THE COLOR REACTION OF DEXTROSE WITH THE ANTHOCYAN PIGMENTS OF FROZEN STRAWBERRIES

by

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INTRODUCTION

The preservation of berries by freezing has become an important method of marketing a large portion of the berries of the Pacific Northwest. These berries are used primarily for preserve making, for which purpose they are well adapted.

This method, as described by Diehl (7) is carried out essentially as follows: The ripe berries are brought from the fields to the freezing plant as quickly as possible after picking; here they are stemmed, washed, sorted, sized and poured into barrels along with the desired quantity of sugar. After the barrel is filled, it is rolled to help mix the sugar with the fruit and then it is removed to the cold room, (0° Fahrenheit), where the contents of the barrel are frozen rapidly enough to avoid all fermentation. After freezing, the barrels are moved to a cold storage room where they may be stored almost indefinitely at a temperature of about 15° Fahrenheit.

After the fresh berries are off the market, these frozen ones provide a constant supply of berries for the preserve makers throughout the entire year.

Sugar is added to the barrel along with the berries because it draws juice from the fruit to form a heavy sirup which protects the berries from enzyme action, prevents oxidation by excluding air, and toughens the fruit by shrinking it to a smaller volume. Another advantage of the use of sugar with berries is that during freezing, storage and thawing, sugar penetrates into the berry, thus improving the flavor.

The packing of frozen berries in the Pacific Northwest is an industry representing a pack of 50,000 to 75,000 barrels annually. This pack at present is frozen entirely with the addition of sucrose and represents an annual consumption of about 7,500,000 pounds of sugar.

Dextrose, which has come on the market in recent years as a chemically pure substance, sells in carload lots at a price which is usually about one dollar less than sucrose per one hundred pound bags. Dextrose would seem to offer a possible saving in the barreling costs if the dextrose could be added to the barrel rather than to the final product. Previous tests made with dextrose in frozen pack berries indicated that dextrose caused color changes, and that it was too insoluble to be used in frozen pack products.

This investigation was carried out to determine the nature of the color changes and the extent to which the low solubility of dextrose is detrimental in frozen pack strawberries.

Dextrose. Dextrose is the most important hexose sugar; it is widely distributed in nature, being found in the juice of most sweet fruits, and is particularly abundant in grapes and honey. Dextrose is a product of the digestion of starches and maltose, and is the normal sugar of the blood in which it circulates to all parts of the body where its oxidation provides heat and muscular energy. It is also synthesized into fats, proteins and other necessary substances.

History of the Commercial Development of Dextrose. A German chemist, Kirchhof (3) in 1811 first demonstrated that the hydrolysis of starch by acid would produce a sweet substance. Other workers quickly verified his findings and due to a sugar shortage in France caused by an embargo against England, a new industry sprang into being. This industry had a short life, however, for Napoleon did away with the continental blockade in 1814 and the new starch sugar factories were forced out of business. When this industry was revived, its main seat was transferred to America. The first patent relating to glucose was taken out by Gossling (3) in 1864, and then one improvement followed another very rapidly. While dextrose manufacture dates back slightly more than a century, it was not until recent years that it has come onto the market as a product which rivals sucrose in purity. Commercial production, in a pure form which began in 1922 with 50,000 pounds, has now risen to 240,000,000 pounds in 1936.

Sweetness of Dextrose. Biester, Wood and Wahlin (4) made a study of the sweetness of sugars; they gave sucrose the arbitrary value of 100. On this scale, dextrose received a value of 74. Invert sugar was found to be 123; levulose

175 and lactose 16. Where sweetness is desired, dextrose is at a disadvantage since more of it must be used to produce the desired results.

In items such as preserves, candies, and other products in which a large proportion of sugars is used, excessive sweetness from the use of sucrose alone may be a disadvantage. This excessive sweetness may be reduced to some extent by replacing a part of the sucrose with dextrose.

Present Status of Corn Sugar. The Pure Food Law of 1906 ruled that the presence of dextrose in a food product must be declared on the label. This ruling was changed in 1930 by the Secretary of Agriculture, Hyde (12) so that the pure refined dextrose can be used without declaring its presence on the label. A few states in which the cane and beet sugar industries are important, still require the label declaration when dextrose is used. Since the two sugars, sucrose and dextrose, are in competition with each other, there naturally is some conflict as to the relative merits of each. The general opinion, however, is that both sugars are equally well suited as a food substance but that the chemical and physical properties of the sugars should determine which of the two is the best for a specific use.

Dextrose is advertised as the quick energy sugar, inferring that dextrose in being the natural blood sugar would naturally be superior to other carbohydrates since it requires no digestive changes.

A report of the American Medical Association, edited by Fishbein (9), states that while dextrose requires no hydrolytic change, there is no evidence that it is better or more quickly absorbed by the body than sucrose is.

Comparison of Food Values. According to Browne (5), sucrose, the anhydride of dextrose and levulose, has slightly higher heat of combustion than that of its two components. The inversion of sucrose in the body provides energy. Further, the differences of the molecule of sucrose and dextrose give sucrose the greater fuel value. This represents a 5% difference since 95 grams of sucrose will be converted into 100 grams of dextrose in the body.

