8-1963

Anthocyanidin Pigments of Southern Peas, *Vigna sinensis*

Jerry L. Graham
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Jerry L. Graham entitled "Anthocyanidin Pigments of Southern Peas, *Vigna sinensis*." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Melvin R. Johnston, Major Professor

We have read this thesis and recommend its acceptance:

John T. Smith, Susan E. McCarty

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

June 7, 1963

To the Graduate Council:

I am submitting herewith a thesis written by Jerry L. Graham entitled "Anthocyanidin Pigments of Southern Peas, Vigna sinensis." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

Melvin L. Knuckles
Major Professor

We have read this thesis and recommend its acceptance:

John T. Smith

Leon E. McCarty

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

ANTHOCYANIDIN PIGMENTS OF SOUTHERN PEAS, *VIGNA SINENSIS*

A Thesis

Presented to

The Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Jerry L. Graham

August 1963

ACKNOWLEDGEMENTS

Sincere appreciation is expressed by the author to Dr. Melvin R. Johnston, Major Professor, and Professor Ivan E. McCarty for their valuable guidance and criticism in the development and preparation of this thesis.

Appreciation is also expressed to Dr. Henry A. Fribourg for his assistance in the operation of the IBM 1620 computer.

Due acknowledgement is made to Mrs. Kathleen McCook for conducting the taste panels and to the panel members who so conscientiously served on the taste panel.

The author wishes to express sincere thanks to the Winter Garden Freezer Company, Bells, Tennessee, for supplying a portion of raw product used in this study.

The author is deeply indebted to his wife, Jeanette Graham, for the typing of the manuscript.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

Southern peas are an important vegetable crop in the Southeastern states. They constitute 11 per cent of the total vegetables packed in this area and are exceeded in volume only by green beans and tomatoes (39).

Southern peas are not widely distributed by retailers throughout the United States. Discoloration during processing is believed to be the major reason preventing wide distribution (13). Also, color is a major factor of quality grade, and deterioration of color in these products results in considerable economic loss to the processor.

With respect to freezing of southern peas, the discoloration occurs during blanching. The water soluble pigments are gradually transferred from the peas to the blanch water. The dissolved pigment adheres to the precipitated solids of the blanch water, and upon sufficient accumulation, the colored solids collect on the surface of the peas.

With these factors in mind, this study was made in an attempt to: (1) determine effects of different blanch water additives on the quality of the product, (2) elucidate the nature of the coloring compounds of southern peas, and (3) study the causitive agents of discoloration in southern peas.

REVIEW OF THE LITERATURE

Pigmentation of Southern Peas

The first study of the pigmentation of southern peas in the United States was conducted by Mann (27) in 1914. He suggested that the great diversity in the coloration of the different varieties of cowpeas could be reduced to two factors: (1) the basal cells contained an extremely uniform pigmentation, ranging in color from yellow to deep copper red and was probably a melanin-like pigment. The differences in tint were caused by differences in quantity rather than the character of the pigment. (2) The palisade cells contained pigments superimposed over the basal layer pigments. The pigments in the palisade layer were of two kinds: (1) a melanin pigment generally identical to the pigment in the basal layer, and (2) an anthocyanin pigment either in the same cell or associated cells. The anthocyanin pigments were red or blue when in an acidic solution and black when in an alkaline solution. The distribution and combination of the different pigments were believed to be responsible for the great variety of colors in cowpeas.

The brown or black discoloration of peas is undesirable from several standpoints. A product which is badly discolored is often considered spoiled and will be discarded. Also, the discoloration is undesirable from the standpoint of U.S. grade because the discoloration usually includes the liquor and cotyledons. Culver and Cain (13) point out that most of the discoloration in canned southern peas was caused by the anthocyanin pigment of the seed coat. They conclude that the discoloration

can be controlled by: (1) use of 3 per cent ammonium alum or aluminum sulfate as a blanching solution, or (2) by the use of a high-temperature and short-time sterilization process. They also noted a reduction in viscosity of the liquor and gelling in the can.

Burns and Winzer (10) report that the seed coat of blackeye-type peas contains two layers of concentrated pigment whereas the purple hull-type peas contain one pigment layer. The pigment layers were located in both the basal and palisade cells of the blackeye peas but only in the basal cells of the purple hull peas. Green, immature blackeye peas were found to contain some pigment in both the palisade and basal cells. However, the large quantity of chlorophyll present in the young peas obscured the visual detection of these pigments. The quantity of pigment was observed to increase with maturity. They suggested the water-soluble eye pigment of southern peas and the pod pigment of purple hull peas were anthocyanins. Their paper chromatograms indicated that one pigmented substance was present in the eye extract. However, several other substances resembling anthoxanthin pigments were present in the pod extracts. The pigment was precipitated during heating and strongly adsorped to the remainder of the pea, therefore, it could not be readily extracted. The addition of sodium chloride (2.5 per cent) during processing was found to increase the rate of precipitation.

In a survey by Bate-Smith and Ribérau-Gayon (5), it was demonstrated that the seed coats of many seeds, especially those of the Leguminosae (peas and beans), contained leucoanthocyanins which were not present in the remainder of the plant. However, in most plants the compounds present in the leaves also were detected in the fleshy parts

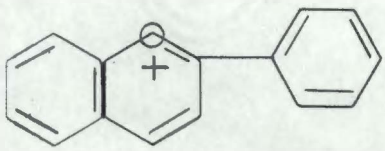
of the fruits. Occasionally the same pigment will be present in the roots and stems (3).

In a study of the behavior of the anthocyanin pigments in canning, Culpepper and Caldwell (12) found the appearance of discoloration in canned products was often due to the reaction of the anthocyanin with tin from the container. Complex metallic salts of anthocyanins were found to be the products of this reaction. The formation of the violet colored complex was favored by low acidity with high acidity depressing or suppressing the reaction.

Chemistry of Anthocyanins

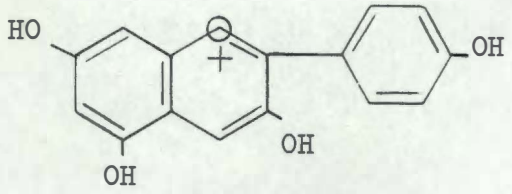
The term "anthocyanin" was initially proposed by Marquart in 1835 to denote the blue pigment of the cornflower and later has been used in a wider sense to include the whole group of similar pigments (22).

According to Willsläatter and Everest (41), the anthocyanins belong to a group of glycosides usually dissolved in the cell sap of flowers, fruits, and other plant organs. The sugar-free portions of these compounds have been named anthocyanidins. The flavylium (2-phenylbenzopyrylium) structure was found to be common to all anthocyanidins.

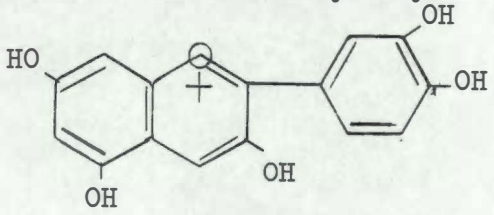


Flavylium Cation

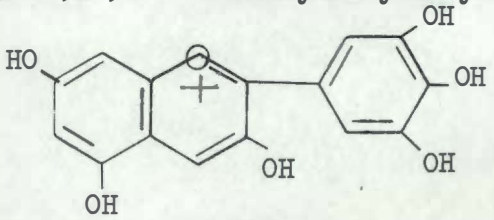
The anthocyanidins were divided into 3 basic groups: (1) pelargonidin, (2) cyanidin, and (3) delphinidin. All are hydroxylated in the 3-, 5-, and 7- positions. The division was made on the basis of hydroxylation of the β ring. The primary factor controlling the color of anthocyanins was observed to reside in the hydroxyl groups attached to the flavylium nucleus; especially those attached to the 2-phenyl group of the molecule. In general, the deepening of visible color was found to be brought about with an increase in the number of hydroxyl groups on the 2-phenyl ring as illustrated by orange-red pelargonidin, deep red cyanidin, and bluish-red delphinidin. Methyl substitution appears to affect the anthocyanin color by increasing the dullness of the compound (22).



Pelargonidin
(3,5,7,4' – Tetrahydroxyflavylium)



Cyanidin
(3,5,7,3',4' – Pentahydroxyflavylium)



Delphinidin
(3,5,7,3',4',5' – Hexahydroxyflavylium)

A great many researches made on the water soluble plant pigments disclosed that the pigments generally occur as anthocyanins, i.e., the

anthocyanidin glycosides containing one or more sugar residues and occasionally various organic acid residues. As a rule, anthocyanins were split into anthocyanidins and sugar components by boiling in 20 per cent hydrochloric acid for 3 minutes. The sugars found ranged from glucose to gentiobiose. The attachment of the sugars was found to be most common at position 3 and less frequently at position 5. Robinson and his school (7,30) are solely responsible for our knowledge on the position of attachment.

Abe and Hayashi (1) report that the action of 20 per cent hydrochloric acid at 70°C on diglycosidic anthocyanins gives a stepwise liberation of sugar residues resulting in the formation of lower glycosidated intermediates. These intermediates may be clearly demonstrated by employing paper-chromatographic analysis during the earlier stages of hydrolysis. For example, they show that cyanin chloride, the 3:5 dimonoside of cyanidin, may be degraded by this treatment into two intermediate products. The formed intermediates were chrysanthemin (3-monoside) and cyanenin (5-monoside), both of which may be further converted into the sugar-free residue cyanidin.

