AN ABSTRACT OF THE THESIS OF

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Title: PROANTHOCYANIDIN CONTENT OF BANANAS AT THREE STAGES OF RIPENESS

Abstract approved: Helen G. Charley

The purpose of this study was to determine the proanthocyanidin content of bananas at the green, the ripe, and the overripe stages. Three bananas from each of three hands at each stage of ripeness, making a total of 27 bananas, were freeze-dried. The ground tissue was dispersed in methanol and the proanthocyanidin in the slurry was converted to anthocyanidin by heating in 0.8 N HCl-n-butanol containing iron ($FeSO_4 \cdot 7H_2O$). The major anthocyanidin was identified as delphinidin with a minor pigment as cyanidin. Two sub-samples of each banana were analyzed in triplicate and the absorbance of anthocyanidin was read at a wavelength of 545 mμ.

The proanthocyanidin content, expressed as milligrams of delphinidin chloride per gram of dried banana, averaged 19.7 for the green, 16.0 for the ripe, and 13.7 for the overripe. Differences in the proanthocyanidin content of the bananas due to ripeness were statistically significant.

Moisture content averaged 71.3% for the green, 73.0% for the ripe, and 74.9% for the overripe. Proanthocyanidin content on a fresh weight basis, expressed as milligrams of delphinidin chloride per gram of banana, averaged 5.63, 4.33, and 3.44 for the green, ripe and overripe, respectively.

The proanthocyanidin content of the bananas in this study was considerably higher than values previously reported. Analysis of the pulp rather than the extractable proanthocyanidin and a more acidic developer and one which contained iron were major factors contributing to the higher values.

While the proanthocyanidin content decreased significantly with ripeness of the bananas, two other groups of workers reported decreases that were proportionately greater. Their data were based on extractable proanthocyanidin and polymerization of these compounds as the fruit ripens could account for their lower values. Conversion of unextractable polymers in the pulp to soluble anthocyanidin could account for the higher values in the present study.

A high proportion of the variance for treatment mean was due to hand and relatively little due to banana on the hand, suggesting that fewer bananas from more hands could have been analyzed. A better method than skin color for assessing ripeness is suggested.

# PROANTHOCYANIDIN CONTENT OF BANANAS AT THREE STAGES OF RIPENESS

by

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# PROANTHOCYANIDIN CONTENT OF BANANAS AT THREE STAGES OF RIPENESS

## INTRODUCTION

In recent years a growing awareness of the importance of proanthocyanidins in foods has stimulated research in this group of polyphenolic compounds.

Astringency in beverages such as wine, tea, cider, and cocoa, which contributes to their unique flavors, is attributed to their proanthocyanidin content (Bate-Smith, 1954a). In fruits such as the banana, astringency decreases with ripening and this is believed to be related to the amount and condition of proanthocyanidin present.

Browning of plant tissue, especially pronounced with some fruits, is believed to be due to oxidation of polyphenols, catalyzed by phenolase when the cells are broken (Craft, 1961; Swain, 1962). Because orthodihydric and trihydric phenolic compounds are acted on by phenolase, Swain (1962) suggested that proanthocyanidins may act as substrates for this enzyme and so may contribute to enzymatic browning.

In analyzing fruits for proanthocyanidin content, the procedure usually has involved the extraction of the proanthocyanidin from the tissue, followed by conversion to anthocyanidin which is measured colorimetrically. Only part of the proanthocyanidin is extracted, with a major

portion remaining in the pulp. In few cases have workers analyzed the whole tissue of plant material for anthocyanidin. Data are available for bananas on the extractable portion only.

In this study the proanthocyanidin content of banana tissue at three stages of ripeness was determined.

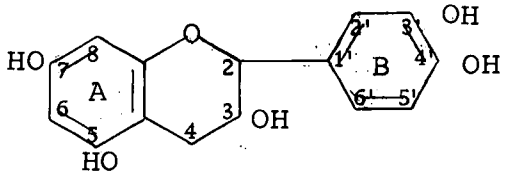
# REVIEW OF LITERATURE

## Nomenclature

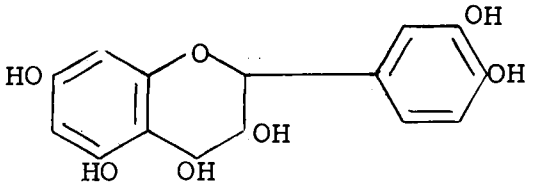
Various terms have been suggested for the colorless precursors which yield anthocyanidin on treatment with hot acid. The term "leucoanthocyanin" was introduced by Rosenheim (1920) and subsequently the terms "leucoanthocyanin" and "leucoanthocyanidin" were used interchangeably to denote compounds of this class. This practice was not in keeping with the fact that the term anthocyanin indicates a glycoside while anthocyanidin is used to indicate an aglycone. In fact, most compounds in the group which yield anthocyanidins are not glycosides (Clark-Lewis, 1962a).

Compounds of this class exhibit properties characteristic of condensed tannins and in many instances have been so designated, a factor adding to the confusion in terminology. One characteristic of tannin-like compounds is that they polymerize on treatment with hot acid to yield "phlobaphene" or tannin reds (White, 1957). "Phlobaphenes" are amorphous reddish brown precipitates formed as a secondary reaction by oxidation when flavan-3,4-diols are heated with mineral acid (Bate-Smith and Swain, 1953). In fact, Bate-Smith and Swain (1953) regarded flavan-3,4-diols as well as flavan-3-ols as possible prototypes of condensed tannins.

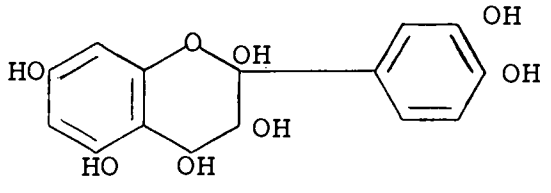
Harris and co-workers (1958) suggested the term "anthocyanogen," while Freudenberg and Weinges (1958), cited by Swain (1962), preferred to call compounds with a flavan-3,4-diol structure "leucoanthocyanin hydrates." The term "leucoanthocyanin" has been used frequently to indicate the the monomeric $C_{15}$ molecule since compounds with a flavan-3,4-diol structure from sources on boiling with mineral acid yield flavylium salts (Roux and Maihs, 1960). However, substances present in plants which give this reaction may be not only monomers but polymers whose structure is uncertain. These polymers may be of any size and could be formed by condensation of flavan-3-ols (catechin):




or the flavan-3,4-diols:




or the flavan-2,3,4-triols (Swain, 1962).



In an attempt to clarify the terminology, Swain (1962) suggested that the polymers or copolymers of the flavan-ols, flavan-diols, and flavan-triols should be called "flavolans" and only those compounds which yield flavylium salts should be termed "flavylans."

Geissman and Dittmar (1965) have suggested that the "leucoanthocyanidins" comprise the following distinct groups. One consists of the flavan-3,4-diols that are converted into the common anthocyanidins, pelargonidin, cyanidin, and delphinidin. Another group comprises the flavan-3,4-diols usually present in woods and barks which are converted into anthocyanidins with unusual hydroxylation patterns. The third includes a group of compounds which consists of two flavan derivatives, forming a 30-carbon-atom structure, which upon conversion yields a flavan-3-ol and an anthocyanidin. The fourth includes an ill-defined group of polymeric substances which can be decomposed by hot acid treatment to yield anthocyanidins.

Freudenberg and Weinges (1960), as cited in 1962, have recently proposed the term "proanthocyanidin" since it has no definite structural implications and refers only to the fact that the compound yields anthocyanidins on treatment with hot acid. The term proanthocyanidin will be used in this report since the actual structure of the flavylium-yielding compounds in the banana is not known.

Proposed Structure of Proanthocyanidins

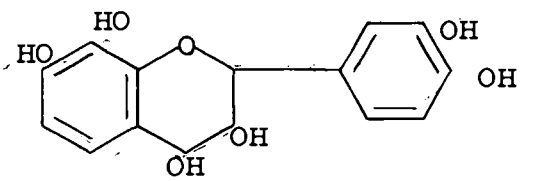
According to Roux (1958, p. 1454) there are few instances in the field of chemical research "when so little fact has given rise to so much speculative theory" as is found with condensed tannins. This observation can be easily understood upon consideration of the following factors concerning condensed tannins: their non-crystalline nature, complex structure, usual occurrence in complicated extract mixtures, and ease of denaturation through oxidation (Roux, 1958).

Rosenheim in 1920 first demonstrated the presence of a water-soluble, colorless precursor of oenidin in grape leaves which he converted, by treating with hot acid, into the corresponding anthocyanidin. He advanced the hypothesis that the precursor was a glycoside of the pseudo-base of anthocyanidin. Subsequently Robinson and Robinson (1933) showed this to be improbable since such pseudo-bases are rapidly converted into the parent anthocyanidin even by cold acids (10 to 15% aqueous HCl). They proposed the name peltogynidin for this precursor since it exhibited characteristic properties distinguishing it from all known substances.

