CHEMICAL CHANGES IN FREEZE-DRIED BANANAS

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Chemical Changes in Freeze-dried Bananas

By

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This work is dedicated to my wife, Lucia, and my daughter, Sandra.

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CHEMICAL CHANGES IN FREEZE-DRIED BANANAS

I. INTRODUCTION.

Bananas belong to the family Musaceae, genus Musa, comprising thirty-two or more distinct species and at least one hundred subspecies (Von Loesecke, 1949). This genus is divided into two broad sections, Eumusa with edible fruit and Physocaulis with inedible fruit. The cultivated varieties of bananas are innumerable and in confusion partly due to bud variation, and partly due to the fact that the same variety is known by different names in various banana-producing regions. The earliest home of bananas is presumed to have been in the humid tropical regions of Southern Asia. In 327 B.C. the armies of Alexander the Great found the fruit abundant in the valley of the Indus (United Fruit Co., 1936). The carbohydrate present in ripe sweet bananas is highly digestible and is well tolerated by people suffering from various intestinal disorders (Simmonds, 1966; Bilenker, 1960). It is one of the important economic crops in several South American countries, especially Brazil and Ecuador.

Approximately half of the bananas of the world are eaten raw and ripe. An alternative means of preparing green bananas is occasionally found in Uganda where the fruit of clones normally devoted to beer making is sliced, dried in the sun to yield chips from which a porridge is made by boiling (Simmonds, 1966). The more important banana products are jam, pastes, flakes, flour, and powder.

Banana powder is made by passing the peeled fruit through a meat chopper to obtain a slurry which may be dried on drums. The slurry can also be spray-dried and this process has been used in Brazil. Banana powder (ripe fruit) is used chiefly in therapeutics and in preparation of malted milk beverage. Banana flour (unripe fruit) has been used more for bread making. Samish and Coussin (1965) reported the production of dehydrated flakes as a means of utilizing surplus bananas. They concluded that the drum-drier method offers an excellent potential for utilization of surplus bananas.

Freeze drying is a modern method for dehydration of foods. The moisture is removed from foods in the frozen state by sublimation under high vacuum. The low temperature of the process inhibits unfavorable chemical and biochemical reactions and minimizes losses of nutrients, vitamins, pigments, and volatile compounds.

The process of freeze-drying is recognized industrially. It has been applied to preserve mushrooms, shrimps, beef steaks,soup mixes, chives, onions, bananas, strawberries, asparagus, etc. The more expensive products are better suited for freeze-drying because they can absorb the high cost of operation. Freeze-drying offers a better future for preserving bananas because of the improved quality and lower shipping cost, compared with traditional methods.

In this work the effect of slow and quick freezing processes and of storage temperature on stability of freeze-dried bananas was investigated. The chemical changes and the deleterious effect of these changes on the quality and nutritive value of the product were evaluated.

The polyphenolic compounds in the freeze-dried bananas were identified by paper chromatography and chromogenic methods.

II. LITERATURE REVIEW.

Freeze-drying.

Freeze-drying of foods is now widely used in commercial operation. Some years ago it was nothing more than a laboratory curiosity. Low temperature evaporation of water under vacuum to produce freezing followed by sublimation of the ice was shown by William Hydee Wollaston to the Royal Society of London in 1813 (Flosdorf, 1949).

The application of freeze-drying to the commercial production of dehydrated meats and orange juice powders was suggested by Flosdorf in 1945.

In 1946 Barker et al. showed that the culinary quality of freeze-dried peas was comparable with that obtained by ordinary freezing methods.

Development of a new method for the production of orange powder was attempted during World War II. The experience of those working in the field of blood-plasma drying was applied. The fundamental approach for drying from the frozen state was used (Sluder et al., 1947).

In 1949 Flosdorf discussed the equipment for food freeze-drying of raw meat, shell fish and others.

The initial work of this process was carried out in 1951 in a plant at Aberdeen by A/S Atlas of Copenhagen (Hanson, 1961). During the subsequent ten years several improvements were made over the other methods of dehydration employing hot air and vacuum. A much faster and better method was developed known as "Accelerated Freeze Drying" (Cotson and Smith, 1963). In October, 1961 there was a symposium at the Borough Polytechnique, London, U.K., to survey the freeze-drying of foods.

In 1963, the U. S. Department of Agriculture published a bibliography of selected references on the freeze-drying of foods. In 1960, the Armour Company of Chicago provided an experimental factory to study the commercial capabilities of the process.

A. Freezing rate.

When foods are frozen, ice crystals of smaller or larger size are formed, depending on whether the freezing rate is rapid or slow. It was stated that the fine pore structure obtained during quick freezing reduced the rate of drying. This opinion is supported by the observation that meat frozen slowly dried faster than meat frozen rapidly (Burke and Decareau, 1964). In rapid freezing, ice crystals of very small size are formed inside the cells. If the freezing rate is slow, larger ice crystals are formed extracellularly.

Rapataz and Luyet (1959) studied the mechanism of ice formation and propagation in beef muscle. They observed that slowly frozen fiber contains large ice masses which completely mar the structure and obliterate the striations. In more rapid freezing, the striations can still be observed. When fibers frozen slowly or rapidly are thawed, their original structural characteristics quickly return, indicating a rapid redistribution of water.

Wang et al. (1954) studied the histology and histo-chemistry of beef dehydration. The morphological pattern of freeze-dried muscle tissue depended upon the cooling rate and the pre-freezing temperature.

Lee et al. (1966) compared freeze-drying, sun-drying, hot-air-dehydration, and dehydro-freezing of peaches. For freeze-drying, the

material was treated by slow freezing, quick freezing and partial osmosis in sugar prior to freeze dehydration. They found that cell wall rupture occurs during conventional freezing. In quick frozen peaches, the size of ice crystals were substantially smaller and cell damage was negligible.

In rapid freezing of biological materials, two closely related problems arise. The first is the dependence of the cooling rate of the sample on its size, shape, and thermal properties. The second is the relation of the size and number of ice crystals to the thermal history of the sample. When the ice crystals grow they release the latent heat of crystallization. This heat release modifies the course of the cooling and consequently the temperature distribution in the sample. Conversely, the rate at which a given ice crystal can grow depends on its size and temperature distribution of the surrounding medium (Stephenson, 1960).

Salt concentration and rates of freezing have been shown to condition the crystal structure of frozen material, thereby influencing the practicable rates of freeze-drying (Rowe, 1960).

Love (1962) studied the mode of growth of ice crystals in pre- and post-rigor muscle of frozen fish. It was established that the ice was mostly intracellular in pre-rigor muscle regardless of freezing rate. Post-rigor muscle freezes extracellularly when the freezing time was longer than eighty minutes. It was concluded that intracellular freezing tends to favor more denaturation in the pre-rigor muscle.

Love (1962) found that the rate at which cod muscle is frozen governs the size and disposition of the ice crystals. When freezing time was longer than eighty minutes, the ice crystals were formed

extracellularly in larger and fewer masses as the freezing time was extended. When freezing time was less than eighty minutes, ice crystals were formed intracellularly. Kuprianoff (1962) studied the effect of freeze-drying on irreversible changes. Ice crystals may cause denaturation of proteins by mechanical action through pressure which results from the greater ice volume. From the standpoint of avoiding freezing damage, quick freezing before drying appears to be advantageous (Kuprianoff, 1962). When consideration is given to the whole freeze-drying process, including reconstitution by adding water, many products show best reconstitution after slow freezing. By this process larger holes are produced which permit better penetration of water and faster escape of the air. Kuprianoff (1962) stated that in most cases there is no marked inactivation of enzyme systems through freeze-drying because the process minimizes protein damage, and dry enzymes usually have good stability at room temperature. During and after reconstitution of the product, the potential for increased enzymic activity is restored. The enzymic activity depends on the water content, processing conditions, storage temperature, and storage time.

Luyet (1962) stated that the rate of initial freezing plays a fundamental role in the structure of frozen material and in the following process of vacuum dessication and rehydration. Significant structural differences are observed between slow and quick frozen muscle. A gradual increase in freezing velocity permits one to obtain ice particles of smaller size.

Burke and Decareau (1964) stated that one disadvantage of quick freezing is the slower rehydration rate because air trapped in the fine pores resists penetration by water.

There are some recommendations to obtain optimum freeze-drying results as follows:

a) determine the point of complete solidification; b) determine the temperature at which melting occurs; c) freeze the food at a temperature of complete solidification; and d) during sublimation the temperature must be kept below the melting point.

Meryman (1962) discussed the kinds of injury caused by freezing. Meryman and Platt (1954) concluded that tissue injury was caused by concentration of solutes rather than mechanical damage by ice crystals.

B. Drying.

In freeze-drying, heat is transferred by conduction inside the product. Once the ice phase has disappeared, the bound water must be removed. This water is retained by capillary forces. The removal of bound water has been called secondary drying (Burke and Decareau, 1964).

In drying the frozen product the slab receives heat from an external source. The heat is then conducted to an ice-dried layer interface. The water vapor sublimes from the interface, moving through the dried layer and finally escaping to the chamber. As drying proceeds, the ice phase recedes inward and becomes surrounded by a layer of porous, dried material (Harper and Chichester, 1962). The drying process involves a balance between the outward flow of vapor and the inward flow of heat. Vapor movement from the ice phase takes place by hydrodynamic flow as a result of a gradient in total pressure and by diffusion resulting from a gradient in partial pressure of water vapor (Harper and Chichester, 1962).

The process of dehydrating a frozen material can be divided into three steps:

a) The introduction of heat to supply the energy necessary for sublimation;

b) The transfer of water vapor from the subliming ice crystal through the already dried shell of the material;

c) The removal of water vapor that reaches the surface of the specimen.

(Meryman, 1960).

The speed of drying in any freeze-drying system depends directly upon the rate of application of the heat necessary to supply the latent heat of vaporization of ice (Greaves, 1960). If the layer of material we are attempting to dry was infinitely thin, the amount of heat that could be applied would be infinitely great, and the speed of drying infinitely fast.

There are five methods by which heat energy may be transferred to the product.

1. inert gas heating
2. convection heating
3. dielectric heating
4. radiant heating
5. conductive heating

Jackson et al. (1957) studied freeze drying of peaches, using four methods of transferring heat to the drying fruit. It was shown that the efficiency of heat transfer limited the drying rates. Maximum rates of drying were obtained by dielectric > infra-red > double plate > single

plate heat. Dielectric heat seems to be most promising for rapid freeze-drying.

Application of dielectric heating to freeze drying has been discussed by Leathermann and Stutz (1962), Jackson et al. (1957) and Copson (1958). The method appears particularly desirable for reducing the time required in freeze drying because in dielectric heating the heat is generated internally directly in the frozen portion of the material being dried. The freeze-drying process is characterized by a long final drying phase, due mainly to the difficulty in supplying heat to the interface of ice with the dry layer.

Methods of application of microwave energy in industrial process was discussed by Gall and Plante (1962) and Decareau (1961).

Heating by radiation from heated plates to loaded trays instead of conduction is a good technique for improving uniformity of heating (Seltzer, 1960).

Problems related to heat and mass transfer is discussed by Lambert and Marshall (1962). The rate of movement of water through the dry cake above the subliming interface depends upon the sample temperature, the structure of dry matter, the mean free path of the escaping gas and the moisture content. Good dryer performance depends on having good thermal container, minimum spacing between evaporator and condenser and adequate pumping capacity.

In order to determine the end point of the process, it is common practice to apply a safety factor, i.e., to dry for a period beyond that considered "probably sufficient". Kan (1962) discussed methods to determine

the end point of the process through thermocouple measurement and vapor pressure.

Meryman (1962) established the causes of injury to foods due to drying under vacuum. The absolute pressure in the vacuum chamber depends on the physical characteristics of the material and on the temperature at which the frozen material must be maintained. In order to maintain a vacuum, either a mechanical vacuum pump or a steam-jet ejector is needed. The vacuum system for freeze-drying has been discussed by Rowe (1960).

The sublimation of ice and the evaporation of water vapor from frozen eutectic mixtures is an important process in freeze-drying. The partial pressure of water vapor at a frozen surface must exceed that at points close to it. In an environment of still air at pressures from atmospheric down to a few torr, collision between evaporating molecules and those of surrounding air retards evaporation and causes accumulation of water molecules in the region of the boundary between the solid and the air (Rowe, 1960).

In freeze-drying the partial pressure of air above the surface is kept at least 10 times lower than the pressure of the water vapor. Low partial pressures of air and water vapor provide conditions favorable for drying. Without a supply of heat, the rate of evaporation would diminish.

When the product is dried, destruction of the vacuum is obtained when dry nitrogen gas is introduced into the chamber containing materials liable to rapid oxidative changes in the dry state.

C. Storage.

Several changes take place in stored freeze-dried foods. Oxidative

deterioration of fats and oils is caused by the oxidation of the unsaturated fatty acids, producing peroxides, aldehydes, ketones and short-chain acids. Potatoes, for example, contain only about 0.001% fat which is thought to be responsible for staling of dehydrated potatoes stored in air (Hanson, 1961). Some of the oxidative reaction takes place more rapidly at lower moisture contents. However high moisture contents accelerate the browning reaction. The low water content has prooxidant effect on autoxidation of lipids (Hanson, 1961).

The actual storage life of a product at a particular temperature is affected by the moisture content, sugar content, and the method of blanching (Hanson, 1961).

The principal reaction leading to the degradation of frozen and dried foodstuffs appears to be the oxidation of pigment, protein, and lipids, and the interaction of compounds containing carbonyl groups with those containing free amino group (Rinfret, 1962). Browning is largely the result of a complex series of chemical reactions initiated by the condensation of reducing sugars and amino acids or proteins.

To protect freeze-dried foods against oxidation, it is necessary to seal them in packages impermeable to oxygen, moisture, and light. During prolonged storage even at low temperatures, there is a slow deterioration in the culinary quality of most dehydrated foods. The storage life of dehydrated foods is reduced by the development of brown pigments. In all foods, conditions that favor browning favor other forms of degradation. The storage life of the product decreases with an increase in storage temperature (Hanson, 1961).

Freeze-dried foods must be rehydrated before serving. Enzymatic action under these conditions becomes very rapid, sometimes even more rapid than in the original material. As an example, unblanched freeze-dried mushrooms darken within seconds after rehydration (Goldblith, 1963). Apples, peaches, pears, bananas as well as other fruits darken on rehydration.

Haas and Stadman (1949) used ion-exchange resins to identify compounds involved in browning in apricots. Storage experiments showed that the overall browning is the result of reactions between the nitrogenous constituent and sugar, between the nitrogenous constituents and organic acids, between sugar and organic acids, and reactions involving only organic acids.

Harper and Tappel (1957) stated that the main deteriorative reaction in dried fruit is believed to be the carbonyl-amine reaction. They observed that the amino nitrogen content decreases when the dried apricot darkens. It was found by Nichols and Reed (1931) that when apples and apricots were dried to a moisture level below 10%, the color stability was improved. The main deteriorative reaction occurring during storage of freeze-dried beef is carbonyl-amine browning (Harper and Tappel, 1957). Huang and Draudt (1964) found several browning intermediates in freeze-dried peaches stored at 1.68% moisture and above, but not at 0.55%. Draudt and Huang (1966) studied banana and the changes during storage related to oxidative and carbonyl-amino browning. They found that in both freeze-dried peaches and bananas, storage at 28°C causes little change at moisture levels below 10%. The peroxidase activity decreased rapidly at all moisture levels during storage at 28°C. The polyphenoloxi-

dase activity was most stable at low moisture levels. The effect of SO_2 on the browning reaction was limited. After a 190-day storage period the Amadori compounds were found in untreated freeze-dried bananas at moisture levels above 0.70%, and in SO_2-treated samples above 3.75% moisture.

Daoud and Luh (1967) studied the effect of moisture, packaging and temperature on stability of freeze-dried bell peppers. At higher storage temperature more rapid loss of ascorbic, carotenoids and vitamins was observed. One of the limiting factors in the storage life of freeze-dried products is non-enzymatic browning. A good way to prevent this type of reaction is to dry to low moisture content (Goldblith et al., 1963). They also stated that oxidation of lipids, pigments, vitamins, and flavor constituents is one of the major problems in retention of quality in freeze-dried foods. Several systems to avoid oxidation in freeze-dried foods have been proposed. Goldblith (1963) reported the extreme sensitivity of freeze-dried food system to oxidation at even extremely low oxygen pressures.

