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The writer discusses a series of investigations to ascertain the important factor or factors influencing the dyeing of cherries with Erythrosine. Substances known to be present in the fruit tissue from previous bleaching treatments are sulfur dioxide and calcium. It is also known that Erythrosine dye is affected by the H-ion concentration, being colorless in very acid solutions. It is shown that sulfur dioxide may readily be removed from the fruit by boiling in water. Calcium is shown to penetrate the cherry tissue, the quantity entering the tissue increasing with the calcium content of the bleach solution. Boiling in water markedly reduces the calcium present in the tissue. Calcium has a retentive effect upon the sulfur dioxide in the fruit. The higher the percentage of calcium present the more difficult it is to free the tissue of the gas. Further, calcium has an influence upon the amount of leaching required due principally to its effect on the H-ion concentration. The indication is that with higher calcium contents of fruit tissue less leaching is necessary. It is shown that the degree of dyeing is correlated with H-ion concentration, the best results being obtained when the fruit tissue had a pH value of 4.0 to 4.4. If the pH value is higher than 4.4, poor coloring usually results. By increasing the H-ion concentration of excessively leached fruit, discoloration can be prevented. It is concluded that H-ion concentration is the dominat factor in influencing the rapidity, uniformity and penetration of the dye.

In view of the results obtained in this investigation, a controlled method of leaching and dyeing is suggested.

INFLUENCE OF SULFUR DIOXIDE, CALCIUM AND HYDROGEN ION CONCENTRATION IN DYEING CHERRIES

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THE INFLUENCE OF SULFUR DIOXIDE, CALCIUM, AND HYDROGEN ION CONCENTRATION IN DYEING CHERRIES.

INTRODUCTION

The use of Royal Ann Cherries for the manufacture of maraschino, fruit salad and glace cherries is increasing. The cherry brining industry was stimulated sharply in 1930 when the Tariff Act of that year, imposing increased duties on imports, became effective. Expansion since then has been rapid. Prior to 1930 the American industry supplied about 20% of the cherries needed by domestic manufacturers and consumers, while 80% of the demand was filled by imports from European sources, mainly Italy. Today the situation is practically reversed. For the years 1927, 1928, 1929, Italian imports averaged 17,500,000 pounds while the United States pack averaged 4,037,000 pounds. In 1933 the Italian imports had dropped to 871,000 pounds while the United States pack had increased to 18,750,000 pounds.

The market for brined cherries is a comparatively inelastic one. The actual average consumption of cherries in brine is about 15,500,000 pounds per year. With an increased number of young trees coming into bearing in the next five years an increase in barreled brined cherries is anticipated. Consequently, there may be more than ample quantities of fruit to supply domestic needs.

The present production of brined cherries is practically limited to the Pacific Coast. California packs about 40% of the total coast

production, while Oregon packs close to 90% of the remainder. The States of New York, Pennsylvania, and Michigan are commencing to brine cherries but only small quantities have been packed to date.

Several methods for brining cherries have been employed commercially. After considerable preliminary experimenting a bleach or brine solution that has proved satisfactory when properly carried out has been developed by Bullis and Wiegand. (3) Most of the cherries brined in Oregon are processed according to their recommendations.

Ponceau 3 R, Amaranth and Erythrosine are artificial red dyes commonly employed in coloring cherries. These three colors are permitted by the pure food laws. Of the three dyes, Erythrosine is preferable. This dye can be set in the fruit tissue so that it will not bleed nor change color. It produces also a bright attractive shade of red. Manufacturers, however, have often experienced difficulty in obtaining a rapid and uniform penetration of the cherries with Erythrosine during the dyeing process.

The method of dyeing used commercially is to leach the cherries in either cold running water or by boiling in water until a point is reached where the fruit will dye. The dye is then boiled into the fruit or applied in cold solution. Variations in the extent of leaching and dyeing are often found.

Numerous factors play a role in influencing the dyeing of cherries with Erythrosine. The type of bleach solution and the method and extent of leaching at once suggest themselves as having an effect on the coloring of the fruit. Any factor which affects the solubility

of the dye or the permeability of the cherry tissue need careful consideration.