Although most proanthocyanidins are not glycosides, Ito and Oshima (1962) found leucodelphinidin-3-glucoside to be the major proanthocyanidin in persimmons. The presence of this compound has been confirmed by

Nakayama and Chichester (1963).

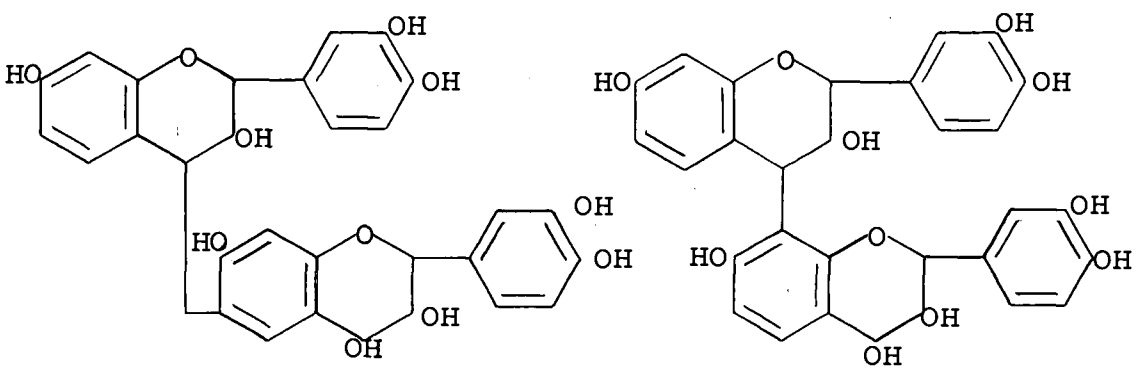
The structure of melacacidin, the first natural proanthocyanidin to be isolated, was elucidated by King and Bottomley (1953). They demonstrated that melacacidin, which they obtained from the heartwood of Australian blackwood (Acacia melanoxylon), possesses typical proanthocyanidin properties and focused attention on the flavan-3,4-diol structure. King and Clark-Lewis (1954) reduced the flavone with the hydroxylation pattern which corresponds to melacacidin and isolated melacacidin in crystalline form. This provided conclusive evidence for the flavan-3,4-diol structure shown below.




A basic flavan-3,4-diol structure does not account for the properties of all isolated proanthocyanidins since several naturally occurring polymeric compounds have been isolated (Forsyth, 1952; Roux, 1957). These compounds yield anthocyanidins when heated with acid; however, they may be polymers of different molecular weights or stereoisomers (Manson, 1960). Since the flavan-3,4-diols have three centers of asymmetry, four racemates and eight optically active forms are possible for each structure. The four racemates are divided into two classes according

to a trans-configuration or cis-configuration which are designated as catechin or epicatechin types, respectively (Clark-Lewis, 1962a, b).

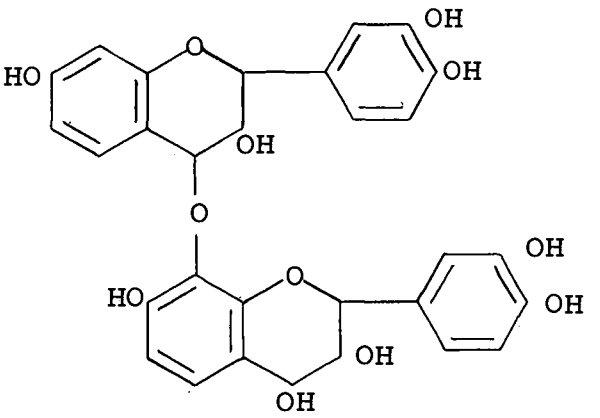
Several pathways for the polymerization of a flavan-3,4-diol are possible. These include an acid-catalyzed mechanism favored by Freudenberg and Weinges (1962) and an oxidative polymerization preferred by Hathway and Seakins (1957a, b). Freudenberg and Weinges (1962) have suggested that the polymers of the flavan-3,4-diols are linked between $C_4$ and $C_6$ or between $C_4$ and $C_8$.



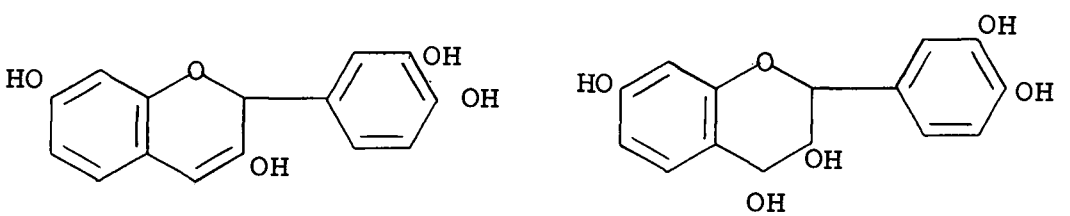
Further condensation could take place and would probably involve condensation at the $C_2$ position of additional flavan-3,4-diol molecules (Haslam, 1966, p. 78).

Another explanation of self condensation of the flavan-3,4-diols has been suggested by Roux and Paulus (1961c). They proposed that the reaction proceeds with the formation of ether linkages between the $C_4$ position of one flavan-3,4-diol and the $C_6$ or $C_8$ of another unit.

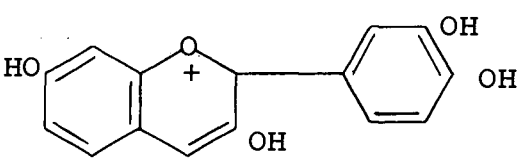
This type of linkage would be expected to separate readily with the formation of anthocyanidin.



To accommodate the observation of Roux (1958) that the polymeric proanthocyanidins yield flavan-3,4-diols or their transformation products on acid treatment, Freudenberg and Weinges (1962) proposed a mechanism for the separation of these polymers in acid. These reactions involve a splitting of the polymer, resulting in the liberation of two $C_{15}$ monomers



with the flavan-3,4-diol being converted to anthocyanidin.



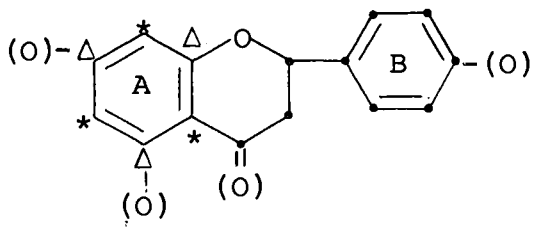
It has been suggested by Creasy and Swain (1965, p. 152) "that condensed tannins of the flavolan type are

complex, branched, three-dimensional structures in which the majority of the repeating units are nuclei derived from hydroxyflavan-3-ols and -3,4-diols." Subsequent changes in the initial polymers could occur by oxidation (Hathway, 1958), by formation of bonds between the $C_2$ and $C_{2'}$ or $C_{6'}$ positions (Freudenberg and Weinges, 1962), and by the formation of ether type linkages (Roux and Paulus, 1961c; Jurd and Waiss, 1964).

Biosynthesis of Proanthocyanidins

Early in the work on the structure of flavonoids certain regularities were noted in the pattern of hydroxyl and methoxyl groups. With few exceptions these substituents in ring A are in the meta position while in ring B they are in ortho position to each other (Grisebach, 1965).

In 1953 an important contribution to biosynthesis was made by Birch and Donovan. They proposed the hypothesis that flavonoids arise from the addition of three acetate units to cinnamic acid or a related compound. It is now certain that ring A is formed by a head-to-tail condensation of three acetyl (malonyl) units while ring B and carbon atoms two, three, and four originate from an intact phenylpropane unit (Grisbach, 1965).




• phenyl propane unit
Δ carboxyl group of acetate (malonate)
* methyl group of acetate (malonate)

The phenylpropane units are formed in higher plants by the shikimic or prephenic acid pathway (Sprinson, 1960).

Some observations support the suggestion that the conversion of a flavonol to the corresponding anthocyanidin may be possible in plants through intermediates such as proanthocyanidins, either directly or indirectly (Bogard, 1958). A second possibility is that a branching occurs at some point during biosynthesis with the formation of related compounds at various oxidation levels (Bogard, 1958). Others consider that the flavan-3,4-diols and the flavan-3-ols are synthesized from a single $C_{15}$ precursor. This theory is based on the co-occurrence in the same plant species of various flavonoid compounds with similar hydroxyl patterns in the aromatic rings (Haslam, 1966, p. 158). Once the $C_{15}$ intermediate is formed it is modified in a variety of ways to yield the different flavonoid types found in nature. At present no precise data is available about these interrelationships (Harborne, 1967, p. 269).

