PIGMENTS IN GREEN BEANS AND THEIR QUALITATIVE AND QUANTITATIVE CHANGES DURING PROCESSING AND STORAGE

by

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INTRODUCTION

In green vegetables, an important and also the most obvious change which takes place during processing is that of color. When green beans are processed, blanching and freezing treatments alter the color to bright green, while canning treatments produce dull olive-green colored beans. Although it is not possible to specify the color which is most desirable for processed green beans, it is important to know why these changes in color take place. Until this information is available, methods of controlling the color of processed beans can be no more than empirical.

Measurement of the concentrations of the pigments does not necessarily give a true measure of the visible color of the vegetable. Since color is the integration of the effects of the pigments, a knowledge of the pigments present, and their changes due to different conditions, is fundamental in controlling the color of processed products.

For these reasons, this study of the qualitative and quantitative changes of the pigments in green beans caused by processing and storage was undertaken. The work was conducted and reported in two parts because the

techniques and manner of conducting the work were distinctly different. In Part I, the pigments in unprocessed, frozen, and canned beans were chromatographically separated and identified, with no attempt being made to obtain quantitative yields of pigments. In Part II, the major pigments and their changes in concentration were quantitatively determined in two varieties of Blue Lake type pole beans. These varieties were Columbia and Associated 92. Two different sizes or maturities of Associated 92 beans were used and the effects of variety, size, blanching, freezing, canning at two different temperatures, storage at two different temperatures, and storage for one and five months were determined.

REVIEW OF LITERATURE

In green vegetables the predominant pigments are the chlorophylls and the carotenoids. Both these types of pigments have large and complex molecular structures making study of their structure, reactions, and degradation products difficult and highly specialized.

Chemistry of Chlorophylls and Carotenoids

Because of the importance of these compounds in photosynthesis and nutrition, they have been studied very extensively and a great volume of knowledge about them has been accumulated.

Between 1906 and 1914, Willstatter (68) prepared pure chlorophyll for the first time and his chemical investigations were the foundations of present knowledge of the structure of the green pigments. Willstatter and coworkers also showed that there were two green pigments, chlorophylls A and B, and separated them by partition between petroleum ether and 90 per cent methanol. Later, Winterstein and Stein devised the method of chromatographic separation on powdered sugar affording an even simpler method of separation.

Spoehr (52) reviewed the information up to 1932 on the function of the chlorophylls in the photosynthetic process, while Steele (54) and Fischer (19) reviewed the

progress in determining the structure of chlorophyll and related compounds up until 1936. Since 1936, the literature on plant pigments has been reviewed by Strain (56), Smith (51), Mackinney (41), Zacheile (72), and Aronoff (1, 2, 3).

The chemistry of chlorophyll is now complete in that both chlorophylls A and B may be synthesized from simple molecules of known structure. Chlorophyll A is the magnesium chelate of 1,3,5,8-tetramethyl-4-ethyl-2-vinyl-9-keto-10-carbomethoxyphorbin phytyl-7-proprionate while chlorophyll B corresponds to chlorophyll A except that the 3 position is substituted by a formyl group rather than a methyl group.

As in the case of the chlorophyll pigments, the carotenoid pigments have been investigated extensively and have been the subject of several reviews. Palmer (45) reviewed the information available up to 1922, while Bogert (9) and Karrer and Helfenstein (33) published reviews in 1932 and 1933 respectively. Since then Strain (56), Karrer (34), and Goodwin (23) have written books in which they have reviewed the literature up until the respective dates of publication.

Briefly, it may be stated that the carotenoids can be related to the parent substance lycopene ($C_{40}H_{56}$), and are generally composed of isoprene residues, usually eight,

arranged in such a way that in the middle of the molecule two methyl groups are present in 1:6 positions, while the other side chain methyl groups occupy 1:5 positions (23, p.1). The xanthophylls are oxygen containing carotenoids and are usually named from the substance from which they were first isolated, e.g. zeaxanthin, which was obtained from Zea Mays.

Separation and Determination of Pigments

A great many methods, most of which vary in detail only, have been used for the separation of the pigments in plant material. All methods involved an initial extraction of the pigments from the plant by means of grinding with a suitable solvent. The choice of solvent was dependent on the particular pigments under study but, in all cases, very mild conditions must be used in order to cause as little alteration of the pigment as possible. After purification of the extract, the pigments were separated. The separation was accomplished by partition between two immiscible liquids (68, p.92), by column chromatography (56, p.63) or by paper chromatography (53, 61). The methods most commonly used at present are those utilizing an adsorption column. The principles of column chromatography, selection of solvents and adsorbents, and reviews of methods utilizing this technique have been discussed

in detail by Strain (60), and by Zechmeister and Cholnoky (70). The most commonly used adsorbents for separation of the chlorophyll pigments are powdered sucrose (61, 74), and inulin (40), while magnesia (56, p.36) and lime (30, 36) are most commonly used for the carotenes. Strain (58) found magnesia and powdered sucrose the most suitable adsorbents for the separation of the xanthophylls.

In the quantitative estimations of the pigments, similar extraction procedures were used. The concentrations of chlorophyll were determined in the purified pigment extract by measurement of the optical density at 660 and 643 mμ (15, 26), and then the carotenes and xanthophylls were obtained separately by adsorption of the pigments on a magnesia-supercel column followed by selective elution. The concentrations of the carotene and xanthophyll fractions were then obtained by measuring the optical densities of the solutions at the appropriate wavelengths (26, 27). A somewhat similar method for determining chloroplast pigments was used by Haskin (31) but a filter photometer, standardized against pure pigments, was used for the final measurements.

When other pigments, which exhibit appreciable absorption at the longer wavelengths, are present, the chlorophylls cannot be measured by the above method.

Pheophytins are the major interfering pigments which would be normally present in plant extracts, especially if the plant material had been subjected to heat or acid treatments. Mackinney and Weast (42) therefore developed a method in which both chlorophylls and pheophytins may be measured in the same extract. This method is, however, of limited accuracy because the four component system is treated as though it contained only two components. Dutton et al. (16) used this method successfully in measuring the pigments in dehydrated spinach. Another method for measuring both chlorophylls and pheophytins in the same extract was presented by Aronoff (3, p.156). In this method, the optical density was measured at 505, 525, 642.5, and 660 mu, where each of the wavelengths corresponded to an optimal maximum for one component. However, Aronoff pointed out that in actual practice the reliability of the results obtainable by this method were severely limited because the rapid change of absorption coefficients in the vicinity of these wavelengths required the use of a spectrophotometer with very isolated spectral regions.

The importance of carotene as a precursor of vitamin A has been responsible for the development of many methods for the determination of this pigment in plant material. Graves (25), however, observed that chemical

determinations of carotene did not necessarily indicate the biological vitamin A activity. He suggested that the biological vitamin A activity in green vegetables was closely correlated with their carotene content but in yellow and orange vegetables it was less directly correlated.

Most of the methods for carotene determination (4, 7, 20, 30, 35, 43, 66) are the same in principle but differ in the choice of extracting solvent, temperature of extraction, or column adsorbent. Although the differences between these methods are minor, it is believed that the use of these different methods probably accounts for some of the conflicting concentrations of carotene reported for various foods. Walsh and Hauge (67) and Pepkowitz (46) found that destruction of carotene in plant material during storage and even during analysis was due to both enzymatic and photochemical action. Pepkowitz advocated the addition of sodium cyanide to the sample during extraction of carotene and showed that this prevented the non-photochemical enzymic destruction of carotene and partially inhibited the photochemical destruction. However, to preserve carotene both types of destruction must be prevented, because prevention of only one leaves more carotene for the other system to destroy. Conversely, Halverson and Hart (29) found that during storage of

dehydrated spinach, broccoli, and carrots, the carotene was well preserved during storage at room temperature by leaving sufficient water (10-12%) in the dried tissue to permit activity of the respiratory enzymes so that the oxygen in the container was removed and replaced by carbon dioxide.

Only one method was found for quantitatively determining the xanthophylls in plant material (26). This was merely a slight modification of the method used for determining carotene.

Vegetable Pigments and Their Changes During Processing and Storage

A survey of the literature revealed that little work had been reported on the identification of pigments in green vegetables. The presence of the two chlorophylls was known and so too was beta carotene which is always found associated with chlorophyll in the chloroplasts. However, the only vegetables in which the xanthophylls had been investigated were of the leafy type such as spinach (56, p.31). Lassen *et al.* (39) investigated the carotenoid pigments of the avocado, but although several pigments in addition to beta carotene were isolated, they did not identify them. Almost all the investigations on the xanthophylls had been conducted on leaves, flowers,

and marine plants.

The changes in the chlorophylls of vegetables due to processing and storage have been frequently investigated because the change in the green color is probably the most obvious change taking place under these conditions. In 1943, Blair and Ayres (8) demonstrated that the degradation of chlorophyll in peas during canning was due to the loss of magnesium from the pigment by hydrolysis. They developed a process, using alkaline substances in a definite sequence, by which the pH of the tissue was kept at a sufficiently high level throughout the process. By this procedure, 60 per cent of the chlorophyll remained unchanged, and at the same time the flavor of the peas was improved and their texture remained satisfactory. Fishbach and Neuberger (17) spectrophotometrically examined the pigments of peas canned by the Blair process and confirmed that the bright green color was due to the retention of unchanged chlorophyll. However, the same authors (18) found that the bright green color of okra canned in the presence of zinc was due not to chlorophyll but to a similar pigment in which the zinc had replaced the magnesium.

Before the advent of the Blair process, several other processes using alkaline treatments for the retention of the green color of fruits and vegetables during

preservation had been patented, but in each case the flavor and/or the texture were adversely affected (11, 49). Apparently, interest is still being shown in processes of this nature because several similar processes were patented between 1943 and 1952 (6, 22, 55).

Changes in chlorophyll in frozen vegetables have also been investigated. Campbell (13) showed that the discoloration produced in frozen peas stored at -6.7° C was due to the conversion of chlorophyll to pheophytin. He illustrated this by preventing this discoloration in duplicate packages stored over ammonia solution. Mackinney and Weast (42) also showed that a substantial part of the chlorophyll in frozen peas and string beans was converted to pheophytin and that during blanching the formation of pheophytin was an interrelated function of time and temperature. They reported that less pheophytin was formed when properly blanched frozen peas and beans were cooked than when the unblanched frozen vegetables were cooked. They therefore suggested that during the blanching process a large portion of the volatile and water-soluble constituents, which would react with the chlorophyll during subsequent cooking, were removed. When properly blanched, the cooked frozen peas retained 40-50 per cent of the chlorophyll. They found no residual chlorophyll in commercially canned beans. Hall et al.

(28), when studying the volatile components of peas and asparagus, found no volatile acids in either vegetable. The beneficial effect of blanching described by Mackinney and Weast, therefore, must be due to leaching of the acidic substances.

Another reason for the loss of chlorophyll during frozen storage of raw peas was suggested by Wagenknecht et al. (65). These authors postulated that the enzymes lipoxidase and lipase together with the large increase in acid number were responsible for chlorophyll destruction.

In an investigation of the changes of pigments and color during the storage of dehydrated spinach, Dutton et al. (16) found that the destruction of chlorophyll was positively correlated with the moisture content and was affected little by the oxygen content of the storage atmosphere. They also reported that the destruction of carotene was greatly dependent upon the oxygen in the atmosphere and independent of the moisture content of the dried product. They concluded that in dehydrated spinach the destruction of chlorophyll may be regarded as an indication of loss of palatability and ascorbic acid.

Kramer et al. (38) showed that the chlorophyll content of asparagus decreased from 195 ppm to 93 ppm during four days' storage at 70° F. After canning, the subjective

color scores for both the samples canned fresh, and those canned after four-day storage, were the same. They concluded that the greater the loss of pigment during storage of the fresh product, the smaller the further loss incurred during processing. They also found that both canning and freezing treatments had detrimental effects on retention of color.

In a qualitative study of the chlorophyll pigments in barley and mallow leaves, Strain (61) found only chlorophylls A and B present in the leaves extracted quickly with acetone or methanol without heating. By killing the leaves by dipping in boiling water, chlorophylls A' and B', in addition to the unaltered chlorophylls, were obtained. When the pigments were extracted by soaking the leaves in methanol or acetone for several hours, only small amounts of chlorophyll A and B were recovered, the major amounts having been converted to the corresponding chlorophyllides or methyl chlorophyllides. In addition to these substances, several other pigments which were derived from the chlorophylls by allomerization, were obtained.

The reports on the effect of cooking and blanching on the carotene concentrations of vegetables are almost unanimous in stating that little or no decrease takes place (4, 5, 71, 73). Bedford and Hand (5) and Zimmerman

et al. (71) also found no losses in carotene occurred when blanched green beans were frozen and stored. Porter et al. (47) reported apparent increases in carotene content when Swiss chard and beet greens were cooked. On the other hand, Zscheile et al. (73) found that although blanched vegetables retain carotene much better during low temperature storage than do unblanched vegetables, the beta-carotene concentration decreased during storage. They noted that the percentage of beta-carotene in the total carotene fraction was fairly constant in all fresh vegetables except green beans and lima beans, which were both low in beta-carotene. They found small inter-varietal differences in the carotene concentration of green beans, and that the younger smaller beans were always higher in carotene concentration than larger more mature beans.

When investigating the changes in composition of the carotene fraction of leafy vegetables due to canning, Kemmerer et al. (37) found that the percentage of beta-carotene was reduced, while the percentages of neo-beta-carotene U and neo-beta-carotene were increased by canning. Beadle and Zscheile (4) also reported that although the total carotene and the percentage beta-carotene did not alter when spinach was blanched for 30 seconds in boiling water, on cooking for 5 or 30 minutes, the total carotene

remained constant but the beta-carotene decreased from 86 to 81 per cent.

Part I. QUALITATIVE SEPARATION AND IDENTIFICATION OF PIGMENTS IN FRESH AND PROCESSED GREEN BEANS

The qualitative separation and identification of the pigments in fresh and processed beans was undertaken in order to determine which pigments were responsible for the changes in color brought about by processing treatments. No comprehensive study of the pigments in green beans or any closely related vegetable had been found during the literature search.

MATERIALS AND PROCEDURES

Collection of samples for both Parts I and II of this study was carried out during the bean harvest and the first set of quantitative analyses had to be done immediately. These analyses were rather time consuming and required full attention, so the samples for the qualitative study were preserved and stored until time was available.

