Development and Use of Color Standards for Egg Yolks

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FOR SOME YEARS poultry scientists have been engaged in nutrition studies on the effects of various grains fed to laying birds. Some of these studies have been concerned with the effect of grain or combinations of grains in determining yolk color. The desired yolk color for home use varies with the geographical location and customs of the people, but in general a fairly light color is preferred. On the other hand users of yolks such as noodle manufacturers and cake bakers pay premium prices for dark colored yolk. It is therefore more profitable for the egg producer if he accurately maintains the yolk color within the desired range for his particular market.

Since verbal color descriptions are ambiguous it is necessary to have standards for egg yolk colors in recording and communicating the results of poultry diet research. The only color chart available in Canada was one which had been produced by the Federal Department of Agriculture based on the work of Heiman and Carver (1935). The colors were painted on watch-glasses mounted on a large black wheel. They had changed markedly through constant use and exposure to direct sunlight and several were missing through breakage. These defects, together with the fact that the wheel was cumbersome, meant that satisfactory comparison of yolks on any standardized basis was practically impossible.

Meanwhile other workers in this field had rejected pigmented coatings as a basis for yolk color measurement. Turner and Conquest (1939) stated that pigments were not translucent, they faded during storage and were not usually mixed in a quantitative manner. Although some of these objections may have been valid at the time, the pigment manufacturers have since then greatly improved the light fastness of their products. Although translucence is troublesome, the correct contour of the colored surface can reduce the problem. It has been found that the flat painted paddles referred to could never duplicate the natural appearance of yolks. The rejection of paint led to the development of methods for chemical estimation of color. The colored matter in the yolks was extracted with acetone and compared, visually at first, and later by colorimeter, Kahlenberg (1949) with solutions of potassium dichromate. Further work along these lines led to the present AOAC method in which the acetone extract is compared to carotene solutions (1960).

These methods, however, have defects

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since they appear to be more applicable to broken yolks such as frozen and dried yolks. In the work reported here it was found that the color of the broken yolk is not the same as the whole yolk. In addition the chemical estimation of color does not always correlate with the visual rating. Carlson *et al.* (1961) report one case where paprika deepened the visual color of yolks without showing any increase by the extraction method. Conversely a high carotene diet doubled the color rating by extraction but had little influence on the visual color.

Because of the defects in both systems, the Poultry Division, Production and Marketing Branch, Department of Agriculture requested the assistance of the Paint Laboratory, Division of Building Research, National Research Council, in preparing a series of standard colors for use by visual comparison which would cover at least the range of yolks produced in Canada. It was initially considered that 15 sets of 15 colors would be sufficient for the Department of Agriculture and other interested parties working on yolk colors. Work on the sets began but before the initial batch was completed, it became evident from inquiries that there was great interest in these color standards, not only in Canada and the United States, but in other countries as well. A further 20 sets were produced but these have not been sufficient to meet the demand. While steps are being taken to make these standards available in quantity, it is considered desirable, due to the extensive interest, that the colors and techniques should be described in a manner which will provide a sound basis for proper reproduction and standardization. This paper, therefore, outlines the trials necessary to achieve a satisfactory set of colors and gives the form which was finally adopted. Since the colors cannot be reproduced exactly by adherence to the particular formulations used, they are described more precisely in color co-ordinates obtained from measurements on a spectrophotometer. Factors affecting the reproduction, maintenance and use of the standards are discussed.

DEVELOPMENT OF TRIAL COLORS

It was considered necessary in order to obtain colors that resemble egg yolks to match colors visually against whole yolks under a standard light source. Instrumental measurements of real yolk colors were not taken because it was not considered feasible to design a reflectance head for a color instrument that would eliminate external light and provide the correct angles of illumination. In addition, it was known that instrumental readings do not always correlate with visual determinations of color.

