to flavor of the meat itself. These results also show that a correlation may be expected between flavor as evaluated in the broth and the meat from which it was prepared.

Soaking in Ice Water

The experiments described above clearly demonstrated that flavor precursors can be extracted from raw chicken meat with water under the laboratory conditions used. As an obvious corollary, two preliminary experiments were conducted to determine how much, if any, flavor loss resulted from the general practice of chilling poultry in slush ice or thawing in water. Half carcasses were used, one half as a control and the other soaked in ice water. The average weight of ice water used for each carcass half was 9 kg. Unsoaked halves were stored in polyethylene bags at 2° C. during the soaking period. Ratios of water to meat used for cooking were based on weights observed before the soaking period. Immersion times for the two experiments were 18 hours in one case and 5 hours in the other. For the former there were six replications; for the latter, three. The results (Table VII) show that broth from the control halves contained significantly more flavor than broth from the halves chilled in ice water. While these results indicate a flavor loss as a result of immersion in ice water, it is emphasized that these experiments were carried out on previously frozen birds. Consequently, conclusions concerning the importance of flavor loss in the commercial chilling of poultry in ice water will require additional experiments with freshly slaughtered unfrozen birds.

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FRUIT COLOR STABILITY

Interaction of Ascorbic Acid, Riboflavin, and Anthocyanin Pigments

DAN E. PRATT, CHARLES M. BALKCOM[1], JOHN J. POWERS, and LIONEL W. MILLS[2]
Food Technology Department, University of Georgia, Athens, Ga.

This study was made to ascertain the effect of ascorbic acid and riboflavin on the loss of color in anthocyanin pigments of strawberry juice and to evaluate the reliability of polarographic methods for quantitatively determining ascorbic acid and riboflavin in mixed solutions. Spectrophotometric determinations demonstrated that as the length of storage was increased there was a corresponding increase in the amount of brown color present. The greatest losses of both ascorbic acid and riboflavin were in samples containing ascorbic acid, riboflavin, and anthocyanin pigments. The retention of riboflavin was greatest when stored in pure solutions; the retention of ascorbic acid was greatest when stored alone or in mixtures containing riboflavin. Ascorbic acid and riboflavin were determined polarographically, with mean errors in the calibration curves of 1.38 and 1.84%, respectively. The results confirm the findings of others that ascorbic acid and anthocyanin pigment react, causing destruction of the pigment, and indicate that riboflavin may contribute to the instability of anthocyanin pigments.

THE STABILITY OF THE COLORED PIGMENTS of strawberry juice has been shown to be affected by the redox constituents present (*6*). Beattie, Wheeler, and Pederson (*2*) suggested that an interaction existed between ascorbic acid and the pigments. Similar observations were made by Pederson, Beattie, and Stotz (*20*) and Nebesky, Esselen, McConnell, and Fellers (*18*). Spectrophotometric determinations by Esselen, Powers, and Woodward (*8*) and Esselen, Powers, and Fellers (*7*) demonstrated that slight color changes were brought about by the use of ascorbic acid in fruit juice, but that the changes in color and flavor were not objectionable.

In a recent article Meschter (*16*) discussed the significant factors to be considered in studying the color deterioration of strawberries. Bauernfeind (*1*) has recently reviewed the uses and limitations of ascorbic acid as an antioxidant in foods.

The purpose of the study reported herein was to determine whether another redox constituent found in many foods—riboflavin—might affect the stability of anthocyanin pigments. Riboflavin has been shown (*19*) to accelerate the oxidation of ascorbic acid. The authors were of the opinion that an interaction might exist among ascorbic acid, riboflavin, and anthocyanin pigments. Strawberries usually contain about 0.07 mg. % riboflavin and 60 mg. % ascorbic acid (*24*).

[1] Present address, Hills Bros. Co., Woodbury, Ga.
[2] Present address, Welch Grape Juice Co., Westfield, N. Y.