It was believed that the same pigments would be present in all horticultural varieties of green beans; therefore, the choice of variety for this study was arbitrary. Because Part II of this work was to be carried out on two varieties, Associated 92 and Columbia, Associated 92 was selected for the qualitative study because it was the more highly pigmented of the two varieties.

Preparation of Samples

In August, 1954, Associated 92 beans from the variety trial plots at the Oregon State College Vegetable Crops Farm were prepared for this study. They were mechanically graded, and commercial sizes 3 and 4 were preserved as follows:

The beans of the combined sizes 3 and 4 were divided into triplicate lots in order to study the pigments in the fresh, blanched and canned beans. Whole unsnipped beans of the first lot were filled into No. 2 plain cans and the cans were evacuated, flushed with nitrogen, evacuated, filled with nitrogen, and seamed. They were then stored at -20° F until opened for analysis. The whole unsnipped beans in the second lot were blanched in steam for three minutes, filled into No. 2 plain cans, evacuated, flushed with nitrogen, evacuated, filled with nitrogen, and seamed. These cans were stored at -20° F until analyzed. The beans in the third lot were snipped, cut, blanched in hot water at 180° F for 1½ minutes, filled into No. 2 plain cans, and the cans filled with hot water, seamed, retorted at 240° F for 20 minutes, cooled, and stored at 34° F until analyzed.

Extraction of Pigments

Extraction of pigments from the beans was necessary in order to separate the pigments from the fiber, protein, sugar, and other constituents so that a relatively concentrated source of pigments would be available for spectrophotometric and chromatographic examination.

Extraction of the pigments was carried out as rapidly as possible and immediately after the cans were opened. When frozen samples were used, the frozen beans were ground while still frozen in an Enterprise meat grinder with a No. 10 screen. When canned samples were used, the liquor was drained off and the beans sliced in a Hobart food slicer.

Approximately 500 g of beans were extracted for each chromatographic examination. The ground or sliced beans were transferred in 150 g portions to a Waring Blendor and 0.5 g of calcium carbonate was added to neutralize any acid present. This was followed by the addition of 200 ml of acetone. Air was displaced by bubbling nitrogen through the mixture, the Blendor was stoppered, and the material blended for 5 minutes. The mixture was then filtered by suction through coarse paper. The combined residues were re-extracted with acetone in the Blendor and filtered. The residue was finally extracted

with a 1:1 mixture of acetone and ether until the residue was visually free of pigment.

The combined filtrates were transferred to a large separatory flask, 500 ml of petroleum ether were added, mixed, and a volume of saturated sodium chloride solution equal to half that of the pigment solution was added. This caused the separation of a petroleum ether layer containing almost all of the pigment. The lower aqueous layer was run off and extracted with three successive 75 ml lots of petroleum ether to remove any remaining pigments. All the petroleum ether fractions were combined.

Further treatments of the extract were determined by the particular group of pigments being studied.

In all cases, extraction of pigments was carried out immediately prior to chromatographic examination.

Absorption Spectra of Total Pigments

Absorption spectra of total pigments in fresh, blanched, and canned beans were determined in order to find the wavelengths at which major changes in light absorption took place when beans were subjected to processing treatments. These absorption spectra were determined in ether solution so that they could be compared with those of chlorophyll and pheophytin.

Aliquots of the acetone extracts from fresh, blanched,

and canned beans were taken and the pigments transferred to ether by the method outlined above where the pigments were transferred to petroleum ether. Acetone was removed from the ether solutions by washing ten times with water in a separatory flask. The ether solutions were then chilled for one hour in a refrigerator to separate as much water as possible. The water was drained off and the ether solutions dried by standing over anhydrous sodium sulfate for one hour. Suitable dilutions were made and the optical densities measured over the wavelength range 400 to 700 mu. Readings were taken every 5 mu, except in the vicinity of maxima or minima where readings were taken every 1 or 2 mu. The absorption spectra obtained are shown in Figure 1.

A Beckman Model DU spectrophotometer was used throughout these studies for optical density measurements. The wavelength calibration of the spectrophotometer was adjusted to check with the hydrogen A line of a hydrogen discharge lamp at 656.3 mu. In all cases, the minimum slit-width which could be used throughout the range of measurements was used to keep the band width as small as possible.

Chromatographic Columns

Column chromatography was used for qualitative

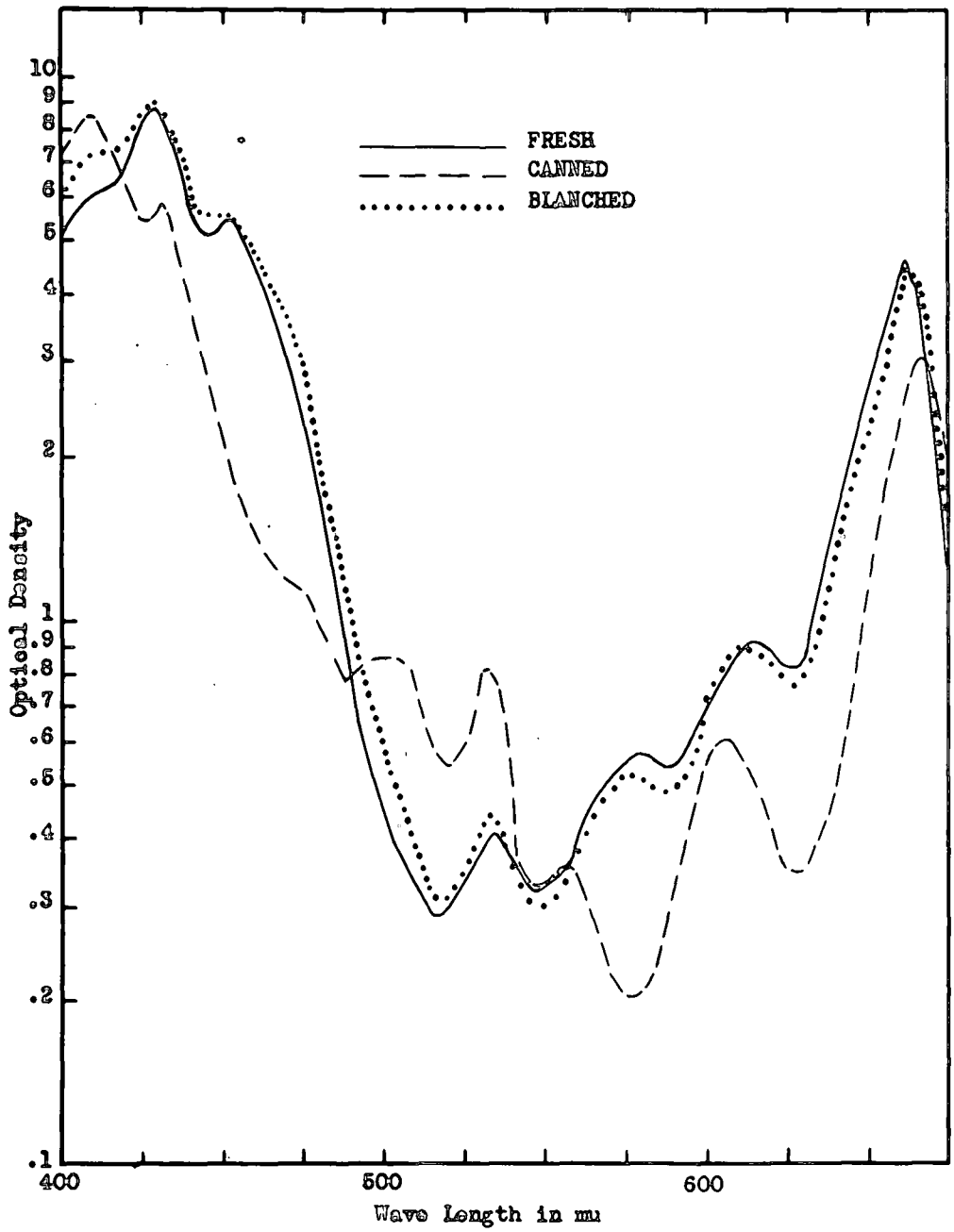

Figure 1. Absorption Spectra of Total Pigments in Beans.

separation of the pigments in beans, because relatively small amounts of pigments were available. Other methods do not bring about separation of such chemically similar substances as the xanthophylls without causing destruction of their original structure.

Preliminary work indicated that a column 25 cm. long and 2 cm. in diameter would be suitable for the amounts of pigments to be used. Moreover, by using a column of these dimensions, many of the difficulties involved in packing the columns uniformly could be eliminated.

For separation of the chlorophyll pigments powdered sugar was used. This material was recommended by Strain (61, p.1222) and was found to produce good separation of pigments, was inexpensive, readily available, and required very little pretreatment. California and Hawaii powdered sugar, containing 3% starch (to prevent caking) was used. This was prepared for use in columns by sieving through a kitchen flour sieve and heating overnight at 95° C under vacuum.

A 1:1 mixture of a specially prepared magnesia (Micron Brand No. 2641, Westvac Chlorine Products Company, Newark, California) and diatomaceous earth (Hyflo Super-cel, Johns-Manville, New York) was used as the column adsorbent for the separation of the carotenes. With this adsorbent separation of alpha-and beta-carotenes was

possible, but the separation of isomers present in smaller amounts required the use of calcium hydroxide or other special adsorbents. Separation of these isomers was not attempted because similar studies in vegetables have already been reported (4, 37, 73).

Both powdered sugar and magnesia-supercel adsorbents have been used for separation of xanthophylls and both were used in this study although the sugar columns appeared to produce better separation of pigments.

The adsorption columns were prepared by placing a cotton plug in the constriction of the tube and adding coarse purified sand to form a layer one centimeter deep at the bottom of the tube. The appropriate adsorbent was added in small amounts, with frequent jarring of the tube, until a solid column of the desired height was obtained. The outlet of the tube was then inserted into the opening of a vacuum desiccator connected to a water aspirator and a 25-inch vacuum drawn. Finally, the adsorbent was pressed into a firm column by applying pressure on the upper surface. After wetting the adsorbent with the desired solvent, the column was ready for use (provided the solvent surface did not fall to the surface of the adsorbent).

Chlorophyll Pigments

The chlorophyll pigments were separated by adsorption from petroleum ether on a sugar column followed by washing with petroleum ether containing n-propanol. It was found that removal of xanthophyll was desirable in order to obtain clear separation of the chlorophyll pigments. The preliminary separations showed that the presence of traces of water in the pigment extract caused serious damage to the sugar column producing very unsatisfactory adsorption and separation of the pigments.

Preparation of Standard Absorption Spectra. During the many preliminary separations, the major pigment bands which were separated were eluted and the solutions of the pigments had been stored under nitrogen in a freezing cabinet. In order to prepare reference spectral curves, the solutions which were believed to contain chlorophyll A were combined and evaporated to dryness under vacuum. The pigment was dissolved in petroleum ether and adsorbed on a sugar column. On washing with petroleum ether containing 0.5% n-propanol, one major highly concentrated band and several faint bands separated. By the time the main band had washed through two-thirds of the column, all the bands were completely separated. The column was then extruded and the major band cut out. The pigment

was eluted with ether plus 20% methanol, and this solution was filtered and evaporated to dryness under vacuum. The pigment was then redissolved in petroleum ether, adsorbed on a sugar column and washed thoroughly. Only one band was then present. This band was cut out, eluted, and the solution filtered and evaporated to dryness under vacuum. The pigment was then dissolved in ether, suitably diluted, and the absorption spectrum determined. The curve obtained is shown in Figure 2.

The pigment in ether was then treated with one drop of concentrated hydrochloric acid and allowed to stand two days in darkness at room temperature. During this time, the magnesium was completely removed from the chlorophyll molecule, thus producing pheophytin. After suitable dilution, the absorption spectrum was determined, and is shown in Figure 2.

The same procedure was used to prepare pure chlorophyll B and pheophytin B. The absorption spectra obtained for these substances are shown in Figure 3.

Willstatter's phase test (68, p.131) was applied to the solutions of chlorophyll A and B. In both cases positive results were obtained.

Unprocessed Beans: Xanthophylls were removed from the petroleum ether solution of the pigments by shaking