Mild conditions of processing and storage will minimize change in proteins and other constituents of food. Brekke and Allen (1967) compared the dehydrated bananas made by the air-blasting drying, drum drying and freeze-drying processes. The effect of sulfuring and storage temperature were studied. Drum dried and freeze-dried bananas that received bisulfite pretreatment were 10 times more stable than the air-dried bananas.

The storage life of dried apricots decreased in proportion to the quantity of oxygen consumed by the fruit. The rate of oxygen consumption is greatly increased by increasing the moisture content over the range

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10-25%, by increasing the partial pressure of oxygen, and by increasing the temperature of storage (Stadman et al., 1949).

Syn and Luh (1965) studied the storage stability at 32, 68, and 98°F of freeze-dried asparagus packed in plastic laminates and aluminum foil pouches.

Landman et al. (1960) stated that high temperatures accelerated the browning rate in freeze-dried beef. Dehydration of bananas was studied by Bathia et al. (1962) and Amin et al. (1962).

Investigation on the packaging and storage of some dehydrated tropical fruits has shown that the best equilibrium relative humidity is 51% for dried pineapple and 60% for dried banana, mango, and papaya. A shelf life of 4-5 months at 37°C, and 8-12 months at 24-30°C may reasonably be expected for dehydrated bananas, pineapples, papaya and mangoes containing about 1,500 ppm SO_2 and 15-20% moisture (Bathia et al., 1962).

Cavendish bananas were converted experimentally into dehydrated flakes in a drum drier. A tasting panel judged flakes produced from bananas which had been steam blanched prior to dehydration to be superior to those prepared from unblanched fruit. Application of SO_2 improved the color of the flakes. The keeping quality of the product was satisfactory when moisture was below 2.6%. For packing, vacuum sealed tin can was the most satisfactory. High storage temperature affected the product (Samish et al., 1965).

The types of materials used for packaging can be classified as metallic and non-metallic (Bowman, 1963). The metallic containers have the advantage of protecting the food from moisture, oxygen, and light. The common non-metallic materials are plastic, paper, and glass.

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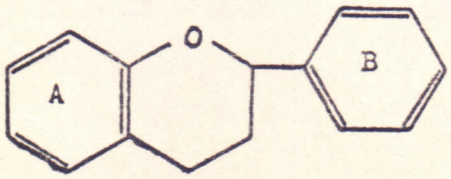
Freeze-dried products are hygroscopic. They must be protected against taking up of moisture during storage. The moisture pick-up initiates caking and undesirable changes. Besides moisture, oxygen must be excluded in containers for freeze-dried foods. The packages must have no vapor transmission and retain their physical integrity under a wide range of storage conditions.

To achieve the required protection in packaging, it is necessary to study the properties of the food product in question. The most important consideration is the fragility of the product, its liability to damage the package, and its tolerance levels to moisture and oxygen (Taylor, 1963).

D. Polyphenols in bananas.

The majority of plant coloring matters are phenols. Such compounds for the commonest and largest class of secondary plant constituents (Swain, 1962). According to Swain (1962) the term "flavonoid" was originally restricted to the compounds having 15-C atoms structurally based on the parent substance, flavone. The basic structure for the 15-C atoms consists of C_6-C_3-C_6 carbon skeleton such as flavones, flavonols, flavanones, isoflavones, chalcones, aurones, flavan 3-ols, flavan 3,4-diol, and flavanonols.



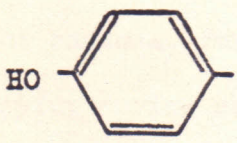
They differ from each other in the degree of oxidation of the central pyran ring.

Bate-Smith (1954) reviewed the nature and chemical constitution of the flavonoid compounds in foods. He stated that most of the flavonoid compounds exist in plants as glycosides. When the molecule is free of sugar, it is called aglycone. According to Grisebach (1965) it is now certain that ring A of the flavonoids is formed by a head-to-tail condensation of three acetyl (malonyl) units, while ring B and carbon atoms 2, 3, and 4 originate from an intact phenyl propane unit.

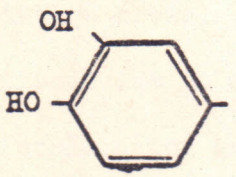
1. Cinnamic acids:

CH = CH - COOH cinnamic acid

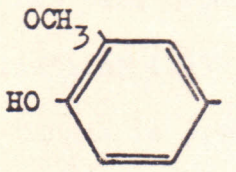
By substitution in the aromatic ring the following result:



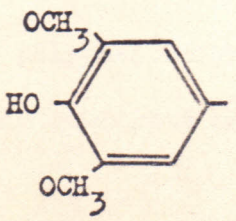
CH = CH - COOH p-coumaric acid



CH = CH - COOH caffeic acid



CH = CH - COOH ferulic acid



CH = CH - COOH sinapic acid

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The acids do not usually occur in the free state but rather as esters. For example, the well-known chlorogenic acid is an ester of quinic and caffeic acids (Neish, 1964). More recently other depsides have been isolated, including neo- and pseudo-chlorogenic acids from peaches and sweet potatoes (Swain, 1962). It has been shown by Williams (1955) that in 2% acetic acid, chlorogenic acid and other cinnamic acid derivatives give two spots due to the separation of the cis- and trans-isomers.

2. Importance.

Polyphenolic compounds are important compounds related to taste, enzymatic browning and color of foods (Swain, 1962). Different types of discoloration have been reported in canned banana products. They include 1) enzymatic browning caused by oxidative enzymes, 2) tannin-browning in canned banana slices with brown lines concentrated mainly on the carpel walls of bananas, formed by interaction of carbonyl compounds in acidified sugar syrup during heating with tannin in the mucilaginous cells, and 3) oxidative browning resulting in grey to black discoloration in the exposed tissue due to oxygen present in the headspace (Agarwal et al., 1963). Pink discoloration in canned pears as related to action of flavonoid compounds has been reported by Luh et al. (1960).

Griffiths (1959) reported that the major browning substrate of the edible banana is 3,4-dihydroxyphenylethylamine. He confirmed the opinion of Simmonds that the leucoanthocyanins present are not acted upon by banana polyphenoloxydase. Siegelman (1955) reported that the principal browning substrate on the skin extract from apple and pear was l-epicatechin.

According to Simmonds (1966) the most abundant phenolic amine in banana is 3,4-dihydroxyphenylethylamine (dopamine) which is abundant in the skin of the fruit (reaching 700 ppm) but relatively sparse in the pulp. Simmonds stated that though dopamine has some physiological activity, as a vasoconstrictor, it is not so active as some other substances also found in bananas. Griffiths (1961) studied a range of clonal varieties to determine whether dopamine was responsible for blackening in other clones or whether other phenolic compounds were implicated in the blackening reaction. Results showed that in all clones investigated the major polyphenoloxidase substrate was dopamine. According to Buckley (1964) dopamine is related to peel discoloration in banana; also peel blackening in later stages of ripening and root discoloration.

Walkes et al. (1958) reported the presence of serotonin (5-hydroxy-tryptamine); it was also indicated that banana contains nor-epinephrine and dopamine. The biological activity of these catecholamines was investigated as further proof of their identity. They also found material with chromatographic properties similar to dopa (3,4-dihydroxy-phenylalanine) and 5-hydroxyindoles substances. Udenfriend et al. (1959) reported the occurrence of these physiologically active arylalkylamines among edible plants. Due to rapidity of metabolism during absorption, when as much as 60 mg of serotonin was administered orally to humans did not cause increase in blood pressure. Block (1958) reported that indoles of biological importance are those which are excreted by animals, probably as a result of tryptophan metabolism. For example, the excretion of serotonin by man may in some way be connected to mental processes.

Enzymatic formation of nor-adrenaline by the banana plant was investigated by Smith and Kirshner (1960). Phenolic compounds in bananas have been investigated by Jones (1965). Swain (1962) reported the following compounds in banana fruit: leucoanthocyanins, flavonoids, ferulic acid, tyramine, dopamine and serotonin.

Robinson (1937) detected delphinidin in the hydrolysis products of the pulp of an edible banana. Simmonds (1954) reported that the bracts of wild banana species are pigmented by glycosides (probably 3-diglucosides) of four combinations of anthocyanidin. He found that leucoanthocyanins are present in most parts of the banana plant; they yield delphinidin and cyanidin in proportions that vary approximately with overall intensity. The leucoanthocyanidins were sought in various parts of the plant and found to be almost universally present.

Phenolic substances are also implicated in the astringency of the unripe banana. The tannins are phenolics with molecules large enough to complex with and precipitate proteins, hence both their tanning properties and astringency in the mouth; but if polymerized to very high molecular weights, these properties decline. This is probably just what happens in various ripening fruits, particularly in bananas (Simmonds, 1966).

De Swardt et al. (1967) reported that polymerization of the phenolic compounds in ripening banana fruit tissue was shown by the decrease of extractable phenolic compounds in absolute methanol and increasing amounts of these substances extractable in aqueous methanol. Low molecular weight tannins may control the activity of enzymes in vivo in preclimacteric fruit and thereby influence ripening in banana. A good review about flavonoid tannins is given by Freudenberg (1962).

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3. Paper Chromatography.

Harborne (1960, 1961) revised the principles involved in chromatography of flavonoid compounds. Seikel (1964) described methods of isolation and identification of phenolic compounds. Seshadri (1962) described methods isolating flavonoid compounds from natural products.

Paper chromatography was first used for the separation of phenols by Bate-Smith (1948). The flavonoid compounds are ideally suited to this technique because of their right range of solubility, characteristic for easy separation, partly because many of them can be seen on chromatograms without the use of chromogenic sprays and partly because they are closely related chemically (Harborne, 1961). The relationship between the structure of the flavonoid compounds and their R_f values has been studied by Bate-Smith and Westall (1950). Seikel (1962) described several solvent systems used in chromatography of polyphenols. A table of R_f values of flavonoid compounds was also compiled.

Extensive reviews on paper chromatography of flavonoid compounds have been written by Geissman (1955), Harborne (1960, 1961), Seikel (1962, 1964), Block et al. (1958), and Bate-Smith (1948, 1964).

A variety of developing solvents have been reported by Seikel (1962, 1964). The solvent system, n-butanol-acetic acid-water introduced by Partridge (1948) for the chromatography of sugars is still the most commonly used (Bate-Smith, 1948).

Harborne (1960) stated that ultraviolet light reveals most of the flavonoid compounds except flavones, isoflavones, catechins and leucoanthocyanins. Aurones, chalcones, anthocyanins and flavonols may be distinguished under normal daylight. Seikel (1962, 1964) and Block

(1958) presented tables with sprays for detection of flavonoid compounds. After the compounds have been detected, they can be isolated by preparative paper chromatography, according to Geissman et al. (1956). By evaluating the R_f values in different solvents, absorption spectra, spectral shifts and alkaline stability of the phenol, color reactions, hydrolysis, it is usually possible to identify with reasonable certainty the chemistry of the compound we have. Seikel (1962) stated that a flavonoid compound can be identified by their physical characteristics: absorption spectra and mobility on paper (R_f value). The spectral properties of flavonoid compounds have been reviewed by Jurd (1962).

III. MATERIALS AND METHODS.

The bananas Musa (Cavendish sub-group) 'Valery' cultivar (Simmonds, 1966) used in this investigation came from Costa Rica. They were ripened with ethylene gas at 20°C for 6 days to stage 5 and 6 according to the United Fruit Company's Guide to the stages of ripeness of bananas (Von Loesecke, 1949). A 5-lb sample was taken for analysis of total acidity, pH, Brix, moisture, firmness, and color. One hundred and twenty-four lbs of bananas were peeled, cut into slices of about 0.5 cm thickness with a stainless steel knife, and then dipped into a 0.5% ascorbic acid solution for one minute (antioxidant). Thirty lbs of the sliced bananas were placed on a sheet of polyethylene in stainless steel trays (61 x 91 cm). They were frozen in a -15°F room for 24 hours. Another 30 lbs of sliced bananas were quick frozen in 2-lb batches in a nylon basket (24 x 20 cm) with Freon 12 for 60 seconds (Fig. 1). The drum (60 x 80 cm) was insulated with polyethylene. A stainless steel chamber containing Freon 11 and dry ice was used to cool the center chamber with Freon 12 (-22°F). The nylon basket with banana slices was dipped into Freon 12 for 60 seconds. The frozen product was sealed in polyethylene bags and kept at -15°F.

Freeze-drying.

Both slow and quick frozen bananas were dehydrated in a Stokes Model 2004 L freeze-dryer. There were two hollow shelves in the drying chamber, each shelf measuring 24" x 36" (Syn and Luh, 1965). The two lower shelves served as condensers, with Freon 12 as the refrigerant. During the drying operation, the pressure in the dryer was maintained at approximately 120 μ Hg by a positive displacement vacuum pump. The pressure was measured

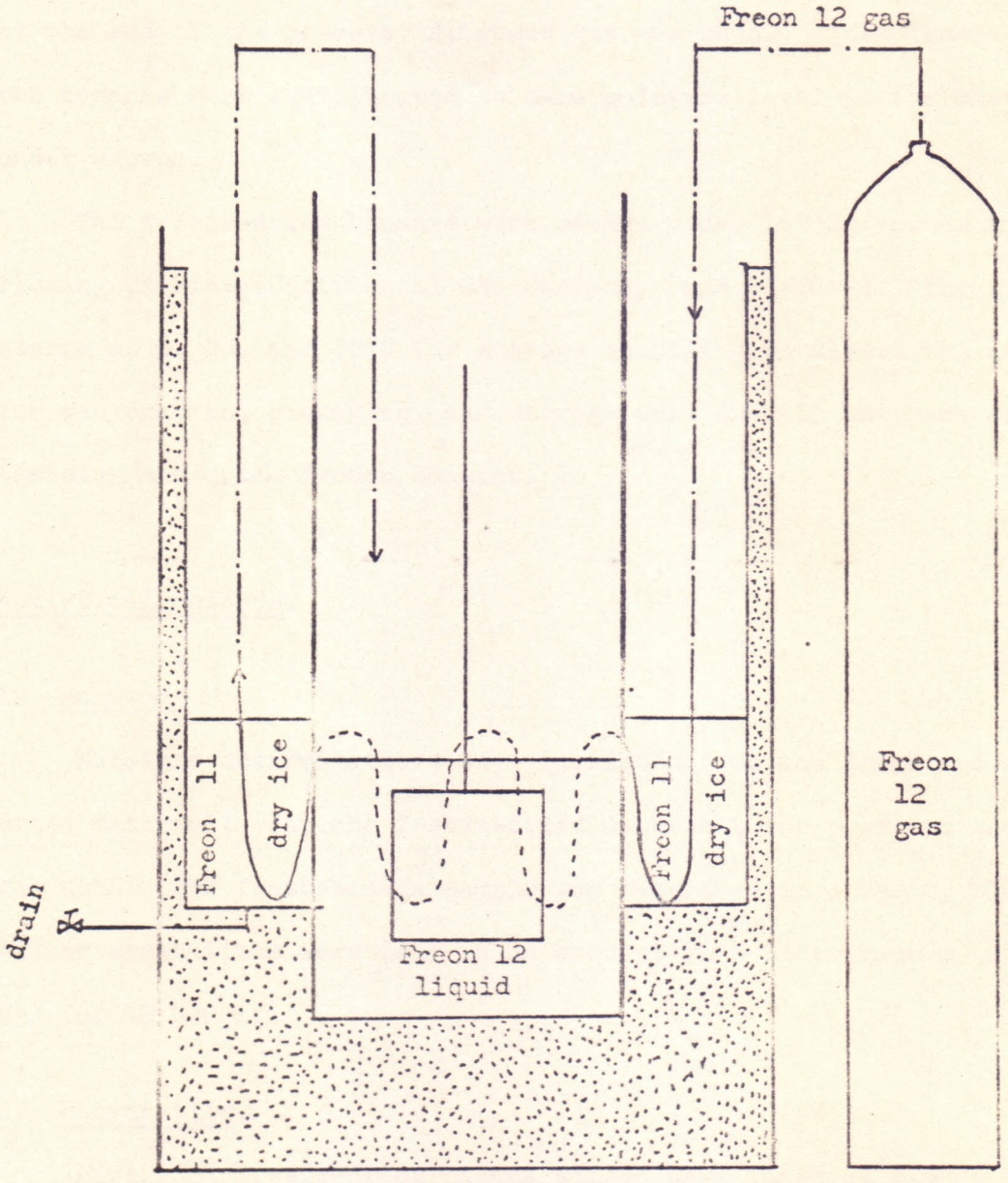

Figure 1. Diagrammatic representation of the quick-freezing process (cross section).

by a McLeod gauge. When the gauge reached 180μ Hg, water at 22°C was circulated through the two upper shelves to supply the heat of sublimation. The drying time was 60 hours. To break the vacuum in the chamber at the end of the process, nitrogen gas was used. After freeze-drying, the bananas were equilibrated to same moisture level in the chamber under vacuum.