Dextrose is usually produced as the hydrate which contains 8% water of crystallization. The calorific value on a pound for pound basis for the two sugars is 13%, in favor of the sucrose.

REVIEW OF THE LITERATURE

A search of the scientific literature produced no information concerning any reaction between dextrose and anthocyanin pigments. For this reason, it was necessary to study the pigments, chemical reactivity of dextrose, and the strawberries.

Pigments Present in Strawberries. Robinson (23) stated that the pigment which he has found in strawberries is the Anthocyanidin, Pelargonidin Monoglycoside, and suggests that he believes the sugar present in the molecule is galactose.

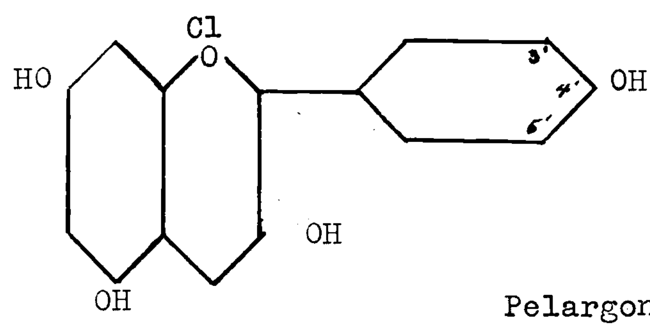
The anthocyan pigments, according to Onslow (18), are a class of pigments to which practically all of the blue, purple and red colors of flowers, fruits and leaves, are due. They occur in solution in the cell-sap and are very widely distributed. They are present in the plant in the form of glycosides, and in this condition they are known as anthocyanins; as glycosides they are readily soluble in water and they are, as a rule, also soluble in alcohol. When hydrolysed, an anthocyanin yields a sugar, and in some cases other residues as well as an anthocyanidin. The anthocyanidins are all derived from three typical substances. The many variations are due to different methyl derivatives.

The wide range of hues obtained in the plants and flowers is partly produced by variations in the acidity of the cell sap; the anthocyanin forms red oxonium salts in acid solutions, which changes through the violet neutral color to the blue metallic salt form as the sap is rendered alkaline. The pigments are unstable in alkaline solution as they are very susceptible to atmospheric oxidation.

Robinson and Robinson (22) state that the differences of color of varieties in a species are not brought about by changes in the pH of the cell saps, but rather by changes in the nature of the anthocyans. By this, they mean the formation of complexes with organic substances and possibly with metals such as iron. Haas and Hill (10) hold a similar view.

Willstatter and Zollinger (24) first observed that the addition of tannin to a solution of the pigment oenin chloride in dilute HCl solution intensified the color and produced a bluer red. Gallic acid had a similar, but weaker, effect.

Armstrong and Armstrong (1) state that Willstatter and his Co-workers were the first to show the anthocyanins to be benzo-pyrylium derivatives, and gave us the formula for the three typical substances, Pelargonidin, Cyanidin, and Delphinidin.



Pelargonidin chloride

The other two fundamental substances vary only in that Cyanidin has an additional hydroxyl group in the 3' position, and Delphinidin has hydroxyl groups in all three, the 3', 4' and 5' positions. The anthocyanins may be monoglycosides, diglycosides or complex diglycosides. In monoglycosides are found glucose (or galactose), and in diglycosides there are two molecules of glucose or one of glucose, and in addition one of rhamnose.

According to Onslow (19), Pelargonin is a diglucoside which yields on hydrolysis, two molecules of glucose and Pelargonidin.

Importance of Pigments in Preserved Foods. Many of our fresh fruits and vegetables owe a large part of their attractiveness to the bright red and purple colors of the anthocyan pigments which are present in the flesh and skins. The extent to which it is possible to retain these natural colors in preserving processes and storage is a large factor in the determination of the value of these products for canning or preserving purposes.

Strawberry pigments are very unstable, with the result that all canned strawberries have more or less of their normal color destroyed by the heat treatment. The trade has become tolerant toward this condition but pays the best prices for berries with the most natural color. Strawberry preserves with small loss of color can be made if the better varieties of berries now available are used and if the proper procedure is carefully followed.

After strawberry preserves are made, they gradually undergo a loss of color. High-class preserves have an average shelf life of about three months. With cool, dark storage, they may retain their color in good condition for six to eight months. The preserves may tend to change from a dark red to a very dark purple during a period of from one to four years. However, some varieties tend to fade to a yellowish brown.

Culpepper and Caldwell (6) find that anthocyan pigments play an important role in the causation of tin can corrosion.

They state that in canned material containing anthocyanins, the metal salts formed by a combination of the acids of the fruit are broken up by a transfer of the metal into a combination of the metal with the pigment, thus releasing the acid to continue attack on the can. The combination of the metal with the anthocyanin causes a change of color. Highly acid fruits generally favor corrosion of the tin cans, but a high acidity will repress the formation of the metal-anthocyanin compounds so that much less perforation occurs in fruits of high acidity than in those with a relatively lower acidity, when anthocyanin pigments are present.

