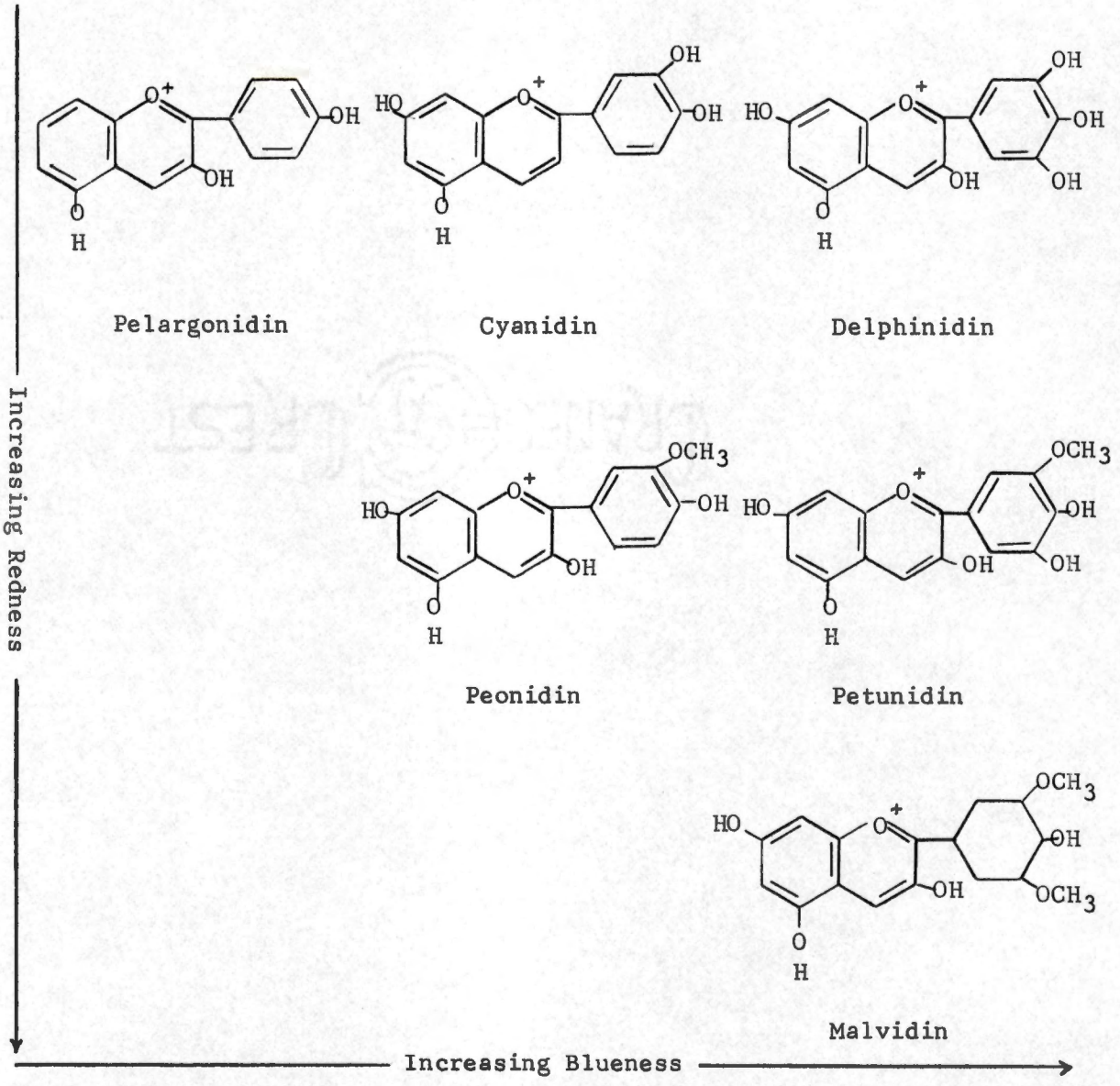


Figure 2. Structures of the more common anthocyanidins arranged so as to show effects of hydroxylation and methoxylation upon color.

substitution is in agreement with the discussion of color theory already presented.

VI. RADIATION

Radiation from radioactive materials is a stream of fast moving particles or waves which comes from atoms. Man-made radiation in the form of X-rays was discovered in Germany by Roentgen in 1895. A year later, in France, natural radioactivity was identified with uranium by Becquered. The invisible rays or particles from radioactive elements were found to be of three kinds, namely alpha, beta and gamma. The alpha particle is identical with the nucleus of the helium atom. A sheet of paper can stop it. The use of it in food preservation therefore is not feasible. The beta particle is the same as an electron and negatively charged which has a little more penetrating power than the alpha particle, but a thin sheet of metal will restrain it. However, it could be successfully utilized in the surface pasteurization of many foods. The gamma ray is not a particle. It is a non-corpuscular electromagnetic radiation of extremely short wavelength.

VII. RADIATION EFFECTS

On Pigments

One undesirable effect of high levels of irradiation is an alteration of food pigments and consequent change in color. A number of workers have reported that irradiation caused either a visible bleaching or a darkening effect on vegetables (38). Huber (47) used a

capacitron electron accelerator to produce sterilization dosages and reported considerable bleaching of carrots and some bleaching of green beans. Usually the major change of darkening produced are by the interaction between the food components during and after treatment. The destruction of chlorophyll in a number of cathode ray irradiated green vegetables was reported by Nickerson et al. (75). A linear decrease in chlorophyll content of green beans and broccoli resulted from increases in gamma radiation dosage over the range of 0.49 to 9.29 megarads (120). Markakis et al. (63) reported that 0.365 megarads of 2 Mev. cathode rays destroyed 55 per cent of the pigment, and dehydration of the juice further increased the radio-resistance of the pigment. The strawberry anthocyanin is more radio-sensitive in pure pigment solutions than in original juice. Sucrose added to juice increased the pigment destruction by gamma radiation. Strawberries irradiated with 2 Mev. cathode rays and also with gamma rays increased bleaching of the fruit as the dose of irradiation increased from 0.093 to 3.72×10^5 rads (63, 106).

Lukton and Mackinney (59) studied the breakdown of carotenoid pigments due to gamma irradiation in a number of products and noted the destruction of carotine and xanthophyll upon irradiation ranged from 5 to 95 per cent in broccoli, 3 to 20 per cent in sweet potatoes and 0 to 5 per cent in carrots. The anthocyanin pigments are easily degraded at high doses of gamma radiation. The carotenoids and chlorophylls appear to be among the more stable plant pigments (104). Strawberries irradiated with 2 Mev. cathode rays in the presence of air

increased the bleaching of fruits as the dosage of irradiation was increased from 0.1 to 4.0 megarep (0.093 to 3.72 megarads) (44). Francis et al. (32) showed that gamma irradiated pigment free residues of green beans, broccoli, sweet potatoes and carrots packed under a variety of conditions and stored for one year had appreciably a different color from those of the frozen controls. Some changes were found during or soon after irradiation and further changes occurred during storage. Green bean residues after eleven months storage, were darker and showed a small hue change from brown to yellow. Samples of broccoli, sweet potatoes and carrots were darker and browner. Residues from green beans, broccoli and carrots irradiated in the frozen state showed less darkening and less hue change than those irradiated at room temperature. Residues from sweet potatoes were darker, but showed less hue change when irradiated under frozen conditions.

According to Naik-Kurade et al. (73), the irradiation-induced browning differed from heat induced browning in that the former involved the formation of reductones as intermediates while the latter involved 5-hydroxymethyl furfural and aldehydes which appeared to be destroyed by ionizing radiation. The reductones were capable of contributing to darkening when the systems were subjected to post-irradiation heating. Hannan (44) reports even at 5×10^5 rep, there was a bleaching of the broad bean pods. Dehydrated carrots treated with 3×10^6 rep did not show appreciable loss of color (15). No color changes were noted in cole slaw exposed to gamma radiation between 50

and 1.5×10^5 rep (14). Radiation dosage of 5×10^5 rep was found to cause darkening of corn. When commercial heat processed cream style corn was treated with 2×10^6 rep and stored for five months at room temperature, the irradiated products showed lighter yellow color than the control. Cucumbers irradiated at 0.5 to 2×10^6 rep indicated no change in the color. Darkening of mushrooms increased with increased level of irradiation dose (15). Peas treated with 2×10^6 rep plus a 12 or 18 minute blanch showed much greener color after the storage for 5 months at room temperature (46). Fresh peas, which had not been blanched, showed a 4 per cent loss of chlorophyll at 1.6×10^6 rep and 9 per cent loss at 3.2×10^6 rep. The amount of pheophytin formed correlated fairly well with the loss of chlorophyll in this work. At 2×10^6 rep a slight bleaching occurs (29). For raw peas freezing seems to preserve the color (44). Sawyer et al. (109) working with Katahdin variety of Irish potatoes showed that irradiation dosage between 2.5 and 12.5×10^3 rep followed by storage at 70°F produced a chip of a lighter color with a tendency toward somewhat darker shades at the higher dosage. Potatoes stored at 50°F gave commercially accaptable chips while those stored at 40°F produced a very dark chip. However storage of potatoes at 70°F for 4 weeks just prior to chipping, regardless of the long term storage temperature, gave commercially acceptable chips (109). Pumpkins that had been blanched, canned and treated with 1.5 to 2.5×10^6 rep and stored for 7 months at 72°F were good in color but slightly duller than frozen pumpkins.

As the radiation dosage increased, the brightness began to increase (15). Apples exposed to 0.5 x 10^6 rep developed a slight brown color (74). Raspberries showed signs of bleaching at 10^5 rep; at 4 x 10^6 rep, the fruits were almost bleached (15). Bleaching of strawberries became noticeable around 5 x 10^5 rep and considerable change in color was noted at 2 x 10^6 rep (85). At 3 x 10^6 rep gamma irradiation, there was a loss of color (15). Prunes treated with doses up to 10^7 rep showed no color changes (93). Commercial prune juice treated at sterilization dosages showed some bleaching, although partial color returned after 20 hours (15). Raisins treated at 3 x 10^6 rep showed bleaching. At 10^7 rep bleaching was complete but the normal color returned after 2 weeks storage at 70° to 80°F (93). The natural color of oranges is retained up to 100,000 rads. Color changes do take place at higher doses and browning becomes evident at over 275,000 rads. Lemons also suffer somewhat the same effect at around 500,000 rads. It appears that development of desirable red pigment in the tomato was retarded in direct relationship to the amount of dose. Tomatoes at the pink stage of maturity deteriorated less rapidly than those irradiated when they were red ripe (124).

Plant pigments respond differently to irradiation treatment in different environments. The presence of free radicals created by radiation appears to be a major factor in degradation of pigments. Pigments separated from fruits or vegetables do not react in the same manner as that of pigments *in vivo*. Work done with dried juices of strawberries indicated that there is no pigment destruction with doses

ranging from 100,000 to 1.5 million rads. It also appears that sucrose and ascorbic acid tends to protect pigments (124) which is not in agreement with the work of Markakis et al. (63).

On Microorganisms Related to Food Preservation

Microorganisms, when subjected to irradiation, are affected by direct radiation or indirectly by free radicals and secondary reactions. The amount of free radicals created is a function of the environment. Low temperatures limit the ability of free radicals to recombine and continue the chemical reaction. As a result, the number of organisms killed is proportional to the temperature (124). The presence of oxygen also increases the kill rate. Radiation in the presence of water tends to create H_2O_2, which destroys the microorganisms.

There are major differences in the resistance of different microorganisms to radiation. It has also been found that different strains of the same microorganisms sometimes have different tolerances.

Thus, a research program on radiation pasteurization of foods should include study of radiation effects on the major microorganisms causing spoilage of each commodity of interest.

Some variables that affect the reaction of microorganisms to radiation are (1) the species of microorganisms, (2) the concentration of the organism, (3) moisture content and temperature of the food, (4) the composition of the medium, (5) the age of the culture, and (6) the type of radiation (124).

Beta and gamma radiation with doses in the 1 to 5 x 10^5 rads range have been shown to retard the growth of spoilage organisms such as bacteria and fungi for appreciable periods of time (114, 67, 103). Hence, the low dose radiation process has been considered as a useful tool for extending self-life of fresh foods, particularly when used in conjunction with refreigeration and other auxiliary methods of preservation. According to Morgan and Reed (71), Clostridium botulinum spores are more resistant to gamma radiation than spores of other food spoilage organisms. Schmidt in 1957 cited by Niven (76) estimated a radiation dose of 4.5 megarad would be required to be equivalent in safety to thermally processed foods. This estimation was based upon resistance of spores of Clostridium botulinum.

Wheaton et al. (119) worked on five strains of Clostridium botulinum and indicated striking differences in radioresistance. Strain 12885A was most resistant and Strain 32B was least resistant. A difference was noted in the survival of spores in five food products he tested (green beans, chicken, codfish, pork and beef). More destruction of organisms was observed with the green beans. He also stated that a given substrate would allow a greater per cent survival of one strain than another. Since the character of the food determines in part the dose required for destruction of clostridium botulinum, no single dose could be reccommoded for all food products.

Beraha and others (7, 8) indicated that fresh fruits are seriously damaged by the molds botrytis which seriously limit the storage

duration after harvest. However, gamma; as well as beta, irradiation appreciably retarded the growth of botrytis for a period of 15-20 days at a dosage of 2×10^5 rads.

Work by Nehemias et al. (74) showed that peaches and cherries treated with radiation and packaged in polyethylene bags underwent a more rapid rate of spoilage than the control. Their work hypothesized that radiation caused either physical or chemical change in the fruit enabling mold to grow more rapidly. In fact, their data indicated a stimulation of growth at doses lower than 1×10^6 rads. Burns (17) suggested that the reason irradiated tomatoes rotted more than the controls might be due to the action of the irradiation upon tissue breakdown due to dipolymerization of pectin found in the middle lemella. Phillips (81) indicated that irradiation produced organic acids from glucose. The production of such acids might stimulate fungus growth on the irradiated foods.

It appears that most radiation pasteurization of fresh fruits and vegetables will be at dose levels under 500,000 rads. Unfortunately with most foods, unfavorable organoleptic effects began to appear at doses somewhat below 500,000 rads. Molds commonly found on strawberries have been inhibited by radiation doses ranging from 200,000 to 500,000 rads. Shelf life of tomatoes appeared to be extended with doses as low as 200,000 rads (124).

Beraha et al. (9) showed that the irradiation dose from 100,000 to 300,000 rep substantially reduced gray mold and rhizopus rot of

strawberries during storage at 75°F for 3 days or at 41°F for 10 days. Doses of 200,000 rep prevented decay of strawberries. Mold inhibition in citrus seems to occur at 150,000 rads and above (124).

Treatment by radiation has posed a few problems relative to destruction of bacteria and bacterial toxins not similar to those of heat processing. These problems are described in detail in numerous reports. The outstanding hazard of bacterial origin is that of Clostridium botulinum and botulinum toxin. The organisms that may escape destruction by heat treatment are usually of the spoilage type so that food could be rejected on this basis. Sterilization by radiation can result in a case where the spoilage organisms may be destroyed but the Clostridium botulinum and its toxin may prevail. Other pathogenic microorganisms such as salmonellae that occur in food can be effectively controlled by low dose radiation treatment. The most resistant strains of this organism can be inactivated with a radiation dose not exceeding 0.5 megarads according to Ley et al. (56).

Radiation-processed foods are as new to this era as thermal-processed foods were in Napoleonic times. More recently, however, a few investigations have turned their attention to a reappraisal of the wholesomeness of these widely accepted preservation techniques. Obviously, no generalizations can be made relevant to nutrient quality and toxicity clearance of foods treated.

On Flavor

One of the major unacceptable characteristics of foods which have received radiation at dose levels above 500,000 rads has been the creation of undesirable flavors and odors (124). Several laboratories have been investigating the effects of radiation on volatiles, a major factor in flavor. Research in the flavor field is difficult and isolation of all the elements creating flavor in a particular food is a problem of considerable dimensions. Some flavor substances are present in very small quantities and often are short-lived. Some of the undesirable flavor changes in irradiated fruits and vegetables at higher doses are possibly due to the destruction of volatile esters and alcohols which are characteristic of the product (104). Other objectionable flavors may also originate from vitamins and pigments, since they are highly susceptible to and easily degraded by gamma radiation. Licciardello et al. (57) showed that the radiation sterilized products stored at 50° and 68°F were acceptable after 10 months storage. Deterioration in quality occurred within one month when samples were stored at 125°F. However, Groninger et al. (43), Pratt and Ecklend (87) calculated a statistical analysis of taste test scores and showed significant off-flavor in each of the irradiated meats and vegetables in sterilization dose levels. In every case, important changes in appearance and flavor developed on storage. Some of these changes might be attributed to enzymatic action. Lemon flavor has been affected by doses of 100,000 to 400,000 rads (124). It is possible to

control undesirable off flavoring changes to some degree when the products are irradiated in the presence of flavor absorbing materials such as activated charcoal. The aeration of fresh fruits and vegetables during irradiation is essential to retain the natural flavor and to extend the storage life. With the supply of oxygen for the normal respiration process, at the same time removal of CO_2 and other gases given out due to the respiration of tissues, dark color development has been found to be inhibited when exposed to gamma radiation (104). However irradiation of many fruits and vegetables (107, 108) produced no detrimental effects on flavor when irradiated at the low doses.

Flavor changes which occur in irradiated food are presumably caused by the action of free radicals which are formed by radiolysis of water. In determining the reactions involved in production of off-flavors, comparative experiments were conducted with gamma rays. This led to the establishment of a series of compounds, the "reactive carbonyl class" as an excellent protection against those mechanisms which are involved in the production of off-flavor (58). It is hoped that continued, detailed work in this field will supply the means to prevent flavor changes.

In some fruits early adverse effects, particularly off-flavors and off-odors, may disappear within a few days. Maxie and Sommer's (64) work with dewberries showed unpleasant flavors and odors one day after irradiation but the observation after seven days storage irradiated fruits rated superior to unirradiated fruits.