Sondheimer (35) reports the anthocyanin of strawberries exists in an equilibrium between a red modification, R^+, and a colorless form, ROH, of the anthocyanins. The equilibrium was observed to be pH dependent.

Bate-Smith and Swain (6) pointed out the only difference in general behavior between catechins and leucoanthocyanins was the latter substance gives red colorations with hot mineral acid treatment. The red colorations were due to the formation of anthocyanidins.

The compounds in plants which form anthocyanidins on hydrolysis with

mineral acid may be monomers or polymers whose structure at present is unknown (22).

Robinson and Robinson (29) devised a scheme of pigment analysis which depends primarily on characteristic color reactions combined with examination of the distribution of pigments between two immiscible solvents. The color reactions were widely used by Robinson and his co-workers to make an extensive survey of the anthocyanins occurring in many familiar plants.

Chromatography of Anthocyanins

Bate-Smith (2,4) introduced the technique of paper chromatography into the study of anthocyanins and other related sap-soluble plant pigments. This technique has become a useful tool for the prompt separation and identification of the individual plant pigments. Bate-Smith reported that rapid and simple identification of anthocyanins was possible employing paper chromatography.

Harborne (18,20) has written two review articles on the chromatographic identification of flavonoid compounds. He outlines the steps of a complete identification procedure employing paper chromatography from extraction through the final step of R_f measurement. The characterization of an unknown pigment depends upon identifying the anthocyanidin produced on hydrolysis and on determining the nature, position of attachment, and number of sugars present. He also points out that with solvents which do not contain mineral acids, it is important that sufficient hydrochloric acid be present in the original extract to keep the anthocyanidin in the chloride form as it traverses the paper.

Wender's group (40) found the banding technique a simple method for obtaining small amounts of chromatography pure flavonoid compounds. The method involves the use of strips or bands instead of spots. The bands were developed, cut apart, and eluted with water and/or alcohol. This method was very useful for procedures where only small amounts of the compounds were desired. However, where larger quantities were desired, column chromatography was found to be more useful.

Column chromatography was first used as a method of separation of anthocyanin isolated from flowers by Karrer (24) in 1936.

Spaeth and Rosenblatt (37) separated a mixture of synthetic anthocyanidins using silicic acid as the inert support. The column consisted of 10 per cent aqueous phosphoric acid fixed in silicic acid. A phenol-toluene mixture was used for development and elution of the column.

Butanol-acetic acid-water was used in conjunction with a silicic acid column by Li and Wagenknecht (26) to separate the anthocyanin pigments of sour cherries.

Sakamura and Francis (31) suggest the use of a cation exchange resin (Amberlite CG-50, Rohm and Haas, Inc., Philadelphia, U.S.A.) to purify the crude anthocyanin extract. They report the red pigments were retained on the column and the brownish pigments passed through. After washing with a large volume of distilled water, the pigments were eluted readily with 95 per cent ethyl alcohol.

Column chromatography offers a method of large-scale isolation and purification of flavonoid compounds. While excellent results have been obtained, the technique is not routinely applicable or easy.

However, ion-exchange resins are extremely useful in the preliminary purification of crude plant extracts (33).

Spectral Analysis on Anthocyanins

Although anthocyanins characteristically exhibit intense absorption in the 500-550 mμ region, the differences in the spectra of the individual compounds are relatively small. For this reason, spectral measurements were not used extensively in the identification of the natural anthocyanins until Bate-Smith (2) introduced effective paper chromatographic procedures for the separation of the pure pigment from co-occurring anthocyanins in crude plant extracts.

The intensity and position of the visible maximum of anthocyanins shifts considerably with changes in solvent (36) and pH (19). The λ_{max} was shown to decrease successively in acidified ethanol, methanol, and water.

Geissman and associates (16) introduced the use of aluminum chloride as a useful reagent in distinguishing certain anthocyanins. The aluminum chloride produces a bathochromic shift of the principle λ_{max} of those anthocyanidin derivatives which contain adjacent hydroxyl groups. The spectra of anthocyanins which did not contain o-dihydroxyl groups was unaffected. This reagent was very useful in distinguishing anthocyanidins such as cyanidin and peonidin which have very similar spectral properties in alcohol.

Harborne (19) has recently published a comprehensive collection of anthocyanin spectra properties and indicates that with the exception of delphinidin and petunidin each of the ten natural anthocyanidins may be

readily distinguished by spectral means.

Glycosidation of the anthocyanidins has been found to shift the absorption maxima toward the shorter wavelengths. The introduction into the 3- or the 3,5-positions produces a hypsochromic shift of 10-15 mμ, whereas an introduction in the 5-position results in a hypsochromic shift of only 7 mμ (23).

Harborne (19) also observed the spectra of those anthocyanins in which the 5-hydroxyl group was free possess a distinct shoulder in the 410-450 mμ region. 5-Glycosides and 3,5-diglycosides showed only an inflection of low intensity in this region. These differences became apparent when the ratio of the optical density at 440 mμ to that at the λ_{max} was calculated. The ratio offers a useful means of distinguishing 3-glycosides and 3,5-diglycosides.

Sensory Evaluation

Taste panel evaluation has become an integral part of most research pertaining to foods. The literature in this field is quite extensive; however, Boggs and Hanson (8) present a comprehensive review on this subject. They suggest that experiments should be designed to: (1) minimize within-sample variation, (2) limit the number of samples and characteristics of each that are judged in one period, (3) submit at one time all the samples for which comparative data are desired, (4) relate experimental samples to control samples, (5) mask all characteristics except the one under consideration, (6) eliminate samples of strong odor or flavor when possible, and (7) judge sufficient replicates to show that trends can be reported or replicate sufficiently that data can be analyzed statistically.

The number of samples which may be served to a flavor panel at one time is important from both an economical and efficiency standpoint. The literature pertaining to this facet of sensory evaluation is not definite. However, Sather and Calvin (32) reported that up to 20 samples may be included in one test period without suffering psychological fatigue, if the products were mild flavored.

For the detection of small differences in the intensity or quality of flavors, three common experimental designs are used. They are: (1) pair tests (which is the stronger? or, which is the regular flavor?); (2) duo-trio tests (which of the two coded aliquots is identical with the third "standard" aliquot?); and (3) triangle tests (which of the three aliquots is odd?).

A study by Gridgeman (17) suggests that pair tests and triangle tests are normally about equally powerful and are superior to duo-trio tests. However, Byer and Abrams (11) found the two-sample tests reflected a higher statistical significance than did the triangle test. These findings demonstrate the necessity of assessment of the different methods under given conditions.

Kramer and Ditman (25) present a simplified variables taste panel method for detecting flavor changes in vegetables treated with pesticides. The method, based on the analysis of variance from the range, eliminates a large part of the computations but retains the greater part of the efficiency of a variables method. His results indicated that this simplified variables method was superior to an attributes method such as the triangle taste test in detecting flavor differences. This method also requires fewer tastings, and at the same time produces additional

information on the direction and importance of the differences.

Filipello (14) in his work on wines noted an insignificant correlation between samples when only a single sample was rated. Having no immediate comparison available, the judge's memory provides a poor measure of standards of quality. Tilgner (38) indicates accuracy in quality scoring could be much greater when objective standards were available to the grader for ready reference in restandardization.

Buch, et al. (9) used an organoleptic evaluation scheme in which the judges were asked to score the samples on a 1 to 10 scale (10 = best and 1 = poorest). A sample of the control, arbitrarily given the score of 5, was included for the purpose of comparison. Fry (15) used a similar method where the panel was requested to score the samples in direct relation to the reference sample. A four-point scale was used, and the products were judged as having the same flavor as the reference, slightly different, moderately different, or extremely different. These judgments were later scored with zero being assigned to the first category of no difference from the reference sample and one, two, and three being assigned to the other categories respectively.

MATERIALS AND METHODS

Experimental Design

Two varieties of southern peas were used for most of this experiment. The varieties used in the blanching experiments were purple hull blackeye and crowder. However, the blackeye variety was included as a third variety in the anthocyanin section.

During the 1961 season, peas were shipped by air freight to the pilot plant from local processors in West Tennessee. In the spring of 1962, two plantings of purple hull blackeye and crowder peas were made on May 9 and May 31. Each planting contained one-fourth acre of two varieties giving a total of one acre. The first bottom near Fort Loudon Lake on the University of Tennessee Cherokee Farm was used as the site. The crop was fertilized at a rate of 500 lb of 6-12-12 per acre, cultivated to control weeds, and irrigated when needed.

The first peas were hand picked on July 23, 1962, followed by additional harvests as maturity level justified. Similar peas were obtained from a grower in Sevier county in order to insure sufficient replication. The peas were considered mature when the pods changed from an immature green color to a reddish purple color, but not allowing the peas to become dry and hard.

Immediately after picking, the peas were transported to the pilot plant and shelled during the 1962 season. However, in 1961 the harvested peas from West Tennessee were transported from the airport and placed in a 40°F cooler until they were shelled the following morning. A Dixie Pea

Huller was used to separate the peas from the hulls. Excess trash was removed with an air cleaner followed by hand sorting. A portion of the shelled peas was immediately steam blanched for 4 minutes and frozen for later anthocyanin determinations. Hulls were also frozen for this purpose. Both the peas and hulls were frozen in 10-lb freezing cans. The remaining fresh peas were allocated to the water blanching experiments.

