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Effect of various post-harvest treatments on the green color and chlorophyll retention of southern peas, Vigna sinensis

Leonard Wayne Russell

To the Graduate Council:

I am submitting herewith a thesis written by Leonard Wayne Russell entitled "Effect of various post-harvest treatments on the green color and chlorophyll retention of southern peas, Vigna sinensis." 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.

J.L. Collins, Major Professor

We have read this thesis and recommend its acceptance:

M.R. Johnston, Ivon E. McCarty, Homer D. Swingle

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

August 5, 1969

To the Graduate Council:

I am submitting herewith a thesis written by Leonard Wayne Russell entitled "Effect of Various Post-Harvest Treatments on the Green Color and Chlorophyll Retention of Southern Peas, Vigna Sinensis." 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.

M. R. Johnston
Major Professor

We have read this thesis
and recommend its acceptance:

Jimmie L. Collins

Homer D. Smingla

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Vice Chancellor for
Graduate Studies and Research

EFFECT OF VARIOUS POST-HARVEST TREATMENTS ON THE GREEN COLOR AND CHLOROPHYLL RETENTION OF SOUTHERN PEAS, *VIGNA SINENSIS*

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Leonard Wayne Russell

August 1969

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ABSTRACT

Tennessee processors of frozen Southern peas have observed a rapid loss of green color in the post-harvested raw product. Color is one of the most important quality factors in grading peas by USDA standards. Therefore, a loss of greenness may result in a lower grade for the frozen product. This study was initiated in order to gain more information on the factors which may be responsible for this loss of green color.

Southern peas (Vigna sinensis, Mississippi Silver var.) were harvested in the mature green stage, shelled and stored at three temperatures (40°, 75°, 90° F) for three time periods (8, 16, 24 hrs.). The effects of illumination and water storage were also incorporated in this experiment. A second experiment investigated the effects of storage under vacuum, nitrogen, air and storage in the pod.

The effect of these treatments was measured by spectrophotometric analysis of chlorophyll extracts, C.I.E. conversion of Color-Eye Colorimeter values, color panel evaluation, pH values, and enzymatic activity.

The data indicated that an increase in time and temperature caused an increased conversion of chlorophyll to pheophytin. Water storage and illumination had no significant effect on chlorophyll retention. Unshelled peas stored at 40° F retained more chlorophyll than all other treatments. The enzymatic activity of peroxidase and lipoxidase was significantly affected by treatments, but was not correlated with chlorophyll retention or color measurements.

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CHAPTER I

INTRODUCTION

Southern peas are becoming one of the most economically important vegetable crops in Tennessee. The largest percentage of the annual crop is produced mainly in the western part of the state, and is processed by freezing. Two types of frozen Southern peas, Black-eye and Crowder, are commonly available on the market.

One of the major problems associated with the freezing of Southern peas is the loss of green color during the post-harvest period prior to freezing. USDA standards for frozen field and Black-eye (Vigna sinensis) peas require that a minimum of 50% of the total count of peas in each package show at least a tinge of green color before "Grade A" may be assigned.

The nature of the degradation of chlorophyll and subsequent loss of greenness in these peas is unknown. This change may be attributed to enzymatic or chemical reaction occurring in the peas. These reactions may be influenced by various physical factors, such as mechanical shelling, time of storage and storage temperatures.

The purpose of this investigation was to investigate the effect of various physical factors and enzymatic activity on the degradation of chlorophyll and changes in the visual appearance of post-harvested Southern peas.

CHAPTER II

LITERATURE REVIEW

I. POST-HARVEST STORAGE EFFECT ON COLOR OF PEAS

Physiological Changes

Wager (48) studied the effects of controlled atmosphere storage and pod-storage on the quality of post-harvested green peas. The shelled peas were stored in various atmospheres ranging from 2.0%-2.5% O_2 and 2.5-15% CO_2, with the remainder being N_2. Other treatments were peas stored unshelled and peas attached to the pod with the pod opened. Gas storage had no significant effect on the quality of the shelled peas. However, peas stored in closed, as well as those stored in opened pods maintained a greener appearance than the shelled peas. The observed differences were theoretically ascribed to a hormone, possibly similar to kinetin in green leaves, which moves from the pod to the pea. The absence of this hormone would lead to an increase in respiration rate in the pea, with an accompanying deterioration of color and quality.

The effects of mechanical shelling vs. not shelling on the quality of frozen peas was investigated by Lee et al (30). Peas frozen in the pod retained more chlorophyll after 62 days storage than mechanically shelled peas. Unblanched frozen peas maintained color over a month when frozen in the pod, and only a week when shelled and frozen.

Working with green beans, Groeschel (18) showed that gas storage did have an effect on color retention. Green beans were held for 14

days at 45° F and at varying concentrations of CO_2 with 3% O_2. Samples stored for 15 days under these optimum conditions lost much less chlorophyll and rated higher on color panels than the air-stored controls. The respiration rate of beans with this treatment was 35% lower than that of the control.

Kertesz (26) measured definite changes in the chemical composition of post-harvested shelled peas. These changes were presumably directly related to respiration. There were slight changes in composition even when the freshly picked peas were stored at -20° C.

The decomposition products of stored peas have been studied by several researchers. Bengtsson (4) used gas chromatography to detect three main volatile compounds in stored peas: acetaldehyde, ethanol, and hexanol. Joslyn (23) attributed the formation of acetaldehyde to enzymatic reactions. Lindquist (28) found evidence of lactic acid production by the activity of Lactobacillus, which could be associated with the conversion of chlorophyll to pheophytin.

Water Storage

Very little research has been conducted on the effect of water storage of peas. Griffith (16) investigated the effect of hydrocooling, refrigeration and top-icing of Southern peas. He found that peas stored at 35° F for 24 hrs. maintained better color than hydrocooled peas, top-iced peas, or peas stored at room temperature. However, the hydrocooled peas were more turgid and appeared somewhat darker in color than peas stored at room temperature. Sulc et al (37) stated that field-vined green peas transported in water-filled tanks maintained better color and quality than those transported with no water.

Storage Temperature

The effect of storage at varying temperatures has been studied by Newman (32). Shelled green peas were stored at 50°, 63°, and 80° F for 10 hrs. before freezing. The chlorophyll loss was pronounced, varying from 0.5-2.0% more for each degree increase in temperature (° F). Cain (8) found that the green color index of blackeyed peas (Vigna sinensis) was significantly influenced by both duration and temperature of storage.

Pod-stored peas at 33° and 75° F. were studied by Eastman et al (12). Color loss was considerably greater at 75° than at 33° F. Stored peas became progressively lighter and more yellow than the controls. The coefficient of correlation between his reflectance measurements and his panel judgements for color was 0.94.

Broccoli stored at room temperature for 48 hrs. decreased in pH, whereas refrigerated storage resulted in little pH change. Differences in the total acid content, however, were greater than the differences in pH. Increases in acidity produced an increased conversion of chlorophyll to pheophytin, resulting in yellowing of the broccoli (13).

II. PROCESSING EFFECT ON COLOR OF PEAS

Canning

Blair and Ayres (5) developed a process for maintaining the natural color in canned peas. This process involved soaking or blanching the peas in a suspension of $CaCl_2$. The addition of the alkaline agent prevents hydrolysis of the Mg^{+2} ion from chlorophyll. Experimentation showed no difference between alkaline-blanched and regular-blanched peas in chlorophyll content, although the alkaline-treated peas appeared

greener. This supports the theory that the Mg^{+2} ion is replaced with the Ca^{+2} ion in the chlorophyll molecule, rather than with H^{+} ions, as in formation of pheophytin.

The temperature at which canned peas are stored affects the chlorophyll retention also. Blair and Ayres (5) compared peas stored for 10 months at seven different temperatures. The pH decreased as the storage temperature was increased, and this resulted in pheophytin production.

Dehydration

Dehydration has been found to result in 26% conversion of chlorophyll to pheophytin in spinach by Dutton et al (11). Contrary to chlorophyll loss in frozen peas, the unblanched dehydrated spinach retained twice as much chlorophyll as the blanched dehydrated spinach. Chlorophyll losses ranged from 5-15% at 2% moisture, and 50-100% at 16% moisture. Foda (14), working with the effect of blanching time and temperature on dehydrated green beans, found that higher blanching temperatures and longer times were more effective in retaining chlorophyll than lower blanching temperatures and shorter times.

Dehydrofrozen peas are peas which have been partially dehydrated prior to freezing. Talburt (40) found that chlorophyll losses were slight where dehydration did not exceed 50% raw weight. A panel of judges could detect little effect of different blanching times on the color of the product.

Freezing

Campbell (9) investigated the effect of frozen storage at -6.7° C on the color retention of green peas. The slow transformation of chlorophyll into pheophytin at this temperature was thought to be a result of the action of acids from the cell sap. A loss of color occurred whether the peas were packed in brine or water, or were packed dry. The presence of O_2 did not prevent color loss, which ruled out the possibility of mold growth. Enzymatic activity was doubtful since the blanching was sufficient to destroy catalase and peroxidase.