On Vitamins

Of the vitamins, only C, B_1, B_2, A and niacin are present in amounts that may be of dietary significance with the fruits and vegetables of interest. Research indicates that irradiation causes degradation of these vitamins in varying amounts, depending on the vitamin and the level of irradiation.

It has already been stressed that the total amount of chemical change which occurs in an irradiated system is limited and with a dose of 200,000 rep, is of the order of a few micromoles per gram. In a foodstuff consisting mainly of water, however, most of the reactions which occur are typically "indirect" reactions, and if one of the constituents is particularly radio-sensitive, it may suffer a large proportion of the total change. If this constituent is present in large amounts the percentage loss is rarely significant, but with trace constituents, such as vitamins, it may represent an appreciable destruction (44).

Irradiation of ascorbic acid has probably received most study and the relevant papers (95, 48, 2) provide a good illustration of the dependence of indirect chemical effects on the nature of the system. Oxidation occurs when the vitamin is irradiated in aqueous media, but one of the products is dehydroascorbic acid; which can be reduced biologically to ascorbic acid without difficulty. Proctor and O'Meara (95) showed that in orange juice irradiated with up to 500,000 rep, more than half of the destruction of L-ascorbic acid was reversible

in this way; the fate of the fraction irreversibly destroyed was not determined but spectrophotometric examination failed to reveal the formation of 2,3-diketogluconic acid, one of the end products with normal oxidative destruction. Nickerson et al. (75) showed a 64 to 92 per cent loss of vitamin C in asparagus, broccoli, green beans, and spinach. Mickaelsen et al. (69) reported that vitamin C content was decreased in both irradiated and non-irradiated potatoes during the first seven months of storage at 40°F; but was restored after this period and that ascorbic acid levels of the radiated samples were higher than those of the control. Proctor and Goldblith (91) observed that there is less relative destruction in more concentrated solutions. For instance, irradiation with 75,000 r of soft X-rays (50 Kv.) caused a destruction of approximately 50 per cent of the ascorbic acid when irradiated in a concentration of 100 mcg. per ml. and a destruction of 74 per cent when irradiated in a concentration of 50 mcg. per ml. Freezing gives some protection at lower than sterilizing doses of gamma irradiation (95). Coleby (22) has demonstrated that ascorbic acid can be formed by irradiating D-glucono-γ-lactone and L-glucono-γ-lactone. The conversion of ascorbic acid to dehydroascorbic is reversible biologically into vitamin C. However, on storage the reduced form is much less stable. Hence, the nutritional value of the irradiated product is dependent upon how soon after irradiation and how long the product is stored prior to consumption.

Clarke (21) and Proctor and O'Meara (95) measured ascorbic acid loss in orange juice and strawberries exposed to varying levels of

irradiation at a dose of 396,000 rads resulted in 26 per cent loss of ascorbic acid in orange juice and 81 per cent loss in strawberries when the dose was decreased to 279,000 rads, 22 per cent of vitamin C loss in orange juice and 63 per cent in strawberries.

Panalakas and Pellefier (78) treated potatoes with gamma at 7.9 Krad, 10.2 Krad and 14.0 Krad and control and observed that the gamma irradiation did not cause consistent or large variation in the ascorbic acid content of potatoes. The main cause of loss of ascorbic acid was storage. Storage at 40°F resulted in lower ascorbic acid values than storage at 68°F. There was no marked differences in the loss of ascorbic acid as were observed between samples stored for 4-1/2 months and for 9 months.

Dunlap and Robbins (28) reported destruction of thiamin by irradiation but the sensitivity did not appear to be great. The irradiation of other B vitamins has been studied mainly by Proctor and Goldblith (40, 90, 92). Riboflavin in dilute solution was found to be appreciably less sensitive than ascorbic acid, and nicotinamide was even less sensitive. In mixtures, however, complex results were recorded; thus ascorbic acid increased the destruction of nicotinamide and was itself protected.

Thiamin may be destroyed in tissues by ionizing radiation which causes a rupture of the molecule resulting in pyrimidine and thiazole moieties. The thiazole undergoes further degradation changes at higher doses of irradiation (123). It is also conceivable, according to

Ziporin et al. (123), that there may be a rupture of either the ring structure or the removal of substituent groups. Goldblith and Proctor (40), using niacin solution treated at 1.6×10^5 rads, demonstrated that there may be decarboxylation of this vitamin and at 6.6×10^5 rads they stated that the pyridine ring was split. Riboflavin is protected by the presence of oxalic acid and still more by added vitamin C (54). Goldblith (39) showed that the inactivation dose for riboflavin in pure solution is about 5×10^9 rads, whereas in evaporated milk it is 3.7×10^{11} rads.

Of the three vitamins in foods, niacin, riboflavin and thiamin, the latter was the most radio-sensitive in five foods assayed (beef, ham, peaches, beets, powdered milk) by Ziporin et al. (123) at both the levels of treatment, 2.8×10^6 and 5.6×10^6 rads.

Greater loss of niacin seems to occur in the presence of ascorbic acid, 44 versus 86 per cent retention of activity at 1.25×10^5 r with and without ascorbic acid, although ascorbic acid alone is more radio-sensitive than niacin (90, 91). Both carotine and vitamin A are quite radio-sensitive. Like the other vitamins, vitamin A radio-sensitivity is dependent upon the media in which it is suspended when irradiated (54). The mechanism of radiation destruction either through indirect action of formed free radicals or by direct hit is not fully understood. Carotine and vitamin A in n-hexane or petroleum ether solution are destroyed by an apparently indirect mechanism (19, 40), a further indication that such indirect effects are not confined to aqueous

solution.

Destruction of vitamin A in irradiated carrots fed to rats does not fully account for lower accumulation of this vitamin in the liver (16). Non-irradiated carrots have a higher percentage of the all-trans-β-carotine. This isomer is the most efficient vitamin A precursor (54). The rate of destruction of vitamin A by gamma rays in the various media occured in the following order: butter > cream cheese > cheddar cheese > cream > margarine (55). Destruction of vitamin A was observed to be threefold greater in butter than in margarine, a very significant difference since 80 per cent of vitamin A content of the butter was lost after exposure to approximately 10^6 rep. The difference in sensitivity is not related to the moisture content of the foods studied; on the other hand, strongly suggesting that the vitamin A is inactivated by a direct hit mechanism. It is more probable that the types of vitamin A ester used to supplement margarine are more resistant to irradiation (15). The susceptibility of carotine and certain other carotenoids to destruction is partially dependent upon its environment during irradiation. Beta carotine in petroleum ether is very easily destroyed by high voltage cathode rays (40) or gamma rays from a cobalt-60 source (62). The result shows that 80 per cent of the beta-carotine in petroleum ether is destroyed by a dose of 0.15×10^6 rep. In tomatoes, the resistance is still greater because 20×10^6 rep caused less than 15 per cent loss.

To summarize the vitamins listed (thiamine, riboflavin, niacin, vitamin A) radiation treatment at a level of 2.79 megarads is no more

destructive in causing vitamin loss for the most part than is heat treatment.

The comparative effects of radiation and heat treatment on the vitamin content of foods as summarized by Read (97) are as follows: loss of thiamin by heat, 60-70 per cent; riboflavin, 18-22 per cent; niacin, 30-35 per cent; vitamin A, 20 per cent; whereas loss by irradiation at 2.79 M. rads thiamin, 55-65 per cent; riboflavin, 6-10 per cent; niacin 0-14 per cent; and vitamin A loss at 440,000 rads was 31-70 per cent.

In general, vitamins in foods are less radio-sensitive than those in pure solution (123). Studies by Pollard (84) have shown that vitamin losses in fruits and vegetables are directly affected by the temperature of irradiation, the rate of radiation, post-irradiation storage temperature, and pre-irradiation treatment with various chemicals. All these factors should be considered in designing studies to measure micronutritional losses due to irradiation.

On Cell Structure and Integrity of Product

Tissue softening has been observed in fruits and vegetables under overdoses of radiation. The reason for this phenomenon is not clearly understood (124). Dr. Kertisz (124), at the New York State Agricultural Experiment Station, has found a straightline relationship between the log irradiation dosage and the per cent decrease in tissue firmness in apples and carrots and also between the log irradiation dosage and per cent decrease in viscosity of solutions of irradiated

pectin, cellulose, and starch (15).

On citrus products, the most extensively reported specific organoleptic effect has been on texture. Rind pitting and internal breakdown of oranges have been observed at doses over 275,000 rads, but the fruit retains a firm texture in the 100,000 range. Lemons appear to retain their natural firmness up to 100,000 rads. However softening is noticeable at doses in the order of 600,000 rads and at 2 million rads a "greasy" surface becomes evident. When the irradiation doses exceeded 100,000 rads strawberries appeared to show some gel breakdown in berries and softening seems to appear when the dose reaches 500,000 rads. Tomatoes subjected to 1 million and 5 million rads per hour showed that the higher rates of irradiation softened the fruits faster than the lower rates. Green tomatoes tend to show cracking at the stem end under doses of over 100,000 rads but this effect does not occur in the pink or the ripe stages. In general, it seems that tissue damage becomes serious between doses of 500,000 and 1 million rads (124). Salunkhes' (104) work showed that apricot varieties treated from 0 (control), 1, 3, and 5×10^5 rads showed softness as the radiation doses were increased. Asparagus subjected to 5×10^5 rads became softer immediately after treatment. However, lower doses of irradiation showed slight softness upon storage at 40°F after six days. The softening at higher doses was attributed to the degradation of pectin, cellulose and lignin whereas the lower doses of irradiation might have accelerated the action of enzymes to produce the softening

within a few days of storage. Strawberries subjected to irradiation dosage of 3×10^5 rads gave a peculiar spongy-soft texture. As the storage period was lengthened the number of spongy berries became more apparent. He relates that the texture changes in fruit during maturation and storage have been associated with the degradation of complex carbohydrates. Because of the importance of pectins and cellulose to the cellular structure of fruit or vegetables, it has been found that textural changes in fruits are directly attributable to radiation degradation of these carbohydrates.

Beets have been reported to be able to withstand 3×10^4 rep as well as 2×10^6 rep followed by 3 months storage at 72°F. In both cases there was a decrease in texture due to the irradiation. The first signs of measurable softening using texturometer were noted at 5.79×10^5 reps. Extrapolation from higher doses indicated that the threshold for softening of this product was at approximately 2.6×10^5 rep (15). The threshold dose for softening of beets has been shown by Glegg et al. (37) to be approximately 300 Krad, a value considerably in excess of that found for apple tissue and approximately double that of carrot tissue.

According to Kertesz et al. (52) pectin appears to be somewhat more susceptible to degradation by ionizing radiation than does cellulose. There is no complete coincidence of the dose response of either pectin or cellulose degradation to that of tissue softening, however, it is possible that the additive effect of the two responses could

account for the resultant softening. Roberts and Proctor (101), using microchemical methods found that irradiating potatoes with 2 to 3 M. rads of cathode rays produced noticeable alterations in the middle lamellae and that these changes were accompanied by a softening of the potatoes. McArdle and Nehemias (66) reported texture measurements on apples and carrots irradiated with gamma rays up to 2.5 M. rads and found that the softening was accompanied by a decrease in both protopectin and total pectins, whereas proportion of soluble pectins and pectates increased. They state that the lowered viscosity of the various extracts indicated a depolymerization of the pectin, pectate, and protopectin.

Salunkhe (105) studied the cell and cell structure of the irradiated lima beans which showed that cells separation increased when the dosage (more than 2×10^6 rep) increased. This can be attributed to the partial or complete destruction of the constituents of the middle lamellae. The starch grains were liberated readily from the cells of unirradiated beans. However, the liberation of starch grains from the cells of radiated beans was inversely proportional to the amount of radiation. The cell walls of the radiated beans were rather elastic and not easy to break open. The protoplasm within the cells appeared unchanged even at the highest dose (100×10^6 rep) of radiation.

On Enzymes

The keeping qualities of fruits and vegetables depend in part upon the effect of the preservation process on the activity of enzymes in the food. An approach to the preservation of food by irradiation

should therefore include a consideration of the effect of irradiation on food enzymes. Most enzymes are affected very little or not at all by pasteurization dose of radiation. It has been found, however, that the enzyme systems of fruits and vegetables are sufficiently disturbed by pasteurization doses to alter the ripening process (124).

Many factors influence the radiation sensitivity of the molecules of a cell. One of the principal factors is the water content of the cell. The water content certainly controls the magnitude of the indirect effect, in which chemically active intermediates formed by the action of ionizing radiation on water diffuse through the cell. They react with, and on occasions inactivate, molecules which serve some important function (83).

The radiation sensitivity of enzymes in general is determined by the states in which they occur and their nature. An individual enzyme may show widely varying sensitivities when irradiated in different states. At the same time, different enzymes in the same state may show widely varying radiation sensitivities simply by virtue of differences in their nature (15). When enzymes are irradiated in dilute aqueous solution, the rate of inactivation will usually decrease with increasing radiation dose. This is due to the fact that inactivated enzyme molecules compete with the active ones for the agents formed from water (24). In many cases the radiation sensitivity is directly related to the molecular weight of the enzyme (83). When compared to inactivation doses for microorganisms, the enzymes, in

general, are much higher (94). In most studies, the data more or less closely fit a straight line when the remaining enzyme activity is plotted against the radiation dose on semilogarithmic paper (23).

Enzymes within whole cells or tissues resist irradiation much more strongly than those in homogenates or in pure solution. Radiosensitivity is higher in vitro than in vivo (18). Bellamy and Lawton's (6) work showed that the mean lethal dose for catalase in crushed potatoes is 5,000,000 rep compared to only 25,000 rep for catalase enzyme in pure solution. Enzymes in cell or cell particulates may in some instances show an increase in activity following irradiation. This phenomenon is believed to be due to a liberation of the enzyme from inactive complexes within the cell (15).

Enzymes in the dry state are inactivated directly by excitation (82) whereas enzymes in solution are inactivated indirectly by OH and OH_2 radicals formed in the solvent. According to Barron (5) the H_2O_2 formed due to irradiation has negligible influence on inactivation of enzymes. Forssberg (31) suggested that H radicals are involved in the inactivation of catalase, since greater inactivation occurred in the absence of oxygen. However, the conclusion does not appear adequate in light of work done by Dale and Russell (25). Thus, chemically produced H radicals do not inactivate catalase (118).

As a general rule, one can say that complete inactivation of enzymes by radiation treatment require in the order of five times the dose required for the destruction of microorganisms and depends on the

temperature, pH, presence of oxygen, concentration of enzyme itself, and the presence of other compounds.

Because of the low sensitivity of enzymes to radiation, and because the sutstrate can be rendered more susceptible to attack by enzymes, while at the same time failing to inactivate them, radiation sterilization can bring about a rapid deterioration of food substances unless enzymes are controlled.

VIII. HISTORY OF BETANIN ISOLATION

The first attempt to isolate a betacyanin pigment appears to have been described by Bischoff (11). He precipitated the pigment from an aqueous alcoholic extract of the berries of phytolacca decandra with lead acetate and regenerated it with alcoholic sulfuric acid. Sixteen years later Haverland (45) again isolated the phytolacca pigment (phytolaccaium) by precipitating it from an alcoholic extract with ether and he followed this with a further precipitation from concentrated aqueous solutions with alcohol.

The next attempt to isolate and purify betacyanin came from Willstatter's laboratory, where Schudel (111) worked with the beet pigment. In this case, he took dried slices of beets and extracted with methonolic hydrochloric acid and precipitated the pigments with ether.

In 1937 Ainley and Robinson (1) investigated the isolation of beet pigment. Beet juice itself was allowed to hydrolyze its pigment. Glucose (betanidin) was isolated by extraction with iso-amyl alcohol

followed by precipitation of pigment with light petroleum. Concurrently Pucher, Curtis and Vickery (96) also developed a procedure for the isolation of betanin. In this case, dried beet slices were extracted with alcoholic hydrochloric acid and the pigments were precipitated with the addition of lithium hydroxide (LiOH) (the reaction being the addition of LiOH causes the conversion of alcohol-soluble hydrochloride of betanin to the alcohol-insoluble lithium salt). The lithium salt was dissolved in water and the pigment again precipitated with lead acetate. Further decomposition of the lead salt with methanolic hydrochloric acid, precipitation with ether and recrystallization from water gave an amorphous betanin sample of about 70 per cent purity.

Chromatographic purification of betanin was attempted by Chmielewska (20) on alumina and by Aronoff and Aronoff (3) on a talc-siliceous earth column into at least eleven colored bands.

Several (41, 121, 80) investigators have reported that beet pigments can be separated on paper by electrophoresis. Schmidt and Schonleben (110) found that betanin migrated toward the anode during electrophoresis when the pH of the buffer was above the isoelectric point (about pH 2). According to Gostalindstedt (41) pigments of red beets were separated into at least seven colored zones upon electrophoresis (6.3 v/cm.) with a 0.1 M. citrate buffer, at pH 5.5. Reznik (99) showed that high-voltage electrophoresis (30-40 v./cm.) was more effective than low-voltage electrophoresis (3193 v./cm.) for resolving beet pigments on paper. Nine colored bands including a major

violet-red zone and major yellow zone, were formed when high-voltage electrophoresis was carried out with a phosphate buffer (pH 6.64, μ= 0.066). With the aid of paper electrophoresis, Wyler et al. (122) found that along with betanin three related violet compounds occurred in the red beet.

CHAPTER III

MATERIALS AND METHODS

Fresh table beets (Beta vulgaris), of the early Wonder variety which were grown for 75 days, were procured from a local farmer. A uniform size of approximately 1½-2 in. diameter were selected for the experiment. The roots so selected were cleaned, washed, trimmed, and packed into one-pound coffee tin cans. The lids were placed on the cans in such a way that all volatile gases could escape during the radiation treatment. The tin cans were labeled and were taken to the Oak Ridge National Laboratory for radiation treatments. The samples were subjected to gamma irradiation levels of 2×10^5, 6×10^5, and 12×10^5 rads at room temperature using a cobalt-60 source with a flux of 8×10^5 rads per hour.