All the products were frozen in a blast freezer at -20°F followed by storage at -10°F.

Blanching Experiments

In an attempt to improve the blanching characteristics of southern peas, the following treatments were used: (1) aluminum sulfate (4 per cent), (2) citric acid (1 per cent), (3) phytic acid (1 per cent), (4) ferric ion (5 ppm), and (5) steam blanch. These treatments were performed with tap water. The various chemicals were combined with 1500 ml of tap water prior to the addition of the peas to the blanch solution.

Steam jacketed kettles were used as the blanching vessel in all the experiments. The blanching solution was heated to boiling followed by the addition of 2.5 lb of peas. This mixture was heated to 210-212°F and simmered 30 minutes keeping the water level constant during this period. After 30 minutes, the peas and blanch water were separated with the latter being returned to the vessel. These peas were discarded. The test peas were added to the blanch solution when the boiling point was attained. The peas were given a 3-minute blanch and were separated from the solution. They were immediately cooled under a running water tap.

The peas were packaged in conventional 10-oz consumer fiberboard cartons, overwrapped, and frozen at -20°F in a blast freezer. The blanch solution was placed in No. 10 cans, sealed and frozen as above. This procedure was used for the five treatments, two varieties, and two seasons. Color photographs and sensory evaluations were used to determine the effects of the treatments on the product.

Sensory Evaluation

The blanched peas were stored at -10°F for six to nine months followed by organoleptic evaluation. The samples were allowed to thaw a few minutes at room temperature, and the panel members were asked to evaluate the color of the uncooked peas. After the raw peas had been evaluated for color, approximately 350 g of peas were placed in three-fourths cup of boiling 0.7 per cent sodium chloride solution. The mixture was again brought to a boil, and the peas were simmered for 30 minutes. After cooking, the peas were placed in small serving dishes and presented to the panel while warm.

At this time, each sample was evaluated for color, flavor, and texture. The method of evaluation used was a slight modification of the technique used by Fry (15). The scale was as follows:

Plus 3 - much better than control
Plus 2 - better than control
Plus 1 - slightly better than control
Zero - same as control
Minus 1 - slightly worse than control
Minus 2 - worse than control

Minus 3 - much worse than control

The panel was instructed to indicate their preference with respect to the control sample for the above mentioned attributes of color, texture, and flavor. All treatments within one replication were judged at one setting. Each of the evaluations were made on individual score sheets in an attempt to minimize prejudice between the separate factors. A glass of water was provided for each panel member to be used at his convenience. The panel was composed of six members (four males and two females).

The taste panel data were statistically analyzed using the analysis of variance and Duncan's multiple range test. The analysis of variance was conducted according to the methods of Snedecor (34) and the Duncan's multiple range according to the tables in Biometrics (21).

The above calculations were made employing an IBM Model 1620 computer.

Anthocyanin Determinations

Spectral analysis and paper chromatography were the two principle methods used to identify the anthocyanidins of southern peas. Methanolic hydrochloric acid (3 per cent), 0.1 N hydrochloric acid, and hot 2 N hydrochloric acid were used to extract the pigments from the product. However, hot 2 N hydrochloric acid was the only successful extractant of pigment from the blanched product. For this reason, it was possible to identify only the hydrolyzed anthocyanins, anthocyanidins, due to the severe hydrolysis by the hot 2 N hydrochloric acid. This procedure was suggested by Bate-Smith (4).

Several methods of pigment purification as suggested by Sakamura and Francis (31) and Pruthi, Susheela, and Lal (28) were tried. The methods were ion exchange purification combined with silicic acid separation as used by the former and lead acetate precipitation followed by picrate crystallization as outlined by the latter workers. However, the simpler and more rapid method of "piling on" ascending paper chromatography as outlined by Harborne (19) was found to be more satisfactory with these particular pigments.

Sample Preparation

Hulls of the two pigmented varieties, purple hull blackeye and crowder, were ground with a hammer mill employing a 0.093-in. sieve while in a frozen state. The frozen ground hulls were maintained at -10°F until they were used in the anthocyanin determinations. The shelled peas were not macerated. Samples of precipitated blancher solids were obtained from a commercial processor in West Tennessee. The samples were frozen until used.

The crude pigment was obtained by extraction with hot 2 N hydrochloric acid. Five hundred g of frozen whole peas, 300 g of frozen ground hulls, and 300 g of blancher precipitate from each variety were placed in 2,000 ml beakers and covered with approximately 400 ml of 2 N hydrochloric acid. Bunsen burners were used to heat the solutions to boiling. After boiling commenced, 3 to 5 minutes were allowed for extraction followed by gravity filtration to remove excess solids. Whatman No. 12 folded filter paper was used. A small quantity of Celite filter-aid was added to the filtrate, and the solution was refiltered through Whatman No. 42 filter paper employing a vacuum.

N-butanol was used to extract the pigment from the aqueous filtrate. Successive extractions were used until most of the pigment was extracted from the filtrate. Three or four extractions were usually sufficient. Distilled water was used to wash soluble material from the alcoholic phase. This phase was evaporated to dryness at room temperature with a Rinco evaporator. The dried pigment was dissolved from the wall of the flask with methanolic hydrochloric acid (1 per cent). The pigment was finally filtered and stored at 32°F.

Paper Chromatography

Ascending paper chromatography employing large sheets (18-1/2 x 22-1/2 inch) of Whatman No. 3 mm filter paper was used to separate the crude pigment into the various fractions. Forestal reagent (water-acetic acid-12 N hydrochloric acid, 10:30:3) was used as the developing solvent.

The various alcoholic pigment solutions were streaked individually along a line 1 inch from the bottom of the filter paper. A conventional hair dryer was used to dry streaks of pigment. The edges of the paper were stapled forming a cylinder. Forestal reagent was placed directly in the bottom of the large chromatography jars (12 x 24 inch). The papers were placed in the irrigated jars and allowed to develop 12-16 hours. At this time, the chromatograms were removed from the chambers and dried at room temperature. A short wavelength ultraviolet lamp was used to aid in the detection of the different fractions. Colors of the fractions and evidence of fluorescence were noted at this time.

The different fractions, as fractionated by Forestal reagent, were clipped out in a manner in which co-pigments were not mixed with the fraction. The fractions were eluted by a simplified rapid method

which consisted of merely tearing the strips into small squares and placing in 250 ml beakers. Approximately 100 ml of methanolic hydrochloric acid (1 per cent) was mixed with the strips and allowed to stand at least 30 minutes with occasional stirring. Harborne (19) suggested a trough elution employing water, methanol, and acetic acid (25:70:5) as the eluting mixture. This procedure was slow and allowed the pigments to oxidize during elution. The leaching procedure yielded a lower per cent recovery of pigment from the strips, but it was found more satisfactory due to the prevention of pigment oxidation and increase in rapidity.

The elutant was evaporated with a Rinco flash evaporator. Methanolic hydrochloric acid (0.01 per cent) was used to dissolve the dry pigment from the flask as suggested by Harborne (18). The pigments were easily oxidized in this weakly acidic solution, and it was found that the substitution of 1 per cent methanolic hydrochloric acid greatly improved the stability of the compounds.

If the fraction appeared homogeneous on the original chromatogram, the sample was spotted both on Whatman No. 3 mm and No. 1. This was done to determine the R_f value of the compound and also as a check of homogeneity of the fraction. However, if the fraction appeared heterogeneous on the original chromatogram, the sample was rechromatogramed until a homogeneous fraction was obtained. The above procedure was followed when the fraction finally appeared to be homogeneous. Once the fractions were spotted on the filter paper, the samples were set aside for spectral analysis since the same samples were used in both determinations.

In order to determine the R_f value of the different fractions,

the various pigments were spotted on Whatman No. 1 sheet chromatography paper. The chromatograms were developed in a Chromatocab employing the following solvents: (1) water-acetic acid-12 N hydrochloric acid (10:30:3 v/v), (2) formic acid-12 N hydrochloric acid-water (5:2:3 v/v), (3) acetic acid-12 N hydrochloric acid-water (5:1:5 v/v). The solvent front was allowed to travel at least 40 cm which usually required approximately 12 hours. Descending chromatography was used in these determinations. R_f corresponds to the ratio of the distance the compound travels to the distance the solvent front moves. The measurements were made to the middle of the pigment spot.

All possible precautions were utilized to prevent excessive light exposure of the purified anthocyanidins since certain fractions were extremely photosensitive.

Spectral Analysis

The same solutions of pigments were used for spectral analysis and R_f determinations. The maximum optical density of the solution was adjusted within the range of 0.8 to 1.2 by dilution, and the pH of the solutions was maintained below 1.0. A Beckman model DB recording spectrophotometer was used to determine the absorption maximum of the different fractions. These results were confirmed with a Beckman model DU spectrophotometer. This procedure was suggested by Harborne (19).

From these determinations, the wavelength exhibiting maximum absorption was noted for each of the fractions. Alcoholic aluminum chloride (5 per cent) was added directly to the cuvette containing the solution. The sample was scanned again to check for a bathochromic shift (15-50 mμ) of the principle wavelength maximum. This reagent,

introduced by Geissman (16), was useful in the detection of anthocyanidins which contain adjacent hydroxyl groups.

