COLOR ESTIMATION OF FROZEN STRAWBERRIES BY REFLECTIONMETER, SPECTROPHOTOMETER, AND VISUAL GRADING

by

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A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1951

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Date thesis is presented December 4, 1950.

Typed by Regina Long

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. Oliver J. Worthington, under whose direction and supervision this work was undertaken, for his helpful criticisms, suggestions and guidance throughout the preparation of this thesis.

Appreciation is expressed to Mr. Edmund Wood for providing the samples and to Dr. Earl M. Litwiller for serving on a scoring panel for the strawberry samples.

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CHAPTER I

INTRODUCTION

The appearance of any product, which includes its color and size, is one of the important factors in the selection of food by the consumer. Color is most evident and is frequently indispensable in describing or identifying any food material. The fundamental reason, besides consumer reaction, is the fact that color is often a good index of quality. Use of color, as one of the important factors, in grading of many agricultural and similar products, indicates the importance of this property. Quality standards have been established for processed and fresh foods, which include color as one of the factors.

The United States Department of Agriculture has tentative and permanent standards for grades of many agricultural products. The Food and Drug Administration, in its minimum quality standards, prescribes a specific shade of red for canned tomatoes. Shades other than the prescribed must be graded as substandard. Many other products in which color is important in quality standards include fats and oils, meats, dairy products, poultry,

eggs, honey, cereals, fresh, canned, frozen and dehydrated fruits and vegetables. In most cases till recently, the formulation of standards described color in subjective terms. At present, the trend among the research workers is to develop standards in objective terms which will eliminate errors due to human factors and make them more uniform.

Color is affected by variety, maturity, growing place, and weather during the growing season. Methods of harvesting, storage, handling, transportation and processing, determine the final color of the product. If bright colored raw material is used with proper care in processing, the color can be retained, but if the color is very poor from the very beginning, the final product cannot be improved.

At present, more and more products are being packaged in transparent containers in order to attract the attention of the consumer.

Since color is the first property observed by a consumer as soon as he opens the container, color evaluation with the eye might be sufficient. Persons under good observing conditions can rival the best photoelectric colorimeter in the detection of small differences. But this is not true for all objects, nor for all persons. For these reasons and because even persons with

normal vision do not see color exactly alike, the control or elimination of personal error from color measurement is always important.

Strawberries are most adapted to freezing and are one of the popular frozen fruits liked by the people. The United States Department of Agriculture grades for frozen strawberries assign 40 points out of a total of 100 to color. Moreover, there is a limiting rule: if the frozen strawberries fall in B or D grade for color, regardless of the total score, the entire sample grades as B or D respectively. When the strawberries are sliced, the interior of the berries must also be well colored and hence the problem of getting uniform color is more important in sliced than in whole berries. The same thing is true in the preserve trade where the preserves must be of uniform color.

With the above factors in mind, the color measurement of frozen strawberries was carried out. The instruments used in this study were Photovolt Photoelectric Reflectionmeter, Model 610, manufactured by Photovolt Corporation, New York 16, and Beckmann Spectrophotometer. Both of these instruments have advantages and disadvantages. The reflectionmeter gives results in terms of per cent reflectance which, by means of proper charts, can be converted to either Munsell notations or I. C. I.

system. Thus, the results can be expressed in numerical value of color as seen by the eye. Spectrophotometric measurements are actual measurements in terms of per cent transmittance at a given wave-length. These measurements can be of qualitative or quantitative nature. These two instruments were chosen in order to compare the results with visual grading and also compare the results between the two instruments and find out whether there is any definite relationship between the visual grading, reflectionmeter and spectrophotometer.

CHAPTER II

REVIEW OF LITERATURE

General aspects of color

In the determination of constituents of any material, certain physical properties of matter are taken into consideration. Color is considered as one of these properties of materials. In reality, no material is colored as such, but we are surrounded with objects and phenomena to which we attribute color. In order to understand the object of color, several related aspects of the subject must be considered. Physicists are interested in the properties of stimulus, and the relative amount of reflectance or transmittance at different wavelengths of light. Chemists are interested in the quality and quantity of the pigments and dyes, and colorimetry as a branch of analytical chemistry. The psychologist is chiefly concerned with the sensation of color.

Methods of color measurements for food products

Color of food products is primarily due to the presence of plant pigments and related compounds. These pigments are classified in different groups, the most important being chlorophylls-green to blue-green; carotenoids-yellow to orange; anthocyanins-pink to red;

flavones-colorless to yellow. Other factors which may affect the general perception are the colloidal nature of the particles, shape of the material, pH, and smoothness of surfaces, source of light. There are two methods for measuring color which are distinctly different. One method specifies the color in terms of the three types of visual receptors of the standard observer. The other measures color indirectly by specifying the stimulus in terms of transmittance at each wave-length. Thus, all the methods available for color measurements can be divided into two distinct classes: (1) Psychophysical and (2) Physical.

(1) Psychophysical

Qualitative: A color of a sample can be compared simply by comparison with other samples. Brightness or darkness, and also hue, etc., can be determined but not the degree of color difference. This group includes numerous color systems and charts such as the Ridgway system, Maerz and Paul "Dictionary of Color". Comparison of color with some definite standards qualitatively include the color chart systems. The Maerz and Paul Dictionary of Color includes a large number, 7056 colors. Each color is given an identifying number (10). In setting color standards for agricultural products, the United States Department of Agriculture makes use of

these charts, because they have the widest number of colors now available. The Lovibond tintometer is another instrument, the principle of which is the matching of a color against the light transmitted by one or more of a set of glasses. It has been used for color measurement of the vegetable oil industry but is very recently being replaced (12).

Quantitative: In quantitative methods of color measurement, it is possible to determine the exact degree of differences. In this method, color is specified by means of three color attributes, namely: hue, value, and chroma or synonimous terms. This system is based on tristimulus specifications, i.e., the color is defined in terms of three primaries: red, blue and green. The Munsell color system is based on the above principles and can be converted to the I. C. I. system, which is a quantitative system. Munsell color charts are used in grading of a large variety of agricultural products.

Nickerson (14) used this procedure in relation to agricultural products. The principle use was made in grading of agricultural crops and products such as cotton, alfalfa, clover, Johnson grass and Prairie hay. Later, this method was employed for such products as tomatoes, tomato pulp, canned corn, as well as grains and bread.

MacGillivary (9) has done work on tomato color, special emphasis being applied to the canning industry. Different regions of tomato fruit--skin, pulp--were studied extensively. Bam (1) determined color of canned and frozen beans using the Munsell disc colorimeter, and found some relationship between quality and color. This method involves personal factors, and is strenuous to the eye.

(2) Physical Methods

There are certain physical characteristics of color which are responsible for giving such qualities to colored materials, in order to be distinguished as different colors, regardless of visual characteristics of any observer, and irrespective of the type of illumination and viewing conditions. The study of these physical characteristics which are responsible for these effects can be achieved by means of spectrophotometric analysis.

Spectrophotometers are instruments for determining the proportion of light of known wave-lengths incident upon a body that is transmitted or reflected by it. A spectrophotometer consists of at least a light source for illuminating the sample, a means for measuring the unabsorbed light, a sample holder and a monochromator for isolation of desired spectral band for the illumination.

Most of the early spectrophotometers were of visual type and they are more or less obsolete. At present, there are other types which are coming into prominence, namely, photographic, radiometric and photometric. In the present review, only the photo-electric group will be considered in detail. There are two general varieties of instruments. One variety makes use of monochromatic light by allowing only a very narrow band of light to pass through the slit. The other measures by allowing the light consisting of a much broader band of wave-lengths. The method of measuring the transmittance varies with different instruments. In some of them, there are two light beams of equal intensities from the illuminant. One illuminates the standard or the solvent and the other unknown.