In a large number of experimental strawberry packs, including two hundred twenty varieties, Culpepper and Caldwell (6) found a very wide variation in the degree of color change taking place. In some cases, the original red color was replaced by varying shades of brown or brownish red; in other cases, the changes of color tended towards the purple. All varieties showed more discoloration in tin than in glass.

Summary of The Color Reactions of Anthocyanin Pigments

1. Anthocyanin pigments are very sensitive to pH change.

2. Organic compounds in some cases act as co-pigments, which modify the colors.

3. Traces of metals such as iron or tin may shift the color from red to blue.

4. Temperature and light cause changes to occur.

Summary of the Color Reactions of Anthocyanin Pigments, (Cont)

5. The anthocyan compounds are basic in nature. They combine with acids very readily by means of oxonium valences.

6. The anthocyanidins are relatively insoluble in water unless they contain a sugar molecule. The anthocyanins are soluble because of the sugar molecules which they contain.

The Composition of Strawberries. Winton (26) gives the composition of strawberries as follows:

Total solids	9.5%
Protein	1.0%
Acid as Citric	1.4%
Reducing Sugars	4.8%
Sucrose	.6%
Ash	.6%

Other substances present in traces, are: pigment, tannin, fiber and pectin. The ash is composed of Potassium, Sodium, Calcium, Magnesium, Phosphorus, and traces of aluminum, copper and zinc.

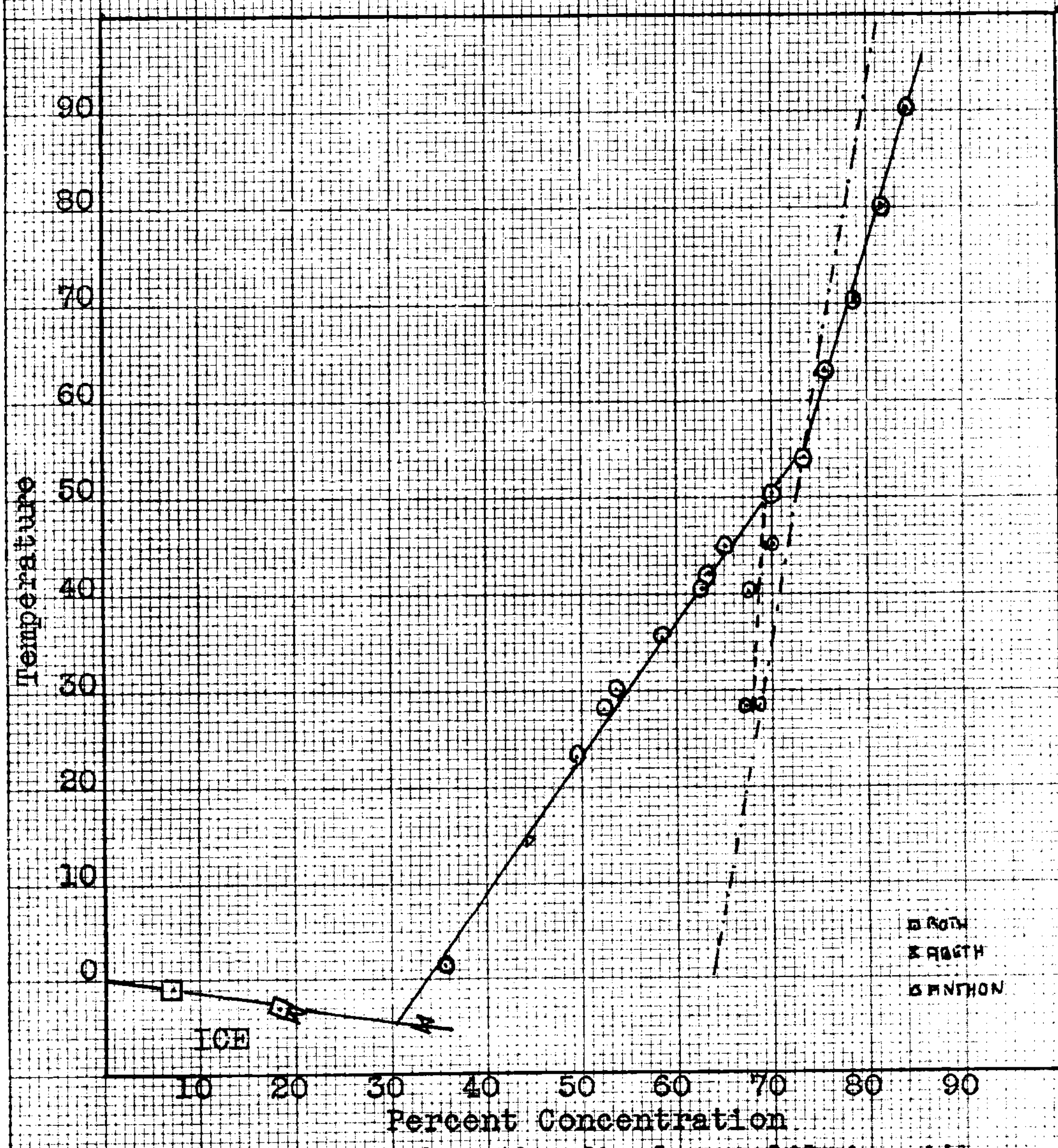
Chemical Properties of Sucrose. Sucrose, the sugar now used almost exclusively in the frozen pack industry, is the most important of the disaccharides. It crystallizes readily, forming very regular clear crystals which are easily soluble in water. Sucrose is sold to the public in a very pure condition. It is readily hydrolysed in acid solution to form dextrose and levulose and is quite stable in basic solution. Sucrose forms saccharates with lime, strontium, or barium hydrate. Sucrose undergoes very few reactions. The only other possibility being salt formation, due to the weak acidic properties of the hydroxyl groups.