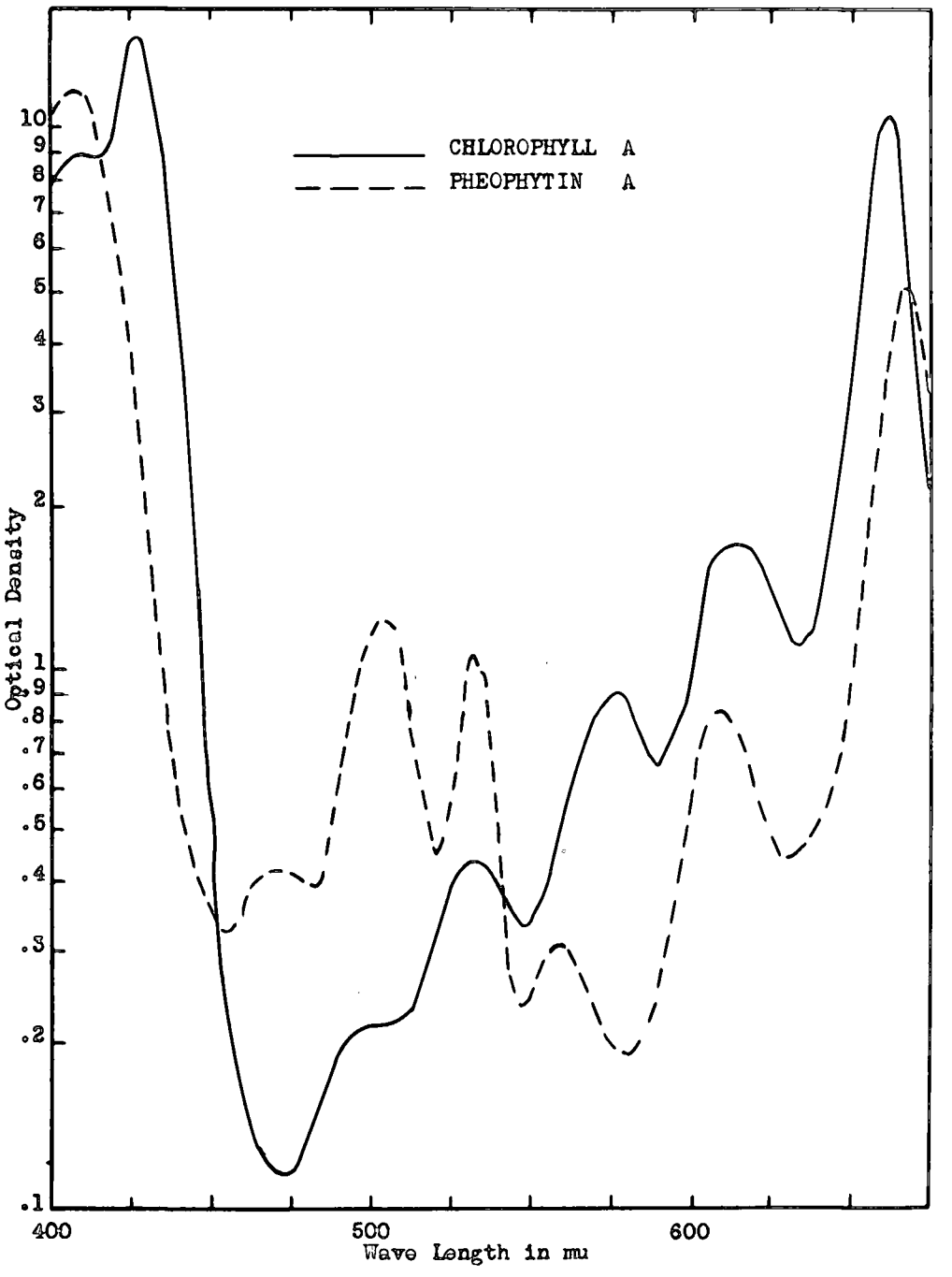

Figure 2. Absorption Spectra of Chlorophyll A and Pheophytin A

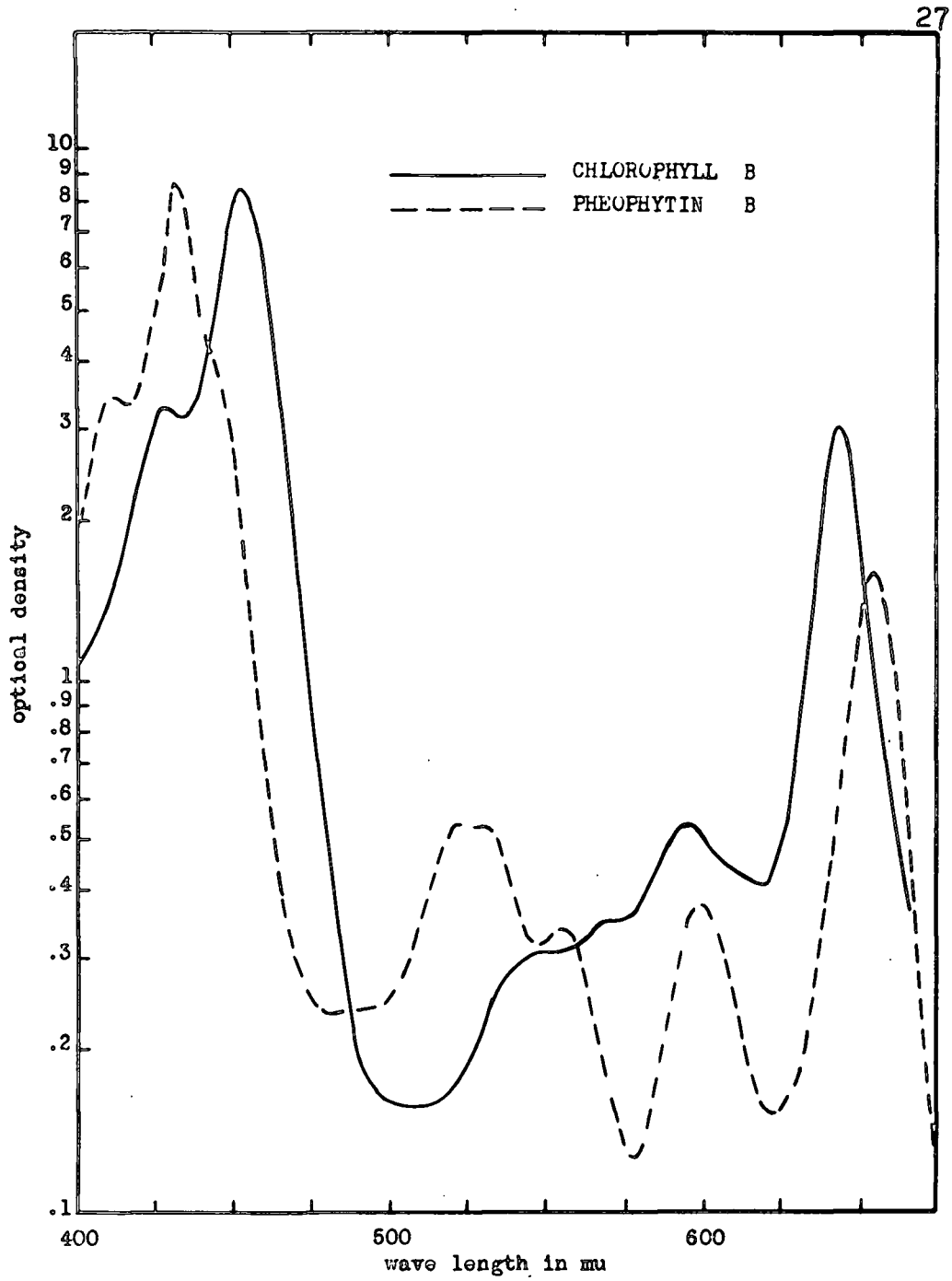

Figure 3. Absorption Spectra of Chlorophyll B and Pheophytin B

with three 40 ml portions of 90% methanol. The combined methyl alcohol extracts were extracted with two 30 ml portions of petroleum ether which were combined with the original petroleum ether solution. This was then washed thoroughly with water to remove acetone and methanol. It was then dried over anhydrous sodium sulfate and finally concentrated to about 50 ml by evaporation under reduced pressure.

The concentrated pigment solution was poured onto a sugar column and washed with petroleum ether. Complete adsorption of the green pigments took place within the top 5 cm. of the column while the carotenes formed a diffuse orange band approximately 6 cm. lower. On continued washing with solvent, no movement of green pigments took place, but the orange zone became more concentrated and slowly moved down the column. This zone then separated into two zones. The lower zone was less concentrated and yellow-orange in color, and the upper zone was of higher concentration and orange in color. As these zones washed down the column, they became more widely separated and were finally washed through and collected separately. The absorption spectra of each of these two solutions were determined, the lower zone showing maxima at 444 and 474 mu, and the upper zone showing maxima at 450 and 478 mu.

After the carotenes had been washed through the

column, development of the chromatogram was continued with petroleum ether containing 0.25% n-propanol. Gradually, several bands washed from the upper layer and after 30 minutes' washing, the column appeared as shown in Figure 4. The column was sucked dry, extruded, and the pigment bands cut out. The pigments in each band were separately eluted with ether containing 20% methanol, and the solutions were then filtered and evaporated to dryness under reduced pressure. The pigments were then dissolved in ether, suitably diluted, and the absorption spectra determined. Band 1 showed maxima at 453 and 644 mu; band 2 showed maxima at 453 and 644 mu; band 3 showed maxima at 428 and 662 mu; and band 4 showed maxima at 429 and 663 mu.

Positive phase tests were obtained with all four pigments.

The small, less dense bands 2 and 4 were apparently the isomers chlorophyll B' and A' noted by Strain and Manning (63, p.275). These were investigated further as reported later.

Blanched Beans: The petroleum ether solution of the pigments from blanched beans was treated as above and the pigments adsorbed on a sugar column. After development the column appeared as shown in Figure 5. Bands 1 and 4

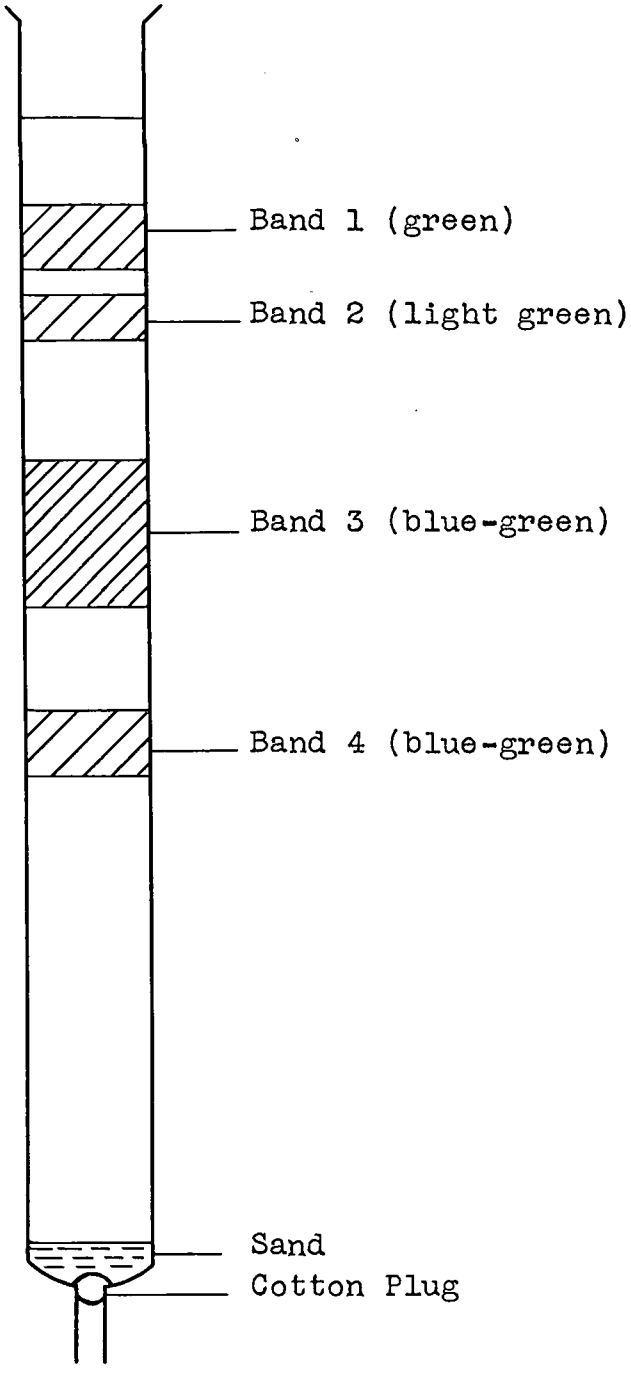

Figure 4. Pigments from Unprocessed Beans on Sucrose

were definite but the bands above and below 4 were rather diffuse and it was believed that they were mixtures of pigments. Therefore the column was extruded and the bands cut out, eluted, filtered, and evaporated to dryness. The pigments from the bands of doubtful purity were redissolved in petroleum ether and readsorbed on separate sugar columns. After development they appeared as shown in Figure 5. These bands were cut out, eluted, filtered, and evaporated to dryness. The pigments from each band were dissolved in ether, suitably diluted, and the absorption spectra determined. The absorption spectra showed maxima at the following wavelengths:

Band 1 (top)	453	644
Band 2	453	644
Band 3	429	662
Band 4	428	662
Band 5	428	662
Band 6	408	668
Band 7	408	670

Canned Beans: The petroleum ether solution of the pigments from canned beans was treated as above and after development the column showed the presence of only two bands. These were well separated and quite distinct. The column was extruded, the bands cut out, eluted, filtered,

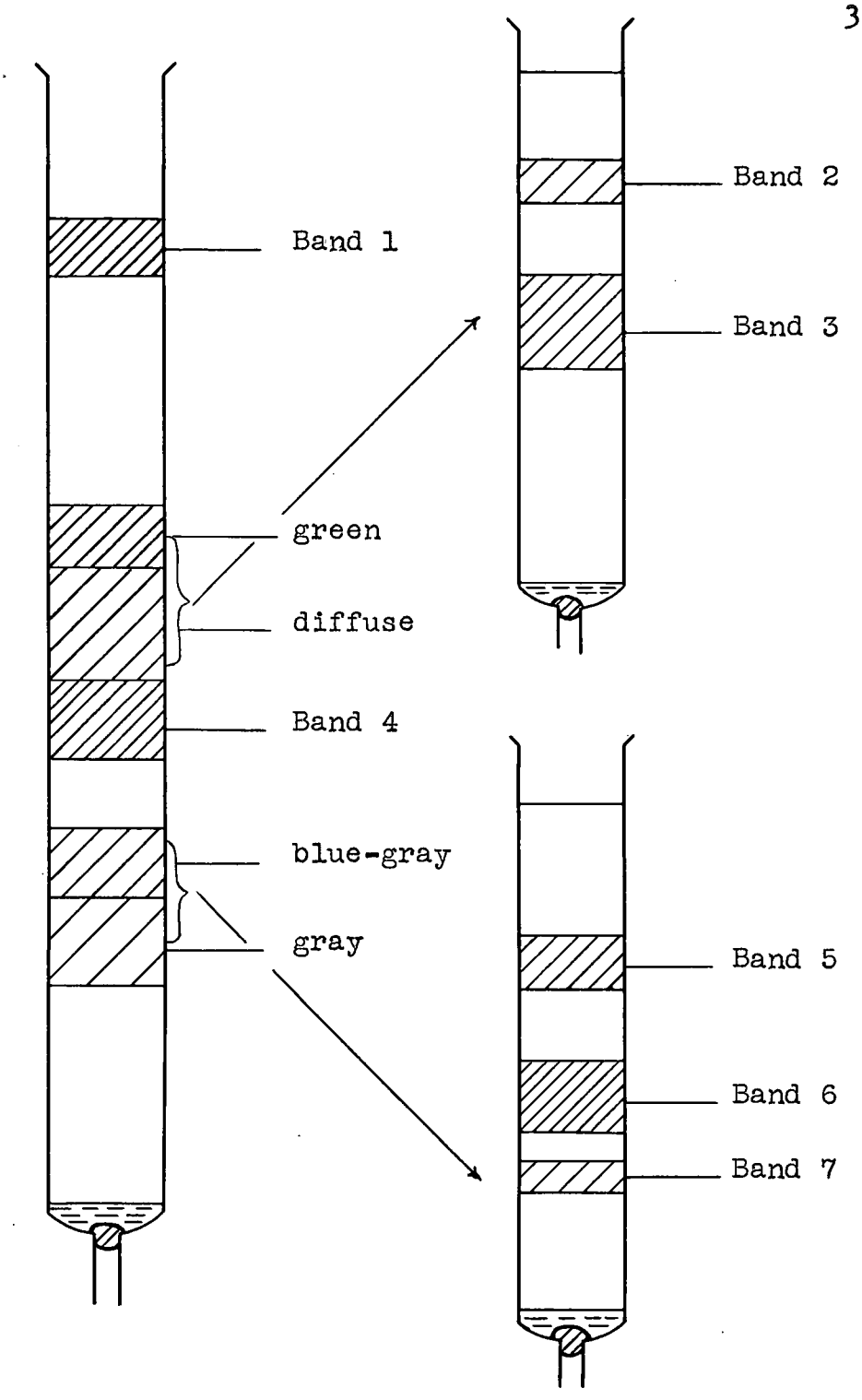

Figure 5. Pigments from Blanched Beans on Sucrose

and evaporated to dryness. The pigment from each band was dissolved in petroleum ether, readsorbed on separate sugar columns, and developed. In each case only a single band was obtained. These bands were cut out, eluted, filtered, evaporated to dryness. The pigments were then dissolved in ether, suitably diluted, and the absorption curves determined.