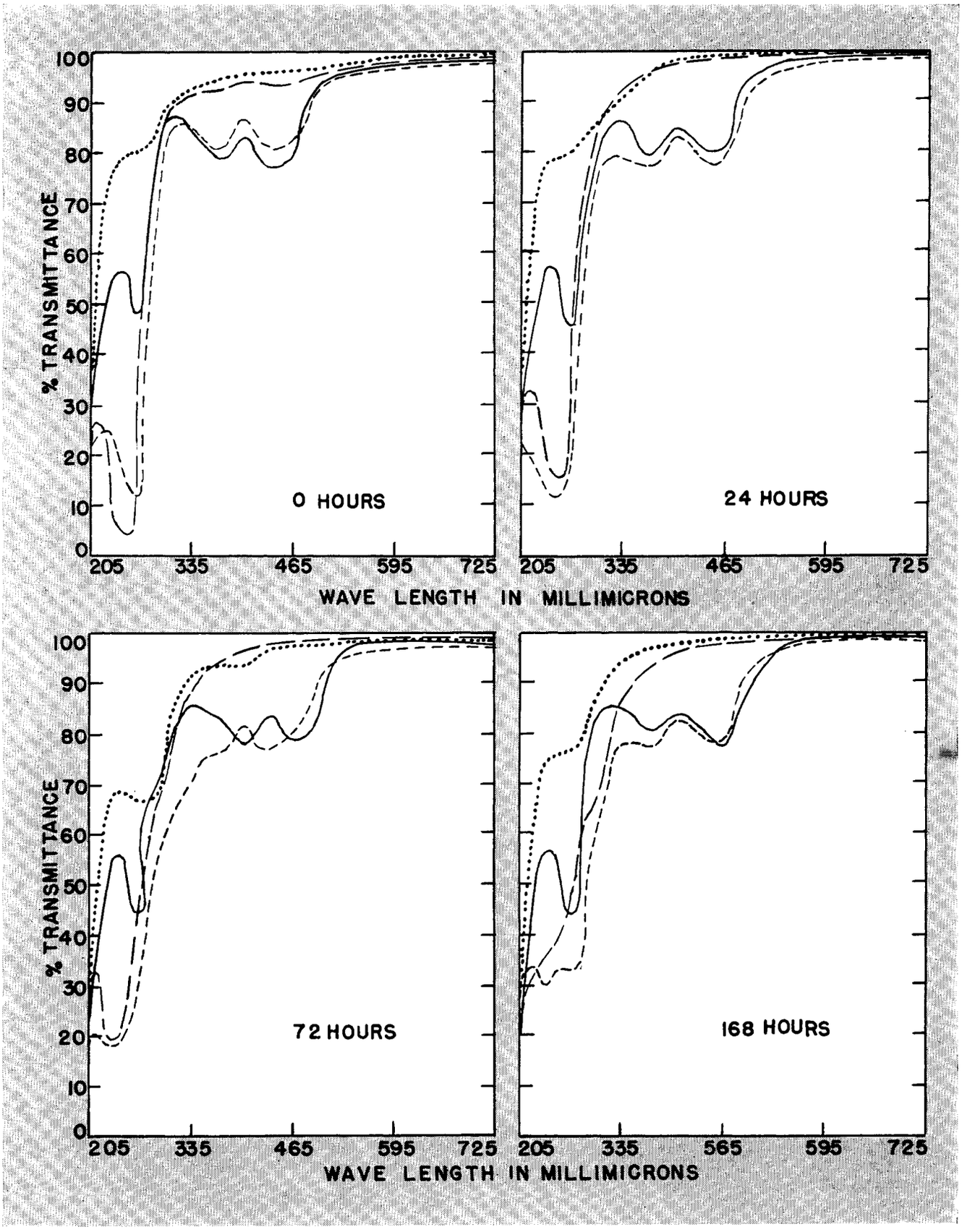

Figure 1. Transmittance curves of anthocyanin pigments isolated from strawberries, adjusted to pH 3.0 with citrate–citric acid buffer

Determinations made with Beckman spectrophotometer, Model DU, at slit width of 0.7 mm.