Spectrophotometers are used extensively in the field of analytical chemistry. In color specification work, we probably do not determine what is present, or its amount. It is always convenient to consider the spectrophotometric measurements in terms of qualitative and quantitative uses. Qualitative application depends upon an object having a definite form of spectral curve, in particular regions. Any conclusion from the curves can be based on these characteristics. Quantitative uses of spectrometry depend upon the fact that the magnitude of

absorptance is a function of the concentration of the absorber. It is customary to make the measurement at the wave-length of the peak of the absorption band, although the point is not always the best.

Even though spectrophotometers are very accurate instruments and have many advantages, they have many drawbacks. If the optical system does not have the same relation to the sample as the eye, the result will not correspond to the light which enters the eye from the sample when it is viewed directly. Two colors having identical spectrophotometric curves may be assumed to be the same under the same condition of illumination, but the reverse is not true. The spectrophotometer is a very delicate instrument and is rather expensive and complicated. It may be very good for fundamental physico-chemical studies, but it is not essential for routine work for measuring color. Operation of the instrument and interpretation of data is also rather difficult. In specifying a single color, it involves measurements of all wave-lengths.

Several workers have made use of the spectrophotometer to study the appearance of several food products. A few instances may be mentioned. Foschback and Newburger (5) studied canned peas, prepared by various processes, to determine the most desirable shade

of green. They reported their results in terms of reflection curves. The possibility of using the spectrophotometer for measurement of color in canned foods was studied by Kramer and Smith (7). The use of suitable solvents for extraction of desired pigments is necessary. The extracted pigment, if not free from turbidity, interferes with the measurement of transmittance and, to compensate for it, an extra step is involved. The results are expressed in terms of per cent transmittance. These results are correlated with color as judged by organoleptic methods. Tentative procedures for tomatoes, snap beans, lima beans, beets, carrots and corn have been described.

McCollum (11) reports curves obtained on samples of tomato juice varying in color. He states that the spectral reflectance curves do not show marked differences between various grades of tomato juice. Extraction of total lycopene pigment gave appreciable difference in the amount of the pigment.

Kramer and Guyer (6) describe a rapid objective method for measuring color of raw and canned tomatoes. Only five grams of sample is necessary. The transmittance is measured at 485 mu. Their results indicate that a relatively simple and inexpensive instrument such as the Lumetron colorimeter may be used in the routine work. Sample procedure is also described, which is important in

order to grade effectively.

Davis (4) used a photoelectric colorimeter for measuring the color index of tomato paste extract which could be used in grading the tomato paste samples. The work was done on laboratory and commercial samples. According to Davis, the color index method is not significantly less accurate than the existing methods and is quick and conveniently applied.

Kramer and Smith (8) have made quantitative measurements of the extractable green pigment from peaches and apricots by using both the spectrophotometer and the fluorometer. Chlorophyll was extracted with acetone and transmittance measured at 665 mu. Transmittance measurements provide a method of measuring extractable pigment in the presence of other pigments. The results are correlated with ripeness and color determined organoleptically.

Sondheimer and Kertsz (15) determined the amount of red anthocyanin quantitatively and qualitatively in strawberries and strawberry products by using the spectrophotometer. The pigment was extracted by means of Sorenson's citrate-hydrochloric acid buffer at a pH 3.4. The transmittance at 500 mu. was determined for this extract at a pH of 3.4 and pH 2.00 at known concentration. They reported that increase in intensity under

such concentration was proportional to the concentration of anthocyanin pigment in the extracted solution. Use of Congo Red dye as a standard was suggested by them. They also indicated that further work in this field should be done by making use of cheaper and simply operated instruments for routine work.

In order to study the color in strawberries, something about the nature of the pigments in the fruit should be known. Robinson (2) reported that the pigment he has found in the strawberries is the anthocyanidin monoglucoside and suggested the sugar present may be galactose. Nair and Robinson (13) found pelargonidin 3-galactoside in wild strawberries (Fragaria vesca). Sondheimer and Kertesz (15) recently reported that they believed the red color in strawberries due to the presence of pelargonidin 3-monoglucoside. There might be present two isomers of pelargonidin. Anthocyanin pigment, according to Onslow (2), is a class of pigments to which practically all of the blue, purple and red color flowers, fruits and leaves belong. The pigment is found chiefly in the cell-sap, though may be present in other plant organs. According to Willstatter and his collaborators (2), these pigments belong to a group of glucosides, the sugar free pigment or aglucone of which are called anthocyanidins. Molisch (2) reported the discovery that

anthocyanins produce well crystallizing products in solution when treated with acids. Subsequently, it was shown the anthocyanins are amphoteric substances which build oxonium salts with acids. A color change towards violet and blue with ferric chloride is shown by aqueous and alcoholic solutions of anthocyanins, which possess two neighboring phenolic hydroxyl groups. Anthocyanin pigments are very sensitive to pH change. Organic compounds in some cases act as co-pigments, which modify the colors. Contact with metals such as iron or tin shifts the color from red to blue. Temperature and light also cause change in color. The anthocyanins are soluble in alcohol and water, but are insoluble in non-hydroxylic solvents such as ether, chloroform and benzene.

CHAPTER III

EXPERIMENTAL PROCEDURES

Description of the samples: During the 1949 berry season, which lasted from May 15 to July 15, samples of Marshall strawberries were collected from different freezing plants located all over the state of Oregon. This is one of the best varieties adapted for freezing and is most popular and widely grown in the Pacific Northwest. The samples were collected from the sorting belts just before the final packing. For the present investigationn berries were packed in one-pound waxed containers and frozen without the addition of sugar in order to measure color in a nearly natural state. A few samples were collected from the berries which were rejected for packing because of poor color. All the samples were frozen and stored at 0°F until ready for examination.

Preparation of the sample: The samples were removed from the 0°F room and kept overnight at 32°F. After 15 to 20 hours, the samples were allowed to thaw out at room temperature. Other methods of thawing at higher temperatures were tried but the thawed product became rather soft and mushy, while when thawed at lower temperatures, berries were very firm and the product much more satisfactory. Thawing at low temperature was adopted for the

present investigation.

After thawing, sample numbers 28 to 49 were graded for color by using the United States Department of Agriculture standards formulated for frozen whole strawberries. The grading was done by four persons and the average results are presented in Table 1. The optimum color density was given 10 points. There were some samples which had a darker color than the best color and hence more than 10 points were given to these samples. The grade of A was given to those samples scoring between 8.25 and 10 points, B grade to samples scoring 7.0 to 8.25, and D grade to samples getting less than 7.0 points.

After the samples were visually graded, a puree of 150 grams of the sample was prepared with the help of the puree machine. It runs at a speed of 5000 r.p.m. and operates on the same principle as a centrifuge, whereby seeds are entirely removed and the puree so obtained is uniform and consistent in character. It was observed during the investigation that when the samples of the same weight were taken, the final weight of the puree remained practically the same in all cases. Purees were also prepared by using a Waring blender and copper screen. The Waring blender incorporates much air and seeds are also present, which gives varied results. The copper screen changes the color of strawberries due to the

contact with copper metal for a long time. Thus, puree prepared with the puree machine was the most satisfactory for the present investigation.