The upper band showed maxima at 433 and 655 mu.

The lower band showed maxima at 408 and 668 mu.

Preparation of chlorophylls A' and B': During the course of the work with fresh and blanched beans, pigment bands were obtained on the columns which showed adsorption maxima identical with those of other bands. At first it was believed that these could be accounted for by assuming the bands exhibiting the same absorption maxima were not separate bands but were merely trailing edges or leading fronts of the one band. Further chromatograms showed, however, that they were quite distinct and separate bands.

Strain and Manning (63, p.275) reported that isomers A' and B' were separated from chlorophylls A and B, and these isomers were less adsorbed on sugar than the parent chlorophylls. Furthermore, they stated that these isomers exhibited the same absorption spectra as the parents, but produced different pheophytins.

Chlorophyll A, which had been purified by two successive adsorptions on sugar columns, was dissolved in n-propanol. The air in the flask was replaced with nitrogen and the solution was heated at 95° C for 2 hours on a water bath. After the addition of 75 ml of ether, the mixture was diluted with 300 ml of water. This caused the separation of the ether layer which contained the pigment. This layer was thoroughly washed with water to remove the propanol. The water was removed by chilling and standing over anhydrous sodium sulfate, and finally the ether was evaporated off under vacuum. The pigment was then dissolved in petroleum ether and poured onto a sugar column. After development with petroleum ether containing 0.25% n-propanol, two distinct and separate bands were present. The column was then extruded, the bands cut out, and the pigments eluted, filtered, and evaporated to dryness. The pigments were dissolved in ether, suitably diluted, and the absorption spectra determined. Both of these pigments showed absorption maxima at 428 and 662 mu.

The same procedure was applied to chlorophyll B, and again two bands were obtained. These bands showed both absorption maxima at 428 and 644 mu.

Each of the pigments, in ether solution, were converted to pheophytins by treatment with hydrochloric acid,

and the absorption spectra were determined.

The pigments from chlorophyll A both showed absorption maxima at 408 and 667, while those from chlorophyll B showed absorption maxima at 432 and 655 mu.

Carotenoid Pigments

The separations of carotenes and xanthophylls were carried out on the same bean extracts. Chlorophylls were the major pigments present in the extracts and separated on the columns dispersed among the xanthophylls. Therefore it was found desirable to remove the chlorophylls before adsorption by saponification with alcoholic potassium hydroxide. The chlorophyll saponification products were soluble in water and were easily removed from the carotenoids by washing with water.

The pigment extract in petroleum ether was shaken for 20 minutes with 35 g. of potassium hydroxide in 80 ml of methanol. After standing in the dark for two hours to ensure complete saponification, 1½ liters of water were added and the aqueous phase containing the saponified chlorophyll was drawn off. This phase was extracted twice with 50 ml portions of petroleum ether which were combined with the phase containing the carotenoids. The carotenoid solution was washed exhaustively with water to remove all remaining chlorophyll products, alkali,

acetone and methanol. Finally, the carotenoid solution was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure.

The pigments were dissolved in the minimum amount of petroleum ether and adsorbed on a magnesia-supercel column. When the column was washed with petroleum ether which contained 10% acetone, the carotenes separated from the xanthophylls and slowly passed down the column, gradually separating into two distinct bands. When the carotene bands were distinctly separated from the xanthophylls and from each other, washing was discontinued, the column was extruded, the bands cut out, and the pigments eluted with ether containing 20% methanol. These solutions were filtered and evaporated to dryness under reduced pressure.

The pigments from the two carotene bands were dissolved in hexane, suitably diluted, and the absorption spectra measured.

In all cases, whether unprocessed, blanched, or canned beans were used, the less adsorbed yellow-orange band had absorption spectra with maxima at 444 and 474 mu, and the more adsorbed orange band had absorption spectra with maxima at 450 and 478 mu. Typical absorption spectra for these two bands are shown in Figure 6.

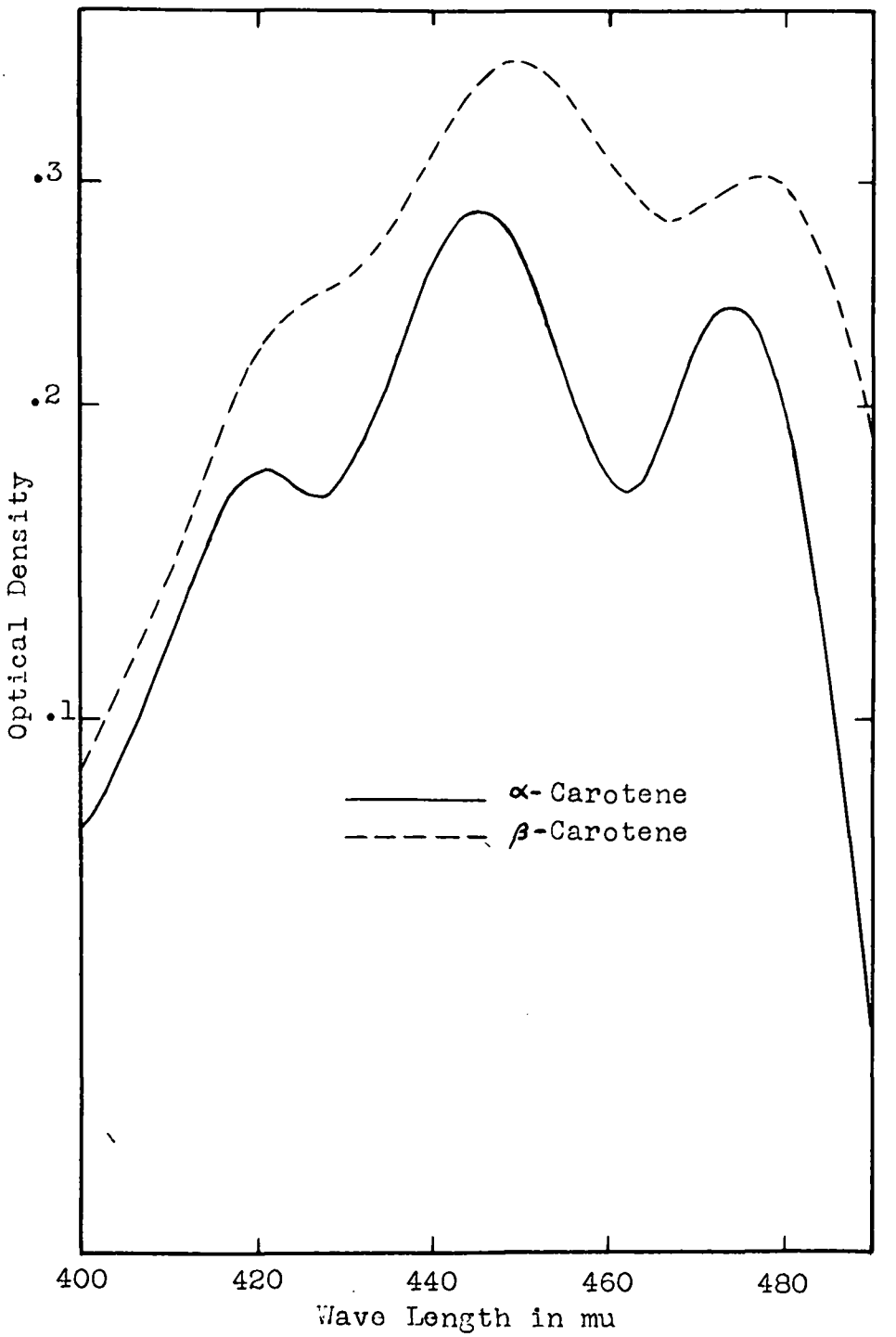

Figure 6. Absorption Spectra of ∝-Carotene and β-Carotene

The pigment from the upper yellow-orange band, which was a mixture of xanthophylls, was dissolved in petroleum ether containing 0.5% n-propanol and adsorbed on a sugar column. After extended washing with the solvent, the xanthophylls washed down the column and separated into bands. The column was extruded, the bands cut out, and the pigments eluted. The solutions were filtered and then evaporated to dryness. The pigments were dissolved in absolute ethanol, suitably diluted, and the absorption spectra determined.

Separation of bands of individual xanthophylls did not take place as readily nor as distinctly as in the case of the chlorophylls or carotenes. Prolonged washing was required, producing diffuse fronts and trailing edges which often merged with neighboring bands. Moreover, the large volume of solvent passing through the column accentuated any irregularities in the density of the column and caused very irregular bands. In many cases, it was extremely difficult to remove a band from the column without including pigments from the adjacent bands. This indistinct separation necessitated using only a thin section from the center of each band so that only a fraction of the pigments present could be used for identification. In many cases, eight to ten sections were cut from a column in order to be sure that a pigment

band was not missed. However, when the absorption spectra of the pigments from each section were plotted, several sections showed absorption maxima at the same wavelengths indicating that only a few distinct xanthophylls were present.

The above procedure for separating the carotenoid pigments was carried out on petroleum ether solutions of the pigments from unprocessed, blanched, and canned beans. The difficulty in producing clear, distinct pigment bands on the columns increased when heat processed beans were examined.

In all samples of beans examined, the two carotene bands with absorption maxima at 444 and 474 mu, and 450 and 478 mu, were consistently separated without difficulty.

Only those bands which were consistently found in repeated chromatographic separations of the xanthophylls are reported below.

The pigment bands which were obtained from unprocessed beans produced absorption spectra with maxima at the following wavelengths:

Band 1 (top)	414	437	466
Band 2	398	422	451
Band 3		443	471 or 472
Band 4 (bottom)	416	441	470

Bands 1 and 3 appeared to contain the highest concentrations of pigments.

When the extracts from blanched beans were examined, the pigments separated into five bands which gave distinct absorption spectra. Two of these bands, 3 and 5, produced absorption spectra with maxima at the same wavelengths as Bands 2 and 3 obtained from the unprocessed samples. The absorption spectra of these five bands showed maxima at the following wavelengths:

Band 1 (top)	399	422	448
Band 2	397	419	445 or 446
Band 3	398	421	451
Band 4		444	473
Band 5 (bottom)		443	471

Chromatographic separation of the pigments from canned beans resulted in diffuse bands making separation of the individual bands difficult. A greater number of bands were obtained from these samples, and although approximately ten were usually cut out, eluted, and examined the maxima of the absorption spectra of several were identical so it was concluded that those sections were merely parts of the same diffuse band. The absorption spectra of the pigments separated from canned beans showed maxima at the following wavelengths:

Band 1 (top)	398 or 399	420	447
Band 2	400	421	449
Band 3	399	421	447
Band 4	423	445	474
Band 5	423	445	470

Two typical absorption spectra of the xanthophylls separated from beans are shown in Figure 7. Only two are shown because the absorption spectra conformed to two types, the spectra in each type being very similar in shape and distinguishable only by the slight differences in maximum wavelengths. The vertical placement of the curves in Figure 7 is not significant because the separations were not quantitative and because the curves are plotted with the optical density on a logarithmic scale so that vertical adjustments of the curve can be made without altering the shape of the curves. The order of placement shown was chosen purely for ease of inspection.