——— Anthocyanin pigments, ascorbic acid and riboflavin

– – – – Anthocyanin pigments and ascorbic acid

- - - - - Anthocyanin pigments and riboflavin

. . . . . Anthocyanin pigments

Kertesz and Sondheimer (*10, 22, 23*) not only demonstrated a method for the isolation of anthocyanin pigments but suggested that the principal anthocyanin pigment of strawberries is pelargonidin 3-glucoside. A chromatographic technique for the extraction of anthocyanin pigments was reported by Nebesky *et al.* (*18*).

The susceptibility of ascorbic acid to polarographic analysis was first demonstrated by Kodicek and Wenig (*12*), who, using a 0.067*N* phosphate buffer, obtained irreversible anodic polarographic waves of ascorbic acid from fruit juice. Müller (*17*) recently stated that the anodic oxidation of ascorbic acid was the only irreversible anodic reaction known. Kirk (*11*) reported cathodic waves were obtained using a 2% metaphosphoric acid solution with better results than in 0.067*N* phosphate buffer, acetate buffer at pH 2.2, or potassium biphthalate–hydrochloric acid buffer at pH 2.2. Gillam (*9*) used four different supporting electrolytes—buffers of biphthalate–0.25% oxalate, phosphate–1.5% metaphosphoric acid. biphthalate–0.25% oxalic acid in 50% ethyl alcohol, and biphthalate–3% metaphosphoric acid—in the determination of ascorbic acid. He made determinations at pH 4.6 and 3.4 for the first two supporting electrolytes but did not list the pH of the latter two electrolytes. He reported the error was about 4% in all determinations. Rogers (*21*) compared the biphthalate buffer method with a procedure using 0.1*M* potassium oxalate and 0.2% oxalic acid in equal portions; no differences were found.

Polarographic waves for riboflavin were obtained cathodically by Lingane and Davis (*13*), using 0.1*M* phosphate buffer with pH values from 4 to 9. They pointed out that the optimum pH was around 7. The diffusion current was demonstrated to be proportional to the concentration at pH 7.2 in tetramethylammonium phosphate buffer at concentrations of 4 to 50 p.p.m. Brdicka and Knobloch (*3–5*), working with riboflavin, reported that curves obtained at concentrations at or above $5 \times 10^{-5}M$ were not centrosymmetrical. At concentrations above this level the wave height was not proportional to the concentrations.

Polarographic Calibration Curves

Ascorbic Acid. A series of 37 calibration curves for six concentrations each was made. Each determination was made anodically using 5.0 ml. of a standard ascorbic acid solution in a 0.35% oxalic acid solution and 20 ml. of supporting electrolyte. The supporting electrolyte consisted of 1 part of 0.35% oxalic acid and 3 parts of 0.1*M* potassium oxalate solutions; in this way the pH was adjusted to approximately 4.2. The ascorbic acid concentration of the resulting solutions ranged from 0.008 to 0.250 mg. per ml.

Riboflavin. Thirty-two calibration curves were made for six concentrations. Each determination was made cathodically using 20 ml. of a standard riboflavin solution in aqueous hydrochloric acid (pH 3.0) and 5.0 ml. of supporting electrolyte. The supporting electrolyte consisted of 1 volume of 0.1*M* sodium dihydrogen phosphate and 4 volumes of 0.1*M* potassium monohydrogen phosphate; this adjusted the pH to about 7.2. The concentration of riboflavin ranged from 0.004 to 0.080 mg. per ml.

Electrolysis cells containing the samples were deaerated 10 minutes by bubbling nitrogen through the solution. In the early stages of this investigation 50-ml. beakers were used; nitrogen was bubbled through a glass tube inserted through the rubber stopper which covered the beaker, according to the method used by Rogers (*21*). The electrolysis cells used in the latter stages were 30-ml. cylindrical vessels with a glass tube connection at the bottom for bubbling nitrogen through the sample. The reference electrode in all polarographic determinations was a saturated calomel electrode.

The half-wave potential and diffusion currents were calculated for each determination. The diffusion coefficient was determined for each polarogram in accordance with the Ilkovič equation corrected for curvature of the electrode surface by Lingane and Loveridge (*14*).