Measurement of color by tristimulus reflectionmeter

A brass cylinder four inches in height and two and three-quarters inches in diameter, as suggested by Dalal (3), was used. It had a transparent removable base of plastic, such as leucite or any other similar and suitable material (it should be optically clear and free from disturbing colors and thick enough to give mechanical strength). The same weight of different samples was used, such that no light passed through the puree when the cylinder was placed on the search unit of the reflectionmeter during the color measurement. The readings were taken by using tristimulus amber, green and blue filters. It was observed, during the preliminary investigation, that some light is absorbed or reflected back due to the two reflecting surfaces of the glass or leucite, and hence it was found necessary to apply certain corrections in order to get accurate results. The correction factor was calculated as follows and applied to all the results, presented in Table 2.

First of all, the tristimulus amber filter was introduced in the search unit. A leucite disk was placed

on a white enamel standardized plaque. The per cent reflectances of the white enamel plaque, as compared to magnesium oxide for the three filters, were recorded on the back of the plaque. The search unit was placed on the leucite disk which was on the plaque. Then the galvanometer was adjusted to the particular value mentioned for the amber filter. The search unit was lifted and the leucite disk removed, then the search unit was placed directly on the enamel plaque and reflectance was noted directly on the galvanometer. This process was repeated for the other two filters, green and blue. These new reflectances, instead of the one mentioned at the back of the plaque, were used in measurement of color of the strawberry puree. There was a reduction of per cent reflectance by the leucite disk due to the reflection at the surfaces, and also absorption due to the thickness of the disk. A thinner leucite disk was tried and because of the thinness of the disk, higher reflectance readings were obtained in comparison to the readings taken when a thick leucite disk was used. For these reasons, it was found necessary to apply corrections in order to get comparative results.

It was observed that in the case of strawberry puree, a smaller sample weighing from 75 to 100 grams was sufficient in comparison to other products like

grapefruit juice and apricot nectar. This is due to the fact that strawberry puree is denser optically, containing more solids. Dalal (3) observed a slight difference in reflectances on stirring apricot nectar samples, due to the suspended nature of the product. In the case of strawberry puree, no difference in per cent reflectance of stirred and unstirred samples was observed. This may be due to the homogenous nature and uniform consistency of the puree.

Table 1

Visual Grading of Frozen Whole Strawberries

Sample No.	Color	Final Grade
28	7.0	B
29	8.5	A
30	6.75	D
32	7.5	B
33	8.75	A
34	4.25	D
35	8.5	A
36	11.0	D
37	7.5	B
38	11.5	D
39	7.5	B
40	6.5	D
41	11.5	D
42	6.5	D
43	8.5	A
44	8.5	A
45	8.5	A
46	5.0	D
47	10.5	D
48	6.75	D
49	9.5	A

Table 2

%A, %G, and %B Reflectance of 49 Samples by Reflectionmeter

No.	%A	%G	%B	No.	%A	%G	%B
1	12.93	6.33	2.53	26	12.60	6.33	2.30
2	14.00	8.07	2.23	27	13.50	7.90	3.60
3	12.53	6.57	2.70	28	12.20	6.07	2.80
4	11.50	5.77	2.70	29	11.10	5.00	2.50
5	11.87	5.73	2.23	30	11.00	6.10	3.70
6	11.00	5.50	2.27	31	11.00	6.00	3.50
7	11.77	5.50	2.20	32	11.00	5.40	2.40
8	12.40	5.93	2.20	33	10.70	5.00	2.20
9	13.87	7.00	3.40	34	15.53	9.07	5.70
10	12.57	6.13	2.90	35	11.67	5.73	2.50
11	12.67	6.33	2.60	36	12.00	6.10	2.80
12	15.73	8.13	3.13	37	11.50	5.90	2.60
13	12.00	5.90	2.80	38	9.60	4.80	2.00
14	11.20	5.70	2.80	39	9.50	4.70	2.00
15	12.00	6.00	2.77	40	10.50	5.80	2.80
16	11.73	6.20	3.23	41	10.20	4.90	2.30
17	11.17	5.77	2.67	42	14.00	7.20	3.00
18	14.17	7.30	3.27	43	12.40	6.30	2.90
19	11.10	5.57	2.40	44	12.80	6.40	2.90
20	12.90	6.10	2.50	45	13.67	6.90	3.60
21	12.20	6.37	2.80	46	16.20	9.00	4.50
22	12.00	6.40	2.80	47	19.87	4.87	2.50
23	12.00	6.03	2.90	48	16.80	8.50	3.80
24	15.20	7.87	3.83	49	11.60	5.70	2.40
25	11.00	5.40	2.50				

Measurement of color by spectrophotometer

Sondheimer and Kertesz (16) have described a method of color measurement of strawberries by means of the spectrophotometer. In the present investigation, the same procedure was followed with slight modifications.

Preparation of the material: Ten grams of the strawberry puree was weighed and 50 ml. of Sorensen's Citrate-hydrochloric acid buffer of pH 3.4 was added. Then the mixture was shaken and a pinch (about 25 mg.) of Pectinol 10M (obtained from Rohn & Hass Co., Philadelphia, Pa.) was added to it. The addition of pectinol was found to be necessary in order to obtain optically clear solution. The mixture was allowed to stay for 30 minutes, and then it was centrifuged and filtered through Whatmann No. 2 filter paper. This was solution A, whose dilution factor was noted. The pH of solution A was measured and kept constant within 3.4 $\pm$ 0.05. Twenty-five ml. of solution A was taken and 25 ml. of buffer solution was added in order to get the optical density within the optimum range of the instrument. The dilution factor of solution B was noted. The pH of solution B should be 3.40 $\pm$ 0.05. All the pH measurements were made with a Beckmann pH meter with glass electrodes.

Forty-five ml. of 0.035N hydrochloric acid was

added to 25 ml. of solution B in order to produce a final pH of 2.00 ± 0.05, and the dilution was such that the readings at 500 mu. were in the most sensitive range. The solution C was allowed to stand for at least one hour before the readings were taken in order to allow full color development. The dilution factor of solution C was noted.

Measurement of optical density: The per cent transmission was measured at 500 mu. for all the samples and expressed as optical density, log Io/I for solution B and C. If the dilution factor of B and C are not equal, the optical density readings should be equalized with respect to dilution.

Preparation of color standard: Congo Red Dye (Congo Red Special, obtained from the National Aniline Division, Allied Chemical and Dye Corporation) dissolved in 0.01N sodium carbonate to give 20 mg. per cent solution was used as a standard. The calibration curve was obtained by diluting this solution with 0.01N sodium carbonate and plotting the optical density against concentration measured at 500 mu. The curve is presented in Diagram 1.

Calculations: After equalizing the optical density readings, optical density of a solution at a pH of 3.4 was

subtracted from the optical density at a pH of 2.00. The net reading or the difference can be converted to milligrams per cent of Congo Red equivalents by using the calibration curve. This mg. per cent should be multiplied by the dilution factor in order to obtain Congo Red equivalent of mg. per cent per 100 grams of the sample. According to Sondheimer and Kertesz, a solution containing 0.825 mg. of Congo Red Special in 0.01N sodium carbonate equals the increase in absorption which occurs when the pH of a purified strawberry anthocyanin chloride solution containing 1.0 mg. per cent is changed from a pH of 3.40 to 2.00. Thus, the Congo Red values multiplied by 1.20 will give the anthocyanin equivalent of the observed absorption. The results are presented in Table 3.

In the present study, only a sample weighing 10 grams was taken. On taking samples of 20 grams and using the same procedure, no appreciable difference was found in the Congo Red equivalent values.

Different congo red dyes have different calibration curves. Sondheimer and Kertesz used special congo red dyes while, in the present case, special congo red dye and ordinary congo red dye were used in order to compare the optical densities of the two dyes having the same concentration at 500 mu. It can be seen from

the diagram (1) and Table 4 that there is an appreciable difference between the two dyes. If congo red equivalent is to be converted to anthocyanin chloride, a factor for conversion has to be calculated.