Lindquist (28) measured the chlorophyll content of frozen green peas stored at -10°, 0°, and 10° F for intervals up to 32 weeks. Both reflectance and extraction methods were used. The chlorophyll loss was found to be directly related to the storage temperature and storage time. As the temperature increased there was a gradual decrease in brightness and purity, and an increase in dominant wavelength. The latter indicates that the higher the temperature, the more yellowness, and more conversion to pheophytin.

Guerrant (19) analyzed vegetables frozen at 10°, 0°, and -20° F for 12 months. Samples stored at 10° and 0° reflected light of a lower intensity and longer wavelength than the sample stored at -20° F. All green vegetables became progressively more yellow at the higher storage temperatures.

Color loss in improperly blanched frozen peas is probably largely enzymatic in nature (10). The rate of chlorophyll loss is affected by both degree of heating and storage temperature. The greatest loss occurred with shorter blanching times and higher storage temperatures (14). Enzymes such as lipoxidase, peroxidase, and lipase have been

associated with color loss and flavor changes (45, 47).

Besides inactivation of enzymes, another function of blanching in preserving pigment content may be the removal of volatile, water-soluble constituents which would react with chlorophyll during subsequent storage and cooking (28). Van Buren (42) stated that blanching brings about changes that make the chlorophyll more readily bleached in the presence of sunlight and O_2.

III. ILLUMINATION EFFECT ON COLOR OF PEAS

Sheppard (35) investigated the loss of chlorophyll in frozen peas stored at 0° and 20° F as a result of illumination. At 0° F conversion to pheophytin showed no clear cut difference between the treatment and control, although there was a significant increase in the converted sample at 20° F. The conversion was actually much less than there appeared to be from visual inspection. The major loss of chlorophyll was confined to the seed coat. Color changes occurred 5-10 times faster at 20° F than at 0° F. Exterior ice formation was more rapid on the illuminated samples than on the samples not illuminated.

Vernon (43) stated that chlorophyll is oxidized to unidentified yellow products on prolonged exposure to light in the presence of O_2. Light catalyzes the following reaction between chlorophyll and Fe:

$$\text{Chlorophyll} + Fe^{+3} \leftrightarrow \text{Chlorophyll}^{+} + Fe^{+2}$$

Further research indicated that light increased the production of pheophytin in the presence of acids.

Bleaching occurred in deoxygenated solutions of chlorophyll when exposed to intense light. The extent of bleaching was proportional to

the square root of the light intensity. The reaction was thought to be reversible (43).

IV. ENZYMATIC EFFECT ON COLOR OF PEAS

Lipoxidase

The presence of lipoxidase in Southern peas has been confirmed by Knapp (27). Ericksson (14) studied the distribution of lipoxidase in green peas. He discovered that the enzyme and substrate exists throughout the pea, but the activity is highest in the cotyledon. At partial pressures of O_2 above 20 mm Hg, the enzyme-catalyzed oxidation of linoleic acid is independent of O_2 supply. Also, the O_2 supply is sufficient throughout the pea for the functioning of lipoxidase.

Lipoxidase activity has been linked to the degradation of chlorophyll by several researchers (22, 47). Lipoxidase action on chlorophyll caused degradation to products of unknown composition. Addition of linoleic acid, the prime substrate of lipoxidase, to an unblanched slurry of peas produced additional losses of chlorophyll until the slurry was bleached colorless within an hour (36).

Holden (22) found that commercial antioxidants prevented bleaching of color in legume seeds due to inhibition of lipoxidase. Siddiqi (34) reported that pea extracts which contained neither fatty acid dehydrogenase nor fatty acid oxidase enzymes still oxidized linoleic acid. Since lipoxidase is specific for linoleic acid, he concluded that lipoxidase was responsible for this oxidation.

The optimum pH for pea lipoxidase activity, as reported by Siddiqi (34), is 6.9; and the optimum temperature for oxidation of linoleic

acid is between 0° and 15° C. The enzyme is unstable at room temperature (33). Activity of lipoxidase is involved normally in the production of hydroperoxides from unsaturated fatty acids, primarily linoleic acid. Only the cis, cis forms of the fatty acid substrate are attacked by the enzyme (33). The hydroperoxides formed give rise to many other O_2 consuming reactions and formation of aldehydes which contribute to off-flavors and off-colors. Commercial antioxidants, such as propyl gallate and ∝-tocopherol, are effective inhibitors.

Chlorophyllase

Chlorophyllase is a specific esterase which catalyzes the hydrolysis of the phytol moiety from chlorophyll, producing the acid chlorophyllide. The enzyme is remarkably active in both polar and non-polar solvents. Optimum temperatures for periods varying from a few minutes to 24 hrs. are as follows: 80% ethanol - 25° C, 70% acetone - 25° C, and water - 75° (31). Boger (6) reported that the greatest activity was in 40% acetone solutions. Chlorophyllase is more resistant to heat than many other enzymes, being active at 66-77° F, but inactivated by boiling (17). Its optimum pH is thought to be 7.2-7.3.

Chlorophyllase activity is analyzed by allowing the reaction to occur in a two-phase system. Chlorophyll is soluble only in the organic phase, and chlorophyllide is only soluble in the aqueous phase. When chlorophyll is acted upon by this enzyme, there is a shift in the adsorption toward the red end of the spectrum (45).

Holden (21) theorized that chlorophyllase may be involved in chlorophyll synthesis as well as chlorophyll degradation. Pea seedlings, when grown in light exhibited four times as much chlorophyllase activity as those grown in the dark.

Neither Wagenknecht (45) nor MacKinney and Weast (31, 49) were able to find evidence of chlorophyllase in green peas. Although this enzyme is found in high concentrations in spinach and parsley, relatively few plants contain much of the enzyme (49).

Peroxidase

Peroxidase is active in Southern peas (29). Peas containing active peroxidase showed greater degradation of chlorophyll than peas in which the enzyme had been inactivated by blanching (25). Samples blanched just adequately for a semi-quantitative peroxidase test maintained better color than samples blanched for shorter or longer times.

The purified enzyme has been isolated from horseradish. It has a molecular weight of 40,000 and an isoelectric point of 7.2, although it is stable at pH values of 4-12 (33). The great heat stability of peroxidase has made it useful as a test enzyme in food processing to determine enzyme activity in general. Joslyn (24) reported that heating 1 min. at 284° F is required for complete destruction of peroxidase in peas.

The activity of peroxidase in peas increases during ripening and decreases with decreased respiration during storage (39). During refrigeration of the raw peas, the enzyme becomes more stable, probably due to loss of moisture from the shelled peas. As more water is lost, the enzyme becomes less sensitive to changes in temperature.

CHAPTER III

MATERIALS AND METHODS

I. SOURCE AND TREATMENT OF PEAS

Southern peas, Vigna sinensis, Mississippi Silver var., were grown at the University of Tennessee Plant Science Farm at Knoxville. This variety has a silvery green, smooth-skinned seed and a light green pod. It is uniform in maturation and resistant to common diseases (20).

The peas were harvested by hand on two different dates. The first crop was harvested on August 6, 1968, and used for the first experiment. The second crop was harvested two weeks later and used for the second experiment. Mature (dry) and very immature peas were removed from each lot. A mechanical pea huller (Dixie Canner Co., Athens, Ga.) was used to shell the peas. Cleaning was performed by a size grader (Chisholm Ryder Co., Niagara Falls, N.Y.), which separated most of the hulls and trash from the peas. The remaining trash was removed by hand. Since the effect of water was studied, the peas were not washed.

The first experiment was performed to study the effect of storage time, temperature, presence of water, and illumination on the color and chlorophyll retention of the peas. Sample lots of 2 lbs. each were stored at 40°, 75° (room temperature), and 90° F for periods of 8, 16, and 24 hrs. Samples stored at 40° F were held in a cooler with circulating air and 85% relative humidity. The 75° F samples were stored at ambient temperature and relative humidity of 70%. Samples stored at 90° F were held in an incubator at approximately 80% humidity.

The effects of light exposure and the presence of water were also studied. The treatments at each level of storage time and temperature were divided into four treatments: illuminated with water, illuminated dry, dark-stored with water, and dark-stored dry. Light was provided by fluorescent lamps each containing two 15-watt fluorescent tubes. Samples exposed to light were held in 2 gal. plastic bags containing water and were laid flat so that as much surface as possible could be exposed. The bags were turned over after each time period to allow light exposure on each side. Dark-stored samples were held in 1/2 gal. metal cans.

At the end of each 8-hr. interval a 200 g. sample was removed from each lot and packaged in 1 qt. plastic bags. These samples were frozen immediately at -20° F for 24 hrs., and stored at -5° F.

The second experiment was designed to study the effect of storage atmospheres upon the retention of color in the peas. The samples were shelled, cleaned, and stored in air-tight 4-gal. pressure cookers under the following atmospheres: nitrogen (5 lbs. pressure), vacuum (15 in. Hg), and still air. A fourth lot was prepared in a similar manner but the peas were unshelled. The treatments were held at room temperature and at 45° F for 24 hrs. At the end of this period, each treatment was divided into three equal lots, packaged in plastic bags and placed in waxed fiberboard boxes. These samples were frozen in the same manner as the samples in the first experiment.