Preparation and Storage of Samples

Standard ascorbic acid solutions were prepared in 0.35% oxalic acid, so that the concentration was 1.5 mg. per ml. Standard solutions of riboflavin were prepared in aqueous hydrochloric acid (pH 3.0) at a concentration of 0.15 mg. per ml. Anthocyanin pigments were separated from strawberry juice by the chromatographic adsorption technique of Nebesky *et al.* (*18*) and brought to the original volume of the juice. The ascorbic acid solution, the riboflavin solution, anthocyanin pigments, and strawberry juice were mixed in all possible combinations; the resulting solutions contained ascorbic acid and riboflavin in concentrations of 0.5 and 0.05 mg. per ml., respectively. The samples were stored at 37.8° C. (100° F.).

The ascorbic acid and riboflavin content was determined polarographically after 0, 24, 72, 168, and 336 hours of storage, by the procedure used in making the calibration curve. For samples containing pigments or strawberry juice, color changes were determined, at each time interval, by transmittance curves using a Beckman spectrophotometer (Model DU) at wave lengths from 205 to 750 mμ.

Calibration Curves

Ascorbic Acid. The errors of the 37 calibration curves ranged from 0.04 to 7.03% calculated by the use of the square of the correlation coefficient, *r*. There was a significant difference among curves obtained by different analysts;

Table I. Effect of Anthocyanin Pigments and Strawberry Juice on Retention of Ascorbic Acid and Riboflavin Stored at 37.8° C. (100° F.)

Constituents of Sample	Hours of Storage: 0	24	72	168 (% Retention of ascorbic acid)	Hours of Storage: 0	24	72	168 (% Retention of riboflavin)
Ascorbic acid[a]	100.2	69.6	64.1	59.7	...	...	...	...
Ascorbic acid[b] and anthocyanin pigments	92.6	74.4	62.6	46.3	...	...	...	...
Ascorbic acid[c] and strawberry juice	91.7	63.5	56.4	46.8	...	...	...	...
Ascorbic acid[a] and riboflavin	97.2	67.5	58.7	59.2	102.0	97.1	93.3	85.5
Ascorbic acid[b], riboflavin, and anthocyanin pigments	91.0	66.2	24.8	13.6	96.5	93.9	88.7	85.2
Ascorbic acid[c], riboflavin, and strawberry juice	96.3	60.0	49.9	50.5	96.5	93.9	97.4	93.0
Riboflavin[a]	...	...	...	...	100.0	100.0	96.3	97.3
Riboflavin[b] and anthocyanin pigments	...	...	...	...	98.3	97.4	93.9	93.9
Riboflavin[c] and strawberry juice	...	...	...	...	97.4	98.3	96.5	93.0

[a] Each value is mean of twelve determinations.
[b] Each value is mean of eight determinations.
[c] Each value is mean of four determinations.