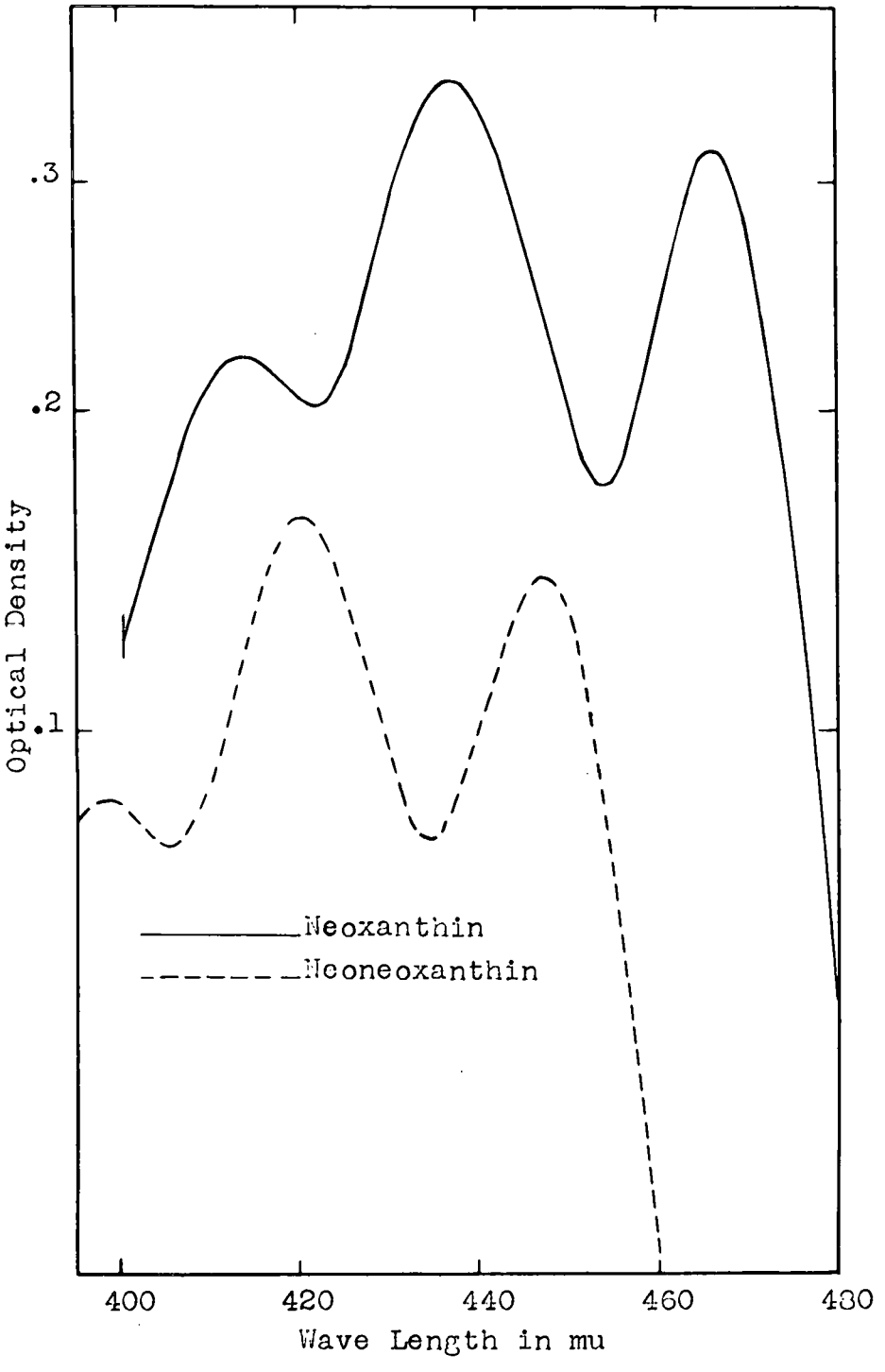

Figure 7. Typical Absorption Spectra of Xanthophyll

RESULTS AND DISCUSSION

Many of the results have been partially reported in the previous section because, in many cases, the sequence of steps in the procedure depended on knowledge of the result of a previous step.

Absorption Spectra of Total Pigments

The absorption spectra of the ether solutions of the total pigments extracted from unprocessed, blanched, and canned beans were shown in Figure 1. Relatively large changes in optical density or absorbence took place when the beans were canned, while only slight changes were caused by the blanching treatment.

Interpretation of the effects of the major changes of absorbence explain the color change when beans are canned. The severe heat treatment, applied in canning, caused a shift of the blue maximum to shorter wavelengths. As a consequence of this increased absorption of blue, the beans would take on a yellowish cast (10, p.263). The increased absorbence between 500 and 540 mu by the pigments in canned beans would result in a substantial reduction of greenness. In addition, the decreased absorption between 550 and 700 mu would tend to introduce a yellow-orange cast. The combined changes in absorbence would

change the color of beans to a dull olive-green color with a yellowish cast.

The great similarity between the absorption spectra of the pigment extract from canned beans and that of pheophytin A (shown in Figure 2) was noted, the major difference being greater absorbance in the region 430 to 490 mu by the bean extract. This was due to the presence of carotenoids which have maximum absorbance in this region.

Chlorophyll Pigments

The absorption spectra obtained for chlorophylls A and B, and pheophytins A and B (shown in Figures 2 and 3) were compared with those reported by Zscheile and Comar (74, pp.471, 472). All of the pigments prepared exhibited maxima at the same wavelengths as the corresponding pigments prepared by these workers.

These curves were used as references with which to compare the pigments separated from the experimental samples.

Unprocessed Beans: The four pigments which were separated from the unprocessed beans were identified by means of the wavelengths of their absorption maxima. The wavelengths of the absorption maxima of Bands 1 and 2

coincided with those of the chlorophyll B reference curve while the wavelengths of the maxima of Bands 3 and 4 coincided with those of the chlorophyll A reference curve.

Band 4 was the same color (blue green) as Band 3, though definitely less concentrated, and was distinctly separated from it by a colorless band of absorbent. When chlorophyll A' was prepared and separated from chlorophyll A on a sugar column, the same adsorption sequence was found as with Bands 3 and 4 and both pigments produced absorption spectra with maxima at the same wavelengths. On this basis, it was concluded that Band 4 was chlorophyll A'. The same relationship was found between Bands 1 and 2 and chlorophylls B and B' hence it was concluded that Band 2 was chlorophyll B'.

Blanched Beans: By comparison of the wavelengths of the absorption maxima of the seven bands separated from blanched beans with the wavelengths of the maxima of the standard curves, and by considering the relative positions of the bands on the column, the pigments in the bands were identified as follows:

Band 1 was chlorophyll B.

Band 2 was chlorophyll B'.

Band 3 was chlorophyll A.

Band 4 was chlorophyll A.

Band 5 was chlorophyll A'.

Band 6 was pheophytin A.

Band 7 was pheophytin A'.

Canned Beans: The separation of the pigments from canned beans was simplified by the presence of only two distinct pigments. These were identified as pheophytin B and pheophytin A, the upper band being pheophytin B.

The presence of only two degradation products of chlorophyll was unexpected in view of the complexity of the chlorophyll molecule and its many relatively reactive centers and side groups. In spite of the severe heating processes, the presence of cell acids, and small amounts of oxygen, the chlorophyll molecule essentially remained intact except for removal of the chelated magnesium atom. The absence of pheophytins A' and B' was unexpected because it is generally recognized that heating accelerates the rate of isomerism. Apparently, during the canning process, the magnesium was removed from the molecule very quickly before isomerization had taken place to any extent.

The absence of pheophytin B in the blanched samples was probably due to both the lower concentrations of chlorophyll B in the extracts and also to the slower rate of conversion of chlorophyll B to pheophytin B (32,

p.1132). It was noted throughout the separations of the chlorophyll pigments that the concentrations of chlorophyll A and pheophytin A were invariably greater than those of the B component. This difference is due to two conditions. Firstly, the concentration of chlorophyll A is approximately three times that of chlorophyll B in higher plants (24, p.849). Also Cemar (14, p.879) found that the chlorophyll in string beans contained 74% of the A component. The average value found during this study was 69%. Secondly, heavy losses of chlorophyll B occurred when the pigment extract was shaken with 90% methanol to remove xanthophylls (40, p.102).

Carotenoid Pigments

It was pointed out earlier that separation of the carotenes from both the chlorophyll pigments and the xanthophyll pigments were invariably readily obtained. The two easily distinguished carotene bands were spectrophotometrically examined but further chromatographic separations were not carried out to separate the minor isomers because such work had already been reported.

The less sorbed and less concentrated carotene band was found to exhibit absorption maxima at the same wavelengths as those reported by Zscheile et al. (72, p.342)

for alpha-carotene, while the more concentrated and more sorbed carotene band was similarly found to be beta-carotene.

The separation of the xanthophylls was carried out repeatedly as distinct separation of the bands was difficult and because a pigment band containing lutein was not obtained. The failure to separate lutein from beans cannot be accounted for when Karrer (34, p.198) states that "it is found together with carotene and chlorophyll in all green parts of plants", and Strain (56, p.77) reports that lutein is the principal component of leaf xanthophyll.

The xanthophylls separated from unprocessed beans were identified by comparison of the maxima wavelengths of the absorption spectra with those reported by other workers.

Band 1 was neoxanthin (56, p.68).

Band 2 was flavoxanthin (56, p.76).

Band 3 was violaxanthin (34, p.195).

Band 4 was taraxanthin (62, p.463).

Similarly, the xanthophylls separated from blanched beans were identified:

Band 1 was neoneoxanthin (64, p.181).

Band 2 was a flavoxanthin (62, passim).

Band 3 was flavoxanthin (56, p.71).

Band 4 was not identified.

Band 5 was violaxanthin (34, p.195).

Two of the pigment bands, violaxanthin and flavoxanthin, which were present in unprocessed beans, were also present after blanching. However, the heat treatment resulted in the failure of the neoxanthin and taraxanthin to appear on the adsorption column. Strain et al. (64, p.185) prepared neoneoxanthin by heating solutions of neoxanthin and also showed that taraxanthin may be converted to a flavoxanthin-like pigment by the action of acid (62, p.464). Hence, it is probable that the two new pigment bands, neoneoxanthin and the flavoxanthin-like pigment (Band 3) were derived from the neoxanthin and taraxanthin originally present in the unprocessed beans. Band 4 was not identified, but from its position on the adsorption column and its absorption maxima, it was suspected to be lutein epoxide which Karrer (34, p.206) reported present in relatively large amounts in leaves of all kinds. The very small amount of pigment available was not sufficient for confirmatory work.

The attempted identification of the pigments from canned beans became increasingly difficult. The apparently greater number of pigments present, the minute amounts of the individual pigments being dealt with, and poorer

separation on the adsorption columns, all contributed to the difficulty. Furthermore, precise data on the effect of heat, acids, and oxidation, on the xanthophylls were not available. Some data were found concerning the effect of these factors on certain xanthophylls (56, pp.85-88), (64, pp.169-191), (62, pp.459-468), but even when the effects of these factors were investigated under carefully controlled conditions, the absorption spectra of the products were found to vary a great deal. For these reasons, only tentative identification of the pigments from canned beans are given, as follows:

Band 1 was neoneoxanthin.

Band 2) were flavoxanthin-like pigments formed as
Band 3) a result of heat treatment.

Band 4) were probably lutein in which the absorption
Band 5) spectra were changed slightly by the heat treatment.

The two flavoxanthin-like pigments were not able to be positively identified because many very similar substances are obtained from the xanthophylls, all of which have almost identical absorption spectra. For example, Strain (62, p.467) isolated eight such compounds by the action of acids on violaxanthin, violeoxanthin, taraxanthin, and tareoxanthin.

The two pigments tentatively identified as lutein, had absorption spectra very similar to that of lutein

which had been subjected to heat treatment (56, p.84). Strain (57, p.3450) obtained two pigments exhibiting absoprtion spectra similar to that of lutein by the action of acid on lutein and an additional two pigments, again similar to lutein, by the action of heat on lutein. Quackenbush et al. (48, p.2940) isolated five pigments from alfalfa silage, and was able to show that they were all derived from lutein by preparing them from pure lutein by treatment with acid.

From these results, it is seen that none of the xanthophyll pigments present in fresh beans remained unaltered after the severe heat treatments given in canning. The heat treatment caused a general change in absorption spectra to those characteristic of the flavoxanthin-like compounds. Such changes have been shown by Strain to be produced by the action of acids on xanthophylls. Zechmeister (69, passim) has also shown that a large number of stereoisomers may be formed from the carotenoids by the action of heat, acids, and light. The overwhelming majority of the carotenoid pigments present in vegetable tissue possess an all-trans configuration and the stereoisomers result from the different degrees of transformation to the final all-cis form. In lutein, for example, 32 theoretically possible sterioisomers may be formed.

The more cis forms possess absorption spectra with progressively decreasing absorption coefficients and maxima at progressively shorter wavelengths. Thus, even without decomposition of the carotenoids during the heat processing of beans, a relatively large number of slightly different pigments may be produced.

The above reported findings may explain why separation and identification of the pigments in the heat treated beans presented such a complex problem.

Part II. QUANTITATIVE DETERMINATION OF THE PIGMENTS IN GREEN BEANS AND THEIR CHANGES DURING PROCESSING AND STORAGE

The qualitative work in Part I of this study showed that the principal changes in the pigments of beans brought about by processing and storage were of the type caused by the action of heat and acids. The purpose of Part II was to determine quantitatively the effect of variety, size, treatment, storage period, and storage temperature on the pigments. It was realized that by investigating the effect of so many variables, accuracy in the effect caused by anyone would be sacrificed. However, because little work of this nature has been reported, it was not known which factors were likely to be of most significance.

MATERIALS AND METHODS

Only the chlorophyll, pheophytin, carotene, and xanthophyll were quantitatively determined because separation of the individual pigments within the groups required extensive chromatographic separations. These separations carried out in Part I showed that the time required for them was far in excess of that available when replicate determinations had to be carried out on a given day. Furthermore, quantitative yields were not

obtained by column chromatography, especially when pure pigments are required as end products.

Selection of Varieties

During work on the quality evaluation of bean varieties it had often been noted that varieties differ in color and also in the color changes produced by heat processing. For this work, it was decided to use two varieties which differ widely in color and in their reaction to processing. The varieties selected were Associated 92, a very dark green bean variety which remains dark on canning, and Columbia, a very light green variety which becomes lighter and yellowish on canning. Both these varieties were available in replicated variety trial plots at the Oregon State College Vegetable Crops Farm.

Preparation of Samples

The two varieties selected for study differed in date of maturity, Columbia being about 10 days earlier than Associated 92. The plots were small, so that the quantity of beans from each plot was limited and the beans from one harvest, after size grading and retention of the selected size, were sufficient for only one complete group of samples. Hence, the samples used in this study

comparing Associated 92, combined sizes 3 and 4, with Associated 92, combined sizes 4 and 5, were obtained from two different dates of harvest of the same plots. This was believed to introduce no harvest variable because Cain and Sidwell (12) had previously reported that the color of a given variety was not altered by date of harvest. Three field replicates of each variety were used and were kept as separate replicates throughout the study.

The beans were harvested from the three replicated field plots and were mechanically graded for size. The desired sizes were snipped by hand and were reduced to 1-inch lengths by passage through a bean cutter.

A 100 g sample was randomly taken for fresh determinations.

An 800 gm sample was randomly taken and blanched in flowing steam for 3 minutes and 100 g were withdrawn for determinations. The remaining 700 g were placed in ½-lb. flat plain cans, evacuated, seamed, and frozen at -20° F. They were stored at this temperature throughout the study.

The remaining cut beans were blanched in water at 180° F for 1½ minutes and then cooled immediately in cold water. Approximately 100 g were withdrawn for

determinations. The remainder were placed in ½-lb. flat plain cans, filled with hot water, and seamed. Half of these cans were retorted at 240° F for 20 minutes, and half at 260° F for 4 minutes. These processes were calculated to have the same Fo value. After retorting, the cans were immediately cooled in cold water. Half of the cans of each of the two heat treatments were stored at 34° F, and half were stored at 100° F.

