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The Chemistry of Colour and Appearance*

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ABSTRACT

The chemistry of colour and appearance is reviewed with emphasis on meat colour.

INTRODUCTION

The sensation of vision, unlike the other sensations discussed in this symposium, is not stimulated by chemical contact with the object. Stimulus for colour perception, for object recognition and its positional relationships within the perceived scene does not reside in the object *per se* but in the light transmitted or reflected from it relative to the light received by the observer from its surroundings. Although 'chemistry' is thus not directly involved in visual perception of objects, its contribution to the three interacting components that generate the sensation is considerable. These are: the mechanism of visual detection which is a sequence of biochemical and neurological events; the appearance characteristics of the object which depend on its external surface properties and internal structure, which are determined by chemical composition; the source of light, especially artificial light, which depends on the chemistry of the emitting device.

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VISION

(a) Photochemistry

The sequence of events culminating in visual sensation starts with the external stimulus, the radiant flux incident on the eye. The primary process is the absorption of a photon by a photoreceptor which acts as a transducer by converting light energy into electrical signals that convey the visual information to the brain (Berridge & Irvine, 1984). The photoreceptors in the human eye are located in multifolded disk-shaped membranous structures in the outer segment of the rods and cones in the retina. A photon passing through the stack of disks has a high probability of being absorbed; this probability is dependent on wavelength and on which photopigment is involved. There are about 125 million rods distributed over the sensitive surface of the retina which colourlessly detect light at scotoptic levels below the cone photoptic threshold. The 5–6 million cones occupy an area of about 1 mm^2, the *fovea centralis*, which is capable of acute photoptic vision in colour. Each photodetector has about ten thousand million molecules of photopigment. Only rhodopsin, the rod pigment, has been isolated and its spectral sensitivity determined directly, but the shapes of the absorption curves of the cone pigments have been constructed with reasonable accuracy from psychophysical experiments (Boynton, 1979). Retinal densitometry is inefficient in measuring the cone pigments but colour matching techniques, using colour blind observers deficient in one pigment, have produced spectral curves for the three pigments (Smith & Pokorny, 1975); probably the best set is that of Estevez (1982). The absorption maximum for rhodopsin is 505 nm and for the three cone pigments is 440, 545 and 580 nm for blue (cyanolabe), green (chlorolabe) and red (erythrolabe), respectively, for light incident on the cornea.

The chemistry of light capture by rhodopsin was established in the 1960s (Wald, 1968). The absorption of a photon causes a conformational change in the molecule which proceeds through a sequence of steps until the pigment bleaches and the chromophore retinal separates from the protein opsin. Regeneration of rhodopsin after bleaching requires reformation of the all-*trans* form of retinal or retinol to the 11-*cis* form either in the eye or via vitamin A in the liver. The first step in the light detecting process is the formation of bathorhodopsin within 6

picoseconds in which the 11-*cis*-retinal of rhodopsin is isomerised to the all-*trans* form to partly emerge from the protein pocket in which it is bound by a Schiff base linkage at the aldehyde end of the chromophore. That the initial vision stimulating event is not the *cis–trans* isomerisation has been questioned because of the time required for full isomerisation to occur. The plausible model of proton translocation proposed by Peters *et al.* (1977) fits with recent information gained using picosecond spectroscopy on the intermediates from the *cis*-forms of rhodopsin to bathorhodopsin (Spalink *et al.*, 1983).

The consequence of photon capture and onset of the conformational change in rhodopsin is a decrease in the permeability of the cell membrane to sodium ions. In the dark, in the unexcited state, the continual leakage of sodium ions into the outer segment membranes is compensated by their removal from the inner segment by a sodium pump mechanism. On photoexcitation the leakage decreases, creating hyperpolarisation of the electrical potential across the cell membrane (Normann & Werblin, 1974). The central question concerning the chemistry of vision is the identity of the biological pathway that results in a sufficient amplification of the initial event of photon capture to achieve the subsequent change in membrane conductance that initiates the neural signal (Berridge & Irvine, 1984; Brown *et al.*, 1984; Fein *et al.*, 1984). This link between photon capture and change in ionic permeability poses the need for an internal transmitter or secondary messenger system. Although not yet demonstrated in vertebrate receptors, inositol triphosphate has been shown to be involved in the control of the signal transduction mechanism of the excitary cascade that opens up to 1000 ionic channels from a signal photon. It is also postulated that inositol triphosphate may have a role in the mechanism that is responsible for the control of visual adaptation.