From preliminary tests four pigments were selected to make yellow, orange, white and black enamels for intermixing to obtain different yolk colors. The particular yellow and orange pigments were chosen because of their resistance to darkening. The resinous binder in the enamels was an air-drying alkyd reported to have good color- and gloss-retention. Baking-type enamels were not used because baking causes a color change which makes it more difficult to estimate what the wet color should be to obtain a certain baked color.

When not in use, eggs which were opened for color matching were stored in glass jars in the refrigerator with the yolks covered with egg white. Unfortunately the yolks tended to change color, especially on the top surface. If the egg was fresh when broken it could be used for about two weeks, but if the egg itself had been stored for some time the yolk began to change 3 to 4 days after breaking. In the latter case it meant that before a good match could be produced, the original color was no longer available. It was also found that if a yolk

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was broken to mix in the color patch on top, the resultant color did not match that of the original whole yolk.

SELECTION OF FORM

First attempts at matching egg yolks soon showed that it was impossible to obtain satisfactory color matches when the enamel was applied to a flat surface. The upper surface of a watchglass, which has a contour similar to an egg yolk on a plate, was found to be very suitable for laboratory use. Glass, however, was not considered acceptable in the field because of possible breakage. The original request was for a flat aluminum sheet with a hole in the center that could be held over the yolk. The Paint Laboratory suggested a convex form similar to the watchglass which would be placed beside the yolk. A second suggestion was to combine both ideas—a convex shape with the center punched out. Six of each of these new types of forms were made from aluminum and trials were run to determine the best shape and the best method of coating them. The Paint Laboratory preferred the solid form because it could be mechanically dip-coated. The Department, however, chose the ring form which had to be sprayed to avoid ridges and sags around the opening. A diagram of the ring form finally adopted is given in Figure 1.

SELECTION OF COLORS

A great many enamel intermixes were made by the Paint Laboratory in the course of trying to match as many different-colored yolks as possible although there was some difficulty in obtaining yolks at the extreme ends of the color range. These intermixes were checked at the Central Experimental Farm when a great many eggs were being broken. From the intermixes, 35 colors were selected which matched one or more yolks, but many of these were too similar for those unfamiliar with color to distinguish. Fifteen of the colors were chosen to cover the range with distinguishable color differences. More colors with correspondingly smaller differences were deliberately placed in the central portion where it was expected most of the egg-yolk colors would fall. A preliminary set of coated watchglasses was submitted to the Poultry Department of the Ontario Agricultural College. They suggested adding a color on each end and dropping two in the middle.

Following these changes, 15 sets of aluminum rings were coated and subjected to field trials at the Ontario Agricultural College, the Central Experimental Farm and the Poultry Marketing Services. A few months later it was reported that lighter colors were needed and that some of the colors were too similar. The latter complaint was easily remedied by removing one color and altering another in the middle of the range. The other difficulty resulted from the difference between using a watchglass or solid form that is placed beside the yolk and a ring held over the yolk thus shading it. Black had to be added to six of the lighter colors to compensate for the shadow cast by the ring. Two very light colors were also included although none of the eggs opened by the Paint Laboratory had ever been that pale.

When modifications were complete the second series of colors was again submitted for field trials. The Department reported that those using the standards thought that two of the numbers should be reversed. Because color is three dimensional it is rather difficult to arrange a one-dimensional system to everyone's satisfaction. On looking at a color, paint chemists tend to think of it in terms of the pigments needed to produce it. Consequently, the yolk colors were numbered by the Paint Laboratory in order of their composition. At one stage in the system, however, some observers thought that one color with more white appeared

