STUDIES ON COLOUR OF EGG YOLK

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ABSTRACT

The effect of varying combinations of irradiation treatment, freezing procedures and duration of storage on the color of naturally and artificially pigmented egg yolk determined by two objective methods was studied. The relationship between the two methods of color evaluation was also determined.

Naturally pigmented yolks were obtained from eggs laid by pullets of a single strain of Single Comb White Leghorn fed a standard diet. Artificially pigmented yolk was prepared by addition of either beta-carotene or canthaxanthin to the naturally pigmented material.

Color of yolk was assessed :

1. On the basis of pigment concentration determined by absorbance of acetone extract and expressed as beta-carotene equivalent (BCE) and

2. On the basis of chromaticity coordinates (x,y), lightness (%Y), dominant wavelength (DWL) and excitation purity (EP) determined by reflectance spectrophotometry.

Both irradiation dose (0, 0.5, 1.0 and 2.0 Mrad) and time of irradiation (before or after freezing) had significant effect on the chromaticity coordinates, BCE values and excitation purity of naturally and artificially pigmented

yolk samples. Higher radiation doses and irradiation before freezing were associated with decreased chromaticity coordinates, BCE values and excitation purity. In artificially pigmented samples increases in irradiation dose and irradiation before freezing resulted in significant decreases in lightness.

Samples frozen and stored at -10 F^{o} had consistently higher mean chromaticity values and lower excitation purity than those at -35 F^{o}. The temperature effect on BCE values was inconsistent among experiments.

After 30 days storage mean x-values were lower and mean y-values were higher than after 10 days storage. These changes were associated with almost no change in DWL or EP.

Nitrogen-packed samples had consistently lower BCE values than air-packed and this difference was significant in all but Experiment 1. No corresponding differences were found in chromaticity coordinates, lightness, DWL or EP.

Correlation analyses revealed highly significant ($P \leqslant 0.01$) linear relationships between BCE and both chromaticity values and lightness ranging from + 0.09 to + 0.79.

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LIST OF ABBREVIATIONS AND DEFINITIONS

rep	Roentgen-equivalent-physical. - The amount of radiation that would release 1 roentgen of energy in 1 gram of tissue.
Roentgen	The quantity of ionizing irradiation that will produce sufficient ions in 1 cc of dry air to carry 1 e.s.u. of electricity.
Rad	Quantity of ionizing radiation which results in the absorption of 100 ergs per gram of irradiated material.
Mrad	10^6 rad.

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INTRODUCTION

Increasing importance is being given to the color of egg yolk when judging the quality of eggs. One of the most striking components of whole egg appearance is the yolk color.

Color in egg yolks is due to the carotenoids which the hens absorb from feed and deposit as pigment. Carotenoids are yellow to red pigments of aliphatic or aliphatic-alicyclic structure composed of isoprene groups, usually 8, linked so that the two methyl groups nearest the center of the molecule are in positions 1:6 and all other lateral methyl groups are in position 1:5, with a series of conjugated C-C double bonds constituting the chromophoric system of the carotenoids. The basic structure is demonstrated by the formula for beta-carotene a symmetrical hydrocarbon with 40 carbon atoms as shown in Fig.1

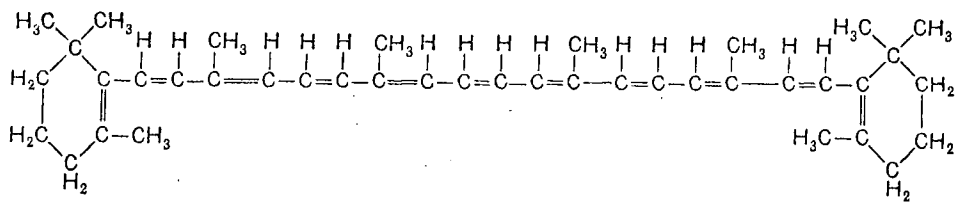

Fig.1. Structural formula of beta-carotene

The major carotenoid subgroups are carotenes and xanthophylls. The former includes all the hydrocarbon carotenoids, and the latter all the hydroxy, epoxy, and oxy-derivatives of the carotenes. Xanthophylls are also frequently esterified, as, for example, physalien, which is the dipalmitoyl ester of zeaxanthin. Many carotenoids were named by their discoverer for some special property or for their source, e.g., carotene (from carrots), cryptoxanthin (hidden pigment), and zeaxanthin (from Zea Maize) (Goodwin, 1954a; Borenstein and Bunnell 1966).

Egg yolk products are traded by the food industry in frozen (plain, salted or sugared), dried or fresh forms and are used in the manufacture of a number of food products including mayonnaise, macaroni and bakery products as an emulsifier and to impart color. Thus, the effect of processing on the color of yolk products is important.

The spectral absorption curves of the carotenoids, particularly in the visible region 400 - 500 nm are widely used for purposes of identification and assay. The official AOAC method of assessing egg yolk color involves comparison of the absorbance (455nm) of an acetone extract with a beta-carotene standard curve (AOAC, 1969). Color comparison charts are also widely used. Yolk color assessment by

reflectance measurement has also been investigated (Richards, 1970).

It was the primary purpose of this research to study the effect of varying combinations of irradiation treatment, freezing methods, and storage time on the color of naturally and artificially pigmented egg yolk determined by two objective methods. A secondary objective was to determine the relationship between the two methods of color evaluation.

REVIEW OF LITERATURE

The stability of food carotenoids during and after processing has received considerable attention. The common unit operations of food processing in general have only minor effects on the carotenoids. The naturally occuring carotenoid-protein complexes apparently are more stable than carotenoids per se. (Takamatsu, 1957). Freezing had little effect on asparagus and lima beans carotenoids (Zimmerman et al., 1941). Cryptoxanthin, and total carotenoids changed only slightly in corn stored for 9 months at 0 F^{o} (Tichenor et al., 1965). Frozen broccoli showed no loss in total carotene during storage at 0 F^{o} for 61 weeks (Martin et al., 1960).

The effect of gamma-irradiation on carotenoids has been studied in a variety of systems. Lukton and Mackinney (1956) found a film of beta-carotene and lycopene in the solid state to be surprisingly stable: 2% loss at 2 million rep. Solutions of beta-carotene were unstable in petroleum ether, methyl stearate, methyl oleate, and methyl linoleate. Stability was greater in stearate than in oleate and linoleate. They concluded that destruction is caused by secondary reactions and depends upon the extent to which free radicals or peroxides, formed in the surrounding medium, are available for reaction with carotenoids. The same workers studied the effects of gamma-irradiation on

tomato purees, whole tomatoes, carrot purees, and prawns. Carotenoid stability was excellent at doses up to 12 x 10^6 rep in vegetable products, but the astaxanthin content of prawns decreased as much as 60% at 4 x 10^6 rep.

Irradiation of green tomatoes retarded the synthesis of lycopene, and at high levels prevented it (Salunkhe et al., 1959). The color of tomatoes fadded at doses of from 5 x 10^5 to 1 x 10^6 rads. Doses up to 3.72 x 10^6 rads had no apparent effect on the carotenoids of canned apricot nector, peach nector, or peach halves (Salunkhe et al., 1959).

Franceschini et al., (1959) studied the effect of gamma-irradiation on the carotenoids of carrots, sweet potatoes, green beans, and broccoli. Green bean carotenoids were unstable when irradiated at 1.86 megarads (Mrads) after freezing, but reasonably stable when irradiated at room temperature. The other vegetables in this study did not exhibit this freezing-radiation interrelatioship. The carotenoids of sweet potatoes showed relatively little destruction from irradiation at 1.86 Mrads. However, visual color changes were greater than pigment changes in storage and highly dependent on storage conditions. Carotenoid destruction of broccoli was 25-50% at 1.86 Mrad. Carotenoid destruction of carrots was moderate at 1.86 Mrad except when the carrots were in an air atmosphere.

Packing in nitrogen improved retention of carotenoid

pigments of irradiated sweet corn (Tichenor et al., 1965). Retention of beta-carotene, cryptoxanthin, and other carotenoids was good at 1.0 mrad but decreased at 3 to 5 mrads.

Lai et al. (1959) gamma-irradiated both a hard red spring wheat and a hard red winter wheat. Total carotenoids per 100g of flour decreased from 13.7 to 10.5mg at 1.0×10^6rep.

Carotenoid addition to foods predated the commercial synthesis of beta-carotene. Carrot extracts, palm oil extracts, annatto extracts, and oleoresin paprika have been used for generations to color cheese, butter, soups, sausage products, etc. The advent of pure synthetic carotenoids has increased interest in coloring foods with these compounds because of the obvious advantages of working with well-controlled, reproducible color sources. Carotenoids are added to foodstuffs for both nutritional enrichment and color improvement (Bunnell et al., 1966).

The major carotenoids, natural and synthetic, used to color foods are, bixin, alpha-carotene, beta-carotene, beta-apo-8-carotenal, canthaxanthin, beta-apo-8-carotenoic acid ethyl ester, capsanthin, and capsorubin (Bunnell et al., 1966).

Beta-carotene is probably the most widely used synthetic carotenoid. The major uses in North America are to color and

fortify margarine, shortening, fruit drinks, popcorn, and baked goods (Bauernfeind et al., 1958). Beta-carotene used in citrus beverages, primary cheese, egg yolk products, ice cream, and cake mixes (Bunnell et al., 1958).

An interest by food processors in standarized egg yolks of darker-color for use in bakery products, macaroni, and mayonnaise has prompted investigation of the addition of beta-carotene to frozen and dried yolk products. The color of yolk products has been expressed in terms of carotene concentration by the technical committee of the National Egg Products Association (NEPA).

NATURAL EGG YOLK EXPRESSED AS CAROTENE UNITS

NEPA yolk color (standards)	Color equivalents in terms of carotene(P.P.M)
1 (very light yellow)	15
2	40
3	70
4	90
5	120
6 (orange)	150

Canthaxanthin, which is not yet approved in the U.S., is used in Europe to color tomato products. Beta-apo-8-carotenoic acid ethyl ester is used in Europe to pigment egg yolk.

A color assay method in which the absorbance of an acetone extract of yolk products is determined at 455 nm and then converted to equivalent beta-carotene concentration is used by the AOAC (1960).

Borenstein and Bunnell, (1966) studied the effect of storage time at -5 F^{o} on the retention of carotenoid content of natural and synthetic beta-carotene pigmented egg yolk and found no loss of carotenoid content after 3 months storage but slight decrease after 15 months.

A very simple and widely used method of measuring color in egg yolks is visual scoring. The Heiman-Carver color rotor (1935) has been used extensively in the U.S. but is unavailable today (Heiman and Carver, 1935). This device consisted of a black wheel containing 24 different yolk shaped color samples so that a color number could be assigned to any yolk after a direct visual comparison.

The Hoffman La Roche color fan is a less cumbersome application of the same idea (Hoffman La Roche & Co. Ltd. 1962). It contains a uniform progression of increasing color from ivory to orange-yellow, numbered 1 to 12. In Canada, an enamelled ring from (NRC discs) is used (Ashton and Fletcher, 1962). Much research was done in preparing the standards, in mixing the enamel to (CIE) tristimulus values, in shaping the surface of the disc so that the method of viewing would be the same for the disc as the actual yolk. This system consists of 15 discs from light yellow to deep orange.

The measurement of color, or in other words the determination of values characterizing a color perception, translated the functions of human vision with the help of physical measuring instruments. The laws of additive color mixing show that any color perception can be produced by mixing three suitable spectral stimuli or primaries in the proper ratio. The human seems to judge the visible part of the electromagnetic spectrum by only three different spectral sensitivity functions, which are experienced by the observer as a single effect or color perception. Thus, three numbers or quantities are required in order to clearly define any one color perception. Therefore, color may be understood as local vectors in a color space. Each point within this color space characterizes color according to hue (wavelength), saturation (proportion of spectral light in the mixture of spectral and white light) and luminosity (intensity of light perception connected with color perception). These are the three properties which any observer with normal vision would attribute to color as a sensory perception (Vuilleumier,1969).