The above treatments were applied to each of the field replicates of Columbia, combined sizes 4 and 5, on August 10; Associated 92, combined sizes 3 and 4, on August 12; and Associated 92, combined sizes 4 and 5, on August 20.

Hereafter size 3-4 and size 4-5 refers to beans of sizes 3 and 4 combined and sizes 4 and 5 combined.

Determinations

On the day of harvest, as soon as possible after preparation of samples, determinations were made on the fresh, water blanched, and steam blanched samples. The following day, determinations were made on the frozen and canned samples. The same determinations were made on the frozen and canned samples after one month and again after five months storage at the temperatures indicated.

The determinations made were total solids, color, chlorophyll, pheophytin, carotene, and xanthophyll.

When the frozen samples were examined, they were thawed in water in the unopened can, then the color measurements were determined on the entire sample. In the case of the canned samples, the liquor was separated from the beans by draining on a No. 12 mesh screen for 2 minutes. The color measurements were determined on the drained beans. After the color was measured, the beans were sliced in a Hobart food slicer, and aliquots were used for total solids and pigment determinations.

Color: The color of the samples was measured in terms of the "Rd", "a", and "b" scales of the Hunter Color Difference Meter. The spinning-cup sample holder as reported by Sidwell and Cain (50) was used.

Total Solids: A 25 g aliquot of sliced frozen beans was weighed accurately in a tared aluminum drying pan. The pan was then placed in a vacuum oven at 95-100° C for 5 hours, after which it was cooled in a desiccator and weighed. The ratio of the weight of residue to weight of original multiplied by 100 was reported as per cent total solids.

Pigments: Before separation and determination of

pigments could be attempted, the pigments had to be separated from the other constituents of beans.

Extraction: A 25 g aliquot of sliced beans was weighed accurately and transferred to a Waring Blendor with 100 ml of acetone. Approximately 0.3 g of calcium carbonate was added to neutralize acidity, and 1 ml of potassium cyanide solution (containing 65 mg potassium cyanide) was added to inhibit both photochemical and enzymic destruction of carotene (46). The mass was blended for 5 minutes and then filtered through coarse paper by suction. The residue was washed with acetone until visually free of pigment. A final wash was given with ether. The filtrate was made to 250 ml.

In a separatory funnel, 100 ml of acetone extract and 50 ml of ether were mixed, followed by the addition of 100 ml of 2.5% sodium sulfate solution. This caused separation of the ether containing the pigment. The aqueous phase was drawn off and extracted twice with 20 ml portions of ether which were combined with the pigment solution and placed in the apparatus shown in Figure 8 and continuously scrubbed with water for 20 minutes to remove acetone. The water was then drawn off, the ether solution chilled in a freezing cabinet for 10 minutes to throw out water, and the ether solution was

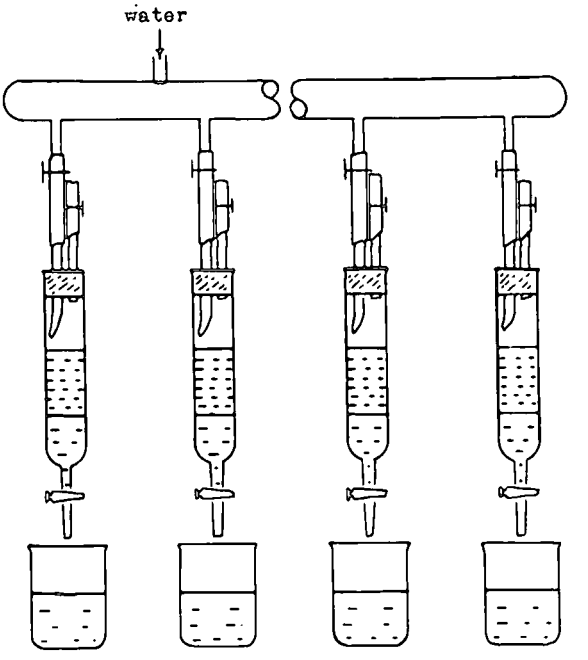

Figure 8. Continuous Washing Apparatus

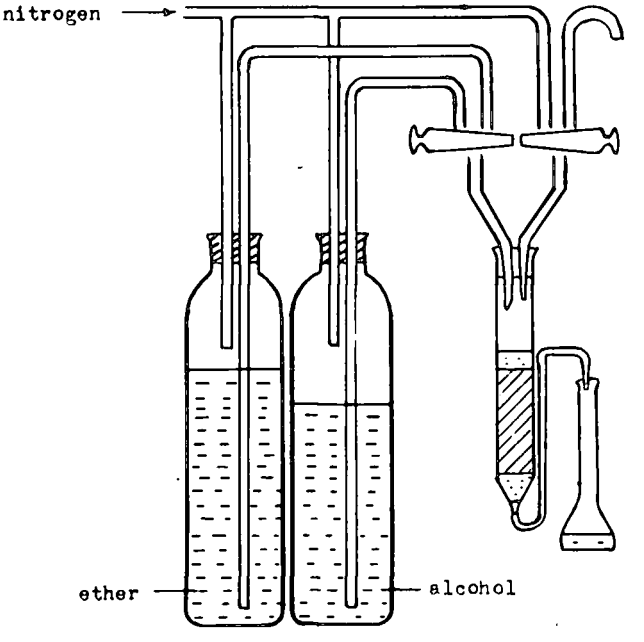

Figure 9. Pressure Chromatographic Apparatus

made to 50 ml in a volumetric flask. Finally, it was dried by standing over anhydrous sodium sulfate for one hour.

Chlorophyll and pheophytin: The chlorophyll and pheophytin concentrations in the ether solution of the pigments were determined by the method of Mackinney and Weast (42). This method was somewhat modified by measuring the optical densities of the ether solution rather than of the acetone solution in which interfering pigments may be present (16). Optical densities were measured at 557 and 533 mu. At 557 mu the specific absorption coefficients of chlorophyll and pheophytin are equal, and at 533 mu, both chlorophyll and pheophytin show a maximum in their specific absorption coefficient curves. These wavelengths were determined in the preliminary work.

Carotenoid determination: Carotene and xanthophyll concentrations were determined on aliquots of the ether solution of the pigments. Separation of these pigments from chlorophyll and from each other was achieved by adsorption on magnesia-supercel columns followed by elution. Poor separations were obtained when the ether solution of the pigments was directly added to the chromatographic column so the ether solution was evaporated to dryness,

the pigments taken up in petroleum ether and then adsorbed on the column.

A 25 ml aliquot of the ether solution was pipetted into a 50 ml beaker, and the ether evaporated off under vacuum. The pigment was dissolved in 5 ml of petroleum ether and this was added to a 6-inch by 1-inch diameter magnesia-supercel column. The beaker was thoroughly rinsed with petroleum ether and the rinsings added to the column. The chromatographic tube was then attached to the apparatus shown in Figure 9, and 3 lb. nitrogen pressure applied. Ether was admitted to the tube to keep the level of the liquid above the surface of the column and to wash the pigment into the column. All pigments except carotene were adsorbed on the top inch of the column while the carotene passed down through the column and was collected in a 25 ml volumetric flask.

After the carotene had been washed through the column, absolute ethanol was admitted to the tube together with ether to elute the xanthophylls which passed down the column as a bright yellow band. Usually, 20% alcohol caused rapid and complete elution of the xanthophylls, but if too high a concentration of alcohol was used, chlorophyll pigments were eluted with the xanthophylls. The column was washed with mixed solvent until all xanthophylls had been washed through, the solution

being collected in a 25 ml volumetric flask.

The carotene and xanthophyll solutions were made to volume with ether and the optical densities of the solutions were measured at 449 and 440 mu respectively. These wavelengths had been found in the preliminary work to be the wavelengths of maximum light absorption for these solutions. At 449 mu the specific absorption coefficient of beta carotene is 240 (26, p.439), from which the carotene concentration in terms of beta-carotene was calculated and reported. In the case of the xanthophylls, because the optical density at 440 mu was the sum of the optical densities for a mixture of xanthophylls of unknown composition, the xanthophyll concentration was expressed merely as the optical density per gram of beans multiplied by 100. Although this figure has no absolute value, it is adequate for comparison of xanthophyll concentrations in different samples.

RESULTS AND DISCUSSION

After completion of the analyses, the pigment concentrations of the samples were calculated from the measured optical densities, and were converted to the dry weight basis in order to eliminate variations due to the different moisture contents of the fresh, blanched, frozen, and canned samples. The results tabulated throughout this chapter are the mean values of the three field replicates. In order to conserve space in the tables, the following abbreviations have been used:

Car.	Carotene, ppm on a dry weight basis
Xan.	Xanthophyll, ppm on a dry weight basis
Pheo.	Pheophytin, ppm on a dry weight basis
Chlor.	Chlorophyll, ppm on a dry weight basis
W. Bl.	Water blanch treatment, 1½ minutes at 180° F
S. Bl.	Steam blanch treatment, 3 minutes at 212° F
Fzn.	Frozen
C240-34	Canned at 240° F for 20 minutes, stored at 34° F
C240-100	Canned at 240° F for 20 minutes, stored at 100° F
C260-34	Canned at 260° F for 4 minutes, stored at 34° F
C260-100	Canned at 260° F for 4 minutes, stored at 100° F

L.S.D.	Least significant difference
N.S.D.	Not significantly different
H.S.	Significant at the .01 level
S.	Significant at the .05 level
Assoc(4-5)	Associated 92, combined sizes 4 and 5
Assoc(3-4)	Associated 92, combined sizes 3 and 4
2da	Analyses conducted within two days
1mo	Analyses conducted after storage for one month
5mo	Analyses conducted after storage for five months

Color of the Experimental Samples

Although the objective of this study was the determination of pigments in different varieties of beans which had been subjected to various processing treatments and storage conditions, it was believed that a general review of the colors of the samples in which the pigment concentrations had been determined would prove informative. It was realized that it would be impossible to correlate the color of the samples with the pigment concentrations because the predominant pigment, chlorophyll, was completely destroyed during the canning processes and replaced by another pigment, pheophytin, which had a color somewhat similar to that of the original chlorophyll.

Two different systems of color notation were used to illustrate the color changes which took place in the beans during the study.

Color differences of samples measured by Hunter dE. The colors of all the samples had been measured in terms of the Hunter "Rd", "a", and "b" scales. Since the color of a sample is rather difficult to visualize when presented in the Hunter notation, it was believed that color differences may be shown more clearly if these readings be converted to a single measure of color difference. The Hunter "Rd" readings were converted to Hunter "L" and the Hunter Color Differences (dE) were calculated by applying the following formula (21, p.7):

$$dE = \sqrt{\overline{dL}^2 + \overline{da}^2 + \overline{db}^2}$$

where dE = difference in color between the fresh and processed beans.

$\overline{dL}$ = difference in "L" scale readings between the fresh and processed beans.

$\overline{da}$ = difference in the "a" scale reading between the fresh and processed beans.

$\overline{db}$ = difference in the "b" scale readings between the fresh and processed beans.

The dE values of the individual samples were

calculated and the mean values of the three field replicates are shown in Table 1.

The dE value is a measure of the difference in color between the fresh and treated sample, but it does not show whether the difference is positive or negative. In the treatments applied to the beans, blanching and freezing in general caused an increase in the visual color, whereas canning reduced the color. These differences in color were not shown adequately by the above dE values.

These values were not statistically analyzed so that apparent differences due to the experimental variables may or may not be significant. However, the following trends were apparent.

When the same size beans were compared, greater differences from the fresh samples were shown throughout all treatments by Columbia variety than by Associated 92. Within the Associated 92 variety all the processing treatments applied caused greater changes of color in the smaller size, 3-4, than in the larger size, 4-5.

Irrespective of the size of bean or the variety, when canned beans were stored at both 34° F and 100° F, and the frozen beans were stored at -20° F, very little change was evident in the dE values after one month and even after five months' storage.

Table 1

Effect of Variety, Size, Treatment, and Storage Conditions on Mean Hunter Color Differences (dE)

Treatment	Columbia			Associated 92 (4-5)			Associated 92 (3-4)		
	2da	1mo	5mo	2da	1mo	5mo	2da	1mo	5mo
W. Bl.	6.79	--	--	3.09	--	--	7.02	--	--
S. Bl.	7.58	--	--	2.12	--	--	6.79	--	--
Fzn.	8.84	8.70	8.94	2.83	2.37	1.90	5.80	5.98	5.59
C240 (34)	10.82	10.34	11.00	7.44	6.58	7.79	8.42	8.65	8.44
C240 (100)	(10.82)	11.20	10.42	(7.44)	7.04	8.16	(8.42)	8.45	7.80
C260 (34)	10.18	10.68	11.19	6.83	6.73	7.95	8.54	9.31	8.40
C260 (100)	(10.18)	12.17	11.11	(6.83)	7.48	8.70	(8.54)	8.94	8.67

The dE values showed that greater changes in color from that of the fresh beans took place when the beans were canned than when the beans were preserved by freezing. This illustrated the deleterious effect of heat on the pigments. Furthermore, retorting of the beans at 240° F for 20 minutes caused slightly greater changes in color than retorting at 260° F for 4 minutes. However, after storage for either one month or five months, the beans canned at 260° F showed a greater difference in color than those canned at 240° F.

No pronounced difference in the effects on the changes of color caused by storing the canned beans at either 34° F or 100° F could be deduced from the dE values.

Color of Samples in Munsell Notation. The Hunter Color Differences (dE) did not prove to be very satisfactory for showing the color changes which took place in the samples during this study. Although the Munsell system of color specification, like the Hunter system, uses three characteristics for color specification, these characteristics are readily visualized, so the Hunter color readings of the samples were converted to the Munsell Notation (21, p.7) (44, p.11). The mean Munsell Notations of the beans from the three field replicates are

shown in Tables 2, 3, and 4.

By examination of these data, the following conclusions may be deduced:

In the same sized beans, Columbia variety was less green in hue, and lighter in value, but with a higher degree of saturation or chroma, than the Associated 92 variety. These differences were shown in the fresh beans and in those immediately after processing.