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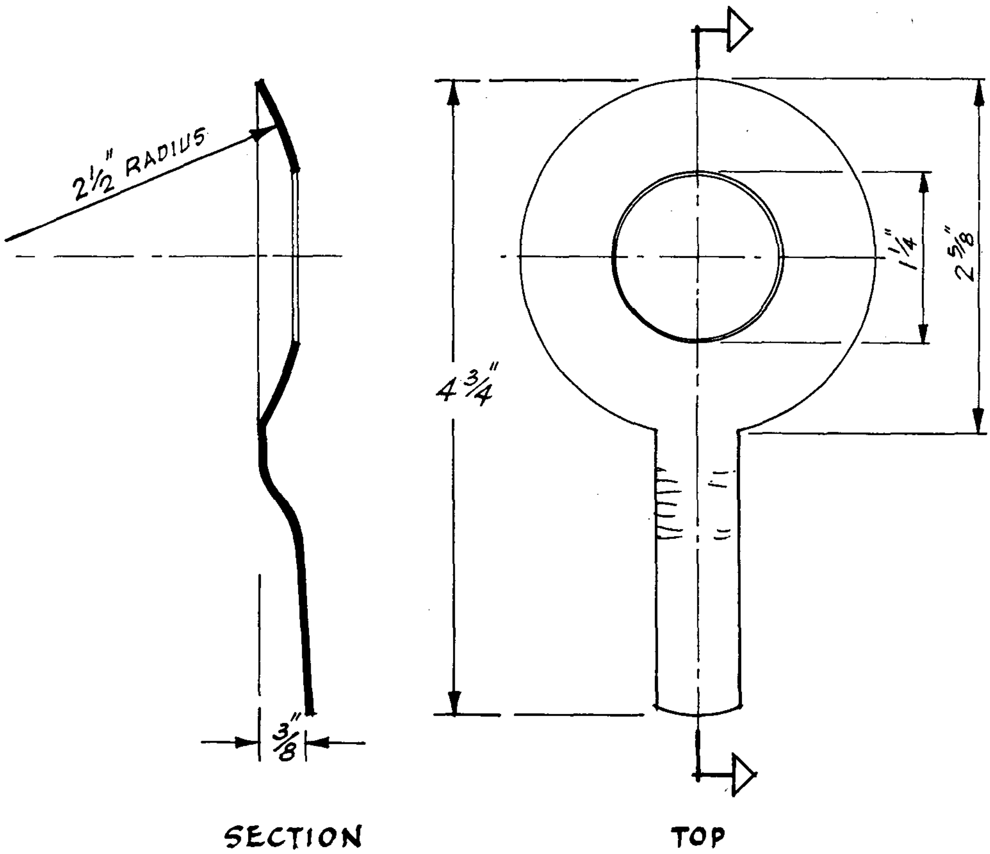

Fig. 1. Diagram of ring form.

darker than the following color. It was considered necessary to change the order so that the users would be satisfied with the arrangement. One of the colors was altered slightly to emphasize the correct order.

COLOR DESCRIPTION

It is not possible for purposes of standardization to describe the colors finally chosen in terms of the formulations and proportions of the basic enamels used. Small differences in dispersing the pigments can lead to differences in color even in successive batches made from exactly the same ingredients. This is well known to paint technologists and, in practice, final color matches are generally obtained by tinting using some standard for comparison. As a matter of interest, however, the proportions of the base enamels used to prepare the final colors are given in Table 1.

Other difficulties in color standardization are that the technique of preparing and the manner of viewing the standards affect the results. The nature of the substrate and base coat, the wet film thickness and the conditions of drying must all be carefully controlled to produce identical colors even from the same batch of enamel. The shape of the surface, the quality of the illumination and the method of viewing can also have an effect on the judgment of color. It is necessary in color standardization, therefore, to define carefully the conditions of

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preparation and the conditions of illumination and viewing.

An objective basis for color standardization can be provided by physical measurements to determine the spectral reflectance curve. Such curves for the final colors were prepared by the Radiation Optics Laboratory, Division of Applied Physics, National Research Council. The instrument was a General Electric recording spectrophotometer using standard illuminant C which corresponds to average daylight. The calculations required to obtain the three-dimensional color readings from the spectral reflectance curves are quite complicated. They were carried out using the weighted ordinate method as described by Judd (1952). The color co-ordinates obtained from measurements on the spectrophotometer for the 15 colors are given in Table 2 in two commonly used systems. One system is that of the International Commission on Illumination (C.I.E.) (Judd, 1952), and the other is the uniform color space proposed by Adams (Judd, 1952). The C.I.E. co-ordinates were transformed into the L, a, b system by use of the formulae given in A.S.T.M. method D1536. Either system may be used as a basis for standardization. At least one authority, however, has stated that the same make of spectrophotometer must always be used for complete standardization.