The tristimulus values of the standard C.I.E. system are defined by the International Commission on Illumination (C.I.E)(1931) as a conventional reference system for color measurements (Mackinney and Little, 1962). The three quantities which characterize a color perception with a defined illuminant are symbolized by X, Y and Z (transformed, unreal primaries). The value of X (or $\bar{x}$ in the case of spectrum

colors) represents the amount of primary which is reddish purple of higher saturation than any obtainable color having this hue. The value of Y (or $\bar{y}$) represents the amount of green primary considerably more saturated than the spectrum color whose wavelength is 520 nm. The value of Z (or $\bar{z}$) represents the amount of blue primary that is considerably more saturated than the spectrum color whose wavelength is 477 nm (Hardy, 1936).

The tristimulus values X, Y and Z of a color were defined as the relative amounts of each of the three unreal primaries needed to effect a color match under specified conditions. The specification of a color in terms of its tristimulus values is cumbersome. It therefore became a matter of convenience to express results more simply. The entire luminosity is ascribed to Y, and the chromaticity can be expressed by co-ordinates x, y and z, such that:

$$x = \frac{X}{X+Y+Z} \quad , \quad y = \frac{Y}{X+Y+Z} \quad , \quad z = \frac{Z}{X+Y+Z}$$

and consequently:

$$x+y+z = 1$$

Therefore a color is uniquely defined by its chromaticity coordinates x and y, and its lightness, the tristimulus Y value. Trichromatic coefficients x, y and z are the proportions of each of the primaries in the total mixture (Mackinney and

Little, 1962).

If the derived values of x and y are plotted in a rectangular coordinate system a very convenient representation according to hue and saturation results. The chromaticity diagram of the C.I.E. is shown in Fig. 2 (Francis, 1969).

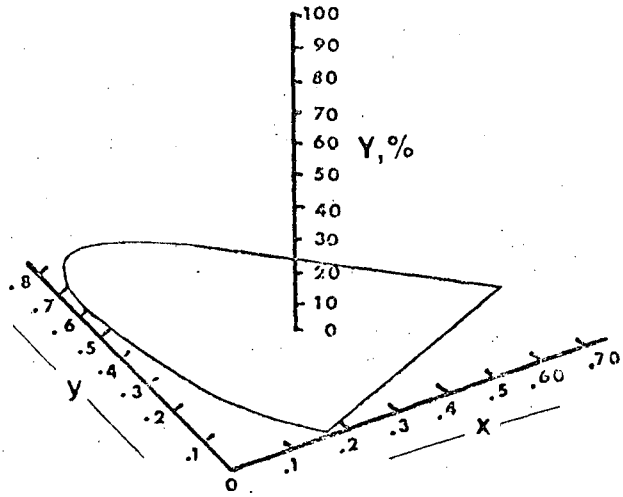

Fig. 2. The C.I.E. horseshoe shaped spectrum locus showing %Y.

The relative brightness of a sample is indicated directly by the value of Y on a scale that represents an absolute black by zero and a perfect white by 100 (Hardy, 1936).

MATERIALS AND METHODS

EXPERIMENT 1

Eggs were obtained from the U.B.C. strain of Single Comb White Leghorn pullets housed in individual cages on the U.B.C. poultry farm. All pullets were fed the same diet during the experiment to obtain as uniform egg yolk color as possible. Eggs were individually broken and egg albumen separated from yolk. All egg yolks were mixed together and 10% sodium chloride (w/w) was added to prevent gelation during freezing and storage. Each experiment required about 200 eggs. Fifty-seven plastic containers (113gm capacity) were filled with the homogeneous yolk material and then randomly assigned to treatment groups. Duplicate samples of 55gm each were drawn and evaluated for each experimental unit (container).

The factors studied in this experiment consisted of: 7 irradiation treatments (zero-dose control, and all combinations of 0.5, 1.0 and 2.0 Mrad administered before or after freezing); 2 freezing and storage temperature (-10 and -35 F^{o}); 2 atmospheres during irradiation (air and nitrogen) and 2 post-treatment storage time (10 and 30 days). The treatments were arranged as a 7 x 2 x 2 x 2 factorial in a completely randomized design. In addition, an untreated sample was evaluated and served as a fresh control.

Gamma-radiation was administered in a Gammacell 220

(Atomic Energy of Canada Ltd.) at a dose rate of about 1.0 Mrad per hour. Unfrozen samples were irradiated under ambient temperatures. An attempt was made to maintain the temperature of frozen samples during irradiation by packing the containers with crushed ice in a doubly insulated vessel.

Initial freezing of samples was accomplished in blast air freezers at -10 or -35 F^{o}. A nitrogen atmosphere was attained by allowing nitrogen gas to flow into the headspace of the containers for about 10 sec. before closing. Samples were stored for 10 or 30 days at -10 or -35 F^{o} after which time yolk color was evaluated by two methods.

The first method, an extraction-colorimetric procedure was carried out according to the method outlined by the Association of Official Agricultural Chemists (AOAC, 1965). In this procedure 2.5gm of liquid yolk was mixed with one to two ml of acetone and stirred to a smooth paste. Approximately 50 ml of acetone, was added and the mixture was washed onto Whatman No. 4 filter paper with successive small portions of acetone. The filtrate was collected in a ground glass-stoppered, 100 ml volumetric flask and diluted to volume with acetone. The contents of the flask were shaken, and the absorbance of an aliquot read at 10 nm intervals between 400 - 500 nm on a previously standarized Bausch and Lomb Spectronic 20. A previously developed standard curve for beta-carotene was used to estimate the beta-carotene equivalent content of the sample.

The second method, an Hitachi, Perkin-Elmer spectrophotometer with a diffuse reflectance attachment was standarized to 100% reflectance against MgO. The sample was determined at 10 nm intervals from 400 - 680 nm. From these values the C.I.E. tristimulus values X, Y and Z, and the trichromatic coefficients x, y and %Y were calculated by the weighted ordinate method incorporating illuminant C at a constant wavelength intervals of 10 nm (Mackinney and Little, 1962). Dominant Wavelength (DWL) and Excitation Purity (EP) were calculated according to the method by McCarley et al., 1965.

EXPERIMENT 2

The methods used were similar to those of Experiment 1 with 2 exceptions. Firstly, a Unicam SP.800 B recording spectrophotometer with reflectance attachment was used for reflectance determinations. The sample was contained in an optically clear petri dish (3.5 x 10 mm). Reflectance was read from the record at 10 nm intervals from 400 - 680 nm.

Secondly, a liquid nitrogen system was used to control temperature during radiation at -10 and -35 F^{o}. In this method liquid nitrogen was forced by compressed air into a container in the radiation drawer. Rubber tube in the container permitted regulation of flow. Temperature was

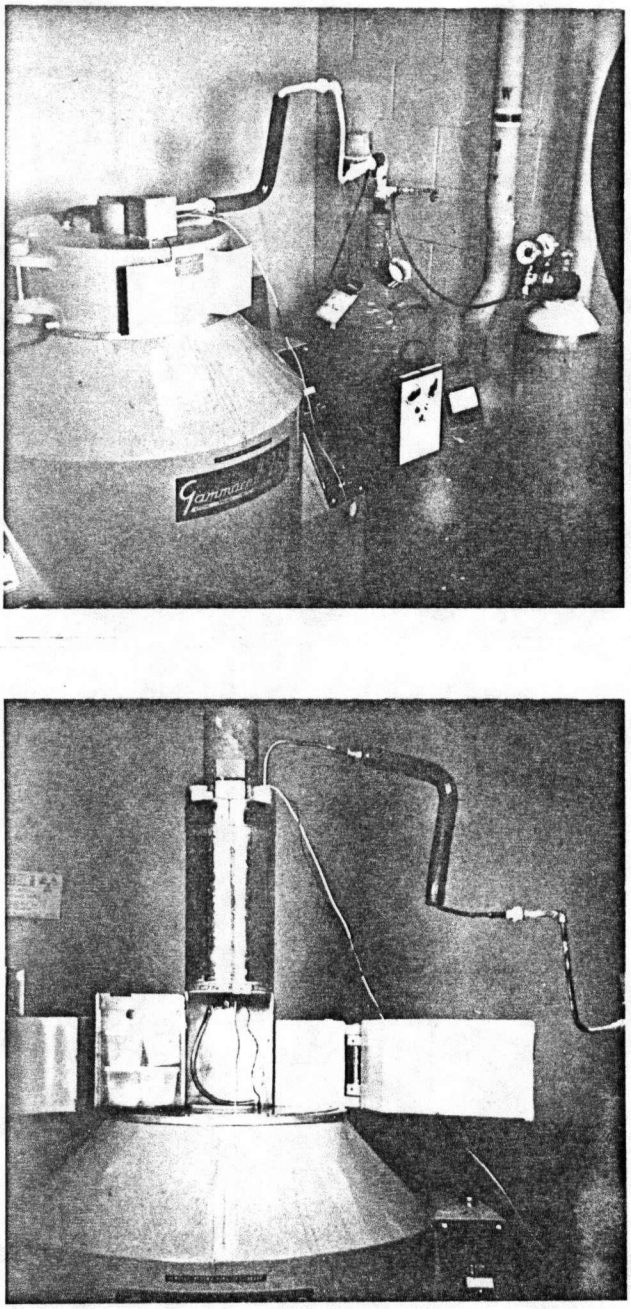

Fig. 3. The instrument for controlling temperature during irradiation.

controlled at -10 and -35 F^{o} to within $\pm$ 2 F^{o}. The system is shown in Fig. 3.

EXPERIMENT 3

The methods were similar to those of Experiment 2. Synthetic beta-carotene was used for coloring egg yolk.

Two and one half gm of 10% beta-carotene water dispersible beadlets were weighed into a small beaker and 25 ml of distilled water was added. An aliquot of 0.53 ml of beta-carotene solution was added per 100 gm of egg yolk.

EXPERIMENT 4

The methods were similar to those of Experiment 2. Canthaxanthin was used to pigment the egg yolk. This was accomplished in the following manner:

Two and one half gm of 10% canthaxanthin water dispersible beadlets were weighed into a small beaker and 25 ml of distilled water was added. An aliquot of 0.23 ml of canthaxanthin solution was added per 100 gm of egg yolk.

STATISTICAL METHODS

The raw reflectance data were reduced to tristimulus values (X,Y,Z) and chromaticity coordinates (x,y,%Y)

calculated by the use of the weighted-ordinate method. The individual color parameters were subjected to analyses of variance and simple linear correlations between each pair of variables was determined. All calculations were performed by an IBM 360 digital computer.

RESULTS

Numerous zero-order interaction terms were found to be significant in these experiments. Most of these interactions involved either radiation treatment or temperature and frequently both. However, graphical examination of the apparent interactions revealed that almost without exception differences in response among the levels of one factor were qualitatively the same for each level of the second factor . Therefore, the means for the main effects were considered to be valuable indications of treatment responses at least qualitatively (Cox, 1958).

REFLECTANCE MEASUREMENTS

Chromaticity Coordinates

Mean values for chromaticity coordinates x and y are shown in tables 1 to 4, 11 and 12. Relationships between means x, y and dose levels are shown in Fig. 1, 2, 3 and 4. Analyses of variance are represented in Tables A1, A2, B1, B2, C1, C2, D1 and D2.

The mean (x) value and mean (y) value of fresh samples were highly significantly ($P \leqslant 0.01$) different than those of corresponding treated samples in all experiments.

Irradiation treatment had a highly significant ($P \leq 0.01$) effect on both mean (x) and (y) value in all experiments. The mean values were significantly higher for control samples than for irradiated samples and higher for samples irradiated after freezing than before freezing. The chromaticity coordinates decreased consistently with increasing dose of radiation.

The mean (x) value was lower after 30 days storage than after 10 days in all experiments. The difference was significant in all but Experiment 2. Conversely, mean (y) values were higher after 30 days storage than 10 days storage.

Freezing and storage at -10 F^{o} resulted in higher mean (x) and (y) values than at -35 F^{o} in all experiments.

Atmosphere had no significant effect on (x) value in any of the experiments. Mean (y) value was significantly higher for nitrogen-packed than air-packed samples in Experiment 1 only. (Atmosphere had no significant effect on (y) value in Experiments 2, 3 and 4)

Lightness

Mean values for (%Y) are shown in Tables 5, 6 and 13. Relationships between mean (%Y) and dose levels are shown in Fig. 1, 2, 3 and 4. Analyses of variance are represented

in Tables A3, B3, C3 and D3.