REVIEW OF THE LITERATURE

A review of the literature reveals that very little work has been done on the factors influencing the coloring of cherries with Erythrosine dye.

Tilgner (14) reported that cherries preserved in sulfurous acid solution only could be readily leached free of sulfur dioxide in cold or boiling water, but that it was much more difficult to liberate the sulfur dioxide from cherries packed in potassium pyrosulfite solutions. No dyeing experiments were performed.

Apparently no investigations have been conducted on the effect of calcium or the intake of Erythrosine dye by the cherry tissue. A great deal of literature, however, is available on the effect of calcium both in animals and plants generally of the lower orders, on the permeability of the cell. While most of the experiments were conducted on various kinds of living tissues and are somewhat unrelated, the accepted general conclusion is that calcium decreases the permeability of the cell. Further, as shown by Osterhout (12) and other investigators, certain salts are antagonistic to one another with respect to their effect on permeability of the cell. Salts of sodium and magnesium particularly show this action towards calcium. The former salts increase permeability while the latter salt decreases it. Stiles (13) reviews the theories that have been advanced to explain these phenomena. H-ions and OH-ions also play a part in permeability. Osterhout (10, 11) showed that H-ions first decreased permeability,

then rapidly increased it, if in sufficient concentrations, due to killing of the cell; while OH-ions continuously increased permeability. Changes in permeability seem to be based upon ion effects. These effects reported on permeability, however, deal with living tissue and may not be analogous for dead cherry tissue.

Gellhorn (6) states that the accumulation of dye in the cells depends not only on the permeability of the cells but also upon the H-ion concentration inside and outside the cell. This factor of H-ion concentration seems of infinite importance. Jeffrey and Cruess (7) definitely showed that the pH value of the cherries and that of the solutions used in the dyeing of the cherries with Erythrosine greatly affected the evenness and rapidity of dyeing. They recommended the addition of sodium bicarbonate to make the cherries slightly alkaline, approximately pH 7.5, and then to be acidified to pH 3.0 to 3.5 by the addition of citric acid. Atkinson and Strachan (2) showed that cherries without the addition of sodium bicarbonate leached to a pH value of 4.4 dyed without the addition of sodium bicarbonate.

Conn (4) states that impurities in any dye sample or in the solvent not only influence the solubility of the dye, but may have a great effect on the intensity of staining. Any impurity may alter the H-ion concentration of the staining fluid; and acid dyes, such as Erythrosine, stain better in acid solutions. However, if a mineral or organic salt does not affect the reaction of the solution, it may effect the intensity of dyeing. Conn and Holmes (5) found that the staining action of fluorane derivatives is influenced by the amount of

mineral salt present. They showed that the intensity with which a dye of this group such as Erythrosine can stain bacteria may be greatly increased by adding a minute amount (0.001 to 0.1%) of some mineral salt such as calcium chloride.

In view of the above facts the writer was led to believe that the factors causing difficulties in dyeing of cherries might be overcome by improved leaching and dyeing methods.

PURPOSE OF THE WORK

This investigation was undertaken to determine the important factors influencing the rapidity and uniformity of dyeing of bleached cherries. More specific information concerning the relative effects of sulfur dioxide, calcium, and H-ion concentrations on the coloring of the cherries with Erythrosine was desired.

EXPERIMENTAL PROCEDURE

The general plan of procedure was as follows: Royal Ann cherries, commercially packed in a bleach solution of approximately known composition, were leached by various treatments and dyed.

Observations were made on the rate of surface dyeing and penetration of the dye. The fruit was analyzed for sulfur dioxide, calcium, and total ash both before and after leaching. The pH value of the leached cherries was determined. A few sulfur dioxide analyses and pH tests were made of the dyed cherries. Microchemical examinations for calcium were made on a few samples of cherry tissue.

One series of 6 two-pound samples of selected Royal Ann cherries packed in quart sealers having the same sulfur dioxide content of 0.95% in the bleach solution but a gradation in calcium content were all leached and dyed in water soltuions in exactly the same manner. The calcium was added to this brine as calcium hydroxide which had been obtained from a weighed portion of calcium oxide by carefully slaking.