The blanching treatments caused an increase in greenness in both varieties, but whereas the darkness (value) and saturation (chroma) of the color increased in the Columbia samples, these two color characteristics remained almost unaltered in the Associated 92 samples. These changes caused by blanching, which increased the greenness (hue) of the samples, appear to be slightly greater with the less severe water blanching treatment than with steam blanching.

The two canning processes caused relatively large changes in color in both varieties. The Columbia beans and the Associated 92 beans became almost equally yellow in hue, but in Associated 92 the color was lighter and less saturated than in Columbia. There seemed to be little difference between the effects on the color of the beans caused by retorting at 240° F for 20 minutes and retorting at 260° F for 4 minutes; after storage, possibly

Table 2

Effect of Treatments and Storage Conditions on the Mean Munsell Notations of Columbia Beans (Size 4-5)

	2 days			1 month			5 months		
	H(GY)	V	C	H(GY)	V	C	H(GY)	V	C
Fresh	7.92	4.60	3.84						
W. Bl.	9.60	3.83	4.30						
S. Bl.	9.46	3.66	4.04						
Frozen	8.94	3.47	3.96	8.92	3.43	4.50	9.42	2.89	3.22
C240 (34)	2.07	3.57	2.41	1.69	3.64	3.12	1.01	3.11	2.33
C240 (100)	(2.07	3.57	2.41)	1.00	3.58	2.99	0.03	3.14	2.62
C260 (34)	1.60	3.67	2.60	1.91	3.53	3.13	0.22	2.92	2.28
C260 (100)	(1.60	3.67	2.60	1.55	3.38	2.83	-0.22[1]	3.19	2.49

H = Hue V = Value C = Chroma

1 - This hue was measured as 9.78Y.

Table 3

Effect of Treatments and Storage Conditions on the Mean Munsell Notations of Associated 92 Beans (Size 4-5).

	2 days	1 month	5 months
	H(GY) V C	H(GY) V C	H(GY) V C
Fresh	8.47/2.96/3.45		
W. Bl.	10.64[1]/3.06/3.76		
S. Bl.	9.73/2.91/3.41		
Frozen	9.85/2.79/3.25	9.73/2.79/3.43	9.6 /2.89/3.22
C240 (34)	2.00/3.01/1.9	1.63/3.07/2.36	0.91/3.11/2.33
C240 (100)	(2.00/3.01/1.90	0.91/3.06/2.36	0.04/3.14/2.60
C260 (34)	4.05/2.82/1.96	1.30/2.98/2.32	0.22/2.92/2.28
C260 (100)	(4.05/2.82/1.96	0.10/2.96/2.34	-0.2[2]0/3.19/2.49

H = Hue V = Value C = Chroma

1 - This hue was measured as 0.64G.

2 - This hue was measured as 9.8Y.

Table 4

Effect of Treatments and Storage Conditions on the Mean Munsell Notations of Associated 92 Beans (Size 3-4)

	2 days			1 month			5 months		
	H(GY)	V	C	H(GY)	V	C	H(GY	V	C
Fresh	8.60	3.60	3.30						
W. Bl.	10.69[1]	2.84	3.28						
S. Bl.	10.46[2]	2.94	2.86						
Frozen	10.0	2.99	3.2	9.52	2.91	3.62	9.17	2.96	3.35
C240 (34)	1.57	3.17	2.11	1.60	2.99	2.50	0.65	3.16	2.60
C240 (100)	(1.51	3.17	2.11)	0.91	3.11	2.63	0.76	3.31	2.51
C260 (34)	1.56	3.16	2.12	1.09	2.92	2.48	0.52	3.22	2.47
C260 (100)	(1.56	3.16	2.12)	0.71	3.04	2.48	0.22	3.28	2.36

H = Hue V = Value C = Chroma

1 - This hue was measured as 0.69G.

2 - This hue was measured as 0.46G.

the beans retorted at 240° F retained a slightly greater greenness (hue), lightness (value), and saturation (chroma).

During storage of the canned samples, the yellowness of hue increased in both varieties. This effect was more pronounced when the beans were stored at 100° F than when they were stored at 34° F. Storage also tended to induce darkening of the color in the Columbia variety while the Associated 92 beans became lighter.

The Associated 92, size 3-4 beans were somewhat greener in hue, lighter in value, and less saturated in chroma than sizes 4-5 beans. However, on canning, size 3-4 became more yellow in hue than the larger size, but still retained greater lightness and saturation. In general, the changes in color in size 3-4 beans, due to different retorting temperature, storage periods, and storage time, followed the same pattern shown by size 4-5, but the effects of these conditions were generally less pronounced in the smaller size.

Pigment Concentrations

The concentrations of carotene, xanthophyll, pheophytin, and chlorophyll found in the experimental samples are shown in Tables 5, 6, and 7.

Table 5

Effect of Treatments and Storage Conditions on Pigment Concentrations in Columbia Beans (Size 4-5).

	2 days				1 month			5 months		
	Car.	Xan.	Pheo.	Chlor.	Car.	Xan.	Pheo.	Car.	Xan.	Pheo.
Fresh	26.3	17.6	--	810	--	--	--	--	--	--
W. Bl.	28.6	18.8	74.7	804	--	--	--	--	--	--
S. Bl.	29.0	16.7	126.7	760	--	--	--	--	--	--
Frozen	28.3	17.6	148.0	745	26.2	17.1	153	34.9	18.3	196
C240 (34)	26.3	11.6	1001	--	26.6	8.9	894	27.6	11.3	906
C240 (100)	(26.3)	(11.6)	(1001)	--	25.9	10.0	861	28.3	12.7	916
C260 (34)	27.6	11.1	960	--	22.9	9.9	833	24.8	9.9	1001
C260 (100)	(27.6)	(11.1)	(960)	--	25.4	9.8	889	27.6	13.1	895

Table 6

Effect of Treatments and Storage Conditions on Pigment Concentrations in Associated 92 Beans (Size 4-5)

	2 days				1 month			5 months		
	Car.	Xan.	Pheo.	Chlor.	Car.	Xan.	Pheo.	Car.	Xan.	Pheo.
Fresh	59.0	39.1	--	1711	--	--	--	--	--	--
W. Bl.	58.7	37.0	125	1454	--	--	--	--	--	--
S. Bl.	57.0	33.9	300	1325	--	--	--	--	--	--
Frozen	67.7	37.8	399	1422	63.1	55.6	420	67.0	41.7	366
C240 (34)	77.7	31.6	2020	--	70.9	37.4	1919	70.5	28.7	2058
C240 (100)	(77.7)	(31.6)	(2020)	--	74.1	28.3	1955	90.3	30.4	2106
C260 (34)	72.7	30.9	1888	--	74.0	28.4	1981	97.6	31.3	2253
C260 (100)	(72.7)	(30.9)	(1888)	--	64.2	28.1	1972	74.5	33.7	2075

Table 7

Effect of Treatments and Storage Conditions on Pigment Concentrations in Associated 92 Beans (Size 3-4)

	2 days				1 month			5 months		
	Car.	Xan.	Pheo.	Chlor.	Car.	Xan.	Pheo.	Car.	Xan.	Pheo.
Fresh	64.0	33.5	--	1785	--	--	--	--	--	--
W. Bl.	39.7	--	131	1574	--	--	--	--	--	--
S. Bl.	51.3	31.8	368	1354	--	--	--	--	--	--
Frozen	53.3	32.4	328	1411	60.6	34.0	353	55.0	35.8	282
C240 (34)	44.0	21.1	1798	--	74.7	17.8	1701	50.0	19.2	1859
C240 (100)	(44.0)	(21.1)	(1798)	--	52.9	19.6	1762	50.9	22.1	1662
C260 (34)	40.7	19.7	1834	--	51.0	19.1	1714	50.3	19.8	1903
C260 (100)	(40.7)	(19.7)	1834	--	48.7	19.1	1759	49.6	22.6	1744

Analyses of variance were carried out on each of the components for each variety, size, and storage period. These data were then grouped and used in determining the effect of variety, size, treatment, and storage period on the pigments.

Effect of Variety on Pigment Concentration. Analyses for the effect of variety on the concentrations of carotene, xanthophyll, pheophytin, and chlorophyll, showed that Associated 92 contained highly significantly greater concentrations of these pigments than Columbia. In most cases, the concentrations of these pigments were significantly altered by processing treatments, and in some cases, the effect of the treatment was dependent on the variety. Moreover, the length of storage time influenced the effects of the different treatments. These general results are examined in detail below.

Carotene: The carotene concentration in the Columbia variety was not affected by the processing treatments, but in Associated 92, it was increased when the beans were frozen and further increased by the canning treatments. This is shown in Table 8.

After storage for one month and five months, the carotene concentrations of both varieties of beans of 4-5 size were not affected by the processing treatments originally

applied.

Xanthophyll: As shown in Table 9, the xanthophyll concentrations of the two varieties, when determined immediately after processing, were reduced by the canning treatments. The same effects were found after storage for one and five months.

Table 8

Carotene Concentrations of Size 4-5 Beans at 2 Days

	Treatment						LSD	LSD
	Fresh	W.Bl.	S.Bl.	Fzn.	C240	C260	.05	.01
Columbia	26.3	28.6	29.0	28.3	26.3	27.6	NSD	NSD
Assoc. 92	59.0	58.7	57.0	67.7	77.7	72.7	3.8	5.4

TxV Interaction Highly Significant

Table 9

Xanthophyll Concentrations of Size 4-5 Beans at 2 Days

	Treatment						LSD	LSD
	Fresh	W. Bl.	S.Bl.	Fzn.	C240	C260	.05	.01
Columbia	17.6	18.8	16.7	17.6	11.6	11.1	2.2	3.2
Assoc. 92	39.1	37.0	33.9	37.8	31.6	30.9	2.1	3.0

TxV Interaction Not Significant

Pheophytin: The treatment-variety interaction with respect to pheophytin concentration was found to be significant at the 5% level on two days' and on five months' storage, but not at the one-month storage interval. The F values were only slightly greater than those required for significance and were very much smaller than the F values obtained for treatments so the interaction effect was not examined further. In the Columbia variety, water blanching caused the production of a small amount of pheophytin, steam blanching and freezing produced a greater amount, and the canning treatments converted all the chlorophyll to pheophytin. Thus, production of pheophytin increased with the severity of the heat treatments. In Associated 92, the same effects were evident, and in addition, over and above the heat treatments, freezing of the beans significantly increased the production of pheophytin (Table 10). The samples stored for one month and five months showed a similar result, i.e., the canned samples contained larger amounts of pheophytins than those which had been frozen.

Chlorophyll: In the Associated 92 samples, the chlorophyll concentrations were reduced by any heat treatment, the amounts of chlorophyll destroyed increasing with the severity of the heat treatments. It is to be pointed

Table 10

Pheophytin Concentrations* of Size 4-5 Beans at 2 Days

	Treatments					LSD	LSD
	W.Bl.	S.Bl.	Fzn.	C240	C260	.05	.01
Columbia	1.860	2.098	2.184	3.001	2.982	.132	.192
Assoc. 92	2.097	2.476	2.601	3.306	3.276	.060	.087

* (Observations Converted to Logarithms)

TxV Interaction Significant

out that when canning treatments were applied, the severe heat treatment completely destroyed the chlorophyll and these data are, therefore, not included in Table 11. Complete destruction of chlorophyll by the canning treatments was also found in the Columbia samples, but in this variety the differences in chlorophyll concentrations, after the various treatments other than canning, were not significant. Probably the chlorophyll concentrations were reduced by the heat treatments, but the combined effect of the low concentrations of chlorophyll with the large error term due to great variation within the samples, did not enable significant differences in chlorophyll concentrations to be shown.

Effect of Bean Size on Pigment Concentration. The effects of the sieve size of the beans on the

Table 11

Chlorophyll Concentrations of Size 4-5 Beans at 2 Days

	Treatments					
	Fresh	W.Bl.	S.Bl.	Fzn.	LSD .05	LSD .01
Columbia	811	804	760	745	N.S.D.	N.S.D.
Assoc. 92	1711	1454	1325	1422	136	206

TxV Interaction Highly Significant

concentrations of the pigments were found to vary with the pigments and the time of storage.

Carotene: The effect of bean size on carotene content was very significant when the beans were first processed and stored, but as the storage time increased this effect was reduced. Size 4-5 contained greater carotene concentrations than size 3-4 (Table 12).

Table 12

Carotene Concentrations in Associated 92 Beans After Storage

	2da	1mo	5mo.
Assoc. 92 (4-5)	73.7	69.3	80.0
Assoc. 92 (3-4)	44.5	52.2	51.2
	H.S.	H.S.	S.

The effects of the various treatments were very significant at the two-day storage interval, but decreased during storage until they were not significant after five months (Tables 13, 14).

Interaction between sizes and treatments was highly significant immediately after processing and during early storage, but was not significant after five months' storage. Where interaction was significant (Tables 13 and 14), the carotene concentrations were increased by canning in size 4-5 but decreased by canning in size 3-4.

Xanthophyll: The effect of bean size on the xanthophyll concentration was highly significant regardless of the treatment applied or the length of time the samples were stored, with size 4-5 beans containing the higher concentrations of xanthophyll. The processing treatments also exerted a significant effect on the xanthophyll concentrations throughout the storage period, and in both size groups examined, the canning treatments caused reductions in the xanthophyll concentrations. These effects on samples analyzed immediately after treatment are shown in Table 15. The same effect of treatments was also found after storage of the samples for one month and five months.

Size-treatment interaction was significant immediately after processing and after storage for one month, but

Table 13

Carotene Concentrations in Associated 92 Beans, Size 4-5

Storage Period	Treatment								LSD .05	LSD .01
	Fresh	W.Bl.	S.Bl.	Fzn.	C240-34	C240-100	C260-34	C260-100		
2 da	59.0	58.7	57.0	67.7	77.7	(77.7)	72.7	(72.7)	3.8	5.4
1 mo	--	--	--	63.1	70.9	74.1	74.0	64.2	8.3	12.1
5 mo	--	--	--	67.0	70.5	90.3	97.6	74.5	NSD	NSD

TxS Interaction highly significant at 2 da and 1 mo, but not at 5 mo.