Alcohol and other solvents were tried for the extraction of the pigment and different results were obtained with each solvent. Slight change in pH or change in time of the contact had a considerable effect on the optical density of the product. It was observed that there was no appreciable change on light absorption if the extractions were stored for 24 hours at 0°F.

Table 3

Anthocyanin Concentration in Strawberries

Samp. No.	Optical Density At pH 3.40	Optical Density At pH 2.00	Optical Density Difference	Dilution Factor	Congo Red Observed, mg. %	Equi. Original sample, mg. %	Anthocyanin chlor. mg. %
1	0.190	0.728	0.538	11.0	1.15	12.65	15.18
2	0.163	0.554	0.391	11.0	0.83	9.13	10.95
3	0.238	0.792	0.554	11.0	1.18	22.11	26.53
4	0.235	1.288	1.053	11.0	2.18	23.98	28.77
5	0.230	1.045	0.815	11.6	1.77	20.53	24.63
6	0.235	0.998	0.763	11.6	1.63	18.91	22.69
7	0.230	0.943	0.713	11.6	1.53	17.75	21.30
8	0.230	0.850	0.620	11.6	1.32	15.41	18.37
9	0.297	1.099	0.802	11.6	1.72	19.95	23.94
10	0.310	1.233	0.913	11.0	1.97	21.67	26.00
11	0.310	1.058	0.784	11.0	1.68	18.48	22.17
12	0.245	0.854	0.609	12.0	1.30	15.60	18.72
14	0.460	1.587	1.127	11.0	2.43	26.73	32.07
16	0.290	0.910	0.620	11.0	1.32	14.52	17.42
17	0.398	1.400	1.002	11.0	2.16	23.76	28.51
18	0.200	0.658	0.458	11.0	0.99	10.39	13.07
19	0.428	1.554	1.126	11.0	2.43	26.73	32.07
22	0.172	0.666	0.494	11.0	1.07	11.77	14.12
23	0.300	1.029	0.729	11.6	1.57	18.21	21.85
25	0.376	1.344	0.968	12.0	2.10	25.20	30.24
28	0.270	0.837	0.567	12.0	1.22	14.64	17.57
29	0.375	1.250	0.875	12.0	1.87	22.44	26.93
30	0.550	1.720	1.170	12.0	2.50	30.00	36.00
32	0.280	0.910	0.630	12.0	1.35	16.20	19.44
33	0.470	1.470	1.000	12.0	2.16	25.92	31.10
34	0.167	0.440	0.273	12.0	0.58	6.96	8.35
35	0.347	1.182	0.835	12.0	1.80	21.60	25.92
36	0.385	1.478	1.093	12.0	2.35	28.20	33.84
37	0.230	1.288	1.058	12.0	2.30	27.60	33.12
39	0.280	1.069	0.789	12.0	1.68	20.16	24.19
40	0.177	0.658	0.481	12.0	1.03	12.36	14.83
42	0.200	0.644	0.444	12.0	0.95	11.40	13.68
43	0.383	1.380	0.997	12.0	2.15	25.80	30.96
44	0.323	1.251	0.928	12.0	2.00	24.00	28.80
45	0.297	1.078	0.781	12.0	1.66	19.92	23.90
46	0.112	0.356	0.244	12.0	0.51	6.12	7.34
47	0.410	1.386	0.976	12.0	2.10	25.20	30.24
48	0.224	0.756	0.532	12.0	1.13	13.56	16.27
49	0.400	1.330	0.920	12.0	1.97	23.64	28.37

Table 4

Optical Density of Two Different Congo Red Dyes at Different Concentration by Beckmann Spectrophotometer

Concentration	Optical Density	
mg. %	Congo Red Special	Congo Red Ordinary
0.25	0.120	0.095
0.50	0.235	0.190
0.75	0.342	0.275
1.00	0.476	0.360
1.25	0.584	
1.50	0.690	
1.75	0.820	
2.00		0.710

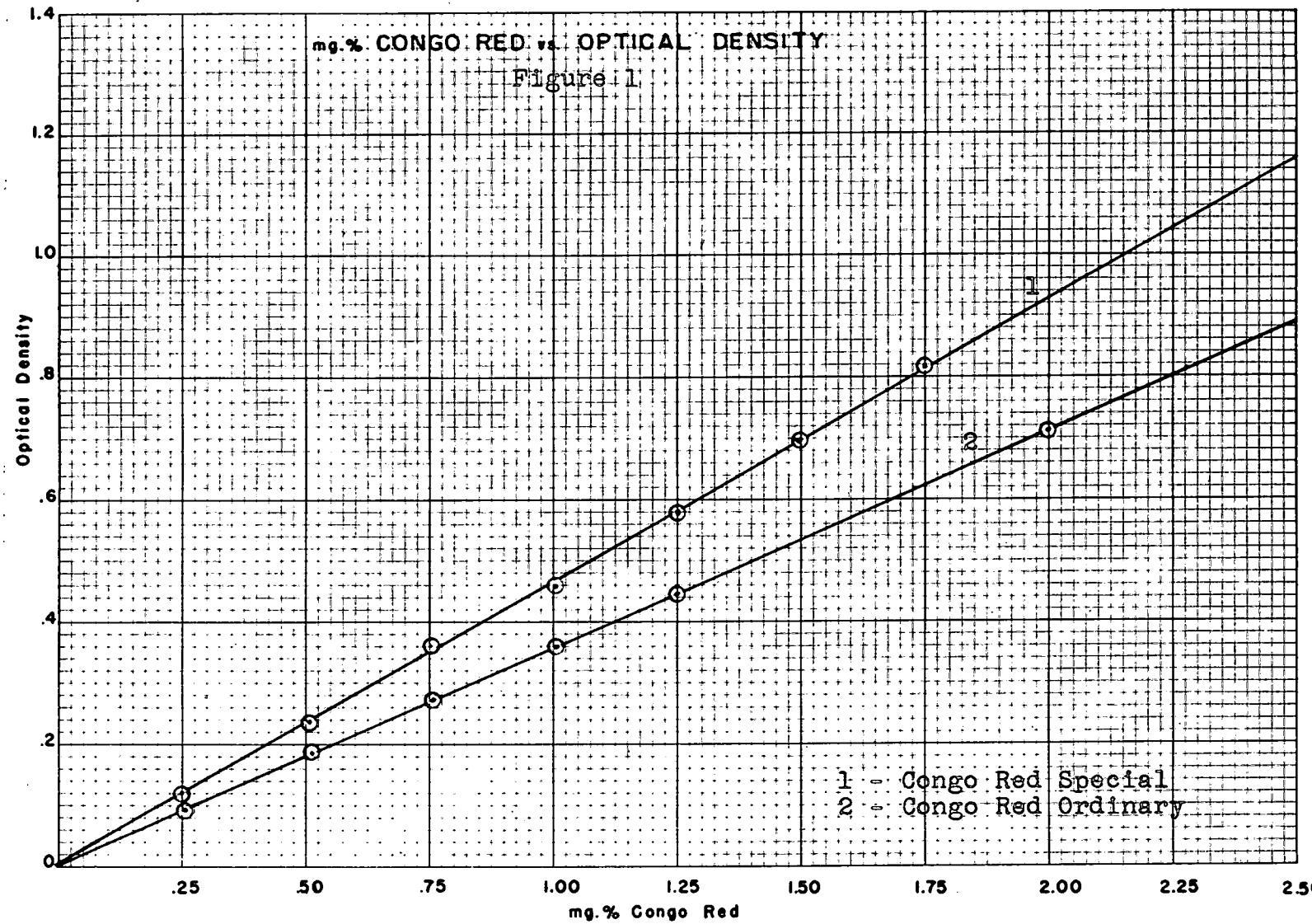

mg.% CONGO RED vs. OPTICAL DENSITY
Optical Density
1.4
1.2
1.0
.8
.6
.4
.2
0
.25
.50
.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
mg.% Congo Red
1
2
1 - Congo Red Special
2 - Congo Red Ordinary

Figure 1

CHAPTER IV

DISCUSSION OF RESULTS

From all the data in the preceding chapter collected so far, it was noticed that there might be a relationship between different methods of color measurement. The data obtained were put to statistical analysis and some light was thrown on the relationship between different factors.