The control samples were treated in a similar manner as that of irradiated except no radiation was applied. The irradiated and control samples of beets were stored at 40°F prior to analysis. Part of the beets were used for analysis the day after irradiation, and the remainder stored for 22 days at 40°F prior to analysis.

Studies were made on pigments, visual color, ascorbic acid, enzymatic activity and histological sections. Histological work was undertaken only for those samples which did not undergo storage treatment.

Data collected were subjected to statistical analysis at the University Computing Center.

Extraction of Pigments

Irradiated beets were cut into small cubes and were lyophilized in a Del-Vac Freeze-Dryer Model 11212 RVM of American Sterilizing Company. The lyophilized material was ground in a Wily mill to pass through a 40 mesh screen and were packaged in air-tight amber colored bottles under nitrogen gas. The bottles were placed in a desiccator and stored at -20°F.

Beet pigment extracts were prepared from lyophilized beet powder throughout the experiment. To one gram of lyophilized beet powder, 10 ml. of 60 per cent ethanol solution was added with stirring. The mixture was allowed to stand for 30 minutes at room temperature. At the end of 30 minutes, the mixture was filtered through a medium porous sintered glass filter under vacuum. The residue was extracted with 10, 5 and 5 ml. portions of 60 per cent ethanol. To the combined filtrate was added 150 ml. of anhydrous ethyl ether in a 500 ml. separatory funnel. It was mixed well and allowed to stand for one hour at room temperature. The concentrated pigment extract was transferred to another separatory funnel containing 10 ml. of 99 per cent methanol. The pigments and methanol were mixed, and 100 ml. of anhydrous ethyl ether was added. The mixture was shaken vigorously and allowed to stand for 15 minutes at room temperature. The pigment

concentrate was collected in test tubes and concentrated by passing dry nitrogen through it for 20 minutes. At the end of this period, all samples were adjusted to exactly 3 ml. with distilled water. The pigment extracts (the betanin) were stored at -20°F in air-tight containers under nitrogen.

Electrophoresis of Pigments

A Research Specialties Model E800-2B electrophoresis system was used in this study. Four paper strips of Whatman No. 3 mm. paper was used for each run. Electrophoresis was conducted at room temperature with a voltage gradiant of 5.2 volts per cm. of paper. Strips were saturated with 0.5 M. pyridin-citric acid buffer of pH 4.5 and were held for at least 15 minutes at room temperature prior to sample application. To each strip 0.04 ml. of concentrated pigment extract (the betanin) was applied, and the instrument was operated for 6½ hours. At the end of this period, four pigments were separated: (1) brown colored pigment; (2) primary purple pigment; (3) secondary purple pigment; and (4) a yellow pigment. The pigments were eluted with 10 ml. of distilled water under nitrogen gas for 3 hours. The pigment solution was filtered and the visible spectrum was scanned and the per cent fluorescence was recorded.

The pigment extract was also separated electrophoretically with potassium biphosphate buffer (KH_2PO_4), pH 4.5. This was necessary to study the absorption spectrum of the pigment in the ultraviolet range. The procedure in this step was exactly the same as with

pyridin-citric acid buffer.

Absorption Spectral Studies

Spectral studies were made exclusively with a Bausch and Lomb spectronic 505 recording spectrophotometer. The instrument was standardized to zero absorbency with distilled water using one cm. Beckman quartz cells.

Pigment dilution of 0.5:100 were prepared with distilled water and the spectrum was scanned from 250 to 650 mμ with absorbance 0 to 1.0.

Further, the pigments separated by electrophoresis using pyridin-citric acid buffer were scanned from 400 to 600 mμ. Similarly, pigments separated with potassium biphosphate buffer (KH_2PO_4) were scanned from 245 to 300 mμ.

The peak absorption values for the different treatments were estimated and the data were subjected to statistical analysis by the analysis of variance.

Fluorescence Studies

The Turner Model 111 fluorimeter with primary filter 7-60 (below 365 mμ) and secondary filter 2A (above 415 mμ) was used in this study. The instrument was set to zero per cent fluorescence with distilled water using Pyrex cuvette at 1 x opening.

A pigment dilution of 0.05:100 ml. of distilled water was prepared and the per cent fluorescence was recorded. Similarly, per cent

fluorescence was measured for the pigments separated electrophoretically with pyridin-citric acid buffer. The data obtained were subjected to statistical analysis by the analysis of variance.

Densitometer Studies

The Model No. 425 Densitometer of the Photovolt Corporation was used to determine the optical density of the pigment spots on the electrophoresis strips. The instrument was adjusted to zero with the blank strip of chromatographic paper and only the peak readings for different groups of pigments were recorded.

Photovolt Studies

Color measurements were made using Photovolt Model No. 610 Reflectometer. The instrument was standardized against National Bureau of Standards SBC 20 (purple) color plate with 45°·0° CIE tristimulus value source for "C" being 37.4 for amber, 33.8 for green and 44.7 for blue filters. The beets were cut radially into halves, blotted with filter paper and the per cent reflectance from each cut surface was taken with each filter.

The tristimulus values X, Y and Z were used to derive the chromaticity coordinate x and y;

$$X = 0.8\ A + 0.18\ B$$

$$Y = G$$

$$Z = 1.18\ B,$$

where A, G, and B are the reflectance values obtained by measuring with the amber, green and blue filters, respectively.

Chromaticity coordinate,

$$x = \frac{X}{\sum X + Y + Z}$$

$$y = \frac{Y}{\sum X + Y + Z}$$

Yellowness index was obtained by substituting the readings for amber, green and blue to the formula A - B/G.

Color Evaluation by Panel

Visual observation on the cut surface of the treated and control beets were made with the limitation to a general description of color. A relative evaluation of color was brought about in such a way that the irradiated and the control samples were compared with a standard set of beets having a relative uniform color.

An arbitrary visual scoring system was adopted to describe the extent of darkness and lightness.

The score description was as follows:

+3 = very much darker in color than standard,

+2 = much darker in color than standard,

+1 = slightly darker in color than standard,

0 = equal to standard,

-1 = slightly lighter than standard,

-2 = much lighter than standard, and

-3 = very much lighter than standard.

Data collected were subjected to statistical analysis by the analysis of variance.

Ascorbic Acid Determination

Fifty-gram samples were osteurized with 0.5 per cent aqueous oxalic acid for exactly 5 minutes. The mixture was filtered through high porosity sintered filter under vacuum. The filtrate was collected and the volume was made up to 100 ml. with 0.5 per cent aqueous oxalic acid. The ascorbic acid content was determined by the reaction with 4-methoxy-2-nitroaniline in acid medium followed by development of a blue color in alkaline solution (72). In this particular analysis, since the sample solution had a large quantity of water, the reaction time prior to the addition of 10 per cent sodium hydroxide was increased to 45 minutes as suggested by Morton et al. (72). The blue color developed was measured at 570 mμ with a Beckman DU Spectrophotometer. The results were expressed as mg. of ascorbic acid per 100 g. samples.

Enzymatic Activity

Irradiated and control samples of beets were tested for enzyme catalase and peroxidase. Beet roots were sliced and catalase activity was tested by placing hydrogen peroxide and benzidin on the cut surface. Presence of peroxidase was identified with guaiacal solution.

Histological Studies

Radiated and control samples of beets were sliced at about one-fourth inch thickness and fixed in FAA (formalin-glacial acetic acid-alcohol) solution (50). After the fixing process, tissues were stored

in 70 per cent ethyl alcohol until ready for the dehydration process.

The dehydration process was carried out by keeping the tissues immersed in series of TBA (tertiary butyl alcohol). The specimens thus prepared were paraffin infilterated and dearated. Dearated samples were embedded in paraffin wax.

Sections of tissue were cut at 10 microns thickness using a Spencer AO rotary microtome and fixed on slides. Slides were stained according to the procedure of fast green and safranin staining schedule (98).

Photomicrographic pictures were taken at low power (48X) and high power (192X) by the use of Polaroid camera for further evaluation of treatmental effects.

CHAPTER IV

RESULTS AND DISCUSSION

I. ABSORPTION SPECTRAL STUDIES OF PIGMENTS

Absorption spectrometry is the measurement of the absorption, by substances, of electromagnetic radiation of definite and narrow wavelength range, approximating monochromatic radiation.

Absorption spectra presented in Figure 3 are typical of those observed in the studies. Control and irradiated pigment extracts (betanin) displayed a maximum absorption peak at about 530 mμ in the visible wavelength. Evidence of a secondary absorption peak was found in the vicinity of 270 mμ. Figure 3 and data in Tables I and II illustrate that as the radiation doses were increased, a significant decrease in optical density occurred at both the wavelengths (530 and 270 mμ).

For convenience of reference, spectral range is roughly divided into the ultraviolet (185 mμ to 380 mμ), visible (380 mμ to 780 mμ), near-infrared (780 mμ to 3000 mμ), and infrared (3 mμ to 4 mμ). The visible and ultraviolet spectrum of a substance generally does not have as high a degree of selectivity as the infrared spectrum. Nevertheless, for many substances, it is a useful means of identification and quantitative assay.

In this study, optical density measurements of pigments in the visible and ultraviolet ranges were used as one of the tools for relative

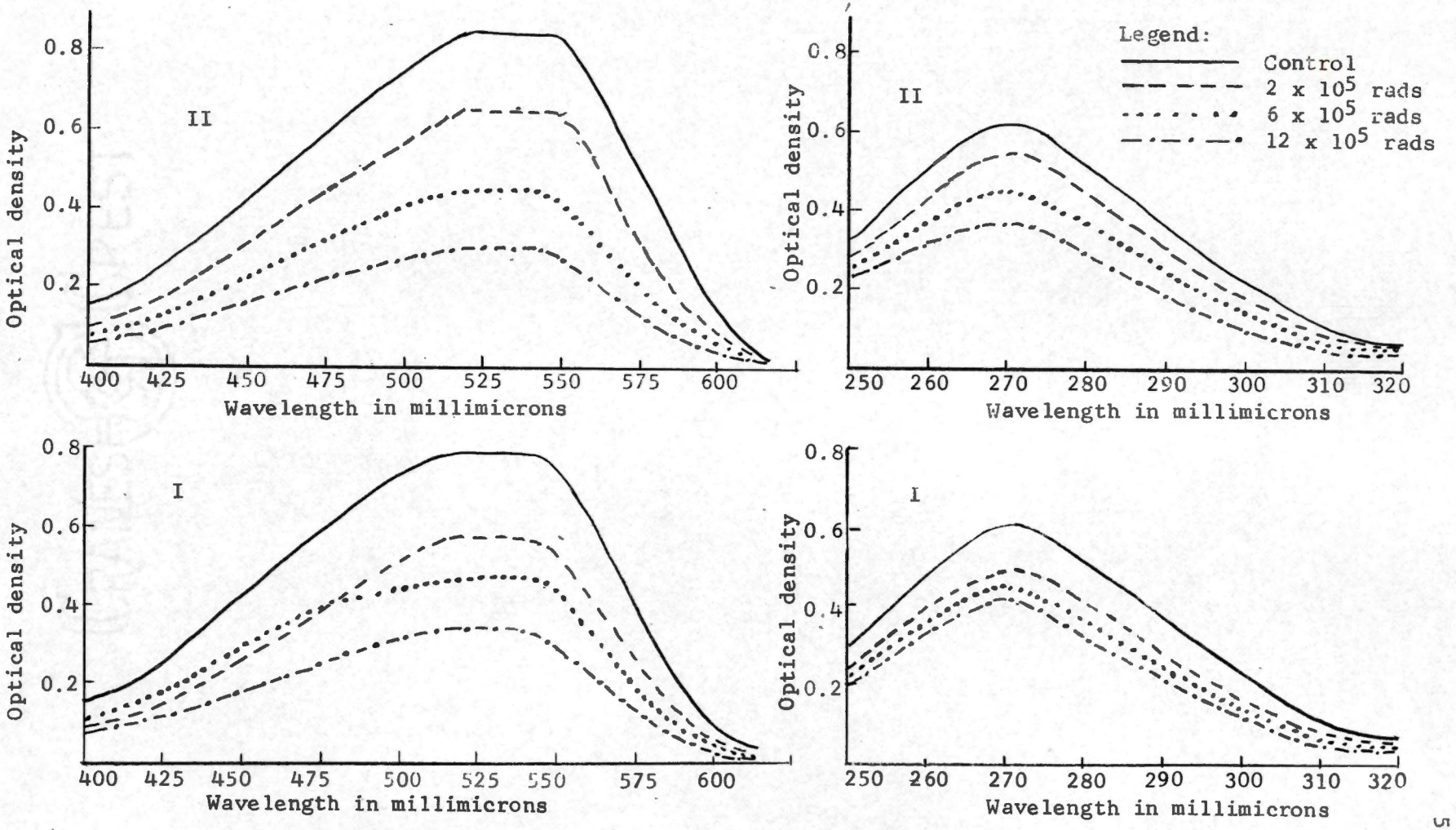


Figure 3. (I) Absorption spectra of betanin prior to storage; (II) absorption spectra of betanin after 22 days storage.

TABLE I

EFFECT OF GAMMA IRRADIATION ON ABSORPTION MAXIMA OF BETANIN AT 530 MILLIMICRONS

Treatment in Rads	Prior to Storage	After 22 Days Storage
Control	0.7980[a]	0.8476[a]
2×10^5	0.5650[b]	0.6456[b]
6×10^5	0.4650[c]	0.4553[c]
12×10^5	0.3420[d]	0.2906[d]

SUMMARY OF ANALYSIS OF VARIANCE

Prior to Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.111993	141.1380**
Error	8	0.000793	

After 22 Days Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.173586	253.1951**
Error	8	0.000685	

[1]Each datum represents the average of three replicates.

[2]Means with different superscripts are significantly different at the 5 per cent level of probability as calculated by Duncan's multiple range test (27).

TABLE II

EFFECT OF GAMMA IRRADIATION ON ABSORPTION MAXIMA OF BETANIN AT 270 MILLIMICRONS

Treatment in Rads	Prior to Storage	After 22 Days Storage
Control	0.6103[a]	0.6233[a]
2×10^5	0.4536[b]	0.5416[b]
6×10^5	0.4480[b]	0.4473[c]
12×10^5	0.4430[b]	0.3533[d]

SUMMARY OF ANALYSIS OF VARIANCE

Prior to Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0196	16.2221**
Error	8	0.0012	

After 22 Days Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0409	26.4141**
Error	8	0.0015	

evaluation of pigment concentration following different levels of gamma radiation. The absorptivity of a substance in the spectrophotometric method is a constant independent of pigment concentration, to the extent that other variables remain constant (variables being wavelength setting, slit-width adjustment, cells and its placement, transmittance levels, etc.). Variation in pigment concentration seems to have a direct relationship to the intensity of the visible purplish red color of betanin. Increase in radiation doses resulted in decrease in intensity of the purplish red color. It appears that radiation treatments caused degradation of pigments which was evident from the optical density readings. This may be attributed to the oxidation of the betanin pigment. Pigment oxidation results in the formation of a brown pigment (86). Data given in Tables IV and V for the brown pigments indicates a considerable increase in the optical density readings with an increase in radiation dosage. Relatively small differences were observed in the optical density readings between pigments extracted prior to storage and those extracted after 22 days of storage.

Further, spectral studies with electrophoretically separated betanin pigments are presented in Figure 4. Of the four groups of pigments separated with 0.5 M. pyridine-citric acid buffer, only one pigment with dark purple color (primary purple) showed an absorption peak at 530 mμ. The other three (secondary purple, yellow and brown colored) pigments did not show an appreciable absorption maximum in the visible region. This may be attributed to the fact that these pigments do not absorb a significant amount of light energy in the visible

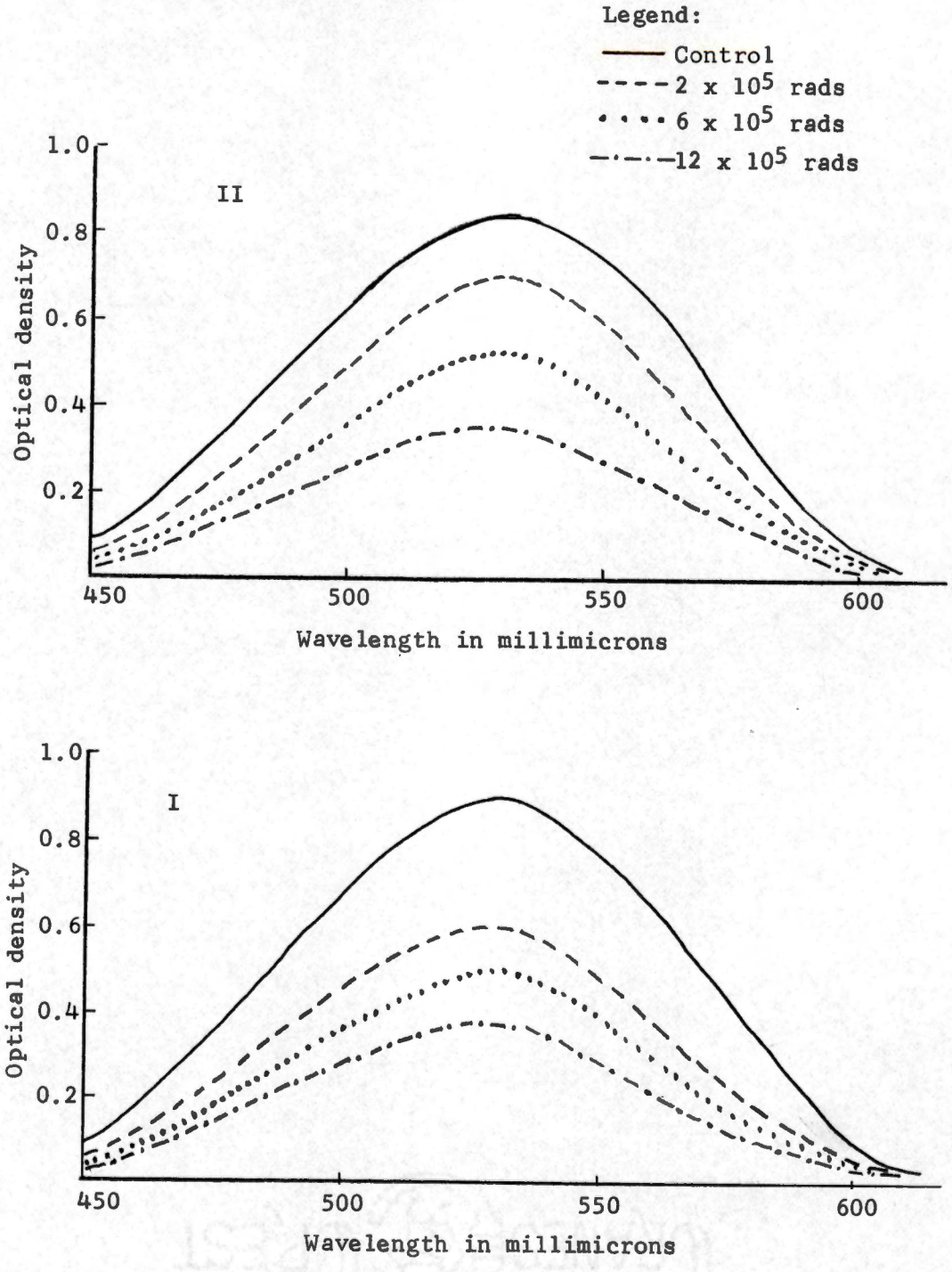


Figure 4. (I) Absorption spectra of primary purple pigment prior to storage; (II) absorption spectra of primary purple pigment after 22 days of storage.

region. Thus, the peak absorption at 530 mμ is primarily due to the presence of primary purple pigment.