II. ANALYTICAL TESTS AND METHODS

Color-Eye Colorimeter (tri-stimulus values)

The Color-Eye Colorimeter (Instrument Development Laboratories, Attleboro, Mass.) was used to measure the tri-stimulus values of all samples prior to freezing. The sample holder used was a small plexiglass cylinder, approximately 3 in. in diameter and 3 in. in height. The bottom surface was packed as uniformly as possible, placed next to the sample viewer and held in place by a cardboard support.

The values for X, Y, Z, and $\bar{x}$ were recorded. These data were used to calculate chromaticity coordinates, dominant wavelengths, and lightness indexes. The dominant wavelength, an indication of hue based on the visible spectrum, is a plot of the chromaticity coordinates (x, y) on the chromaticity diagram, which is shown in Figure A-1, Appendix. Formulas for the calculation of chromaticity coordinates and lightness index are given in Table A-2, Appendix.

Chlorophyll Determination

The method and formulas of Vernon (44) were used to calculate total pheophytin, chlorophyll a, chlorophyll b, and the percentage retention of chlorophyll a, chlorophyll b, and total chlorophyll. The formulas for these calculations are given in Table A-1, Appendix.

Twenty-five grams of sample were blended with 87 ml. of distilled acetone for 10 min. in a Waring Blendor with a rheostat setting of 110. The resultant mixture was approximately 80% acetone. One gram of filter aid was added before extraction. Approximately 50 ml. of extract was filtered three times through Whatman No. 1 filter paper, followed by filtering through No. 44 filter paper.

A converted sample was prepared for each sample by extracting as outlined above, placing 2 ml. of saturated oxalic acid in a 50 ml. volumetric flask and bringing to volume with the extract. Unconverted samples were prepared in the same manner by substituting 2 ml. of 80% acetone for the oxalic acid. The chlorophyll of the acid-treated sample was converted completely to pheophytin within 2 hrs., and this served as a standard on which to base calculations of percentage conversion of chlorophyll to pheophytin in the unconverted sample.

The absorbance curves of the converted and unconverted samples were recorded with a Perkin-Elmer 202 recording spectrophotometer between the wavelengths of 536 and 666 nm. The absorbance of the converted samples at 536, 649, 655, and 666 nm, and the difference in absorbance between converted and unconverted samples at 645 and 662 nm were recorded from spectral curves plotted by the instrument.

Peroxidase Determination

Peroxidase activity was analyzed for all samples in the second experiment. Twenty-five grams of peas plus 1 g. of filter aid were blended in 50 ml. of precooled (0° C) 2% aqueous NaCl solution for 3 min. The slurry was filtered through linen cloth, and then through Whatman No. 1 paper on a Buchner funnel (2).

Twenty ml. of distilled water plus 0.1 ml. filtrate were mixed in a large test tube. One ml. each of 0.5% guaiacol in 50% ethyl alcohol and 0.05% H_2O_2 were added to the tube (24). After mixing thoroughly the solution was poured into a cuvette and the absorbance was measured at 480 nm in a Bauch and Lomb Spectronic 20 Colorimeter. The instrument was previously adjusted to zero absorbance with a blank solution

containing water, filtrate, and guaiacol. The change in absorbance was recorded every 30 sec. for 5 min.

The change in absorbance values vs. time (min.) were plotted on arithmetic graph paper. The activity was expressed as change in absorbance per min. per g. of tissue (dry weight basis), calculated from the linear portion of the graph. The formula for calculation of peroxidase activity is as follows:

$$\text{Peroxidase activity} = \frac{\text{optical absorbance}}{\text{min. x g. (dry weight)}}$$

Chlorophyllase Determination

The method for quantitative analysis of chlorophyllase was a modification of the procedure described by Aardo and Vennesland (1) and MacKinney and Weast (49). A 50 g. sample was extracted by blending and filtering three times in 80% aqueous acetone. This was done in order to remove as much of the chlorophyll as possible. The residue was suspended in 50 ml. of distilled water and centrifuged for 5 min. at 3000 r.p.m. One g. of residue was suspended in 8 ml. of 80% acetone containing a small quantity of chlorophyll a. The chlorophyll a was prepared by paper chromatography of an extract from turnip greens.

The suspension of chlorophyll a and crude enzyme extract was incubated in a stoppered 50 ml. flask at room temperature for 3 hrs. while shaking gently on a mechanical shaker. The flasks were wrapped in aluminum foil to prevent exposure to light. At the end of the incubation period the suspension was filtered through Whatman No. 1 paper. Five ml. of the extract were decanted into a 10 ml. test tube and shaken gently with 5 ml. petroleum ether. As the bi-phase formed, the chlorophyll was contained in the petroleum ether layer and the

chlorophyllide, if formed, remained in the aqueous layer.

The absorbance of both layers was measured on the Perkin-Elmer 202 recording spectrophotometer between 600 and 700 nm. Chlorophyll a showed a distinct peak at 660 nm and chlorophyllide a, at 663 nm.

Lipoxidase Determination

Lipoxidase activity was determined by the method described by Surrey (38). The extraction procedure was a modification of the procedure outlined by Ericksson (14). The enzyme extract was prepared by blending 50 g. of the sample with 100 ml. petroleum ether for 3 min. in a Waring Blendor. The ether was decanted and the residue was blended for 3 min. with 100 ml. of phosphate buffer at pH 7.0. The slurry was centrifuged for 10 min. at 3000 r.p.m. before filtering on a Buchner funnel through Whatman No. 1 paper.

A substrate solution was prepared by mixing 0.2 ml. linoleic acid with 2.5 ml. of Tween 20 (Fisher Scientific Co., Atlanta, Ga.) in a 100 ml. volumetric flask (14). This mixture was brought to volume with the phosphate buffer. Fresh substrate was prepared each day to prevent error due to oxidation of the linoleic acid.

The enzyme activity was determined by measuring the change in absorbance at 234 nm when the pea extract was mixed with the substrate solution. Prior to measurement the substrate solution was oxygenated by bubbling air through it for approximately 5 min. Three ml. of the substrate solution were mixed with 0.5 ml. of the pea extract and the absorbance was measured immediately. The Perkin-Elmer 202 spectrophotometer recorded the absorbance at 234 nm for a period of 8 min.

Lipoxidase activity was expressed as units per g. dry tissue, one unit being the amount of enzyme producing an increase in absorbance of 0.001 nm per min.

Color Panel Evaluation

A panel of six members scored each of the samples for color according to the following 10-point descriptive scale:

1 - Bright green	6 - Greenish brown
2 - Green	7 - Pale brown (beige)
3 - Pale green	8 - Brown
4 - Greyish green	9 - Dark brown
5 - Brownish green	10 - Black

Each sample was scored on three different occasions.

pH Measurement

The pH of each sample was measured with a Beckman Zeromatic pH Meter. A 50 g. sample was blended with 50 ml. of deionized water for 1 min. The slurry was mixed with a magnetic stirrer when the pH was determined.

III. STATISTICAL METHODS

The first experiment was a 3x3x2x2x3 factorial design (storage time, temperature, water storage, and illumination). The second experiment was a 4x2x3 factorial design (storage treatment and temperature). The number of replications was three.

All data were analyzed by the analysis of variance method, using the ANOVAR computer program (7) modified for use by The University of

Tennessee Computer Center. Significant differences between means were determined by Duncan's Multiple Range Test. The coefficients of correlation between chlorophyll concentration, color measurements, and enzyme activity were determined by the computer program, BMD02D (3). The values used for determination of correlation coefficients in Experiment One were the interactions of time and temperature, as calculated by the ANOVAR computer program. Each value was an average of twelve observations. The total number of values used for each variable (x, y) was nine. For Experiment Two, the values for the interactions of storage treatment and time were used to determine the correlation coefficient. The number of observations for each value was three, and the total number of values used for each variable was eight.

CHAPTER IV

EXPERIMENTAL RESULTS

Experiment One

Experiment One was conducted to study the effect of storage time, storage temperature, storage in water, and illumination on the color and chlorophyll retention in peas. The analysis of variance summary for the effect of these variables on the percentage retention of chlorophyll a, chlorophyll b, and total chlorophyll is shown in Table 1. The factors and interactions for time and temperature were significant at the 0.01 level of probability. The factors and interactions of water and light were not significant.

Table 2 shows the effect of time and temperature on the percentage retention of total chlorophyll, chlorophyll a, and chlorophyll b. Both variables caused a significant decrease in chlorophyll retention. A greater loss of total chlorophyll occurred during the first 8 hr. period than during the 16 hr. and 24 hr. periods. Retention of chlorophyll b was greater than that of chlorophyll a. After 8 hrs. storage there was 67% retention of chlorophyll b, whereas chlorophyll a retention was 58%. Decreases in both total and fractional chlorophyll were significant among all levels of time and temperature.