An analysis of fresh frozen Royal Ann cherries was carried out for ash and calcium.

Methods of Leaching.

Preliminary leaching tests were conducted by treating several 1,000 gram samples of cherries from the same pack as follows:

1. Cold running water (temperature 49° F)
2. Hot running water (temperature of 100° F and 132° F)
3. Chemical pretreatment and cold running water.

(a) Samples of brined cherries were treated in 1%, 0.5% and 0.25% solutions of hydrochloric and citric acids respectively, for four hours at 100° F. The fruit was then taken out of the acid and leached in cold water for eight hours.

(b) Samples of cherries were leached in cold water for twelve hours, then treated for two hours with the same acids employing the same concentrations as enumerated above in (a). The fruit was then removed from the acid solutions and leached in cold water for four hours.

(c) Samples of cherries which had been leached with hot water at 132° F for twelve hours and contained approximately 150 ppm sulfur dioxide were boiled (1) for periods of five, ten, and fifteen minutes in water only; (2) for the same periods in a solution of citric acid having a pH 4.4; and (3) for a period of ten minutes in solutions of citric acid of concentrations of 1%, 0.5%, and 0.25%.

4. The rate of flow of water for leaching was varied.

A few samples of fruit were only rinsed in water and then subjected to three ten-minute vigorous boils in water or slightly acidified during the third boil.

In the later experiments the cherries were leached overnight in cold running water, then boiled in water for ten-minute periods changing the water each time, till the cherry tissue reached a predetermined pH value, discussed later in this paper.

Method of Dyeing.

The Erythrosine* No. 773 (517) used analyzed 93.87% pure dye. One hundred and fifty to three hundred gram samples of leached cherries were employed in the dyeing tests. The majority of these samples were dyed at the rate of 0.0468 grams Erythrosine per 100 grams of pitted fruit. For a few samples 0.0312 grams of Erythrosine was employed per 100 grams of pitted fruit to determine if the effects of different treatments on dyeing could be more readily detected in a weaker concentration of dye. The dye was dissolved in 300 to 500 ml of water or solution acidified with formic, acetic, or citric acid to a pH of 4.4 using a Leeds and Northrup quinhydrone electrode indicator to determine the pH. The cherries were added to the solution and gently boiled for one hour. The volume of solution was maintained as constant as possible by the addition of water or acidified solution. The dye was set in the fruit by adding 1 ml of 25% citric acid for each 100 ml of dye solution.

Methods of Analysis.

The fruit for analysis was prepared as follows: (1) removed from the solutions, broken open and shaken to remove adhering moisture, (2) approximately 300 gram samples were ground in an ordinary food chopper. After mixing thoroughly samples were withdrawn for analysis. The fruit for calcium analysis was prepared as above with the exception that the cherries which were preserved in brine were thoroughly washed in distilled water after removal from the brine. This procedure

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* Manufactured by the National Aniline and Chemical Company

of rinsing in water removed any calcium that might have been adhering to the fruit.

The series of specially packed cherries were taken from the bleach solution, carefully and thoroughly rinsed in distilled water, then stemmed, pitted, and prepared for analysis as above.

All above analyses were made in duplicate or triplicate and the results are calculated on the basis of fresh tissue.

Sulfur dioxide was determined on 32 gram samples according to the method of Nichols and Reed (9). The strength of iodine solution employed was 0.1 N or 0.05 N depending upon the quantity of sulfur dioxide present in the tissue.

Moisture content was determined by drying 100 gram samples in an electric oven at 55-60°C.

Total ash was determined on the residue from the moisture determination by ashing in a muffle furnace according to the Official Methods (1). When no moisture estimations were made, 25 gram samples of pulp were taken for ash analysis.

Calcium was determined volumetrically according to the Official Methods. (1)

Determinations of pH were made upon the expressed juice from the pulped tissue with the Leeds and Northrup quinhydrone electrode hydrogen ion indicator.