Roux and Paulus (1961a), following a study of the

distribution of flavonoid compounds in various heartwoods, Acacia mollisima, Schinopsis species (1961b), and Robinia pseudacacia (1962b), have suggested that the flavan-3,4-diols have a central position in flavonoid biosynthesis, are the first formed, and subsequently are converted to other flavonoids by a combination of reduction and dehydrogenation reactions.

The most widely accepted theory for the formation of proanthocyanidins is "that condensed tannins are derived by oxidative or simulated acid catalysed condensation of flavan-3,4-diols" (Haslam, 1966, p. 160).

Extraction of Proanthocyanidins

The isolation of pure compounds is complicated since many proanthocyanidins are accompanied in plants by closely related substances and are susceptible to polymerization and oxidation. Whatever method of extraction is used the final extract usually contains phenolic compounds of low molecular weight as well as polyphenolic compounds (Clark-Lewis, 1962a).

Although the analysis of proanthocyanidins has usually been on the extractable portion, three cases have been reported in which the proanthocyanidins remaining in the pulp were analyzed. Hillis and Swain (1959) extracted fresh plum tissue exhaustively with absolute methanol, followed by extraction with 50% aqueous methanol, and

reported that proanthocyanidins were retained in the marc. Craft (1961) extracted polyphenols of Elberta peaches with 70% ethanol and determined the proanthocyanidin content in both the filtrate and slurry. He found that an appreciable amount of proanthocyanidin remained in the pulp. The proanthocyanidin content of extract, marc, and slurry of Bartlett pears was analyzed by Smathers and Charley (1967). They reported that 95% ethanol extracted approximately one-fourth, methanol one-third, and 70% acetone (ethanolic) almost two-thirds of the proanthocyanidin of the pears. However, Joslyn and Goldstein (1964a) found acetone less efficient than methanol or ethanol in extracting proanthocyanidins from astringent tissue such as sulfited persimmons.

Polymerization of the proanthocyanidins during the ripening of fruit results in a reduction of the extractable portion. Other changes occurring on ripening which may affect the extraction of proanthocyanidins include an increase in the polysaccharide constituents and the water content of the cells (Goldstein and Swain, 1963). The peach undergoes considerable changes in texture on ripening, but no observable reduction in proanthocyanidins takes place (Craft, 1961). In contrast, textural changes are less drastic in the banana, but the extractable proanthocyanidins are reduced to a large extent (Barnell and Barnell, 1945; De Swardt, Maxie, and Singleton, 1967).

However, in the banana and persimmon there are special localized "tannin cells" while in the peach the proanthocyanidins are distributed throughout the tissue (Goldstein and Swain, 1963). Goldstein and Swain (1963) observed that a larger quantity of proanthocyanidin was extracted from the unripe than from the ripe banana with absolute methanol while the reverse was true when 50% methanol was the solvent.

Based on their solubility in anhydrous and aqueous organic solvents, Hillis and Swain (1959) showed that proanthocyanidin of plums can be divided into three classes. The first two were successively extracted with absolute methanol followed by aqueous methanol while the third remained in the residue, not being extracted by these or other solvents. The first fraction was believed to be composed of monomeric and oligomeric compounds, mainly mobile on paper chromatograms. The second fraction, extracted by aqueous methanol, contained mainly components that were non-mobile on the chromatograms. These were believed to have a higher molecular weight than the first group, since the polymers had a high affinity for cellulose and usually remained near the point of application (Roux and Evelyn, 1958). This fraction was not necessarily insoluble in absolute methanol but could be bound by hydrogen bonds to the cell walls or proteins in the plant material and were released only when the aqueous solvent

broke the bonds. In a similar way the third fraction possibly contained proanthocyanidins which were not really insoluble but were more firmly bound to the cell wall polysaccharides or other polymers (Hillis and Swain, 1959).

Hillis and Swain (1959) and subsequently Goldstein and Swain (1963) assumed the monomers and oligomers of proanthocyanidin were soluble in absolute methanol but the more highly polymerized products were not. They used the difference in extraction of phenolics with 100 and 50% methanol as a measure of the changes in molecular size.

Conversion of Proanthocyanidin to Anthocyanidin

The quantity of proanthocyanidin present in plant tissue is determined by conversion to anthocyanidin when the extract or pulp is heated with acid. The yield varies with the conditions of the treatment and the state of the proanthocyanidins in solution (Bate-Smith and Metcalfe, 1957). In early work the Robinsons (1933) used ten percent aqueous HCl while Bate-Smith and Lerner (Bate-Smith, 1954b; Bate-Smith and Lerner, 1954) used hot aqueous 2 N HCl in a hot water bath.

Pigman et al. (1953) devised a semiquantitative test for the estimation of proanthocyanidins which was based on spectrophotometric measurement of anthocyanidins produced by hot acid. A calculation of the concentration of anthocyanidin, based on the extinction coefficient of

cyanidin, indicated that the yield was only 7 to 20% of the theoretical. Instead of aqueous or methanolic-HCl solutions, Pigman et al. (1953) introduced the use of boiling solutions of n-propanol-HCl for the conversion. Swain and Hillis (1959) used n-butanol (b.p. 117.7° C.) in place of the more volatile n-propanol (b.p. 82.3° C.).

In the early work the low yield of anthocyanidin was believed to be due to the difficulty in isolating the proanthocyanidins (Robinson, 1937). In most cases considerable "phlobaphene" develops when proanthocyanidins are converted by boiling with acid and particularly aqueous acid (Clark-Lewis, 1962a). According to McFarlane and Vader (1962, p. 256) the yield of anthocyanidin from proanthocyanidin "depends primarily on the concentration of HCl and to (sic) the presence of an oxidation catalyst such as iron, and to a lesser degree on the solvent employed." By using a special adsorbent for purification of the proanthocyanidins in beer, a higher concentration of acid could be used, resulting in a higher yield of anthocyanidin without the formation of a reddish brown precipitate of "phlobaphene."

The presence of a small amount of iron or copper in the n-butanol-HCl solution was found by McFarlane (1961) to improve the yield of anthocyanidin converted from proanthocyanidin and results were more reproducible. Relatively large amounts of iron (10 ppm) were required

to obtain a maximum color intensity as compared with copper (0.2 ppm). However, considerable fading was apparent with the copper, and the iron was preferred. Also, McFarlane (1961) observed that the iron did not alter the optical density of the anthocyanidin. The accelerated conversion of proanthocyanidin to anthocyanidin was believed to be due to the formation of an iron chelate complex during the initial stages of the reaction. Govindarajan and Mathew (1965) disagreed with this suggestion that a chelate of iron was formed with the proanthocyanidin, since the iron was effective only when the developing reagent which contained the iron and the proanthocyanidin were heated to bring about the conversion.

Maximum conversion of proanthocyanidin resulted with a concentration of three milligrams of iron (using $FeSO_4 \cdot 7H_2O$) per 100 milliliters of reagent (McFarlane, 1961; Govindarajan and Mathew, 1965). Comparisons were made by Govindarajan and Mathew (1965) on aqueous extracts of fresh and dried plant material, using a developing reagent of 40% (5 N) HCl-*n*-butanol containing the iron with a conversion time of 15 minutes and a developing reagent of 5% (0.6 N) HCl-*n*-butanol for 40 minutes. The results varied with the different plant extracts, but the reagent with the iron and the shorter conversion time consistently yielded higher values. In the case of the dried green Cavendish banana the yield was twice as high.

Using the two levels of acidity, 5 and 40%, Govindarajan and Mathew (1965) compared the yield of cyanidin from two synthetic leucocyanidins, areca leucocyanidin and leucofisetinidin. Conversions were effected at both levels of acidity with and without iron in the reagent. Highest yield of cyanidin was obtained with 40% HCl-n-butanol containing iron, and lowest yield with 40% HCl without the iron. In all four instances, maximum absorbancy occurred at a wavelength of 545 mμ. The areca leucocyanidin exhibited a definite peak at 450 mμ also, and leucofisetinidin showed a small second peak. The absorbancy at the shorter wavelength may possibly be explained by the presence of a free 5-hydroxy group (Harborne, 1958) and an increased production of "phlobaphene" polymers from acid-catalyzed self-condensation (Hathway and Seakins, 1955; Swain and Hillis, 1959).

In the conversion of pear proanthocyanidin to anthocyanidin, Smathers and Charley (1967) used several normalities, 0.025, 0.05, 0.1, and 0.6, of HCl in n-butanol. They observed the greatest yield of anthocyanidin at 0.025 N HCl when the marc was dispersed in 70% ethanol, but when dispersed in 70% acetone (ethanolic) the greatest yield was at 0.6 N HCl.