Fresh samples were significantly lighter in color (higher mean %Y) than treated samples in all experiments. Similarly, irradiated samples were darker than unirradiated controls in all experiments.

In Experiments 3 and 4 the darkness of samples increased significantly with increasing irradiation dose, and samples irradiated before freezing were significantly darker than those irradiated in the frozen state. Similar insignificant trends were evident in Experiment 2.

The effect of storage time on lightness was inconsistent among experiments. In Experiments 1 and 4 samples stored for 10 days were significantly darker than those stored for 30 days. In Experiments 2 and 3 the samples stored for 10 days were lighter but this effect was significant only in Experiment 2.

In Experiment 1 and 2 samples stored at -35 F^{o} were significantly lighter than samples stored at -10 F^{o}. No significant effect of temperature was found in Experiments 3 and 4.

In Experiment 3 a highly significant interaction between storage time and temperature was found. This may account for the lack of significance of the 2 corresponding main effects in this experiment.

Atmosphere had no significant effect on lightness in any of the experiments.

Dominant Wavelength (DWL)

Mean DWL was almost invariable among treatments within experiments (Table 16). The values ranged only from 577 to 590 nm.

Excitation Purity (EP)

EP was markedly affected by dose level. Increasing the dose level resulted in a decrease in EP. Also samples which were irradiated after freezing or stored at -10 F^{o} had higher EP values than corresponding samples irradiated before freezing or stored at -35 F^{o}. EP tended to decline with length of storage but the effect was generally slight. Atmosphere had no effect on EP (Table 17).

ABSORBANCE MEASUREMENTS

Mean values for beta-carotene equivalent (BCE) at 450 and 460 nm are shown in Tables 7 to 10, 14 and 15. Relationships between mean BCE at 450, 460 nm and dose level are shown in Fig. 8 and 9. Analyses of variance are represented in Tables A4, A5, B4, B5, C4, C5, D4 and D5.

Reading at 450 nm

Beta-carotene equivalent (BCE) values of fresh samples were highly significantly ($P \leq 0.01$) different than corresponding treated samples in all experiments.

Irradiation treatment had a highly significant ($P \leq 0.01$) effect on BCE. Mean BCE values were higher for control than for irradiated samples and higher for samples irradiated after freezing than before. Mean BCE values decreased consistently with increasing dose of radiation.

The effect of storage time on BCE value was not consistent among experiments. BCE values were higher after 30 days than 10 days storage in Experiments 1 and 4 but the opposite was true in Experiments 2 and 3. The differences were highly significant ($P \leq 0.01$) in the first 3 experiments.

Samples frozen and stored at -35 F^{o} had significantly ($P \leq 0.01$) higher average BCE values than corresponding samples at -10 F^{o} in Experiments 2 and 4. No significant temperature effect was found in Experiments 1 and 3.

Mean BCE values were higher for air-packed than nitrogen-packed samples in all experiments and these differences were significant ($P \leq 0.05$) in Experiments 2, 3 and 4.

Reading at 460 nm

The results at 460 nm were similar to those at 460 nm.

CORRELATION MATRIX

Simple linear correlation coefficients among absorbance and reflectance variables are shown in Tables A6, B6, C6 and D6 for Experiments 1, 2, 3 and 4 respectively. The correlation coefficients were highly significant ($P \leqslant 0.01$) in all experiments except those in Experiment 1 between primary Y and BCE values at 450 and 460 nm.

Table 1. Mean (x) value for fresh samples and all treated samples by experiment.

Experiment	Fresh (n=2)	Other (n=112)
1	0.4700	0.4420**
2	0.4690	0.4427**
3	0.4997	0.4930**
4	0.5235	0.4954**

** Means are significantly different at $p \leqslant 0.01$ (see Tables A1, B1, C1 and D1

Table 2. Mean (x) value for control samples and treated samples by experiment.

Experiment	Control (n=16)	Treated (n=96)
1	0.4637	0.4384**
2	0.4699	0.4381**
3	0.5092	0.4982**
4	0.5134	0.4924**

** Means are significantly different at $P \leqslant 0.01$ (see Tables A1, B1, C1 and D1

Table 3. Mean (y) value for fresh samples and all treated samples by experiment.

Experiment	Fresh (n=2)	Other (n=112)
1	0.4770	0.4609**
2	0.4770	0.4653**
3	0.4325	0.4220**
4	0.4125	0.3957**

** Means are significantly different at $P \leqslant 0.01$ (see Tables A2, B2, C2 and D2

Table 4. Mean (y) value for control samples and treated samples by experiment.

Experiment	Control (n=16)	Treated (n=96)
1	0.4684	0.4596**
2	0.4748	0.4637**
3	0.4377	0.4316**
4	0.4077	0.3937**

** Means are significantly different at $P \leqslant 0.01$ (see Tables A2, B2, C2 and D2

Table 5. Mean (%Y) for fresh samples and all treated samples by experiment.

Experiment	Fresh (n=2)	Other (n=112)
1	44.56	35.72**
2	44.10	30.98*
3	42.57	28.22**
4	26.96	23.00**

** Means are significantly different at $P \leq 0.01$
* Means are significantly different at $P \leq 0.05$
(see Tables A3, B3, C3 and D3)

Table 6. Mean (%Y) value for control samples and treated samples by experiment.

Experiment	Control (n=16)	Treated (n=96)
1	37.08	35.49NS
2	35.00	30.31*
3	33.04	27.42**
4	24.73	22.71**

** Means are significantly different at $P \leq 0.01$
* Means are significantly different at $P \leq 0.05$
NS Means are insignificant
(see Tables A3, B3, C3 and D3)

Table 7. Mean beta-carotene equivalent at 450 nm for fresh samples and all treated samples by experiment.

Experiment	Fresh (n=2)	Other (n=112)
1	0.5070	0.2742**
2	0.5070	0.3790**
3	1.4575	1.0982**
4	1.1980	0.7830**

** Means are significantly different at $P \leqslant 0.01$ (see Tables A4, B4, C4 and D4)

Table 8. Mean beta-carotene equivalent at 450 nm for control samples and treated samples by experiment.

Experiment	Control (n=16)	Treated (n=96)
1	0.4258	0.2489**
2	0.6251	0.3380**
3	1.2894	1.0663**
4	1.0731	0.7346**

** Means are significantly different at $P \leqslant 0.01$ (see Tables A4, B4, C4 and D4)

Table 9. Mean beta-carotene equivalent at 460 nm for fresh samples and all treated samples by experiment.

Experiment	Fresh (n=2)	Other (n=112)
1	0.4680	0.2591**
2	0.4680	0.3559*
3	1.4075	1.0734**
4	1.2000	0.7948**

** Means are significantly different at $P \leq 0.01$
* Means are significantly different at $P \leq 0.05$
(see Tables A5, B5, C5 and D5)

Table 10. Mean beta-carotene equivalent at 460nm for control samples and treated samples by experiment.

Experiment	Control (n=16)	Treated (n=96)
1	0.3996	0.2357**
2	0.5825	0.3181**
3	1.2563	1.0430**
4	1.0724	0.7486**

** Means are significantly different at $P \leq 0.01$ (see Tables A5, B5, C5 and D5)

Table 11. Mean value for chromaticity coordinate (x). Experiments 1, 2, 3 and 4.

Irradiation Dose (Mrad)*	Experiment	0.0	0.5	1.0	2.0
	1	0.4637^{A}	0.4509^{B}	0.4411^{C}	0.4231^{D}
	2	0.4699^{A}	0.4533^{B}	0.4422^{C}	0.4189^{D}
	3	0.5092^{A}	0.5038^{B}	0.4989^{C}	0.4917^{D}
	4	0.5134^{A}	0.5022^{B}	0.4934^{C}	0.4817^{D}
Irradiation Time		Before Freezing		After Freezing	
	1	0.4348		0.4420^{**}	
	2	0.4299		0.4464^{**}	
	3	0.4963		0.5000^{**}	
	4	0.4905		0.4944	
Storage Time		10 days		30 days	
	1	0.4427		0.4412^{*}	
	2	0.4433		0.4420^{ns}	
	3	0.5025		0.4970^{**}	
	4	0.5016		0.4892	
Temperature		-10 F°		-35 F°	
	1	0.4532		0.4308^{**}	
	2	0.4485		0.4369^{**}	
	3	0.5057		0.4937^{**}	
	4	0.5013		0.4896	
Atmosphere		Air		Nitrogen	
	1	0.4421		0.4419^{ns}	
	2	0.4427		0.4426^{ns}	
	3	0.4995		0.4999^{ns}	
	4	0.4952		0.4957^{ns}	

* Duncan new multiple range test for dose mean values; any two means not sharing the same letter are significantly different at $P \leq 0.01$.

** Means are significantly different at $P \leq 0.01$.

* Means are significantly different at $P \leq 0.05$.

ns Means are insignificant.

(see Tables A1, B1, C1 and D1).

Table 12. Mean value for chromaticity coordinate (y). Experiments 1, 2, 3 and 4.

Irradiation Dose (Mrad)*/	Experiment	0.0	0.5	1.0	2.0
	1	0.4684^{A}	0.4664^{B}	0.4619^{C}	0.4506^{D}
	2	0.4748^{A}	0.4707^{B}	0.4670^{C}	0.4534^{D}
	3	0.4377^{A}	0.4343^{B}	0.4319^{C}	0.4287^{D}
	4	0.4077^{A}	0.4010^{B}	0.3942^{C}	0.3860^{D}
Irradiation Time		Before Freezing		After Freezing	
	1	0.4567		0.4626^{**}	
	2	0.4616		0.4658^{**}	
	3	0.4304		0.4329^{**}	
	4	0.3918		0.3956	
Storage Time		10 days		30 days	
	1	0.4608		0.4609^{ns}	
	2	0.4637		0.4668^{**}	
	3	0.4321		0.4330^{**}	
	4	0.3942		0.3972	
Temperature		-10 F^{o}		-35 F^{o}	
	1	0.4678		0.4540^{**}	
	2	0.4683		0.4622^{**}	
	3	0.4341		0.4310^{ns}	
	4	0.3961		0.3953^{ns}	
Atmosphere		Air		Nitrogen	
	1	0.4600		0.4617^{*}	
	2	0.4651		0.4655^{ns}	
	3	0.4325		0.4326^{ns}	
	4	0.3957		0.3957^{ns}	

*/ Duncan new multiple range test for dose mean values; any two means not sharing the same letter are significantly different at $P \leqslant 0.01$.

** Means are significantly different at $P \leqslant 0.01$.

* Means are significantly different at $P \leqslant 0.05$.

ns means are insignificant.

(see Tables A2, B2, C2 and D2).

Table 13. Mean value for (%Y). Experiments 1, 2, 3 and 4.

Irradiation Dose (Mrad)*	Experiment	0.0	0.5	1.0	2.0
	1	37.08^{A}	35.18^{A}	35.40^{A}	35.89^{A}
	2	35.00^{A}	31.50^{A}	31.09^{A}	28.34^{A}
	3	33.04^{B}	28.92^{AB}	28.04^{A}	25.30^{C}
	4	24.73^{A}	24.03^{AB}	23.29^{B}	20.82^{C}
Irradiation Time		Before Freezing		After Frrezing	
	1	35.96		35.01^{ns}	
	2	29.62		31.00^{**}	
	3	26.84		28.00^{**}	
	4	21.86		23.57	
Storage Time		10 days		30 days	
	1	34.91		36.53^{**}	
	2	33.01		28.95^{ns}	
	3	28.48		28.00^{**}	
	4	22.35		23.66	
Temperature		-10 F°		-35 F°	
	1	31.00		40.43^{**}	
	2	29.21		32.75^{*}	
	3	28.44		28.00^{ns}	
	4	23.24		22.76^{ns}	
Atmosphere		Air		Nitrogen	
	1	35.63		35.80^{ns}	
	2	31.31		30.66^{ns}	
	3	28.31		28.14^{ns}	
	4	23.03		22.97^{ns}	

*/ Duncan new multiple range test for dose mean values; any two means not sharing the same letter are significantly different at $P \leqslant 0.01$.

** Means are significantly different at $P \leqslant 0.01$.

* Means are significantly different at $P \leqslant 0.05$.

ns Means are insignificant.