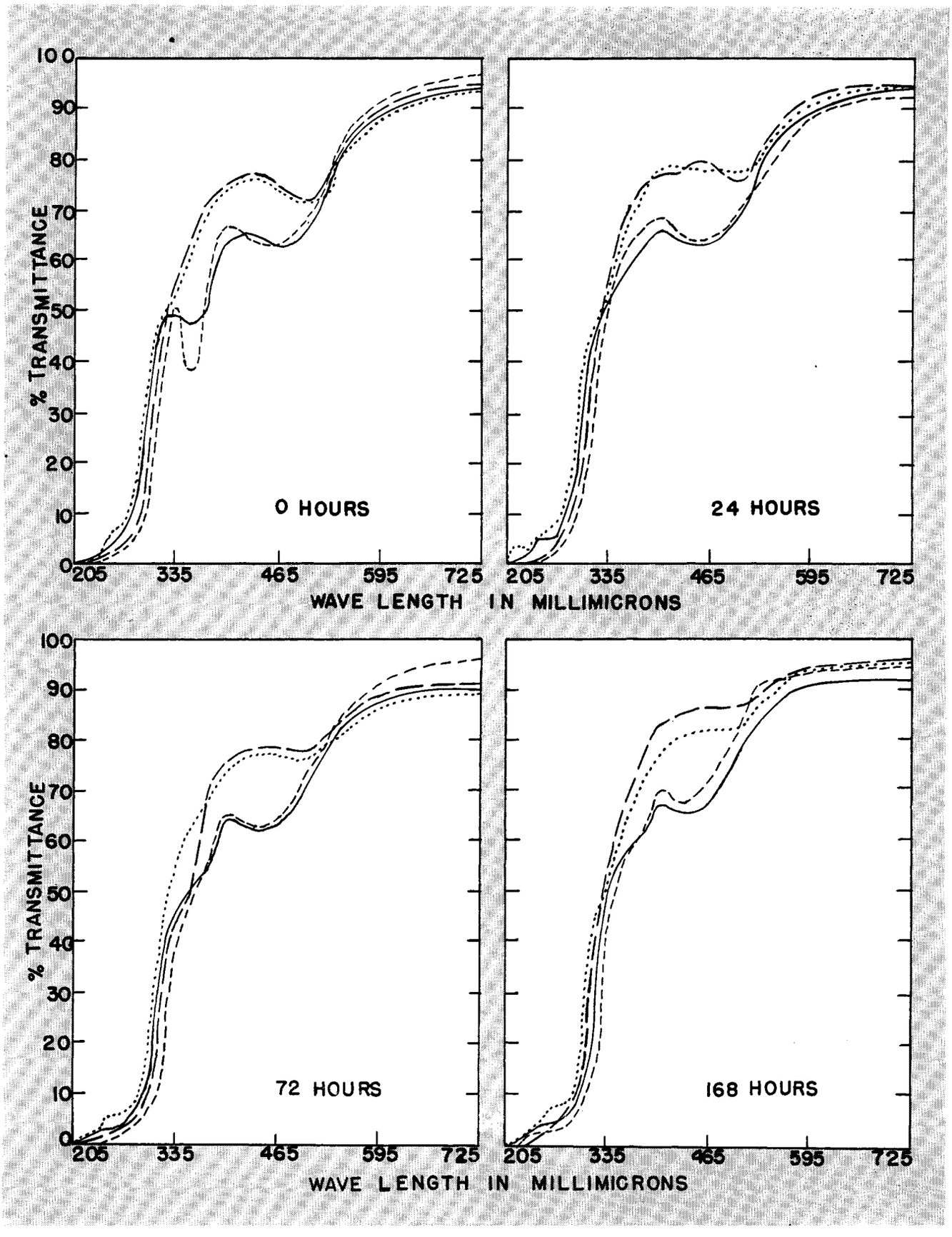

Figure 2. Transmittance curves of strawberry juice, adjusted to pH 3.0 with citric acid buffer

Determinations made with Beckman spectrophotometer, Model DU, at slit width of 0.7 mm.

——— Strawberry juice, ascorbic acid, and riboflavin
– – – – Strawberry juice and ascorbic acid
- - - - - Strawberry juice and riboflavin
. . . . . Strawberry juice

however, statistical methods failed to show a significant difference between curves obtained by a single analyst. Two curves varied significantly (5% probability level) from the origin. The r^2 value of the pooled 37 curves was 0.9862, showing a mean error of 1.38%. The mean diffusion coefficient for all curves was 6.82×10^{-6} sq. cm. per second. Confidence limits (5% probability level) applied to diffusion coefficients indicated that the true diffusion coefficient for ascorbic acid in the oxalate–oxalic acid buffer was between 6.29×10^{-6} and 7.35×10^{-6} sq. cm. per second. The diffusion currents used to calculate diffusion coefficients were adjusted values calculated by the regression equation, $Y = a + bX$. The formula for the combined calibration curves was $i_d = 0.033 - 5.084\ X$ (X equals the concentration of ascorbic acid in millimoles per liter). The half-wave potentials, $E_{1/2}$, of the polarograms varied widely, because there were moderate variations in the pH values of the samples. Variations of 0.05 to 0.1 pH unit did not affect the wave height significantly. The half-wave potentials of polarograms at pH 4.20 ranged from -0.082 to -0.087 volt with a mean $E_{1/2}$ of -0.084 volt.