Relationship between Per Cent A and Per Cent G reflectance

The results obtained by the measurement of color by reflectionmeter were subjected to statistical analysis in order to find any relationship between per cent A and per cent G reflectance. In the present case, linear regression was used.

A scatter diagram was drawn in order to find an approximate distribution of 49 samples of strawberries examined. Figure 2 indicates that there might be a linear relationship between per cent A and per cent G reflectance. The per cent A on the horizontal axis represents per cent A reflectance with tri-stimulus amber filter and per cent G on the vertical axis represents per cent G reflectance with tri-stimulus green filter.

The next step is to find out the values of

regression coefficient, b, and a, the ordinate of the point where the line crosses the Y axis (X = 0). The value of b is 0.6008 and a is -1.115. The estimated line of regression is

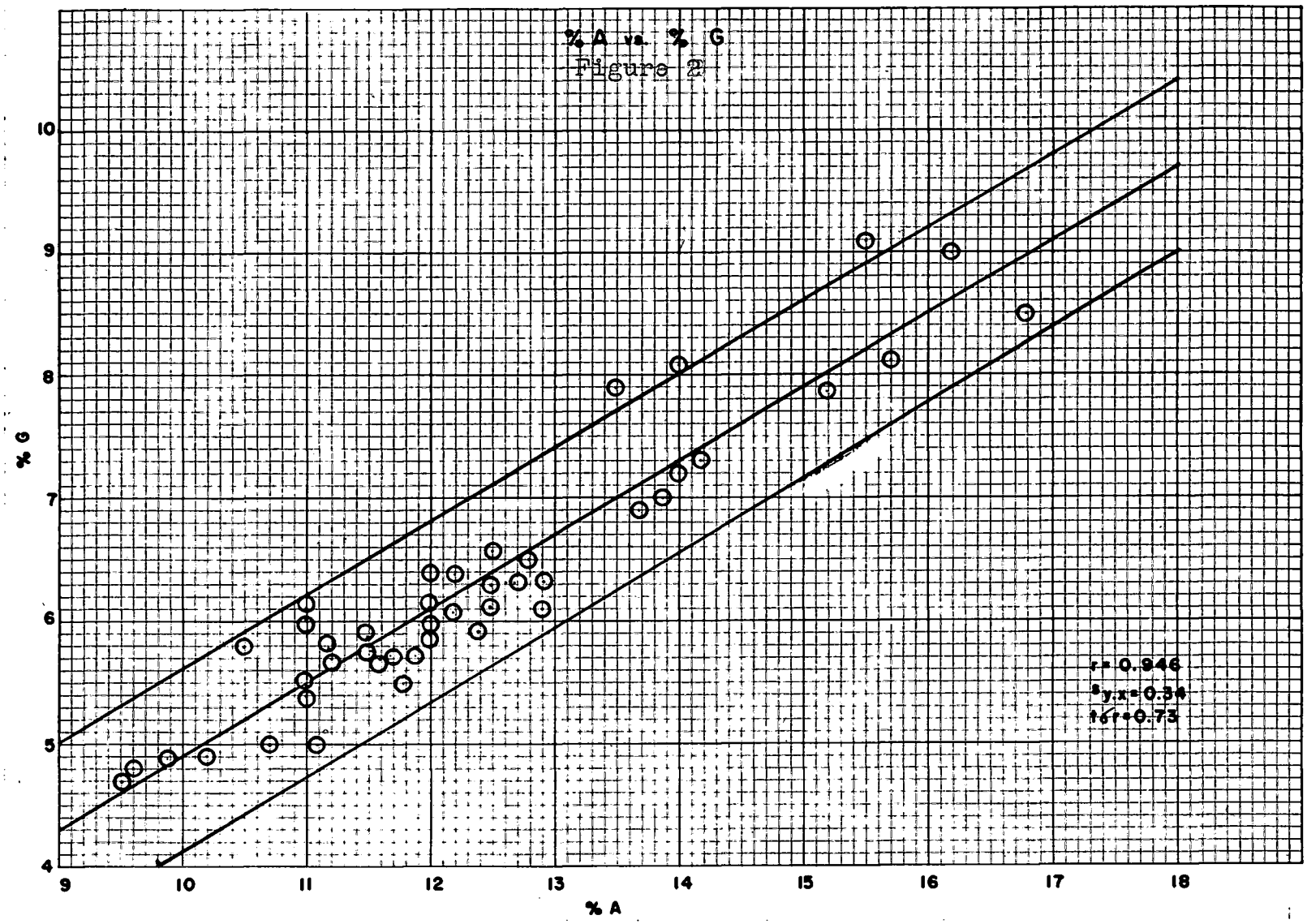
$$Y_X = b.x + a$$

$$= 0.6008(\%A) + (-1.115)$$

where Y_X is the sample estimate of the population means of the array of y. The regression coefficient, b, is an unbiased estimate of the population regression coefficient B. It represents the slope of the line of regression and, consequently, measures the rate of change of the mean of an array of y with respect to x. The line of regression is drawn bwtween the x values of 9 and 18. The regression line is a kind of moving average, passing among the points that determine it. It is a better estimate than the average value. Deviations from the regression are depicted clearly by vertical distances from the line to the observation points, positive deviations above the line and negative below. These deviations measure the failure of the sample to confirm to the estimate trend. The standard error of the estimate, $s_{y \cdot x}$, is a good index of deviation. If all the points are on the line of regression, $s_{y.x}$ is equal to zero. The value of $s_{y.x}$ is 0.343, which shows that the points are close to the estimated line of regression.

The linear relationship is confirmed by the calculation of correlation coefficient, r. The value of r may range from -1 to +1. When all the points fall on a line, the values of r equals -1 or + 1. If the points deviate a little from the line, the values of r also change depending upon the amount of deviation. The value of r in this case is 0.946, which shows a very high degree of linear correlationship. It can be easily seen from the above relationship that either tri-stimulus amber or tri-stimulus green filter can be used for measuring color of strawberries if the results are not to be expressed in Munsell notations, with a fair degree of accuracy.

Two lines were drawn parallel to the line of regression, these confidence limits giving us the limits between which we have a 95 per cent chance of being correct in our prediction. For example, we would like to know what would be the corresponding value of per cent G for 12 per cent A reflectance. We can look up the diagram and can say that the value of per cent G will lie between the limits of 6.10 $\pm$ 0.728, i.e., it will range from 5.37 to 6.83, for these 49 samples.