TABLE 1.—*Composition of intermixes by weight*

Color Number	Yellow Enamel	Orange Enamel	White Enamel	Reduced Black Enamel
1	100	2	250	5
2	100	0.5	160	3
3	100	1.25	125	3
4	100	1.8	100	3
5	100	0.5	80	2
6	100	1.5	60	2
7	100	0.75	45	2
8	100	0.5	30	0.5
9	100	1.8	35	0.75
10	100	1.25	20	0.1
11	100	2.5	15	0.1
12	100	4	20	0.1
13	100	4	3.5	—
14	100	12	15	0.25
15	100	18	10	0.1

TABLE 2.—*Color co-ordinates*

Color	C.I.E. System			Uniform Color Space		
	x	y	Y	L	a*	b**
1	.401	.420	63.66	82.51	−3.8	48.5
2	.413	.437	63.9	82.64	−5.4	56.9
3	.423	.442	60.94	81.03	−3.3	59.7
4	.431	.444	58.15	79.46	−1.6	61.4
5	.435	.455	60.33	80.68	−3.4	67.1
6	.446	.456	55.83	78.12	−0.3	68.5
7	.451	.464	55.35	77.83	−1.2	72.9
8	.460	.471	56.2	78.34	−0.4	79.4
9	.465	.462	53.08	76.48	3.6	75.5
10	.478	.467	53.21	76.44	5.6	83.0
11	.487	.464	48.17	73.4	9.0	82.2
12	.488	.454	46.84	72.55	12.0	77.3
13	.510	.456	41.65	69.01	16.6	85.8
14	.515	.429	36.89	65.52	24.8	70.7
15	.534	.418	32.38	61.96	31.2	70.2

* Negative values of "a" signify green colors, positive values red.

** Positive values of "b" indicate yellow colors, negative values blue.

A further complication in standardization of these colors was introduced by the adoption of the curved rings. Only flat, uniform surfaces can normally be used in a spectrophotometer. The readings in Table 2 apply to the films produced on flat forms and cannot be obtained from films produced on the curved forms. It is necessary, therefore, to specify and to control carefully the method of preparation and drying of the films on both surfaces in order that the colors finally produced on the curved forms may be properly controlled in relation to the physical measurements specified which have to be made on flat forms.

The methods of film preparation, together with the complete details of the formulations, which may be of use to paint technologists who may be called upon to produce the colors described, are given in another paper (Ashton, 1961).

USE OF STANDARD COLORS

Use of the forms is illustrated in Figure 2. Because the original color matches were made against unbroken egg yolks illuminated by artificial daylight, the colors must be used with the same type of illumination

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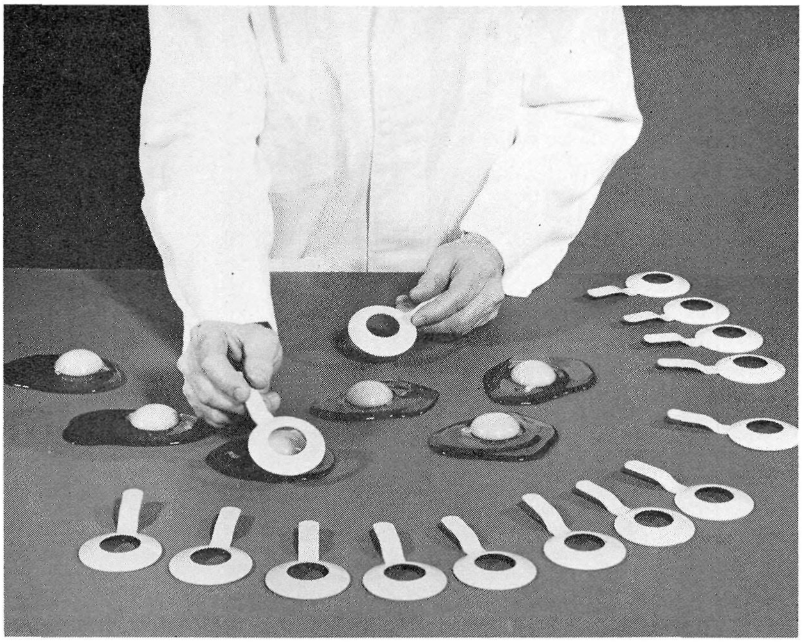