Solubility of Dextrose and Sucrose. One of the most important characteristics of dextrose, which must be considered when it is used, is its solubility. *Figure I taken from the Bureau of Standards (14) and Browne (5), shows the maximum solubility of dextrose and sucrose at various temperatures. A knowledge of the saturation points of the sugars is very important to the manufacturer who makes jams, jellies, preserves or confections. Figure I also shows that the solubility of dextrose increases rapidly as the temperature increases. Sucrose, on the other hand, is quite soluble at low temperatures but increases only a small amount as the temperature is increased.

Preserves must contain a minimum of 68% soluble solids. Dextrose alone is greatly super-saturated at this concentration under normal storage conditions. Sucrose is at the saturation point in preserves when the temperature of storage is below 30° C. However, during preserve making, a part of the sucrose is usually inverted which wards off crystallization. In some cases, where preserves are slightly over concentrated and the storage temperature rather low, crystallization of the sucrose will result. The use of low percentage of dextrose will tend to prevent this crystallization of the sucrose. * Figure 1 on following page.

<u>FIGURE I</u>

The Solubility of Dextrose and Sucrose
in Water (14) (5).



The solid curves show the final equalibria with respect to the solid phases, ice, dextrose, hydrate and anhydrous dextrose. All data are expressed as Anhydrous Dextrose.

The short broken curve shows the equalibria for the anhydrous form of dextrose.

The long broken curve shows the solubility of sucrose.

A mixture of the two sugars in solution is more stable than either sugar alone at a given concentration provided the proportions of the two are correct. According to Jackson and Silsbee (13), at 30° C. 100 grams of water will dissolve 213.6 grams of sucrose, but 280.1 grams of the two sugars will go into solution; an increase of 31%.

Chemical Characteristics of Dextrose. Dextrose is now prepared quite readily by hydrolysis of starch under pressure with a small amount of HCl. Dextrose crystallizes either as the hydrate, or in the anhydrous form, depending upon the temperature. Unlike sucrose, it seldom forms well-defined regular crystals, making it a more difficult product to purify. Dextrose contains a potential aldehyde group which allows it to undergo the reactions of aldehydes. The normal structure of dextrose which is now accepted, according to Armstrong (2) is that established by Haworth as that of a 1:5 glucopyranose ring with a primary alcohol group as a side chain.

The unstable nature of the pyranose ring allows dextrose to undergo a number of reactions. Mathews (17) states that acids combine with dextrose at the double bonded oxygen of the aldehyde, thus exhibiting weak basic properties of the same type that is shown by aldehydes and ketones. In these combinations, the oxygen is tetravalent, opening up two residual valences; thereby being able to unite with acids just as ammonia does.

Armstrong (1) states, "Glucose hydrate almost certainly has the structure of the oxonium hydroxide." Thus, the "H" and "OH" group of the molecule of hydration is bound to the lactonic oxygen by the residual or oxonium valences. Armstrong (1) further explains the muta rotation of dextrose in solution by a process in which the dextrose molecule loses the hydrated water molecule, forming a temporarily unsaturated molecule which adds water on again in either of two ways to form either the Alpha or the Beta form of dextrose.

The aldehyde properties of dextrose manifests itself in various ways. Ling (16) found that dextrose will add on ammonia, forming an additive compound of the aldehyde ammonia type. Ramsey, Tracy and Ruehe (24) found that dextrose reacts with the amino acids of Casein or Albumin when solutions of these proteins are heated together. This reaction resulted in a colored protein sugar compound which caused off-color difficulties when condensed milk was sweetened with dextrose.

Fellers and Mack (8) in 1929 put up cold pack strawberries with dextrose. They noticed a purplish red-off-color in the dextrose packs, and explained it as follows: "Dextrose is bleached with sulphur dioxide in the process of manufacture. It is this residual sulphur dioxide in the dextrose that we attribute this discoloration. The purple color is formed by the interaction of the anthocyans of the fruit with tin. The sulphur dioxide accelerates this corrosive action and thus impairs both flavor and color."

Off-color Problems of a Similar Nature to This Dextrose Strawberry Problem. Red apples, during storage, sometimes develop very dark blue spots on the skin. Pentzer (19) found that these spots are caused by a lowering of the acidity of the apples during storage with the result that the pigments in localities of a low acidity changed from the red acid form to the dark purple neutral form. Pentzer produced these spots artificially by subjecting normal apples with ammonia vapors. pH determinations made of material from the discolored spots averaged about 4.7; whereas, the normal pH was 3.8.