The freeze-dried bananas were packed under 16" Hg vacuum in a vacuum closing machine (Continental Can Company, type 23-DS-4). The product was stored at 0, 20, and 30°C for storage stability studies. The conditions for dehydration, packaging, and storage were exactly the same for both the slow and quick frozen bananas.

Analytical methods.

1. Moisture.

Moisture determinations were carried out on the fresh and freeze-dried material. For the freeze-dried material, the powdered sample was used. The fresh banana sample was macerated in a Waring blender. All determinations were done in an oven at 70°C under vacuum (29 inches Hg) for 12 hours.

2. Rehydration.

Five g of freeze-dried banana slices were taken at random from each can. The samples were weighed with a Mettler balance and soaked in 250 ml of distilled water at room temperature. At five-minute intervals, samples were removed, drained on an 8-mesh screen for one minute, and then re-weighed. The weight of water absorbed per g of freeze-dried

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material was calculated up to 40 minutes of soaking.

3. Ascorbic acid.

The 2,6-dichlorophenol indophenol photometric method (Loeffler and Ponting, 1957; Townsend et al., 1956) was used for determination of ascorbic acid in freeze-dried bananas. Five g of the powdered sample was homogenized for 120 seconds with 250 ml of 1% HPO_3. The resulting mixture was centrifuged for 15 minutes in an International centrifuge. The supernatant was filtered through a Whatman No. 1 filter paper. The filtrate was used for the determination of ascorbic acid. The dye was prepared by dissolving 13 mg of sodium 2,6-dichlorophenol indophenol in 950 ml of warm, copper-free distilled water, cooled, filtered, and diluted to 1 liter. A Klett-Summerson photoelectric colorimeter with a No. 54 green filter was used. Nine ml of the dye was pipetted into 1 ml of 1% metaphosphoric acid. The absorbance of the well-mixed solution was read (L 2). Nine ml of dye was then added to a Klett tube containing 1 ml of filtered sample. The absorbance of the mixture was recorded (L 1). A blank was prepared by mixing 9 ml of blue dye with 1 ml of the sample and a few crystals of l-ascorbic acid. In this way a blank was obtained which compensates for the turbidity of the sample.

4. Folin-Denis test.

Tannin-like substances containing aromatic hydroxyl groups were measured by the method described by Luh et al. (1958) with some modifications.

Ten g of sample was blended with 50 ml of 6% HPO_3 and 50 ml 95% ethanol. The total volume was made to 200 ml with 70% ethanol. The

macerated product was centrifuged in an International centrifuge for 10 minutes. The supernatant was filtered through Whatman No. 1 filter paper. To one ml of the filtrate was added 50 ml of distilled water, 5 ml of Folin-Denis reagent and 20 ml of 1 M Na_2CO_3 and then diluted to 100 ml with distilled water. The mixture was allowed to stand for 90 minutes and then filtered through Whatman No. 1 paper. The absorbance of the blue colored solution was measured in a Klett-Summerson photoelectric colorimeter with a No. 66 blue filter. The results were expressed as mg of tannic acid per 100 g of freeze-dried bananas.

5. Color.

A Gardner color-difference meter was used to evaluate the color of the freeze-dried banana powder. Used as reference was a white porcelain plate (Rd=82.7, a=-2.1, b=+2.8). For 9 and 12 months a Gardner automatic difference meter, Model AC-3, was used.

6. Water-soluble pigments.

Water-soluble pigments in freeze-dried bananas were determined by the colorimetric method described by Luh et al. (1958). Five g of powdered sample was soaked for 30 minutes in 95 ml of 1% HPO_3, blended for 3 minutes in a Waring blender and then centrifuged at 1,400 rpm for 20 minutes. The supernatant was filtered through Whatman No. 1 filter paper. Twenty ml of the filtrate was mixed with an equal volume of 95% ethanol and filtered through Whatman No. 1 filter paper. The absorbance of the filtrate was measured in a Klett-Summerson photoelectric colorimeter with a No. 42 blue filter.

7. Extraction of polyphenoloxidase from freeze-dried bananas.

The extraction was done at 32°F. All equipment and reagents were cooled to 32°F before use. Ten g of powdered sample was blended for 5 minutes in a Waring blender with 100 ml of 0.01 M acetate buffer, pH 5.65. The slurry was centrifuged at 3,500 rpm. The supernatant was decanted. Two volumes of acetone -15°F was slowly added to the supernatant. The mixture was allowed to stand for five minutes and centrifuged at 3,500 rpm for 10 minutes. The supernatant was discarded. The precipitated enzyme was dissolved in 50 ml of 0.01 M acetate buffer, pH 5.65. The enzyme was precipitated again with two volumes of cold acetone (-15°F) and allowed to stand for 5 minutes. The mixture was again centrifuged for 10 minutes at 3,500 rpm. The supernatant was decanted. The enzyme (precipitate) was dissolved in 30 ml of 0.01 M acetate buffer, pH 5.65. The yellowish preparation was then centrifuged at 4,000 rpm for 20 minutes in order to remove any cell debris. The enzyme preparation was kept in rubber-stoppered test tubes at 32°F with a few drops of toluene. This partially purified preparation of polyphenoloxidase was stable for at least seven weeks.

8. Polyphenoloxidase activity.

The activity of the enzyme preparation was measured by the method described by Ponting and Joslyn (1948). A colorimeter (Bausch and Lomb, Spectronic 20) was used to measure the optical density of the yellowish preparation at a wavelength of 420 mμ. A 0.075 M pyrocatechol solution was used as substrate. The reaction mixture was made in a 125-ml Erlenmeyer flask in the following way:

2 ml enzyme (1 volume diluted with 4 volumes of 0.01 M acetate, pH 5.65)

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5 ml 0.075 M pyrocatechol

43 ml 0.1 M citrate-0.2 M phosphate buffer, pH 6.20

The reaction was done at 30 ± 0.1°C. The buffer and substrate were mixed and incubated at 30°C for 10 minutes. The enzyme at 30°C was then added with a rapid delivery pipette. The blank was the reaction mixture containing the enzyme which was boiled for 10 minutes and reconstituted to its original volume with distilled water. After the addition of the enzyme, the material was shaken for 2 seconds and the absorbance was measured in a Spectronic 20 at 420 mμ at 60-second intervals. The results are expressed as increase in optical density per minute per ml enzyme.

9. Effect of pH on polyphenoloxidase activity.

The rate of catechol oxidation by banana polyphenoloxidase was studied in the pH range 4.0-7.0. Two ml of diluted enzyme (1 volume enzyme preparation + 4 volumes of 0.01 M acetate buffer, pH 5.65), 5 ml of 0.075 M pyrocatechol and 43 ml of 0.1 M citrate-0.2 M phosphate buffer of desired pH values were used. This was equivalent to 0.125 g freeze-dried banana in 50 ml reacting mixture. To compare slow and quick-freezing processes, enzyme extracts of the samples right after freeze-drying were compared for polyphenoloxidase activity at various pH values.

10. Total acidity.

Five g of freeze-dried banana powder was mixed with 250 ml of distilled water. The mixture was titrated to pH 8.0 with 0.1 N NaOH in a Beckman Model K automatic titrator. The results are reported as percent malic acid. For fresh bananas, 20 g of representative sample was blended

with 150 ml of distilled water. The resulting mixture was titrated with 0.1 N NaOH to pH 8.0.

11. pH value.

Ten g of freeze-dried banana powder was diluted with 30 ml of distilled water. The pH of the mixture was measured with a Corning Model 12 Research pH meter.

12. Organoleptic evaluation.

One part of freeze-dried banana powder was rehydrated with 3 parts of distilled water containing 0.5% ascorbic acid to inhibit browning. A panel of 12 members scored the color, aroma, and flavor of 4 samples on a 1-10 hedonic scale as follows: excellent, 9-10; good, 7-8; fair, 5-6; poor, 3-4; and very poor, 1-2. The average color, aroma, and flavor scores were evaluated for statistical significance by analysis of variance and least significant differences.

13. Total carotenoids.

Thirty g of freeze-dried banana powder was blended with 100 ml of petroleum ether in a Waring blender for 2 minutes. The macerate was centrifuged at 2,000 rpm for 10 minutes. The extraction process was repeated twice. The combined supernatants were filtered and then washed several times with distilled water. The petroleum ether extract was treated with anhydrous sodium sulphate to remove water and concentrated to 50 ml. The absorbance of the extract was measured in a Beckman DB recording spectrophotometer using 1 cm cuvettes. The results were expressed by increase in optical density.

14. Pressure test.

A Magness-Taylor pressure tester equipped with a 5/16" plunger and

pressure range of 0 to 10 lbs was used to determine the firmness of the fresh fruit. Pressure readings were taken on the ends and center of the fresh fruit, both with and without peel. The average of 10 determinations was reported.

15. Soluble solids.

The soluble solid content of the macerated fresh fruit was determined at 20°C with a Zeiss-Opton Refractometer.

16. Polyphenolic compounds in freeze-dried bananas.

The material used for the present study consisted of freeze-dried bananas made by the slow and quick frozen processes. The products were kept at 0°C in No. 2½ cans under a vacuum of 16 in Hg.

Reagents

a) $FeCl_3$-$K_3Fe(CN)_6$ reagent. The reagent was prepared just before use by mixing equal volumes of aqueous 0.5% $FeCl_3$ and 0.5% $K_3Fe(CN)_6$ (W/V). It gives blue color with catechol and pyrogallol types of polyphenols (Keppler, 1957).

b) Diazotized p-nitroaniline (DPNA) reagent. It was prepared by mixing in an ice bath 0.5% p-nitroaniline in 2 N HCl, 5% sodium nitrite, and 20% sodium acetate (W/V) in a ratio of 10:1:30. The reagent gives characteristic colors with phenolic acids (Swain, 1953).

c) Hoepfner reagent. This was prepared by mixing equal volumes of 5% (V/V) acetic acid and 5% (W/V) sodium nitrite just before use. This gives characteristic colors with phenolic acids (Walker, 1962).

d) Vanillin reagent. A 1% ethanolic solution of vanillin was mixed with an equal volume of concentrated hydrochloric acid immediately

before use. It gives a pink to orange-red color with flavonoid compounds containing phloroglucinol nuclei (Swain and Hillis, 1959).

3) Ethanolic aluminum chloride. A 5% ethanolic aluminum chloride solution was used for spectral studies.

f) Leucoanthocyanin reagent. The reagent was made from conc. HCl and n-butanol in a ratio of 5:95 (V/V). It was used to convert leucoanthocyanidins to anthocyanidin (Swain and Hillis, 1959).

g) Folin-Denis solution. The procedure described in the Official Methods of Analysis (A.O.A.C., 1960) was used for quantitative determination of polyphenolic compounds.

h) Serotonin reagent. 5-hydroxytryptamine (serotonin) was developed with a reagent composed of 9 volumes of 0.1% potassium dicromate and 1 volume of 40% formaldehyde. The sprayed chromatogram is heated for 5 minutes at 100-110°C and is viewed under an ultraviolet light source, where the compound produces a golden yellow fluorescent spot (Block, 1958).

i) Ehrlich reagent. Two g of p-dimethylaminobenzaldehyde is dissolved in 100 ml of concentrated HCl. One volume of the solution was mixed with 9 volumes of acetone. The reagent was made up just before use. The chromatograms are dipped into this reagent and then dried at 40°C. The sensitivity of this reagent is 0.05 µg for serotonin and other indoles (Block, 1958).

j) Ninhydrin reagent. A 0.3% ninhydrin solution in 95% ethanol was used. The color was developed at room temperature in the dark for 18 hours (Block, 1958).

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k) Detection of chlorogenic acid. The chromatogram was sprayed with a saturated solution of 3,5-dinitrosalicylic acid in 50% ethanol containing 2% NaOH. A bright yellow spot was produced (Block, 1958).

17. Extraction of polyphenols with ethyl acetate.

Sixty g of freeze-dried bananas was blended for 5 minutes with 300 ml of 70% methanol containing 0.2% HCl under a nitrogen atmosphere. The macerate was centrifuged and the supernatant was filtered in a Buchner funnel through a Whatman No. 1 filter paper. The residue was extracted 3 times more with the same amount of solvent. The combined supernatants were stored overnight at 0°C under a nitrogen atmosphere, filtered through Whatman No. 1 filter paper, and concentrated in a flash evaporator under vacuum to remove the methanol. The residue was suspended in a sufficient quantity of distilled water and extracted twice with petroleum ether to remove carotenoids. The aqueous solution was saturated with sodium chloride and extracted 4 times with 250-ml portions of ethyl acetate. The ethyl acetate extracts were combined, dried with anhydrous sodium sulphate, filtered, and evaporated to a dryness in a flash evaporator under vacuum. The residue was dissolved in methyl alcohol.

18. Two-dimensional paper chromatography.

Two-dimensional, descending paper chromatography was done to determine the number of polyphenolic compounds in the extracts and for measuring the relative quantity of the polyphenolic compounds. Fifty microliters of the extract was spotted on the upper left corner of a Whatman No. 1 paper (46 x 57 cm). The chromatograms were developed in two dimensions at 20°C, with BAW (4:1:5) as the first solvent for 18 hours and 2% acetic acid as

the second solvent for 3.5 hours.

Harborne (1960) reported that the upper phase of the mixture of n-butanol-acetic acid-water (4:1:5, by volume) is most valuable for separating all types of flavonoids, both glycosides and aglycones. R_f values determined in BAW often vary more than those measured in other kinds of solvent. Equally good separation of phenols are obtained with the n-butanol-acetic acid-water mixture, if used fresh, or after it has been kept for several days; R_f values, however, vary with the time of equilibration (Harborne, 1961).

The air-dried chromatograms were first examined under ultraviolet light and examined again on exposure to ammonia vapor (Swain, 1953). One chromatogram was sprayed with freshly prepared $FeCl_3$-$K_3Fe(CN)_6$ reagent. The chromatogram was rinsed with 2% HCl and then with distilled water. The blue spots represent the location of polyphenolic compounds. A second chromatogram was sprayed with diazotized p-nitroaniline (Swain, 1953), and the color characteristic of the spots were noted. A third chromatogram was sprayed with the vanillin reagent. The R_f values of the spots were measured.

19. Identification of polyphenols.

The methyl alcohol extract was applied on Whatman No. 3 MM paper. The papers were chromatographed in a descending direction in two dimensions in the same manner as described above. The spots showing identical R_f values and color reaction were cut, combined, and extracted with 95% ethanol overnight on a mechanical shaker. The eluates were concentrated to dryness in a flash evaporator under vacuum.

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The compounds were spotted on Whatman No. 1 papers. The papers were chromatographed at 20°C with BAW (4:1:5) in a descending direction the same way as described before. The color reactions of each spot with various reagents were observed and the R_f values were measured. Spectral measurements were made with a Beckman DB recording spectrophotometer, using 1 cm silica cuvettes. For the purpose of measuring a bathochromic shift, three drops of 5% aluminum chloride in 95% ethanol was added to the cuvettes. Measurements of the shifts in absorption spectrum were made after standing for one minute.