The optical density at 440 mμ and the wavelength of maximum absorption were noted in order to establish another criterion of identification. A ratio of the two optical densities was calculated as a percentage.

Color Reactions

Color tests were determined on the various fractions of the products as outlined by Robinson and Robinson (29). The same solutions were used for the color tests as the spectral analysis and R_f determinations. However, the solutions were transferred to an aqueous 1 per cent hydrochloric acid solution prior to execution of the tests. The transfer was made by evaporating the methanol from the pigment and adding back 1 per cent hydrochloric acid. These solutions were used as the stock pigments for all the color tests.

RESULTS AND DISCUSSION

Blanching Experiments

Results of the blanching experiments are shown in Tables I through XII. In general, the various treatments did not improve the color of either the uncooked or cooked southern peas as evaluated by the taste panel. Flavor and texture were also evaluated by the taste panel. These indices were included as a measure of any off flavors or changes in texture resulting from the various treatments. In general, the treated peas gave a lower average score than the control when evaluated for flavor and texture.

Since the discoloration is believed to occur during blanching, various blanching solutions were tried: (1) aluminum sulfate (4 per cent) was used to toughen the seed coat of the pea and prevent diffusion of soluble solids from the pea to the blanch solution; (2) citric acid (1 per cent) was used to chelate the iron from the blanch solution, thereby preventing the formation of the typical dark colored iron-anthocyanin complex; (3) phytic acid (1 per cent) was also used as a chelating agent; (4) steam blanching was used as a comparative method to the conventional water blanch; (5) ferric chloride (5 ppm) was added to determine the effect of additional ferric ions in the blanch solution.

Color of Uncooked Peas

All treatments were found to affect the color of the uncooked peas. Analysis of variance of the data presented in Table III indicates that treatment was significant at the .01 level, and the individual was significant at the .05 level. Replication and variety were not significant.

TABLE I

EFFECT OF VARIOUS TREATMENTS ON THE ORGANOLEPTIC EVALUATION OF COLOR OF UNCOOKED CROWDER PEAS

Individual	Average Score of 6 Replications					
	Aluminum Sulfate	Citric Acid	Ferric Chloride	Steam Blanch	Phytic Acid	Control
1	- 3.0	-2.2	0.0	0.0	-0.3	0.0
2	- 3.0	-2.5	0.0	+0.3	-0.5	0.0
3	0.0	+0.7	+0.3	-0.2	-0.2	0.0
4	- 2.5	-0.7	0.0	0.0	+0.7	0.0
5	- 2.8	-1.7	-0.7	-0.5	-0.2	0.0
6	- 0.2	+0.8	-0.5	0.0	+0.5	0.0
Total	-11.50	-5.60	-0.90	-0.40	0.00	0.00
Mean	- 1.92	-0.94	-0.15	-0.07	0.00	0.00
Statistical significance at 0.05 level						

TABLE II

EFFECT OF VARIOUS TREATMENTS ON THE ORGANOLEPTIC EVALUATION OF COLOR OF UNCOOKED PURPLE HULL BLACKEYE PEAS

Individual	Average Score of 6 Replications					
	Citric Acid	Phytic Acid	Aluminum Sulfate	Control	Ferric Chloride	Steam Blanch
1	+0.7	0.0	+0.3	0.0	+0.2	+0.5
2	-0.8	-0.2	0.0	0.0	-0.2	0.0
3	-0.2	+0.2	-0.3	0.0	+0.5	+0.5
4	-1.2	0.0	-0.7	0.0	+0.3	+0.7
5	-0.7	-0.3	-1.0	0.0	-0.2	0.0
6	-1.3	-0.7	+1.0	0.0	0.0	+0.5
Total	-3.50	-1.00	-0.70	0.00	+0.60	+2.20
Mean	-0.59	-0.17	-0.12	0.00	+0.10	+0.36
Statistical significance at 0.05 level						

TABLE III

ANALYSIS OF VARIANCE OF THE ORGANOLEPTIC
EVALUATION OF COLOR OF UNCOOKED SOUTHERN PEAS

Source	D.F.	M.S.	F. Ratio
Total	431		
Treatment	5	17.19	6.99**
Replication	5	3.39	1.79
Individual	5	6.83	3.08*
Variety	1	20.89	3.51
T x R	25	1.70	1.75*
T x I	25	2.22	2.13**
R x I	25	1.32	1.80*
T x V	5	7.42	1.91
R x V	5	5.70	5.14**
I x V	5	4.74	2.62*
T x R x I	125	0.73	0.71
T x R x V	25	1.21	1.17
T x I x V	25	2.61	2.53**
R x I x V	25	1.00	0.97
T x R x I x V	125	1.03	

TABLE IV

EFFECT OF VARIOUS TREATMENTS ON THE ORGANOLEPTIC EVALUATION OF COLOR OF COOKED CROWDER PEAS

Individual	Average Score of 6 Replications					
	Aluminum Sulfate	Citric Acid	Phytic Acid	Steam Blanch	Ferric Chloride	Control
1	- 1.5	-0.8	-0.5	-0.7	-0.2	0.0
2	- 2.0	-1.3	-1.3	-0.3	0.0	0.0
3	- 1.3	-1.2	-0.5	-1.0	-0.7	0.0
4	- 2.2	-1.7	-0.7	+0.2	+0.2	0.0
5	- 2.3	-1.3	-0.3	-0.2	+0.5	0.0
6	- 2.5	-1.7	0.0	+0.5	0.0	0.0
Total	-11.80	-8.00	-3.30	-1.50	-0.20	0.00
Mean	- 1.97	-1.33	-0.55	-0.25	-0.04	0.00
Statistical significance at 0.05 level						

TABLE V

EFFECT OF VARIOUS TREATMENTS ON THE ORGANOLEPTIC EVALUATION OF COLOR OF COOKED PURPLE HULL BLACKEYE PEAS

Individual	Average Score of 6 Replications					
	Ferric Chloride	Aluminum Sulfate	Control	Steam Blanch	Phytic Acid	Citric Acid
1	-0.5	+0.7	0.0	-0.7	+0.7	+1.3
2	-0.5	+0.7	0.0	0.0	+0.3	+1.2
3	-0.3	+0.8	0.0	+0.5	+0.5	+0.5
4	-0.2	-0.8	0.0	0.0	+0.2	+0.3
5	+0.5	-1.0	0.0	+0.3	+0.2	+0.5
6	-1.2	-1.0	0.0	0.0	+0.2	+0.3
Total	-2.20	-0.60	0.00	+0.10	+2.10	+4.10
Mean	-0.37	-0.10	0.00	+0.02	+0.35	+0.68
Statistical significance at 0.05 level						

TABLE VI

ANALYSIS OF VARIANCE OF THE ORGANOLEPTIC EVALUATION OF COLOR OF COOKED SOUTHERN PEAS

Source	D.F.	M.S.	F. Ratio
Total	431		
Treatment	5	10.41	2.78*
Replication	5	0.73	0.44
Individual	5	8.70	4.16**
Variety	1	66.89	6.18*
T x R	25	2.00	2.99**
T x I	25	2.76	3.58**
R x I	25	1.31	1.56
T x V	5	17.42	22.05**
R x V	5	1.66	1.66
I x V	5	2.68	2.09
T x R x I	125	0.70	0.71
T x R x V	25	0.50	0.51
T x I x V	25	1.07	1.08
R x I x V	25	1.49	1.51
T x R x I x V	125	0.99	

TABLE VII

EFFECT OF VARIOUS TREATMENTS ON THE ORGANOLEPTIC EVALUATION OF TEXTURE OF COOKED CROWDER PEAS

Individual	Average Score of 6 Replications					
	Aluminum Sulfate	Citric Acid	Phytic Acid	Steam Blanch	Control	Ferric Chloride
1	- 1.2	-0.8	-0.2	-0.7	0.0	0.0
2	- 2.2	-1.5	-1.5	-0.7	0.0	-0.5
3	- 1.8	-0.5	-0.3	-1.2	0.0	+0.5
4	- 2.0	-1.0	-0.3	-0.3	0.0	-0.2
5	- 1.7	-1.7	-0.5	+0.3	0.0	+1.3
6	- 2.0	-0.5	0.0	-0.2	0.0	+0.7
Total	-10.90	-6.00	-2.80	-2.80	0.00	+1.80
Mean	- 1.82	-1.00	-0.47	-0.47	0.00	+0.30
Statistical significance at 0.05 level						

TABLE VIII

EFFECT OF VARIOUS TREATMENTS ON THE ORGANOLEPTIC EVALUATION OF TEXTURE OF COOKED PURPLE HULL BLACKEYE PEAS

Individual	Average Score of 6 Replications					
	Aluminum Sulfate	Citric Acid	Steam Blanch	Phytic Acid	Control	Ferric Chloride
1	-1.2	-0.7	0.0	-0.2	0.0	+0.5
2	-1.2	-1.2	0.0	-0.5	0.0	+0.3
3	-1.7	-0.5	-0.3	0.0	0.0	+0.2
4	-1.8	-0.5	-0.5	+0.2	0.0	+0.3
5	-1.5	-0.7	-0.5	-0.5	0.0	+0.5
6	-1.7	-0.7	-1.0	+0.2	0.0	-0.3
Total	-9.10	-4.30	-2.30	-0.80	0.00	+1.55
Mean	-1.52	-0.72	-0.39	-0.14	0.00	+0.25
Statistical significance at 0.05 level						