Hartman and Bullis (10) in a study of the canning qualities of new cherry varieties, found that in some cases cherries which were nearly colorless before canning, changed during the canning process to a dark blue. A study of this condition proved that the change came about because of a lower acidity than that amount necessary to keep the pigments in the acid form. This color change could be prevented by adding sufficient acid to lower the pH from 4.14 to 3.90.

Leo (15) observed the color change of strawberries with dextrose at some time previous to this investigation. He suggested that the use of dextrose probably caused a pH change.

The literature offers no definite clue as to the cause of the off-color, but suggests the following possibilities:

1. The sensitivity of the anthocyan pigments to small amounts of metals such as iron and tin, and the effects of organic compounds suggest that impurities of either an organic or an inorganic nature might be a factor in this color change.

2. Dextrose is a more chemically active substance than the sucrose which is normally used with berries. This suggests that the dextrose may either react with the pigments or be active in the production of the pigments.

3. The extreme sensitivity of the anthocyan pigments to pH changes suggests that dextrose may have the effect of changing the hydrogen ion concentration.

4. The high concentration of dextrose may cause enzyme changes to take place, which might have an effect upon the pigments.

PROCEDURE

During the 1936 berry season, two thousand lbs. of berries were put up in tin, glass jars and fifteen-gallon barrels for this investigation. Dextrose, sucrose, and varying mixtures of both sugars were used. These samples were put up in the following ratios: 2 plus 1, 3 plus 1, 4 plus 1, and 5 plus 1 berries to fruit, following the common commercial procedure. Berries were also frozen without sugar to provide a source of berries in a nearly natural state for use during this investigation. In all packs, the berries and sugars were well mixed. The berries were frozen and stored at 10° Fahrenheit until ready for examination. For laboratory tests, small samples were stored at 30° Fahrenheit in a refrigerator. pH determinations were made with a quinhydrone apparatus. Juice was extracted from the berries by allowing the frozen berries to thaw in, and drip from, a cloth bag. The freezing and thawing broke up the cells sufficiently to release the juice. Color comparisons were made by the use of a Klett Colorimeter.

EXPERIMENTAL

In order to determine the differences in the effects of the two sugars on strawberries, the following experiments were conducted.

Mashed Berries. Ten pounds of mature Marshall strawberries were well mashed to form a pulp of a uniform consistency. This pulped material was divided into five glass jars. Sugars were added and well mixed, as indicated in the following table:

TABLE 1

Wt. of Berries	Wt. of Sucrose	Wt. of Dextrose
1 lb.	8 oz.	0
1 lb.	4 oz.	4 oz. Regular
1 lb.	0	8 oz. Regular
1 lb.	0	8 oz. Anhydrous
1 lb.	0	0

In mixing the sugars with the berries, it was noted that the sucrose went into solution very rapidly; whereas the dextrose failed to dissolve completely. The Anhydrous Dextrose showed a marked tendency to form lumps as it came into contact with the mashed berries. The undissolved dextrose caused the samples containing dextrose to appear much lighter in color than the sucrose samples. These mixtures were stored at 30°Fahrenheit for fifteen hours, then examined for differences of appearance and color changes.

TABLE 2.

Appearance After 15 Hours

Sucrose pack	-	Normal red, characteristic of strawberries.
Sucrose-dextrose	-	Color lighter than normal.
Regular dextrose	-	Light red with a slight bluish-gray cast.
Anhydrous dextrose	-	Light red with a slight bluish-gray cast.
Berries without sugar	-	Normal red, characteristic of strawberries.

These samples were then frozen and stored at 12° Fahrenheit for eight days and examined again for color changes.

TABLE 3.

Appearance After 8 Days

Sucrose Pack	-	No change.
Sucrose-dextrose	-	Light red with slight bluish-gray cast.
Regular dextrose	-	Light blue color predominated the light red.
Anhydrous dextrose	-	Light blue color of undissolved dextrose predominated the red color of the berries.
Berries without sugar	-	No change.

After the storage period, the samples were allowed to thaw at room temperature and stand until all of the dextrose had dissolved. This resulted in a change of color in the dextrose samples. The blue disappeared and a normal red color took its place. The relative insolubility of the dextrose appeared to be an important factor in the appearance of the off-color.

Whole berries: The experiment just described was repeated except that whole berries were used instead of the mashed ones. The sugar was well mixed with the berries by shaking the jar after adding it. This resulted in good contact between the sugar and the outer surface of the berries.

TABLE 4.

Appearance After Mixing

Sucrose	- Juice begins to come from the berry and forms a heavy sirup with the sucrose.
Sucrose-dextrose	- Solution begins to take place but more slowly than in the sucrose pack.
Regular dextrose	- No appreciable amount of dextrose is dissolved.
Anhydrous dextrose	- No appreciable amount of dextrose is dissolved.
Berries only	- Normal red color.

TABLE 5.

Appearance After 15 Hours at 30° F.

Sucrose	- Color normal; solution of dextrose is now very nearly complete. Berries seem somewhat shrunken.

A - Berries normal in color; sugar completely dissolved.

B - Very little dextrose has dissolved; very little juice has been drawn from the berries; some discoloration where berries contact sugar.

C- A layer of undissolved, discolored dextrose is present, with traces of the discolored dextrose throughout the sample.

D - Berries normal in color; sugar completely dissolved.