Riboflavin. The 32 calibration curves had errors ranging from 0.10 to 7.68%. Only one curve varied significantly from the origin, although the slopes of the curves obtained by different workers varied significantly. The mean error of all the curves was 1.84%. The mean diffusion coefficient for the curve was 3.67×10^{-6} sq. cm. per second. The lower and upper confidence limits (5% probability level) of the diffusion coefficient were 3.27×10^{-6} and 4.07×10^{-6} sq. cm. per second, respectively. The formula for the combined calibration curve for riboflavin was $i_d = 0.002 - 3.791X$ (concentration of riboflavin in millimoles per liter). The half-wave potential of polarograms at pH 7.20 varied from -0.415 to -0.473 volt with a mean $E_{1/2}$ of -0.444 volt.

As reported by Brdička and Knobloch (*3–5*), several polarograms of riboflavin at the higher concentration demonstrated a second current voltage wave; however, contrary to their findings, the wave height of second curves reported herein remained in direct proportion to the concentration.

Storage Studies. The effects of strawberry juice and anthocyanin pigments on the retention of ascorbic acid and riboflavin stored for various lengths of time at 37.8° C. (100° F.) are shown in Table I. The application of analysis of variance demonstrated very highly significant differences (0.1% probability level) in all samples due to the length of storage and to the differences in constituents of the sample. The percentage destruction of ascorbic acid was greater in all combinations, except that of ascorbic acid and riboflavin, than in pure ascorbic acid solutions. Riboflavin likewise was more stable in pure solution than when with other constituents.

The transmittance curves of samples containing strawberry juice and pigments are shown in Figures 1 and 2. A pronounced loss in red color was encountered during storage of samples composed of strawberry juice plus ascorbic acid and strawberry juice alone. Riboflavin used in conjunction with the juice or the juice and ascorbic acid either masked or prevented the loss of the red color; however, as the length of storage increased there was a sharp increase in the amount of brown color. There was a slight fading of the yellow color in samples containing only riboflavin and juice, but this fading could not be detected when ascorbic acid was present in the samples.

Red color was lost in the first 24 hours for samples containing ascorbic acid plus the pigment. The loss of red color in the remaining samples was very slight. The transmittance curves indicated that all samples containing pigments turned brown.

Discussion

The 1.38% error for the composite calibration curve of ascorbic acid demonstrates the acceptability of the method for analytical determinations. The oxalate–oxalic acid buffer at pH 4.20 was shown to be a satisfactory supporting electrolyte for anodic polarographic measurements of ascorbic acid. It has been applied to other foods with comparable precision. The applicability of polarographic quantitative analysis for riboflavin, using phosphate buffer at pH 7.20, is also shown by the error of the composite calibration curves. Contrary to the findings of Brdička and Knobloch (*3–5*), the diffusion currents for riboflavin polarograms that gave a second current-voltage wave remain directly proportional to the concentration, showing that differences existed between the two methods.

The results reported herein on the losses occurring in ascorbic acid and red color in anthocyanin pigments and strawberry juice agree with the findings of earlier investigators (*2, 7, 8, 15, 18, 20*).

The findings of Pederson, Beattie, and coworkers (*2, 20*) that an interaction exists between the readily reducible pigments and ascorbic acid were verified. The losses of ascorbic acid were greatest in samples containing ascorbic acid, riboflavin, and pigments. Riboflavin tended to be least stable when in combination with ascorbic acid in synthetic mixtures. Even though the riboflavin was used at a concentration much greater than normally present in strawberry juice, it had little tendency to be less stable in the presence of pigment than in pure solution. Spectrophotometric determinations failed to confirm that an interaction existed among all three components.

The studies on pigments and juice also agree with the work of Beattie *et al.* (*2*) that as the length of storage is increased a corresponding increase occurs in the amount of brown color present.

Summary

In the quantitative polarographic determination of ascorbic acid and riboflavin the mean errors of the linear calibration curves were 1.38 and 1.84%, respectively.