20. Relative amount of polyphenols.

Ten Whatman No. 3 papers were spotted each with 50 microliters of the methyl alcohol extract. The papers were chromatographed in a descending direction in two dimensions as described previously. The spots shown under ultraviolet light or by spraying with $FeCl_3$-$K_3Fe(CN)_6$ reagent on a separate two-dimensional chromatogram were cut, combined, and eluted with 95% ethanol. The eluates were concentrated in a flash evaporator. Seven ml of distilled water and 0.5 ml of Folin-Denis reagent were added. After standing for 3 minutes, 1.0 ml of 20% (W/V) Na_2CO_3 solution was added. The mixture was diluted with distilled water to make 10 ml, kept in the dark for 1 hour, and then centrifuged. The absorbance of the blue solution was measured in a Klett-Summerson photoelectric colorimeter, using a No. 66 red filter (Swain and Hillis, 1959).

21. Indoles in freeze-dried bananas.

A portion of the methanolic extract was applied to papers and developed in a descending direction using the following solvents:

iso-propanol: NH_4OH: H_2O 8:1:1 (V/V)

iso-propanol: NH_4OH: H_2O 10:1:1 (V/V)

n-propanol: NH_4OH: H_2O 6:3:1 (V/V)

iso-propanol: NH_4OH: H_2O 20:1:1 (V/V)

Occasionally ascending chromatography was done. The time for development was 13 hours. The air-dried chromatograms were first examined under ultraviolet light. One chromatogram was sprayed with the serotonin reagent, another with the Ehrlich's reagent. The R_f values and color reactions were recorded (Block, 1958).

22. Leucoanthocyanidin.

To investigate the presence of such compounds, banana powder was homogenized in 5 volumes of acetone-2 N HCl (12:1) and centrifuged.

The supernatant was concentrated to a small volume in a flash evaporator. A portion of the extract was applied to Whatman No. 1 paper and the chromatograms developed in two dimensions with BAW (4:1:5 V/V) and 2% acetic acid in the same way as described above. A part of the acetone extract was refluxed with the leucoanthocyanidin reagent which caused the development of a pink to brownish-red color.

The hydrolysate was chromatographed on Whatman No. 1 and 3 MM filter papers in BAW (4:1:5 V/V) in a descending direction for 18 hours. The Forestal solvent (acetic acid-water-conc HCl, 30:10:3 V/V) was used to irrigate the paper for 16 hours in a descending direction.

IV. RESULTS AND DISCUSSION.

The chemical changes in freeze-dried bananas made by the slow and quick-freezing processes when stored at 20 and 30°C were studied. Samples obtained just after the freeze-drying process were used as references.

1. Evaporation ratio.

The fresh Valery bananas contained 71.74% moisture and the freeze-dried product 2%. The relation between the moisture content, M (percentage on the wet basis), and the moisture ratio, T (weight of water in grams per gram of dry matter), is as follows:

$$T = \frac{M}{(100-M)} .$$

The initial moisture ratio (T_o) was 2.53 and the final moisture ratio (T_f) 0.020. The evaporation ratio (grams of fresh bananas required to yield one gram of dried product) was calculated as follows:

$$\text{Evaporation ratio} = \frac{T_o+1}{T_f+1} = \frac{2.53+1}{0.020+1} = 3.46 \text{ g}$$

The relatively low evaporation ratio could be explained by the high solid content in the fresh bananas. These values are presented in Table 1.

2. Moisture content.

Freeze-dried banana is hygroscopic. It will absorb moisture rapidly from the air. Thus the permeability of the packaging material to moisture is an important factor to be considered. Sealed tin can is an excellent barrier against moisture penetration. The moisture content before and after freeze-drying are shown in Table 1. There was no uptake of moisture during storage.

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Table 1. Moisture content of fresh and freeze-dried Valery bananas.

Property measured	Values
Moisture content ... fresh (%)	71.74
Moisture content ... freeze-dried (%) ave.	2.00
Evaporation ratio	3.46
Moisture ratio (T_o) g water/g dry substance	2.53
Moisture ratio (T_f) g water/g dry substance	0.02

3. Rehydration.

One of the desirable qualities of freeze-dried foods is a rapid rate of rehydration. It was found that moisture uptake in freeze-dried banana slices was more rapid during the first few minutes of rehydration. Figure 2 illustrates that, right after freeze-drying, the sample made by the slow-freezing process rehydrated more readily than that made by the Freon-immersion (quick-freezing) process.

Figure 3 shows a decrease in rehydration capacity of the product (slow-freezing) during storage. The samples stored at 20°C were better in rehydration capacity than those at 30°C. Figure 4 shows the effect of storage and temperature on rehydration of freeze-dried product (quick-freezing). Storage at 30°C caused loss in rehydration capacity. The slow-freezing product showed a better rehydration capacity than the quick-freezing product. The percentage of rehydration was 62% for the quick-freezing and 68% for slow-freezing product. That 100% rehydration was

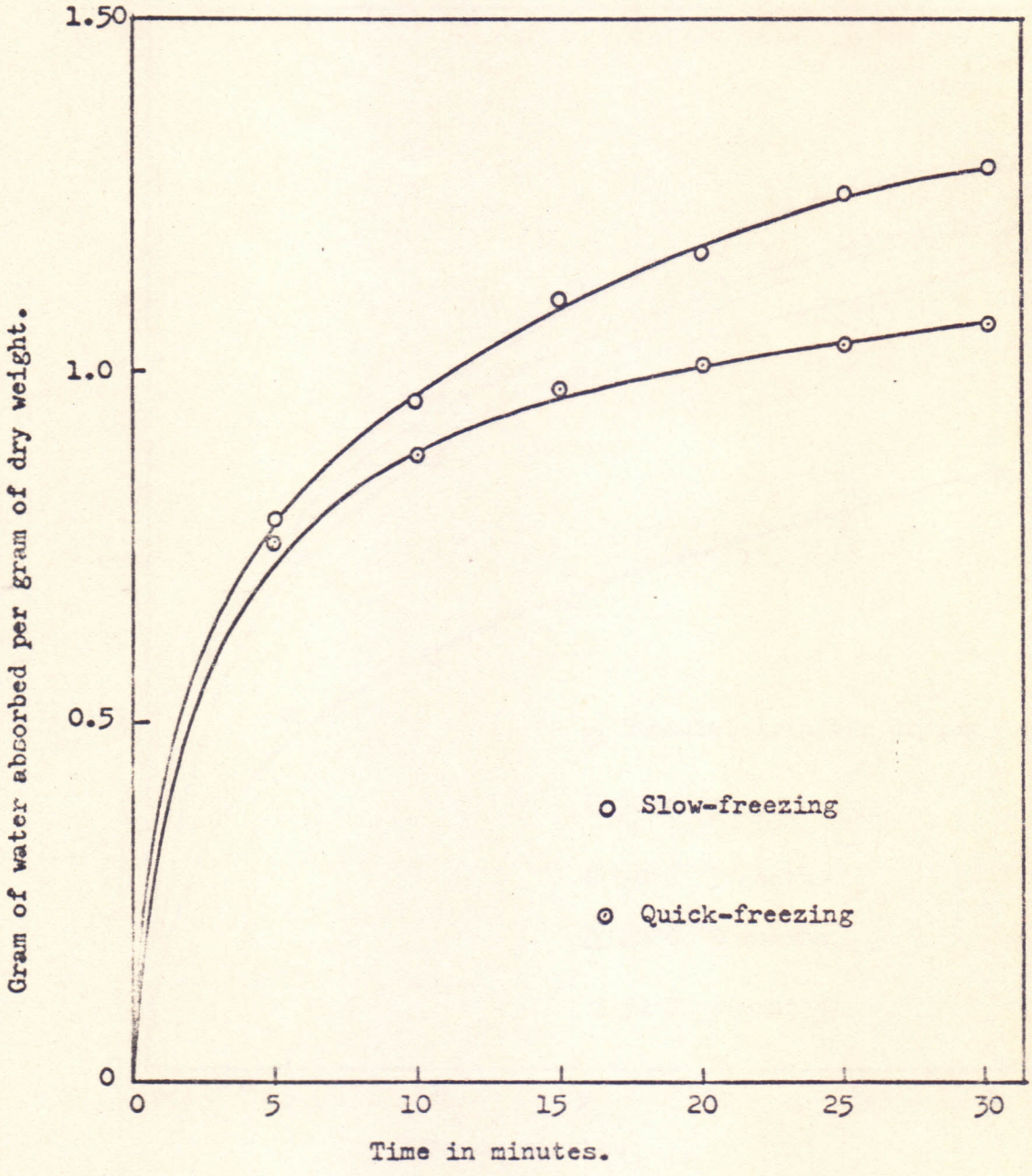

Figure 2. Rehydration of freeze-dried bananas immediately after drying. Effect of freezing rates.

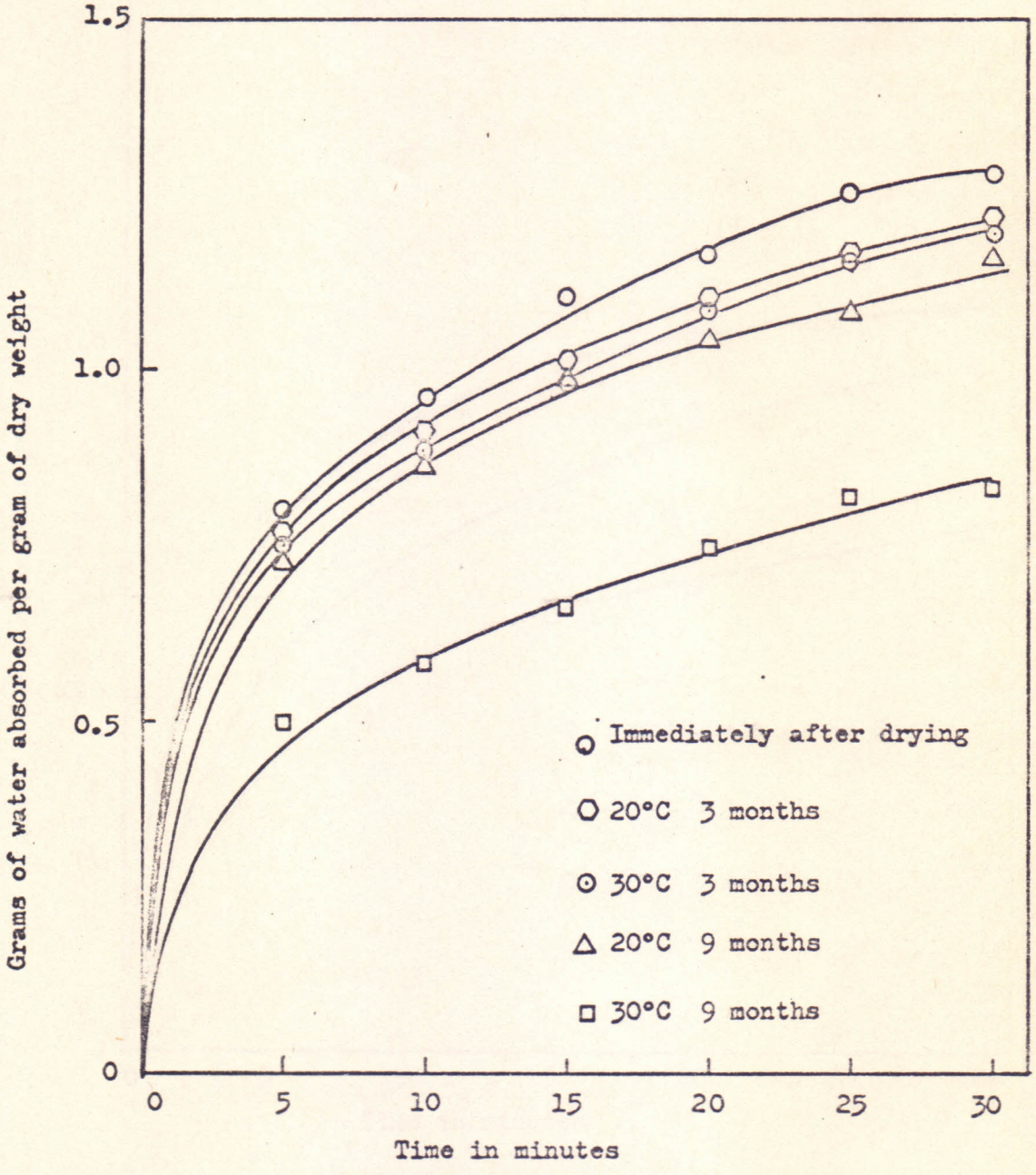

Figure 3. Rehydration of freeze-dried bananas made by the slow-freezing process. Effect of temperature and storage time.

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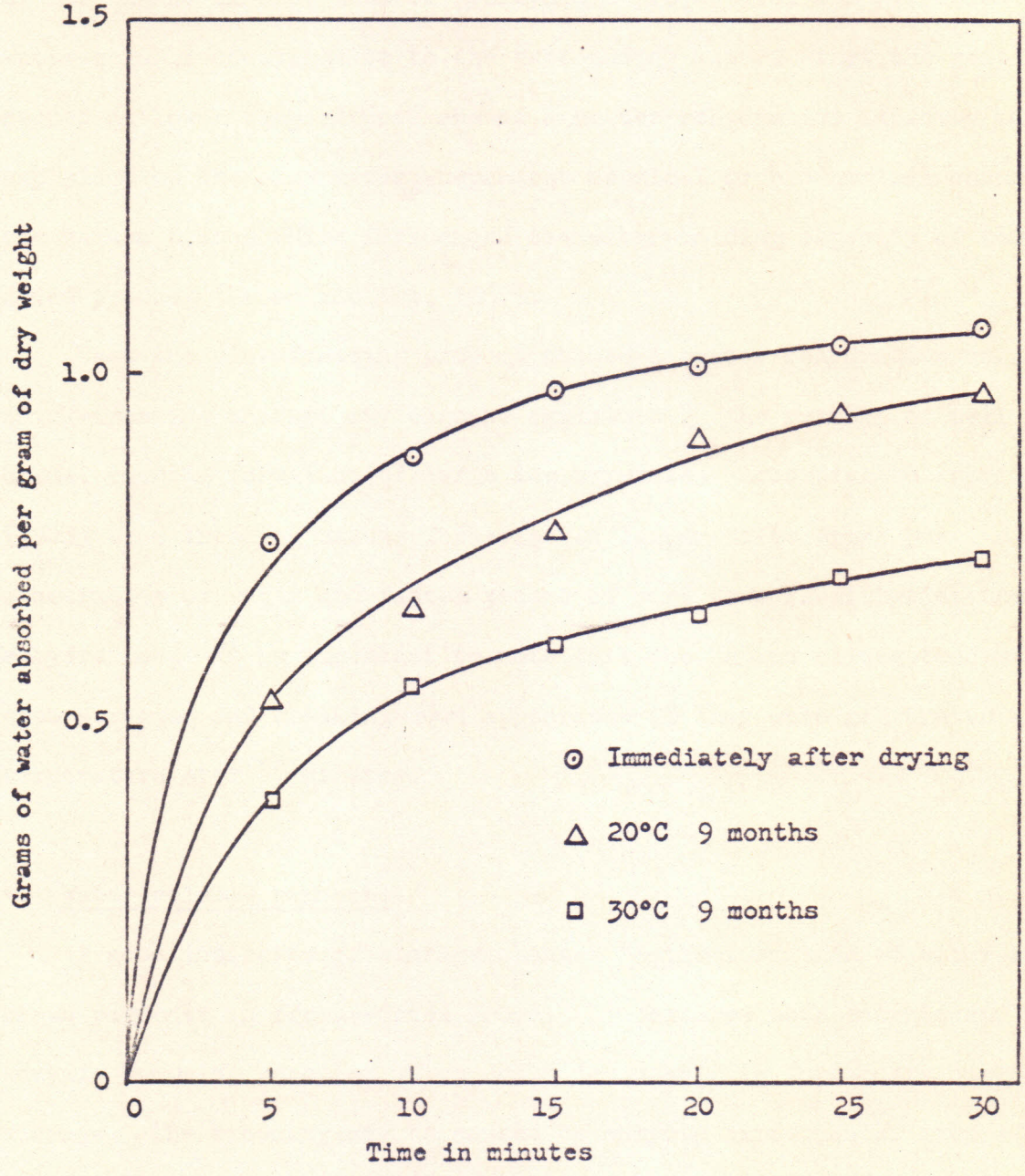

Figure 4. Rehydration of freeze-dried bananas made by the quick-freezing process. Effect of temperature and storage time.

never achieved may be explained by the changes in structural characteristics of the banana tissue, loss of hydrophilic properties, and diffusion of water-soluble constituents to the surrounding water. That the samples stored at lower temperatures showed a better rehydration capacity may be explained by the temperature-dependent chemical or biochemical changes in the banana tissue which influenced the water-holding capacity of the freeze-dried product (Daoud and Luh, 1967).