Microchemical tests for calcium were performed on a few samples of cherries. Cross sections of the fruit were cut by hand, treated with 5% sulfuric acid or 2% oxalic acid. The prepared slides were

examined under the microscope for the formation of crystals of calcium sulfate or calcium oxalate.

PRESENTATION OF EXPERIMENTAL RESULTS

Removal of Sulfur Dioxide.

The figures recorded in Table 1 show the effect of the temperature of the leaching water on the rapidity and extent of liberation of sulfur dioxide in parts per million from cherries packed in calcium hydroxide bleach solution.

Table 1.

EFFECT OF TEMPERATURE OF WATER ON RATE OF REMOVAL OF SULFUR DIOXIDE

Duration of leach in hours	Sulfur Dioxide Content, ppm		
	Temperature of Water		
	49° F	100° F	132° F
0	1491	1491	1491
3		933	
6	989	683	
9		553	
12	807	418	129
18	611		
24	485	212	
30	416		
36	316		
48	160	46	
60	108		
96	55		

It may be observed that leaching with cold water (49°) did not remove all the sulfur dioxide from the fruit even after ninety-six hours. At that time the tissue contained about 55 ppm. The most rapid loss of sulfur dioxide took place during the first six hours in the case of cold water and the first three hours with water at 100° F. It will be noted that with hot water the liberation of sulfur dioxide was much more rapid. At 100° F it took only forty-eight hours to reduce the sulfur dioxide content of the fruit to 46 ppm. At 132° F the sulfur dioxide content was reduced to 129 ppm. in twelve hours. Thus, at 100° F the rate of removal of the sulfur dioxide was twice as fast as in cold water, while at 132° F it was approximately four times as rapid.

In Table 2 are recorded the results of treatment of the cherries (1) in warm acid solution followed by cold water leach and (2) in cold water, then warm acid solution, followed by cold water leach again.

Table 2.

EFFECT OF TREATMENT WITH ACIDS ON

THE REMOVAL OF SULFUR DIOXIDE

Acid	Concentration %	Hours Leached in			Total Hours Leached	Sulfur Dioxide Content ppm
		Water 49° F	Acid 100° F	Water 49° F		
Citric	0.25		4	8	12	521
	0.50		4	8	12	501
	1.00		4	8	12	501
Citric	0.25	12	2	4	18	456
	0.50	12	2	4	18	515
	1.00	12	2	4	18	460
Hydrochloric	0.25	12	2	4	18	296
	0.50	12	2	4	18	263
	1.00	12	2	4	18	208

The treatment of cherries in warm acid solutions while increasing the speed of removal of sulfur dioxide over that of cold water (Table 1) appears of little practical value for several reasons. It introduces another factor that needs control. Furthermore, with the higher concentrations of acids used the cherries were softened or discolored. This was particularly true of hydrochloric acid. Both the 1% and 0.5% solutions of hydrochloric acid decidedly softened the tissue of the fruit and produced "pink" discoloration

(the blush of the cherry reappearing). It was possible to remove the discoloration by leaching in cold water for about forty-eight hours. The cherries also seemed to firm up to some extent. The hydrochloric acid was more effective in freeing the tissue of sulfur dioxide than the citric acid. However, the strength of the particular acid within the limits employed made no apparent difference.

Cherries leached to a sulfur dioxide content of 129 ppm were boiled in water or acid solutions for a specified time. The results of this experiment are presented in Table 3.

Table 3.

EFFECT OF BOILING IN WATER AND IN ACIDIFIED SOLUTIONS

Boiling Solution	Sulfur Dioxide Content ppm		
	Boiling Periods, in Minutes		
	5	10	15
Water	77	63	70
Citric Acid (pH 4.4)	83	65	72
Citric Acid (0.25%)		72	
(0.50%)		70	
(1.00%)		76	

The data incorporated in the above table show that with the boiling periods used, water alone was as effective as the acidified

solutions in freeing the tissue of the last traces of sulfur dioxide.

The rate of flow of water during the leaching process, so long as it provided free circulation, had very little appreciable effect on the speed of removal of sulfur dioxide. The flow used was generally at the rate of 150 ml. of water a minute for 1,000 gram samples of fruit. For a few of the hot water leached samples the rate of flow was doubled, but little if any more sulfur dioxide was removed in the same length of time.