Storage studies demonstrated that the greatest losses of ascorbic acid and riboflavin were encountered in samples which contained ascorbic acid, riboflavin, and anthocyanin pigments. The retention of riboflavin was greatest when stored in pure solution; the retention of ascorbic acid was greatest when stored alone or in mixtures containing riboflavin.

Spectrophotometric color determinations and polarographic measurement of ascorbic acid demonstrated an interaction in the breakdown of ascorbic acid and the color pigments of strawberry juice.

Polarographic analysis appeared to indicate that riboflavin played a role in this interaction; however, this was not confirmed by spectrophotometric studies.

Acknowledgment

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ANTIBIOTICS IN FOOD PROCESSING

Experimental Preservation of Fish and Beef with Antibiotics

H. L. A. TARR, JOHN W. BOYD, and H. M. BISSETT
Pacific Fisheries Experimental Station, Vancouver 2, B. C., Canada

The need for a simple inexpensive method of reducing losses of flesh foods due to bacterial spoilage prompted an investigation of the value of antibiotics. Spoilage of whole eviscerated fish was retarded markedly by ices containing 1 to 4 p.p.m. of chlorotetracycline (Aureomycin hydrochloride), by holding 6 days at −1° C. in sea water containing 2 p.p.m., or by 1-minute immersion in solutions containing 50 or 100 p.p.m. of the antibiotic prior to icing. The effect of different conditions on stability of chlorotetracycline in flesh foods was studied. These treatments have value in protecting fish from bacterial degradation. Owing to presence of interfering substances, it was not possible to detect, by the usual microbiological assay procedure, chlorotetracycline in flesh of fish so iced.

PRESERVATION OF FISH AND MEATS with antibiotics was reviewed recently, and it was shown that chlorotetracycline was more effective in preserving such foods than any of fourteen other antibiotics studied (*5*). Another investigation, conducted at about the same time, led to similar conclusions regarding the relative effectiveness of chlorotetracycline (*1*). The present report is concerned with possible practical methods of application of chlorotetracycline in retarding bacterial spoilage of fish, and its stability in flesh foods in the presence and absence of certain additives and on application of heat. Data concerning the antimicrobiological activity of puromycin and thiolutin in flesh materials are also included.

Materials and Methods

Ices containing chlorotetracycline or chlorotetracycline plus potassium dihydrogen phosphate were prepared by continuous addition of freshly prepared solutions to water which was frozen in a North Star type of flake ice machine. The resulting ices, in which the chlorotetracycline was uniformly distributed, were stored at about −20° C. until required. The fish were iced in 30 × 17 × 17 inch galvanized tanks equipped with drain spouts, four tanks being enclosed in a large box with an approximately 2-inch thickness of glass wool as insulation. The fish were sampled under clean conditions by removing a center steak about 1 inch thick from each fish, and blending a representative 200-gram portion with 600 ml. of water.

Experience in this laboratory has indicated that direct determinations of the total bacterial populations of samples of similar fish stored under comparable conditions are usually a good indication of their general state of preservation as judged organoleptically. For this reason total bacterial counts of the fish have been used arbitrarily throughout the present work as an indication of their comparative keeping quality. In the first experiment with iced fish the skin was removed from the steaks before blending with three volumes of water for a direct bacterial count, and in the second the skin was included. Bacterial counts were made by a direct method (*4*) using aniline methylene blue stain (*3*). Chlorotetracycline was determined by a pad-plate assay procedure (*2*). The pads containing the solutions (usually 0.1 ml.) were dried in vacuo over concentrated sulfuric acid before being placed on the assay agar. In some experiments vegetative cells of *Bacillus cereus* ATCC 10702 were used in the seed layer of agar. However, with this inoculum a response was not obtained with less than about 0.03γ of chlorotetracycline per pad. A similar assay, using spores of *Bacillus mycoides* (Lederle No. PCI 213) instead of vegetative cells, was found to be somewhat more sensitive, exhibition zones being obtained with between 0.005 and 0.01 γ of chlorotetracycline per pad. This assay was used in attempts to demonstrate chlorotetracycline in flesh of whole fish which had been stored in ices containing chlorotetracycline.

For determination of chlorotetracycline in flesh samples a standard curve was obtained by adding the required