(b) Signal processing

There is ample evidence from physiological studies to indicate that the signals generated by the cones undergo two important transformations before transmission along the optic nerve to the visual cortex where final interpretation is made in the brain. The sensations of space, object recognition and movement are then perceived by the observer as though projected back into the world from which they originated. All is coloured, but the sensation of colour exists only in the mind and is not

intrinsic to objects in the scene (Wright, 1967). The pre-optic nerve transformations are thought to occur in the horizontal and bipolar cells in the retina. In Hunt's (1982) comprehensive model for predicting colour appearance, the first transformation is to compress the dynamic range of the red (R), green (G) and blue (B) signals, represented in the model as square-root functions. The second stage (Hunt & Pointer, 1985) is the formation of an achromatic signal:

$$A = 2R^{1/2} + G^{1/2} + (1/20)B^{1/2}$$

and three opponent colour difference signals:

$$C_1 = R^{1/2} - G^{1/2}$$
$$C_2 = G^{1/2} - B^{1/2}$$
$$C_3 = B^{1/2} - R^{1/2}$$

The achromatic signal is weighted for the relative abundance of the cones, and the opponent processes are consistent with Hurvich's (1981) scheme for the generation of response functions from cone absorption. The model provides a colour order system with good prediction of the unique hues, red, green, blue and yellow, and of constant hues and saturation compared with the Munsell and Swedish Natural Colour Systems. The model also includes a chromatic adaptation transformation which means that tristimulus values can be converted to appearance specification relative to daylight and tungsten illumination.

(c) Colour specification

The trichromatic principle of three primary response mechanisms, the spectral sensitivities for cone vision, is the basis for all systems that specify colour numerically. The factors required are the spectral power distribution of the illuminant, the spectrum of the object and the colour matching functions of a standard observer. The mathematical procedure for two visual fields (2° and 10°), standard light sources and presentation geometry is specified by the Commission Internationale de l'Eclairage (CIE, 1971; Wright, 1980; Wyszecki & Stiles, 1982). Instead of using the 'real' primaries R, G and B, they are transformed into imaginary primaries, X, Y and Z, in such a way that Y is equal to the observer's overall spectral sensitivity function and values of the primary colour matching functions X, Y and Z are all positive. This system, which uniquely locates colours in three-dimensional space, was first

constructed in 1931 but has undergone many modifications to adjust its scaling to make it more nearly visually uniform. In 1976 the CIE proposed adoption of two colour spaces (Robertson, 1977) and standardised colour terminology (CIE, 1978; Hunt, 1978). The two spaces are known as CIELUV and CIELAB; the former has the property that linear additivity of coloured lights lies on straight lines and the latter approximates to the visual spacing of the Munsell colour solid. These spaces were chosen because of their proven merit in practice. The colour examples in this paper have all been calculated in CIELAB where:

$$L^* = 166(Y/Y_0) - 16$$
$$a^* = 500[(X/X_0) - (Y/Y_0)]$$
$$b^* = 200[(Y/Y_0) - (Z/Z_0)]$$

The tristimulus values of the measured object are X, Y and Z and X_0, Y_0, and Z_0 are the values for white relative to the chosen illuminant. L^* is the psychometric correlate of lightness but depends significantly on the nature of the surround and luminance (Hunt, 1977). The psychometric correlates of perceived hue, $h^* = \tan^{-1} b^*/a^*$, and chroma, $C^* = (a^{*2} + b^{*2})^{1/2}$, are also dependent on the viewing conditions. Hue is that attribute of colour described in colour values, red, orange, yellow, green, etc., and chroma is the term that describes the colourfulness of the object relative to its surroundings.

ABSORPTION AND SCATTER

According to Nassau (1983) there are fifteen separate physical and chemical causes of colour. The physical causes range from colours produced by vibrations and simple excitations, e.g. incandescence and some lasers, to those produced by geometrical and physical optics, e.g. refraction, diffraction, interference and scattering. The chemical causes can be divided into three main groups, those involving ligand field effects, energy bands and molecular orbitals. Ligand field colours are typified by transition metal coloured compounds and the ruby laser while those involving transitions in energy bands include the colours of pure metals and the phosphors used for fluorescent lamps. It is, however, the 'molecular orbital' colour cause that includes virtually all the biochrome pigments and synthetic dyes important in food. These include the non-cyclic conjugated polyene carotenoids, the cyclic non-benzenoid

ring polyenes, chlorophyll and the porphyrin-containing proteins, haemoglobin and myoglobin, and also the donor–acceptor brilliant plant pigments, the flavones and anthocyanins. The chemistry of these pigments has been recently reviewed, the anthocyanins by Timberlake & Bridle (1980), the synthetic carotenoids by Counsell (1980), the haemoproteins by Ledward (1984) and other natural colours by Taylor (1984). The Maillard reaction, important as the cause of brown colours and discoloration in foods, has been the subject matter of two recent symposia and a book in which the mechanism of the formation of these brown compounds was reviewed (Vernin & Parkanyi, 1982) and, for example, the formation of coloured compounds by the interaction of glycine and xylose (Nursten & O'Reilly, 1983), the effect of sulphur dioxide on retarding colour development of glycine/glucose and glycine/ascorbic acid solutions (McWeeny, 1981) and the effect of storage on colour development in plantation white sugar (Cheng *et al.*, 1983) have all been reported. Although the chemistry of the above naturally occurring food colours and the analysis of synthetic colours used in food (Wadds, 1984) have been fully covered in recent years, there is sparse information on the visual effect of these colours in their respective foodstuffs. A recent exception is the work of Petriella *et al.* (1985) on the Maillard reaction, where colour change due to non-enzymatic browning in model food systems is reported in CIE tristimulus values and metric chroma. It is not the purpose of this paper to review again these colour-producing compounds but to discuss the relationship that pigment absorption and light scatter have on the calculated visual stimulus and how it is altered, for example, in a transparent highly pigmented beverage and in a variable light-scattering food.