(see Tables A3, B3, C3 and D3).

Table 14. Mean value for beta-carotene equivalent at 450 nm. Experiments 1, 2, 3 and 4.

Irradiation Dose (Mrad)*	Experiment	0.0	0.5	1.0	2.0
	1	0.4258^{A}_{A}	0.3042^{B}_{B}	0.2568^{C}_{C}	0.1857^{D}_{D}
	2	0.6251^{A}_{A}	0.4121^{B}_{B}	0.3373^{C}_{C}	0.2646^{D}_{D}
	3	1.2894^{A}_{A}	1.1707^{B}_{B}	1.0655^{C}_{C}	0.9628^{D}_{D}
	4	1.0731^{A}	0.8502^{B}	0.7336^{C}	0.6199^{D}
Irradiation Time		Before Freezing		After Freezing	
	1	0.2128		0.2849^{**}_{**}	
	2	0.2921		0.3839^{**}	
	3	1.0367		1.0959^{**}	
	4	0.7045		0.7647	
Storage Time		10 days		30 days	
	1	0.2582		0.2901^{**}_{**}	
	2	0.4123		0.3458^{*}	
	3	1.1120		1.0844^{ns}	
	4	0.7727		0.7932	
Temperature		-10 F°		-35 F°	
	1	0.2689		0.2794^{ns}_{*}	
	2	0.3656		0.3925^{ns}	
	3	1.1006		1.0958^{**}	
	4	0.7492		0.8167	
Atmosphere		Air		Nitrogen	
	1	0.2782		0.2702^{ns}_{*}	
	2	0.3908		0.3672^{**}	
	3	1.1169		1.0795^{**}	
	4	0.8173		0.7486	

*/ Duncan new multiple range test for dose mean values; any two means not sharing the same letter are significantly different at $P \leqslant 0.01$.

** Means are significantly different at $P \leqslant 0.01$.

* Means are significantly different at $P \leqslant 0.05$.

ns Means are insignificant.

(see Tables A4, B4, C4 and D4).

Table 15. Mean value for beta-carotene equivalent at 460 nm. Experiments 1, 2, 3 and 4.

Irradiation Dose (Mrad)*	Experiment	0.0	0.5	1.0	2.0
	1	0.3996^{A}	0.2867^{B}	0.2453^{C}	0.1749^{D}
	2	0.5825^{A}	0.3864^{B}	0.3172^{C}	0.2508^{D}
	3	1.2563^{A}	1.1412^{B}	1.0396^{C}	0.9481^{D}
	4	1.0724^{A}	0.8617^{B}	0.7473^{C}	0.6367^{D}
Irradiation Time		Before Freezing		After Freezing	
	1	0.2012		0.2702^{**}	
	2	0.2718		0.3645^{**}	
	3	1.0182		1.0677^{**}	
	4	0.7214		0.7757	
Storage Time		10 days		30 days	
	1	0.2462		0.2720^{**}	
	2	0.3867		0.3251^{ns}	
	3	1.0850		1.0619^{ns}	
	4	0.7847		0.8049^{ns}	
Temperature		-10 F°		-35 F°	
	1	0.2565		0.2617^{ns}	
	2	0.3378		0.3740^{**}	
	3	1.0770		1.0699^{ns}	
	4	0.7606		0.8290	
Atmosphere		Air		Nitrogen	
	1	0.2626		0.2556^{ns}	
	2	0.3674		0.3444^{*}	
	3	1.0893		1.0576^{**}	
	4	0.8283		0.7613	

*/ Duncan new multiple range test for dose mean values; any two means not sharing the same letter are significantly different at $P \leq 0.01$.

** Means are significantly different at $P \leq 0.01$.

* Means are significantly different at $P \leq 0.05$.

ns Means are insignificant.

(see Tables A5, B5, C5 and D5).

Table 16. Dominant Wavelength (DWL) of the mean values for factors and level within experiments.

Factor	Level	Experiment 1	2	3	4
Fresh		577	577	584	589
Dose (Mrad)	0.0	578	577	584	589
	0.5	577	577	584	589
	1.0	577	577	584	589
	2.0	577	577	584	590
Irradiation Time	Before Freezing	577	577	584	590
	After Freezing	577	577	584	589
Storage Time	10 days	577	577	585	590
	30 days	577	577	584	589
Temperature	-10 F°	577	577	585	590
	-35 F°	577	577	584	589
Atmosphere	Air	577	577	584	589
	Nitrogen	577	577	584	589

Table 17. Excitation Purity (EP) of the mean values for factors and levels within experiments.

Factor	Level	Experiment 1	2	3	4
Fresh		82.03	85.76	82.30	82.72
Dose (Mrad)	0.0	82.04	85.41	86.30	78.77
	0.5	78.09	79.91	83.94	74.19
	1.0	74.32	76.05	81.93	70.33
	2.0	66.60	66.42	79.04	64.71
Irradiation Time	Before Freezing	71.23	71.46	80.82	68.33
	After Freezing	74.75	76.77	82.47	70.89
Storage Time	10 days	74.42	75.39	82.63	72.23
	30 days	74.07	75.94	81.37	69.44
Temperature	-10 F°	79.08	78.01	83.85	72.39
	-35 F°	69.48	73.35	80.11	69.30
Atmosphere	Air	74.04	75.63	82.23	71.17
	Nitrogen	74.47	75.72	82.38	71.34

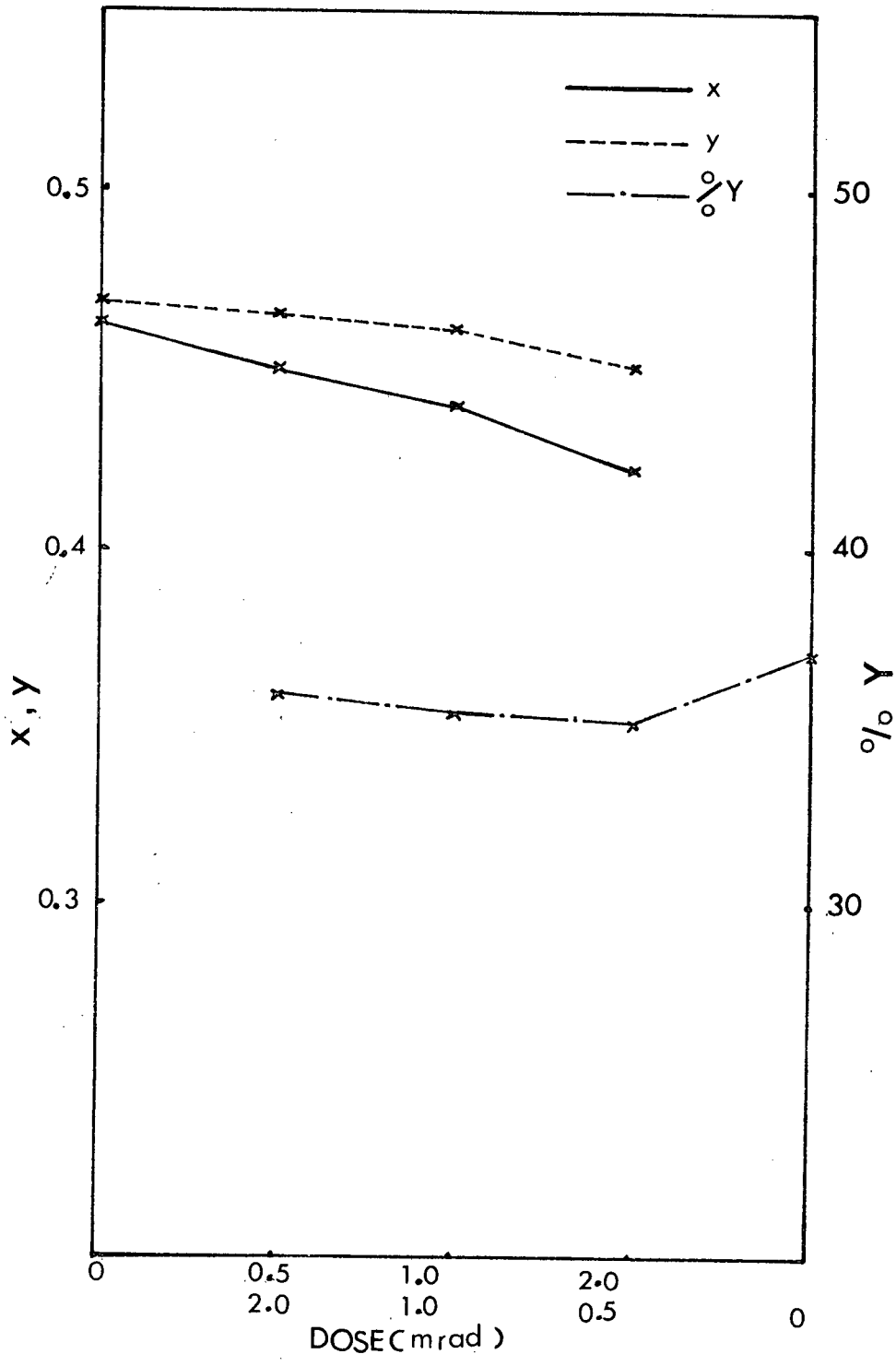

Fig. 4. Relationships between mean x, y, %Y and dose levels. Experiment 1.

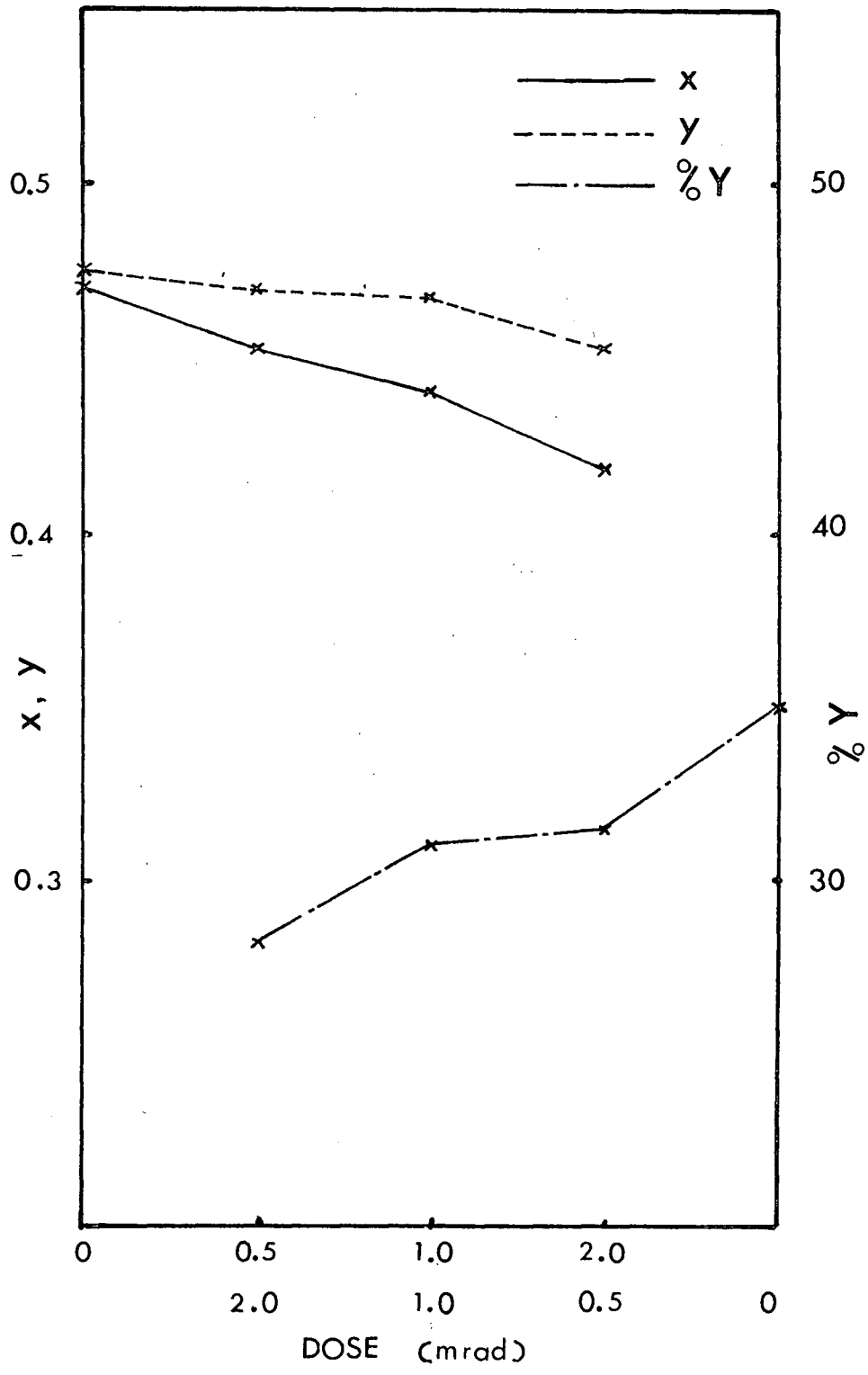

Fig. 5. Relationships between mean x, y, %Y and dose levels. Experiment 2.