Penetration of Calcium.

With respect to the entry of calcium into the cherry tissue it may be noted from Table 4 that calcium does penetrate the fruit tissue and in amounts depending upon the quantity of whiting or slaked lime added to the sulfurous acid bleach solution. The calcium is calculated as milligrams of the element present in 100 grams of tissue.

Table 4.

PENETRATION OF CALCIUM INTO CHERRY TISSUE

Form in Bleach Solution	Amount of Compound in Solution lbs./100 gal.	Calcium Calculated in Solution mg/100 g	Calcium In Tissue mg/100 g	Calcium In Bleach Solution mg/100 g
$Ca(OH)_2$	5.00	323.85	163.30	134.00
$Ca(OH)_2$	6.25	405.42	200.15	
$CaCO_3$	6.50	311.86	184.58	

An analysis of fresh Royal Ann cherries showed them to have a calcium content of only 19.05 mg and an ash content of 0.588%. It is interesting that in the lot of brined cherries in which the amount of calcium in the bleach solution was estimated that the calcium content of the tissue was higher than that of the solution. The calculated amount of calcium added to the bleach solution is slightly excessive since the chemicals used are only 96 to 97% pure.

Microchemical tests made on sections of cherry tissue indicated that calcium penetrated into the tissue. An improved technique will be necessary before it can be stated whether the calcium diffused evenly through the tissue or was congregated in any particular place, such as in the epidermal layer.

Removal of Calcium.

Data incorporated in Table 5 show the effect of boiling on the ash and calcium content of fruit leached overnight in cold running water.

Table 5.

EFFECT OF BOILING ON REMOVAL OF CALCIUM

Sample	Number of Ten-Minute Boiling Periods	Ash %	Calcium mg/100g
$CaCO_3$	Unleached	0.776	184.58
	1	0.383	131.41
	4	0.348	109.41
$Ca(OH)_2$	Unleached	0.729	200.15
	6	0.185	76.23

From the data in the above table it will be noted that boiling markedly reduced the ash and calcium content of the cherries.

Effect of the Form of Calcium.

In Table 6 are recorded figures indicating the effect of the presence of calcium carbonate and calcium hydroxide respectively in the bleach solutions on the removal of sulfur dioxide and lowering of the H-ion concentration so that the cherries may be dyed. All samples of cherries were leached sixteen hours in cold water and then subjected to boiling in water for the various periods indicated.

Table 6.

INFLUENCE OF CALCIUM CARBONATE

AND CALCIUM HYDROXIDE

Number of Ten Minute Boiling Periods	Calcium Carbonate		Calcium Hydroxide	
	Sulfur Dioxide ppm	pH	Sulfur Dioxide ppm	pH
Unleached	5,231		4,461	
1	1,464	3.82	126	3.76
3		4.00	10	4.15
4	405	4.47		4.21
5				4.32
6				4.47

The results tabulated in the above table show that in these particular samples at least the sulfur dioxide was much more readily removed from the calcium hydroxide brined cherries than the calcium carbonate treated ones. It may be stated here that the calculated available calcium content of the calcium hydroxide bleach solution is higher than that of the calcium carbonate solution, the proportion being approximately 3.38 to 2.6. A greater amount of leaching was required to raise the pH value of the cherries which had been packed in calcium hydroxide bleach solution.

In the dye tests, the calcium carbonate cherries boiled for four ten-minute periods testing pH 4.47, colored well and were of an attractive bright red shade. The calcium hydroxide treated fruit

raised to the same pH, dyed at a slower rate although there was a greater final penetration and they showed the characteristic purple tinge of overleaching. The whiting packed cherries received three ten-minute boils, dyed well and were of excellent color. Samples boiled only one ten-minute period were too acidic and caused precipitation of the dye early during the dyeing period. The calcium hydroxide packed cherries, however, receiving only one boil, colored very well, although a shade darker than the calcium carbonate treated cherries.

Effect of the Calcium Concentration.