The rate of conversion of three purified "leucoanthocyanidins" was observed by Joslyn and Goldstein (1964c) at several temperatures and various heating periods. They

used a developing solution of 0.66 N HCl-n-butanol for the conversion of grape, cacao, and melacacidin "leucoanthocyanidins." The cyanidin converted from the "leucoanthocyanidins" varied from 0.1% at 50° C. for five minutes to near 10% for 60 minutes at 90° C., with the greatest yield from the grape "leucoanthocyanidin." Smathers and Charley (1967) compared various heating times, up to 40 minutes. A greater conversion of proanthocyanidin from pear tissue was noted with the longer heating time when 70% acetone was the dispersing medium and conversion was effected with 0.6 N HCl-n-butanol, although 94% of the conversion had occurred in the first ten minutes. When the marc was dispersed in 70% ethanol and heated with 0.025 N HCl, only 92% of the anthocyanidin developed in 40 minutes.

Pigman et al. (1953) reported that the conversion of proanthocyanidins in n-propanol-HCl was reduced in rate and extent by the presence of water. These results were confirmed and extended by Roux and Bill (1959). They reported yields of approximately 40% with anhydrous 0.03 and 0.02 N HCl-n-propanol in comparison to a 3% yield in aqueous 3 N HCl. Roux and Bill (1959) proposed that the conversion of proanthocyanidin to anthocyanidin occurs by dehydration at the diol group and also reported a greater yield of anthocyanidin when moisture was excluded from the reaction mixture. In a more recent study Govindarajan and Mathew (1965) reported that up to 20% water did not

adversely affect the yield of anthocyanidin. They further stated that the optimum amount of water is 10%, the amount usually introduced when fresh tissue is analyzed.

Roux and Paulus (1962a) determined the effect the degree of polymerization of the proanthocyanidin had on the yield of anthocyanidin. The conversion of leucofisetinidin from the heartwood of black wattle was reported to be approximately 24% for the monomeric leucofisetinidin, decreasing to approximately 7% for the trimeric and to 5% for the more highly polymerized components.

Relationship Between Proanthocyanidins and Astringency

Astringency is believed to be due to the presence of tannins (Bate-Smith, 1954a) and its loss is one of the major changes that takes place during the ripening of many fruits (Goldstein and Swain, 1963). Some fruits such as bananas (Barnell and Barnell, 1945) and persimmons (Joslyn and Goldstein, 1964a) exhibit a marked loss of astringency on ripening while others such as peaches (Craft, 1961) do not. The relationship between tannin content and astringency is not completely understood (Goldstein and Swain, 1963).

The naturally occurring phenolic compounds have long been associated with astringency (Joslyn and Goldstein, 1964a). In 1953 Bate-Smith and Swain proposed that proanthocyanidins were responsible for astringency as well

as "phlobaphene" formation. Swain (1954) later found the monomeric proanthocyanidins to be only slightly astringent while the oligomers were definitely so.

Astringency is a dry, puckering sensation in the mouth following the consumption of unripe fruit and probably arises from the cross-linking of proteins and glycoproteins by tannins (Bate-Smith, 1954a). The low molecular weight phenolic compounds, including the precursors of tannins, are apparently too small to form effective cross-links and are not conspicuously astringent, while the highly polymerized components are either too insoluble or too large to fit between the protein molecules. Maximum astringency is probably due to tannins of intermediate size. Thus in ripening fruits it may be expected that changes in astringency are the result of changes in the molecular size of the tannins (Goldstein and Swain, 1963).

Hillis and Swain (1959) found that as plum fruit matures the proanthocyanidin content decreases on a fresh weight basis with probably net synthesis ceasing during the process of ripening. A continuation of the polymerization process, leading to an increased proportion of higher molecular weight phenols in the fully ripe fruit, has been suggested by Goldstein and Swain (1963). Changes of this type appear to take place in the banana, with the "tannin cells" being reported to appear "caked" in the ripe fruit (Barnell and Barnell, 1945).

The extent of polymerization needed to produce astringency is not known. Astringent proanthocyanidins isolated from cocoa, apples, peaches, bananas, and other fruit apparently are polymers, while the proanthocyanidins found in wood are monomers (Joslyn and Goldstein, 1964a).

Proanthocyanidins in the Banana

The cultivated or edible bananas are vegetatively parthenocarpic and have their origin in the wild seedy fruit that requires pollination (Simmonds, 1966, p. 42). The two principal edible clones are the Gros Michel and Cavendish, both derived from the wild species Musa acuminata (Simmonds, 1966, p. 63).

The proanthocyanidins in the banana are found in the latex system which consists of the largest and most conspicuous cells of the fruit. Within the latex cells is a watery fluid that is milky white when fresh but on standing in contact with air turns a light brown due to the proanthocyanidin content (Barnell and Barnell, 1945; Von Loseche, 1950, p. 35). Histochemical investigations by Barnell and Barnell (1945) indicated that the latex vessels in the pulp are at right angles to the long axis of the fruit and also outline the individual carpels.

The Barnells (1945) used the diastase inactivation method to estimate a tannin fraction which they assumed was responsible for astringency in the unripe fruit.

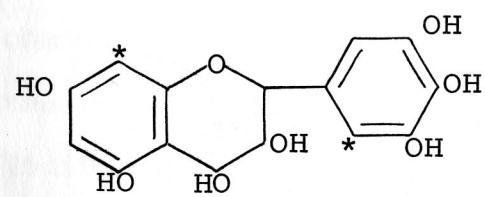
This fraction decreased sharply on ripening and was approximately one-fifth of the amount found in green fruit; the fraction was three to five times more abundant in the peel than in the pulp. This trend corresponds to the change in astringency that is generally detectable by taste. During the ripening process, Barnell and Barnell (1945) observed that the cells in the latex vessels became "tanniferous," the intensity of the staining reaction declined, and the latex in the vessels gelled and shrank and no longer filled the cells. These authors and subsequently Young (1965) found that when the latex cells were broken in green fruit the tannin diffused into the surrounding tissue but on ripening the tannin became a nondiffusible gel.

Bananas exposed for long periods to low temperatures have about twice the concentration of proanthocyanidin compared to fruit ripened in the usual manner (Barnell and Barnell, 1945). The Barnells suggested that the permeability of the "tannin cells" in the chilled fruit had been altered so that proanthocyanidin spread into the surrounding tissue. When this occurs, bananas do not undergo the changes that lead to "normal" loss of astringency with ripening.

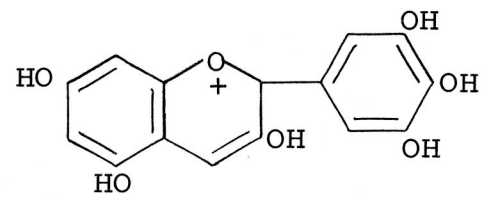
Changes in the color of the banana peel are often used to determine the stage of ripeness of the fruit. However, the visual changes from green to yellow are

often not rapid enough to be used as the only criterion for the physiological state of the fruit (De Swardt, Maxie, and Singleton, 1967; Maxie and Sommer, 1968) since the ripening process proceeds from the center of the fruit outward (Simmonds, 1966, p. 227). The skin may appear green when the banana is more than half way through the ripening process. Because the banana is a climacteric fruit in which the respiratory rate follows a definite pattern in ripening, the respiratory rate could be used to determine the physiological state of the banana (De Swardt, Maxie, and Singleton, 1967; Maxie and Sommer, 1968).

Robinson in 1937 suggested that the banana contained proanthocyanidins which yielded delphinidin. More recently Simmonds (1954) in a chromatographic survey observed that the proanthocyanidin in the fruit yielded mainly delphinidin and a lesser amount of cyanidin. Thus the precursor of delphinidin has definitely been identified in the banana fruit (Jones, 1965; Simmonds, 1966, p. 453; De Swardt, Maxie, and Singleton, 1967). The $C_{15}$ flavan-3,4-diol, with possible sites of polymerization and the flavylium ion of delphinidin are shown.




Flavan-3,4-diol




Delphinidin

Although information about the amount and the condition of proanthocyanidin in the banana is meager, a decrease in astringency of the banana to an acceptable level on ripening is believed to be associated with polymerization of the proanthocyanidins. With the exception of studies by Swain and Hillis (1959), Craft (1961), and Smathers and Charley (1967), values for the proanthocyanidin content of fruits have been based on the extractable portion rather than that in the entire tissue with considerable proanthocyanidin being retained in the pulp. Young (1965) reported, on the basis of microscopic studies, that the total proanthocyanidin in the banana remains constant, but that polymerization which occurs on ripening is accompanied by a decrease in the extractable fraction.

Because of the possible relationship of proanthocyanidins in fruits both to astringency and to susceptibility to browning, and because of the limited information concerning the proanthocyanidins in the banana, this project was undertaken.

# METHOD

## Selection and Preparation of Bananas for Analysis

Ripeness Levels of Bananas. Bananas[1] used in this study were obtained from the same shipping lot from a local wholesaler. Fruits at three stages of ripeness, green, ripe, and overripe, were analyzed. Ripeness of the fruit was judged by the color of the banana skin, using a commercial color chart showing eight stages of ripeness (Von Loesecke, 1950). Fruit representing the green stage had an all-green skin matching the No. 2 stage of ripeness, that of the ripe stage had a yellow skin with green tips (No. 5), and the overripe fruit had a yellow skin with brown flecks (No. 7). For each stage of ripeness three adjacent bananas from each of three hands, making a total of 27 bananas, were obtained and held in the laboratory until the desired stage of ripeness was reached.