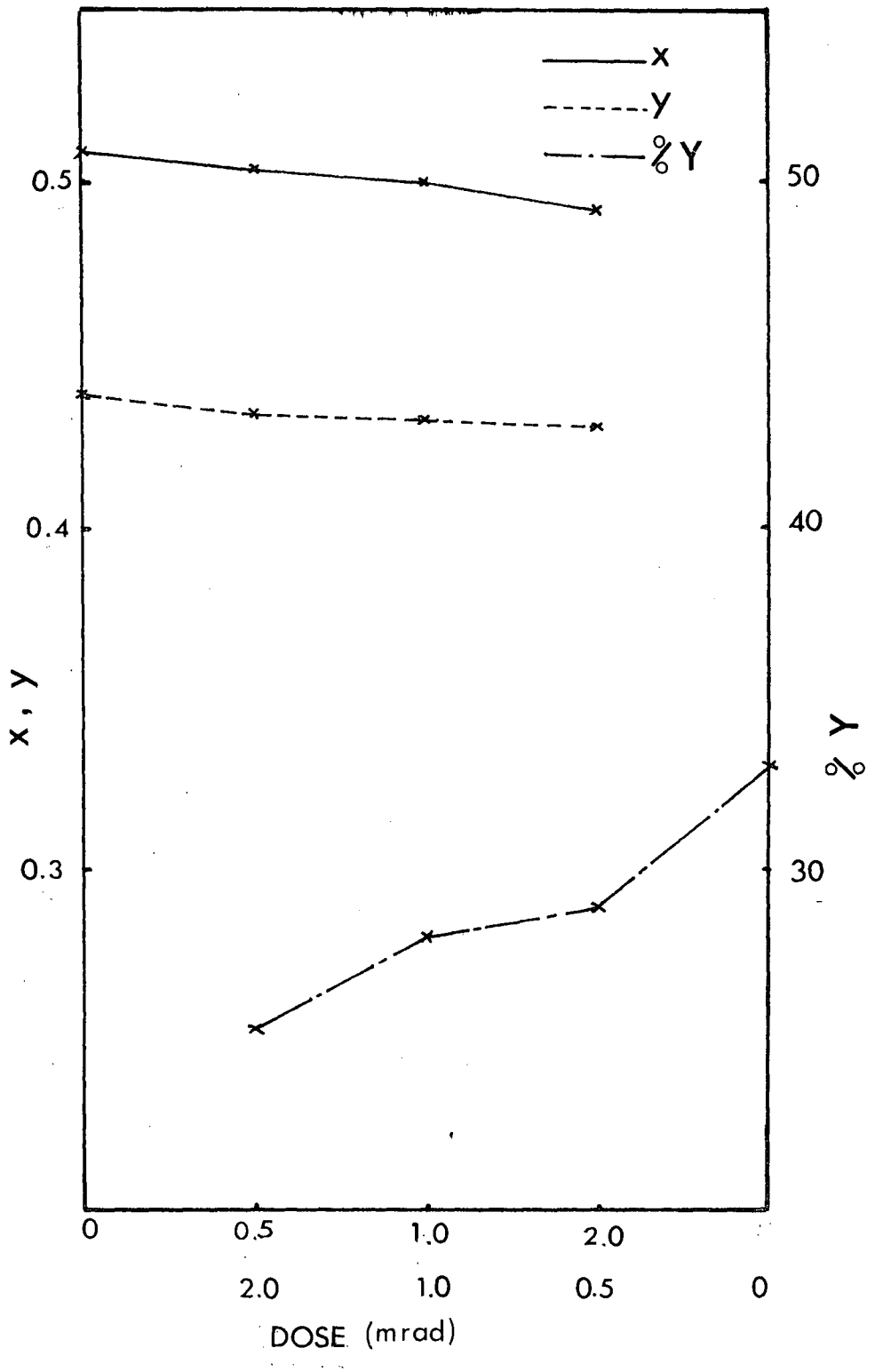

Fig. 6. Relationships between mean x, y, %Y and dose levels. Experiment 3.

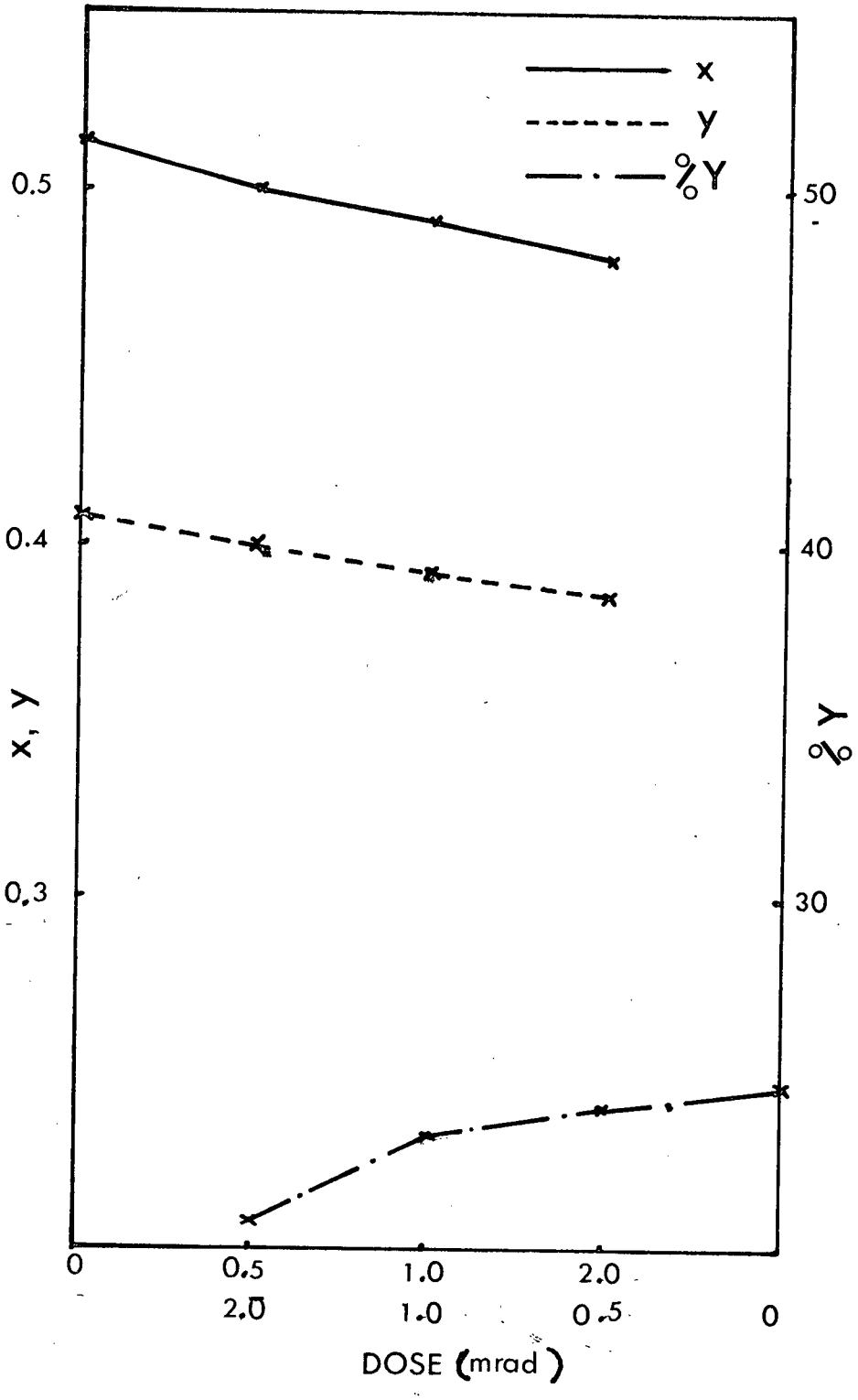

Fig. 7. Relationships between mean x, y, %Y and dose levels. Experiment 4.

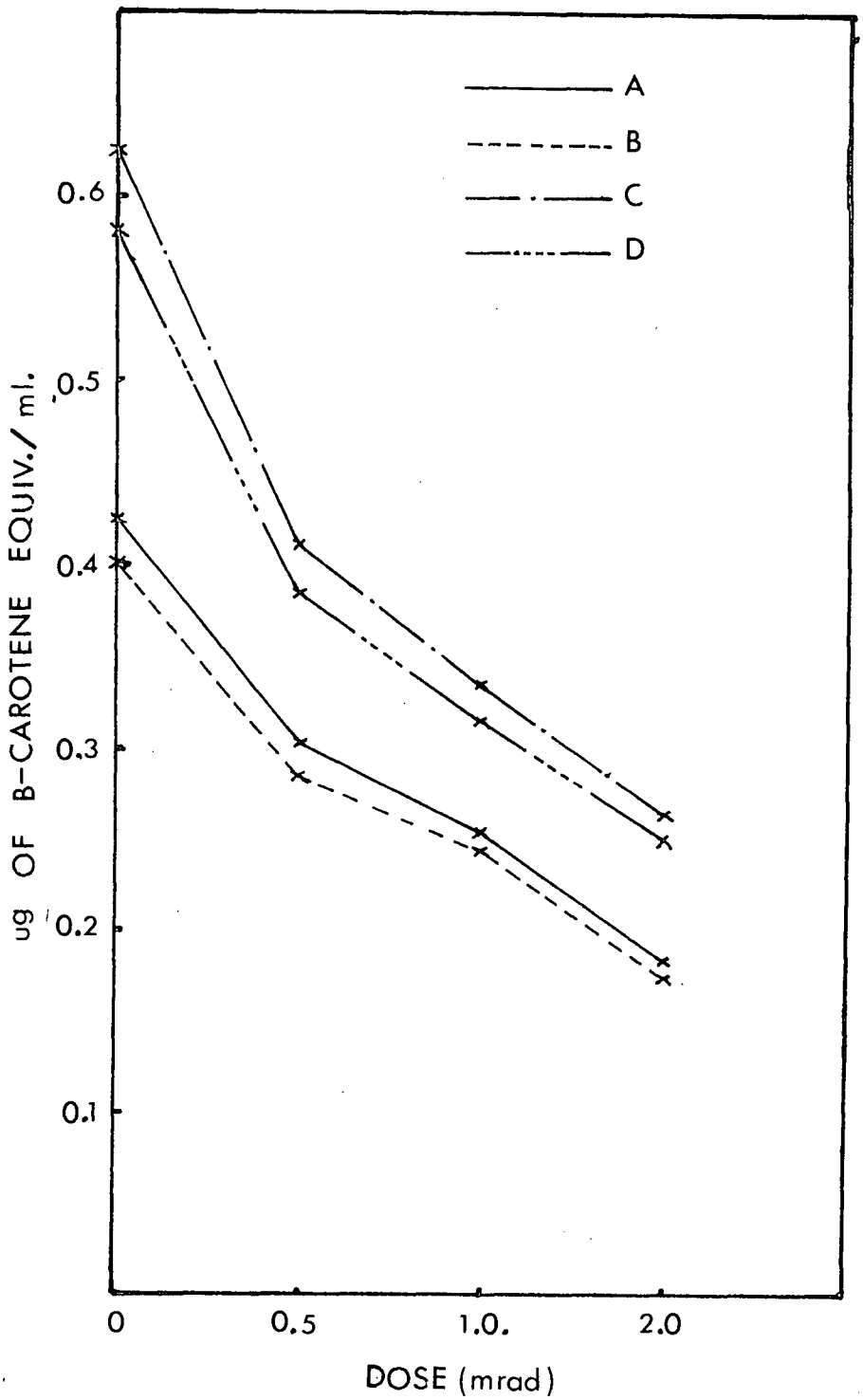

Fig. 8. Relationships between mean beta-carotene equivalent and dose levels; A&B= beta-carotene equivalent at 450 and 460 nm respectively. Experiment 1; C&D= beta-carotene equivalent at 450 and 460 nm respectively. Experiment 2.