The influence of the quantity of calcium present in the fruit tissue on the pH value and retention of sulfur dioxide by the leached tissue is shown in Table 7. How these factors affect the rapidity and penetration of the dye is also recorded. The cherries employed in this experiment were from the six samples of specially packed fruit of the same sulfur dioxide content but varying calcium content. The samples of fruit were leached overnight in cold running water and received three ten-minute vigorous boils in water. The degree of dyeing is scored on the basis of allowing ten points for sample number one which was thoroughly dyed.

Table 7.

EFFECT OF CALCIUM CONCENTRATION

Sample Number	CaO Added to Bleach Solution	Ca in Unleached-ed Tissue mg/100 g	Leached Tissue		Degree of Dyeing
			SO_2 ppm	pH	
1	3.75	173.13	286	4.21	10.0
2	3.37	162.91	179	4.1	8.3
3	3.0	134.74	186	4.1	8.3
4	2.62	133.76	157	4.03	6.6
5	2.25	113.86	144	3.92	5.0
6	1.85	97.39	85	3.74	1.7

It is apparent that the cherries with the higher calcium content are more retentive of the sulfur dioxide. Further, the fruit with the highest calcium content had the highest pH value in the leached tissue. There was a gradual decrease in pH or in other words an increase in H-ion concentration with a decrease in calcium.

The degree of dyeing appears to be correlated with the H-ion concentration. Penetration of the dye was practically complete in the fruit of the highest pH and calcium content. There was a continuous decrease in penetration which was particularly marked in the sample of the lowest pH and calcium content. In this sample the color was bright and attractive, as were the other samples, but of a lighter shade. The dye only penetrated the surface layers of the

fruit. Precipitation of the dye solution occured early during the process of dyeing. The results of the tests indicate that fruit packed in solutions of rather low calcium content will require more leaching in order to raise the pH value to a point where the dye may take. It is probable that the calcium neutralizes some of the organic acids naturally present in the cherry. It is possible that the higher calcium content had some effect also on the intensity of dyeing. In the range of the pH values of leached tissue found in the above experiment no difference was noted in the rate of the first taking of the dye by the surface layers of the fruit.

The results recorded in Table 7 corroborate those presented in Table 4 in showing that calcium penetrates the tissue in accordance with the amount present in the bleach solution.

Effect of Acid.

In some preliminary experiments, cherries were acidified during the third boil of the leaching process or during dyeing with citric, acetic or hydrochloric acid, usually in sufficient quantities to bring the solution to a pH value of 4.4. None of these acids increased the penetration of the dye. There was some indication though that in certain cases the acids increased the speed and evenness of surface dyeing. Acetic acid gave the best results. The acids had an influence upon the color of the dye. Acetic acid produced a deeper, duller shade of red. On setting the dye with citric acid, however, all the fruits took on the same bright color.

Later experiments showed that if the proper amount of leaching was accorded the cherries of similar quality, acidification had no apparent effect on the dyeing. Excessive leaching was found to be detrimental to dyeing. It was in the treatment of these cherries that the influence of the acid was most marked.

Fruit that was overleached became discolored, showing a grey or purplish color. This was found to appear as a rule when the fruit was leached to a sulfur dioxide content below 150 ppm. Continued leaching caused increased discoloration. The addition of an alkali produced the same result in fruit of low sulfur dioxide content. By the addition of an acid it was possible to restore to the fruit its natural bleached color. The addition of excessive quantities of acid produced a pink tinge to the fruit. These changes in coloration with changes in H-ion concentration are due to the anthocyanin pigments in the tissue of the fruit. These pigments are not destroyed by sulfur dioxide but are rendered colorless.

Excessively leached cherries dyed more slowly and more unevenly than those properly leached. The color was dull and unattractive, and had a tendency to remain poor after setting of the dye. Even the use of an excess of acid and heating did not always restore the fruit to a bright attractive color, although it invariably improved it. By the addition of an acid such as citric to excessively leached cherries, showing marked discoloration, it was possible to increase

the speed and uniformity of dyeing and produce cherries of an attractive red color.