Freeze-drying. In the preliminary work, slices of banana heated in acidified butanol developed a deep red color in the center of the slices and along the outlines of the individual carpels. Because of this uneven distribution of proanthocyanidin and also to arrest further changes once the desired stage of ripeness was reached, the bananas were freeze-dried prior to reducing the individual bananas to a powder.

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[1] Gros Michel, Valorie Brand, from Panama.

Before freeze-drying the bananas it was determined with a synthetic leucocyanidin[2] that the addition of sodium metabisulfite (Ito and Joslyn, 1965) did not alter the conversion of leucocyanidin to cyanidin. The bananas were sliced with a stainless steel knife, dipped in a one percent sodium metabisulfite solution (Brekke and Allen, 1967), and frozen in an air-blast freezer at -35° C. The freeze-drying[3] occurred under 30 microns of mercury at a temperature of 35° C. for approximately 62 hours, resulting in a dried product with a final moisture content of less than one percent. Each freeze-dried banana was placed in an air-tight glass container and stored in a freezer until analyzed.

Moisture Determination. The moisture content of each banana was determined in duplicate. As each banana was prepared for freeze-drying, a slice of approximately ten grams was removed from the center section prior to slicing the banana for freezing. The slice was macerated until homogeneous and then divided into two portions. The green banana slices were difficult to macerate and were first minced with a stainless steel knife. The macerated banana was put into a pre-weighed aluminum container and weighed to the fourth decimal place. Approximately three

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[2] Fluka, AG, Buchs SG, Switzerland.

[3] Hull Corporation, Hatboro, Pennsylvania.

milliliters of methanol were sprayed on the banana to prevent browning before the sample was frozen. The samples were kept frozen until they were dried in an oven under 24 inches of vacuum at 50° C. for 24 hours. The dried samples were removed from the vacuum oven to a desiccator for equilibration to room temperature before reweighing.

Grinding the Banana to a Powder. Each banana was ground for three minutes with a household blender[4] attached to a variable transformer set at 100 volts. The sides of the blender were scraped down and the banana powder ground for an additional three minutes. Grinding was done in a freezer, a precaution employed to avoid uptake of moisture by the hydroscopic banana powder. The powder was stored in a tightly capped glass container in the freezer.

Analyzing Bananas for Proanthocyanidin

Preparation of the Slurry. When the sample was to be analyzed, the banana powder was transferred to a weighing bottle. Each sample was weighed in duplicate on an analytical balance with an effort to keep the weight of the sample near 0.4500 gram. Weighed samples were covered with parafilm since the powder absorbs moisture and agglomeration results.

Methanol was used as the dispersing medium. The use of 70% acetone (methanolic) as a dispersing medium was

[4]Waring Blender, Waring Products Corporation, New York, New York.

investigated in the preliminary work. Smathers and Charley (1967) had observed the greatest yield of anthocyanidin from proanthocyanidin in pears with 70% acetone as the dispersing agent. According to Joslyn and Goldstein (1964a) acetone is not superior to methanol or ethanol in extracting proanthocyanidin from dried, astringent tissue. In this work when 70% acetone (methanolic) was compared to absolute methanol as a dispersing agent for synthetic leucocyanidin, little difference in the yield of cyanidin was obtained. Therefore, methanol was selected as the dispersing medium.

To disperse the powder one milliliter of methanol was added to the weighed sample followed by one milliliter of water for rehydration. Water was used since better suspension of the powder resulted when the remainder of the methanol (20 milliliters) was added. Although various workers (Pigman et al., 1953; Roux and Bill, 1959) have advocated the use of anhydrous conditions to obtain a greater conversion of proanthocyanidins to anthocyanidins, more recently Govindarajan and Mathew (1965) reported that 20% water (with 10% water as optimum amount) did not affect the yield.

The suspension of banana powder in methanol was boiled for five minutes to deactivate enzymes. The ground tissue from the green banana was easily dispersed; that from the ripe and overripe bananas tended to lump. Suspensions of

the last two were transferred to the blender, using 15 milliliters of methanol, and then blended for two minutes at 100 volts, followed by three additional minutes at 90 volts. Once dispersed, each sample was transferred to a 100-milliliter volumetric flask and made to volume. The ground banana powder suspended in the dispersing solvent will hereafter be referred to as the slurry.

Conversion of Proanthocyanidin to Anthocyanidin. The conversion of proanthocyanidin in the banana was first attempted with 2 N HCl-n-butanol containing $FeSO_4 \cdot 7H_2O$ to give iron at a concentration of three milligrams per 100 milliliters. With this reagent considerable "phlobaphene" was developed, so comparisons were made using various normalities of acid with and without the iron in the reagent. When the acidity of the developer was reduced to 1.4 N HCl and the conversion time was ten minutes, some "phlobaphene" developed while at normalities of 0.1, 0.2, 0.4, 0.6, and 0.8 only the color typical of anthocyanidin resulted. Conversion of proanthocyanidin in banana slurry with the developer acidified to 0.2, 0.4, 0.6, and 0.8 N HCl were compared at five minute intervals up to 30 minutes. A greater yield of anthocyanidin resulted at all normalities when the developing reagent contained iron, and the yield was greatest at the highest normality. When synthetic leucocyanidin (rather than leucodelphinidin, which was unavailable) was used in place of banana slurry,

a maximum yield of anthocyanidin was obtained when the developer containing the iron was 0.8 N with respect to HCl, compared to 0.4 N, and when the conversion time was 20 minutes. Therefore, 0.8 N HCl-n-butanol containing iron with a conversion time of 20 minutes was used in subsequent work.

Each banana was analyzed in duplicate with three conversions for each analysis, making a total of six determinations per banana. For the conversion one milliliter of the slurry was pipetted into a 7" by 1" test tube provided with a ground-glass, solid penny head stopper. A pipette with a wide aperture was used for measuring the slurry. Getting a representative sample was difficult due to the tendency of the powder to settle. Ten milliliters of the developing reagent was pipetted into the tube containing the sample. The developer was used to wash down any of the pulp on the sides of the test tube. For the conversion the tubes were placed unstoppered in a boiling water bath. After three minutes the stoppers were placed in the test tubes and the cover of the water bath was secured to exclude light. After a total heating time of 20 minutes the stoppers were removed and the test tubes were transferred from the water bath. The contents of each tube were filtered through glass wool to remove the banana pulp. The filtrate was stored in the dark until it reached room temperature.

Chromatographic Study of Developed Anthocyanidins. To identify anthocyanidins obtained from banana, they were analyzed by paper chromatography with known anthocyanidins. Synthetic cyanidin chloride from two sources[5,6], synthetic delphinidin chloride[6], and anthocyanidins developed from the banana by heating a portion of the powder in 0.4 N HCl in methanol were spotted on No. 1 Whatman filter paper. Four chromatograms with the pigments were developed with Forestal reagent (water, glacial acetic acid, 12 N HCl; 10:30:3 v/v).

The major part of the banana anthocyanidin migrated on the chromatogram at the same rate as the slower moving delphinidin chloride (with three hydroxyl groups in the B ring) and a smaller, faster moving pigment migrated the same distance as the cyanidin chloride. This confirms the report by Simmonds (1966, p. 453) that delphinidin chloride is the major pigment converted from the proanthocyanidin in the banana with a lesser amount of cyanidin chloride, although Jones (1965) and De Swardt, Maxie, and Singleton (1967) reported delphinidin chloride only in the banana.

Measuring the Concentration of Anthocyanidin. The absorbancy of the solution of anthocyanidin developed from

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[5]School of Forestry, Oregon State University, Corvallis, Oregon.

[6]Fluka, AG, Buchs SG, Switzerland.

proanthocyanidin was determined with a colorimeter[7] at a wavelength of 545 mμ. A portion of the unheated developing reagent was used as the blank since heating it made no difference in the absorbance. The concentration of anthocyanidin was read from a standard curve of delphinidin chloride made with the reagent used in the conversion.

The data for plotting the slope of the standard curve were obtained in the following manner. Five milligrams of delphinidin chloride were weighed on an analytical balance and made to 50 milliliters with methanol. Working standards made with the developing reagent were prepared from two such stock solutions. These contained 2, 4, 5, 8, 10, and 15 micrograms of delphinidin chloride per milliliter. The absorbancy of each working standard was read at a wavelength of 545 mμ. Averages of the two readings were plotted against the concentration to get the slope of the curve which is shown in Figure 1.

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[7] Beckman/Spinco Spectro-colorimeter, Beckman Instruments, Inc., Fullerton, California.