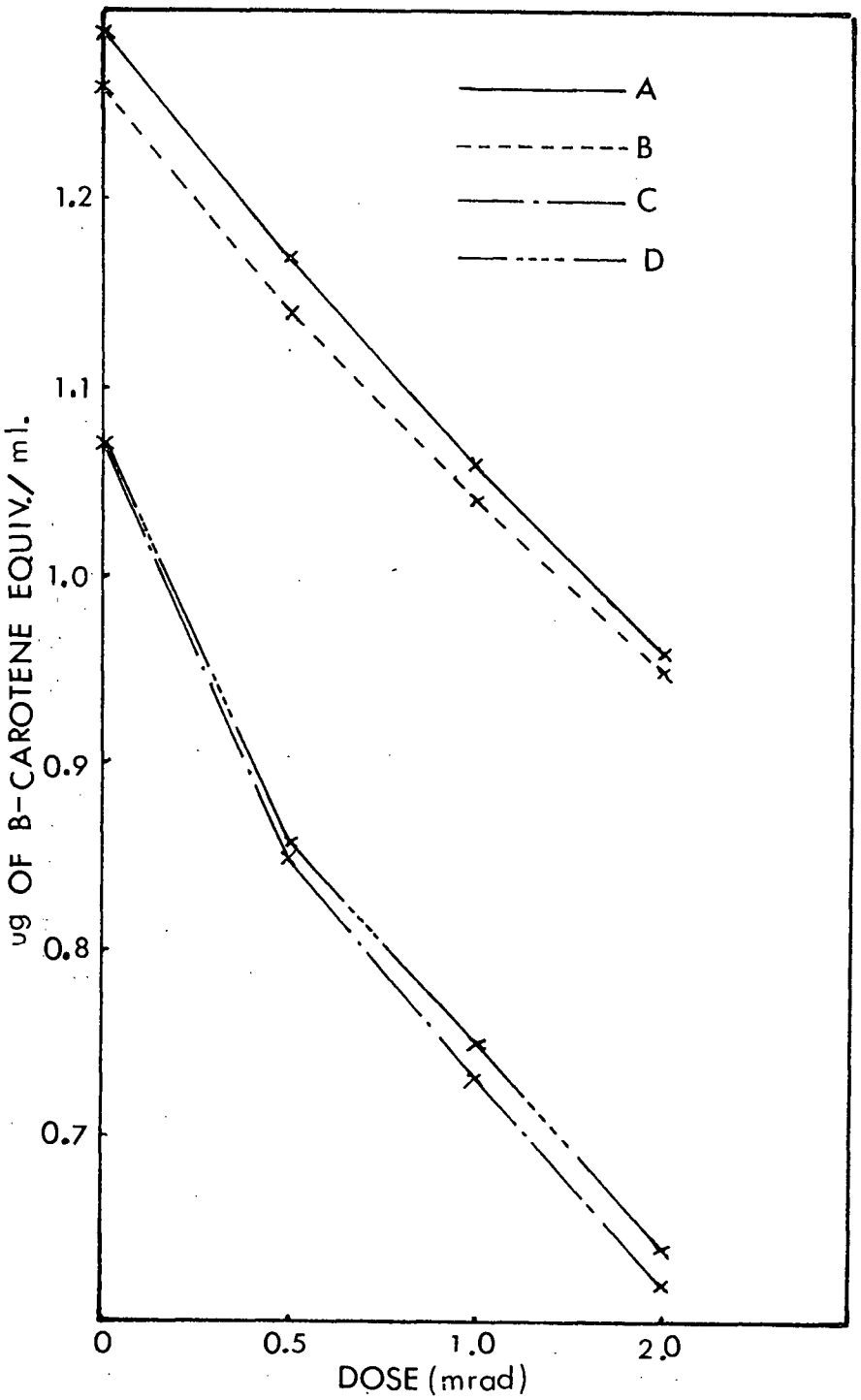

Fig. 9. Relationships between mean beta-carotene equivalent and dose levels; A&B= beta-carotene equivalent at 450 and 460 nm respectively. Experiment 3; C&D= beta-carotene equivalent at 450 and 460 nm respectively. Experiment 4.

DISCUSSION

Both irradiation dose and time of irradiation had marked effects on chromaticity coordinates , lightness and BCE values. Changes in the chromaticity coordinates (x and y) indicated a decrease in excitation purity (decline in both x and y) but little change in dominant wavelength (DWL) in all experiments. The results of Experiments 3 and 4 clearly indicate that increasing irradiation dose and irradiation before freezing results in an increase in darkness of samples. In these two experiments beta-carotene and canthaxanthin respectively were added to the yolk as colorants. The lack of a significant effect of irradiation dose and time on darkness in the first two experiments suggests that either the pigments added in Experiments 3 and 4 are more radio-sensitive *per se* than the natural colorant of yolk or that the natural colorants are made more radioresistant by interaction with components of the milieu at least to modifications resulting in changes in lightness (%Y).

The marked and significant decrease in BCE values with increasing radiation dose and the lower BCE values for samples irradiated before freezing in all experiments clearly implicate changes in the pigments themselves, or in their extractability by acetone in the color changes evident by reflectance measurements.

The effects of irradiation dose and time are consistent with the hypothesis that free radicals and peroxides are induced by radiation in proportion to dose level and react with the carotenoids to an extent dependent upon physical state (frozen or unfrozen) during irradiation. The results indicate that low doses of radiation and irradiation in the frozen state minimize the color and pigment changes induced.

The effect of temperature on chromaticity coordinates was consistent for all experiments. Samples frozen and held at -10 F^{o} had consistently higher mean (x) and (y) values than those at -35 F^{o}. Only the naturally-pigmented samples (Experiments 1 and 2) showed a corresponding darkening at -10 F^{o} compared to -35 F^{o}. However, highly significant interactions between storage temperature and irradiation treatment occured in both Experiments 3 and 4 for lightness and may account for the lack of a significant temperature effect. The interactions indicated that darkness increased more rapidly with irradiation dose at -10 F^{o} than at -35 F^{o}.

Similarly, the presence of interactions involving temperature in the Experiment 3 may account for the lack of a significant temperature effect on BCE in this experiment.

The effect of storage time on the mean chromaticity values was consistent in all experiments but the effects on lightness and BCE were not. However, mean lightness and BCE values were positively related. Similar significant correlations

from analysis of individual sample data indicate that pigment destruction or modification (lower BCE value) can be expected to produce a decrease in lightness of samples regardless of pigment source although the relationship is far from perfect.

SUMMARY AND CONCLUSIONS

Egg yolk color was studied in relation to radiation dose, radiation time, freezing procedure, storage time and headspace atmosphere.

1. Irradiation dose had a highly significant effect on yolk color. Significant differences were found in reflectance and absorbance measurements among doses in all experiments.

2. Samples irradiated after freezing had higher mean chromaticity values, BCE values and excitation purity than samples irradiated before freezing in all experiments.

3. After 30 days storage mean x-values were lower and mean y-values were higher than after 10 days storage. These changes were associated with almost no change in DWL or EP.

4. Samples stored at -10 F^{o} had higher mean chromaticity values and lower excitation purity in all experiments. Naturally-pigmented samples showed a corresponding darkening (lower %Y) at -10 F^{o} compared to -35 F^{o}.

5. With only one exception, atmosphere had no significant effect on mean x, y and %Y. Samples under air atmosphere had consistently higher mean BCE values than those under nitrogen atmosphere. These differences were significant in Experiments 2, 3 and 4.

6. Dominant wavelength was not affected by the varying treatments within experiments. Mean DWL values were 577-578 and 589-590 nm for naturally and artificially pigmented

samples respectively.

7. Correlation analyses revealed highly significant ($P \leqslant 0.01$) linear relationships between BCE and both chromaticity coordinates and lightness ranging from + 0.09 to + 0.79.

8. It is concluded that irradiation dose and physical state of the product have important effects on the acetone-soluble pigments and color of naturally or artificially pigmented egg yolk such that the effect increases with dose and is greater for irradiation of liquid compared to frozen yolk.

9. It is further concluded that although statistical significance ($P \leqslant 0.01$) of the relationships between BCE and each of the chromaticity coordinates and lightness was demonstrated the magnitude of the correlation coefficients is not great enough to permit accurate prediction.

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APPENDIX A

Table A1. Analysis of variance for chromaticity coordinate (x). Experiment 1.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.15434×10^{-2}	$<5 \times 10^{-7}$
Radiation Treatment	6	0.38676×10^{-2}	$<5 \times 10^{-7}$
Control vs Treatment	1	0.88015×10^{-2}	$<5 \times 10^{-7}$
Dose	2	0.63822×10^{-2}	$<5 \times 10^{-7}$
Irradiation Time	1	0.12615×10^{-2}	$<5 \times 10^{-7}$
Dose x Irradiation Time	2	0.18884×10^{-3}	0.0002
Storage	1	0.67580×10^{-4}	0.0389
Temperature	1	0.14018×10^{-1}	$<5 \times 10^{-7}$
Atmosphere	1	0.10804×10^{-5}	0.7773
Radiation Treatment x Sto.	6	0.17893×10^{-4}	0.3240
Radiation Treatment x Temp.	6	0.50792×10^{-4}	0.0120
Radiation Treatment x Atmo.	6	0.23512×10^{-5}	0.9831
Storage x Temperature	1	0.97232×10^{-5}	0.4262
Storage x Atmosphere	1	0.21438×10^{-4}	0.2343
Temperature x Atmosphere	1	0.94723×10^{-4}	0.0165
Error	25	0.14535×10^{-4}	0.0016
Error between duplicates	57	0.56404×10^{-5}	
Total	113		

Table A2. Analysis of variance for chromaticity coordinate (y). Experiment 1.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.50978×10^{-3}	$<5 \times 10^{-5}$
Radiation Treatment	6	0.10526×10^{-2}	$<5 \times 10^{-7}$
Control vs Treatment	1	0.10625×10^{-2}	$<5 \times 10^{-7}$
Dose	2	0.21128×10^{-2}	$<5 \times 10^{-7}$
Irradiation Time	1	0.84609×10^{-3}	$<5 \times 10^{-5}$
Dose x Irradiation Time	2	0.90594×10^{-4}	0.0050
Storage	1	0.32143×10^{-6}	0.8522
Temperature	1	0.53213×10^{-2}	$<5 \times 10^{-7}$
Atmosphere	1	0.82286×10^{-4}	0.0205
Radiation Treatment x Sto.	6	0.23839×10^{-5}	0.9799
Radiation Treatment x Temp.	6	0.35265×10^{-4}	0.0437
Radiation Treatment x Atmo.	6	0.10348×10^{-4}	0.6114
Storage x Temperature	1	0.38893×10^{-4}	0.1004
Storage x Atmosphere	1	0.15750×10^{-4}	0.2937
Temperature x Atmosphere	1	0.91429×10^{-5}	0.4262
Error	25	0.13669×10^{-4}	0.0002
Error between duplicates	57	0.43158×10^{-5}	
Total	113		

Table A3. Analysis of variance for (%Y).
Experiment 1.