That the slow-freezing product showed a better rehydration than the quick-freezing product may also be explained by the rupture of cell membrane, causing formation of large ice crystals. According to Kuprianoff (1962) slow freezing causes formation of larger holes which permits better penetration of water and faster escape of air, thus facilitating the rehydration. It is important to note that the banana slices tended to show a ragged and breaking-down appearance if they were rehydrated at room temperature over 40 minutes.

4. Water-soluble pigments.

A good indicator of storage changes is the formation of water-soluble brown pigments in freeze-dried foods. It measures both enzymic and non-enzymic browning reaction which occur frequently in foodstuffs during storage. The browning may be caused by enzymic oxidation of natural polyphenols or by chemical reactions between reducing sugars and amino acids. Figure 5 shows the effect of storage temperature on the water-soluble pigments in freeze-dried bananas. Samples stored at 30°C showed a more rapid formation of water-soluble pigments than those stored at 20°C. Thus storage temperature is an important factor influencing the rate of

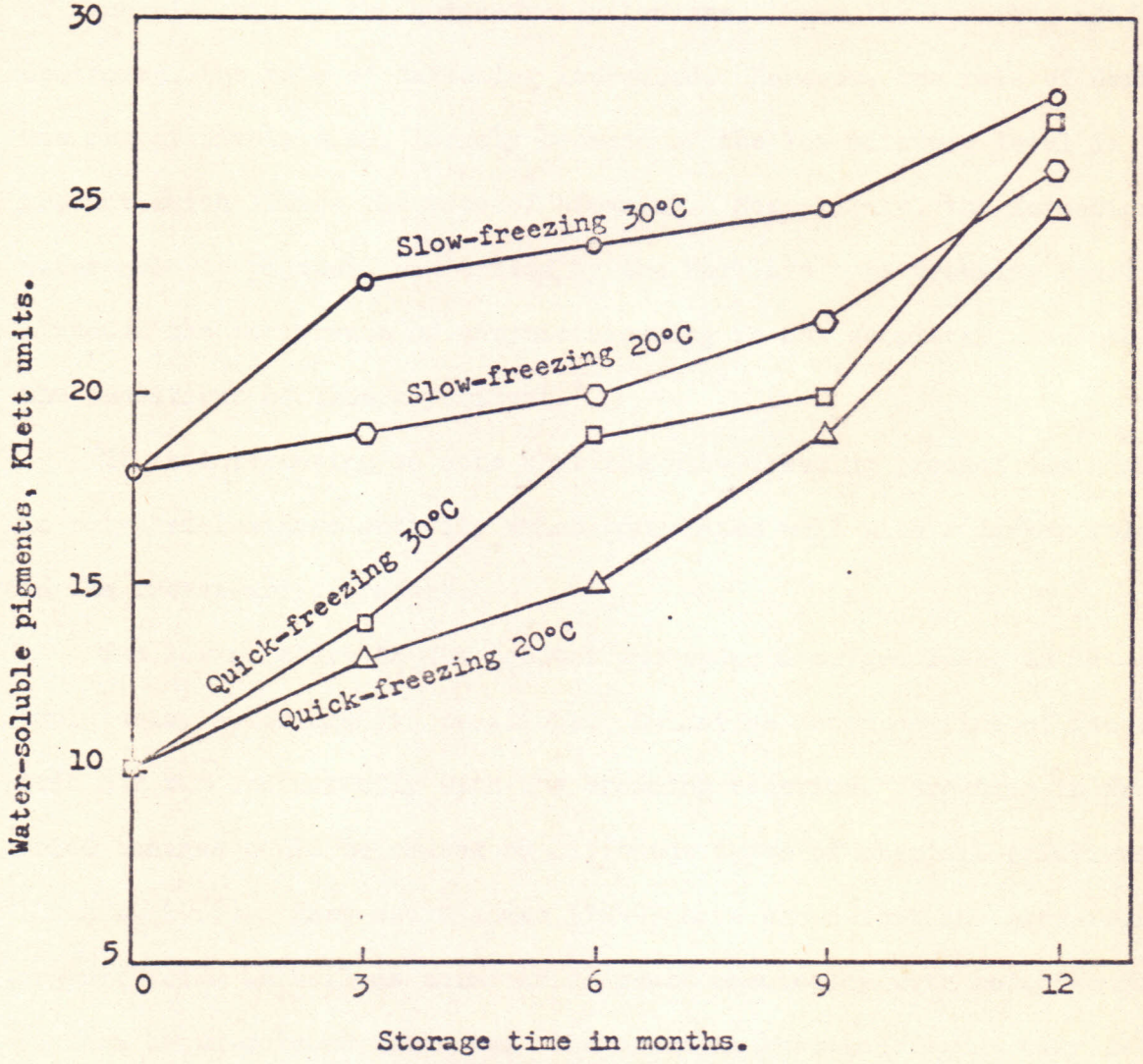

Figure 5. Effect of storage temperature on formation of water-soluble pigments in freeze-dried bananas.

formation of water-soluble pigments. We can see that the slow-freezing process causes more darkening in the product than the quick-freezing process. This phenomenon may be explained by more tissue-cell disruption in the slow-freezing process, causing more contact between the enzyme and the substrate. This may also be explained by the greater retention of ascorbic acid in the quick-frozen bananas. When the ascorbic acid was destroyed, the rate of darkening increased. However, the rate of darkening was comparatively slow, largely because of the low moisture level in the product, which limits the rate of browning. Most likely, the formation of water-soluble pigments was caused by the Maillard type browning reaction, although the occurrence of enzymic browning is not completely excluded under the conditions of this experiment.

It is interesting to note that the slow-freezing product was higher in polyphenoloxidase activity which correlates well with a darker color in the freeze-dried product.

The low moisture in the product and reduced oxygen level in the can would delay deteriorative reactions. Oxidative decomposition of ascorbic acid may run concurrently with the browning reaction. Browning in freeze-dried bananas could be caused by different types of chemical reactions (Draudt, 1966). Hass and Stadman (1949) have shown that in dried fruits, organic acids as well as amino acids react chemically with reducing sugars to form brown-colored products. The browning reactions cause many deleterious effects in foods. Deterioration in texture and failure to reconstitute properly may occur simultaneously with the browning reaction. In addition to these undesirable effects, the loss in nutritive value could accompany the browning reaction.

5. Polyphenoloxidase activity.

Figure 6 shows the activity of banana polyphenoloxidase (PPO) on catechol at 30°C and pH 6.2. The slow-freezing process seems to cause a higher PPO activity in the product than the quick-freezing process. The freeze-drying process did not inactivate the PPO enzyme which showed a high activity in the extract. Dilutions with buffer were made to obtain a proper concentration of the enzyme for the investigation. The fact that the slow-freezing process caused more PPO activity in the bananas may be explained by the formation of more PPO by the cells during the freezing procedure or by liberation of the enzyme through cell disruption. In the Freon-immersion process (quick-freezing), the time for freezing was only 60 seconds, which allows practically no time for the cells to form PPO enzyme.

6. Effect of pH on polyphenoloxidase activity.

The effect of pH on the banana polyphenoloxidase activity in 0.1 M citrate-0.2 M phosphate buffer is shown in Figure 7. The enzyme was most active in the pH range of 5.7 to 6.3.

Reyes and Luh (1960) reported that the type of buffer affects the pH optimum of peach polyphenoloxidase. In the present investigation, citrate-phosphate buffer was used to avoid differences in PPO activity due to different types of buffer. The optimum pH for the partially purified polyphenoloxidase was 6.1.

Griffiths (1959) stated that the browning reactions of banana fruit result from enzymic oxidation of dopamine (3,4-dihydroxyphenylethylamine). Palmer (1961) found more banana PPO activity on dopamine in the pH range 6.0 to 7.0. The optimum pH was at 7.0. He showed that enzyme preparations from bananas could also oxidize other ortho-diphenols such as

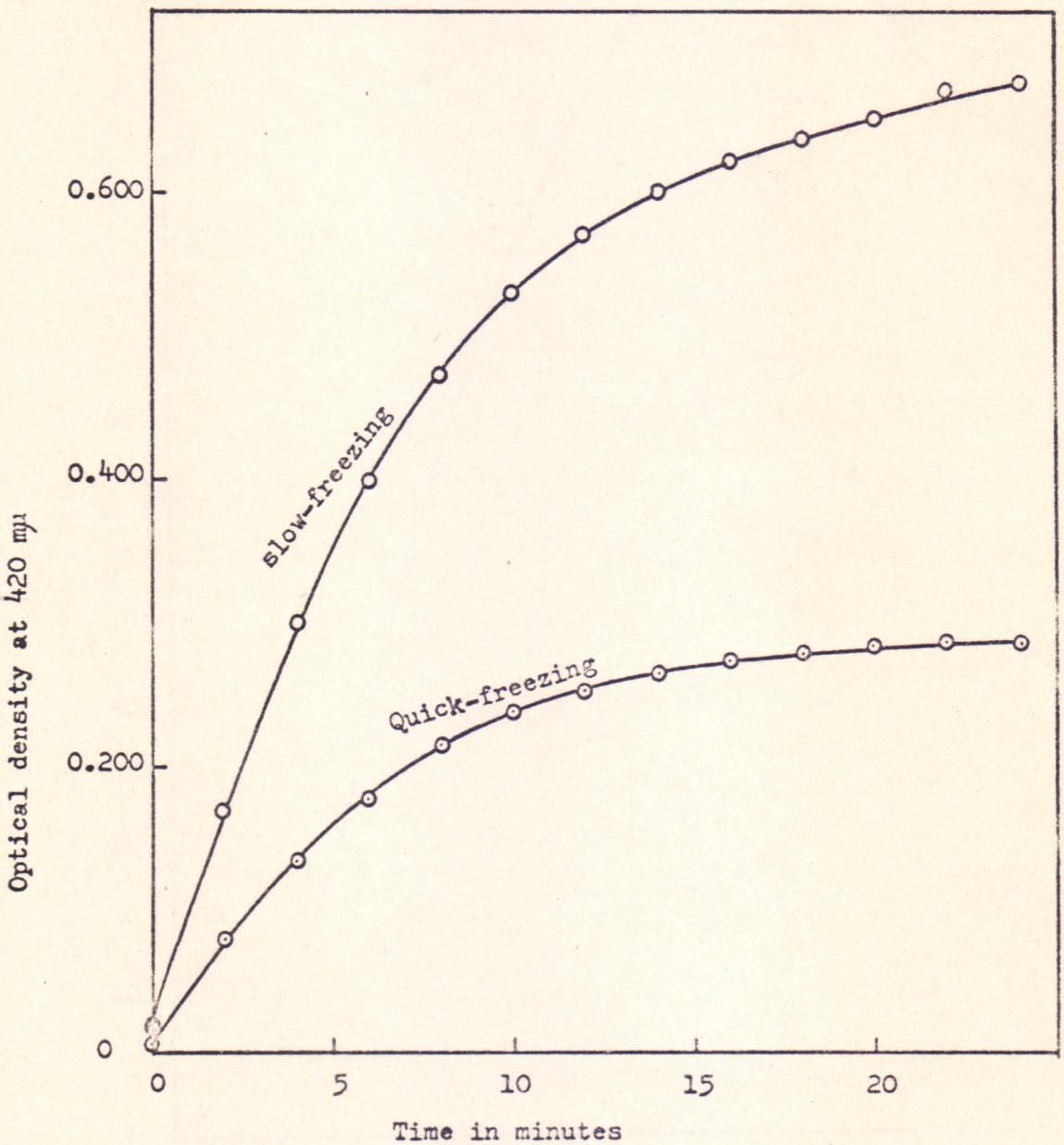

Figure 6. Polyphenoloxidase activity in freeze-dried bananas at pH 6.2 and 30°C in 0.075 M pyrocatechol.

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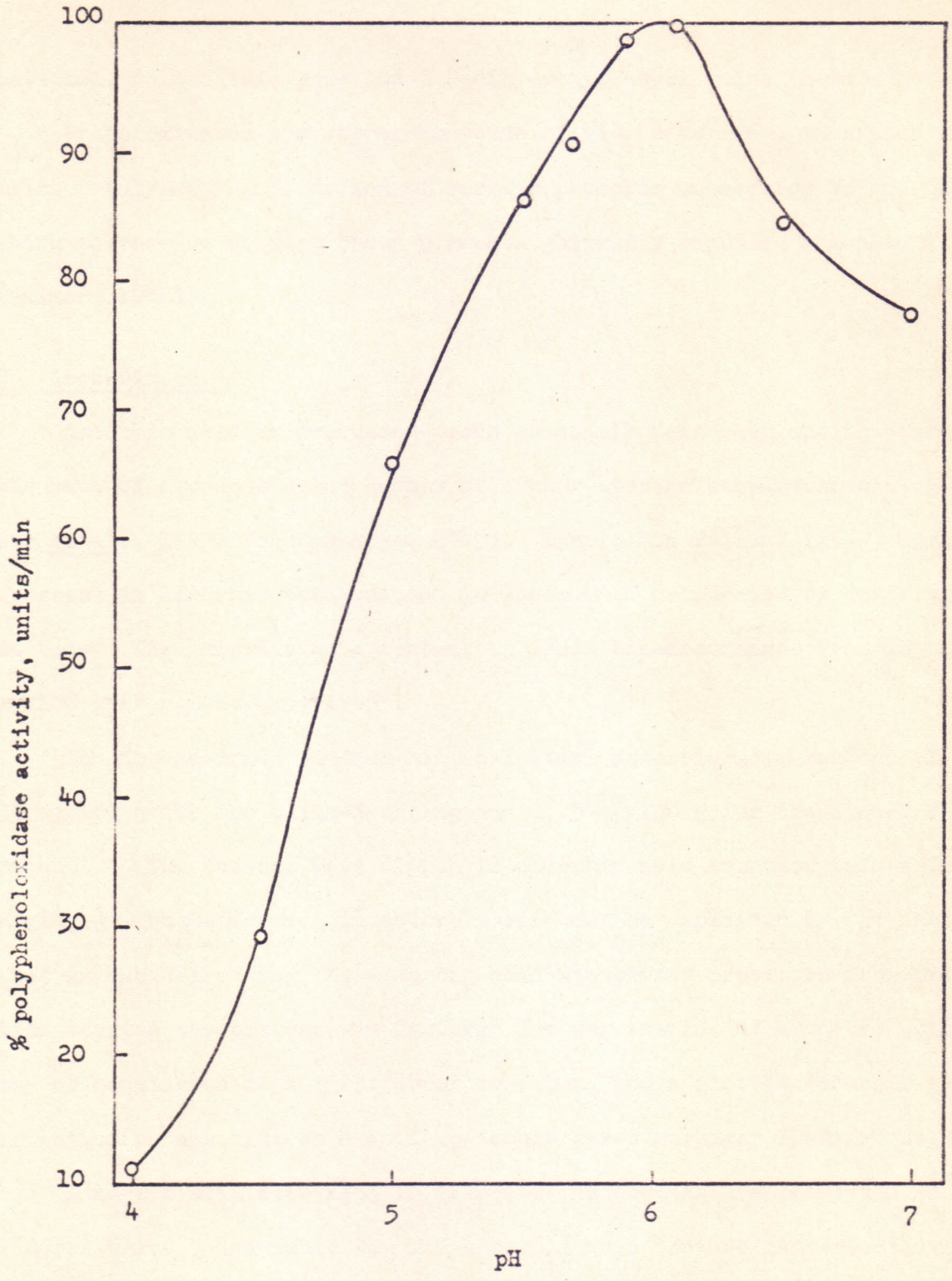

Figure 7. Effect of pH on banana polyphenoloxidase activity in 0.1 M citrate-0.2 M phosphate buffer at 30°C. Substrate 0.075 M pyrocatechol.

catechol, chlorogenic acid and 3,4-dihydroxyphenylalanine (dopa).

Phenoloxidases are copper proteins of wide occurrence in nature which catalyzes the oxidation of certain phenolic substrates to quinones which polymerize to dark brown pigments generally known as melanins (Palmer, 1966).