The intensity of primary purple pigment imparts a purplish red color to betanin. The data in Table III shows the effect of radiation treatments on primary purple pigments. Increase in radiation dosages resulted in a significant decrease in optical density. The use of pyridine-citric acid buffer was limited to the spectral studies in the visible region due to the absorption of pyridine in the ultraviolet range. Thus potassium biphosphate buffer was used for scanning the pigments in the ultraviolet range.

The absorption spectrum of the pigment separations obtained with potassium biphosphate buffer are presented in Figures 5 and 6 respectively. It is of interest to note that the maximum absorption appeared to be at wavelength 258 mμ instead of 270 mμ. The shift in the wavelength of maximum absorbancy may be related to the changes in the buffer and/or the process of electrophoresis.

Data presented in Tables IV and V were the optical density readings for primary purple, secondary purple, yellow and brown pigments at 258 mμ. These data reveal that the pigment degradation at the pasteurization dose level (2×10^5 rads) was not significant in comparison to the control. But there was a significant decrease in optical density readings at the dose levels of 6×10^5 and 12×10^5 rads.

In general the pigment loss at a pasteurization dose of 2×10^5 rads gamma irradiation was considerably less in comparison to a higher dosage (6×10^5 and 12×10^5 rads).

TABLE III

EFFECT OF GAMMA IRRADIATION ON ABSORPTION MAXIMA OF PRIMARY PURPLE PIGMENT AT 530 MILLIMICRONS

Treatment in Rads	Prior to Storage	After 22 Days Storage
Control	0.9060[a]	0.8376[a]
2×10^5	0.6133[b]	0.7166[a]
6×10^5	0.5486[c]	0.5296[b]
12×10^5	0.3780[d]	0.3466[c]

SUMMARY OF ANALYSIS OF VARIANCE

Prior to Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.1452	194.6650**
Error	8	0.0007	

After 22 Days Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.1389	35.6572**
Error	8	0.0038	

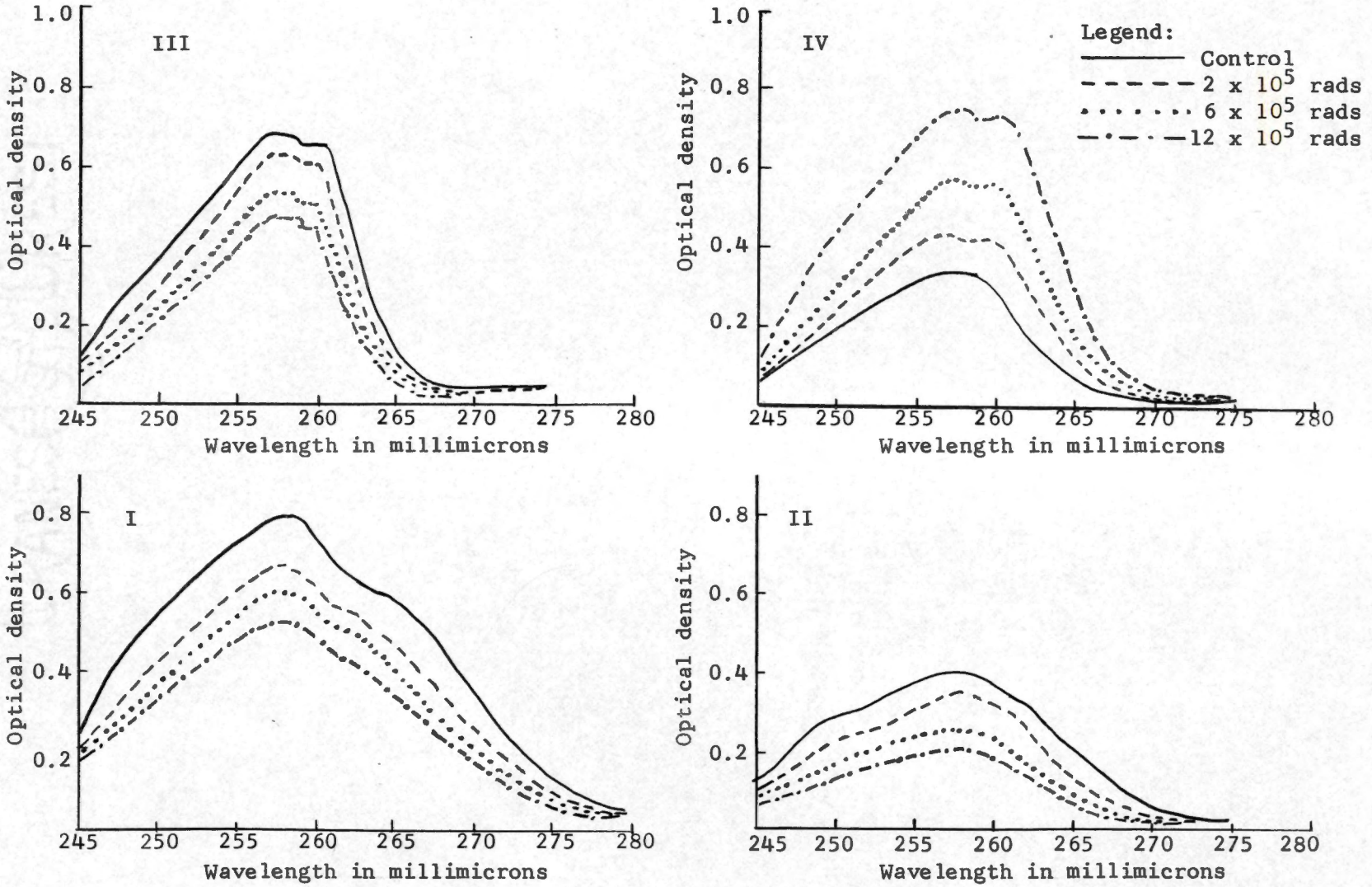


Figure 5. Absorption spectra of (I) primary purple; (II) secondary purple; (III) yellow, and (IV) brown pigments prior to storage.

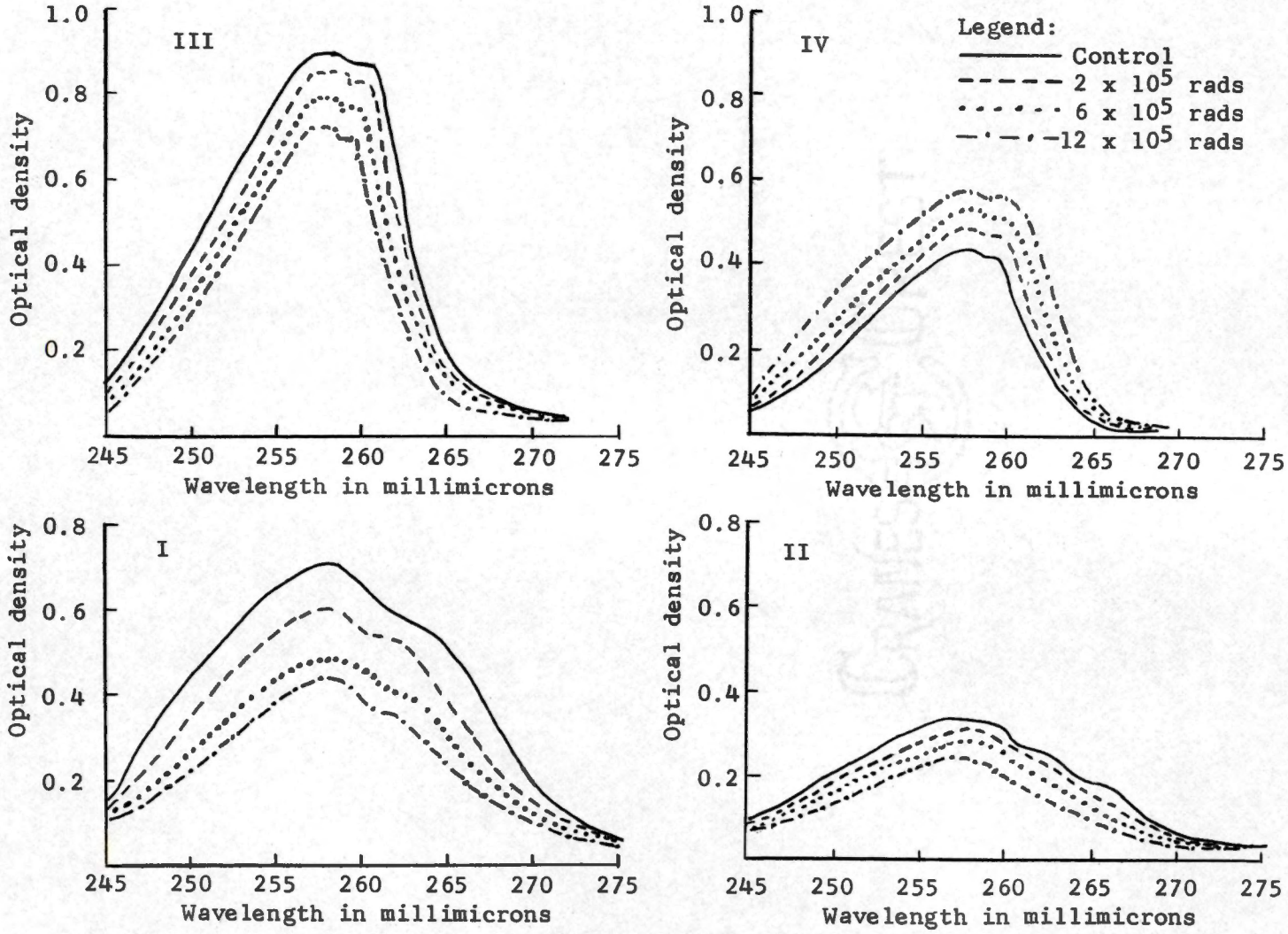


Figure 6. Absorption spectra of (I) primary purple; (II) secondary purple; (III) yellow, and (IV) brown pigments after 22 days storage.

TABLE IV

EFFECT OF GAMMA IRRADIATION ON ABSORPTION MAXIMA OF VARIOUS FRACTIONS OF BETANIN AT 258 MILLIMICRONS[1]

Treatment in Rads	Primary Purple	Secondary Purple	Yellow	Brown
Control	0.7900[a]	0.4000[a]	0.6793[a]	0.3480[a]
2×10^5	0.6796[a,b]	0.3593[a]	0.6303[a]	0.4230[a,c]
6×10^5	0.6163[b]	0.2653[b]	0.5250[a,b]	0.5700[b,c]
12×10^5	0.5333[b]	0.2190[b]	0.4693[b]	0.7523[b]

SUMMARY OF ANALYSIS OF VARIANCE

Primary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0351	5.4959*
Error	8	0.0063	

Secondary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0208	24.1609**
Error	8	0.0008	

Yellow Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0276	3.9326
Error	8	0.0070	

TABLE IV (continued)

Brown Pigment			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0959	11.1428**
Error	8	0.0086	

[1]Pigment extracts obtained prior to storage.

TABLE V

EFFECT OF GAMMA IRRADIATION AND STORAGE ON ABSORPTION MAXIMA OF VARIOUS FRACTIONS OF BETANIN AT 258 MILLIMICRONS[1]

Treatment in Rads	Primary Purple	Secondary Purple	Yellow	Brown
Control	0.7000[a]	0.3383[a]	0.8906[a]	0.4403[a]
2×10^5	0.6000[a,b]	0.3293[a]	0.8573[a]	0.4966[a,c]
6×10^5	0.4830[b]	0.2866[b]	0.7933[b]	0.5383[b,c]
12×10^5	0.4450[b]	0.2460[c]	0.7223[c]	0.5733[b]

SUMMARY OF ANALYSIS OF VARIANCE

Primary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0408	43.1251**
Error	8	0.0009	

Secondary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0054	13.8979**
Error	8	0.0004	

Yellow Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0165	22.8851**
Error	8	0.0007	

TABLE V (continued)

Source	Degrees of Freedom	Mean Square	F Ratio
	Brown Pigment		
Treatment	3	0.0098	8.2223**
Error	8	0.0011	

[1]Pigment extracts obtained after 22 days of storage at 40°F.

II. FLUORESCENCE STUDIES ON PIGMENTS

The emission of light from matter under the influence of an exciting agent is termed fluorescence. Many substances are capable of receiving radiant energy of short wavelength and transforming it into radiant energy of a visible wavelength. This kind of excitation includes several processes by which energy is introduced into and/or released from particles.

The intensity of fluorescent radiation is often directly related to the concentration of the emitting substances and therefore serves as a basis for many sensitive analytical procedures. With this idea in mind, the fluorescence studies were conducted on pigment dilutions to determine the fluorescent property of pigments and its changes due to radiation. The data obtained from fluorescence studies are presented in Tables VI through VIII.

Data in Table VI represents the per cent fluorescence for pigment betanin, extracted prior to and after 22 days of storage. From the data, it is evident that the radiation treatments significantly decreased the per cent fluorescence of betanin pigment. This is in agreement with the optical density studies.

The per cent fluorescence of the four electrophoretically-separated groups of betanin, extracted prior to and after 22 days of storage, are tabulated in Tables VII and VIII. The per cent fluorescence of primary purple (Table VII) shows a significant increase in fluorescence at the radiation doses of 2×10^5 and 6×10^5 rads. A

TABLE VI

EFFECT OF GAMMA IRRADIATION ON PER CENT FLUORESCENCE OF PIGMENT BETANIN

Treatment in Rads	Prior to Storage	After 22 Days Storage
Control	92.0000[a]	67.6666[a]
2×10^5	53.0000[b]	58.6666[b]
6×10^5	49.6666[b,c]	47.6666[c]
12×10^5	47.0000[c]	35.3333[d]

SUMMARY OF ANALYSIS OF VARIANCE

Prior to Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	1348.0833	376.2093**
Error	8	3.5833	

After 22 Days of Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	586.0000	121.2414**
Error	8	4.8333	

TABLE VII

EFFECT OF GAMMA IRRADIATION ON PER CENT FLUORESCENCE OF VARIOUS FRACTIONS OF BETANIN

Treatment in Rads	Primary Purple	Secondary Purple	Yellow	Brown
Control	50.0000[a]	70.3333[a]	41.0000[a]	79.0000[a]
2×10^5	58.3333[b]	50.0000[b]	54.0000[b]	65.3333[b]
6×10^5	61.3333[b]	63.0000[c]	94.0000[c]	73.0000[c]
12×10^5	48.6666[a]	58.0000[d]	86.3333[c]	82.0000[a]

SUMMARY OF ANALYSIS OF VARIANCE

Primary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	115.6389	25.6975**
Error	8	4.5000	

Secondary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	219.3333	57.2174**
Error	8	3.8333	

Yellow Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	1934.3334	116.6432**
Error	8	16.5833	

TABLE VII (continued)

Brown Pigment			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	162.3333	45.3023**
Error	8	3.5833	

TABLE VIII

EFFECT OF GAMMA IRRADIATION AND STORAGE ON PER CENT FLUORESCENCE OF VARIOUS FRACTIONS OF BETANIN

Treatment in Rads	Primary Purple	Secondary Purple	Yellow	Brown
Control	50.0000[a]	54.0000[a]	45.3333[a]	71.6666[a]
2 x 10^5	47.6666[a]	49.0000[b]	51.0000[b]	66.3333[b]
6 x 10^5	48.0000[a]	40.6666[c]	52.0000[b]	64.0000[b]
12 x 10^5	66.0000[b]	56.0000[a]	69.3333[c]	76.0000[c]

SUMMARY OF ANALYSIS OF VARIANCE

Primary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	231.4167	32.6706**
Error	8	7.0833	

Secondary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	140.0833	25.0896**
Error	8	5.5833	

Yellow Pigment

Source	Degree of Freedom	Mean Square	F Ratio
Treatment	3	322.5279	133.4598**
Error	8	2.4167	

TABLE VIII (continued)

Brown Pigment			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	87.2222	32.7083**
Error	8	2.6667	

further increase in irradiation dosage up to 12 x 10^5 rads showed a decrease in per cent fluorescence. Primary purple pigment, obtained from pigment extract after storage, showed no significant differences at the irradiation dose of 2 x 10^5 and 6 x 10^5 rads; whereas, 12 x 10^5 rads showed a significant increase in the per cent fluorescence.

Secondary purple pigments indicated a significant decrease in per cent fluorescence as the irradiation doses were increased, with the exception of pigment extract obtained from 12 x 10^5 rads treatment after storage.