E - Very little dextrose in solution, but all of it is discolored,

F - Both sugars completely dissolved; color is normal.

TABLE 5 (Cont.)

Appearance After 15 Hours at 30° F.

Sucrose-dextrose	- Marked bluish discoloration of the undissolved sugar; very little shrinkage of the berries.
Regular dextrose	- Marked discoloration of the dextrose wherever the juice of the berry has come in contact with it. Practically no dextrose has dissolved.
Anhydrous dextrose	- Marked discoloration of the dextrose near the surface of the berry. The berries have lost very little juice and practically no dextrose has dissolved.
Berries with no sugar	- Normal red color.

Samples were then stored for seven days at 12° Fahrenheit.

TABLE 6.

Appearance After 7 Days' Storage

Sucrose	- Solution is complete. Berries are surrounded by a heavy sirup. Color is normal red. Sirup has large ice crystals but the sirup is not frozen completely.
Sucrose-dextrose	- Slightly more off-color than was present at previous examination; otherwise unchanged.
Regular dextrose	- No appreciable change.
Anhydrous dextrose	- Slightly more off-color; otherwise no change.
Berries without sugar	- Very solidly frozen, but appearance normal.

The berries themselves were not discolored by the application of dextrose; the off-color only appeared when the juice of the berries was in contact with undissolved dextrose.

Impurities of Commercial Dextrose. Previous to this study, an analysis of commercial dextrose showed only minute traces of sulphur dioxide. This low amount of sulphur and the fact that the color shows up as well in glass as in tin, indicates that factors other than tin and sulphur are responsible for the off-color. Presumably the dextrose has been improved since the investigations of Fellers and Mack (8) in 1929.

Close examination of the commercial grades of dextrose showed that some samples were not a pure white color. These samples dissolved in water gave a distinctly brown-colored liquid, indicating the presence of impurities.

Ash determinations proved difficult to make due to the small percentage of ash present and the swelling of the dextrose when heat was applied.

TABLE 7.

Ash Determinations

No. 1	.08% Ash	Regular dextrose
No. 2	.07% Ash	Regular dextrose

Qualitative analysis of the ash showed the presence of iron and NaCl. It seemed unlikely that such small amounts of mineral substances would cause the marked discoloration which was found when dextrose came in contact with the berry juices.

Comparison of the Off-Color Formed by Dextrose From Different Sources. It was decided to test samples of dextrose from several sources to see if the amounts of off-color could in any way be correlated with the amounts of impurities.

Samples of dextrose of different crystal sizes and different degrees of purity were tested by adding them to the ripe strawberries in the ratio of 3 parts of berries to 1 of dextrose. These packs were compared, after storage for three days, at 30° Fahrenheit. The samples used were obtained from the following sources:

Merck's Reagent Dextrose. (Ash .05%).
Baker's C. P. Dextrose. (Ash .004%).
Baker and Adamson Dextrose. (Ash .03%).
Regular Commercial Dextrose.
B. H. Dextrose (an especially pure commercial dextrose).
Coarse Dextrose (Commercial Dextrose, large crystals).

All packs produced the blue color to an almost equal extent. The differences were so slight that they could be accounted for by individual differences of the berries except in the case of the coarse dextrose which produced somewhat less off-color. This was probably due to a decrease in the crystal surface.

Further packs were put up using dextrose samples especially prepared, as follows:

1. Dextrose - prepared by crystallizing a hydrolysed starch solution furnished by the Corn Products Refining

Company. No purification of this material was carried out; therefore, the sample was quite brown in appearance.

2. Dextrose - prepared by the hydrolysis of pure sucrose according to the method described by Plimmer (20).

3. Dextrose - prepared by separation from granulated honey, according to the method described by Browne (5).

4. Merck's Reagent Dextrose - which had been re-purified by five successive recrystallizations from 80% alcohol.

These samples of dextrose were added to berries in small jars, and were stored for three days at 30° Fahrenheit before comparing the color changes.

All samples of dextrose became discolored to an almost equal extent, indicating quite conclusively that the degree of purity had little or no effect on the color change.

Strawberry Color Changes With pH Changes. The off-color is a very dark blue; sometimes having a violet shade. This color is characteristic of anthocyanin pigments in the neutral and alkaline forms.

A drop of normal NaOH placed on the surface of a ripe berry changed the color from red to a very dark blue. The addition of normal acid changes the blue back to a normal red. The addition of acid to a ripe berry brightens the normal red color.

pH determinations made on several varieties of berries show that the pH of ripe berries ranges from 3.5 to 4.3, depending upon the variety and the ripeness. The pH goes up quite rapidly as the berries ripen. As they become overripe, they lose their bright red color and change toward the blue, resulting in a darker red. The pH seems to be the factor which determines the color of the pigments.

The pH of five packs of each, sucrose and dextrose, were determined. The berries were all from the same lot in both packs. The sucrose packs were normal in appearance in each sample. The dextrose packs all had much off-color before thawing, but it was not present when the pH determination was made at 20° C.