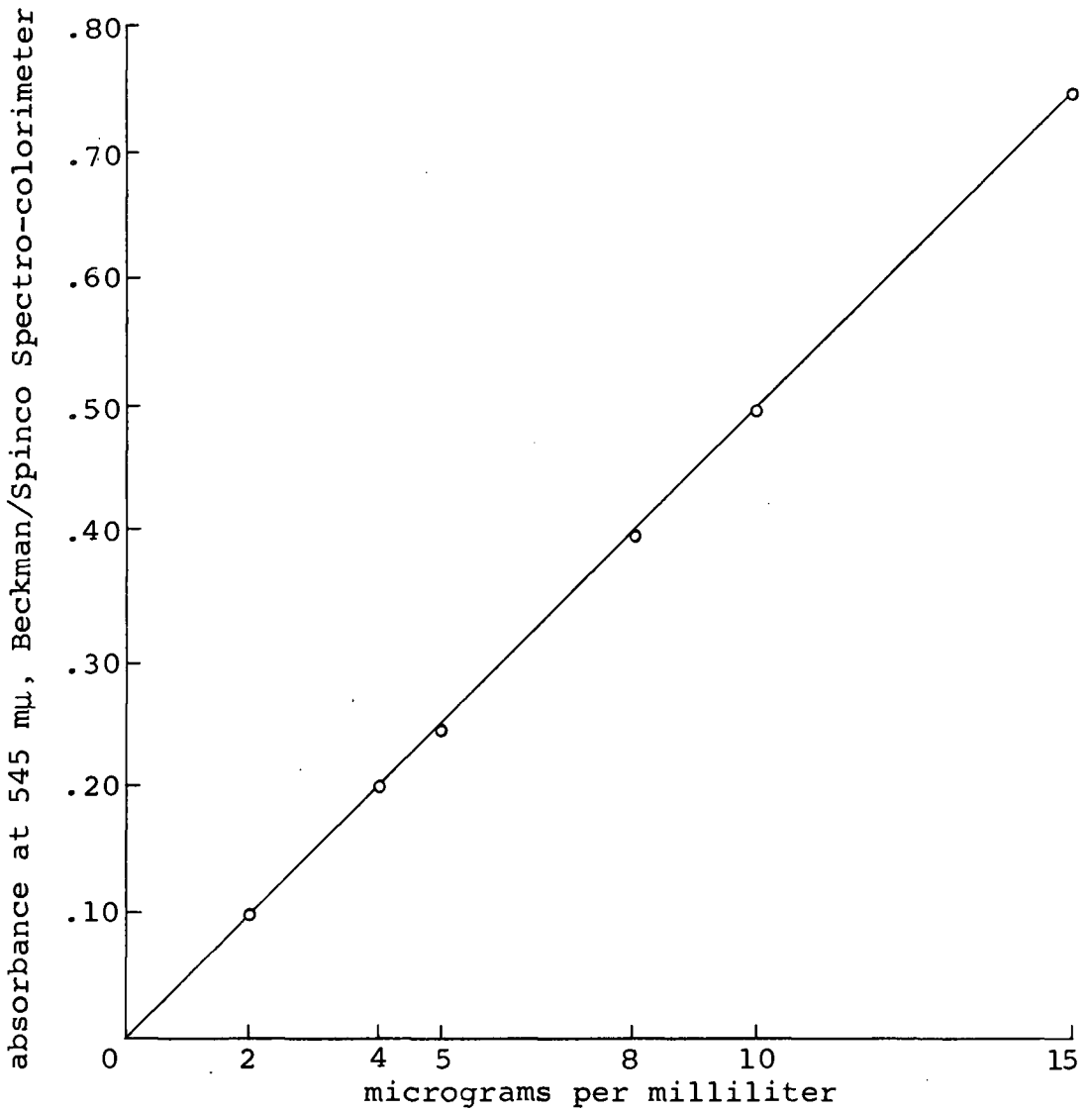


Figure 1. Standard curve for delphinidin chloride (in n-butanol-0.8 N HCl-$FeSO_4 \cdot 7H_2O$).

# RESULTS AND DISCUSSION

## Yield of Anthocyanidin from Proanthocyanidin of Banana Powder

The yields of anthocyanidin from proanthocyanidin of freeze-dried banana powder are given in Table I. The average value for the nine green bananas, expressed as milligrams of delphinidin chloride per gram of powder, is 19.7; that for the nine ripe bananas, 16.0; and for the nine overripe bananas, 13.7. Analysis of variance of the data[8] by random block design (Mendenhall, 1967, p. 282) gave a calculated F value of 14.02 which exceeds the value of 10.92 for the F distribution with two and six degrees of freedom at the 1% level. Thus the ripeness of the banana resulted in a significant difference in the proanthocyanidin content.

The higher proanthocyanidin content of the green bananas compared to ripe is in accordance with that found by Goldstein and Swain (1963). These authors reported concentrations of 9.0 milligrams per gram in unripe and 0.6 milligrams per gram in the ripe banana, based on the amount extractable by absolute methanol, and 1.5 milligrams per gram for unripe and 4.7 milligrams per gram for ripe banana when the extractant was 50% methanol. Thus the total extract from unripe banana yielded approximately

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[8]The author is indebted to Dr. K. E. Rowe for statistical advice and for supervising the analysis of the data.

TABLE I. PROANTHOCYANIDIN CONTENT OF BANANAS AT THREE STAGES OF RIPENESS ON A DRY WEIGHT BASIS (as milligrams delphinidin chloride per gram dried banana)

| HAND | BANANA | SAMPLE* | GREEN | | RIPE | | OVERRIPE | |
|---|---|---|---|---|---|---|---|---|
| I | 1 | a | 18.9 | | 16.0 | | 10.7 | |
| | | b | 18.7 | | 15.9 | | 10.9 | |
| | av. | | | 18.7 | | 15.9 | | 10.8 |
| | 2 | a | 20.9 | | 15.8 | | 11.6 | |
| | | b | 21.2 | | 16.0 | | 11.5 | |
| | av. | | | 21.0 | | 15.9 | | 11.5 |
| | 3 | a | 18.2 | | 16.2 | | 12.6 | |
| | | b | 18.0 | | 16.2 | | 12.2 | |
| | av. | | | 18.1 | | 16.2 | | 12.4 |
| Av. | | | | 19.3 | | 16.0 | | 11.6 |
| II | 1 | a | 20.6 | | 16.3 | | 15.3 | |
| | | b | 20.3 | | 16.3 | | 15.2 | |
| | av. | | | 20.4 | | 16.3 | | 15.3 |
| | 2 | a | 19.6 | | 16.9 | | 16.2 | |
| | | b | 19.2 | | 17.0 | | 16.4 | |
| | av. | | | 19.4 | | 16.9 | | 16.3 |
| | 3 | a | 19.9 | | 16.6 | | 17.0 | |
| | | b | 19.1 | | 16.6 | | 16.5 | |
| | av. | | | 19.5 | | 16.6 | | 16.8 |
| Av. | | | | 19.8 | | 16.6 | | 16.1 |
| III | 1 | a | 19.3 | | 14.6 | | 13.3 | |
| | | b | 18.7 | | 14.7 | | 13.1 | |
| | av. | | | 19.0 | | 14.7 | | 13.2 |
| | 2 | a | 20.2 | | 15.9 | | 13.5 | |
| | | b | 20.2 | | 15.8 | | 13.1 | |
| | av. | | | 20.2 | | 15.8 | | 13.3 |
| | 3 | a | 20.3 | | 15.8 | | 14.1 | |
| | | b | 20.5 | | 15.8 | | 13.9 | |
| | av. | | | 20.4 | | 15.8 | | 14.0 |
| Av. | | | | 19.9 | | 15.4 | | 13.5 |
| Grand Av. | | | | 19.7 | | 16.0 | | 13.7 |

* Each sample value an average of three determinations

twice the quantity of anthocyanidin as did that from ripe (10.5 versus 5.3 milligrams per gram). Differences between the values for the green and ripe bananas in the present study, while highly significant, are not as great as those reported by Goldstein and Swain (1963). The yields in the present study were twice as high as those reported by the above authors for the green banana and three times as high for the ripe. A number of factors possibly contributed to the different values obtained in the two studies. The values reported by Goldstein and Swain (1963) were based on the proanthocyanidin in the extract rather than in the pulp; the normality of the developer was 0.6 rather than 0.8 and it lacked the iron; and their values were reported as cyanidin chloride rather than delphinidin chloride. Furthermore, the criteria they used for ripeness were not stated.