FIG. 2. Determining yolk color with standard color form (Canada Department of Agriculture).

or with natural north daylight. With other light sources such as incandescent bulbs the color may not resemble actual yolks. Extremely yellow lights, *e.g.* Macbeth horizon yellow, reduce the apparent differences between successive colors.

When being used, the enamelled forms should be handled with care so that they may retain their original color. The most common soil is egg white which is easily removed if washed with warm water before it has dried. More resistant stains may be washed with a mild detergent solution followed immediately by rinsing and drying. Brushes, abrasive cleaners or solvents should not be used for cleaning. The forms should not be left in strong sunlight longer than necessary and should not be exposed to high temperatures. Immediate replacement in a covered container will help avoid unnecessary contamination or exposure.

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The Effect of Acrylic Acid Salts on Growth of Chicks

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INTRODUCTION

STUDIES which reported that certain polar birds and animals have "bacteriologically sterile" intestinal contents were reviewed by Sieburth (1961a). In an attempt to determine if "bacteriologically sterile" birds do exist, and if so what factors were responsible, a study on the gastrointestinal microflora and dietary materials of Antarctic birds was conducted during the 1957-1958 Argentine Antarctic Expedition (Sieburth, 1959a). Although bacteriologically sterile birds were not found, typical lactose-fermenting strains of *Escherichia coli* were not observed in pygoscelid penguins and in certain specimens the non-lactose-fermenting coliform microflora was suppressed in the anterior gastrointestinal contents. A substance which inhibited the anterior gastrointestinal microflora of pygoscelid penguins was traced to the phytoplankton-laden stomach contents of euphausids (*Euphausia superba*) which were the sole diet of the penguins studied. During the trip into the Weddell Sea a green mucilaginous colonial alga, which occurred in half the water samples, was found to possess a heat-stable, water-soluble antibacterial substance which inhibited both gram-positive and gram-negative test organisms (Sieburth, 1956b).

Field studies conducted aboard the Hydrographic Ship ARA *Chiriguano* during the 1958-1959 Argentine Antarctic Expedition (Sieburth and Burkholder, 1959) confirmed the antibacterial activity of certain phytoplankton blooms, identified the responsible alga as *Phaeocystis pouchetii,* and yielded material needed to isolate the active principle. This acidic volatile substance which polymerizes on concentration to form inactive residues has been extracted as the sodium salt and indentified chemically, physically, and biologically as acrylic acid, $CH_2 = CH - COOH$ (Sieburth, 1960). The antibiotic properties *in vitro* and *in vivo* of acrylic acid have been described (Sieburth, 1961b). The broad spectrum of activity at concentrations of 0.012-12.0 mg./ml. by filter paper disc assays was enhanced by acid reactions approximating those of the avian gut. Chick trials conducted in an attempt to explain the suppression of *Escherichia coli* in pygoscelid penguins indicated that acrylate feed levels as low as one-fifth (0.01%) of those estimated to be ingested by penguins under natural conditions suppressed *E. coli* and permitted its partial replacement by *Aerobacter aerogenes*. Acrylate feed levels be-

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