Source	DF	Mean Square	Probability
Fresh vs All	1	153.69	0.0006
Radiation Treatment	6	14.15	0.2267
Control vs Treatment	1	34.83	0.0653
Dose	2	4.22	0.6544
Irradiation Time	1	21.71	0.1415
Dose x Irradiation Time	2	9.97	0.3702
Storage	1	73.55	0.0101
Temperature	1	2491.2	$< 5 \times 10^{-7}$
Atmosphere	1	0.79	0.7678
Radiation Treatment x Sto.	6	4.52	0.8240
Radiation Treatment x Temp.	6	3.97	0.8633
Radiation Treatment x Atmo.	6	2.32	0.9572
Storage x Temperature	1	6.22	0.4335
Storage x Atmosphere	1	36.89	0.0584
Temperature x Atmosphere	1	192.15	0.0002
Error	25	9.59	$< 5 \times 10^{-7}$
Error between duplicates	57	0.90	
Total	113		

Table A4. Analysis of variance for beta-carotene equivalent at 450 nm. Experiment 1.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.10653	$<5 \times 10^{-7}$
Radiation Treatment	6	0.13303	$<5 \times 10^{-7}$
Control vs Treatment	1	0.42930	$<5 \times 10^{-7}$
Dose	2	0.11379	$<5 \times 10^{-7}$
Irradiation Time	1	0.12478	$<5 \times 10^{-7}$
Dose x Irradiation Time	2	0.82726×10^{-2}	0.0079
Storage	1	0.28608×10^{-1}	0.0002
Temperature	1	0.31291×10^{-2}	0.1438
Atmosphere	1	0.17920×10^{-2}	0.2681
Radiation Treatment x Sto.	6	0.29993×10^{-2}	0.0833
Radiation Treatment x Temp.	6	0.13751×10^{-2}	0.4585
Radiation Treatment x Atmo.	6	0.53491×10^{-2}	0.0078
Storage x Temperature	1	0.68014×10^{-3}	0.4988
Storage x Atmosphere	1	0.15557×10^{-3}	0.7377
Temperature x Atmosphere	1	0.27032×10^{-3}	0.6670
Error	25	0.13998×10^{-2}	0.0582
Error between duplicate	57	0.84382×10^{-3}	
Total	113		

Table A5. Analysis of variance for beta-carotene equivalent at 460 nm. Experiment 1.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.85756×10^{-1}	$< 5 \times 10^{-5}$
Radiation Treatment	6	0.11719	$< 5 \times 10^{-7}$
Control vs Treatment	1	0.36867	$< 5 \times 10^{-7}$
Dose	2	0.10225	$< 5 \times 10^{-7}$
Irradiation Time	1	0.11426	$< 5 \times 10^{-7}$
Dose x Irradiation Time	2	0.78665×10^{-2}	0.0105
Storage	1	0.18721×10^{-1}	0.0014
Temperature	1	0.75089×10^{-3}	0.4821
Atmosphere	1	0.13580×10^{-2}	0.3416
Radiation Treatment x Sto.	6	0.25522×10^{-2}	0.1431
Radiation Treatment x Temp.	6	0.12832×10^{-2}	0.5139
Radiation Treatment x Atmo.	6	0.45692×10^{-2}	0.0183
Storage x Temperature	1	0.24891×10^{-2}	0.1966
Storage x Atmosphere	1	0.13729×10^{-3}	0.7532
Temperature x Atmosphere	1	0.28289×10^{-3}	0.6637
Error	25	0.14318×10^{-2}	0.0155
Error between duplicates	57	0.71409×10^{-3}	
Total	113		

Table A6. Correlation matrix and mean value. Experiment 1.

Variable	x	y	% Y	450nm	460nm	Mean
x	1.0000					0.4425
y	0.9434**	1.0000				0.4612
Y	-0.5047	-0.5394	1.0000			3587.0
450 nm	0.6574**	0.5644**	0.1265	1.0000		0.2782
460 nm	0.6764**	0.5833**	0.0906	0.9888**	1.0000	0.2628

** Correlation coefficients are significantly different at $P \leqslant 0.01$.

APPENDIX B

Table B1. Analysis of variance for chromaticity coordinate (x). Experiment 2.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.13622×10^{-2}	$<5 \times 10^{-5}$
Radiation Treatment	6	0.75903×10^{-2}	$<5 \times 10^{-7}$
Control vs Treatment	1	0.13816×10^{-1}	$<5 \times 10^{-7}$
Dose	2	0.98300×10^{-2}	$<5 \times 10^{-7}$
Irradiation Time	1	0.65836×10^{-2}	$<5 \times 10^{-7}$
Dose x Irradiation Time	2	0.27413×10^{-2}	$<5 \times 10^{-7}$
Storage	1	0.50223×10^{-4}	0.1182
Temperature	1	0.37607×10^{-2}	$<5 \times 10^{-7}$
Atmosphere	1	0.72321×10^{-6}	0.8284
Radiation Treatment x Sto.	6	0.10589×10^{-3}	0.0011
Radiation Treatment x Temp.	6	0.90973×10^{-4}	0.0027
Radiation Treatment x Atmo.	6	0.29307×10^{-4}	0.2196
Storage x Temperature	1	0.72321×10^{-6}	0.8284
Storage x Atmosphere	1	0.25080×10^{-4}	0.2681
Temperature x Atmosphere	1	0.15009×10^{-4}	0.3938
Error	25	0.19587×10^{-4}	$<5 \times 10^{-7}$
Error between duplicates	57	0.15351×10^{-5}	
Total	113		

Table B2. Analysis of variance for chromaticity coordinate (y). Experiment 2.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.26963×10^{-3}	$<5 \times 10^{-5}$
Radiation Treatment	6	0.15995×10^{-2}	$<5 \times 10^{-7}$
Control vs Treatment	1	0.16942×10^{-2}	$<5 \times 10^{-7}$
Dose	2	0.26472×10^{-2}	$<5 \times 10^{-7}$
Irradiation Time	1	0.41251×10^{-3}	$<5 \times 10^{-5}$
Dose x Irradiation Time	2	0.10980×10^{-2}	$<5 \times 10^{-7}$
Storage	1	0.26414×10^{-3}	$<5 \times 10^{-5}$
Temperature	1	0.10443×10^{-2}	$<5 \times 10^{-7}$
Atmosphere	1	0.35714×10^{-5}	0.4991
Radiation Treatment x Sto.	6	0.29018×10^{-4}	0.0066
Radiation Treatment x Temp.	6	0.65655×10^{-4}	$<5 \times 10^{-5}$
Radiation Treatment x Atmo.	6	0.10363×10^{-4}	0.2503
Storage x Temperature	1	0.60357×10^{-5}	0.3773
Storage x Atmosphere	1	0.91429×10^{-5}	0.2754
Temperature x Atmosphere	1	0.10321×10^{-4}	0.2462
Error	25	0.73600×10^{-5}	$<5 \times 10^{-7}$
Error between duplicates	57	0.80702×10^{-6}	
Total	113		

Table B3. Analysis of variance for (%Y).
Experiment 2.

Source	DF	Mean Square	Probability
Fresh vs All	1	338.29	0.0111
Radiation Treatment	6	111.68	0.0521
Control vs Treatment	1	301.18	0.0157
Dose	2	94.47	0.1442
Irradiation Time	1	45.73	0.3269
Dose x Irradiation Time	2	67.10	0.2466
Storage	1	461.39	0.0039
Temperature	1	352.11	0.0098
Atmosphere	1	11.93	0.6182
Radiation Treatment x Sto.	6	32.95	0.6347
Radiation Treatment x Temp.	6	13.16	0.9355
Radiation Treatment x Atmo.	6	18.95	0.8610
Storage x Temperature	1	101.23	0.1444
Storage x Atmosphere	1	4.06	0.7603
Temperatur x Atmosphere	1	1.39	0.8390
Error	25	45.42	$< 5 \times 10^{-7}$
Error between duplicates	57	0.4970	
Total	113		

Table B4. Analysis of variance for beta-carotene equivalent at 450 nm. Experiment 2.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.32184×10^{-1}	0.0048
Radiation Treatment	6	0.28803	$<5 \times 10^{-7}$
Control vs Treatment	1	0.11306×10^{-1}	$<5 \times 10^{-7}$
Dose	2	0.17391	$<5 \times 10^{-7}$
Irradiation Time	1	0.20240	$<5 \times 10^{-5}$
Dose x Irradiation Time	2	0.23670×10^{-1}	0.0038
Storage	1	0.12382	$<5 \times 10^{-5}$
Temperature	1	0.20304×10^{-1}	0.0203
Atmosphere	1	0.15604×10^{-1}	0.0391
Radiation Treatment x Sto.	6	0.54923×10^{-2}	0.1790
Radiation Treatment x Temp.	6	0.35904×10^{-2}	0.4081
Radiation Treatment x Atmo.	6	0.21043×10^{-2}	0.7096
Storage x Temperature	1	0.87509×10^{-2}	0.1155
Storage x Atmosphere	1	0.92893×10^{-2}	0.1053
Temperature x Atmosphere	1	0.15156×10^{-2}	0.5149
Error	25	0.33626×10^{-2}	0.0026
Error between duplicates	57	0.13674×10^{-2}	
Total	113		

Table B5. Analysis of variance for beta-carotene equivalent at 460 nm. Experiment 2.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.24687×10^{-1}	0.0115
Radiation Treatment	6	0.24953	$< 5 \times 10^{-7}$
Control vs Treatment	1	0.95840	$< 5 \times 10^{-7}$
Dose	2	0.14697	$< 5 \times 10^{-7}$
Irradiation Time	1	0.20628	$< 5 \times 10^{-5}$
Dose x Irradiation Time	2	0.19295×10^{-1}	0.0088
Storage	1	0.10640	$< 5 \times 10^{-5}$
Temperature	1	0.36577×10^{-1}	0.0029
Atmosphere	1	0.14766×10^{-1}	0.0439
Radiation Treatment x Sto.	6	0.48347×10^{-2}	0.2380
Radiation Treatment x Temp.	6	0.25612×10^{-2}	0.6064
Radiation Treatment x Atmo.	6	0.19386×10^{-2}	0.7454
Storage x Temperature	1	0.66343×10^{-2}	0.1685
Storage x Atmosphere	1	0.74263×10^{-2}	0.1456
Temperature x Atmosphere	1	0.64129×10^{-3}	0.6684
Error	25	0.33524×10^{-2}	0.0027
Error between duplicate	57	0.13680×10^{-2}	
Total	113		

Table B6. Correlation matrix and mean value.
Experiment 2.

Variable	x	y	%Y	450nm	460nm	Mean
x	1.0000					0.4431
y	0.9291**	1.0000				0.4655
%Y	0.4130**	0.2238**	1.0000			31.21
450 nm	0.7654**	0.5895**	0.5304**	1.0000		0.3813
460 nm	0.7459**	0.5650**	0.5449**	0.9959**	1.0000	0.3579

** Correlation coefficients are significantly different at $P \leq 0.01$.

APPENDIX C

Table C1. Analysis of variance for chromaticity coordinate (x). Experiment 3.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.89290×10^{-4}	0.0010
Radiation Treatment	6	0.76426×10^{-3}	$< 5 \times 10^{-7}$
Control vs Treatment	1	0.16878×10^{-2}	$< 5 \times 10^{-7}$
Dose	2	0.11825×10^{-2}	$< 5 \times 10^{-7}$
Irradiation Time	1	0.31901×10^{-3}	$< 5 \times 10^{-5}$
Dose x Irradiation Time	2	0.10689×10^{-3}	$< 5 \times 10^{-5}$
Storage	1	0.86358×10^{-3}	$< 5 \times 10^{-7}$
Temperature	1	0.40200×10^{-2}	$< 5 \times 10^{-7}$
Atmosphere	1	0.47232×10^{-5}	0.4002
Radiation Treatment x Sto.	6	0.32054×10^{-5}	0.7988
Radiation Treatment x Temp.	6	0.23051×10^{-4}	0.0099
Radiation Treatment x Atmo.	6	0.28482×10^{-5}	0.8389
Storage x Temperature	1	0.26722×10^{-3}	$< 5 \times 10^{-5}$
Storage x Atmosphere	1	0.20089×10^{-5}	0.5847
Temperature x Atmosphere	1	0.89286×10^{-8}	0.9213
Error	25	0.63304×10^{-5}	$< 5 \times 10^{-5}$
Error between duplicate	57	0.13596×10^{-5}	
Total	113		

Table C2. Analysis of variance for chromaticity coordinate (y). Experiment 3.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.15664×10^{-3}	$< 5 \times 10^{-5}$
Radiation Treatment	6	0.19734×10^{-3}	$< 5 \times 10^{-7}$
Control vs Treatment	1	0.50060×10^{-3}	$< 5 \times 10^{-7}$
Dose	2	0.25517×10^{-3}	$< 5 \times 10^{-7}$
Irradiation Time	1	0.14504×10^{-3}	$< 5 \times 10^{-5}$
Dose x Irradiation Time	2	0.14042×10^{-4}	0.0095
Storage	1	0.23223×10^{-4}	0.0053
Temperature	1	0.26722×10^{-3}	$< 5 \times 10^{-7}$
Atmosphere	1	0.22321×10^{-6}	0.7599
Radiation Treatment x Sto.	6	0.51815×10^{-5}	0.0913
Radiation Treatment x Temp.	6	0.78065×10^{-5}	0.0197
Radiation Treatment x Atmo.	6	0.12649×10^{-5}	0.7975
Storage x Temperatur	1	0.21437×10^{-4}	0.0069
Storage x Atmosphere	1	0.15089×10^{-5}	0.4492
Temperature x Atmosphere	1	0.80357×10^{-7}	0.8361
Error	25	0.24889×10^{-5}	0.0016
Error between duplicates	57	0.96491×10^{-6}	
Total	113		

Table C3. Analysis of variance for (%Y).
Experiment 3.