The effect of the interaction of time and temperature on the percentage retention of total chlorophyll, chlorophyll a, and chlorophyll b is given in Table 3. At the 40° F and 75° F temperatures, total chlorophyll decreased significantly among all three storage periods.

Table 1. Analysis of variance summary for the effect of storage treatments on the percentage retention of total chlorophyll, chlorophyll a, and chlorophyll b in Mississippi Silver Southern peas.

Source of Variation	D.F.	Mean Squares Chlorophyll		
		Total	a	b
Time	2	0.80**	0.72**	0.53**
Temperature	2	0.23**	0.21**	0.16**
Time x Temp.	4	0.06**	0.06**	0.04**
Water	1	0.06	0.07	0.04
Light	1	0.01	0.01	0.00
Water x Light	1	0.01	0.01	0.01
Replication	2	0.00	0.00	0.00
Residual Error	94	0.02	0.00	0.00

** Significant at the 0.01 level of probability

Table 2. Effect of storage time and temperature on the percentage retention [1] of total chlorophyll, chlorophyll a, and chlorophyll b of Mississippi Silver Southern peas.

Time				Temperature			
	Mean Percentage Chlorophyll[2,3]				Mean Percentage Chlorophyll		
Hours	Total	a	b	°F	Total	a	b
8	71[a]	58[a]	67[a]	40	64[a]	52[a]	61[a]
16	54[b]	44[b]	51[b]	75	54[b]	44[b]	52[b]
24	41[c]	34[c]	39[c]	90	47[c]	38[c]	45[c]

[1] Percentage actual chlorophyll of the original concentration

[2] Means of 36 observations

[3] Means within a column followed by different letters are significantly different at the 0.05 level of probability

Table 3. Effect of the interaction of storage time and temperature on the percentage retention [1] of total chlorophyll, chlorophyll a, and chlorophyll b of Mississippi Silver Southern peas.[2,3]

Time (hrs.)	Total Chlorophyll			Chlorophyll a			Chlorophyll b		
	40°F	75°F	90°F	40°F	75°F	90°F	40°F	75°F	90°F
8	83^{d}	75^{d}	54^{b}	68^{d}	62^{d}	44^{bc}	79^{d}	72^{d}	52^{bc}
16	63^{c}	49^{b}	49^{b}	51^{c}	40^{b}	40^{b}	60^{b}	47^{b}	47^{b}
24	45^{ab}	38^{a}	39^{a}	37^{b}	31^{a}	32^{a}	43^{ab}	36^{a}	37^{a}

[1] Percentage actual chlorophyll of the original concentration

[2] Means of 12 observations

[3] Means within each chlorophyll value followed by different letters are significantly different at the 0.05 level of probability

Chlorophyll a and b decreased likewise. The samples stored at 90° F showed no significant decrease in total or fractional chlorophyll between the 8 hr. and 16 hr. periods. However, there was a significant decrease after 24 hrs. at this temperature.

Analysis of variance summaries for the effect of storage treatments on the chromaticity coordinates and lightness index are shown in Table 4. The chromaticity coordinates (x,y) were calculated from the Color-Eye measurements (Table A-2, Appendix), and the dominant wavelengths were derived by plotting these coordinates on the chromaticity diagram (Figure A-1, Appendix). There was significance for the interaction of time and temperature on both coordinates, and for the effect of temperature on the "y" coordinate. Lightness index mean squares were significantly different for all treatments except for the interaction of light and water.

The effect of storage time and temperature on the chromaticity coordinates and dominant wavelength of the color of peas is shown in Table 5. The "y" coordinates showed greater differences due to treatment than the "x" coordinate. Dominant wavelengths increased due to an increase in storage time.

Table 6 shows the lightness index as affected by the four variables of time, temperature, water and light. Lightness index is the square root of the "Y" tri-stimulus value measured directly by the Color-Eye Colorimeter. This index expresses the degree of lightness of hue in the sample. Each treatment variable produced significant differences in lightness index. An increase in storage time produced a significantly darker hue between the 16 hr. and 24 hr. periods. An increase in temperature also produced a darker hue.

Table 4. Analysis of variance summaries for the effect of storage treatments on the chromaticity coordinates and lightness index values of Mississippi Silver Southern peas.

Source	D.F.	Mean Squares		
		x Coordinate	y Coordinate	Lightness Index
Time	2	0.0001	0.0003	0.6**
Temperature	2	0.0003	0.0012*	1.2**
Time x Temp.	4	0.0070**	0.0021**	0.7**
Water	1	0.0001	0.0093**	0.5*
Light	1	0.0005	0.0011	10.7**
Water x Light	2	0.0001	0.0006	0.1
Replication	2	0.0005	0.0001	0.1

* Significant at 0.05 level of probability

** Significant at 0.01 level of probability

Table 5. Effect of storage time and temperature on the chromaticity coordinates and dominant wavelengths [1] of Mississippi Silver Southern peas.[2,3]

Time (hrs.)	Temperature (°F) 40 x	40 y	40 D.W.[4]	75 x	75 y	75 D.W.	90 x	90 y	90 D.W.
8	0.388[a]	0.381[bc]	580	0.383[a]	0.387[d]	578	0.360[c]	0.385[a]	573
16	0.387[a]	0.377[b]	581	0.381[a]	0.381[bc]	579	0.380[a]	0.384[a]	578
24	0.375[ab]	0.365[a]	582	0.385[a]	0.372[b]	582	0.378[b]	0.375[b]	580

[1] Calculated from Color-Eye tri-stimulus values

[2] Means within each variable followed by different letters are significantly different at the 0.05 level of probability

[3] Means of 12 observations

[4] Dominant wavelengths (nm.)

Table 6. Effect of time, temperature, water, and illumination on the lightness index [1] of Mississippi Silver Southern peas.

Time		Temperature		Water		Illumination	
Hrs.	Mean[2,3]	°F	Mean	Tmt.[4]	Mean	Tmt.	Mean
8	4.6[a]	40	4.7[a]	Wet	4.6[a]	Light	4.2[a]
16	4.6[a]	75	4.5[b]	Dry	4.4[b]	Dark	4.8[b]
24	4.3[b]	90	4.3[c]				

[1] Calculated from Color-Eye tri-stimulus measurements

[2] Means of 12 observations

[3] Means within each variable followed by different letters are significantly different at the 0.05 level of probability

[4] Treatment

The effect of illumination and water storage was also significant for lightness index. Illumination resulted in a significantly darker color than storage in the dark. Samples stored in water were significantly lighter than samples stored dry. The effect of illumination was more pronounced than any of the other treatments. (See Table 4 and 5.)

The analysis of variance summary for the effect of the variable factors on the color panel scores is shown in Table 7. The factors and interactions of time and temperature were significant at the 0.05 level of probability. Water storage was significant at the 0.01 level of probability.

Table 8 shows the effect of time, temperature, and water on the panel scores for color. There were significant differences among all means at all levels of each treatment variable. The greatest difference was between the mean scores for the water-stored and dry treatments. The dry samples received a 6.5 (pale brown) mean score, whereas the water-stored samples received a 4.1 (greyish green) mean score.

The effect of temperature showed greater differences between means than the effect of time. In both cases peas from the longer storage times and higher temperatures received a significantly higher panel score (less acceptable). However, there was less difference in mean scores between the 16 hr. and 24 hr. periods than between the 8 hr. and 16 hr. periods.

Analysis of the interaction of time and temperature on the color panel scores is shown in Table 9. These means indicated no significant increase in mean scores due to time of storage at 40° F and little

Table 7. Analysis of variance summary for the effect of storage treatments on the color panel scores of Mississippi Silver Southern peas.

Source of Variation	D.F.	Mean Squares
Time	2	7.1*
Temperature	2	24.2*
Time x Temp.	4	3.2*
Water	1	151.9**
Light	1	0.2
Water x Light	1	2.2
Replication	2	0.3
Residual Error	94	0.5

* Significant at the 0.05 level of probability

** Significant at the 0.01 level of probability

Table 8. Effect of time, temperature, and water on the color panel scores [1] for Mississippi Silver Southern peas.

Time		Temperature		Water	
Hrs.	Mean[2,3]	°F	Mean	Treatment	Mean
8	4.8[a]	40	4.4[a]	Wet	4.1[a]
16	5.5[b]	70	5.4[b]	Dry	6.5[b]
24	5.6[c]	90	6.0[c]		

[1] Scores based on a 10-point descriptive scale, 1-10 (bright green-black)

[2] Means of 12 observations

[3] Means within a column followed by different letters are significantly different at the 0.05 level of probability

Table 9. Effect of the interaction of time and temperature on the color panel scores [1] for Mississippi Silver Southern peas.[2,3]

Time (hrs.)	Temperature (°F) 40	70	90
8	4.37[a]	4.83[ab]	5.20[b]
16	4.45[a]	6.02[c]	6.05[c]
24	4.50[a]	5.43[bc]	6.90[d]

[1] Scores based on a 10-point descriptive scale, 1-10 (bright green-black)

[2] Means of 12 observations

[3] Means followed by different letters are significantly different at the 0.05 level of probability

increase at 70° F. However, there were significant increases between the mean scores for all three storage times at 90° F. Only at 24 hrs. were there significant differences among the mean scores for all three temperatures. The mean score increased from 4.5 at 40°, to 5.4 at 70° and 6.9 at 90° F.