In a later study, data for the proanthocyanidin content of bananas at seven stages of ripeness were reported by De Swardt, Maxie, and Singleton (1967) (who used the method of Goldstein and Swain). The bananas were analyzed at three stages preclimacteric, three stages postclimacteric, as well as the climacteric peak. Values for proanthocyanidin extractable with absolute methanol, expressed as milligrams per gram of cyanidin chloride on a dry weight basis, decreased steadily from the very green stage to the climacteric peak (2.05 to 1.58), but dropped

precipitously to 0.14 one-half day after the climacteric peak and was at a low value (0.06) at five and one-half days after the climacteric peak. Proanthocyanidin content extractable with 50% methanol was at a low level (0.06) at all three stages preclimacteric and increased slowly to 0.21 at five and one-half days postclimacteric. The changes in the proanthocyanidin content are in the same direction and of approximately the same magnitude in both studies; however, De Swardt and co-workers (1967) reported lower values than Goldstein and Swain (1963) and both reported lower values than those found in this study. Both groups of workers measured the extractable rather than total proanthocyanidin in the pulp.

The marked difference in proanthocyanidin content between ripe and overripe bananas reported by De Swardt, Maxie, and Singleton (1967) was not observed in the present study, although the differences due to ripeness were significant. De Swardt and co-workers (1967) used the respiratory rate to determine the level of ripeness, a more accurate criteria than skin color, since ripening is initiated in the center of the banana (Simmonds, 1966, p. 227) and the skin may appear green when the fruit is half-way through the ripening process (Maxie and Sommer, 1968).

A factor that may be involved in the high values of proanthocyanidin in bananas in the present study may be that the tissue was analyzed after freeze-drying.

Swain and Hillis (1959) suggested that part of the proanthocyanidin might be adsorbed by constituents of the cell walls, and Goldstein and Swain (1963) stated that this might account for the lower values they obtained with absolute methanolic extracts of ripe compared to unripe bananas. Young (1965) found that when fresh tissue was ground to break the latex cells, proanthocyanidin diffused throughout the tissues and was adsorbed by constituents of the cell walls, especially the protein. This did not occur when the tissue was freeze-dried prior to grinding.

Although freeze-drying the tissue prior to grinding may have contributed to the higher yield of proanthocyanidin from the bananas in the present study compared to yields reported by Goldstein and Swain (1963) and De Swardt, Maxie, and Singleton (1967), the major factor was that the pulp was analyzed rather than the extract. Apparently the concentration of proanthocyanidin in the banana changes less with ripening than the data of these two groups of workers suggest. Instead, the proanthocyanidin polymerizes and so becomes less extractable on ripening (De Swardt, Maxie, and Singleton, 1967). Conversion of unextractable polymers in the pulp to soluble anthocyanidins could account for the high values in the present study, particularly in the case of the ripe and overripe fruit in which it has been suggested that polymerization of proanthocyanidins frees enzymes which are necessary

for the ripening process (De Swardt, Maxie, and Singleton, 1967).

Yield of Anthocyanidin as Influenced by Hand of Banana. Average values for the three hands of green bananas were 19.3, 19.8, and 19.9 milligrams of delphinidin chloride per gram of banana powder. Average values for the three hands of ripe bananas were 16.0, 16.6, and 15.4 milligrams per gram. Hands of overripe bananas showed greater variation with average values of 11.6, 16.1, and 13.5 milligrams per gram for the three hands. The total variance for treatment mean was 0.6314, and that due to hand was 0.5664. The high proanthocyanidin content of the bananas in the one overripe hand suggests that judging ripeness by skin color, subjectively, is an imprecise index to the ripeness of the fruit (De Swardt, Maxie, and Singleton, 1967; Maxie and Sommer, 1968), and that the three bananas in this hand may have been less ripe than they appeared.

Yield of Anthocyanidin as Influenced by Bananas in a Hand. The yields of delphinidin chloride from the three bananas of a single hand were close. The lowest and highest values differed by not more than 1.6 milligrams per gram of banana powder except for hand I of the green bananas where the difference was 2.9. Of the total variance for the treatment mean, that due to banana was only 0.0624. Thus it appears that bananas adjacent to each

other on a hand are similar in proanthocyanidin content and attain approximately the same stage of ripeness. This suggests the possibility of decreasing the number of bananas per hand and analyzing additional hands since most of the variation was due to hand.

Moisture of the Bananas

The moisture contents of the bananas at three stages of ripeness are listed in Table II. The average moisture content was lowest for the green bananas (71.3%), higher for the ripe (73.0%), and highest for the overripe (74.9%). These values are not out of line with a range of 62 to 78% and an average of 70% reported for bananas by Simmonds (1966, p. 253).

Variation in moisture content among bananas from the same hand was insignificant, except for those in ripe hand I, and among hands at the same stage of ripeness, except for overripe hand II. Bananas from this hand had a lower moisture content than those from the other two hands and also a higher yield of anthocyanidin on a dry weight basis, suggesting that the fruit may have been less ripe than that of the other two hands. This re-emphasizes the need for a better criterion than skin color for determining ripeness (Maxie and Sommer, 1968).

TABLE II. MOISTURE CONTENT OF BANANAS AT THREE STAGES OF RIPENESS (average of two determinations)

| BANANA SAMPLE | | GREEN | RIPE | OVERRIPE |
|---|---|---|---|---|
| Hand | Banana | % | % | % |
| I | 1 | 71.0 | 74.3 | 75.9 |
| | 2 | 71.9 | 72.0 | 75.6 |
| | 3 | 71.8 | 71.8 | 75.6 |
| | av. | 71.6 | 72.7 | 75.7 |
| II | 1 | 71.6 | 72.4 | 73.6 |
| | 2 | 71.9 | 71.5 | 73.9 |
| | 3 | 71.7 | 71.6 | 73.6 |
| | av. | 71.7 | 71.8 | 73.7 |
| III | 1 | 70.4 | 74.8 | 74.9 |
| | 2 | 70.6 | 74.6 | 75.4 |
| | 3 | 70.8 | 74.2 | 75.5 |
| | av. | 70.6 | 74.5 | 75.3 |
| Grand Av. | | 71.3 | 73.0 | 74.9 |

Proanthocyanidin of Bananas on a Fresh Weight Basis

Proanthocyanidin content of bananas calculated on a fresh weight basis from the moisture content of each banana and expressed as milligrams of delphinidin chloride per gram of banana are recorded in Table III. Average values for the three stages of ripeness were 5.63, 4.33, and 3.44 milligrams per gram for the green, ripe, and overripe, respectively. Analysis of variance gave a calculated F value of 15.32 which for two and six degrees of freedom is significant at the 1% level. Thus, on both the fresh weight as well as the dry weight basis there was a significant difference in the proanthocyanidin content of the bananas due to ripeness.

TABLE III. PROANTHOCYANIDIN CONTENT OF BANANAS AT THREE STAGES OF RIPENESS ON A FRESH WEIGHT BASIS (as milligrams delphinidin chloride per gram banana)

| HAND | BANANA | SAMPLE* | GREEN | | RIPE | | OVERRIPE | |
|---|---|---|---|---|---|---|---|---|
| I | 1 | a | 5.46 | | 4.10 | | 2.59 | |
| | | b | 5.35 | | 4.09 | | 2.63 | |
| | av. | | | 5.41 | | 4.10 | | 2.61 |
| | 2 | a | 5.87 | | 4.41 | | 2.82 | |
| | | b | 5.96 | | 4.48 | | 2.80 | |
| | av. | | | 5.91 | | 4.44 | | 2.81 |
| | 3 | a | 5.12 | | 4.56 | | 3.06 | |
| | | b | 5.07 | | 4.56 | | 2.96 | |
| | av. | | | 5.09 | | 4.56 | | 3.01 |
| Av. | | | | 5.47 | | 4.37 | | 2.81 |
| II | 1 | a | 5.83 | | 4.51 | | 4.05 | |
| | | b | 5.75 | | 4.49 | | 4.03 | |
| | av. | | | 5.79 | | 4.50 | | 4.04 |
| | 2 | a | 5.50 | | 4.82 | | 4.22 | |
| | | b | 5.38 | | 4.83 | | 4.28 | |
| | av. | | | 5.44 | | 4.83 | | 4.25 |
| | 3 | a | 5.61 | | 4.72 | | 4.49 | |
| | | b | 5.38 | | 4.71 | | 4.37 | |
| | av. | | | 5.50 | | 4.72 | | 4.43 |
| Av. | | | | 5.58 | | 4.68 | | 4.24 |
| III | 1 | a | 5.74 | | 3.67 | | 3.33 | |
| | | b | 5.56 | | 3.72 | | 3.29 | |
| | av. | | | 5.65 | | 3.75 | | 3.31 |
| | 2 | a | 5.95 | | 4.04 | | 3.31 | |
| | | b | 5.94 | | 4.03 | | 3.22 | |
| | av. | | | 5.94 | | 4.04 | | 3.27 |
| | 3 | a | 5.92 | | 4.07 | | 3.44 | |
| | | b | 5.99 | | 4.07 | | 3.41 | |
| | av. | | | 5.96 | | 4.07 | | 3.42 |
| Av. | | | | 5.85 | | 3.95 | | 3.33 |
| Grand Av. | | | | 5.63 | | 4.33 | | 3.44 |

* Each sample value an average of three determinations

# SUMMARY

Bananas at three stages of ripeness were analyzed for proanthocyanidin content. Three hands with three adjacent bananas per hand at the green, the ripe, and the overripe stages were analyzed, making a total of 27 bananas. Individual bananas were freeze-dried to arrest further ripening and ground to provide a more representative sample for analysis. The ground tissue was dispersed in methanol and the proanthocyanidin content of the resulting slurry was measured. The developer used to convert the proanthocyanidin to anthocyanidin was 0.8 N HCl-n-butanol containing iron ($FeSO_4 \cdot 7H_2O$). The major anthocyanidin was identified as delphinidin and a minor pigment as cyanidin. Two samples of each banana were analyzed in triplicate, making a total of six determinations for each banana. The absorbance of the solution of anthocyanidin so obtained was measured at a wavelength of 545 mμ.