TABLE 8.

pH of 3 plus 1 Sucrose Packs	pH of 3 plus 1 Dextrose Packs
pH = 3.6	pH = 3.7
pH = 3.9	pH = 3.6
pH = 3.8	pH = 3.7
pH = 3.7	pH = 3.8
pH = 3.8	pH = 3.7
Average pH = 3.76	Average pH = 3.70

This shows that the dextrose in solution has no significant change in the pH of berries.

Sodium bicarbonate was mixed with dextrose in the ratio of 1 to 100; this mixture added to the surface of ripe strawberries produced the blue color more quickly than dextrose alone. In a similar manner, small amounts of $NaHCO_3$, Na_2CO_3, and $Ca(OH_2)$ caused the production of the blue color when mixed with starch. As strawberry juice diffused through starch alone, the juice remained a normal red and where the $NaHCO_3$ or other alkalies listed above were present in the starch, the mixture turned blue. This blue was very near the same shade as that produced by dextrose and the pigments, indicating that the off-color may be due to a condition at the crystal surface which tends to produce a more alkaline condition than is normal for the pigments in the red form.

Effects of Other Sugars. An experiment was conducted to test the effects of other sugars on cold pack berries. In this experiment, berries of uniform ripeness were selected and coated with the various sugars by rolling the berries in a shallow pan with the sugar, then packing them into 200 cc. beakers and adding more sugar to make up the 3 plus 1 ratio. The samples were all stored at 30° Fahrenheit for forty-eight hours. Seventy-five grams of berries to twenty-five grams of sugar was used in each case, except for mannose wherein only twenty-four grams of berries to eight of sugar was used:

TABLE 9.

Sugar	Result	Color
Sucrose	Solution complete	Normal red
Dextrose	Very little solution	Sugar-all off-color (blue)
Maltose	Solution complete	Lighter than normal
Galactose	Very little solution	Normal red
Lactose	Very little solution	Normal red
Levulose	Solution complete	Normal red
Mannose	Solution complete	Normal red
Xylose	Partial solution	Normal red

None of these sugars showed signs of discoloration comparable to that caused by dextrose. Mannose and levulose are both very soluble, which fact would prevent them under the conditions of this experiment from forming any blue color of the same nature as that formed with dextrose.

The Effect of Heat on the Formation of the Off-Color. Four hundred grams of berries which had been frozen without sugar were thawed and heated to boiling, then divided equally into two beakers. One hundred grams of dextrose was added to one beaker while the berries were still hot. The other beaker was cooled to 32° Fahrenheit before adding the dextrose. Mixing of the berries and sugar was avoided so that the dextrose would not go into solution completely. The beakers were stored for forty-eight hours at 30° Fahrenheit to allow time for the development of the blue color.

The blue color only developed in the sample which had been cooled before adding dextrose, showing that the amount of dextrose in solution acts as an inhibiting factor for the off-color reaction. The heat treatment had little or no effect upon the pigments, also indicating that inactivation of the enzymes has no noticeable effect upon the off-color change.

Comparison of the Depth of Color in Sucrose and Dextrose Berry Juice Solutions. Two hundred cc. of berry juice were divided into two equal portions. To one portion, 50 grams of sucrose was added; to the other, 50 grams of anhydrous dextrose was added. After the sugar had dissolved in both samples, the depth of color of the two solutions was compared in a Klett Colorimeter.

The color of the two solutions proved to be the same within the limits of experimental error, indicating that dextrose in solution has no appreciable effect upon the color of the pigments in solution.

The Action of Berry Juice Pigment with Undissolved Dextrose. Two hundred grams of berry juice were cooled to 35° Fahrenheit so that dextrose could be added to it with only a slight amount going into solution. One hundred grams of dextrose was added without stirring and the mixture stored at 30° Fahrenheit for twenty-four hours. During this time, the undissolved dextrose became blue in color.

Berry juice, as prepared by dripping from a cloth bag containing frozen berries, contains considerable colloidal material such as pectin, protein and fiber. This juice was filtered through paper pulp, which removed most of these colloidal materials leaving a clear solution of the pigment containing the fruit sugars and fruit acids.

This clarified juice produced the blue color in the same manner as the unclarified juice in the experiment previously described. The blue from clarified juice was somewhat less dark, due probably to the removal of some of the pigments by filtration.

Concentration of clarified berry juices by boiling under vacuum greatly reduced the intensity of the off-color formed with dextrose. This lessened reactivity can be explained by the fact that the natural berry acid and the sugars present are both concentrated to the same extent as the pigment, and both seem to have a tendency to inhibit the blue color formation.

The Effect of Acid Washes on the Off-color Formation. Fresh berries were soaked two minutes in dilute HCl solutions of the following concentrations: .1%, .5%, 1%, 5% and 10%. After soaking, these berries were removed from the acid solution and put in quart jars along with dextrose. One pound of berries to one-half a pound of dextrose was used in each case. The acid resulted in a brightening of the normal red color in the 1%, 5% and 10% solutions; the .1% and .5% solutions had very little effect.

These samples were frozen and stored at 10° Fahrenheit for forty-eight hours. Examination showed off-color in all packs, indicating that none of the berries had adsorbed enough acid to inhibit the reaction.