% A vs. % G
Figure 2
% G
% A
r = 0.946
$s_{y.x}$ = 0.34
$t_{6}r$ = 0.73

Relationship between spectrophotometric and reflectometric measurements

On comparing the results obtained by both the instruments presented in Table 5, it can be observed that there might be some relationship between the per cent anthocyanin chloride and per cent A or per cent G reflectance. Linear regression was used to find out any possible relation. All the calculations were performed as mentioned previously and the final results are tabulated in the following Table 5:

Table 5

Statistical Relationship between Spectrophotometric and Reflectometric Measurements

Measurement	r	$s_{y.x}$
% Anthocyanin vs. % G reflectance	-0.653	0.820
% Anthocyanin vs. % A reflectance	-0.590	1.00

It can be seen from the value of r that a higher linear correlationship exists between per cent anthocyanin and per cent G reflectance. The estimated standard error of the arrays, $s_{y.x}$, is 0.820 which is comparatively low.

A distribution between per cent anthocyanin and

per cent G reflectance is presented in Figure 3, 39 samples being represented in the diagram. The estimated line of regression is drawn with x values of five and 40. The minimum significant correlation coefficient at five per cent level of significance for 37 degrees of freedom according to Fisher's table is 0.317. In the present case, the value of r for 37 degrees of freedom is 0.653. This value is significantly high. This shows that the linear correlationship is not merely due to chance. In order to substitute one method of analysis by another, the value of r must be very high to justify the replacement. In this case, even though we have a fairly high value of r, it is not high enough to justify our replacement of one method by the other. Perhaps more data or certain refinements or limitations would improve the correlation. It will be noted that per cent A reflectance gives a lower correlation coefficient than per cent G. The reason for this is not clear.

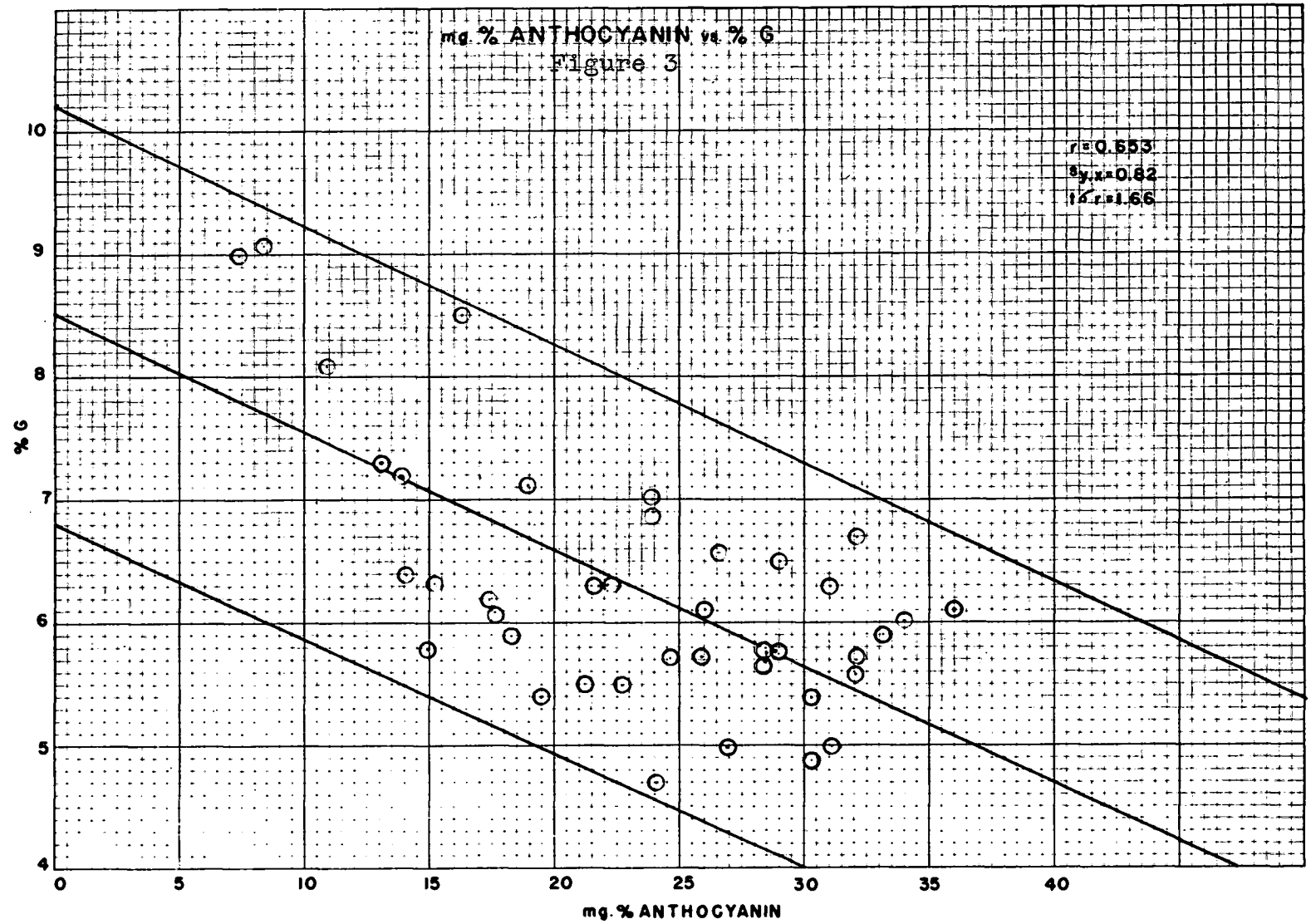

mg.% ANTHOCYANIN vs. % G
r = 0.553
$s_{y.x}$ = 0.82
$1\sigma_r$ = 1.66
% G
mg.% ANTHOCYANIN
10
9
8
7
6
5
4
0
5
10
15
20
25
30
35
40

Figure 3

Table 6

Comparison of Spectrophotometric and Reflectometric Results

Sample No.	% Anthocyanin chloride	Reflectance	
		% A	% G
1	15.18	12.93	6.33
2	10.95	14.00	8.07
3	26.53	12.53	6.57
4	28.77	11.50	5.77
5	24.63	11.87	5.73
6	22.69	11.00	5.50
7	21.30	11.77	5.50
8	18.37	12.40	5.90
9	23.94	13.87	7.00
10	26.00	14.47	6.13
11	22.17	12.67	6.33
12	18.72	15.73	8.13
14	32.07	11.20	5.70
16	17.42	11.73	6.20
17	28.51	11.17	5.77
18	13.07	14.17	7.30
19	32.07	11.10	5.57
22	14.12	12.00	6.40
23	21.85	12.00	6.03
25	30.24	11.00	5.40
28	17.57	12.20	6.07
29	26.93	11.10	5.00
30	36.00	11.00	6.10
32	19.44	11.00	5.40
33	30.10	10.70	5.00
34	8.35	15.53	9.07
35	25.92	11.67	5.73
36	33.84	12.00	6.10
37	33.12	11.50	5.90
39	24.19	9.50	4.70
40	14.83	10.50	5.80
42	13.68	14.00	7.20
43	30.96	12.50	6.30
44	28.80	12.80	6.50
45	23.90	13.67	6.90
46	7.34	16.20	9.00
47	30.24	9.87	4.87
48	16.27	16.80	8.50
49	28.37	11.60	5.70

Relationship between visual color grades and per cent reflectances

After finding that tri-stimulus amber and green filters are able to detect differences between samples of strawberry puree, it was decided to find the relationship between per cent A or per cent G reflectance and visual color grade; also, to show how reliably visual color grading can be predicted. Seventeen samples were graded visually by four persons and then the color was measured with the reflectionmeter using tri-stimulus amber and green filters. It was decided to use linear regression to find out the linear correlationship between visual color grades and per cent A or per cent G reflectance. The following table gives the statistical value for 17 graded samples:

Table 7

Measurement	r	$s_{y.x}$
% A	-0.793	1.26
% G	-0.833	1.14

It can be seen from Table 7 that a better correlation exists between per cent G and visual color grades. This is indicated by the correlation coefficient, r, being higher and standard error of the estimate of an

array, $s_{y \cdot x}$, being lower.