Significant differences were observed in the proanthocyanidin content of the bananas at the three stages of ripeness. The average values, expressed as milligrams of delphinidin chloride per gram of dried banana, were 19.7 for the green, 16.0 for the ripe, and 13.7 for the overripe.

The moisture content of each banana was determined in duplicate. The average for the green was 71.3%, for the

ripe 73.0%, and for the overripe 74.9%. These values are in line with the previously reported range of 62 to 78% with an average of 70%. Proanthocyanidin content of the bananas on a fresh weight basis, expressed as milligrams of delphinidin chloride per gram of banana, averaged 5.63, 4.33, and 3.44 for the green, ripe, and overripe, respectively.

The proanthocyanidin contents of the bananas in this study were considerably higher than values previously reported. Analysis of the pulp, rather than the extractable proanthocyanidin and a more acidic developer and one which contained iron were major factors contributing to the higher values.

While proanthocyanidin content decreased significantly with ripeness of the bananas, the differences were not as great as reported in two earlier studies. Polymerization of the proanthocyanidins as the fruit ripens could account for their lower values which were based on extractable proanthocyanidins. Conversion of unextractable polymers in the pulp to soluble anthocyanidin could account for the proportionately higher values in the present study.

For future work a better method than skin color for assessing ripeness is recommended. Statistical analysis of the data showed that of the total variance for treatment mean (ripeness), a high proportion was due to hand

and relatively little due to banana on the hand. Bananas adjacent to each other on a hand were similar in proanthocyanidin content and presumably in ripeness, but it is possible that the three overripe hands were not equally overripe. This suggests the desirability of analyzing fewer bananas from a hand and more hands.

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# APPENDIX

TABLE IV. PROANTHOCYANIDIN CONTENT OF INDIVIDUAL BANANAS* ON A DRY WEIGHT BASIS (as milligrams of delphinidin chloride per gram dried banana)

| HAND | BANANA | GREEN | | RIPE | | OVERRIPE | |
|---|---|---|---|---|---|---|---|
| | | a | b | a | b | a | b |
| I | 1 | 19.2 | 18.7 | 16.1 | 15.9 | 11.0 | 11.1 |
| | | 19.0 | 18.4 | 16.1 | 15.9 | 10.7 | 11.1 |
| | | 18.4 | 18.3 | 15.7 | 15.9 | 10.4 | 10.6 |
| | 2 | 21.3 | 21.5 | 16.0 | 16.1 | 11.8 | 11.6 |
| | | 20.8 | 21.1 | 15.8 | 16.0 | 11.5 | 11.5 |
| | | 20.5 | 21.0 | 15.5 | 15.9 | 11.4 | 11.3 |
| | 3 | 18.4 | 18.3 | 16.2 | 16.2 | 12.9 | 12.6 |
| | | 18.1 | 18.1 | 16.2 | 16.2 | 12.9 | 12.6 |
| | | 18.1 | 17.5 | 16.2 | 16.2 | 12.2 | 11.7 |
| II | 1 | 20.7 | 20.5 | 16.6 | 16.4 | 15.4 | 15.3 |
| | | 20.6 | 20.3 | 16.2 | 16.3 | 15.3 | 15.3 |
| | | 20.5 | 20.1 | 16.2 | 16.2 | 15.3 | 15.1 |
| | 2 | 19.7 | 19.3 | 17.0 | 17.2 | 16.6 | 16.5 |
| | | 19.7 | 19.1 | 16.9 | 16.9 | 16.0 | 16.3 |
| | | 19.4 | 19.1 | 16.8 | 16.8 | 16.0 | 16.3 |
| | 3 | 20.0 | 19.1 | 16.9 | 16.8 | 17.2 | 16.7 |
| | | 19.9 | 19.1 | 16.6 | 16.7 | 17.0 | 16.5 |
| | | 19.7 | 19.0 | 16.4 | 16.4 | 16.9 | 16.4 |
| III | 1 | 20.6 | 18.8 | 14.8 | 14.9 | 13.4 | 13.3 |
| | | 18.8 | 18.7 | 14.5 | 14.7 | 13.3 | 13.1 |
| | | 18.6 | 18.6 | 14.5 | 14.6 | 13.2 | 13.0 |
| | 2 | 20.2 | 20.3 | 16.0 | 15.8 | 13.7 | 13.2 |
| | | 20.2 | 20.2 | 15.8 | 15.8 | 13.7 | 13.2 |
| | | 20.2 | 20.2 | 15.8 | 15.8 | 13.2 | 13.0 |
| | 3 | 20.4 | 20.8 | 15.8 | 15.9 | 14.2 | 14.0 |
| | | 20.2 | 20.5 | 15.8 | 15.9 | 14.1 | 13.9 |
| | | 20.2 | 20.2 | 15.7 | 15.5 | 13.8 | 13.9 |

* Three determinations on each of two sub-samples

TABLE V. PROANTHOCYANIDIN CONTENT OF INDIVIDUAL BANANAS* ON A FRESH WEIGHT BASIS (as milligrams delphinidin chloride per gram banana)

| HAND | BANANA | GREEN | | RIPE | | OVERRIPE | |
|---|---|---|---|---|---|---|---|
| | | a | b | a | b | a | b |
| I | 1 | 5.56 | 5.43 | 4.13 | 4.10 | 2.65 | 2.67 |
| | | 5.50 | 5.34 | 4.13 | 4.10 | 2.60 | 2.67 |
| | | 5.32 | 5.29 | 4.05 | 4.07 | 2.52 | 2.55 |
| | 2 | 5.98 | 6.04 | 4.47 | 4.51 | 2.87 | 2.83 |
| | | 5.84 | 5.92 | 4.43 | 4.47 | 2.81 | 2.80 |
| | | 5.78 | 5.91 | 4.34 | 4.45 | 2.78 | 2.77 |
| | 3 | 5.18 | 5.16 | 4.56 | 4.56 | 3.14 | 3.07 |
| | | 5.09 | 5.11 | 4.56 | 4.56 | 3.07 | 2.97 |
| | | 5.09 | 4.93 | 4.56 | 4.56 | 2.98 | 2.85 |
| II | 1 | 5.86 | 5.82 | 4.58 | 4.52 | 4.08 | 4.04 |
| | | 5.84 | 5.72 | 4.48 | 4.49 | 4.04 | 4.04 |
| | | 5.80 | 5.71 | 4.46 | 4.46 | 4.04 | 4.00 |
| | 2 | 5.54 | 5.42 | 4.85 | 4.90 | 4.32 | 4.31 |
| | | 5.53 | 5.36 | 4.83 | 4.81 | 4.17 | 4.26 |
| | | 5.44 | 5.36 | 4.79 | 4.78 | 4.17 | 4.26 |
| | 3 | 5.65 | 5.39 | 4.81 | 4.76 | 4.53 | 4.41 |
| | | 5.62 | 5.39 | 4.71 | 4.73 | 4.50 | 4.36 |
| | | 5.57 | 5.37 | 4.65 | 4.65 | 4.45 | 4.33 |
| III | 1 | 6.12 | 5.59 | 3.72 | 3.76 | 3.35 | 3.34 |
| | | 5.58 | 5.55 | 3.65 | 3.71 | 3.33 | 3.27 |
| | | 5.53 | 5.53 | 3.65 | 3.69 | 3.30 | 3.25 |
| | 2 | 5.95 | 5.97 | 4.08 | 4.03 | 3.35 | 3.24 |
| | | 5.95 | 5.93 | 4.02 | 4.03 | 3.35 | 3.24 |
| | | 5.95 | 5.93 | 4.02 | 4.03 | 3.24 | 3.19 |
| | 3 | 5.96 | 6.08 | 4.08 | 4.10 | 3.48 | 3.43 |
| | | 5.90 | 6.00 | 4.08 | 4.10 | 3.45 | 3.40 |
| | | 5.90 | 5.89 | 4.04 | 4.00 | 3.38 | 3.40 |

* Three determinations on each of two sub-samples