The pH of peas from all treatments was measured to determine if there was a relationship between the pH value and chlorophyll loss. Table 10 shows the analysis of variance summary for the effect of the variable factors on the pH values. The factors of storage time and water storage had a significant effect at the 0.01 level of probability.

The pH means as affected by time of storage and by storage in water are given in Table 11. A significant decrease in the mean pH values due to storage time occurred between the 8 and 16 hr. periods, but the 24 hr. period the pH had increased to the same as that at the 8 hr. period. The mean pH (6.19) for the sample stored in water was significantly lower than the mean pH (6.62) for the sample stored without water.

Table 12 shows the correlation coefficients between the means of percentage chlorophyll retention, color measurements, and pH. The means compared were those for the interaction of time and temperature as calculated by the BMD02D computer program. Panel scores correlated significantly with dominant wavelengths (0.82) and with lightness index values (0.78). The correlation coefficient between dominant wavelengths and percentage chlorophyll retention was significant at the 0.05 level of probability. The pH values did not correlate significantly with any of the other values.

Table 10. Analysis of variance summary for the effect of storage treatments on the pH value of Mississippi Silver peas.

Source of Variation	D.F.	Mean Squares
Time	2	0.40**
Temperature	2	0.01
Time x Temp.	4	0.02
Water	1	4.82**
Light	1	0.01
Water x Light	1	0.09
Replication	2	0.01
Residual Error	94	0.04

* Significant at the 0.01 level of probability

Table 11. Effect of time and water on the pH value of Mississippi Silver Southern peas.

Time		Water	
Hrs.	Mean[1,2]	Treatment	Mean
8	6.47[a]	Dry	6.62[a]
16	6.29[b]	Wet	6.19[b]
24	6.46[a]		

[1] Means of 36 observations

[2] Means within a column followed by different letters are significantly different at the 0.05 level of probability

Table 12. Correlation coefficients between chlorophyll concentration and various measurements for color of Mississippi Silver Southern peas.

	Dominant Wavelength	Lightness Index	Panel Scores	pH
% Total Chlorophyll	-0.68*	0.39	-0.64	-0.10
Dominant Wavelength	---	-0.78**	0.82**	-0.05
Lightness Index	---	---	-0.80**	0.35
Panel Scores	---	---	---	0.09

* Significant at the 0.05 level of probability

** Significant at the 0.01 level of probability

Experiment Two

Experiment Two was a study of the effect of storing shelled peas under vacuum, nitrogen, and air (control). The fourth treatment was unshelled peas exposed to air. These samples were stored for 24 hrs. at 40° and 75° F.

The analysis of variance summary for the effect of the variable factors on the concentration of total chlorophyll and its a and b components is given in Table 13. The factors and the interaction for each test indicated a significant effect at the 0.01 and 0.05 level of probability.

Table 14 gives the effect of storage treatment and temperature on the concentration (mg/L) of total chlorophyll, chlorophyll a, and chlorophyll b. The refrigerated, unshelled sample retained the highest concentration of total chlorophyll (12.3 mg/L). There were smaller differences among the other treatments stored at this temperature. The sample held under vacuum at room temperature retained more chlorophyll (11.2 mg/L) than all other treatments at room temperature. Differences between the concentration of chlorophyll a and chlorophyll b were greater for the unshelled treatment than for the other treatments. Refrigerated samples showed greater differences between chlorophyll a and chlorophyll b than did samples stored at room temperature, with the exception of the nitrogen stored sample.

The analysis of variance summary for the effect of storage treatments and temperatures on the chromaticity coordinates is given in Table 15. Each treatment was significant at the 0.01 level of probability and the interactions, at the 0.05 level.

Table 13. Analysis of variance summary for the effect of storage treatment and temperature on the concentration of total chlorophyll, chlorophyll a, and chlorophyll b of Mississippi Silver Southern peas.

Source of Variation	D.F.	Mean Squares Chlorophyll		
		a	b	Total
Treatment	3	8.74**	1.57*	12.07**
Temperature	1	3.24**	4.95**	2.18*
Tmt. x Temp.	3	8.44**	2.54**	6.66**
Replication	1	0.02	0.05	0.64
Residual Error	1	0.05	0.06	0.17

* Significant at the 0.05 level of probability

** Significant at the 0.01 level of probability

Table 14. Effect of storage treatment and temperature on the total chlorophyll, chlorophyll a, and chlorophyll b [1] of Mississippi Silver Southern peas.[2,3]

Treatment	Total Chlorophyll		Chlorophyll a		Chlorophyll b	
	40°F	75°F	40°F	75°F	40°F	75°F
Unshelled	12.3^{e}	10.4^{c}	8.9^{e}	6.3^{d}	3.3^{b}	4.2^{c}
Control	7.9^{b}	7.2^{a}	4.2^{b}	3.6^{a}	3.7^{b}	3.6^{b}
Vacuum	7.2^{a}	11.2^{d}	3.5^{a}	5.8^{c}	3.2^{e}	5.4^{d}
Nitrogen	8.1^{b}	7.6^{ab}	4.3^{b}	4.7^{b}	3.6^{b}	2.8^{a}

[1] Expressed as mg/L

[2] Means of two observations

[3] Means for each chlorophyll class followed by different letters are significantly different at the 0.05 level of probability

Table 15. Analysis of variance summary for the effect of storage treatment and temperature on the chromaticity coordinates of the color of Mississippi Silver Southern peas.

Source of Variation	D.F.	Mean Squares	
		x Coordinate	y Coordinate
Treatment	3	0.0004**	0.0009**
Temperature	1	0.0008**	0.0007**
Tmt. x Temp.	3	0.0003*	0.0001*
Replication	2	0.0001	0.0000
Residual Error	14	0.0000	0.0000

** Significant at the 0.01 level of probability

* Significant at the 0.05 level of probability

Table 16 shows the mean chromaticity coordinates and dominant wavelengths as affected by the storage treatment alone. The storage treatments showed significant differences among all "x" coordinates, but not among all "y" coordinates. The unshelled treatment had a dominant wavelength of 571 nm (greenish yellow), which was lower than all other treatments. This indicates that the unshelled treatment was greener than the other treatments. Although there was a narrow range of dominant wavelengths, the highest wavelength 578 nm (yellow), was that of the control sample.

The significant effects of the storage treatments and temperatures on the color scores by a panel are shown in the analysis of variance summary in Table 17. Both factors and their interaction gave a significant test at the 0.01 level of probability and at the 0.05 level respectively.

Table 18 gives the effect of the interaction of treatment and temperature on the panel scores for color. The lowest mean score, 1.1 (bright green), was given to the refrigerated unshelled treatment and the highest, 8.3 (dark brown), to the unrefrigerated vacuum stored treatment. All mean scores were significantly lower at 40° F than at room temperature. This indicated that the lower temperature maintained a greener color. The largest differences due to temperature occurred within the vacuum stored treatment, which had 3.3 units difference between temperatures, and the control treatment, which had 2.2 units difference. There was no significant difference between the vacuum and nitrogen stored treatments at 40°, whereas the same treatments stored at 75° received a significantly higher mean score for the vacuum than for the nitrogen treatment.

Table 16. Effect of storage treatment on the chromaticity coordinates and dominant wavelengths of the color of Mississippi Silver Southern peas.

Treatment	Mean [1,2]		
	x	y	Dominant Wavelength (nm)
Control	0.386[a]	0.385[a]	578
Nitrogen	0.381[b]	0.399[b]	576
Vacuum	0.372[c]	0.388[a]	576
Unshelled	0.369[d]	0.412[c]	571

[1] Means of four observations

[2] Means for each variable followed by different letters are not significantly different at the 0.05 level of probability

Table 17. Analysis of variance summary for the effect of storage treatment and temperature on the color panel scores of Mississippi Silver Southern peas.

Source of Variation	D.F.	Mean Squares
Treatment	3	33.3**
Temperature	1	1.3**
Tmt. x Temp.	3	6.9**
Replication	2	0.0
Residual Error	14	0.1

** Significant at the 0.01 level of probability

Table 18. Effect of the interaction of storage treatment and temperature on the color panel scores [1] of Mississippi Silver Southern peas.[2,3]

Storage Treatment	Temperature	
	75°F	40°F
Control	6.5[b]	4.7[e]
Nitrogen	6.3[c]	5.7[d]
Vacuum	8.3[a]	5.6[d]
Unshelled	2.1[f]	1.1[k]

[1] Scoring range 1-10 (bright green-black)

[2] Means of two observations

[3] Means followed by different letters are significantly different at the 0.05 level of probability

The analysis of variance summary for the effect of storage treatment and temperature on the pH values is shown in Table 19. The effect of temperature and the interaction of time and temperature were significant at the 0.01 level of probability.