7. Ascorbic acid.

Ascorbic acid in processed foods generally decreases during storage, the rate of decrease being higher at higher storage temperatures (Wagner et al., 1947; Von Loesecke, 1949). Harris and Polland (1939) found a decrease in ascorbic acid content in foods when dehydrated by different methods. The ascorbic acid content in fresh bananas ranges from 10-12 mg/100 g (Von Loesecke, 1949).

The freeze-dried bananas had an initial ascorbic acid content of 35 mg/100 g for the quick-freezing and 21.5 mg/100 g for the slow-freezing product. (The bananas were dipped in ascorbic acid solution before freeze-drying.) The difference in ascorbic acid may be explained by the fact that on quick-freezing the ascorbic acid was better protected from oxidation because the process was faster. The degradation of ascorbic acid may be considered as a first order reaction, and a plot of ascorbic acid retention versus time on a semi-log scale gives straight line graphs.

Ascorbic acid retention in freeze-dried bananas are presented in Figures 8 and 9 and Table 2. The ascorbic acid content decreased during storage. The quick-freezing product showed a better ascorbic acid retention during storage. After 12 months at 20°C, a retention of 31.7% was observed in the quick-freezing sample. High retention of ascorbic acid

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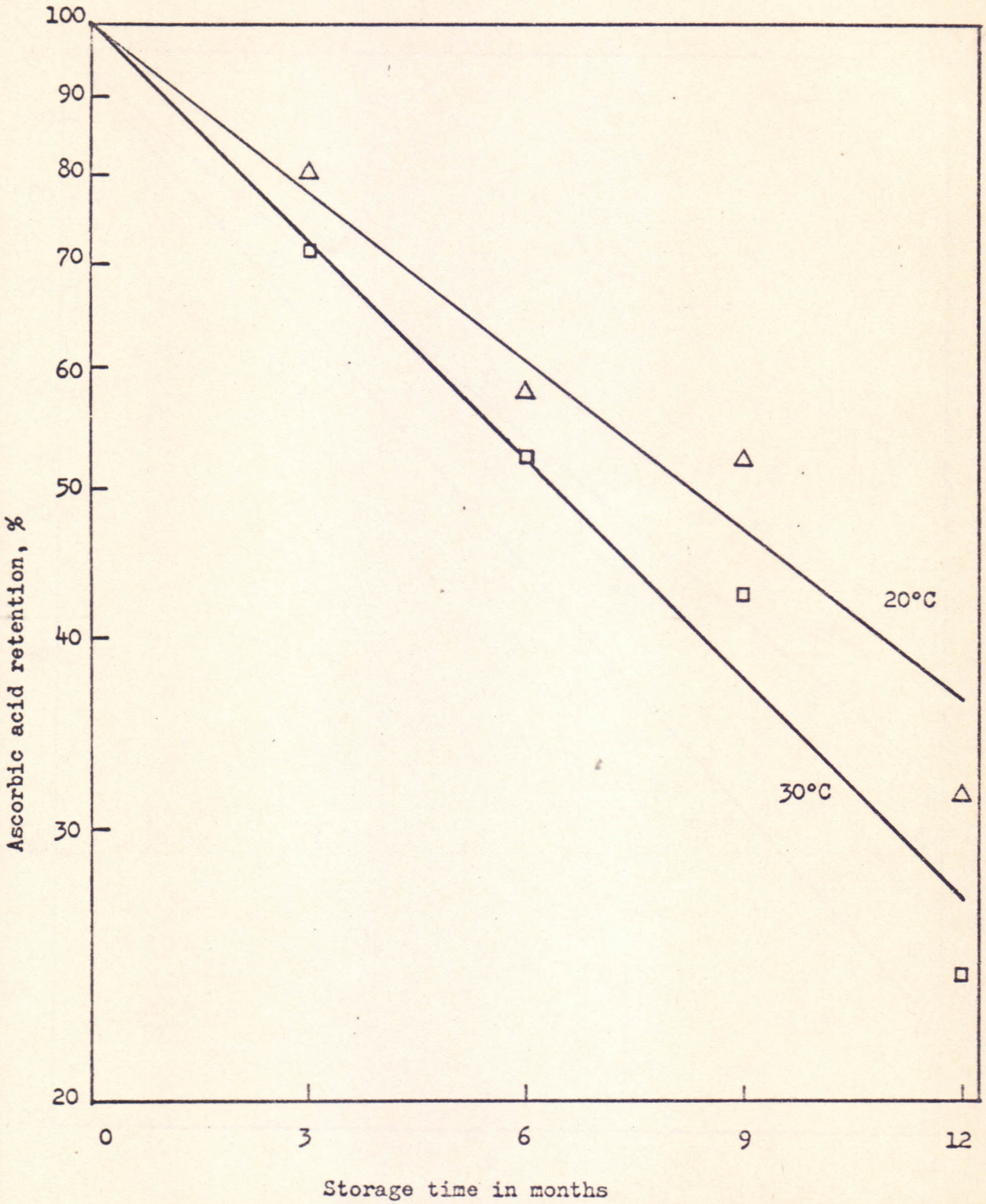

Figure 8. Effect of storage at 20 and 30°C on ascorbic acid retention in freeze-dried bananas made by the quick-freezing process.

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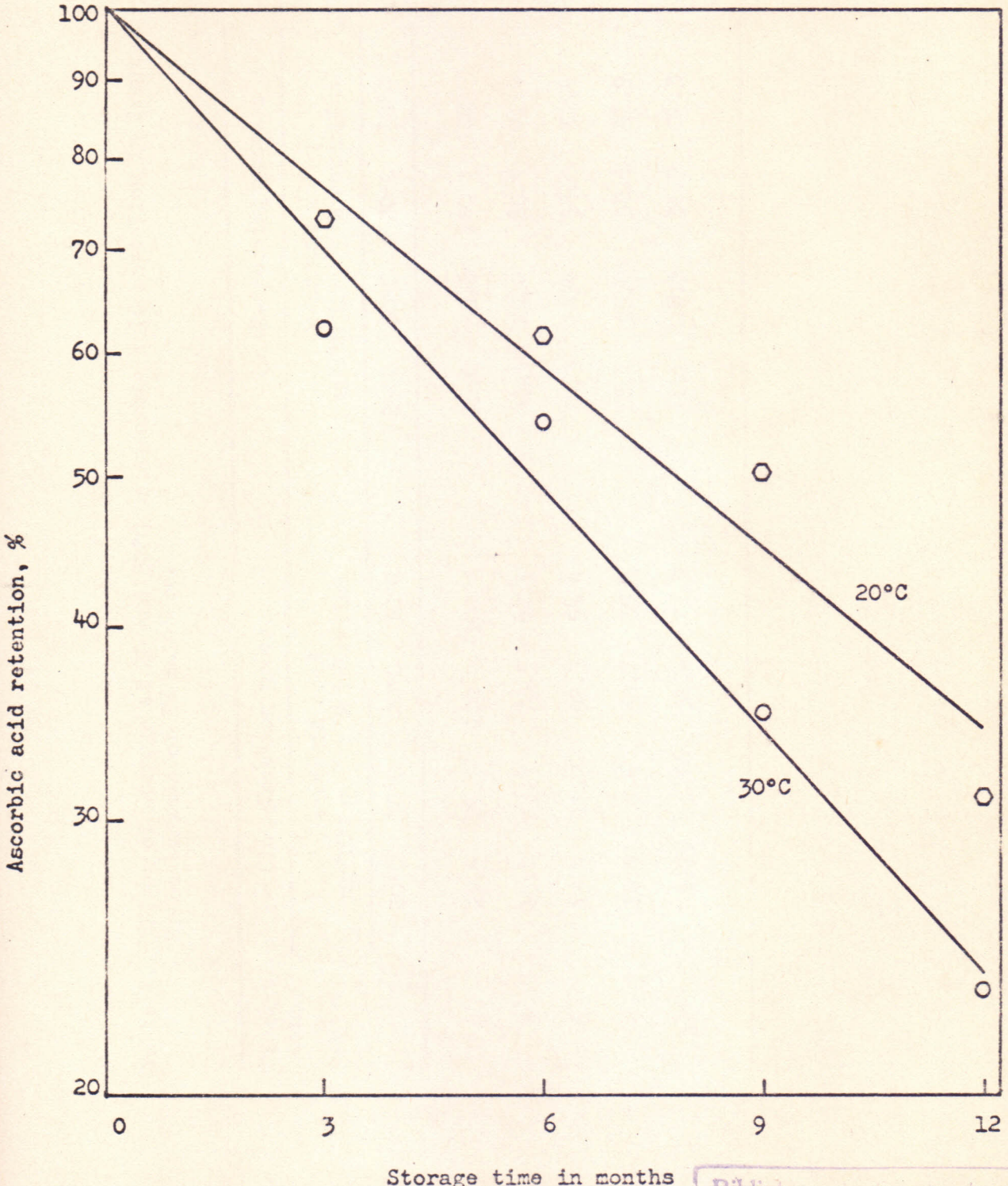

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Figure 9. Effect of storage at 20 and 30°C on ascorbic acid retention in freeze-dried bananas made by the slow-freezing process.

Table 2. Effect of storage at 20 and 30°C on ascorbic acid retention in freeze-dried bananas of 2% moisture.

Storage time, months	Slow-freezing process				Quick-freezing process			
	ascorbic acid mg/100 g		% retention		ascorbic acid mg/100 g		% retention	
	20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C
0	21.5	21.5	100	100	35.0	35.0	100	100
3	15.8	13.4	73.5	62.3	28.3	25.0	80.9	71.4
6	13.4	11.7	62.3	54.4	20.3	18.4	58.0	52.6
9	10.9	7.6	50.7	35.4	18.4	15.0	52.6	42.9
12	6.75	5.06	31.4	23.5	11.1	8.5	31.7	24.3

in the product may be explained by the low moisture in the samples, and the protection provided by the vacuum packed cans. Draudt et al. (1966) reported that the loss of ascorbic acid in freeze-dried bananas during storage is moisture dependent. They reported 57.1% retention of ascorbic acid in freeze-dried bananas of 3.43% moisture after storage at 28°C for 178 days.

The half life of ascorbic acid in the freeze-dried banana was calculated as follows:

$$t_{1/2} = \frac{0.693}{K}$$

where K = specific reaction velocity in reciprocal days; K was calculated by using the following equation:

$$K = \frac{2.303}{t_2 - t_1} \log \frac{C_1}{C_2}$$

where t = time in days,

C_1 = ascorbic acid retention at time t_1

C_2 = ascorbic acid retention at time t_2.

The half-life and velocity constant for the samples are presented in Table 3.

At 20°C, ascorbic acid retention was higher than at 30°C. A higher half-life was observed in the quick-freezing sample stored at 20°C, followed by the slow-freezing product stored at 20°C. In both treatments, high storage temperature caused a more rapid loss of ascorbic acid. The effect of storage temperature and the freezing rate on ascorbic acid retention are manifested in the present investigation. The test is useful for tracing the deteriorative changes in the product during storage.

Table 3. Velocity constants and half-life of ascorbic acid in freeze-dried bananas made by the slow and quick-freezing processes.

Process	Storage temperature °C	Velocity constant reciprocal days	Half-life, days
Slow-freezing	20	2.94×10^{-3}	235.70
Slow-freezing	30	3.92×10^{-3}	176.00
Quick-freezing	20	2.81×10^{-3}	246.61
Quick-freezing	30	3.63×10^{-3}	190.90

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8. Folin-Denis test.

The Folin-Denis test has been applied by Syn et al. (1965) as an indicator for storage changes in freeze-dried asparagus. The basis for this test is the formation of a blue color by the alkaline tungstate-phosphomolybdate reagent with the melanoidin-reductones formed during storage. The intensity of the blue color was measured with a Klett-Summerson phtocolorimeter. The effect of storage temperature on reductone formation in freeze-dried bananas is shown in Figure 9a. A more rapid increase in Folin-Denis value of the slow-freezing sample stored at 30°C was observed. Draudt (1966) found little change in tannic acid in freeze-dried bananas after 183 days' storage, and a relatively low increase in tannic acid also in freeze-dried peaches. Luh et al. (1958) found an increase in tannin-like substances in tomato paste during storage. Luh and Tsiang (1965) also reported a rapid increase in Folin-Denis value in tomato ketchup stored at high temperatures. Thus the deleterious effect of high storage temperature appears to be reflected by an increase in Folin-Denis values. Higher readings were obtained in samples stored for a longer period. The Folin-Denis test proves to be a good indicator for quality evaluation of freeze-dried bananas.

9. Color.

Table 4 shows the effect of storage on Gardner color difference meter readings of the freeze-dried bananas. The samples were powdered just before the color evaluation. This test measures the color of the freeze-dried product as it would appear to the consumer before rehydration. The Gardner color values are reported as "Rd", "a", and "b" values.

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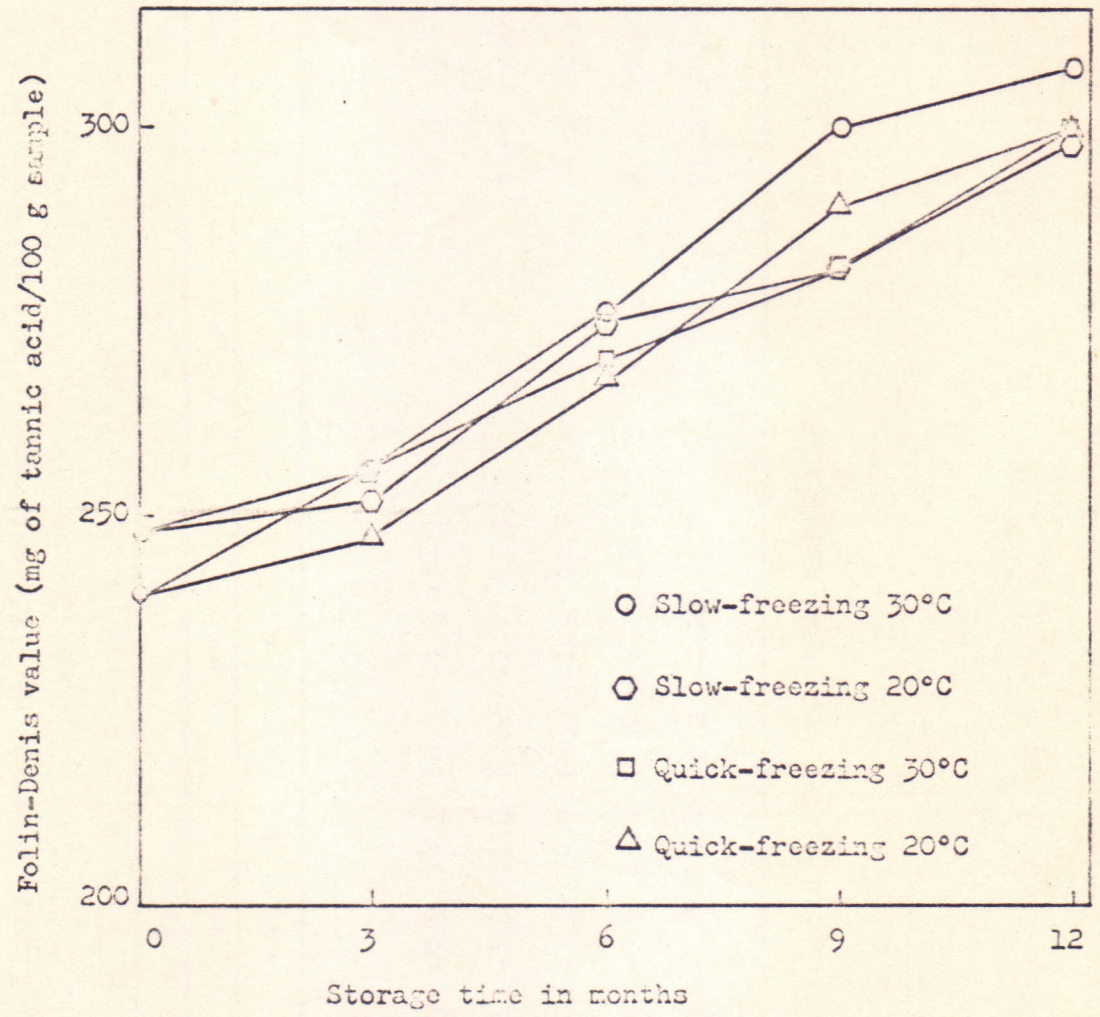

Figure 9a. Effect of storage temperature on the Folin-Denis value of freeze-dried bananas made by the slow and quick-freezing processes.