Table 14

Carotene Concentrations in Associated 92 Beans, Size 3-4

Storage Period	Treatment								LSD .05	LSD .01
	Fresh	W.Bl.	S.Bl.	Fzn.	C240-34	C240-100	C260-34	C260-100		
2 da	46.0	39.7	51.3	53.3	44.0	(44.0)	40.7	(40.7)	5.8	8.4
1 mo	--	--	--	60.6	47.7	52.9	51.0	48.7	5.6	8.2
5 mo	--	--	--	55.0	50.0	50.9	50.3	49.6	NSD	NSD

TxS Interaction highly significant at 2 da and 1 mo, but not at 5 mo.

Table 15

Effect of Treatments on
Xanthophyll Concentrations (2 days)

	Treatment						LSD	LSD
	Fresh	W.Bl.	S.Bl.	Fzn.	C240	C260	.05	.01
Assoc. 92 (4-5)	39.1	37.0	33.9	37.8	31.6	20.9	2.1	3.0
Assoc. 92 (3-4)	33.5	--	31.8	32.4	21.1	19.7	2.9	4.2

Size effects highly significant

Table 16

Pheophytin Concentrations* in
Associated 92 Beans After Storage

	2da	1mo	5mo
Size 4-5	2.751	3.158	3.173
Size 3-4	2.743	3.100	3.091
	N.S.D.	H.S.	S.

* (observations converted to logarithms)

after five months' storage it was no longer significant. The loss of xanthophyll due to canning processes was more severe in size 3-4 beans than in size 4-5 beans.

Pheophytin: Size of the beans showed no effect on the pheophytin concentrations immediately after processing, but the effect of size was highly significant after

storage for one month, and significant after storage for five months. As shown in Table 16, beans of size 4-5 contained the greater concentrations of pheophytin after storage.

Up to one month's storage the processing treatments caused highly significant effects on the pheophytin concentration, but after five months' storage the effects of the original treatments were significant only at the 5% level. In both size classifications, immediately after processing, the pheophytin concentrations increased significantly with increasing severity of the heat treatments. In size 4-5, even the mere freezing of the processed beans caused a significant increase of the pheophytin concentration. After storage, all canning treatments resulted in the same pheophytin concentration which was highly significantly greater than that of the frozen samples. These effects are shown in Tables 17 and 18. TxS interaction was highly significant after storage for two days and one month, but was not significant after storage for five months.

Chlorophyll: Size of the beans was found to have no effect on the chlorophyll concentrations. However, the treatments applied to the beans caused highly significant changes in the chlorophyll concentrations, the loss of

Table 17

Pheophytin Concentrations* of Associated 92 Beans (Size 4-5)

Storage Period	Treatment							LSD .05	LSD .01
	W.Bl.	S.Bl.	Fzn.	C240-34	C240-100	C260-34	C260-100		
2 da	2.097	2.476	2.601	3.306	(3.306)	3.276	(3.276)	.060	.087
1 mo	--	--	2.623	3.283	3.291	3.297	3.295	.055	.080
5 mo	--	--	2.562	3.311	3.323	3.352	3.317	.033	.047

* (observations converted to logarithms)

Table 18

Pheophytin Concentrations* of Associated 92 Beans (Size 3-4)

Storage Period	Treatment							LSD .05	LSD .01
	W.Bl.	S.Bl.	Fzn.	C240-34	C240-100	C260-34	C260-100		
2 da	2.116	2.564	2.516	3.255	3.255	3.263	3.263	.042	.061
1 mo	--	--	2.547	3.230	3.246	3.233	3.245	.037	.054
5 mo	--	--	2.449	3.267	3.220	3.279	3.242	.054	.079

* (observations converted to logarithms)

chlorophyll increasing with severity of the heat treatments. These effects are shown in Table 19. As pointed out previously, the canning processes caused complete degradation of chlorophyll to pheophytin, thus the chlorophyll concentrations are not included for the canning treatments in this table.

Table 19

Chlorophyll Concentrations in Associated 92 Beans (2 days)

	Treatment					
	Fresh	W.Bl.	S.Bl.	Fzn.	LSD .05	LSD .01
Size 4-5	1711	1454	1325	1422	136	206
Size 3-4	1785	1574	1354	1411	193	303

Effect of Storage on Pigment Concentration. The concentrations of carotene, xanthophyll, and pheophytin in the frozen and canned samples of both varieties and both size groups, were statistically analyzed to determine if the length of storage had any effect on the concentrations of these pigments.

Carotene: The carotene concentrations of the two varieties in size 4-5 were unaltered by storage for up to five months at either 34° F or 100° F storage temperature. However, in Associated 92, size 3-4, storage period did

exert a very significant effect. As shown in Table 20, carotene concentration increased during the early part of the storage period after processing but was not significantly different after one month and five months' storage.

Table 20

Carotene Concentrations in Associated 92 Beans (Size 3-4)

2 da	1 mo	5 mo	LSD .05	LSD .01
44.5	52.2	51.2	3.6	6.0

In addition, the carotene concentrations were influenced by the original treatments, the frozen samples possessing higher concentrations of carotene than the canned samples irrespective of storage period (Table 21).

Table 21

Carotene Concentrations in Associated 92 Beans (Size 3-4)

	Treatment				LSD	LSD
Fzn.	C240-34	C240-100	C260-34	C260-100	.05	.01
56.3	47.2	49.3	47.3	46.3	3.8	5.4

TxP Interaction not Significant

Xanthophyll: In the Columbia variety, storage period did not exert any influence on the xanthophyll

concentrations. Treatment was the only factor causing significant differences between the samples. Xanthophyll concentrations were lower in the canned samples than in those frozen. In addition, canned samples stored at 34°F suffered greater losses of xanthophyll than those stored at 100° F (Table 22).

Table 22

Xanthophyll Concentrations in Columbia Beans (Size 4-5)

	Treatment				LSD	LSD
Fzn.	C240-34	C240-100	C260-34	C260-100	.05	.01
17.7	10.5	11.4	10.3	11.4	.83	1.2

TxP Interaction not Significant

In Associated 92, size 4-5, the effect of storage periods was significant at the 5% level with the concentrations of xanthophyll being lower after one month's storage than immediately after processing or after five months' storage (Table 23).

In Associated 92, size 3-4, the effect of storage periods was highly significant. The xanthophyll concentrations decreased during storage for one month, and then after five months' storage had increased to a higher level than immediately after processing (Table 23).

Table 23

Xanthophyll Concentrations in Associated 92 Beans

	2 da	1 mo	5 mo	LSD .05	LSD .01
Size 4-5	32.5	30.3	33.1	1.8	3.0
Size 3-4	22.8	21.9	23.9	.74	1.23

In both size groups of Associated 92, the effect of treatments on the xanthophyll concentrations was highly significant, with the frozen samples containing higher concentrations than the canned samples. Moreover, in size 4-5 treatment-storage period interaction was significant. These effects are shown in Table 24 and 25.

Pheophytin: The effect of storage on the pheophytin concentrations was found to be different in the two varieties. In Columbia beans, storage resulted in significant changes in the pheophytin concentrations; the pheophytin level falling during storage for one month and then increasing during further storage (Table 26).

In both size groups of Associated 92, the storage periods did not affect the pheophytin concentrations.

Table 24

Xanthophyll Concentrations in Associated 92 Beans (Size 4-5)

Storage Periods	Treatment					LSD .05	LSD .01
	Fzn.	C240-34	C240-100	C260-34	C260-100		
Total	39.3	29.6	30.1	30.2	30.9	1.8	3.0
2 da	37.8	31.6	31.6	30.9	30.9	1.6	2.3
1 mo	38.4	28.4	28.3	28.4	28.1	3.9	5.7
5 mo	41.7	28.7	30.4	31.3	33.7	4.5	6.7

TxP Interaction Significant

Table 25

Xanthophyll Concentrations in Associated 92 Beans (Size 3-4)

Storage Periods	Treatment					LSD .05	LSD .01
	Fzn.	C240-34	C240-100	C260-34	C260-100		
Total	34.0	19.4	20.9	19.5	20.5	2.2	3.3

TxP Interaction not Significant

Table 26

Pheophytin Concentrations* in Columbia Beans

2da	1mo	5mo	LSD .05	LSD .01
2.826	2.787	2.832	.022	.037

*(observations converted to logarithms)

Effect of Canning Processes and Storage Temperature on Canned Beans

Up to this stage, the analyses of variance had shown that in most pigments the effects of freezing and canning treatments were different but no differences between the two canning processes or between the two storage temperatures had been revealed. The effects of these two variables were investigated by analyses of variance of the data on the concentrations of the pigments in samples which had been subjected to these treatments. The following effects were found.

Carotene: Neither the canning treatments nor the storage temperatures caused significant differences in the carotene concentrations in either of the two varieties or the two size groups. However, in both size groups of Associated 92 beans, the canning process-storage temperature interaction was highly significant. This is shown in Table 27.

Table 27

Carotene Concentrations in Associated 92 Beans

	Treatment			
	C240-34	C240-100	C260-34	C260-100
Size 4-5	73.0	80.7	81.4	70.5
Size 3-4	47.2	49.3	47.3	46.3

Xanthophyll: In both Columbia variety and Associated 92, size 3-4, the two canning processes produced no significant differences in the xanthophyll contents, but the effect of storage temperature was significant with storage at 100° F retaining the greater amounts of xanthophyll (Table 28).

Table 28

Xanthophyll Concentrations of Columbia and Associated 92 Beans (Size 3-4)

	Treatment			
	C240-34	C240-100	C260-34	C260-100
Columbia	10.5	11.4	10.3	11.4
Assoc. 92 (3-4)	19.4	20.9	19.5	20.5

In Associated 92, size 4-5, both the effects due to canning processes and storage temperatures were

significant at the 5% level; canning at 260° F for 4 minutes and storage at 100° F causing the retention of greater amounts of xanthophyll. These effects are shown in Table 24.

Pheophytin: In the Columbia variety, neither the canning processes nor the storage temperatures exerted significant effects on the pheophytin concentration. In Associated 92, size 4-5, the same result was found but the interaction between these two variables was significant at the 5% level (Table 29).

Table 29

Pheophytin Concentrations* of Associated 92 Beans (Size 4-5)

Treatment			
C240-34	C240-100	C260-34	C260-100
3.300	3.307	3.308	3.296

In Associated 92, size 3-4, the canning processes exerted significant effects at the 5% level, and the storage temperature effect was highly significant. Canning at 260° F for 4 minutes produced greater concentrations of pheophytin than canning at 240° F for 20 minutes. Similarly, storage at 34° F resulted in greater production of pheophytin than storage at 100° F. These effects are

shown in Table 30.

Table 30

Pheophytin Concentrations* of Associated 92 Beans (Size 3-4)

Treatment			
C240-34	C240-100	C260-34	C260-100
3.251	3.240	3.258	3.250

When the results shown above were reviewed, several anomalies were revealed and thus the true validity of these cases is doubted. These particular results may have been obtained by chance because of the great variability of the fresh beans and because only three replicates were used. For example, in Table 20, it was shown that the concentration of carotene in Associated 92, size 3-4, beans increased on storage; this appears unlikely. In Table 23, it was shown that the xanthophyll concentration in both size groups of Associated 92 fluctuated during storage; it is felt unlikely that the xanthophyll concentration could increase on storage after an initial decrease. In Table 26, it was shown that the pheophytin concentration in the Columbia beans fluctuated during storage and in Table 30 it was shown that the two different canning processes and the two different storage

temperatures resulted in different pheophytin concentrations in Associated 92, size 3-4, beans.

As it had been shown previously that all the chlorophyll in the samples had been converted to pheophytin by canning, it would not be possible to show effects of storage, canning temperature, and storage temperature, on the concentrations of pheophytin.

SUMMARY AND CONCLUSIONS

A study of the pigments in fresh and processed Blue Lake type pole beans was made.

In Part I of the study, the pigments from unprocessed, blanched, and canned beans were separated by column chromatography and then identified by means of the absorption spectra. The conclusions drawn from these qualitative separations were as follows:

1. In unprocessed green beans the major pigments were chlorophylls A and B. Small amounts of the isomers, chlorophylls A' and B', occurred with the parent chlorophylls.
2. Mild heat treatments, such as blanching, caused partial conversion of chlorophyll A to pheophytin A.
3. Severe heat treatments, such as canning, caused complete conversion of chlorophylls A and B to pheophytins A and B.
4. Both alpha-and beta-carotenes were present in unprocessed beans and were not decomposed by the heat treatments normally applied in the processing of beans.
5. Unprocessed beans contained the following xanthophylls: taraxanthin, violaxanthin, flavoxanthin, and neoxanthin. When heat treatments were applied to the beans, these pigments were readily

converted to flavoxanthin-like pigments which exhibit absorption spectra with maxima at shorter wavelengths than the original pigments.

In Part II of the study, the concentrations of the pigments chlorophyll, pheophytin, carotene, and xanthophyll were determined in Columbia and Associated 92 variety green beans. The effects of bean size, processing treatments, storage temperature, and storage time, on the pigment concentrations were determined. The results indicated that in biological material of this nature, with variables whose effects on the pigments are so closely related, no confident and definitive statement can be made concerning the effect of any one variable on the pigments without associating it with specific levels of the other variables. Recognizing these limitations, the following general conclusions were reached:

1. Associated 92 beans contained higher concentrations of the pigments chlorophyll, carotene, and xanthophyll than Columbia beans.
2. The larger size beans of Associated 92 variety contained higher concentrations of the pigments investigated than the small size beans.
3. The effects of the processing treatments on the pigment concentrations varied with the individual pigments.
    a. Processing treatments did not change the

carotene concentrations of the beans, but there was a tendency for blanching treatments to produce apparent increases in carotene concentration.

b. Blanching and freezing did not affect the xanthophyll concentration, but canning caused an appreciable loss of xanthophyll.

c. Any heat processing applied to the beans caused reduction of the chlorophyll concentration, the magnitude of the reduction increasing with severity of the heat treatment. Canning caused complete conversion of chlorophyll to pheophytin.

4. The pigment concentrations were not affected by the length of time the processed beans were stored.

5. The same effects on the pigment concentrations were produced by retorting at 240° F for 20 minutes as by retorting at 260° F for 4 minutes.

6. The temperature at which canned beans were stored did not affect the carotene or pheophytin concentrations, but storage at 100° F caused retention of higher concentrations of xanthophyll than storage at 34° F.

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