Per cent fluorescence studies on the yellow group of pigments (prior to storage) showed significant increase up to a dose level of 6 x 10^5 rads. A further increase in radiation dose did not seem to increase the per cent fluorescence. No significant difference occurred between 2 x 10^5 and 6 x 10^5 rads treatment levels.

Data obtained for the brown group of pigments (prior and after storage) revealed that there was a decrease in per cent fluorescence at the irradiation doses of 2 x 10^5 and 6 x 10^5 rads in comparison to control. A further increase in radiation dosage (12 x 10^5 rads) resulted in an increased per cent fluorescence.

In general, a slight discordance was noted in per cent fluorescence of the electrophoretically-separated groups of pigments (primary purple, secondary purple, yellow and brown colored). This may be related to factors involved in conducting pigment studies such as purity of pigments, age source, pigment particle size, water content, and experimental errors might have occurred in the process of electrophoresis.

These pigments appear to be very sensitive to temperature, oxygen, and light.

The most interesting feature of this study was the yellow group of pigments. As the radiation doses were increased there was a decrease in optical density while the per cent fluorescence readings increased.

III. DENSITOMETER STUDIES ON PIGMENTS

The densitometer readings for fractions of betanin were measured and were expressed in terms of optical density readings. The data thus collected are tabulated in Tables IX and X. Data in Table IX indicates on those samples before storage; whereas, Table X indicates on those stored for 22 days after irradiation.

From the data obtained it shows that under both the conditions (prior to and after storage) the densitometer readings decreased with increase of radiation dosage. This clearly indicates that the radiation treatment effected pigment degradation.

Relative evaluation of the instruments used to measure pigments, concentration in terms of optical density, the Spectronic 505 was much more sensitive and gave more precise values of the pigment concentration than the densitometer. The densitometer readings were the optical density for a given spot, but it was not a quantitative evaluation of pigment concentration. Spot densitometer readings were likely to vary with the type of buffers used, method of electrophoresis, and also behavior of certain pigments. During the process of electrophoresis, it was possible for the pigments to move in concentrated

TABLE IX

INFLUENCE OF GAMMA IRRADIATION ON PIGMENT CONCENTRATION[1]

Treatment in Rads	Optical Density = Reading x 10			
	Primary Purple	Secondary Purple	Yellow	Brown
Control	3.4166[a]	1.1000[a]	0.3333[a]	0.8500[a]
2×10^5	2.3333[b]	0.6000[b]	0.2000[a]	0.5000[b]
6×10^5	2.1333[b]	0.4500[b]	0.1500[a]	0.5000[b]
12×10^5	1.8666[b]	0.2333[c]	0.1500[a]	0.4000[b]

SUMMARY OF ANALYSIS OF VARIANCE

Primary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	1.3879	57.9333**
Error	8	0.0239	

Secondary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.4068	63.0000**
Error	8	0.0064	

Yellow Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0225	10.8000**
Error	8	0.0020	

TABLE IX (continued)

Brown Pigment			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0985	15.2581**
Error	8	0.0064	

[1]Each datum represents the average of three replicates measured with densitometer for fractions of betanin.

TABLE X

INFLUENCE OF GAMMA IRRADIATION AND STORAGE ON PIGMENT CONCENTRATION

Treatment in Rads	Optical Density = Reading x 10			
	Primary Purple	Secondary Purple	Yellow	Brown
Control	3.1000[a]	0.8000[a]	0.2666[a]	1.1000[a]
2×10^5	2.6333[b]	0.8000[a]	0.2500[a,b]	0.5000[a]
6×10^5	2.0000[c]	0.7666[a]	0.1500[b]	1.1333[a]
12×10^5	1.8333[c]	0.6166[a]	0.1500[b]	0.8000[a]

SUMMARY OF ANALYSIS OF VARIANCE

Primary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	1.0252	32.3772**
Error	8	0.0316	

Secondary Purple Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0275	2.0625
Error	8	0.0133	

Yellow Pigment

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0118	4.3846*
Error	8	0.0027	

TABLE X (continued)

Brown Pigment			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.2268	2.1311
Error	8	0.1064	

spots, thus giving erroneous results. Consequently, the use of the densitometer in this type of study may be limited to the desired precision of experimental results.

IV. OBJECTIVE COLOR EVALUATION WITH PHOTOVOLT READINGS

On the basis of the trichromatic system of color measurements established by the CIE (51), the surface color of a product can be specified by its chromaticity coordinates (x and y) and luminance. In this study, the reflectance values of the samples from different treatments were measured under three filters (amber, green and blue) by the use of the Photovolt and the average readings are tabulated in Tables XI and XII. From those readings, the chromacity coordinates x, y and Y (yellowness) were calculated.

The values of x, y and Y on samples irradiated and not subjected to storage ranged from 0.4933 to 0.5319, 0.2445 to 0.2659 and 1.4800 to 1.8319 respectively, whereas the x, y, and Y values on samples irradiated and stored for 22 days ranged from 0.5004 to 0.4570, 0.2932 to 0.2654 and 1.4301 to 1.2361 respectively. Photovolt readings taken on samples irradiated and stored indicated a significant decrease in readings at the treatment level of 12×10^5 rads.

The excitation purities of different treatments are given in Tables XIII and XIV. These values ranged from 59.00 to 70.76 per cent for the samples before storage, whereas those after storage for 22 days varied from 43.90 to 54.68 per cent. This clearly indicates that a considerable variation occurred on excitation purity due to

TABLE XI

EFFECT OF GAMMA IRRADIATION ON COLOR NOTATION OF BETA VULGARIS[1]

Treatment in rads	Amber Filter	Green Filter	Blue Filter	x	y	Yellow-ness
Control	13.8333[a]	6.0000[a]	5.5000[a]	0.4933[a]	0.2445[a]	1.4800[a]
2 x 10^5	16.0000[a]	7.0000[a]	5.1666[a]	0.5118[a]	0.2600[a]	1.6037[a]
6 x 10^5	14.3333[a]	5.6666[a]	4.0000[a]	0.5391[a]	0.2519[a]	1.8523[a]
12 x 10^5	15.1666[a]	6.5000[a]	4.1666[a]	0.5319[a]	0.2659[a]	1.8319[a]

SUMMARY OF ANALYSIS OF VARIANCE

Amber Filter

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	10.8889	1.6990
Error	44	6.4091	

Green Filter

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	4.0833	2.0037
Error	44	2.0379	

Blue Filter

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	6.5278	5.7064**
Error	44	1.1439	

TABLE XI (continued)

x			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0051	1.9443
Error	44	0.0026	

y			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0010	0.6507
Error	44	0.0016	

Yellowness			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.3922	1.2244
Error	44	0.3202	

[1]Each datum represents the average of three replicates obtained from the photovolt readings.

TABLE XII

EFFECT OF GAMMA IRRADIATION AND STORAGE ON COLOR NOTATION OF BETA VULGARIS

Treatment in Rads	Amber Filter	Green Filter	Blue Filter	x	y	Yellow-ness
Control	12.1666[a]	6.0000[a]	3.5833[a]	0.5004[a]	0.2932[a]	1.4301[a]
2×10^5	9.8333[a]	5.0000[a]	3.3333[a]	0.4835[a]	0.2894[a]	1.3611[a]
6×10^5	11.0000[a]	5.1666[a]	4.1666[a]	0.4857[a]	0.2629[a]	1.3583[a]
12×10^5	5.6666[b]	2.8333[b]	2.5000[b]	0.4570[a]	0.2654[a]	1.2361[a]

SUMMARY OF ANALYSIS OF VARIANCE

Amber Filter

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	96.2222	23.7853**
Error	44	4.0455	

Green Filter

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	21.8889	27.2579**
Error	44	0.8030	

Blue Filter

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	5.7431	24.6531**
Error	44	0.2330	

TABLE XII (continued)

x			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0039	1.3056
Error	44	0.0030	

y			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0030	1.0185
Error	44	0.0029	

Yellowness			
Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	0.0781	0.2877
Error	44	0.2716	

TABLE XIII

INFLUENCE OF GAMMA IRRADIATION ON CALCULATED CIE COLOR NOTATIONS

Treatment in Rads	Dominant Wavelength Millimicron	Per Cent Purity
Control	496	64.00
2×10^5	494	59.00
6×10^5	494	70.76
12×10^5	494	64.70

TABLE XIV

INFLUENCE OF GAMMA IRRADIATION AND STORAGE ON CALCULATED CIE COLOR NOTATIONS

Treatment in Rads	Dominant Wavelength	Per Cent Purity
	Millimicron	
Control	492	43.90
2×10^5	493	46.57
6×10^5	495	54.68
12×10^5	496	50.00

storage treatment. This suggests that the reaction contributing to the color development was active during the storage period.

There was no marked change noticed for the calculated CIE dominant wavelengths due to treatment. Values of x and y were made use of in a chromaticity diagram of the ICI system (Figure 7) to locate the visual color of the product. The samples irradiated and stored indicated a visual color of pink, whereas those before storage showed a purplish red color.

V. VISUAL COLOR EVALUATION

The mean scorings for different treatments are given in Table XV. Data on samples evaluated soon after the irradiation had scores ranging from +0.7333 to -1.3000 units, whereas the samples evaluated after 22 days of storage ranged from +0.5333 to +2.7666 units. Irradiated and stored samples were much darker than samples evaluated soon after irradiation. The intensity of the dark color appeared to increase with the increase in radiation dosage. The maximum darkness occurred at the dose of 12×10^5 rads. The darkness in color which appeared was not in any way related to the concentration of pigments but was due to the degradation of pigments. The color appearance was more or less a bluish black and had a dull luster.

Samples examined soon after the irradiation showed bleaching of red color, but there was no significant difference between the treatments.

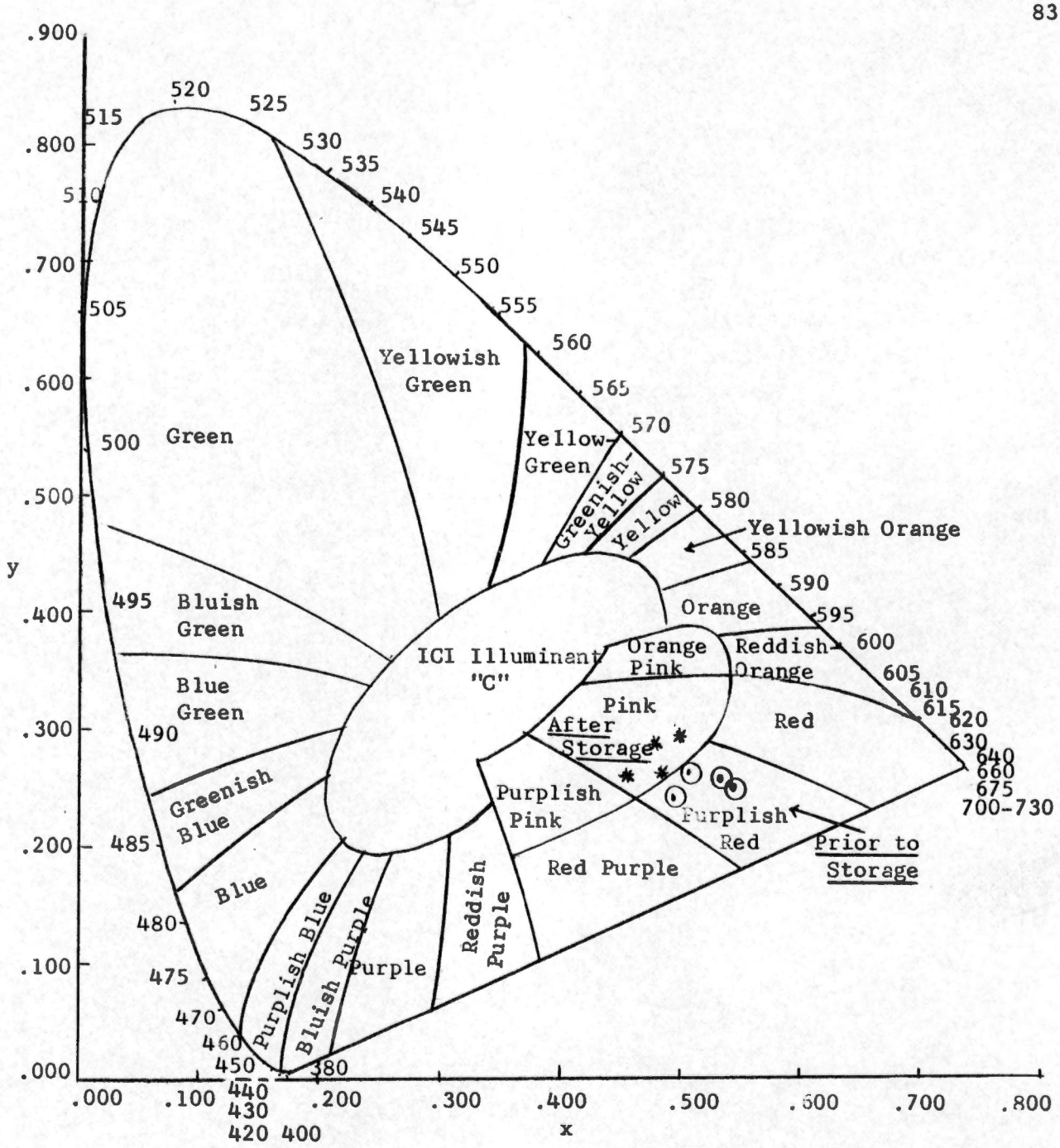


Figure 7. The (x, y)-chromaticity diagram of the ICI system (51) showing the locus of colors.

TABLE XV

INFLUENCE OF GAMMA IRRADIATION ON VISUAL COLOR OF BETA VULGARIS[1]

Treatment in Rads	Prior to Storage	After 22 Days Storage
Control	+0.7333[a]	+0.5333[a]
2×10^5	-0.1000[a]	+1.1000[a,b]
6×10^5	-1.3000[a]	+1.8666[a,b]
12×10^5	-0.3000[a]	+2.7666[b]

SUMMARY OF ANALYSIS OF VARIANCE

Prior to Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	20.9416	9.5189**
Error	80	2.2000	

After 22 Days Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	28.1555	30.1660**
Error	80	0.9333	

[1]Each datum represents the average of three replicates scored by ten panel members.

VI. STUDIES ON ASCORBIC ACID RETENTION

Ascorbic acid determinations were made on the various treatments and are expressed (Table XVI) as mg. of ascorbic acid per 100 g. sample.

Ascorbic acid content of samples analyzed soon after irradiation showed no significant difference between the control sample and the pasteurization dose level (2×10^5 rads). The samples stored for 22 days after the irradiation showed a significant decrease in ascorbic acid content at the pasteurization dose (2×10^5 rads) level.

Even though the data in Table XVI show that a decrease in ascorbic acid content occurred with an increase in irradiation dose, both before and after storage, the most drastic loss of ascorbic acid occurred in samples analyzed after 22 days of storage. This clearly illustrates that the storage loss after irradiation was much greater than loss due to radiation only.

In this study, the maximum loss of ascorbic acid occurred at the dose level of 12×10^5 rads.

VII. STUDIES ON ENZYMATIC ACTIVITY

A major concern with irradiation treatment was the inactivation of enzymes present in the tissues. Enzymes that are usually concerned with fruits and vegetables are catalase and peroxidase.

In this study, qualitative evaluations showed that irradiation doses used did not seem to accomplish total destruction of enzymes (catalase and peroxidase). Attempts were made to study the enzymatic

TABLE XVI

EFFECT OF GAMMA IRRADIATION ON ASCORBIC ACID CONTENT OF BETA VULGARIS

Treatment in Rads	Prior to Storage	After 22 Days Storage
	mg./100 g.	mg./100 g.
Control	7.3333[a]	6.5333[a]
2×10^5	6.7333[a]	4.2333[b]
6×10^5	4.7666[b]	2.9666[c]
12×10^5	4.2000[b]	2.4333[c]

SUMMARY OF ANALYSIS OF VARIANCE

Prior to Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	6.8430	53.6710**
Error	8	0.1275	

After 22 Days Storage

Source	Degrees of Freedom	Mean Square	F Ratio
Treatment	3	9.9875	69.6802**
Error	8	0.1433	

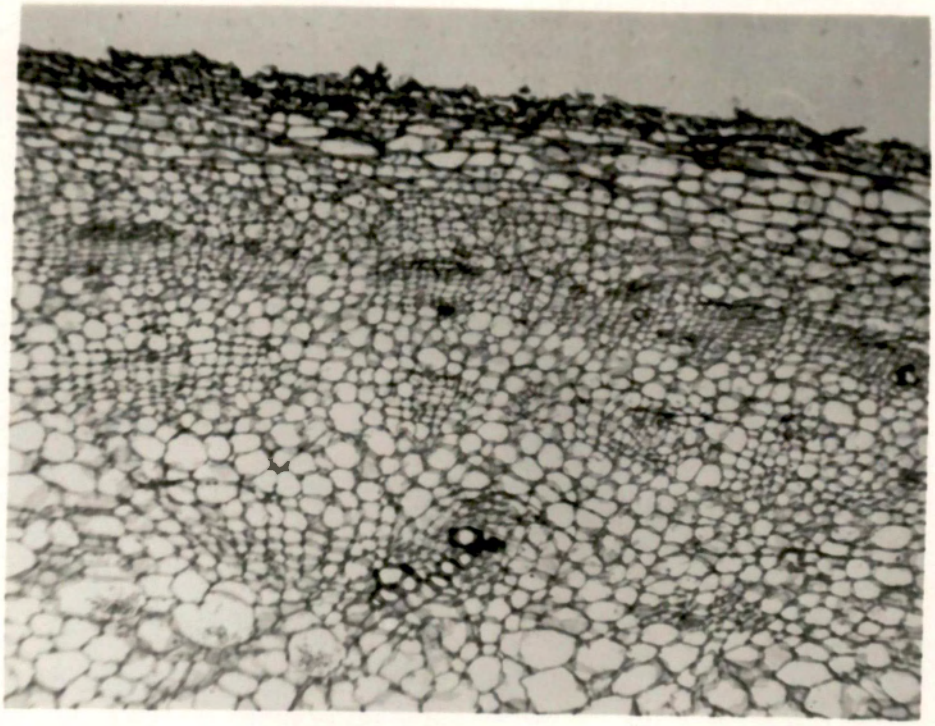
activity on samples irradiated and stored for 22 days. However it was impossible to distinguish the change of color due to enzyme reaction. At the end of the storage period, all the irradiated samples attained a bluish black color which failed to show the distinguishing color reactions. On the other hand, the control sample did show the presence of enzymes.