The scatter diagram, Figure 4, shows the approximate distribution between per cent G reflectance and visual grades. A regression line is drawn with x values of 9.5 and 17.

The minimum significant correlation coefficient at the five per cent level of significance for 15 degrees of freedom from the Fisher's table is 0.482. In the present case, the value of r is 0.833. This is highly significant. Human factors and other environmental factors are involved in subjective grading of any biological material and, hence, it is not possible to get a perfect correlation. In the diagram, samples having D grade for color are distributed in two areas. One area at the bottom of the diagram has five samples having #s 30, 34, 42, 46 and 48. Similarly, on the upper left hand there are three samples graded as D bearing #s 38, 41 and 47, while most of A and B grades are in the central part of the graph. There is a slight overlapping of grades. This may be due to the difficulty of grading by visual means. The per cent G reflectance for A and B grade samples ranges from 5.0 to 6.5, while for D grade it is from less than five on one side and more than 6.5 on the other side of the diagram. Samples having color darker than the best have a lower reflectance, while those

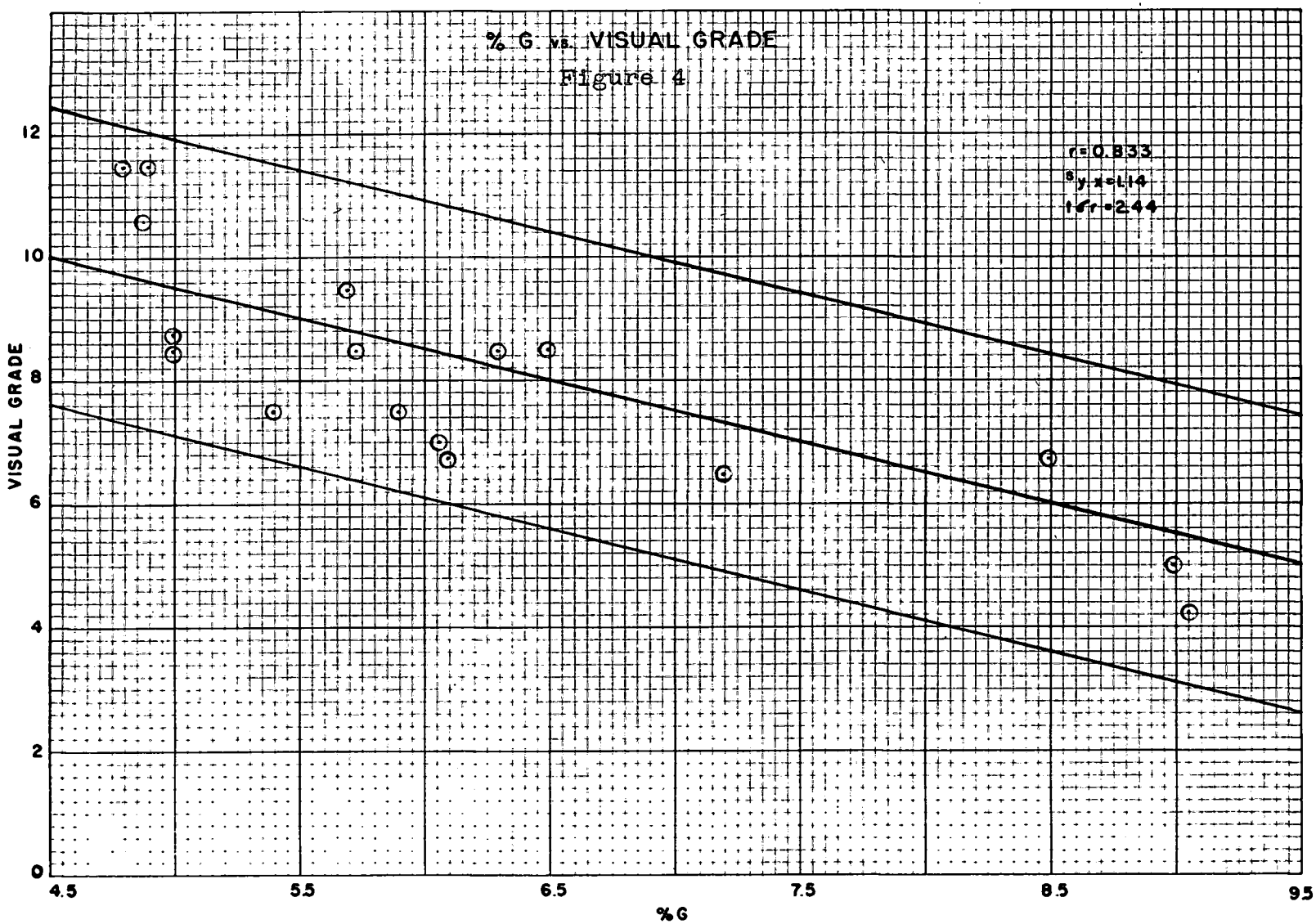

% G. vs. VISUAL GRADE
r = 0.833
$s_{y.x}$ = 1.14
$t_{\sigma r}$ = 2.44
VISUAL GRADE
0
2
4
6
8
10
12
4.5
5.5
6.5
7.5
8.5
9.5
%G

Figure 4

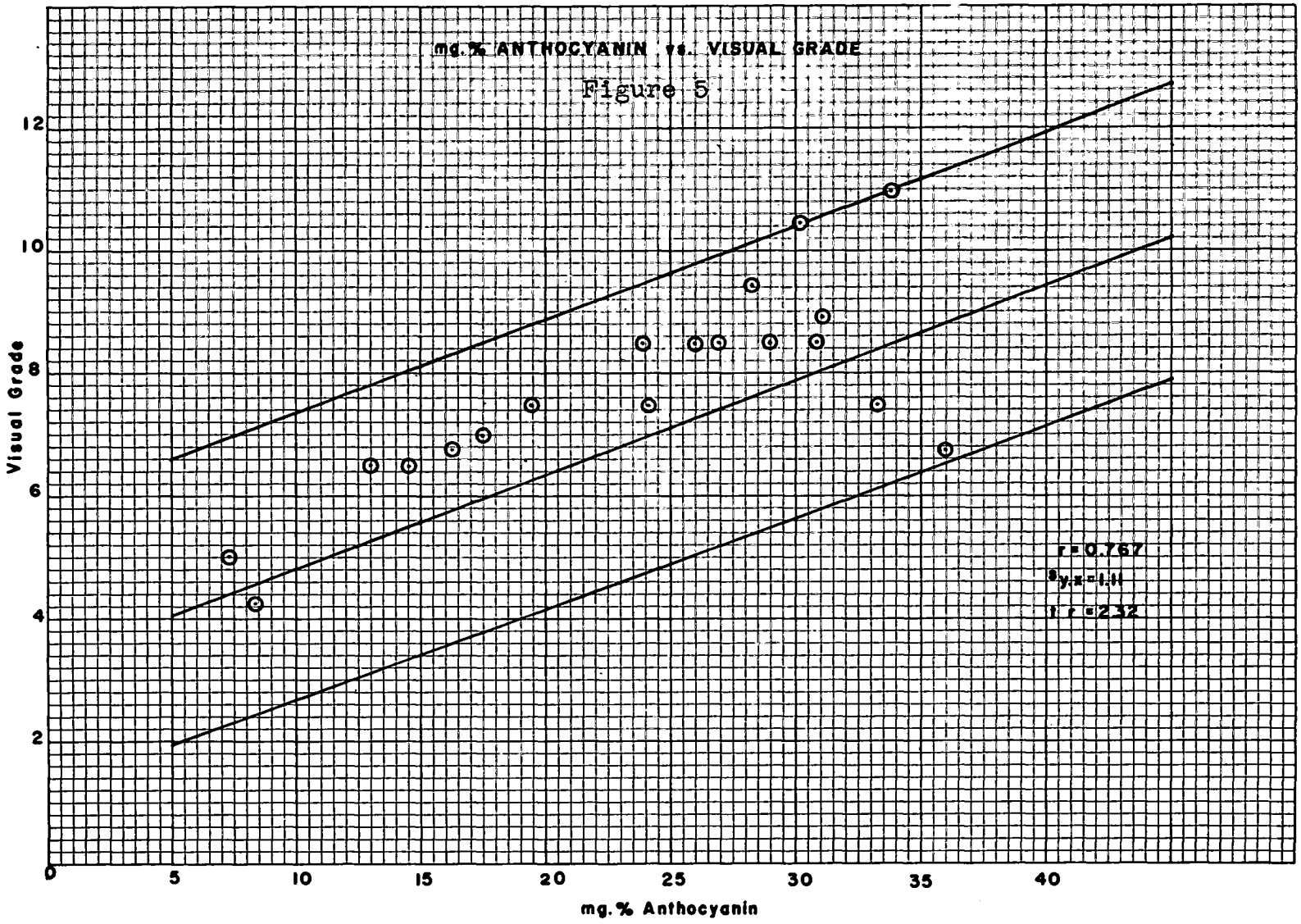
lighter in color have a high per cent G reflectance with tri-stimulus green filter and hence have a reflectance higher than 7.00 per cent.