TABLE IX

ANALYSIS OF VARIANCE OF THE ORGANOLEPTIC
EVALUATION OF TEXTURE OF COOKED SOUTHERN PEAS

Source	D.F.	M.S.	F. Ratio
Total	431		
Treatment	5	33.82	33.16**
Replication	5	1.28	1.19
Individual	5	8.24	6.81**
Variety	1	2.67	1.87
T x R	25	0.96	1.55
T x I	25	1.17	1.86*
R x I	25	1.08	1.69*
T x V	5	0.56	0.85
R x V	5	1.69	2.45*
I x V	5	2.03	2.90*
T x R x I	125	0.62	1.48*
T x R x V	25	0.64	1.52
T x I x V	25	0.67	1.60
R x I x V	25	0.73	1.74**
T x R x I x V	125	0.42	

TABLE X

EFFECT OF VARIOUS TREATMENTS ON THE ORGANOLEPTIC EVALUATION OF FLAVOR OF COOKED CROWDER PEAS

Individual	Average Score of 6 Replications					
	Aluminum Sulfate	Citric Acid	Steam Blanch	Phytic Acid	Control	Ferric Chloride
1	- 2.8	-1.8	-1.0	-0.7	0.0	+0.2
2	- 2.5	-1.5	-0.3	-0.8	0.0	0.0
3	- 2.0	-0.5	0.0	-0.8	0.0	+0.7
4	- 2.5	-2.2	-1.2	-0.7	0.0	0.0
5	- 1.3	+0.3	-0.7	+0.2	0.0	+0.2
6	- 0.8	-1.2	-0.5	0.0	0.0	+0.3
Total	-11.90	-6.90	-3.70	-2.28	0.00	+1.40
Mean	- 1.99	-1.15	-0.62	-0.47	0.00	+0.23
Statistical significance at 0.05 level						

TABLE XI

EFFECT OF VARIOUS TREATMENTS ON THE ORGANOLEPTIC EVALUATION OF FLAVOR OF COOKED PURPLE HULL BLACKEYE PEAS

Individual	Average Score of 6 Replications					
	Aluminum Sulfate	Citric Acid	Phytic Acid	Ferric Chloride	Steam Blanch	Control
1	-2.5	-1.2	-0.7	0.0	-0.3	0.0
2	-2.2	-0.8	-0.5	-0.2	-0.3	0.0
3	-1.3	-0.7	0.0	-0.3	+0.2	0.0
4	-1.5	-0.5	+0.3	+0.2	-0.3	0.0
5	-1.0	-0.3	+0.3	-0.3	0.0	0.0
6	-1.0	0.0	-0.5	-0.2	+0.2	0.0
Total	-9.50	-3.50	-1.10	-0.80	-0.50	0.00
Mean	-1.58	-0.59	-0.19	-0.14	-0.09	0.00
Statistical significance at 0.05 level						

TABLE XII

ANALYSIS OF VARIANCE OF THE ORGANOLEPTIC EVALUATION OF FLAVOR OF COOKED SOUTHERN PEAS

Source	D.F.	M.S.	F. Ratio
Total	431		
Treatment	5	33.60	22.86**
Replication	5	2.79	1.81
Individual	5	5.20	4.16**
Variety	1	4.69	2.76
T x R	25	1.60	1.88*
T x I	25	1.25	1.67*
R x I	25	1.32	1.65*
T x V	5	1.94	2.33
R x V	5	2.31	2.33
I x V	5	0.84	1.22
T x R x I	125	0.79	1.41*
T x R x V	25	1.13	2.01*
T x I x V	25	0.54	0.96
R x I x V	25	0.85	1.52
T x R x I x V	125	0.56	

Average taste panel scores are shown in Tables I and II for crowder and purple hull blackeye peas respectively. All the treated crowder peas were given a lower average score than the control; however, aluminum sulfate and citric acid were the only treatments which were statistically lower (.05 level). The uncooked color of the purple hull blackeye pea responded slightly different to the treatments. The ferric chloride and steam blanch treatments gave higher average scores than the control, but the only treatments which gave statistically (.05) different scores were citric acid and steam blanch.

The effect of the various treatments on the color of the two varieties is shown in Plates I and II. The aluminum sulfate and citric acid treatments gave the peas an unnatural bleached yellow-orange color. The steam blanch and ferric chloride treatments evidenced no appreciable change in color of the product. The phytic acid treatment imparted a very dark color to the product. Plate I gives an indication of the reduction of precipitated solids in the blanch water with aluminum sulfate, citric acid, and phytic acid treatments. Note the difference in settled solids between the control and above mentioned treatments.

Color of Cooked Peas

The analysis of variance of the data shown in Table VI indicates treatment and individual to be significant at the .05 and .01 levels respectively. Replication was not significant.

Results of the color evaluation of crowder peas were similar to those reported for the uncooked color factor. All treatments gave lower average scores than the control. Aluminum sulfate and citric acid were the only treatments with statistically (.05) lower average scores than

Treatments

A.	Control	D.	4 per cent Aluminum Sulfate
B.	Steam Blanch	E.	1 per cent Citric Acid
C.	5 ppm Ferric Chloride	F.	1 per cent Phytic Acid

PLATE I

COLOR PHOTOGRAPH SHOWING THE EFFECT OF VARIOUS BLANCH WATER ADDITIVES ON THE COLOR OF CROWDER PEAS

Treatments

A. Control
B. Steam Blanch
C. 5 ppm Ferric Chloride
D. 4 per cent Aluminum Sulfate
E. 1 per cent Citric Acid
F. 1 per cent Phytic Acid

PLATE II

COLOR PHOTOGRAPH SHOWING THE EFFECT OF VARIOUS BLANCH WATER ADDITIVES ON THE COLOR OF PURPLE HULL BLACKEYE PEAS

the control (Table IV).

Citric acid, phytic acid, and steam blanch treatments resulted in higher average scores than the control sample with the purple hull blackeye variety (Table V). A significant difference (.05) was exhibited only between the ferric chloride and citric acid treatments with the latter giving the highest average score.

The results obtained for the two factors, uncooked color and cooked color, were very similar except for the reversing effect shown by the purple hull blackeye variety. The significant effect of variety on the analysis of variance (Table VI) is also exemplified by the interaction due to variety. The acid treatments provided the purple hull blackeye peas with a cleaner appearing, more desirable cooked color.

Texture of Cooked Peas

Treatment and individual were significant at the .01 level as shown by the analysis of variance (Table IX).

Aluminum sulfate and citric acid treatments gave significantly (.05) lower scores than the control with both varieties. The ferric chloride treatment indicated a nonsignificant higher average score than the control. The other treatments were not significantly (.05) different from the control treatment (Tables VII and VIII).

Aluminum sulfate and citric acid treatments resulted in significantly (.05) lower average texture scores than the control, but the other treatments had no significant effect on texture of the peas. Aluminum sulfate and citric acid treatments did toughen the seed coat of the pea, but at the concentrations used, the treatments presented an adverse effect on the quality of the peas.

Flavor of Cooked Peas

Analysis of variance (Table XII) shows treatment and individual to be significant at the .01 level. Replication and variety were not significant.

With respect to the crowder variety, all treatments gave lower average scores than the control except the ferric chloride treatment which gave a nonsignificant higher average score than the control (Table X). Steam blanch, citric acid, and aluminum sulfate treatments resulted in significantly lower average scores than the control.

The purple hull blackeye variety responded differently to the treatments than the crowder variety. The aluminum sulfate treatment was the only treatment which resulted in significantly (.05) lower average score than all other treatments.

In conclusion, the analysis of variance indicates treatment and individual to have a significant effect on average taste panel scores with each of the four quality attributes. Duncan's analysis of means demonstrates that the aluminum sulfate and citric acid treatments gave significantly lower average scores for all quality attributes of both varieties except the cooked color of the purple hull blackeye variety. In this case, the citric acid treatment gave a preferred cooked color.

The treatments discussed above could be useful in the reduction of discoloration in southern peas. However, the concentrations of certain additives used in these experiments adversely affected the texture and flavor of the final product.

Anthocyanin Determinations

The nature of coloring matter of southern peas has never been elucidated. Several attempts were made in this laboratory to extract the anthocyanins from the blanched products; however, the extractives contained extremely low concentrations of pigment and high concentrations of impurities. Due to these circumstances, the complete identification procedure was abandoned. A 2 N hydrochloric acid extraction procedure was adapted in order that anthocyanidin identification would be possible.

In an attempt to determine the pigment source of the dark colored blancher precipitate, samples of whole peas, ground hulls, and blancher precipitates of both varieties were analyzed in the following manner. The anthocyanidins extractives from the above sources were purified employing a paper chromatographic technique with Forestal reagent as the developing solvent. The fractions obtained by this method were concentrated for use in later determinations.

Paper Chromatography

The various fractions were chromatogramed employing the descending technique. R_f values of the various fractions were determined utilizing three different solvent systems (Table XIII). Published R_f values for known anthocyanidins are shown in Table XIV. A composite chromatogram depicting the location of the various pigments is shown in Figure 1.