The pH measurements for storage treatments and temperatures are shown in Table 20. Peas stored under vacuum at 40° F had the highest mean pH. The lowest mean pH occurred in the nitrogen stored peas at 40° F. The pH of the vacuum stored sample was lower at 75° F than at 40° F, whereas the pH of the nitrogen stored sample was lower at 40° F than at 75° F. Within the unshelled and the control samples there was little difference in the pH between the two temperatures.

Table 21 shows the analysis of variance summary for the effect of storage treatment and temperature on the activity of lipoxidase and peroxidase enzymes. Each factor and the interaction of both indicate that there was significance at the 0.01 level of probability for both enzymes.

Table 22 gives the activity of the enzyme lipoxidase as affected by storage treatment and temperature. The highest activity was found in the refrigerated unshelled treatment. Both the nitrogen and control sample showed no activity at room temperature. These samples also had the least activity of all treatments at 40° F. All treatments stored at room temperature had a lower activity than comparable treatments stored at 40° F.

The activity of peroxidase as affected by storage treatment and temperature is shown in Table 23. Temperature of storage had the same effect on peroxidase activity as upon lipoxidase activity, that is

Table 19. Analysis of variance summary for the effect of storage treatment and temperature on the pH value of Mississippi Silver Southern peas.

Source of Variation	D.F.	Mean Squares
Treatment	3	0.136**
Temperature	1	0.004
Tmt. x Temp.	3	0.231**
Replication	2	0.024
Residual Error	14	0.014

** Significant at the 0.01 level of probability

Table 20. Effect of storage treatment and temperature on the pH of Mississippi Silver Southern peas.[1,2]

Storage Treatment	Temperature	
	75°F	40°F
Control	6.23^{bc}	6.13^{ab}
Nitrogen	6.43^{c}	6.00^{a}
Vacuum	6.27^{bc}	6.77^{d}
Unshelled	6.27^{bc}	6.40^{c}

[1] Means of two observations

[2] Means followed by different letters are significantly different at the 0.05 level of probability

Table 21. Analysis of variance summaries for the effect of storage treatment and temperature on the activity of peroxidase and lipoxidase in Mississippi Silver Southern peas.

Source of Variation	D.F.	Mean Squares	
		Peroxidase	Lipoxidase
Treatment	3	0.071**	8.996**
Temperature	1	0.222**	3.994**
Tmt. x Temp.	3	0.039**	18.587**
Replication	2	0.001	0.079
Error	14	0.005	0.097

** Significant at the 0.01 level of probability

Table 22. Effect of storage treatment and temperature on the lipoxidase activity [1] of Mississippi Silver Southern peas.[2,3]

Storage Treatment	Temperature	
	75°F	40°F
Control	0[e]	23[a]
Nitrogen	0[e]	19[a]
Vacuum	6[b]	32[f]
Unshelled	12[c]	82[g]

[1] Units activity per g. dry weight

[2] Means of two observations

[3] Means followed by different letters are significantly different at the 0.05 level of probability

Table 23. Effect of storage treatment and temperature on the peroxidase activity [1] of Mississippi Silver Southern peas.[2,3]

Storage Treatment	Temperature	
	75°F	40°F
Control	0.52[b]	0.67[a]
Nitrogen	0.27[c]	0.63[ab]
Vacuum	0.52[b]	0.57[ab]
Unshelled	0.28[c]	0.43[b]

[1] Units activity per g. dry weight

[2] Means of two observations

[3] Means followed by different letters are significantly different at the 0.05 level of probability

activity was greater at 40° than at 75° F. There was very little significant difference between the treatments stored at 40° F. At room temperature, activity was lowest for the unshelled and nitrogen stored samples. Unshelled peas had the least activity of all samples at 40°.

The correlation coefficients between the various measurements in Experiment Two are shown in Table 24. Dominant wavelengths correlated significantly with chlorophyll concentration and color panel scores. However, panel scores did not correlate significantly with total chlorophyll concentration. Peroxidase and lipoxidase activity had no significant correlation with any of the other measurements.

A quantitative analysis for the activity of chlorophyllase was conducted. No positive indication of the presence of this enzyme was confirmed. The analysis was repeated numerous times, using samples from Experiment Two. Only once were positive results indicated. Since several researchers (30, 48) were unable to find evidence of chlorophyllase in green peas, it may be assumed that the same holds true of Southern peas.

Table 24. Correlation coefficients between chlorophyll concentration, various color measurements, and enzyme activity of Mississippi Silver Southern peas.

	Dominant Wavelength	Panel Score	Lipoxidase	Peroxidase
Total Chlorophyll	-0.79*	-0.62	-0.41	0.33
Dominant Wavelength	---	0.76*	0.42	-0.28
Panel Score	---	---	0.37	0.31
Lipoxidase	---	---	---	-0.17

* Significant at the 0.05 level of probability

CHAPTER V

DISCUSSION

Experiment One

Time of storage had the greatest effect on the conversion of chlorophyll to pheophytin. The conversion exceeded 50% for all samples stored for 24 hrs. Apparently the greater loss of chlorophyll occurred due to the longer period for conversion of chlorophyll to pheophytin. The rate of reaction decreased as the storage time was increased. Most of the conversion occurred during the first 8 hrs. of storage. These results indicate that Southern peas should be processed as soon as possible after shelling.

Temperature of storage affected the chlorophyll conversion significantly. The increase in conversion with an elevation of temperature may indicate an increased rate of the conversion reaction. Since there was less conversion of chlorophyll at the 40° F temperature than at 75° and 90° F, this would be nearer to the preferred storage temperature.

Dominant wavelengths calculated from Color-Eye Colorimeter measurements indicated greater yellowness due to an increase in temperature. The visual appearance of the peas was more green than the color indicated by the dominant wavelength. Lindquist (28), who studied storage temperatures of frozen green peas, found that an increase in temperature caused a decrease in brightness and purity, and an increase in dominant wavelength.

Lightness index values calculated from Color-Eye Colorimeter measurements showed a darker color for all samples after 24 hrs. of storage. Higher temperatures also produced a darker hue. Water stored samples were lighter than dry stored samples. The non-illuminated samples were lighter than those which were illuminated. These differences were clearly observable prior to freezing of the samples.

The darker hue in these samples may have resulted from dehydration of the seed coat. This dehydration would account for the darker hue of the dry samples and those held at higher temperatures. Removal of the darkened seed coat revealed a green cotyledon. Sheppard (35), who investigated the loss of chlorophyll in frozen peas as affected by illumination, showed that the major loss of chlorophyll was confined to the seed coat. The conversion of chlorophyll in the entire pea was actually much less than there appeared to be from visual inspection.

Color panel scores correlated significantly with dominant wavelengths and lightness index values, but not with percentage chlorophyll retention. This correlation may be explained by the observation that the color and appearance of the seed coat was not an indication of the chlorophyll retention in the cotyledon.

The panel members were able to distinguish only a rather narrow range of color differences, which was 4.4 (8 hrs., 40° F) to 6.9 (24 hrs., 90° F). This range was less than the range for chlorophyll conversion to pheophytin, which was 38% (24 hrs., 75° F) to 83% (8 hrs., 40° F). Although the water stored peas were scored as greyish green (4.1) and the dry stored peas were scored as brown (6.5), there was no significant difference between the two samples in percentage chlorophyll retention.

The pH values of all treatments were measured to determine if a change in pH had occurred, and if this change was correlated with chlorophyll conversion to pheophytin. All pH values were slightly acid (6.19-6.62). A decrease in pH occurred after 16 hrs., and then increased again after 24 hrs. of storage. This variation may be attributed possibly to various reactions within the pea. Water stored peas had a lower pH than dry stored peas. The imbibition of water could have increased the chemical or enzymatic reactions which might affect the pH value.

Experiment Two

The retention of total chlorophyll and chlorophyll a was highest for refrigerated unshelled peas and lowest for the control (air stored) peas. It appeared that the unshelled peas were better protected against reactions causing chlorophyll degradation. This observation may possibly be explained by the presence of a respiration-inhibiting hormone, as postulated by Wager (48), which is transferred from the pod to the pea. Lee *et al* (30) reported that peas frozen unshelled, even when unblanched, retained more chlorophyll after 62 days storage than shelled peas.

Peas held under vacuum at room temperature had the highest retention of chlorophyll of all treatments at room temperature. This retention may have been related to the rapid dehydration of the peas, which were held under a 15 in. Hg vacuum. Talburt (40) found that dehydration reduced chlorophyll loss. Dehydrofrozen peas, which had been partially dehydrated prior to freezing, had only slight chlorophyll losses when dehydration did not exceed 50%.

Since Color-Eye measurements were taken prior to freezing, dominant wavelengths calculated from these values are probably a more accurate measure of treatment effects. The dominant wavelength of the control sample was the highest, or the most yellow in hue. Refrigerated unshelled peas had the greenest hue, as indicated by a lower dominant wavelength. These results agree with the findings of Wager (48).