Relationship between per cent anthocyanin chloride and visual color grade

A scatter diagram in Figure 5 shows the distribution between per cent anthocyanin chloride and visual color grade. Different statistical values were calculated to find the linear correlationship between per cent anthocyanin and visual color grades. The value of r is 0.767, and the value of $s_{y \cdot x}$ is 1.11. The grading of the samples visually was done only from the outside surface, while in measuring the anthocyanin content of the fruit, the character of all the parts, outside and inside, were included by blending. This may be responsible for a little lower value of r and a higher value of $s_{y \cdot x}$. Better correlationship may be obtained by visual grading the puree instead of the whole fruit. In order to replace a subjective method by an objective method, the value of r in the present case seems to be high enough.

This was a preliminary experiment with only 19 samples, only one variety of strawberries being examined. In order to establish standards of grades of a product, a much larger sampling should be used. The most important

factor is to decide whether anthocyanin pigment is a good index of quality of strawberries. There was a wide variation between samples in the amount of anthocyanin pigment in the present study, although all of the samples were of the same variety. The values range from 7.34 to 34 mg. per cent. The berry samples which were light in color had a lower anthocyanin content, while those having a bright red color had high pigment content. There were some samples which were sun scorched and had an off color in that they were darker. They were not acceptable because of their bitter taste. The amount of anthocyanin present in these samples was much higher than in the best samples. Therefore, there is an optimum concentration of anthocyanin which is less than maximum concentration found.


mg.% ANTHOCYANIN vs. VISUAL GRADE
Figure 5
Visual Grade
mg.% Anthocyanin
0
2
4
6
8
10
12
5
10
15
20
25
30
35
40
r = 0.767
$s_{y.x}$ = 1.11
t r = 2.32

CHAPTER V

SUMMARY AND CONCLUSION

Until recently, the color of strawberries has been generally estimated by subjective means such as visual grading by experienced inspectors. But this method is not very accurate and results differ between individuals. Thus, need for a rapid, inexpensive and accurate method is felt in the industry, because in the grading of strawberries, 40 points out of a total of 100 are assigned to color in the most widely used scoring system, namely Production and Marketing Administration grades.

The Photovolt Photoelectric Reflectionmeter, Model 610, and the Beckmann Spectrophotometer, Model DU Quartz, with one cm. cells were used in the present study in order to compare methods which could be used in estimating color of strawberries. This reflectionmeter is a comparatively cheap, easily operated, fairly accurate instrument which can be used for field work as well as the plant, whereas the spectrophotometer is an expensive and accurate instrument and needs a trained person to operate it successfully. The results of the former instrument can be expressed in numerical value of color as seen by the eye, while the latter instrument measures

color qualitatively and quantitatively in terms of optical density or per cent transmittance. The spectrophotometric method was that of Sondheimer and Kertesz (16).

Forty-nine samples of Marshall strawberries without added sugar or syrup were studied. These samples were graded by four experts according to P.M.A. standards, and their averages ranged from substandard to A grade. Results obtained by the two instruments and visual grading indicated that there might be a relationship between these different methods of color measurement or evaluation. Linear regression and correlation coefficient were applied to find out the relationship statistically.

1. In the case of per cent A and per cent G reflectance, the value of correlation coefficient, r, was 0.96, and estimated standard error, $s_{y \cdot x}$, was 0.343. This shows a high degree of correlation between tri-stimulus amber and tri-stimulus green filters when used with the above reflectionmeter. In the case of measuring color with the instrument, either green or amber filter could be used with reasonable accuracy if the results are not being expressed in the Munsell system.

2. It was found that a better correlationship existed between concentration expressed as mg. per cent anthocyanin and per cent G reflectance ($r=-0.65$) than

between the concentration and the per cent A reflectance (r_s=-0.59).

3. Reflectionmeter readings for the tri-stimulus amber and tri-stimulus green filters ranged from 9.60 to 16.20 per cent, and 4.87 to 9.07 per cent respectively. The visual grades ranged from 4.2 to 11.5 (with 10 optimum or ideal). There is a gradual decrease in grade with an increase in per cent reflectances with slight overlapping.

4. For the same samples, the spectrophotometric results showed an even wider range (7.34 to 36 mg. %) for the amount of anthocyanin pigment. The results indicated an improvement in visual grade with increasing concentration of the anthocyanin pigment. This relationship holds true only up to a certain limit (30 mg. %) beyond which the berries were graded as substandard due to their poor color and off flavor.

5. The statistical relationship of the two different objective methods with the subjective method of visual grading is summarized as follows:

	r	$s_{y.x}$	t r	N
% A vs Visual grades	-0.793	1.26		17
% G vs Visual grades	-0.833	1.14	2.44	17
% Anthocyanin vs Visual grades	0.767	1.11	2.32	19

The values of r are all significant at the five per cent level. Considering the value of $s_{y \cdot x}$, the error involved is comparatively high and hence the range in predicting the exact grade will be very large. The error of estimation may be reduced by examining a larger number of samples, but particularly by improving the reliability of visual grading. The correlation coefficients would be higher if the visual grading were done on purees instead of whole berries as was done in instrumental grading.

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APPENDIX

Table 8

Measurement of Color of Strawberries by Reflectionmeter Expressed in Munsell Notations

Sample Number	Reflectance % A	% G	% B	Munsell Notations
1	12.93	6.33	2.53	6.06 R 2.95/ 8.92
2	14.00	8.07	3.23	8.69 R 3.32/ 7.19
3	12.53	6.57	2.70	6.73 R 3.00/ 8.00
4	11.50	5.77	2.40	6.37 R 2.81/ 8.30
5	11.87	3.73	2.23	6.44 R 2.80/ 8.90
6	11.00	3.50	2.27	6.50 R 2.75/ 8.36
7	11.77	5.50	2.20	6.08 R 2.75/ 9.27
8	12.40	5.93	2.20	6.56 R 2.85/ 9.16
48	14.12	8.50	4.28	5.22 R 3.40/ 9.47