The chromatograms indicate two anthocyanidins were present in each of the sources selected regardless of variety. Also, the crowder peas and blancher precipitates contained two pigments in common as did the purple hull blackeye variety. The hull pigments of both varieties

TABLE XIII

R_f VALUES OF ANTHOCYANIDINS FROM SOUTHERN PEAS*

Source	Solvent		
	Forestal	Formic Acid	Acetic Acid
	Acetic Acid-Water-Conc. HCl (30:10:3-v/v)	Formic Acid-Conc. HCl-Water (5:2:3-v/v)	Acetic Acid-Conc. HCl-Water (5:1:5-v/v)
Crowder pea Frt. I	0.35	0.13	0.24
Crowder pea Frt. II	0.55	0.24	0.41
Crowder ppt** Frt. I	0.35	0.13	0.26
Crowder ppt Frt. II	0.53	0.24	0.41
Crowder hull Frt. I	0.54	0.23	0.41
Crowder hull Frt. II	0.70	0.57	0.66
phbe*** pea Frt. I	0.35	0.13	0.24
phbe pea Frt. II	0.55	0.24	0.39
phbe ppt Frt. I	0.36	0.14	0.26
phbe ppt Frt. II	0.53	0.25	0.42
phbe hull Frt. I	0.53	0.24	0.41
phbe hull Frt. II	0.71	0.58	0.67
Blackeye pea Frt. I	0.36	0.13	0.25
Blackeye pea Frt. II	0.53	0.23	0.40
cyanidin	0.60	0.27	-

*The R_f values were determined by descending paper chromatography measuring to the middle of pigment spot.

**ppt - blancher precipitate

***phbe - purple hull blackeye

TABLE XIV

R_f VALUES OF ANTHOCYANIDINS

Anthocyanidin	Solvent		
	Forestal*	Formic Acid*	Acetic Acid**
hirsutidin	0.78	0.36	-
malvidin	0.60	0.27	0.43
petunidin	0.46	0.20	-
delphinidin	0.32	0.13	0.22
rosinidin	0.76	0.39	-
peonidin	0.63	0.30	0.50
cyanidin	0.49	0.22	0.34
pelargonidin	0.68	0.33	0.55
luteolinidin	0.61	0.35	-
apigeninidin	0.75	0.44	-

*(18)

**(1)

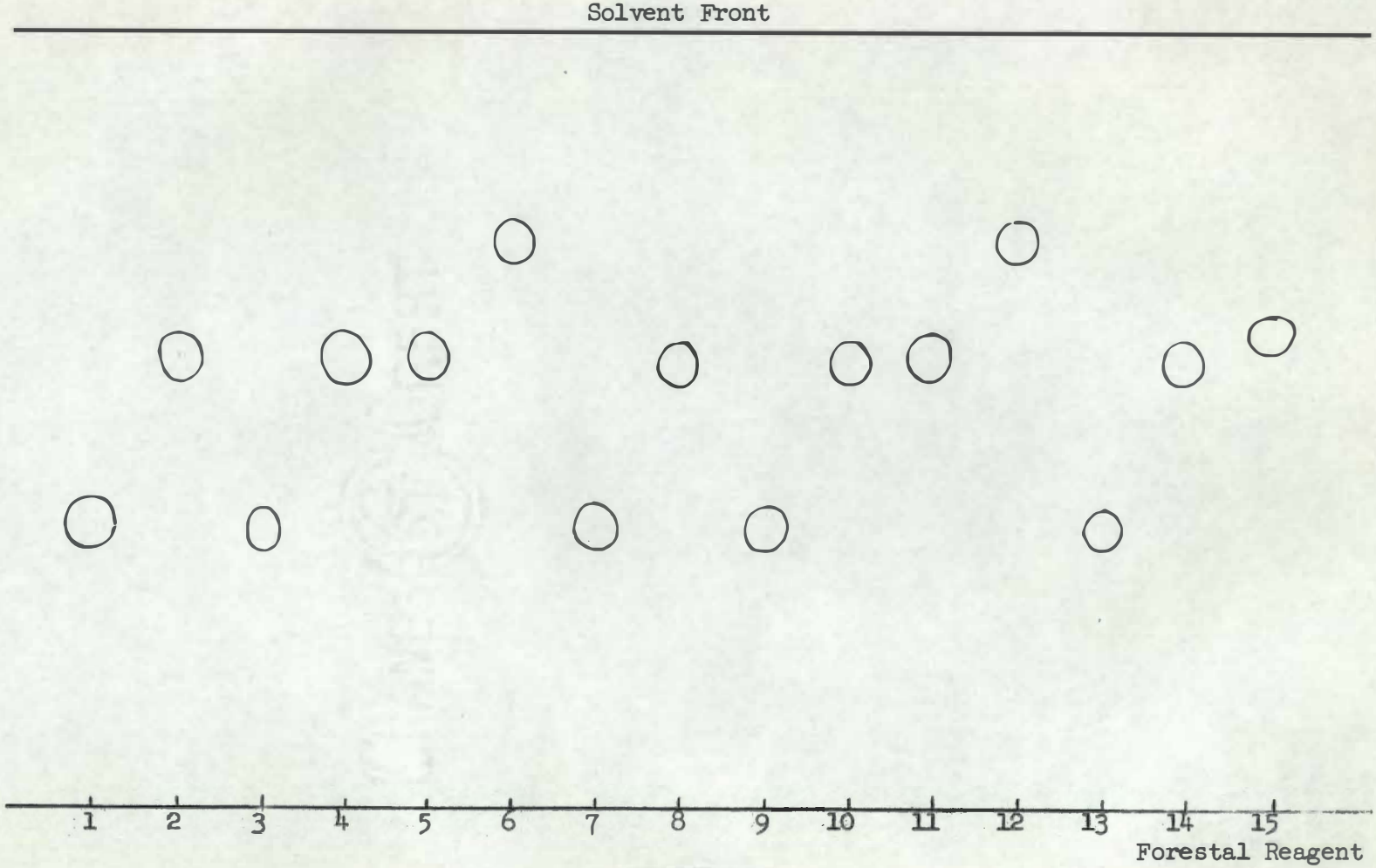


FIGURE 1

TYPICAL COMPOSITE CHROMATOGRAM OF ANTHOCYANIDINS FROM SOUTHERN PEAS

*See Table XV for Legend.

appeared to be identical irrespective of varietal effect. The hulls contained one pigment in common with the peas and another pigment not observed in the peas (Figure 1). These data suggest that the pigment of the blancher precipitate must originate in the pea. Blackeye peas were observed to have identical pigmentation as the other two varieties. The hull of the blackeye variety is not pigmented.

The anthocyanidins may be grouped irrespective of variety as follows: (1) peas and precipitate fraction I, (2) pea and precipitate fraction II and hull fraction I, and (3) hull fraction II.

Group 1 pigment gave R_f's of 0.35, 0.13, and 0.24 in Forestal, formic acid, and acetic acid reagents respectively (Table XIII). The pigment exhibited a purple color with visible light and a fluorescent mauve color under short wavelength ultraviolet light (Table XV). R_f values are difficult to reproduce from one laboratory to another since temperature, quality of solvents, type of paper, and many other factors influence the R_f value. However, a comparison of the data with the reported R_f values in Table XIV indicates that the group 1 pigment is probably delphinidin. The identification is not definite since an authentic sample of delphinidin was not available to use for comparison with the different solvents.

The group 2 pigment gave R_f values of approximately 0.54, 0.24, and 0.43 in Forestal, formic acid, and acetic acid solvents respectively (Table XIII). A comparison of the data indicates this pigment could be either peonidin or malvidin. The R_f values for this compound were very similar to the R_f values for cyandin; however, when the compounds were chromatogramed in unison, the authentic cyanidin gave a slightly higher

TABLE XV

LEGEND AND CHARACTERISTIC COLORS OF FRACTIONS SHOWN IN FIGURE 1

Legend	Visible Color	Ultraviolet Color
1. Crowder pea Frt. I	purple	fluorescent mauve
2. Crowder pea Frt. II	pink	fluorescent pink
3. Crowder ppt Frt. I	purple-pink	fluorescent mauve
4. Crowder ppt Frt. II	pink	fluorescent pink
5. Crowder hull Frt. I	pink	fluorescent pink
6. Crowder hull Frt. II	pink	non-fluorescent
7. phbe pea Frt. I	purple	fluorescent mauve
8. phbe pea Frt. II	pink	fluorescent pink
9. phbe ppt Frt. I	purple	fluorescent mauve
10. phbe ppt Frt. II	pink	fluorescent pink
11. phbe hull Frt. I	pink	fluorescent pink
12. phbe hull Frt. II	pink	non-fluorescent
13. Blackeye pea Frt. I	purple	slightly fluorescent mauve
14. Blackeye pea Frt. II	pink	fluorescent pink

value than the pigment in question. The color reactions, which will be discussed later, indicated the group 2 pigment was not cyanidin.

R_f values of the group 2 pigment were approximately 0.70, 0.57, and 0.66 in Forestal, formic acid, and acetic acid solvents respectively (Table XIII). The only pigments with approximately the same R_f values are rosinidin, hirsutidin, and pelargonidin. Pelargonidin was co-chromatogramed with group 2 pigment. The results demonstrated that the pigment was not identical with pelargonidin. Also, the visible color of the two pigments was different. Group 3 pigment exhibited a pink color in the visible range, whereas, pelargonidin was orange pink. Examination under ultraviolet light provided additional differences between the two pigment groups; pelargonidin was fluorescent, whereas, group 3 pigment was nonfluorescent. Examination of the spectral data (Table XVI and XVII) indicates the group 3 pigment exhibited similar spectral properties as rosinidin. From these data, it can be concluded that group 3 pigment appears to resemble rosinidin more closely than either of the other two pigments.