According to Bellamy and Laxoton (6), the mean lethal dose for enzyme catalase inactivation was about 5,000,000 rep *in vivo*, and 25,000 rep *in vitro*; whereas in this study, the highest dosage used was 12 x 10^5 rads which was much lower than their dosage levels.

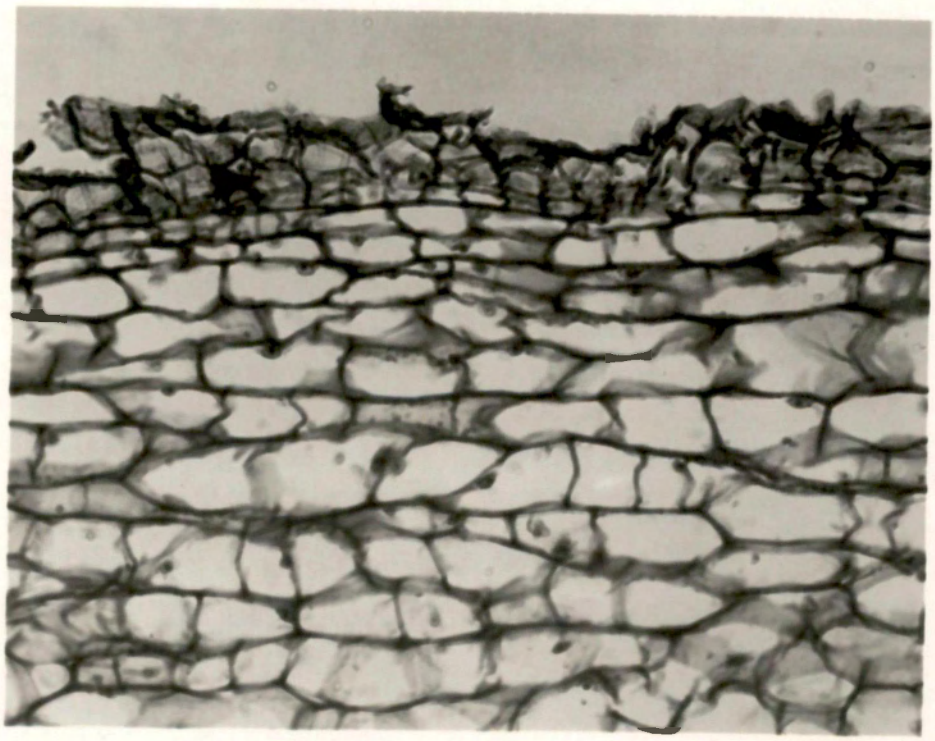
VIII. HISTOLOGICAL STUDIES

The photomicrographs (Plates I through IV) shown in this study are the cross-section of beet tissue. Plate I indicates the control samples under low (48X) and high (192X) powers. Plates II and III are the samples that were irradiated at 2 x 10^5 and 6 x 10^5 rads respectively. The photomicrographs clearly illustrate the cell damage due to the radiation treatment. The cell damage was relatively pronounced at the dose of 6 x 10^5 rads, and shows that damage occurs not only at the epidermis layer but also deep into the endodermis layer. The cell damage which occurred at 2 x 10^5 rads treatment was limited to localized spots on the epidermal layer only.

Destruction of cells was much greater at the dose level of 12 x 10^5 rads and is clearly noticed in Plate IV. The damage was evident not only in the areas of the epidermis but also deep into the endodermis,

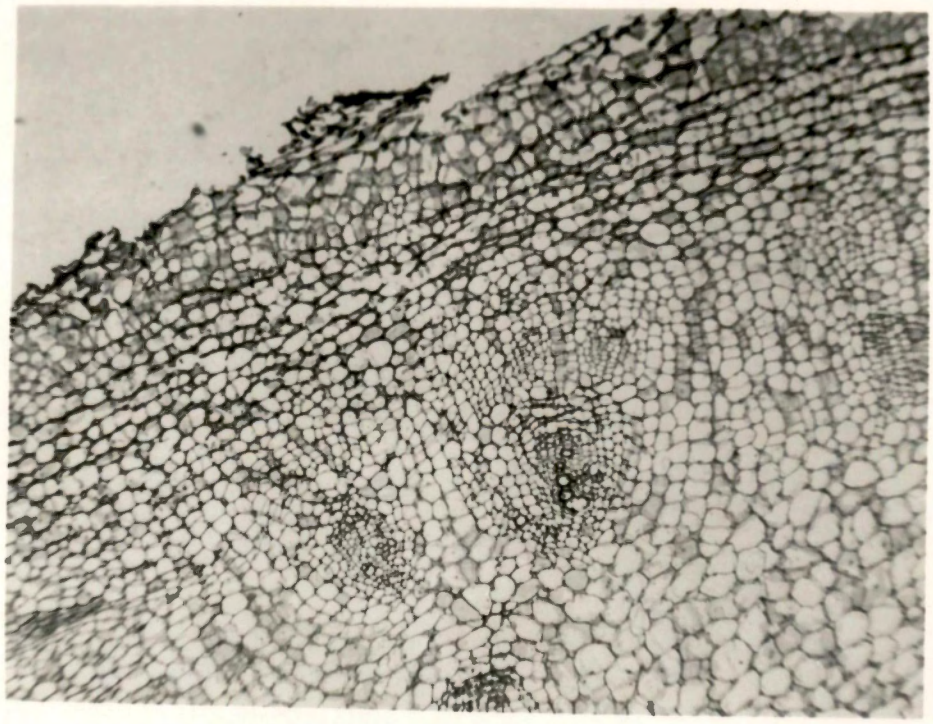


(a) 48X Low Power

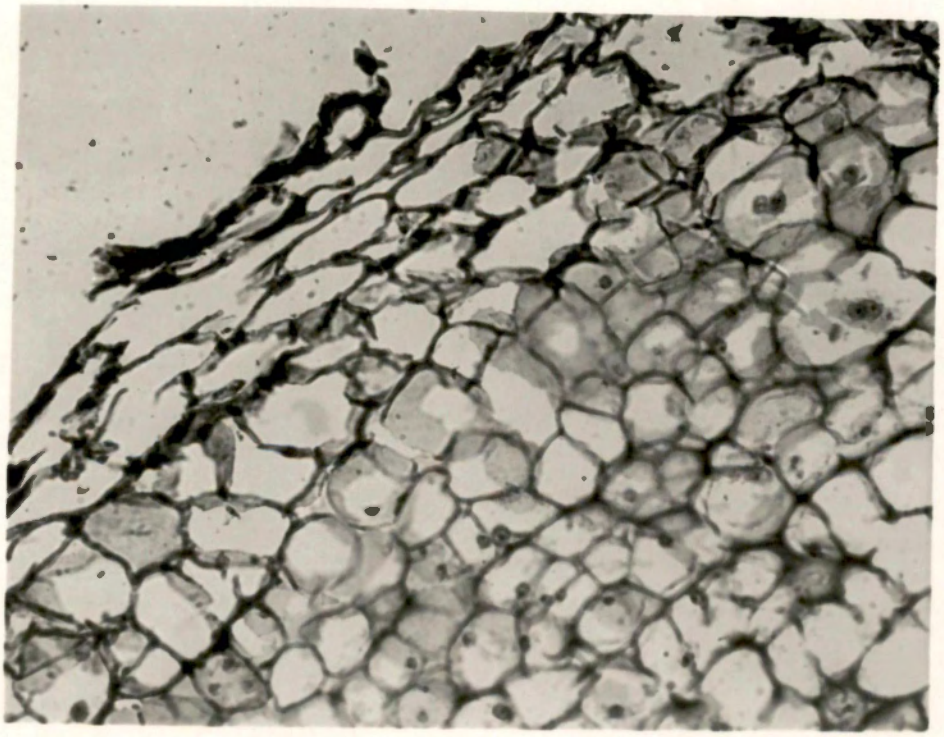


(b) 192X High Power

Plate I. Photomicrograph of beet tissue--control.

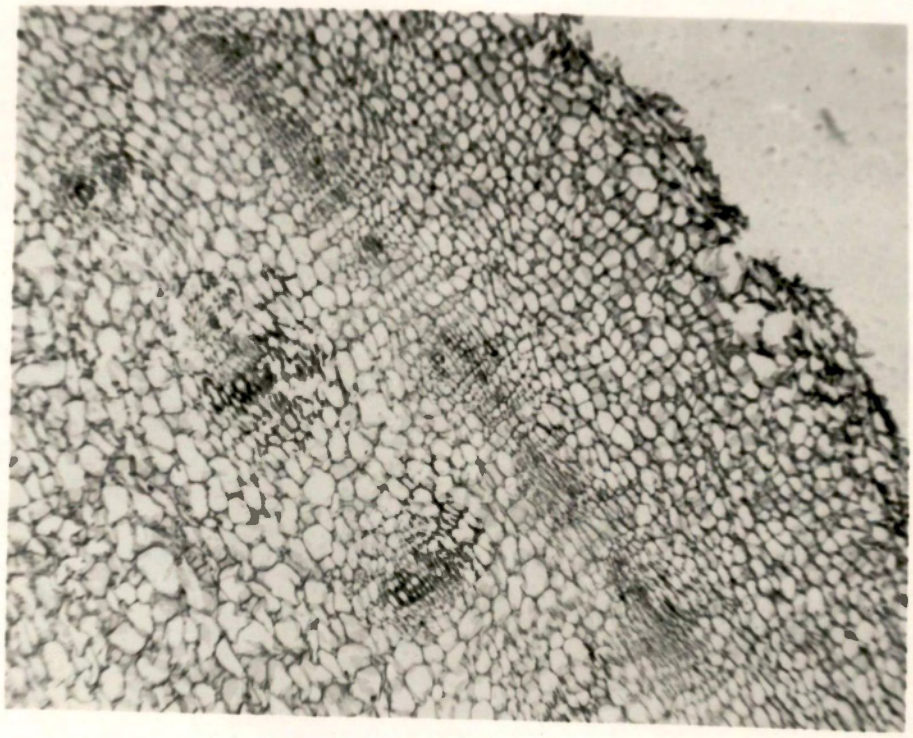


(a) 48X Low Power

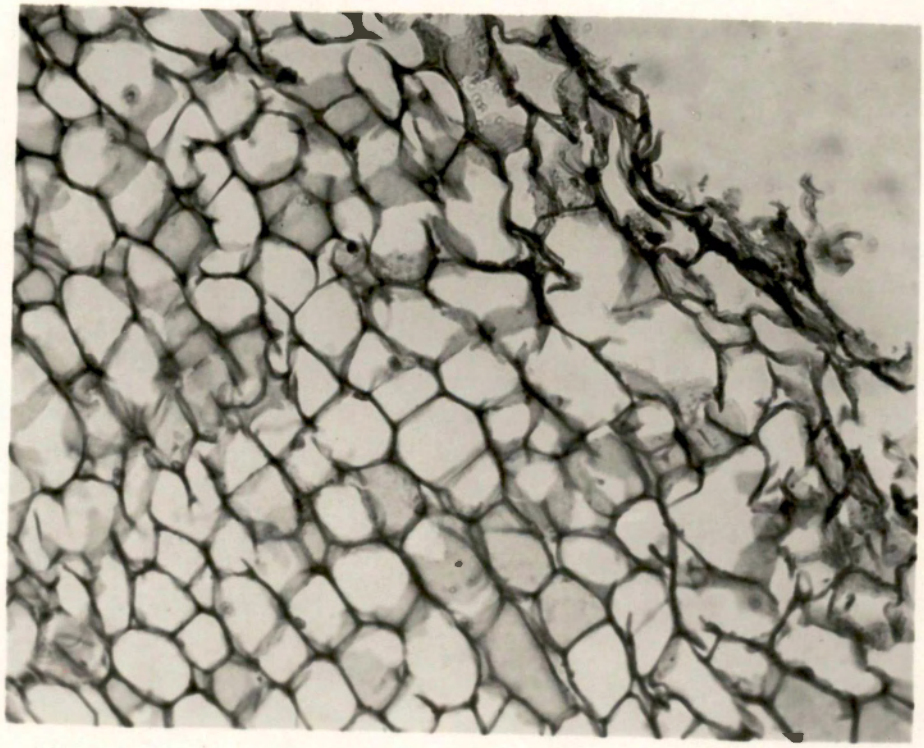


(b) 192X High Power

Plate II. Photomicrograph of beet tissue--2 x 10^5 rads.

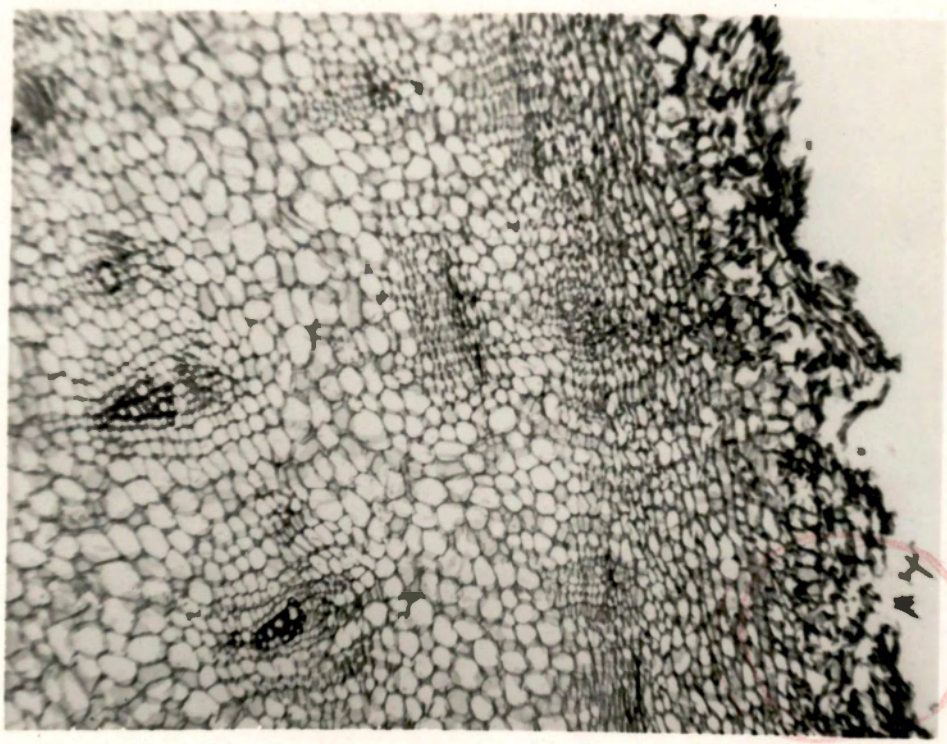


(a) 48X Low Power

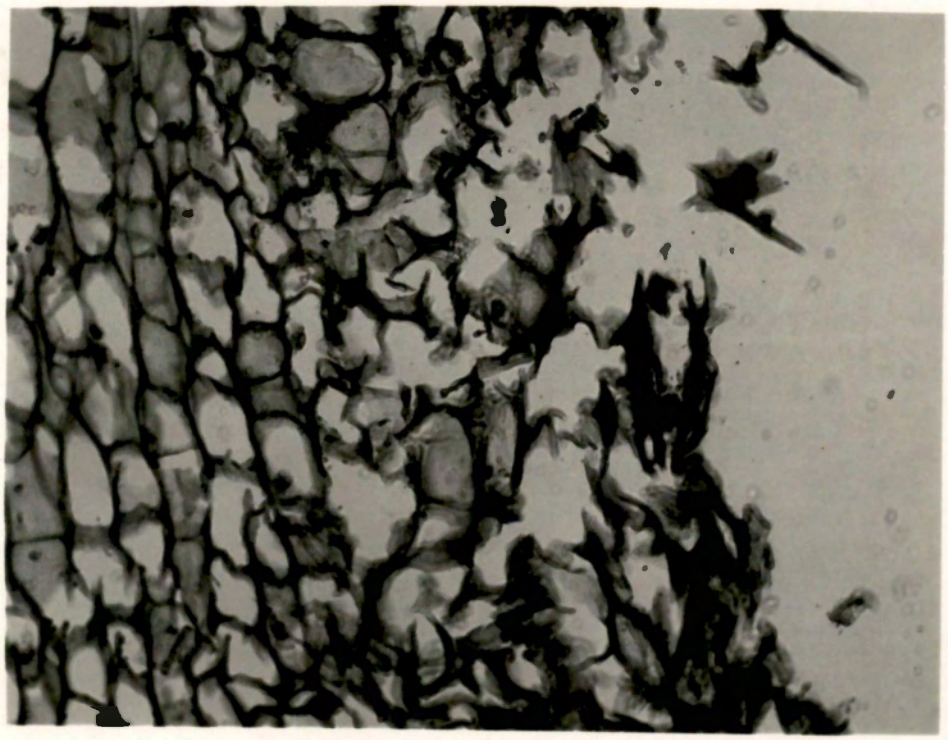


(b) 192X High Power

Plate III. Photomicrograph of beet tissue--6 x 10^5 rads.



(a) 48X Low Power



(b) 192X High Power

Plate IV. Photomicrograph of beet tissue--12 x 10^5 rads.

and the intensity of damage was relatively more than those observed at both the dose 2×10^5 and 6×10^5 rads. No cell damage was evident beyond the cortex (epidermis and endodermis) region.