There was some variation in the pH at which discoloration appeared as may be noted in the presentation of results in Table 6. The indications are that both the form and the quantity of calcium present has an influence on the degree of dyeing. Generally, leaching to a pH value between 3.9 and 4.4 is satisfactory. The amount of sulfur dioxide present at these values is usually sufficient to prevent discoloration.

Method for Leaching and Dyeing.

As a result of the preceding experiments the following procedure for leaching and dyeing of cherries was evolved. The fruit is first leached overnight in cold running water. This removes the readily soluble sulfur dioxide. Then the cherries are vigorously boiled for one to three ten-minute periods in water, employing fresh water for each boil. Generally three ten-minute boils are the most satisfactory. The number of boilings required depends upon the nature of the fruit and the calcium content which in turn affects the H-ion concentration. The amount of leaching necessary may be controlled by the pH value of the cherry tissue. The color of the tissue is also a fair index as to whether the fruit is sufficiently leached. If the leaching has been excessive the addition of citric acid to the dye solution to decrease the pH value to between 4.0 and 4.4 will increase the rapidity and uniformity of the dyeing as well as insure a

good color.

If the batch of fruit being processed is unevenly graded -- that is if it contains many broken or badly cracked cherries, it may be found advisable to acidify the solution to enhance uniformity in dyeing. The broken or cracked cherries when mixed with good fruit will in all probability be leached excessively.

Dyeing of the cherries is best accomplished by gently boiling the fruit in the water solution or in special cases, acidified solutions of the dye, for an hour. If penetration of the tissue is not complete at the end of heating, the cherries may be left in the dye solution overnight before setting of the color.

By the method as described above, an attractive product is obtained. Furthermore, the fruit is almost completely freed of sulfur dioxide, containing not more than 50 ppm of the gas, and in most cases considerably less. The sulfur dioxide content of cherries after dyeing, having been leached overnight and subjected to three ten-minute boilings, is recorded in Table 8. The small quantity of sulfur dioxide remaining in the tissue will be lost during the candying process.

Table 8.

SULFUR DIOXIDE CONTENT OF DYED CHERRIES

Sample Number	Calcium in Unleached Tissue mg/100 g	Sulfur Dioxide, ppm	
		Bleached Cherries	Dyed Cherries
1	175.13	3,085	51
2	162.91	3,068	41
3	134.74	2,454	41
4	133.76	2,581	9
5	113.86	2,512	22
6	97.39	2,203	5

The differences shown in the above table by the samples in retention of sulfur dioxide can be accounted for by the varying calcium contents of the fruit. The samples are listed in the descending order of their calcium content.

DISCUSSION OF RESULTS

The liberation of sulfur dioxide from brined cherries is apparently affected by the amount of calcium present. Larger quantities increase the difficulty of removing the last traces of the gas. This is due probably to the formation in the tissues of calcium bisulfite or possibly calcium sulfite. The free sulfur dioxide is much more readily removed as has been reported by Tilgner (14). Acids of sufficient strength, stronger than those which would be practical to use, aid in the liberation of the sulfur dioxide. When acids strong enough to markedly increase liberation of the gas are employed, it is necessary, due to increased H-ion concentration to remove them by leaching in order to dye the fruit. By (1) leaching the brined cherry overnight, then (2) by boiling vigorously for three ten-minute periods in water, changing the water after each boil, then (3) by boiling the dye into the fruit for one hour, the sulfur dioxide present in the tissue is reduced to only a few parts per million and by the time the fruit has been made into the finished product, is probably within the tolerance of the food laws of any state.

When this investigation was begun, some doubt was expressed as to the actual penetration of calcium into the cherry tissue. The result of this study conclusively shows that calcium does enter the fruit tissue and in amounts corresponding to the quantity of calcium salt added to the bleach solution. The only reference to work of this nature discovered in the literature was that of Kahlenberg and Truxler

(8). They found that green cherries when immersed in salt or acid solutions were more permeable to the solutions than ripe cherries. Certain lithium salts passed into the green fruit in detectable amounts.

The Royal Anns employed for bleaching purposes are, from an edible viewpoint, immature. This immaturity may have some bearing on the penetration of the calcium. What appears to be more probable, however, is the effect of the sulfur dioxide. This gas in solution tends to greatly soften the cherry tissue and thus very likely increases its permeability to salts.