The data obtained from the paper chromatographic analysis are not conclusive. However, the data provide the following information: (1) the pigment present in blancher solids resulted from a continual removal of pigment from the peas rather than the pigment being carried into the blancher superficially by the peas from the hulls, (2) identical anthocyanidins were found in all three varieties of southern peas, and (3) the hulls of the crowder purple hull blackeye varieties contained identical anthocyanidins.

TABLE XVI

SPECTRA OF ANTHOCYANIDINS FROM SOUTHERN PEAS*

Source	Methanol-HCl	$AlCl_3$ Shift (mμ)
Crowder pea Frt. I	544	0
Crowder pea Frt. II	536	0
Crowder ppt** Frt. I	542	0
Crowder ppt Frt. II	536	0
Crowder hull Frt. I	536	0
Crowder hull Frt. II	526	0
phbe*** pea Frt. I	544	0
phbe pea Frt. II	536	0
phbe ppt Frt. I	542	0
phbe ppt Frt. II	536	0
phbe hull Frt. I	536	0
phbe hull Frt. II	526	0
Blackeye pea Frt. I	542	0
Blackeye pea Frt. II	536	0

*The spectra was determined with a Beckman DB recording spectrophotometer and verified with a Beckman DU spectrophotometer.

**ppt - blancher precipitate.

***phbe - purple hull blackeye.

TABLE XVII

SPECTRA OF ANTHOCYANIDINS IN THE VISIBLE REGION*

Anthocyanidin	Methanol-HCl	$AlCl_3$ Shift (mμ)
hirsutidin	536	0
malvidin	542	0
petunidin	543	14
delphinidin	546	23
rosinidin	524	0
peonidin	532	0
cyanidin	535	18
pelargonidin	520	0
luteolinidin	493	52
apigeninidin	476	0

*(19)

Spectral Analysis

The same fractions were used in these determinations as were used in the paper chromatography analysis.

As would be expected, the spectral analysis also demonstrated three major anthocyanidins in the various fractions (Table XVI). The aluminum chloride reagent did not give a bathochromic shift for any of the fractions.

The different fractions may be grouped into the same three groups as presented in the above section on paper chromatography. The group 1 pigments, fraction I from all varieties of peas and blancher solids, exhibited a maximum absorption in the 542-544 mμ region and a negative aluminum chloride shift. A comparison of these data with the reported spectral data in Table XVII indicates that three anthocyanidins show maximum absorption in this region. They are malvidin, petunidin, and delphinidin. The paper chromatography data eliminate malvidin and petunidin leaving delphinidin as the best choice for the group 1 pigment. However, the desired bathochromic shift was not detected in the group 1 pigments. This facet may be accounted for in that this pigment was very unstable and difficult to purify resulting in spectral curves which were difficult to interpret. Also, co-occurring flavonoid pigments may have chelated the aluminum chloride reagent before it could react with the dilute anthocyanidin pigment.

The group 2 pigment, fraction II from peas and precipitates and fraction I from the hulls, showed maximum absorption in the 536 mμ region (Table XVI). The pigments exhibiting maximum absorption in this region are peonidin, cyanidin, and hirsutidin. Malvidin may also be included in this group since the paper chromatography data indicate this pigment

as a possibility. A bathochromic shift was not detected from this pigment. The paper chromatography data eliminate cyanidin and hirsutidin. Cyanidin may also be eliminated by the color reactions (Table XVIII) which will be discussed later. On the basis of these data, the group 2 pigment appears to be either peonidin or malvidin with peonidin as the best possibility when spectral data are considered singularly.

The group 3 pigment, fraction II from the hulls, exhibited maximum absorption in the 526 mμ region (Table XVI). This pigment also gave a negative aluminum chloride shift. Comparison of the data in Tables XVI and XVII indicates rosinidin as the pigment possessing properties most similar to the unidentified group 3 pigment.

Ratios of the optical density at 440 mμ to the peak optical density were calculated, but the data were of little value since large variations were present from one replication to another.

Absorption curves are presented in Figure 2. Only representative curves from each of the groups are depicted in this figure. These curves and the data presented in Tables XVI and XVII demonstrated that only three different pigments were isolated from the entire selection of fractions from all sources. These data correspond with data presented in the section on paper chromatography. However, the data presented does not positively identify the three pigments.

Color Reactions

The data obtained from the color reactions are presented in Table XIX. Comparisons were difficult to make since Table XVIII is incomplete. The group 1 pigment, fraction I from purple hull blackeye precipitate gave identical results with delphinidin for all criterion

TABLE XVIII

COLOR REACTIONS OF REPRESENTATIVE ANTHOCYANIDINS FROM SOUTHERN PEAS

Source	Reagent				
	Cyanidin Reagent	Delphinidin Reagent	Oxidation Test	Color Test	
				Na Acetate	Ferric Chloride
phbe ppt* Frt. I	partly extracted	partly extracted	destroyed	blue	no change
Crowder pea Frt. II	extracted rose-pink color	extracted	slightly changed	reddish-violet	no change
phbe hull Frt. II	not extracted	slightly extracted	very slightly changed	pink-blue	no change
cyanidin	partly extracted rose-red color.	partly extracted	stable	red-violet	violet
pelagonidin	not extracted	slightly extracted	stable	rose-red	no change

*Blancher precipitate from purple hull blackeye peas.

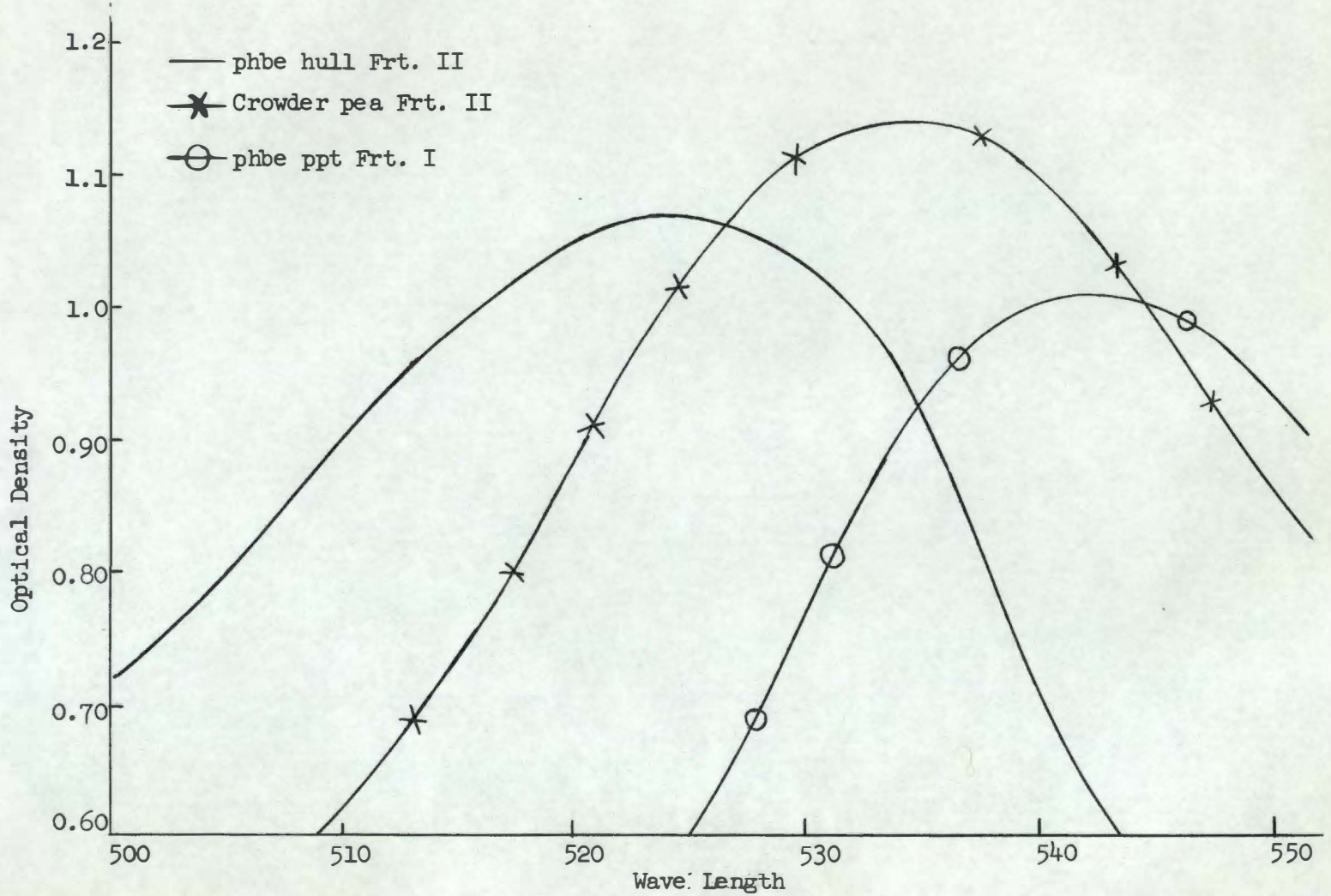


FIGURE 2

TYPICAL SPECTRAL CURVES OF REPRESENTATIVE ANTHOCYANIDINS FROM SOUTHERN PEAS

TABLE XIX

CHARACTERISTIC COLOR REACTIONS OF REPRESENTATIVE ANTHOCYANIDINS

Pigment	Reagent				
	Cyanidin Reagent	Delphidinidin Reagent	Oxidation Test	Color Test	
				Na Acetate	Ferric Chloride
pelargonidin	mostly extracted	completely extracted	not destroyed	violet-red	no change
cyanidin	imparts a rose-red color	not completely extracted	fairly stable	reddish-violet	changes to blue
malvidin	not extracted	completely extracted	almost unchanged	bluish-violet	no change
petunidin	not extracted	not extracted	destroyed	violet-blue	no change
delphinidin	not extracted	not extracted	destroyed	blue	unchanged

except the delphinidin reagent. This reagent was found to be almost useless due to the difficulty of determining if the pigment was extracted. The oxidation test showed the pigment to be very unstable to oxidation. This property was noted throughout the entire study.