Table 4. Color of freeze-dried bananas stored at different temperatures and measured with the Gardner color difference meter.

Storage time, (mos.)	Slow freezing									Quick freezing								
	0°C			20°C			30°C			0°C			20°C			30°C		
	Rd	a	b	Rd	a	b	Rd	a	b	Rd	a	b	Rd	a	b	Rd	a	b
0	+69.7	-0.6	+14.5							+70.0	-0.9	+12.3						
3				66.9	-0.9	14.1	66.3	-1.0	+14.1				67.0	-0.9	+12.1	66.9	-1.1	+13.00
6				68.1	-2.0	+11.2	67.2	-1.7	12.1				68.2	-2.0	10.2	67.9	-2.1	11.7
9				71.4	-2.2	14.9	68.0	-1.4	14.7				68.3	-2.3	13.9	69.7	-2.1	14.1
				*73.6	-0.6	13.1	72.6	-0.1	13.1				72.7	-0.60	12.2	73.6	-0.75	11.83
12				*72.3	-0.32	13.6	70.2	+0.22	13.7				71.6	-0.28	11.8	71.63	-0.31	12.86

*another apparatus, Gardner automatic difference meter Model AC-3.

Rd is defined as 100 times the amount of light reflected by a sample divided by that reflected by a perfectly diffusing sample. A completely absorbing specimen would have a Rd value of zero, and a perfect diffusing white one would have an Rd value of 100. The a and b values are defined in terms of tristimulus values x, y, and z as follows:

$$a = 175\ fy\ (1.02x-y) \text{ and}$$

$$b = 70\ fy\ (y-0.847z)$$

A plus value of "a" indicates redness, and a minus value, greenness. A plus value of "b" indicates yellowness, and a minus value, blueness.

From Table 4 we can see that the Rd value of all samples increased slightly during storage. The "a" value changed very slightly. The "b" value showed some fluctuations during storage. The sample made by the slow-freezing process was darker in color than that made by the quick-freezing process. However, there was only a small difference in color after the 12-month storage period.

The freeze-dried banana slices were nearly white in color, much lighter than the fresh banana slices. Color evaluation of the samples after 9 and 12 months' storage was made with another Gardner color difference meter in the Department of Pomology because the apparatus used originally was out of order at that time.

10. Organoleptic evaluation.

The color, aroma and flavor scores of freeze-dried bananas as judged by a panel of 11 persons are shown in Table 5. Just after dehydration the slow-freezing product was scored higher in color and flavor. After storage for 9 months the quick-frozen product was scored higher

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Table 5. Effect of storage temperature on organoleptic quality of freeze-dried bananas. The samples were rehydrated prior to evaluation.

Freezing rate	Storage temp. °C	Average organoleptic scores														
		0 months			3 months			6 months			9 months			12 months		
		color	aroma	flavor	color	aroma	flavor	color	aroma	flavor	color	aroma	flavor	color	aroma	flavor
Slow-freezing		7.6	6.7	7.3												
Quick-freezing		6.0	6.7	6.8												
Slow-freezing	20				7.8	6.8	7.5	7.2	7.0	7.5	7.1	6.5	6.3	7.4	7.4	6.9
Slow-freezing	30				6.7	5.4	6.0	6.4	6.0	6.5	5.4	5.3	5.3	5.4	6.3	6.2
Quick-freezing	20				7.0	6.2	6.8	7.5	7.3	7.3	7.9	7.0	7.4	7.7	7.8	7.4
Quick-freezing	30				6.0	5.6	6.4	6.8	6.6	6.9	6.9	5.4	6.4	5.8	6.7	6.1
LSD at P = .05		2.60	NS	NS	1.07	NS	NS	NS	NS	NS	0.94	0.99	0.92	1.07	1.03	0.99

Evaluations were based on a hedonic scale of 1-10; excellent, 9-10; good, 7-8; fair, 5-6; poor, 3-4; very poor, 1-2.

than the slow-freezing product stored at 20°C and 30°C. The preference for the slow-freezing product at the beginning may be explained by the influence of Freon on the quick-freezing process.

Even after storage for 12 months the samples were acceptable to the judges. Some difficulty was confronted due to the darkening of the samples after rehydration, even though ascorbic acid was used as an antioxidant.

11. pH and total acidity.

The pH and titratable acidity of freeze-dried bananas as influenced by storage temperature are shown in Table 6. An increase in total acidity during storage was observed. The samples stored at higher temperatures were higher in acidity. This increase in acidity may be caused by degradation of the reducing sugars to acidic products or by enzymic formation of acids. No appreciable difference in pH between the samples was observed. Table 7 shows the chemical and physical properties of the fresh bananas used in this work. The Brix value, moisture content, and pressure test indicate that the bananas used in the present work were at a good stage of ripeness for processing.

12. Total carotenoids.

Figure 10 and Table 8 show the absorption spectra of the total carotenoids present in freeze-dried bananas. There was a significant difference in carotenoids content of the products made by the slow and quick-freezing processes. By comparison of the absorption spectra, we can see that the slow-freezing product retained more carotenoids than the one made by the Freon-immersion process. The phenomenon may be explained

Table 6. Effect of storage temperature on pH and total acidity of freeze-dried bananas.

Sample	Storage condition	pH					Total acid (malic) %			
		Storage time in months								
		0	3	6	9	12	0	3	6	12
Slow	0°C	4.90					1.545			
Quick	0°C	4.91					1.544			
Slow	20°C		4.90	4.90	4.90	4.87		1.534	1.545	1.610
Slow	30°C		4.88	4.87	4.88	4.87		1.532	1.568	1.657
Quick	20°C		4.86	4.85	4.87	4.87		1.534	1.582	1.610
Quick	30°C		4.89	4.86	4.90	4.85		1.561	1.610	1.671

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Table 7. Chemical and physical properties of fresh 'Valery' bananas.

Pressure test 5/16 lbs	without peel		with peel	
	center	end	center	end
	1.49	1.40	9.65	9.10
Soluble solids at 20°C (Brix)	20			
pH	4.78			
Acidity as malic acid g/100 g	0.443			
moisture %	71.74			

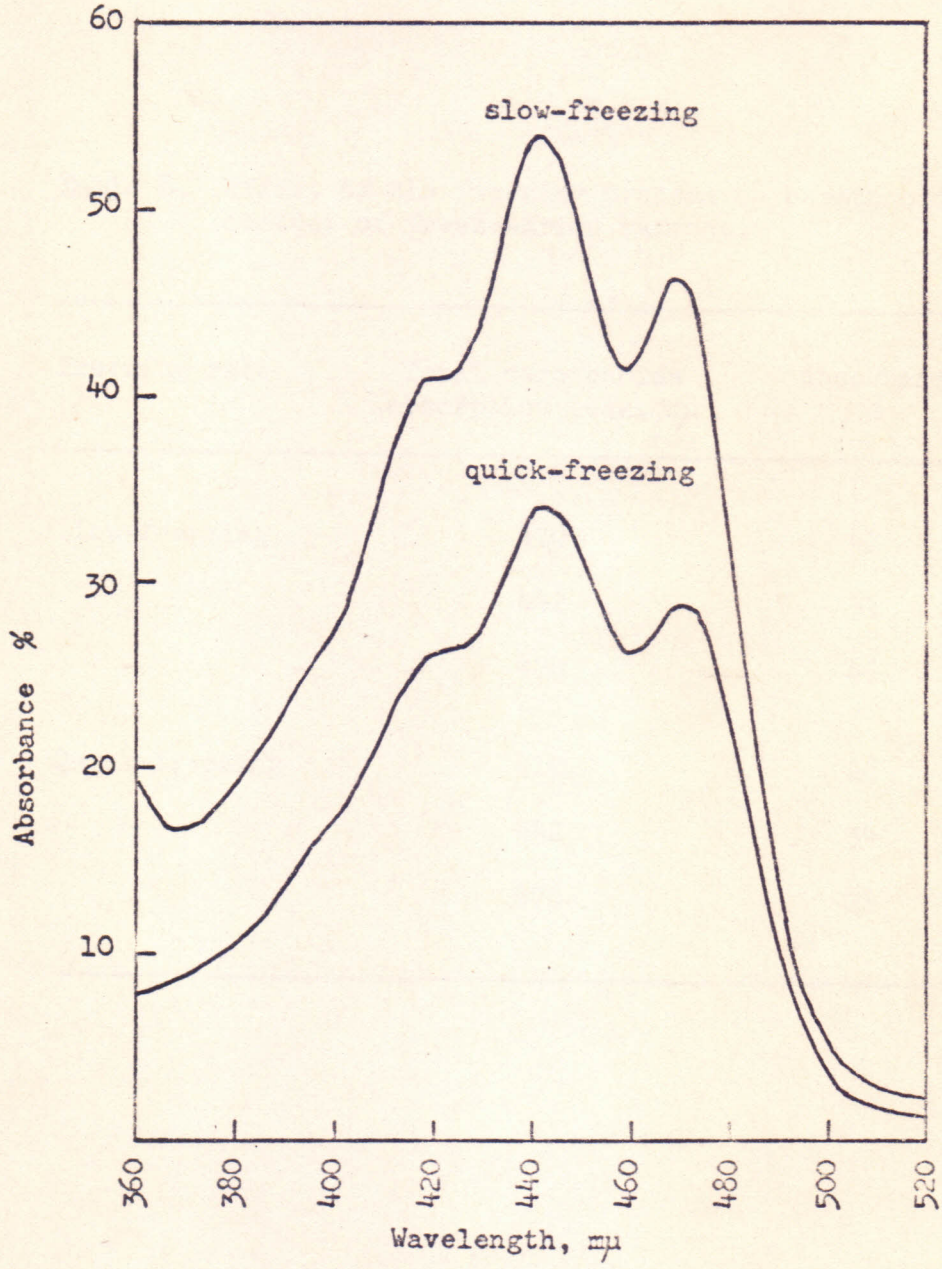

Figure 10. Absorption spectra of carotenoids extracted from freeze-dried bananas. Effect of freezing rates.

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Table 8. Effect of the freezing process on carotenoid content of freeze-dried bananas.

Freezing rate	Total carotenoids absorption peak, mμ	Absorbance %
Slow-freezing	410	41
	442	54
	470	46
Quick-freezing	410	26
	442	34
	470	28

by the fact that the Freon may extract some carotenoids from the bananas. The mixed chlorofluoromethanes, known as Freons, are colorless, non-inflammable, and non-corrosive. Freon is produced commercially from carbon tetrachloride and antimony fluoride.

On rehydration the quick-freezing samples appeared lighter than the slow-freezing samples. This may be explained by the enzymic oxidation of natural polyphenols in banana slices by the polyphenoloxidase which causes enzymic browning during the slow-freezing process.

13. Polyphenolic compounds.

The polyphenolic compounds were satisfactorily extracted from freeze-dried banana with 70% methanol containing 0.2% conc. HCl (V/V). Figure 11 shows a two-dimensional paper chromatogram of the polyphenolic compounds present in the extract of freeze-dried banana made by the slow-freezing process. A similar pattern was obtained with freeze-dried bananas made by the quick-freezing process. No difference was observed regarding the type of polyphenols present in the two types of products. A total of seventeen blue-colored spots were detected when the chromatogram was sprayed with the $FeCl_3$-$K_3Fe(CN)_6$ reagent. Spots marked with full lines were present in larger amount than those marked with broken lines. The aqueous portion of the extract after extraction with ethyl acetate, when refluxed with leucoanthocyanidin reagent changed to red color. This indicates the presence of leucoanthocyanidin in the aqueous portion.

Table 9 shows the R_f values of the polyphenolic compounds extracted from freeze-dried bananas in two solvent system. Table 10 shows the color reactions of these compounds on a two-dimensional chromatogram

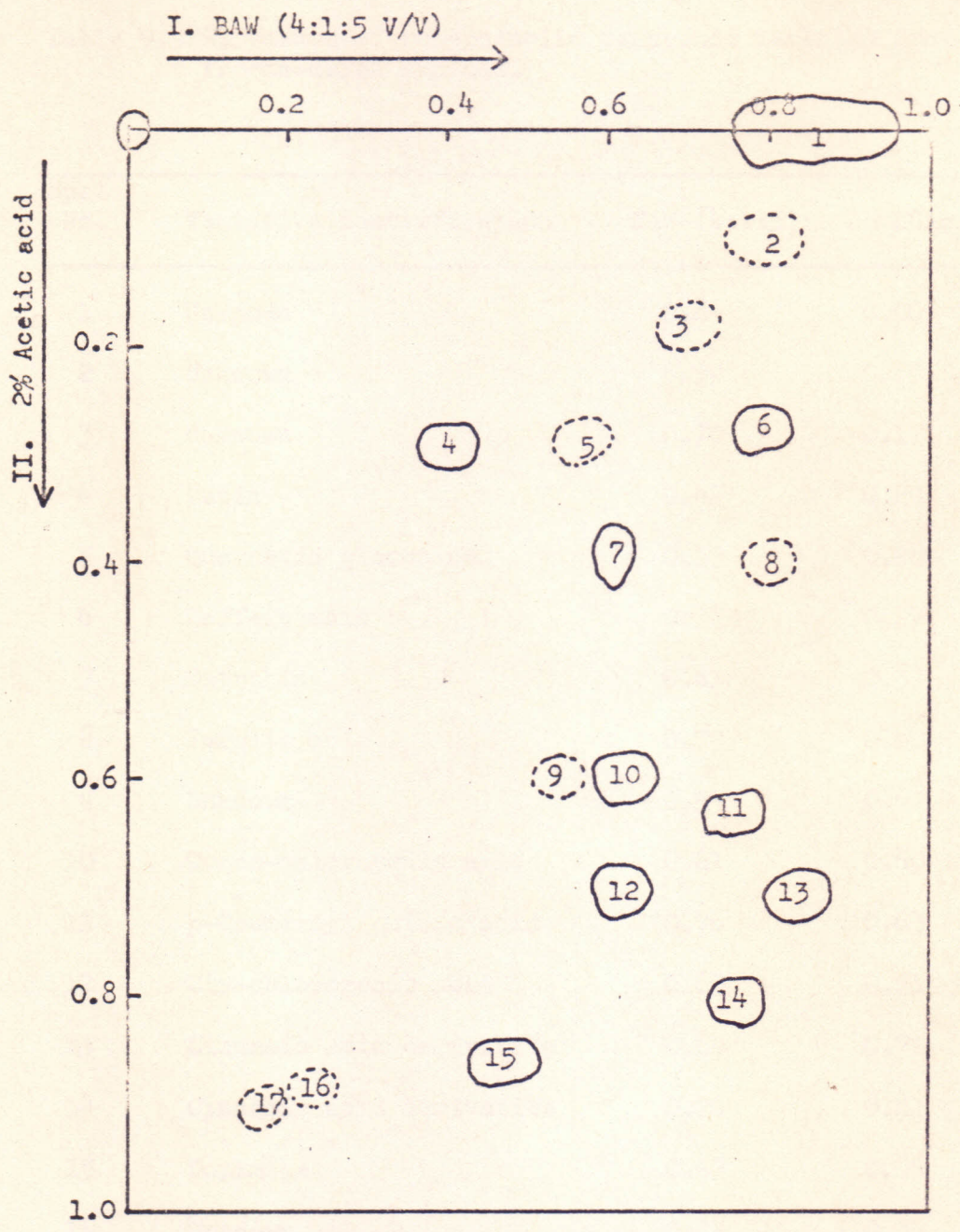

Figure 11. Two-dimensional paper chromatogram of polyphenolic compounds in freeze-dried bananas, made by the slow-freezing process.

Table 9. R_f values of polyphenolic compounds isolated from freeze-dried bananas.