Panel scores showed a significant correlation with dominant wavelengths. Refrigerated unshelled samples were scored greener than all other treatments. This sample had a pronounced greenness, as shown in the color photographs in Figure A-2, Appendix. Vacuum stored samples were scored as most brown at both temperatures, even though the chlorophyll loss was less than that in the other samples. The judges may have been influenced by the shrivelled appearance of the peas.

The pH values were significantly affected by temperature and by the interaction of storage time and temperature. The means for the interaction of time and temperature did not correlate with any of the other measurements. However, it was observed that the samples with the lowest average pH also had the lowest average chlorophyll content, with the exception of the unshelled treatment. This observation would indicate that a decrease in pH, possibly attributed to the release of plant acids, affected the conversion of chlorophyll to pheophytin.

The activity of lipoxidase and peroxidase was analyzed for the treatments in Experiment Two. The units of activity of lipoxidase in this investigation were lower than those reported by Ericksson (14) in fresh green peas. The difference may be attributed to the effect of the storage treatments and differences between the two types of peas.

Lipoxidase activity was significantly different between various treatments. The activity was highest in the refrigerated unshelled samples. The lower activity in the other treatments may have been due to the decrease in stability of the enzyme in the shelled peas. Activity was lower at room temperature than at 40° . Reed (33) stated that lipoxidase is very unstable at room temperature. Therefore, storage of the peas at room temperature would probably result in decreased activity. The relatively higher activity of the vacuum stored samples may have been affected by dehydration of the peas. Low levels of free water sometimes prevents diffusion of the enzyme or substrate (33).

Although peroxidase has not been definitely associated with chlorophyll degradation in peas, it serves as an indication of general enzyme activity. As with lipoxidase, the activity of peroxidase was greater in samples held at 40° than samples held at 75° F. Peroxidase is very stable, and therefore instability due to higher temperatures is unlikely. Other factors, such as physiological and respiratory changes, may be involved in this observed difference. Activity in the unshelled pea was the lowest of all storage treatments. This may indicate that shelling of the peas increased activity.

Neither peroxidase or lipoxidase activity correlated significantly with chlorophyll loss. Therefore, it cannot be stated that these enzymes function in this manner. However, since lipoxidase has been linked with chlorophyll degradation in green peas, confirmation of its presence in Southern peas may prompt further research.

Correlation coefficients were significant between dominant wavelength and chlorophyll concentration, and between dominant wavelength and panel scores. These coefficients were also significant in Experiment One. Dominant wavelength, therefore, appeared to be a good measure of color as well as of chlorophyll conversion.

CHAPTER VI

SUMMARY

This study was conducted to investigate the effect of certain storage treatments on the degradation of chlorophyll and subsequent loss of color in post-harvested Southern peas. Experimental variables in Experiment One consisted of storage time (8, 16, 24 hrs.), temperature (40°, 75°, 90° F), water storage, and illumination. Experiment Two was a study of the effect of various storage mediums (nitrogen, vacuum, air, storage in the pod) on chlorophyll and color loss.

Based on the results of this study, the following conclusions were reached:

Experiment One

(1) Chlorophyll degradation was affected more by the length of storage period than by the storage temperature.

(2) An increase in both time and temperature of storage caused an increased loss of chlorophyll and apparent greenness.

(3) Water storage and illumination had no significant effect on chlorophyll retention.

(4) Water stored peas were lighter in hue than dry peas. Peas stored in the dark were lighter than those which were illuminated.

(5) Panel scores for color were based on the appearance of the seed coat, and did not correlate with overall retention of chlorophyll.

Experiment Two

(1) Peas stored unshelled at 40° F maintained the greenest appearance and the highest chlorophyll retention of all treatments.

(2) Partial dehydration due to vacuum storage at room temperature resulted in a relatively high retention of chlorophyll.

(3) Activity of lipoxidase and peroxidase did not correlate with the color measurements or with chlorophyll retention.

(4) Dominant wavelength appeared to be a good measure of visual color and chlorophyll retention.

(5) Enzymatic analysis indicated no chlorophyllase activity.

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APPENDIX

Table A-1. Formulas for calculation of chlorophyll concentration and percentage retention of chlorophyll.

A. Percentage chlorophyll retention:

(1) $$\text{Percentage total chl.} = \frac{18.80\ (\Delta\ A662)^{1} + 34.02\ (\Delta\ A645)\ X\ 100}{6.90\ (A666) + 26.72\ (A655)}$$

(2) $$\text{Percentage chl. b} = \frac{30.38\ (\Delta\ A645) - 6.58\ (\Delta\ A662)\ X\ 100}{32.74\ (A655) - 13.75\ (A666)}$$

(3) $$\text{Percentage chl. a} = \frac{25.38\ (\Delta A662) + 3.46\ (\Delta A645)\ X\ 100}{20.65\ (A666) - 6.02\ (A655)}$$

B. Chlorophyll concentration (mg./L.)

(1) Total chl. = 11.63 (A665) - 2.39 (A649)

(2) Chl. b = 20.11 (A649) - 5.18 (A665)

(3) Chl. a = 11.63 (A665) - 2.39 (A649)

[1] Difference in absorbance between converted and unconverted samples

Source: Vernon, L. P. 1960. Spectrophotometric determination of chlorophylls and pheophytin in plant extracts. Anal. Chem. 32, 1144

Table A-2. Formulas for calculation of C.I.E. values from Color-Eye colorimeter tri-stimulus data (X, Y, Z, $\bar{x}$).

(1) X-CIE = (0.783) X + (0.197) $\bar{x}$

(2) Y-CIE = Y

(3) Z-CIE = (1.18) Z

(4) Chromaticity coordinates:

$$x = \frac{\text{X-CIE}}{\text{X-CIE} \quad \text{Y-CIE} \quad \text{Z-CIE}}$$

$$y = \frac{\text{Y-CIE}}{\text{X-CIE} \quad \text{Y-CIE} \quad \text{Z-CIE}}$$

(5) Lightness index = $\sqrt{\text{Y}}$

Source: Hardy, A. C. 1936. "Handbook of Colorimetry," The Technology Press, Cambridge, Mass.

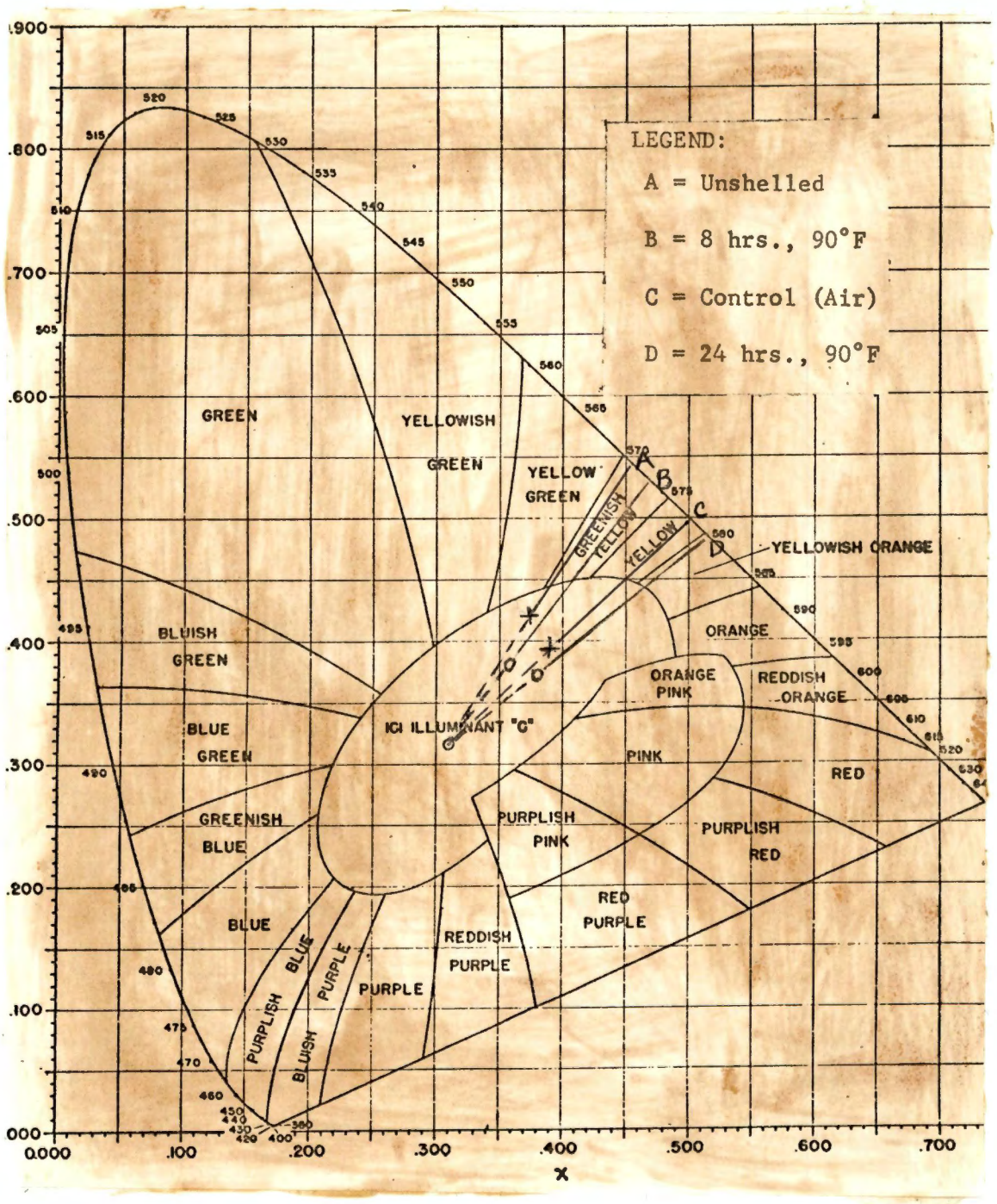
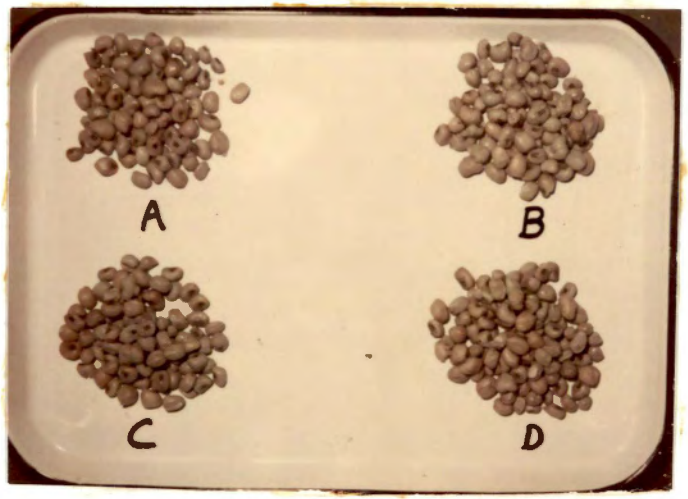