Color reactions were useful in differentiating between cyanidin and group 2 pigments as represented by fraction II from crowder peas. Cyanidin gave a violet color when ferric chloride was added to the sodium acetate and pigment mixture, whereas, the pea pigment did not.

Color reactions were not conclusive in the identification of the group 3 pigment represented by fraction II purple hull blackeye hulls.

The color reactions were of limited value since the tables do not include certain of the methylated derivatives of the basic anthocyanidins.

SUMMARY

A study has been made to evaluate the effect of certain blanch water additives on the quality of southern peas and to elucidate the nature of southern pea pigmentation.

Under conditions of the experiment reported in this paper it was found that:

(1) A reduction of discoloration in southern peas was possible with the additives used, but the texture and flavor of the final product were adversely affected by the aluminum sulfate and citric acid treatments.

(2) The pigment present in the blancher solids resulted from a continual removal of pigment from the peas.

(3) Identical anthocyanidins were found in all three varieties of southern peas.

(4) The hulls of the crowder and purple hull blackeye varieties contained identical anthocyanidins.

(5) The anthocyanidins were not conclusively identified; however, a tentative identification shows the group 1 pigment to be delphinidin, the group 2 pigment to be either malvidin or peonidin and the group 3 pigment to be rosinidin.

BIBLIOGRAPHY

Bibliography

1. Abe, Y., and K. Hayashi. 1956. Further studies on paper chromatography of anthocyanins, involving an examination of glycoside types by partial hydrolysis. Botan. Mag. (Tokyo) 69, 577.

2. Bate-Smith, E. C. 1948. Paper chromatography of anthocyanins and related substances in petal extracts. Nature 161, 835.

3. Bate-Smith, E. C. 1954a. Flavonoid compounds in food. Advances in Food Research 5, 261.

4. Bate-Smith, E. C. 1954b. Leucoanthocyanins. I. Detection and identification of anthocyanidins in plant tissues. Biochem. J. 58, 122.

5. Bate-Smith, E. C., and P. Ribérau-Gayon. 1958. Symposium on biochemistry and taxonomy. Plant phenolics as taxonomic guides. Proc. Linn. Soc. London 169, 198.

6. Bate-Smith, E. C., and T. Swain. 1953. Identification of leucoanthocyanins as "tannins" in foods. Chem. and Ind. (London) p. 377.

7. Bell, J. G., and R. Robinson. 1934. Experiments on the synthesis of anthocyanins. XXIII. Glucosides of petunidin chloride. J. Chem. Soc. p. 1604.

8. Boggs, M. B., and H. L. Hanson. 1949. Analysis of foods by sensory difference tests. Advances in Food Research 2, 219.

9. Buch, M. L., E. C. Dryden, C. H. Hills, and J. R. Oyler. 1956. Organoleptic evaluation of applesauce fortified with essence and citric acid. Food Technol. 10, 560.

10. Burns, E. E., and J. W. Winzer. 1962. Pigment characteristics of the southern pea, Vigna sinensis. Proc. Am. Soc. Hort. Sci. 80, 449.

11. Byer, A. J., and D. Abrams. 1953. A comparison of the triangular and two-sample taste-test methods. Food Technol. 7, 185.

12. Culpepper, C. W., and J. S. Caldwell. 1927. The behavior of the anthocyan pigments in canning. J. Agri. Research 35, 107.

13. Culver, W. H., and R. F. Cain. 1952. Nature, causes, and correction of discoloration of canned blackeye and purple hull peas. Texas Agr. Expt. Sta. Bull. No. 748.

14. Filipello, F. 1957. Organoleptic wine-quality evaluation. II. Performance of judges. Food Technol. 11, 51.

15. Fry, P. C. 1959. Flavor evaluation of potatoes grown in soils treated with pentachloronitrobenzene. J. Ag. Food Chem. 7, 201.

16. Geissman, T. A., and L. Jurd. 1955. The anthocyanin of Spirodela oligorrhiza. Arch. Biochem. Biophys. 56, 259.

17. Gridgeman, N. T. 1955. Taste comparisons: two or three? Food Technol. 9, 148.

18. Harborne, J. B. 1958a. The chromatographic identification of anthocyanin pigments. J. Chromatog. 1, 473.

19. Harborne, J. B. 1958b. Spectral methods of characterizing anthocyanins. Biochem. J. 70, 22.

20. Harborne, J. B. 1959. The chromatography of the flavonoid pigments. J. Chromatog. 2, 581.

21. Harter, H. L. 1960. Critical values for Duncan's new multiple range test. Biometrics 16, 671.

22. Hayashi, Kôzô. 1962. The anthocyanins. "The Chemistry of Flavonoid Compounds." (T. A. Geissman, ed.) The Macmillan Company, New York, p. 248.

23. Jurd, L. 1962. Spectral properties of flavonoid compounds. "The Chemistry of Flavonoid Compounds." (T. A. Geissman, ed.) The Macmillan Company, New York, p. 107.

24. Karrer, P., and F. M. Strong. 1936. Reindarstellung von anthocyanen durch chromatographische analyse. Helv. Chim. Acta 19, 25.

25. Kramer, A., and L. P. Ditman. 1956. A simplified variables taste panel method for detecting flavor changes in vegetables treated with pesticides. Food Technol. 10, 155.

26. Li, K. C., and A. C. Wagenknecht. 1955. The anthocyanin pigments of sour cherries. J. Am. Chem. Soc. 78, 979.

27. Mann, A. 1914. Coloration of the seed coat of cowpeas. J. Agri. Research 2, 33.

28. Pruthi, J. S., R. Susheela, Girdhari Lal. 1961. Anthocyanin pigment in passion fruit rind. J. Food Sci. 26, 385.

29. Robinson, G. M., and R. Robinson. 1931. A survey of anthocyanins. Biochem. J. 25, 1687.

30. Robinson, R., and A. R. Todd. 1932. Experiments on the synthesis of anthocyanins. XVI. A synthesis of malvin chloride. J. Chem. Soc. p. 2299.

31. Sakamura, S., and F. J. Francis. 1961. The anthocyanins of the American cranberries. J. Food Sci. 26, 318.

32. Sather, L. A., and L. D. Calvin. 1960. The effect of number of judgements in a test on flavor evaluations for preference. Food Technol. 14, 613.

33. Seikel, M. K. 1962. Chromatographic methods of separation, isolation and identification of flavonoid compounds. "The Chemistry of Flavonoid Compounds." (T. A. Geissman, ed.) The Macmillan Company, New York, p. 34.

34. Snedecor, G. W. 1956. "Statistical Methods." 5th ed. Iowa State University Press, Ames, Iowa, p. 237.

35. Sondheimer, E. 1953. On relation between spectral changes in pH of the anthocyanin pelargonidin-3-monglucoside. J. Am. Chem. Soc. 75, 1507.

36. Sondheimer, E., and Z. I. Kertesz. 1948. The anthocyanin of strawberries. J. Am. Chem. Soc. 70, 3476.

37. Spaeth, E. G., and B. H. Rosenblatt. 1950. Partition chromatography of synthetic anthocyanidin mixtures. Anal. Chem. 22, 1321.

38. Tilgner, D. J. 1962. Anchored sensory evaluation tests - a status report. Food Technol. 16, 47.

39. Williams, F. W., and M. B. Allen. 1962. The Southeastern vegetable processing industry: location and number of plants - composition, volume, and value of pack, 1960. U.S.D.A. Marketing Research Report No. 527.

40. Williams, B. L., and S. H. Wender. 1952. The isolation and identification of kaempferol and quercetin from strawberries Fragaria chiloensis. J. Am. Chem. Soc. 74, 5919.

41. Willstätter, R., and A. E. Everest. 1913. Untersuchungen uber die anthocyane. I. Uber den farbstoff der kornblume. Ann. Chemie 401, 189. (abstract)

VITA

The author was born May 25, 1938, in Maysville, Missouri. He was graduated from Maysville High School, Maysville, Missouri in 1956. He was enrolled at the University of Missouri from September, 1956 until he received a B.S. degree in Agriculture in June, 1960. On July 1 of the same year he was appointed Assistant in Food Technology at the University of Tennessee, Knoxville. Since that time, he has been working to complete the requirements for the degree, Master of Science.