Source	DF	Mean Square	Probability
Fresh vs All	1	404.36	$< 5 \times 10^{-7}$
Radiation Treatment	6	118.11	$< 5 \times 10^{-7}$
Control vs Treatment	1	432.82	$< 5 \times 10^{-7}$
Dose	2	113.93	$< 5 \times 10^{-7}$
Irradiation Time	1	31.93	0.0018
Dose x Irradiation Time	2	8.03	0.0606
Storage	1	7.37	0.0995
Temperature	1	5.41	0.1562
Atmosphere	1	0.78	0.5930
Radiation Treatment x Sto.	6	19.41	0.0001
Radiation Treatment x Temp.	6	17.36	0.0003
Radiation Treatment x Atmo.	6	0.47	0.9774
Storage x Temperature	1	21.47	0.0077
Storage x Atmosphere	1	0.20	0.7742
Temperature x Atmosphere	1	0.18	0.7832
Error	25	2.58	$< 5 \times 10^{-7}$
Error between duplicates	57	0.36	
Total	113		

Table C4. Analysis of variance for beta-carotene equivalent at 450 nm. Experiment 3.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.25367	$< 5 \times 10^{-5}$
Radiation Treatment	6	0.24488	$< 5 \times 10^{-7}$
Control vs Treatment	1	0.68270	$< 5 \times 10^{-7}$
Dose	2	0.34551	$< 5 \times 10^{-7}$
Irradiation Time	1	0.83958×10^{-1}	0.0002
Dose x Irradiation Time	2	0.58167×10^{-2}	0.2629
Storage	1	0.21285×10^{-1}	0.0306
Temperature	1	0.62229×10^{-3}	0.7014
Atmosphere	1	0.39001×10^{-1}	0.0050
Radiation Treatment x Sto.	6	0.13629×10^{-1}	0.0157
Radiation Treatment x Temp.	6	0.12288×10^{-1}	0.0248
Radiation Treatment x Atmo.	6	0.31194×10^{-1}	0.0001
Storage x Temperature	1	0.26846×10^{-1}	0.0166
Storage x Atmosphere	1	0.19223×10^{-2}	0.5082
Temperature x Atmosphere	1	0.17286×10^{-4}	0.9050
Error	25	0.41324×10^{-2}	0.2112
Error between duplicate	57	0.32033×10^{-2}	
Total	113		

Table C5. Analysis of variance for beta-carotene equivalent at 460 nm. Experiment 3.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.21927	$< 5 \times 10^{-5}$
Radiation Treatment	6	0.21484	$< 5 \times 10^{-7}$
Control vs Treatment	1	0.62421	$< 5 \times 10^{-7}$
Dose	2	0.29856	$< 5 \times 10^{-7}$
Irradiation Time	1	0.58658×10^{-1}	0.0014
Dose x Irradiation Time	2	0.45271×10^{-2}	0.3814
Storage	1	0.14858×10^{-1}	0.0778
Temperature	1	0.14429×10^{-2}	0.5826
Atmosphere	1	0.28289×10^{-1}	0.0181
Radiation Treatment x Sto.	6	0.14006×10^{-1}	0.0203
Radiation Treatment x Temp.	6	0.12906×10^{-1}	0.0287
Radiation Treatment x Atmo.	6	0.26599×10^{-1}	0.0006
Storage x Temperature	1	0.30956×10^{-1}	0.0140
Storage x Atmosphere	1	0.17286×10^{-2}	0.5477
Temperature x Atmosphere	1	0.91429×10^{-5}	0.9167
Error	25	0.44952×10^{-2}	0.1463
Error between duplicate	57	0.32083×10^{-2}	
Total	113		

Table C6. Correlation matrix and mean value.
Experiment 3.

Variable	x	y	%Y	450nm	460nm	Mean
x	1.0000					0.4996
y	0.8760**	1.0000				0.4325
%Y	0.4420**	0.5628**	1.0000			28.47
450 nm	0.5502**	0.6836**	0.6867**	1.0000		1.104
460 nm	0.5547**	0.6812**	0.6797**	0.9938**	1.0000	1.079

** Correlation coefficients are significantly different at $P \leqslant 0.01$.

APPENDIX D

Table D1. Analysis of variance for chromaticity coordinate (x). Experiment 4.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.15484×10^{-2}	$<5 \times 10^{-5}$
Radiation Treatment	6	0.23442×10^{-2}	$<5 \times 10^{-7}$
Control vs Treatment	1	0.60121×10^{-2}	$<5 \times 10^{-7}$
Dose	2	0.33538×10^{-2}	$<5 \times 10^{-7}$
Irradiation Time	1	0.37604×10^{-3}	0.0007
Dose x Irradiation Time	2	0.48470×10^{-3}	$<5 \times 10^{-5}$
Storage	1	0.43003×10^{-2}	$<5 \times 10^{-7}$
Temperature	1	0.38189×10^{-2}	$<5 \times 10^{-7}$
Atmosphere	1	0.60357×10^{-5}	0.6306
Radiation Treatment x Sto.	6	0.53696×10^{-4}	0.0799
Radiation Treatment x Temp.	6	0.18556×10^{-3}	0.0001
Radiation Treatment x Atmo.	6	0.18869×10^{-4}	0.6071
Storage x Temperature	1	0.14629×10^{-3}	0.0215
Storage x Atmosphere	1	0.51429×10^{-5}	0.6560
Temperature x Atmosphere	1	0.35714×10^{-5}	0.7067
Error	25	0.24730×10^{-4}	$<5 \times 10^{-7}$
Error between duplicate	57	0.23421×10^{-5}	
Total	113		

Table D2. Analysis of variance for chromaticity coordinate (y). Experiment 4.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.55363×10^{-3}	$< 5 \times 10^{-5}$
Radiation Treatment	6	0.11734×10^{-2}	$< 5 \times 10^{-7}$
Control vs Treatment	1	0.27040×10^{-2}	$< 5 \times 10^{-7}$
Dose	2	0.17973×10^{-2}	$< 5 \times 10^{-7}$
Irradiation Time	1	0.36038×10^{-3}	0.0003
Dose x Irradiation Time	2	0.19053×10^{-3}	0.0007
Storage	1	0.24014×10^{-3}	0.0017
Temperature	1	0.15750×10^{-4}	0.3767
Atmosphere	1	0.53291×10^{-13}	0.9502
Radiation Treatment x Sto.	6	0.30810×10^{-4}	0.1859
Radiation Treatment x Temp.	6	0.25875×10^{-4}	0.2723
Radiation Treatment x Atmo.	6	0.31833×10^{-4}	0.1716
Storage x Temperature	1	0.12893×10^{-4}	0.4248
Storage x Atmosphere	1	0.51429×10^{-5}	0.6146
Temperature x Atmosphere	1	0.15750×10^{-4}	0.3767
Error	25	0.19159×10^{-4}	0.2081
Error between duplicate	57	0.14798×10^{-4}	
Total	113		

Table D3. Analysis of variance for (%Y).
Experiment 4.

Source	DF	Mean Square	Probability
Fresh vs All	1	30.85	0.0012
Radiation Treatment	6	51.81	$< 5 \times 10^{-7}$
Control vs Treatment	1	55.93	0.0001
Dose	2	90.17	$< 5 \times 10^{-5}$
Irradiation Time	1	69.77	$< 5 \times 10^{-5}$
Dose x Irradiation Time	2	2.41	0.3639
Storage	1	48.18	0.0001
Temperature	1	6.50	0.1002
Atmosphere	1	0.12	0.8061
Radiation Treatment x Sto.	6	4.93	0.0808
Radiation Treatment x Temp.	6	10.02	0.0037
Radiation Treatment x Atmo.	6	0.87	0.8835
Storage x Temperature	1	1.25	0.4726
Storage x Atmosphere	1	0.13×10^{-1}	0.8991
Temperature x Atmosphere	1	1.32	0.4598
Error	25	2.28	$< 5 \times 10^{-7}$
Error between duplicates	57	0.14	
Total	113		

Table D4. Analysis of variance for beta-carotene equivalent at 450 nm. Experiment 4.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.33848	$<5 \times 10^{-7}$
Radiation Treatment	6	0.42518	$<5 \times 10^{-7}$
Control vs Treatment	1	0.15717×10^{-1}	$<5 \times 10^{-7}$
Dose	2	0.42426	$<5 \times 10^{-7}$
Irradiation Time	1	0.86941×10^{-1}	$<5 \times 10^{-5}$
Dose x Irradiation Time	2	0.21968×10^{-1}	0.0058
Storage	1	0.11870×10^{-1}	0.0716
Temperature	1	0.12764	$<5 \times 10^{-5}$
Atmosphere	1	0.13241	$<5 \times 10^{-5}$
Radiation Treatment x Sto.	6	0.19887×10^{-2}	0.7447
Radiation Treatment x Temp.	6	0.50075×10^{-2}	0.2322
Radiation Treatment x Atmo.	6	0.34273×10^{-2}	0.4487
Storage x Temperature	1	0.29251×10^{-3}	0.7648
Storage x Atmosphere	1	0.14401×10^{-3}	0.8205
Temperature x Atmosphere	1	0.80072×10^{-2}	0.1356
Error	25	0.34328×10^{-2}	0.0543
Error between duplicates	57	0.20463×10^{-2}	
Total	113		

Table D5. Analysis of variance for beta-carotene equivalent at 460 nm. Experiment 4.

Source	DF	Mean Square	Probability
Fresh vs All	1	0.32258	$< 5 \times 10^{-7}$
Radiation Treatment	6	0.39170	$< 5 \times 10^{-7}$
Control vs Treatment	1	0.14380×10^{-1}	$< 5 \times 10^{-7}$
Dose	2	0.40538	$< 5 \times 10^{-7}$
Irradiation Time	1	0.70742×10^{-1}	0.0002
Dose x Irradiation Time	2	0.15343×10^{-1}	0.0279
Storage	1	0.11482×10^{-1}	0.0879
Temperature	1	0.13111	$< 5 \times 10^{-5}$
Atmosphere	1	0.12583	$< 5 \times 10^{-5}$
Radiation Treatment x Sto.	6	0.29024×10^{-2}	0.5955
Radiation Treatment x Temp.	6	0.54812×10^{-2}	0.2279
Radiation Treatment x Atmo.	6	0.32203×10^{-2}	0.5354
Storage x Temperature	1	0.36800×10^{-2}	0.3316
Storage x Atmosphere	1	0.22857×10^{-5}	0.9294
Temperature x Atmosphere	1	0.68829×10^{-2}	0.1832
Error	25	0.37257×10^{-2}	0.0479
Error between duplicates	57	0.21772×10^{-2}	
Total	113		

Table D6. Correlation matrix and mean value.
Experiment 4.

Variable	x	y	%Y	450nm	460nm	Mean
x	1.0000					0.4959
y	0.6365**	1.0000				0.3960
%Y	0.4559**	0.7545**	1.0000			23.07
450 nm	0.6153**	0.7906**	0.6198**	1.0000		0.7902
460 nm	0.6070**	0.7810**	0.6164**	0.9980**	1.0000	0.8019

** Correlation coefficients are significantly different at $P \leqslant 0.01$.