The Limiting pH for The Reaction. Five pounds of berries were mashed to a uniform consistency, divided into five one-pound batches and an equal weight of dextrose added to each batch. HCl was added to the mixtures in varying quantities. These samples were stored for two days at 30° C. to allow time for color formation. The resulting colors and the pH determinations are shown in the following table:

TABLE 10.

cc. of 15% HCl Added.	Color	pH
1 cc.	Blue	3.6
2 cc.	Blue	3.5
3 cc.	Normal red, no off-color	3.4
4 cc.	Bright red, no off-color	3.2
0 cc.	Blue	3.7

A pH of 3.4 will completely inhibit the formation of the blue color. The amount of acid necessary to inhibit this reaction is about .1 of one percent of the amount the berries used.

A similar experiment was made using Citric Acid; this failed to inhibit the blue color until a concentration of 1% was reached.

Examination of Strawberries Barreled With Dextrose and Sucrose. Six 15-gallon barrels were packed and frozen in June, 1936. They were stored until March, 1937, examined and allowed to thaw, then they were made into preserves using both the open kettle and the vacuum pan. The barrels were put up with the ratios three plus one and four plus one, as follows:

TABLE 11.

No. of Bbls.	Lbs. of Fruit	Lbs. of Sugar	Kind of Sugar
2	100	33.3	½ Dextrose & ½ Sucrose
3	104	26.0	½ Dextrose & ½ Sucrose
1	100	33.3	Sucrose only

All of the barrels containing dextrose showed marked off-color formation, and in the frozen condition they were very solid, due to the absence of the heavy sirup normally present in the frozen pack. The off-color was most concentrated in the undissolved dextrose near the surface of the berries. The berries retained their normal shape with very little shrinkage, but they showed no evidence of having adsorbed any of the sugar.

The barrel in which only sucrose and berries were present contained berries with a normal red color. The sucrose was completely dissolved, forming a heavy sirup which was only partially frozen. These barrels were allowed to thaw at room temperature for three days. During the thawing, the blue color of the dextrose packs disappeared with the exception of about five pounds of dextrose which remained undissolved in the bottom of the barrels containing the higher proportion of dextrose.

Some of the berries in the barrels with the higher proportion of dextrose contained a few berries which were discolored on the surface. This can be explained by the penetration of the dextrose into the berries before freezing and then the dextrose recrystallized after freezing and caused the discoloration of the surface. This blue color disappeared more slowly than the off-color in the dextrose which was outside of the berries.

These six barrels of berries were made up into preserves by adding an additional amount of sucrose to make a ratio of fifty pounds of berries to fifty pounds of sugar. These mixtures were cooked down to a solids content of 68% and sealed in glass jars.

The preserves containing dextrose were satisfactory in every respect when compared with the sucrose preserves. The preserves containing dextrose could be detected due to a slight difference in sweetness, but to some persons this is an advantage rather than a disadvantage.

Loganberries and Raspberries Frozen with Dextrose. A pound of loganberries plus one-half pound of dextrose was frozen at 15° Fahrenheit. After two days, an examination showed that a few of the very ripe berries with a pH above 3.5 produced a blue color quite similar to that produced by strawberries. Raspberries given similar treatment failed to produce the blue color.

SUMMARY

1. The use of dextrose, to replace sucrose in frozin pack strawberries, results in a color change. This color change is from the red to the blue, and occurs wherever the juice of the berries is in contact with crystalline dextrose.

2. The relatively low solubility, and the tendency which dextrose has to recrystallize at low temperatures, makes it difficult to use dextrose without having a portion

of the dextrose remain in the crystalline form.

3. The off-color occurs on the surface of the undissolved dextrose crystals. The berries themselves are discolored when dextrose recrystallizes after having penetrated the surface of the berry.

4. Comparisons made with dextrose of different degrees of purity showed that the impurities in the commercial product are not a factor in the production of the blue color.

5. The anthocyan pigment of the strawberry is very sensitive to pH change. As the hydrogen ion concentration is lowered, the color shifts from the red toward the blue.

6. The undissolved dextrose shifts the color of the strawberry pigment toward the blue; however, no pH difference could be demonstrated.

7. Dextrose was the only sugar which produced a color change when undissolved crystals were in contact with the berries. Dextrose, discolored by contact with strawberry pigments in solution, may be changed back to the normal red by the addition of a small amount of acid.

8. Colorimetric comparisons of berry juices containing sucrose and juices containing dextrose show that the dextrose in solution has no different effect than the sucrose.

9. Loganberries packed with dextrose will produce the same color change provided they are very ripe so that the pH condition of the fruit is not too low.

10. The blue color was never observed at a pH of less than 3.4.

11. Strawberry preserves made from berries packed in sucrose-dextrose mixtures were satisfactory in every respect when the sucrose replacement by dextrose was not greater than 20% of the sugar added. The lack of sweetness and the low solubility of the dextrose limit the replacement of sucrose to 20%.

CONCLUSION

This study indicates conclusively that the color change is not due to impurities or chemical combinations between the dextrose and the anthocyan pigment. The results of these experiments show that this change in color is brought about by a lowering of the active acidity of the juices by dextrose crystals. Indications are that the strawberry anthocyan pigments change from the red oxonium form to the neutral and basic forms when in contact with the surface of the dextrose crystals.

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