The samples irradiated at 6×10^5 and 12×10^5 rads appeared to show localized spots of mold growth and softening of tissue after 22 days of storage. This may be related to the death of cells caused by the higher levels of irradiation. Control and 2×10^5 rads (pasteurization dose level) treatments did not show any evidence of mold growth or tissue softening.

According to Glegg et al. (37), the threshold dose for softening of beets is 300 krad, a value considerably in excess of that found for apple tissue and approximately double that of carrot tissue. Salunkhe's (105) work with irradiated lima beans showed cell separations which increased when the dosage was above 2×10^6 rep. A survey of the literature on this topic shows no indication of histological studies related to cell damage on account of radiation.

CHAPTER V

SUMMARY

The experiment was designed to study the effects of gamma radiation upon intensity of color and pigment concentration, ascorbic acid content, enzymatic activity, and histological structure of beets (Beta vulgaris). Pasteurization dose levels of 2×10^5 rads and above (6×10^5 and 12×10^5 rads), revealed that an increase in radiation doses produced a decrease in pigment concentration due to the reduction of the reddish purple pigment.

Electrophoretic studies showed that the pigment betanin consists of four distinct groups of pigments with visual colors of (1) dark purple (primary purple) which makes up the major portion of the pigment betanin), (2) secondary purple which is less darker in color than the primary purple, (3) yellow, and (4) brown.

Spectral studies revealed that the pigment betanin has two absproption peaks, 530 mu and 270 mu. The peak absorption at 530 mu was primarily due to the presence of the primary purple pigment group. Secondary purple, yellow and brown groups of pigments showed absorption maxima at 258 mu. Fluorescence studies indicated a decrease in per cent fluorescence of pigments with an increase in radiation dosage, with the exception of the yellow pigment group.

Spectrophotometric, fluorometric, and densitometer studies on pigment concentration showed a relatively small difference between the

samples evaluated soon after treatment and 22 days after treatment, with the exception of the treatment at 12 x 10^5 rads. Photovolt studies showed that irradiation and storage caused change in color from purplish red to pink. Visual color observations showed that the higher doses of radiation imparted bluish black color to the beet tissue during storage, which appeared to increase with increased duration.

Ascorbic acid content was decreased with a rise in radiation doses. Irradiation and storage resulted in a greater loss of ascorbic acid than radiation treatment alone. Enzymatic activity was not appreciably altered even at the highest dose level used (12 x 10^5 rads). Cellular damage was apparent at the lowest dose level (2 x 10^5 rads) and the most drastic cell damage occurred at the treatment level of 12 x 10^5 rads.

In general, it appears that the loss of color, ascorbic acid and cell damage observed at the pasteurization dose level (2 x 10^5 rads) were relatively very small. Thus radiation sterilization methods could be a very useful tool for prolonging the shelf life of products. In order to evaluate the potential value of radiation pasteurization, it would be necessary to make a comparative study with conventional methods of preservation. Its popularity depends on its ability to compete with numerous other conventional methods of preservation.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Ainley, A. D., and R. Robinson. 1937 Nitrogenous anthocyanins. Part III. Preliminary experiments with betanidin. J. of Chem. Soc., 446-449.

2. Anderson, R. S., and B. Harrison. 1943 The quantitative effects of X-rays on ascorbic acid in simple solution and in naturally occurring substances. J. Gen. Physiol. 27: 69.

3. Aronoff, S., and E. M. Aronoff. 1948 Thermal degradation of dehydrated beets. II. Chromatographic separation of red beet root pigment. J. Food Research 13: 59-65.

4. Bate-Smith, E. C. 1954 Flavonoid compounds in foods. Advances in Food Research 5: 261-300.

5. Barron, E. S. G. 1954 The role of free radicals and oxygen in reactions produced by ionizing radiation. Radiation Research 1: 109.

6. Bellamy, W. D., and E. J. Lawton. 1954 Problems in using high voltage electrons for sterilization. Nucleonics 12: 4.

7. Beraha, L., G. B. Ramsey, M. A. Smith, and W. R. Wright. 1957 Gamma radiation of possible control of post-harvest diseases of apples, strawberries, grapes and peaches. Phytopathology 47: 4.

8. Beraha, L., G. B. Ramsey, M. A. Smith, and W. R. Wright 1959 Effects of gamma radiation on brown rot and Rhizopus rot of peaches and causal organism. Phytophathology 49: 354-356.

9. Beraha, L., G. B. Ramsey, M. A. Smith, and W. R. Wright 1961 Gamma radiation in the control of decay in strawberries, grapes and apples. Food Technology 15: 94-98.

10. Bergmeyer, H. U. 1963 Methods of enzymatic analysis. Academic Press, New York and London.

11. Bischoff, Inaug 1876 Diss. Tubingen; cited in A. S. Dreidling. 1961 In The betacyanin, a class of red pigments in centrospermae. Recent developments in the chemistry of natural phenolic compounds. Proceedings of the Plant Phenolic Group Symposium, pp. 194-211.

12. Blank, F. 1947 The anthocyanin pigments of plants. Botanical Rev. 13: 241-317.

13. Bonner, J. F. 1950 Plant biochemistry. Academic Press, Inc., New York, pp. 421-437.

14. Brownell, L. E. December 1954 Utilization of gross fission products. Prog. Rept. No. 7, U. S. Atomic Energy Commission Contract No. AT(11-1)-162, University of Michigan.

15. Bruce, H. Morgan, and R. G. H. Siu. August 1, 1957 Action of ionizing radiations on individual foods. Radiation preservation of food. U. S. Army Quartermaster Crops, PB-151493, pp. 268-288.

16. Bubl, E. C., and I. J. Tinsley. June 1961 Wholesomeness of radiation sterilized foods (utilization of carotine by the rat), Proceedings of the Seventh Contractors Meeting, Quartermaster Corps, Radiation Preservation of Food Project, p. 153.

17. Burns, E. E. 1956 Maturation changes in tomato fruits induced by ionizing radiation. Ph. D. Thesis, Purdue University, Lafayette, Indiana.

18. Byers, S. O., A. A. Tytell, and M. A. Logan. 1949 The production and some properties of clustridium perfringens hyaluronidase. Arch. Biochem. 22: 66.

19. Chalmers, T. A., T. W. Goodwin, and R. A. Morton. 1945 Action of ionizing radiation on carotine and vitamin A. Nature (London) 155: 513.

20. Chmielewska, I. 1938 Investigations on the coloring matter of red beets, Beta vulgaris L. Roczniki. Chem. 18: 1-8.

21. Clarke, I. D. 1959 Possible applications of ionizing radiations in the fruits, vegetables and related industries. Int. J. Appl. Rad. Isotopes 6: 175.

22. Coleby, B. January 1957 Formation of ascorbic acids by ionizing radiation, Chemistry and Industry, pp. 111-112.

23. Dale, W. M. 1940 The effect of X-rays on enzymes, J. of Biochem. 34: 1367-1373.

24. Dale, W. M., W. J. Meredith, and M. C. K. Tweedie. 1943 Mode of action of ionizing radiation on aqueous solutions. Nature 151: 280-281.

25. Dale, W. M., and C. Russell. 1956 Irradiation of catalase by ionizing radiation in the presence of cysteine, cystine and glutathione. J. of Biochem. 62: 50.

26. Dreiding, A. S. 1961 The betacyanins, a class of red pigments in the centrospermae. Recent developments in the chemistry of natural phenolic compounds. Proceedings of the Plant Phenolic Group Symposium, pp. 194-211.

27. Duncan, E. B. 1955 Multiple range and multiple F test. Biometrics 11: 1-42.

28. Dunlap, C. E., and F. C. Robbins, 1943 The effect of roentgen rays, radon and radioactive phosphorus on thiamin chloride. Amer. J. Roentgenol Rad. Ther. 50: 641.

29. Fargerson, I. S. 1955 Effect of radiation on pigmented foods used in ration for the armed forces. Prog. Rept. U. S. Quartermaster Corps, Contract No. DA19-129-GM-321, University of Massachusetts.

30. Folsdorf, Earl William. 1949 Freeze drying, drying by sublimation. Reinhold Publishing Corporation, New York.

31. Forssberg, A. 1947 Mechanism of the action of X-rays on enzymes in water solution. Nature 159: 308.

32. Francis, F. J., G. E. Livingston, R. Franceschini, and T. Wishnetsky. 1960 Color changes in pigment-free residues from gamma irradiated green beans, broccoli, sweet potatoes and carrots. Food Research 25: 739-749.

33. Franceschini, R., F. J. Francis, G. E. Livingston, and I. S. Fagerson. 1959 Effect of gamma ray irradiation on carotenoid retention and color of carrots, sweet potatoes, green beans and broccoli. Food Technol. 13: 358-365.

34. Frutton, J. S., and S. Simmonds. 1953 General biochemistry. John Wiley and Sons, Inc., New York, pp. 578-583.

35. Geissman, T. A., and Elly Hinriener. 1952 Theories of biogenesis of flavonoids compounds (Part I). Botanical Rev. 18: 77-164.

36. Geissman, T. A. 1952 Theories of the biogenesis of flavonoid compounds (Part II). Botanical Rev. 18: 165-244.

37. Glegg, R. E., F. P. Boyle, L. W. Tuttle, D. E. Wilson, and Z. I. Kertesz. 1956 Effect of ionizing radiations on plant tissues. I. Quantitative measurements of the softening of apples, beets and carrots. Radiation Research 5: 127.

38. Goldblith, S. A. 1963 Exploration in future processing techniques. The M. I. T. Press, Massachusetts Institute of Technology, Cambridge, Massachusetts.

39. Goldblith, S. A. 1955 Preservation of foods by ionizing radiations. J. Am. Dietet. Assoc. 31: 243.

40. Goldblith, S. A., and B. E. Proctor. 1949 Effect of high voltage X-rays and cathode rays on vitamins (riboflavin and carotine). Nucleonics 5: (No. 2) 50.

41. Gostalindstedt. 1956 Electrophoresis of the red beet pigment. Acta Chem. Scand. 10: 698-699.

42. Graham, D., and H. A. Lubs. 1955 The chemistry of synthetic dyes and pigments. Reinhold Publishing Company, New York, pp. 662-688.

43. Groninger, H. S., A. L. Tapple, and F. W. Knapp. 1956 Some chemical and organoleptic changes in gamma irradiated meats. Food Research 21: 555-564.

44. Hannan, R. S. 1956 Science and technology of food preservation by ionizing radiations. Chemical Publishing Company, New York.

45. Haverland, Inaug. 1892 Diss Erlangen, cited by A. S. Drieding. 1961 In The betacyanin, a class of red pigments in centrospermae. Recent developments in the chemistry of natural phenolic compounds. Proceedings of the Plant Phenolic Group Symposium, pp. 194-211.

46. Heinen, J. M. 1956 Radiation sterilization of canned foods. Prog. Rept., U. S. Army Quartermaster Corps, Contract No. 24, Continental Can Co.

47. Huber, W. A. 1948 Electronic preservation of food. Electronics 21 (3): 24-29.

48. Huber, W. A., A. Brasch, and A. Astrack. 1950 Effect of ionizing radiation on ascorbic acid and other vitamins. Amer. Chem. Soc. Abstr. Paper, 117th.

49. Jan, Bures, P. Mojamir, and Z. Jozef. 1962 Electrophysiological methods in biological research. Academic Press, New York-London.

50. Johansen, D. A. 1940 Plant microtechnique. McGraw-Hill Book Company, Inc., New York, p. 41.

51. Judd, B. Deane. 1950 Colorimetry. National Bureau of Standards Circular No. 478, p. 7.

52. Kertesz, Z. I., R. E. Glegg, F. P. Boyle, G. F. Pearsons, and L. M. Massey, Jr. 1964 Effect of ionizing radiation on plant tissues. III. Softening and changes in pectins and cellulose of apples, carrots and beets. J. Food Sci. 29 (1): 40-48.

53. Koltz, I. M. 1947 The effects of salts and proteins on the spectra of some dyes and indicators. Chem. Rev. 41: 373-399.

54. Kraybill, H. F. October 1961 The effect of ionizing radiation on the vitamins and other physiologically active compounds. F. A. O. of the United Nations Technical Meeting on the Evaluation of Wholesomeness of Irradiated Foods. Palais des Congres, Brussels, Belgium, pp. 23-30.

55. Kung, H., E. L. Gaden, Jr., and C. G. King. 1953 Vitamins and enzymes in milk. Effect of γ-irradiation on activity. J. Agric. and Food Chem. 1: 142.

56. Ley, F. J., B. M. Freeman, and B. C. Hobbs. 1963 The use of gamma irradiation for the elimination of salmonellae from various foods. J. Hyg. Camb. 61: 515-529.

57. Licciardello, J. J., J. T. R. Nickerson, B. E. Proctor, and C. L. Campbell. 1959 Storage characteristics of some irradiated foods held at various temperatures above freezing. I. Studies with chicken meat and sweet potatoes. Food Technol. 13: 398-404.

58. Littman, F. E., and A. P. Brady. 1957 Flavor changes induced by radiation sterilization. Review Quick Frozen Foods 20: 48.

59. Lukton, A., and G. Mackinney. 1956 Effect of ionizing radiation on carotenoid stability. Food Technol. 10: 630-632.

60. Lusas, E. W., A. C. Rice, and K. G. Weckel. February 1960 Changes in the color of canning beets. University of Wisconsin, Madison, Research Bulletin No. 218.

61. Mackinney, G., and A. C. Little. 1962 Color of foods. The Avi Publishing Company, Inc., Westport, Connecticut, pp. 220-231.

62. Mackinney, G., and A. Lukton. 1955 Effect of ionizing radiation on carotenoid stability. Prog. Rpt., U. S. Army Quartermaster Corps, Contract No. DA19-129-QM-254.

63. Markakis, P., G. E. Livingston, and I. S. Fagerson. 1959 Effects of cathode ray and gamma ray irradiation on the anthocyanin pigments of strawberries. Food Research 24: 520-528.

64. Maxie, E. C., and N. F. Sommer. 1964 Irradiation of fruits and vegetables. Proceedings of an International Conference, Publication No. 1273, pp. 27-30.

65. Mayer, F., and A. H. Cook. 1943 The chemistry of natural coloring matters. Reinhold Publishing Company, New York.

66. McArdle, F. J., and J. W. Nehemias. 1956 Effect of gamma radiation on the pectic constituents of fruits and vegetables. Food Technol. 10: 599.

67. McArdle, F. J., R. C. Nicholas, and D. E. Wiant. 1957 Rays show promise in curbing fruit decay. Food Eng. 29 (4): 74-76.

68. Meyer, L. H. 1960 Food chemistry. Reinhold Publishing Company, New York.

69. Mickaelsen, K., H. Brenna, and L. Roer. 1955 Effect of gamma rays on sprouting and growth during storage in carrots and potatoes. Proc. of International Conf. on the Peaceful Uses of Atomic Energy 12: 208-210.

70. Milan, Bier. 1959 Electrophoresis theory, methods and applications. Academic Press, Inc., New York.

71. Morgan, B. H., and J. M. Reed. 1954 Resistance of bacterial spores to gamma irradiation. Food Research 19: 357.

72. Morton, Schmall, Charles W. Pifer, and Ernest G. Wollish. 1953 Determination of ascorbic acid. A new colorimetric reaction. Anal. Chem. 25: 1486-90.

73. Naik-Kurade, A. G., G. E. Livingston, F. J. Francis, and I. S. Fagerson. 1959 Effects of cathode ray and gamma ray irradiation on some organic acid-carbohydrate systems. Food Research 24: 618-632.

74. Nehemias, J. V., L. E. Brownell, and H. A. Harlin. 1954 Radiation pasteurization of fresh fruits. Food Manuf. 29: 431-433.

75. Nickerson, J. T. R., B. E. Proctor, and S. A. Goldblith. 1953 Public health aspects of electronic food sterilization. American J. Pub. Health 43: 554-560.

76. Niven, C. F. 1958 Microbiological aspects of radiation preservation of food. Ann. Rev. of Microbiology 12: 507.

77. Noller, C. R. 1951 Textbook of organic chemistry. W. B. Saunders Company, Philadelphia, Pennsylvania.

78. Panalakas, T., and O. Pellefier. 1960 The effect of storage on ascorbic acid content of gamma radiated potatoes. Food Research 25: 33-36.

79. Peterson, R. G., and M. A. Joslyn. 1958 Nature of betanin, the pigment of red beet. Nature 182: 45-46.

80. Peterson, R. G., and M. A. Joslyn. 1960 The red pigment of the root of the beet (Beta vulgaris) as a pyrrol compound. Food Research 25: 429.

81. Phillips, G. O. 1954 Action of ionizing radiation on aqueous solutions of carbohydrates. Nature 173: 1044-1045.

82. Pollard, E. 1951 Ionizing radiation as a test of molecular organization. Am. Scientist 39: 99.

83. Pollard, E. C., W. R. Guild, F. Hutchinson, and R. B. Setlow. 1955 The direct action of ionizing radiation on enzymes and antigens. Progr. Biophys. and Biophys. Chem. 5: 72-108.

84. Pollard, L. H. August 1956 Studies on radiation pasteurization and radiation sterilization of fruit and vegetable products. Progr. Rept. No. 3, U. S. Army Quartermaster Corps, Contract No. DA-19-129-QM-539.

85. Pomerantz, R., M. Rayman, D. Calloway, F. J. Pilgrien, and K. Woods. 1955 Survey of the effect of radiation on selected foods. Prog. Rept., U. S. Army Quartermaster Corps, March 1-October 15.

86. Powrie, W. D., and O. Fennema. 1963 Electrophoretic separation of beet pigments. J. Food Science 28: 214-220.

87. Pratt, G. B., and O. F. Ecklend. 1956 Organoleptic studies of irradiated foods. Food Technol. 10: 496-499.

88. Price, J. R., and R. Robinson 1937 Nitrogenous anthocyanins. Part IV. The coloring matter of Bougainvillaea glabra. J. Chem. Soc. (London), 449.