Spot No.	Tentative identification	BAW (4:1:5)	2% HOAc
1	Unknown	0.84	0.00
2	Unknown	0.79	0.10
3	Unknown	0.70	0.17
4	Rutin	0.42	0.29
5	Quercetin glucoside	0.57	0.28
6	Caffeic acid	0.78	0.27
7	Catechin	0.61	0.39
8	Ferulic acid	0.79	0.40
9	Unknown	0.54	0.60
10	Trans-chlorogenic acid	0.61	0.60
11	p-Coumaroyl quinic acid	0.76	0.63
12	Cis-chlorogenic acid	0.63	0.71
13	Cinnamic acid derivative	0.84	0.71
14	Cinnamic acid derivative	0.76	0.81
15	Dopamine	0.47	0.86
16	Unknown	0.24	0.89
17	Unknown	0.18	0.90

Table 10. Color reactions of polyphenolic compounds isolated from freeze-dried bananas.

Spot No.	Tentative identification	U.V. light	U.V. + NH_3	visible light + NH_3	$FeCl_3$ $K_3Fe(CN)_6$	DPNA	Hoepfner reagent
1	Unknown	Y	Y	-	B	OY	Y
2	Unknown	B	BG	-	B	-	-
3	Unknown	B	B	-	-	-	-
4	Rutin	P	Y	Y	B	OY	Y
5	Quercetin glucoside	P	Y	-	B	Y	-
6	Caffeic acid	B	B	C	B	R	-
7	Catechin	C	C	C	B	-	-
8	Ferulic acid	B	B	C	B	-	-
9	Unknown	fBG	fYG	-	fB	-	-
10	Trans-chlorogenic acid	BG	YG	Y	B	OY	-
11	p-Coumaroyl quinic acid	SB	Br B	C	B	-	-
12	Cis-chlorogenic acid	BG	YG	Y	B	OY	-
13	Cinnamic acid derivative	BG	YG	C	B	-	-
14	Cinnamic acid derivative	BG	YG	C	B	-	-
15	Dopamine	D	-	C	B	PBn	-
16	Unknown	-	-	-	B	P	-
17	Unknown	-	-	-	B	P	-

Y: Yellow B: Blue P: Purple R: Red O: Orange T: Tan
C: Colorless l: Light f: Faint Br: Bright Bn: Brown
S: Slight D: Dull G: Green

when examined under ultraviolet radiation with or without exposure to ammonia vapor, under visible light with ammonia vapor, and when sprayed with various chromogenic reagents. Table 11 shows the color reactions and Table 12 the R_f values of authentic polyphenolic compounds.

Spots 10 and 12 showed blue fluorescence under ultraviolet radiation. Their color changed to yellowish green fluorescence in the presence of ammonia vapor. When the chromatogram was sprayed with the $FeCl_3$-$K_3Fe(CN)_6$ reagent they appeared as blue spots. On separate chromatograms they appeared as tan spots when sprayed with the DPNA reagent. The spots showed a yellow color when sprayed with a saturated solution of 3,5-dinitrosalicylic acid in 50% ethanol containing 2% NaOH. The color reaction of these compounds resembled those of caffeic acid derivatives reported by Luh et al. (1967) and Cartwright et al. (1955). These spots (10 and 12) in BAW (4:1:5) and 2% acetic acid show values comparable to those of trans-and cis-chlorogenic acid respectively (Williams, 1955; Rivas, 1966). Therefore compounds 10 and 12 were tentatively identified as trans- and cis-chlorogenic acids respectively.

Spot 4 showed a purple color when examined under ultraviolet radiation which changed to yellow when exposed to ammonia vapor. Its R_f and color reactions with chromogenic reagents were similar to those of an authentic rutin sample. This compound showed absorption at 260 mμ (shoulder) and inflection at 360 mμ. After addition of 3 drops of aluminum chloride, a bathochromic shift of 12 mμ was observed. The solution turned deep yellow, giving a complex with aluminum. The aluminum ions complex with polyphenolic compounds that have a free 5 or 3-hydroxyl group (Jurd, 1962).

Table 11. Color reactions of authentic polyphenolic compounds

Compound	U.V.	U.V. and NH_3	visible light and NH_3	$FeCl_3$ $K_3Fe(CN)_6$	DPNA	Hoepfner reagent	Ninhydrin
Rutin	P	Y	Y	B	OY	Y	-
Caffeic acid	B	B	C	B	O	R	-
Ferulic acid	B	B	C	B	R	YBn	-
Chlorogenic acid	YB	YG	Y	B	T	Y	-
Quercetin	Y	Y	-	B	Y	Y	-
Dopamine	-	-	-	B	PBn	-	P

Y: Yellow B: Blue P: Purple R: Red O: Orange
C: Colorless l: Light f: Faint Br: Bright Bn: Brown
S: Slight D: Dull G: Green T: Tan

Table 12. R_f values of authentic polyphenolic compounds.

Compound	Observed		Reported	
	BAW (4:1:5)	Acetic acid (2%)	BAW (4:1:5)	Acetic acid (2%)
Rutin	0.42	0.30		
Caffeic acid	0.75	0.28	0.80	0.31
Chlorogenic acid	0.65	0.60	0.58	0.62
Ferulic acid	0.83	0.39	0.80	0.32-0.46
+ Catechin			0.67	0.45
nor-Adrenaline			0.28	
Quercetin	0.70	0.00		
Serotonin	0.42	0.54		
DOPA	0.20	0.83		
Dopamine	0.50	0.87		
L-Epinephrine	0.40	0.79		
p-Coumaroyl quinic acid			0.76	0.66
Quercetin glucoside			0.64	0.20

Spot No. 4 was therefore tentatively identified as rutin.

Spot 6 was tentatively identified as caffeic acid as its R_f values in various solvents and color reactions were exactly the same as an authentic compound.

Spot 15 was tentatively identified as dopamine because its behavior is similar to that of an authentic sample.

Spot 11 showed a slight fluorescence under ultraviolet radiation. It changed to a bright royal blue fluorescence when the chromatogram was examined under ultraviolet radiation in the presence of ammonia. This spot was tentatively identified as p-coumaroylquinic acid.

Compounds 13 and 14, under ultraviolet radiation either with or without ammonia vapor showed color reactions similar to chlorogenic acid. The color reaction of these compounds were similar to chlorogenic acids. They were tentatively identified as cinnamic acid derivatives (Cartwright et al., 1955).

Spot 5 was identified tentatively as quercetin glucoside as evidence by its color reaction, behavior under ultraviolet radiation, and R_f values (El-Sayed, 1966).

Based on the R_f values in BAW (4:1:5) and 2% HOAc, Spot 7 was identified tentatively as catechin. It did not show fluorescence under ultraviolet radiation. It did not show a pink color with vanillin reagent, probably because the quantity present was relatively small (El-Sayed, 1966).

Spot 8 has R_f comparable to authentic ferulic acid and was tentatively identified as such.

Leucoanthocyanidin

According to Clark-Lewis (1962), plant leucoanthocyanidins can be classified into three groups: (a) condensed polymers, those that are insoluble in water and the usual organic solvents, or give only colloidal solutions; (b) glycosides or diglycosides, those readily soluble in water and not extractable by ethyl acetate; (c) monomers, including flavan-3,4-diols, those which may be extracted from aqueous solution with ethyl acetate. It seems that the leucoanthocyanidin in the present work belongs to the second group.

Clark-Lewis (1962) reported that when leucoanthocyanidins were boiled with hydrochloric acid, two reactions happened at the same time, the formation of anthocyanidin and polymeric condensation of flavan-3,4-diols to yield "phlobaphenes".

The spot detected on the paper after the application of the hydrolysate had an R_f of 0.43 in BAW (4:1:5) and 0.30 in Forestal solvent. The compound was pinkish when viewed under visible light, purple after spraying with $AlCl_3$, and mauve under ultraviolet light. Harborne (1958) found that delphinidin has a R_f of 0.32 in Forestal solvent and 0.42 in BAW (4:1:5). Bate-Smith (1954) reported that delphinidin has a R_f value of 0.30 when irrigated with the Forestal solvent. Based on the above information, the pigment obtained from the hydrolysis of acetonic extract was identified as delphinidin. Robinson (1937) also detected delphinidin in the pulp of edible banana. The application of acetonic extract to paper showed that after the chromatogram had been developed and dried the leucoanthocyanidin stays on the base line and was detected after spraying the chromatogram with the vanillin reagent.

14. Relative amounts of polyphenolic compounds.

Results on the quantitative determination of the polyphenolic compounds present in freeze-dried bananas, as determined by the Folin-Denis method, are presented in Table 13. Compounds 5, 9, and 17 were not determined because they were present in trace amounts. Spots 7 and 17 were not determined because of the difficulty in locating them on the paper chromatogram without the chromogenic reagent. It was assumed that the elution of the compounds was complete and that a linear relationship exists between absorptivity and concentration (Swain and Hillis, 1959). The Folin-Denis reagent, according to Singleton and Rossi (1965) determines only the aromatic hydroxyl groups in the polyphenols. Therefore this procedure indicates the relative amount of polyphenols based on the color reaction. Spot 1 appeared in largest concentration, followed by chlorogenic acids (Spots 10 and 12).

Unidentified compounds.

The presence of Spot 1 has been reported by Tsiang (1964), Hsu (1966), and Rivas (1966). The compound presents an absorption peak at 272 mμ and an inflection at 396 mμ. This compound is yellow in color under visible light.

Spots 2 and 3 showed a slight green fluorescence under radiation from ultraviolet in the presence of ammonia. Spot 9 could be a chlorogenic acid isomer due to its color under ultraviolet. However, it was present in very low concentration. Compound 16 could be identified as nor-adrenaline because its R_f value coincides with the R_f reported in the literature (Block, 1958) and it reacts with DFNA and ninhydrin. However, we did not have an authentic compound for comparison.

Table 13. Relative amount of polyphenolic compounds in freeze-dried bananas.

Compound	Tentative identification	Absorbance Klett units	% of total polyphenols
1	Unknown	480	23.8
2+6+3+8	Unk+Caffeic+Unk+Ferulic	238	11.8
4	Rutin	130	6.5
10+12	Trans and Cis-chl. acid	360	17.8
11	p-Coumaroyl quinic acid	180	8.9
13	Cinnamic derivative	160	7.9
14	Cinnamic derivative	245	12.2
15	Dopamine	220	10.9

15. Indoles in freeze-dried bananas.

This part of the study was done with the purpose of finding serotonin in freeze-dried bananas. Four compounds reacted with the Ehrlich reagent (para-dimethylaminobenzaldehyde). The chromatographic properties of these spots are listed in Tables 14 and 15. The compounds that react with Ehrlich reagent were classified as indoles (Block, 1958). Tryptamine derivatives give the general indole test with p-dimethylaminobenzaldehyde (Seikel, 1964). Compound 1' has color reaction and R_f value similar to 5-OH tryptophan as reported by Block (1958). It was tentatively identified as such. Compound 2' was tentatively identified as L-tryptophan because its R_f and color reaction matches those of an authentic sample. Spot 3' shows color reaction and R_f value close to an authentic sample of tryptamine. Spot 4' shows a blue color after reacting with the Ehrlich reagent. Its R_f values in different solvents were similar to an authentic sample of serotonin. The presence of serotonin was also evidenced by the positive reaction with a reagent composed of nine volumes of 0.1% potassium dicromate and one volume of 40% formaldehyde. After spraying the chromatogram, a yellow fluorescent spot was detected. This reagent, according to Block (1958), is specific for serotonin. The compound was identified as serotonin (5 hydroxytryptamine). This compound appeared in very low concentration. Further determination of each spot is required.

Table 14. R_f value of indoles isolated from freeze-dried bananas.

Compound	Tentative identification	Solvent System 1	2	3	4
1'	5-OH tryptophan	0.11	0.12	-	0.09
2'	L-tryptophan	0.40	0.27	0.70	0.27
3'	Unknown	0.76	0.68	0.89	-
4'	Serotonin	0.69	0.50	-	0.58
Authentic					
Serotonin		0.68	0.52	0.83	0.56
Tryptamine		0.80(0.70*)	0.71	0.92	0.77
L-tryptophan		0.43	0.28	-	0.29
REPORTED (Block, 1958)					
Tryptophan		0.42	0.18		0.24
5-OH trypt.		-	0.11		-
Tryptamine		0.82	0.65		0.82
Serotonin		0.65	0.52		0.57

1 Iso-propanol:NH_4OH:H_2O 8:1:1 (V/V)
2 Iso-propanol:NH_4OH:H_2O 10:1:1 (V/V)
3 N-propanol:NH_4OH:H_2O 6:3:1 (V/V)
4 Iso-propanol:NH_4OH:H_2O 20:1:2 (V/V)

*R_f when applied with the crude extract.

Table 15. Color reactions of indoles isolated from freeze-dried bananas.

Compound	Tentative identification	Color reaction p-dimethylaminobenzaldehyde reagent
1	5-OH Tryptophan	pink
2	L-Tryptophan	pink
3	Unknown	pink bluish
4	Serotonin	bluish

V. SUMMARY.

The effect of storage temperature and freezing rate on chemical changes in freeze-dried 'Valery' bananas was investigated. Banana slices were frozen by dipping in Freon 12 for 60 seconds (quick-freezing) and by slow-freezing. The samples were freeze-dried in a Stokes freeze-drier, sealed under 16" vacuum in No. 2 1/2 cans, and then stored at 20 and 30°C for storage stability tests. Samples were taken at 3-month intervals up to 12 months for storage stability tests. Changes in ascorbic acid, pH, acidity, color, Folin-Denis value, polyphenoloxidase activity, water-soluble pigments, carotenoids and rehydration were used as objective tests.

1. Samples stored at 20°C showed a better rehydration capacity than those stored at 30°C. The slow-freezing samples showed a better rehydration capacity than the quick-freezing samples at the same temperature.
2. Ascorbic acid retention was used as an objective test for storage changes in freeze-dried bananas. A more rapid loss of ascorbic acid was found when the product was stored at 30°C than at 20°C.
3. The Folin-Denis test was applied to detect changes in tannin-like substances in freeze-dried bananas as influenced by storage temperature. There was an increase in tannin-like substances through the 12-month storage.
4. The formation of water-soluble pigments served as a good criterion of storage changes in freeze-dried bananas. Samples stored at 30°C showed a more rapid formation of water-soluble

pigments than those stored at 20°C. Storage temperature is an important factor influencing the rate of formation of water-soluble pigments.

5. Polyphenoloxidase activity was found to be higher in the slow-freezing than in the quick-freezing product. The optimum pH of banana polyphenoloxidase on pyrocatechol was 6.1 at 30°C.
6. The total acidity of freeze-dried bananas increased during storage.
7. The Gardner color difference meter was used to evaluate the color changes in freeze-dried bananas. The color of the slow-freezing product was slightly darker than that of the quick-freezing product.
8. Organoleptic evaluation of the products stored at 20 and 30°C up to 12 months was made after rehydration. The products retained their quality well, especially those stored at 20°C. There were some differences in quality between the slow- and quick-freezing products.
9. The total carotenoid content of freeze-dried bananas was shown to be present in larger amounts in the slow-freezing product than in the quick-freezing process. This was explained by the extraction of carotenoids by Freon.
10. The polyphenolic compounds in freeze-dried bananas were investigated. The presence of a Quercetin-glucoside, Caffeic acid, Catechin, Ferulic acid, Chlorogenic acids, Cinnamic acid derivatives, Rutin, and Dopamine in freeze-dried bananas was indicated.

The polyphenols were detected by their R_f values in different solvents, color reaction with chromogenic sprays, and fluorescence under ultraviolet radiation with and without NH_4OH. Quantitative determination of the flavonoids by the Folin-Denis method showed that the unknown Spot 1 was present in largest concentration, followed by the chlorogenic acid isomers (Spots 10 and 12).

11. The presence of leucoanthocyanidin as precursor of delphinidin in freeze-dried bananas was evidenced by paper chromatographic methods, using the Forestal and Butanol: acetic acid: water (4:1:5) solvents for development.

12. Four compounds that show positive reaction with the Ehrlich reagent were found in the ethyl acetate extract. They were tentatively identified as 5-OH tryptophan, L-tryptophan and serotonin (5-OH tryptamine). The color reaction and R_f values of Spot 3 was similar to an authentic sample of tryptamine.

One can detect the deleterious effect of storage at 30°C on quality changes in the freeze-dried product. Products made by the slow and quick-freezing processes were of good quality even after 12 months' storage at 20°C in hermetically-sealed cans.

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