There are strong indications that H-ion concentration is the dominant factor influencing the rapidity, uniformity and penetration of the dye. Practically no dyeing will occur if the leached tissue has a pH value below 3.7. At that pH only a very light surface coloring may be obtained. Excessive leaching causes discoloration, again resulting in poor dyeing. Penetration of the dye may be increased in overleached fruit. In most instances the best dyeing of the fruit is accomplished when the pH of the leached tissue is 4.0 to 4.4.

The data recorded in Table 7 suggest that calcium may have an influence on the intensity of dyeing. It is just possible for the salt to be in such a concentration as to render that degree of solubility to the dye that would increase its staining properties as noted by Conn and Holmes (5) in their bacteriological studies. Even if this were true, it is still necessary to have a favorable H-ion

concentration. A more likely explanation of the occurence of the highest pH value with the highest calcium content is that the calcium neutralized more of the natural organic acids present in the fruit.

Under the conditions studied, the experiments indicate that calcium, in the process of firming the cherry tissues, does not reduce their permeability. Thus, in this respect, it does not appear to be a factor influencing dye absorption.

The intensity and rapidity of dyeing seems to be a function of the solubility of the dye. In these studies solubility was primarily affected by H-ion concentration although the amount of calcium present in the fruit may have had some effect.

SUMMARY

This paper presents the results of an investigation to determine the influence of sulfur dioxide, calcium and H-ion concentration in the dyeing of bleached Royal Ann cherries with Erythrosine.

The data show that H-ion concentration is the dominant factor influencing the rapidity, uniformity and penetration of the dye.

A controlled method of leaching and dyeing is suggested.

LITERATURE CITED

(1) Association of Official Agricultural Chemists. Official and tentative methods of analysis, third ed., p. 278, 104, Washington, D. C., 1930.

(2) Atkinson, F.E. and Strachan, C. C. Cherry processing. Part II. Leaching sulfured stock. Part III. Glaceing of Royal Ann Cherries. Fruit Products Jour., 14 : 6 : 174 - 175; 14 : 7 : 206 - 208, 221, 1934.

(3) Bullis, D. E. and Wiegand, E. H. Bleaching and dyeing Royal Ann Cherries for maraschino or fruit salad use. Ore. Agr. Exp. Sta. Bul. 275, 1931.

(4) Conn, H. J. Biological Stains. Second Edition. Commission on standardization of biological stains. Geneva, N. Y., 1929.

(5) Conn, H. J. and Holmes, W. C. Certain factors influencing the staining properties of fluorescein derivatives. Stain Technology, 3 : 3 : 94 - 104, 1928.

(6) Gellhorn, E. Vital staining and permeability. II Communication. Protoplasma, 12 : 66 - 78, 1931.

(7) Jeffrey, R. N. and Cruess, W. V. Effect of hydrogen - ion concentration in the dyeing of cherries. Ind. and Eng. Chem. 21: 12: 1268 - 1269, 1929.

(8) Kahlenberg, L. and Truxler, B. On the passage of boric acid and certain salts into fruits and vegetables. Jour. Plant Phys., 11 : 39 - 54, 1927.

(9) Nichols, P. F. and Reed, H. M. Distillation methods for the determination of sulfur dioxide. Ind. and Eng. Chem., Anal. Ed., 4 : 1 : 79 - 84, 1932

(10) Osterhout, W. J. V. Effect of alkali on permeability. Jour. Biol. Chem., 19 : 335 - 343, 1914.

(11) _____ Effect of acid on permeability. Jour. Biol. Chem., 19 : 493 - 501, 1914

(12) _____ Injury, recovery, and death in relation to conductivity and permeability. J. B. Lippincott Co., Philadelphia, 1922.

(13) Stiles, W. Permeability. New Phytologist Reprint, No. 13, Wheldon and Wesley, Ltd., London, 1924

(14) Tilgner, Damazy Jersy. Maraschino cherry manufacture. The Canner, p. 18, July 30, 1932.