89. Pridham, J. B. 1960 Phenolics in plants in health and disease. Proceedings of Plant Phenolics Group Symposium held at Bristol, April 1959, Symposium Publications Division, Pergamon Press, Oxford-London-New York-Paris.

90. Proctor, B. E., and S. A. Goldblith. 1948 Effect of high-voltage X-rays and cathode rays on vitamins (niacin). Nucleonics 3: 32.

91. Proctor, B. E., and S. A. Goldblith. 1949 Effect of soft X-rays on vitamins (niacin, riboflavin and ascorbic acid). Nucleonics 4: 56.

92. Proctor, B. E., and S. A. Goldblith. 1949 Effect of soft X-rays on vitamins (niacin, riboflavin and ascorbic acid). Nucleonics 5: 56.

93. Proctor, B. E., and S. A. Goldblith. 1949 The effect of super-voltage cathode rays on the nonenzymatic browning reaction of dried fruits and on chemical compounds pertaining to rats. Science 109: 519.

94. Proctor, B. E., and S. A. Goldblith. 1951 Food processing with ionizing radiation. Food Technol. 5: 376.

95. Proctor, B. E., and J. P. O'Meara. 1951 Effect of high-voltage cathode rays on ascorbic acid. Ind. and Eng. Chem. 43: 718-721.

96. Pucher, G. W., L. C. Curtis, and H. B. Vickery. 1938 The red pigment of the root of the beet. J. of Biological Chemistry 123: 61-70.

97. Read, M. S. October 1960 Current aspects of the wholesomeness of irradiated food. J. of Agric. and Food Chem. 8: 342-349.

98. Reid, M. Brooks, Muriel V. Bradley and Thelda I. Anderson. 1956 Plant microtechnique manual. Second printing, Department of Pomology, University of California, Davis, p. 22.

99. Reznik, H. 1957 Die pigments der centrospermae als systematisches. Element II. Unter suchungen uber das Ionophorelische Verhalten Planta 49: 406. Cited by W. D. Powrie and O. Fennema, 1963, in Electrophoretic separation of beet pigments. J. Food Science 28: 214-220.

100. Richter, G. H. 1952 Textbook of organic chemistry. 3rd edition. John Wiley and Sons, Inc., New York.

101. Roberts, E. A., and B. E. Proctor. 1955 The comparative effect of ionizing radiation and heat upon the starch-containing cell of potato tubers. Food Research 20: 254.

102. Robinson, G. M., and R. Robinson. 1934 A survey of anthocyanins. Biochemical J. 28: 1712.

103. Ryer, R. 1956 III. Influence of radiation preservation of foods on military feeding. Food Technol. 10: 516-519.

104. Salunkhe, D. K. 1961 Gamma radiation effects on fruits and vegetables. Econ. Bot. 15: 28-56.

105. Salunkhe, D. K. 1957 Histological and histochemical changes in gamma irradiated lima beans. Nature 179: 585-586.

106. Salunkhe, D. K., R. K. Gerber, and L. H. Pollard. 1959 Physiological and chemical effects of gamma radiation on certain fruits and vegetables and other products. Proc. Amer. Soc. Hort. Sci. 74: 423-429.

107. Salunkhe, D. K., L. H. Pollard, and R. K. Gerber. 1959 Effect of gamma radiation dose, rate, and temperature on the taste preference and storage life of certain fruits, vegetables, and their products. Proc. Amer. Soc. Hort. Sci. 74: 414-422.

108. Salunkhe, D. K., and M. Simon. 1960 Further studies on effects of gamma radiation on fruit and vegetables. Food Technol. 14 (4): 28.

109. Sawyer, R. L., S. L. Dallyn, and D. J. Cotter. 1955 Some physiological aspects of irradiated potatoes. Conference on Biological, Physical and Industrial Aspects of Potato Irradiation, Brookhaven National Laboratory, Long Island, New York, May 25.

110. Schmidt, O. T., and W. Schonleben. 1956 Zur Kenntnis der Farbstoffs der Roten Rube. Naturwissinschaften 43: 159.

111. Schudel, G. 1918 The anthocyanins of Beta vulgaris L. and Raphans sativus L. Doctoral dissertation, Eidgenoss. Tech. Hochschule, Zurich, pp. 1-64.

112. Seshadri, T. R. 1951 Biochemistry of natural pigments (exclusive of haeme pigments and carotenoids). Ann. Rev. of Biochem. 20: 487-509.

113. Seybold, A. September 1942 Pflanzenpigmente und Lichtfeld als Physiologisches, Geographisches und landwirtschaftlichforsliches problem. Ber. Dtsch. Bot. Ges. 60: 64-85. Supplement.

114. Shea, K. G. 1958 Food preservation by radiation. Food Technol. 12 (8): 6-16. Supplement.

115. Smith, Ivor. 1960 Chromatographic and electrophoretic techniques. 2nd edition. Interscience Publishers, London, Heinemann, New York.

116. Snedecor, G. W. 1956 Statistical methods. The Iowa State University Press, Ames, Iowa.

117. Spragg, S. P. 1960 The mobilization of betanin in beet root. Proceedings of Plant Phenolics Group Symposium held at Bristol, April 1959, pp. 17-24.

118. Sutton, H. C. 1952 Radiation chemistry. Faraday Society Discussions 12: 281.

119. Wheaton, E., G. B. Pratt, and J. M. Jackson. 1961 Radioresistance of five strains of Clostridium botulinum in selected food products. J. Food Sci. 26: 345.

120. Wishantskey, T., G. E. Livingston, F. J. Francis, and I. S. Fagerson. 1959 Effect of gamma ray irradiation on color and chlorophyll retention in green beans and broccoli. Food Technol. 13: 352-357.

121. Wyler, H., and A. S. Dreiding. 1957 Kristallisiertes betanin. Helv. Chim. Acta, 40: 191.

122. Wyler, H., G. Vincenti, M. Mercier, G. Sassu, and A. S. Dreiding. 1959 Zur konstitution des randenfarbstoffes betanin. Helv. Chim. Acta 42: 1696.

123. Ziporin, Z. Z., H. F. Kraybill, and H. J. Thach. 1957 Vitamin content of foods exposed to ionizing radiation. J. of Nutrition 63: 201-209.

124. Radiation preservation of selected fruits and vegetables. Stanford Research Institute, Menlo Park, California. US-AEC Contract No. AT(04-3)-115, Project Agreement No. 23, January 1961.

APPENDIX

OPTICAL DENSITY READINGS FOR THE PIGMENT BETANIN

Replication	Treatment	At 530 mμ		At 270 mμ	
		Prior to Storage	After 22 Days Storage	Prior to Storage	After 22 Days Storage
1	Control	0.7400	0.8360	0.5630	0.5650
2		0.8350	0.8720	0.6370	0.6550
3		0.8190	0.8350	0.6310	0.6500
1	2×10^5 rads	0.5500	0.6000	0.4510	0.4850
2		0.5740	0.6890	0.5000	0.5730
3		0.5710	0.6480	0.5200	0.5670
1	6×10^5 rads	0.4450	0.4390	0.4100	0.4100
2		0.4800	0.4670	0.4650	0.4620
3		0.4700	0.4600	0.4690	0.4670
1	12×10^5 rads	0.3470	0.2800	0.4100	0.3680
2		0.3480	0.3000	0.4390	0.3420
3		0.3310	0.2920	0.4500	0.3500

OPTICAL DENSITY READINGS AT 530 mμ

Replication	Treatment	Primary Purple Pigment	
		Prior to Storage	After 22 Days Storage
1	Control	0.9390	0.8700
2		0.8800	0.8020
3		0.8990	0.8410
1	2×10^5 rads	0.6050	0.7200
2		0.6250	0.7200
3		0.6100	0.7100
1	6×10^5 rads	0.5680	0.4600
2		0.5800	0.4699
3		0.4980	0.6600
1	12×10^5 rads	0.3800	0.3000
2		0.3750	0.3700
3		0.3790	0.3700

OPTICAL DENSITY READINGS FOR ELECTROPHORETICALLY SEPARATED PIGMENTS AT 258 mμ

Replication	Treatment	Brown Pigment	Primary Purple Pigment	Secondary Purple Pigment	Yellow Pigment
		Prior to Storage			
1	Control	0.3300	0.8600	0.4100	0.7880
2		0.3740	0.7300	0.4250	0.5900
3		0.3400	0.7800	0.3650	0.6600
1	2×10^5 rads	0.3350	0.7690	0.3980	0.7820
2		0.4740	0.6600	0.3400	0.5530
3		0.4600	0.6100	0.3400	0.5560
1	6×10^5 rads	0.7300	0.7200	0.2300	0.5250
2		0.5000	0.5470	0.2710	0.5200
3		0.4950	0.5820	0.2950	0.5300
1	12×10^5 rads	0.8500	0.5850	0.2150	0.4500
2		0.7570	0.4420	0.2370	0.4580
3		0.6500	0.5730	0.2050	0.5000
		After 22 Days Storage			
1	Control	0.4500	0.7300	0.3450	0.9200
2		0.4500	0.7300	0.3300	0.8720
3		0.4210	0.6400	0.3400	0.8800
1	2×10^5 rads	0.5200	0.6200	0.3280	0.8750
2		0.5250	0.6200	0.3400	0.8720
3		0.4450	0.5780	0.3200	0.8250
1	6×10^5 rads	0.5500	0.5000	0.2700	0.7900
2		0.5800	0.4890	0.2900	0.7750
3		0.4850	0.4600	0.3000	0.8150
1	12×10^5 rads	0.5800	0.4500	0.2100	0.7520
2		0.5800	0.4350	0.2500	0.7270
3		0.5600	0.4500	0.2780	0.6880

PER CENT FLUROESCENCE ON PIGMENT BETANIN

Replication	Treatment	Prior to Storage	After 22 Days Storage
1	Control	92.0	66.0
2		90.0	66.0
3		94.0	70.0
1	2×10^5 rads	53.0	59.0
2		55.0	57.0
3		51.0	60.0
1	6×10^5 rads	50.0	48.0
2		48.0	45.0
3		51.0	50.0
1	12×10^5 rads	49.0	35.0
2		45.0	38.0
3		47.0	33.0

PER CENT FLUORESCENCE ON ELECTROPHORETICALLY SEPARATED PIGMENTS

Replication	Treatment	Prior to Storage: Brown Pigment	Primary Purple Pigment	Secondary Purple Pigment	Yellow Pigment
1	Control	79.0	50.0	73.0	44.0
2		81.0	48.0	70.0	40.0
3		77.0	52.0	68.0	39.0
1	2×10^5 rads	65.0	57.0	52.0	54.0
2		64.0	60.0	50.0	51.0
3		67.0	58.0	48.0	57.0
1	6×10^5 rads	73.0	62.0	63.0	96.0
2		72.0	64.0	65.0	98.0
3		74.0	58.0	61.0	88.0
1	12×10^5 rads	81.0	49.0	58.0	81.0
2		85.0	50.0	57.0	88.0
3		80.0	47.0	59.0	90.0
		After 22 Days Storage			
1	Control	73.0	52.0	54.0	45.0
2		70.0	48.0	58.0	47.0
3		72.0	50.0	50.0	44.0
1	2×10^5 rads	66.0	47.0	49.0	50.0
2		66.0	50.0	50.0	51.0
3		67.0	46.0	48.0	52.0
1	6×10^5 rads	64.0	46.0	40.0	52.0
2		62.0	48.0	42.0	54.0
3		66.0	50.0	40.0	50.0
1	12×10^5 rads	74.0	66.0	54.0	68.0
2		78.0	70.0	58.0	69.0
3		76.0	62.0	56.0	71.0

DENSITOMETER READINGS FOR ELECTROPHORETICALLY SEPARATED PIGMENTS

Replication	Treatment	Prior to Storage: Brown Pigment	Primary Purple Pigment	Secondary Purple Pigment	Yellow Pigment
1	Control	0.85	3.30	1.00	0.30
2		0.90	3.50	1.20	0.30
3		0.80	3.40	1.10	0.40
1	2×10^5 rads	0.40	2.50	0.50	0.20
2		0.50	2.40	0.60	0.20
3		0.60	2.10	0.70	0.20
1	6×10^5 rads	0.40	2.30	0.45	0.15
2		0.50	2.10	0.50	0.20
3		0.60	2.00	0.40	0.10
1	12×10^5 rads	0.50	2.00	0.30	0.15
2		0.40	1.90	0.20	0.20
3		0.50	1.70	0.20	0.10
		After 22 Days Storage			
1	Control	1.00	3.10	0.80	0.30
2		1.20	3.20	0.70	0.30
3		1.10	3.00	0.90	0.20
1	2×10^5 rads	0.50	2.50	0.80	0.25
2		0.60	2.60	0.70	0.20
3		0.55	2.80	0.90	0.30
1	6×10^5 rads	0.60	2.00	0.80	0.20
2		1.20	2.10	0.90	0.15
3		1.60	1.90	0.60	0.10
1	12×10^5 rads	0.40	2.00	0.50	0.20
2		0.80	2.00	0.60	0.15
3		1.20	1.50	0.70	0.10

Optical Density = Reading x 10.

PHOTOVOLT READINGS

Replication	Treatment	Prior to Storage											
		Filters											
		Green				Amber				Blue			
		1st Half		2nd Half		1st Half		2nd Half		1st Half		2nd Half	
		1	2	1	2	1	2	1	2	1	2	1	1
1	Control	7	7	5	5	14	14	15	15	5	5	5	5
2		9	9	5	5	19	19	11	11	9	9	4	4
3		4	4	6	6	14	14	10	10	5	5	5	5
1	2×10^5 rads	8	8	6	6	21	21	16	16	6	6	5	5
2		9	9	8	8	17	17	14	14	6	6	5	5
3		5	5	6	6	16	16	12	12	4	4	5	5
1	6×10^5 rads	5	5	6	6	16	16	13	13	4	4	3	3
2		7	7	5	5	16	16	15	15	4	4	5	5
3		6	6	5	5	12	12	14	14	4	4	4	4
1	12×10^5 rads	9	9	6	6	14	14	17	17	5	5	3	3
2		5	5	8	8	19	19	14	14	3	3	5	5
3		5	5	6	6	15	15	12	12	5	5	4	4
		After 22 Days Storage											
1	Control	6	6	5	5	10	10	12	12	3	3	4	4
2		6	6	5	5	9	9	12	12	3	3	4	4
3		7	7	7	7	15	15	15	15	4	4	4	4
1	2×10^5 rads	4	4	5	5	10	10	7	7	3	3	4	4
2		6	6	4	4	8	8	13	13	3	3	4	4
3		5	5	6	6	10	10	11	11	3	3	3	3
1	6×10^5 rads	5	5	6	6	11	11	10	10	4	4	4	4
2		6	6	4	4	10	10	12	12	4	4	4	4
3		4	4	6	6	9	9	14	14	4	4	5	5
1	12×10^5 rads	3	3	2	2	4	4	6	6	2	2	3	3
2		2	2	4	4	8	8	4	4	3	3	3	3
3		4	4	2	2	8	8	4	4	2	2	2	2

VISUAL COLOR SCORES

Replications	Treatment	Individual Scores									
		1	2	3	4	5	6	7	8	9	10
					Prior to Storage						
1	Control	+1	+1	-3	-2	-1	+1	+3	+1	+1	-2
2		0	0	+1	+2	+1	+1	0	-1	0	0
3		+2	+1	+2	+3	+1	+2	+3	+3	+2	-1
1	2×10^5 rads	-1	0	-1	-1	-1	0	0	+2	0	0
2		0	0	0	+2	0	+1	+1	0	-1	+1
3		-1	-1	0	0	0	-1	-1	0	-2	+1
1	6×10^5 rads	-2	-2	-2	-3	-2	-3	-3	-1	-1	+3
2		-2	-1	-1	-2	-2	-2	-1	-1	-2	+1
3		-2	-1	-1	-1	-1	-1	-1	-2	-1	+1
1	12×10^5 rads	-3	0	-3	-3	-2	-3	-1	-3	-2	+2
2		+1	0	+1	+2	0	+1	+1	0	0	-1
3		+2	+2	-2	+2	+3	+3	-2	-3	-3	+2
					After 22 Days of Storage						
1	Control	+3	+1	+1	+1	+1	+2	+1	+1	+1	+1
2		+2	+1	+2	+2	+1	+1	0	+1	+1	-1
3		-2	0	+1	0	-1	-1	0	-1	-1	-1
1	2×10^5 rads	+1	+1	+2	+2	+2	+3	+1	+1	+1	+2
2		+1	0	+1	+1	+1	0	+1	+1	0	0
3		0	+1	+2	+1	+2	+3	0	0	+1	0
1	6×10^5 rads	+2	+1	+2	+2	+1	+2	+2	+1	+1	+2
2		+3	+1	+2	+2	+2	+3	+1	+2	+2	+2
3		+3	+1	+3	+2	+2	+3	+1	+1	+2	+2
1	12×10^5 rads	+3	+3	+3	+3	+2	+3	+3	+3	+3	+3
2		+3	+3	+3	+3	+3	+3	+3	+3	+3	+3
3		-3	+3	+3	+3	+3	+3	+3	+3	+3	+3

MILLIGRAMS OF ASCORBIC ACID PER 100 GRAMS OF TISSUE

Replication	Treatment	Prior to Storage	After 22 Days Storage
1	Control	7.8	6.8
2		7.0	6.2
3		7.2	6.6
1	2×10^5 rads	7.0	4.2
2		6.7	4.0
3		6.5	4.5
1	6×10^5 rads	5.2	2.9
2		4.8	3.5
3		5.3	2.5
1	12×10^5 rads	4.5	2.0
2		4.0	2.8
3		4.1	2.5

VITA

The author was born in Coorg, India, on June 2, 1938. In 1960 he received B. Sc. (Agri) degree from the University of Karnatak, Dharwar, India. In 1962 he was admitted to the Graduate School of The University of Tennessee, Knoxville. He received a M. S. degree with a major in Horticulture and a minor in Botany in 1963. In 1964 he registered in the Department of Food Technology. Since then he has been working toward the degree of Master of Science, with a major in Food Technology.