Figure A-1. The ICI chromaticity diagram showing plots of the coordinates of various treatments.

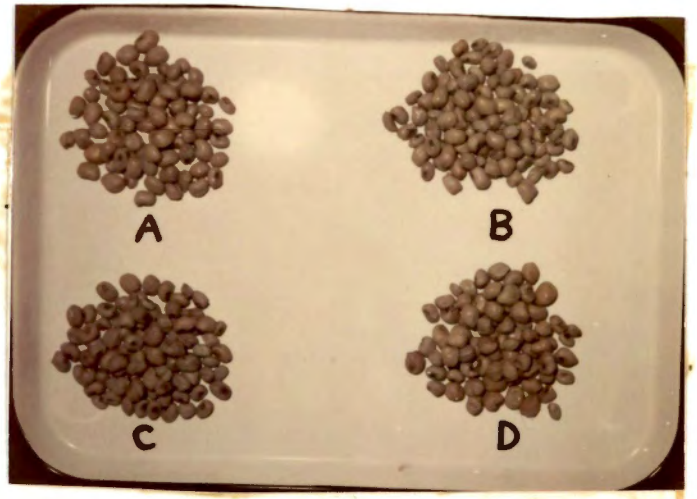
Source: National Bureau of Standards Cir. 553, (1955) U. S. Dept. of Commerce.

Figure A-2. Color photographs showing the effect of various storage mediums on the color of Mississippi Silver Southern peas.

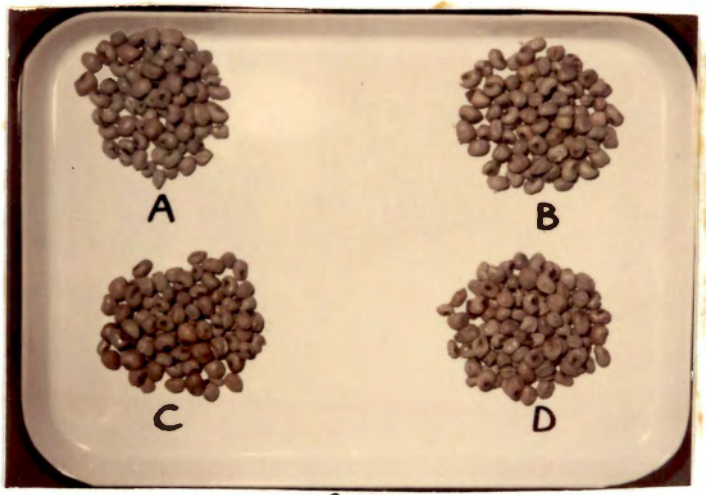
Erratum: "Vacuum, RT" and "Vacuum, 45" labels should be reversed.



40° F



70° F

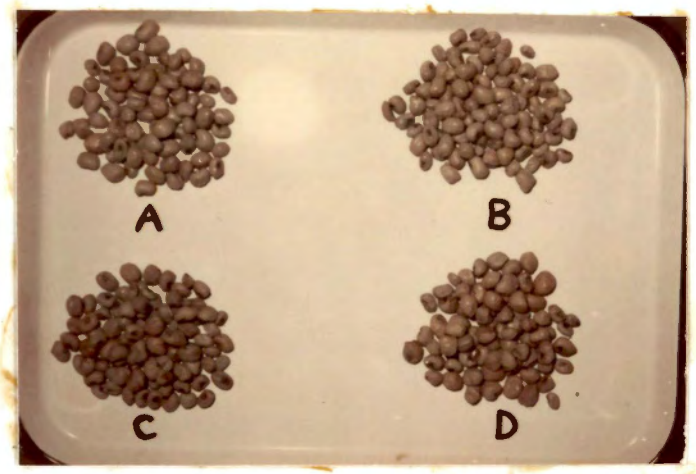


90° F

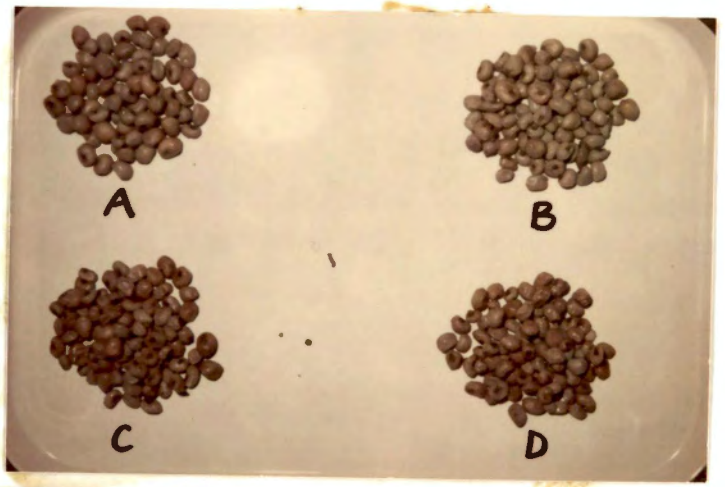
LEGEND:

A=Wet, Illuminated
B=Wet, Dark
C=Dry, Illuminated
D=Dry, Dark

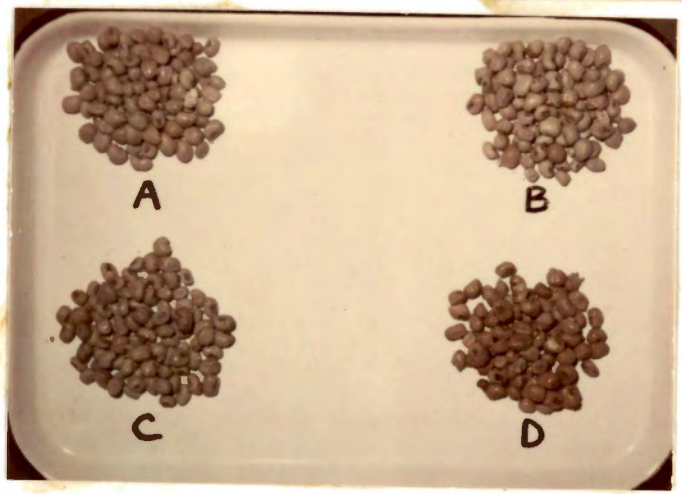
Figure A-3. Color photographs showing the effect of various treatments on the color of Mississippi Silver Southern peas after 8 hrs. storage.



40° F



70° F



90° F

LEGEND:

A=Wet, Illuminated
B=Wet, Dark
C=Dry, Illuminated
D=Dry, Dark

Figure A-4. Color photographs showing the effect of various treatments on the color of Mississippi Silver Southern peas after 24 hrs. storage.

VITA

Leonard Wayne Russell was born in Warner Robbins, Georgia, on December 21, 1945. He attended elementary school in Crossville, Tennessee, and was graduated from Cumberland County High School in 1963. The following September he enrolled at The University of Tennessee, and in June, 1967, he received a Bachelor of Science degree in Agriculture. In the same month he accepted an assistantship in Food Technology at the same institution. Since that time he has been studying for completion of the requirements of the Master of Science degree.