The transmittance and colour of transparent materials depend on pigment concentration, the absorption spectrum of the pigment or mixture of pigments, thickness of the product and the emission spectrum of the light used for viewing. The reflectance of opaque or translucent materials, in addition to attenuation by absorption, depends on light scatter produced by differences in refractive index at the boundaries between the light reflecting particles in the system, their concentration and size relative to the wavelength of light. Common to both transparent and light-scattering materials is the effect on appearance of surface reflection at the air/object interface. The surface may range from flat, wet and shiny, although few foods in their natural state are flat, to rough, dry and matt. In opaque materials the nature of the surface

influences the amount of light reflected in both external and internal directions. Increase in reflectance by inclusion of more scattering elements in the system affects the attenuation by pigment absorption in a much more complex manner than that which occurs by simple dilution in transparent media. In transparent media the Beer–Lambert law applies. Variation in internal transmittance, T_i, with thickness, X, and concentration, c, of a non-light-scattering pigment (dye) is given by:

$$T_i = e^{-cK_1X}$$

where K_1 is the absorption coefficient for unit concentration ($c = 1$). In opaque and translucent materials both absorption and scatter affect reflectance. The Kubelka–Munk analysis is the most commonly used technique for separating the relative contributions of K, the absorption coefficient, and S, the scattering coefficient; the technique is used in colorant layer formation, e.g. in the paint industry, because of the simple additivity of the coefficients. The relationship of reflectivity, R_∞, the reflectance at infinite thickness, to K and S is given by:

$$K/S = (1 - R_\infty)^2/2R_\infty$$

where K and S are determined from reflectance of thin layers on white and black backgrounds. This two-variable procedure of Kubelka & Munk (Kubelka, 1948; Allen, 1978) is fully illustrated by Judd & Wyszecki (1975).

Modern colorimetry, with its visually based colour scales, is now such an effective tool in those industries where colour matching and colour pass–fail judgements are critical that it can be used in conjunction with computer match prediction techniques to replace much of the visual judgements that were once required, although more complex formulae than those recommended by the CIE may be necessary (McDonald, 1985). For many colour applications where change in colour, e.g. ripening or fading of foods, is to be related to orderly colour arrangement in visually uniform space, CIELAB is an ideal colour space; it is recommended for following the course of colour reactions or describing the magnitude of colour differences (McLaren, 1985). For most transparent or highly opaque materials it is possible to construct colour diagrams from transmittance or reflectance spectra to determine the visual magnitude of colour change in pigment absorption and degree of light scatter. The following two examples illustrate the use and limitations of the system.

(a) Red wine

Anthocyanin pigments are responsible for the red colour of red wine. Intensity of redness is a desired characteristic ranging from near deep blood-like purple–red to the delicate pink of rosé. To illustrate the potential of colour space interpretation from transmission spectra, a sample of 'appelation controllé' claret was measured over a range of thicknesses between 1 and 50 mm pathlength, which represents every possible observation from a full glass to that produced by transmitted light observed near the rim at the meniscus in a tall glass held at an angle. There is a progressive increase in transmission at lower wavelengths as the pathlength is decreased (Fig. 1). However, not until between 5 and

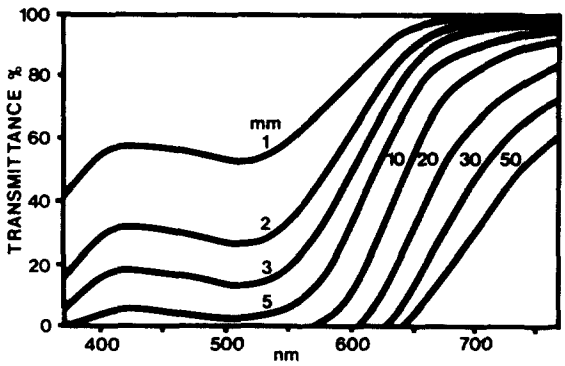

Fig. 1. Spectral transmittance of a sample of red wine between 1 and 50 mm pathlength.

10 mm is there any contribution to transmittance from wavelengths shorter than the maximum absorbance at 520 nm, and then there is an increasing contribution from the 'blue' end of the spectrum at 420 nm with very large increases occurring from 3 to 2 to 1 mm. If Beer–Lambert's law is obeyed for clear solutions, the relationship of log concentration (or, in this case, log pathlength) to log absorbance (log log $1/T$) should be a straight line (Judd & Wyszecki, 1975). This was found to be so, as the values at 500, 600 and 700 nm in Fig. 2 confirm. Thus, from one spectrum it is possible to construct the entire transmittance set and the associated calculated tristimulus values provided the spectrum is so chosen that the absorbance (extinction) can be accurately determined over the complete visible wavelength range.

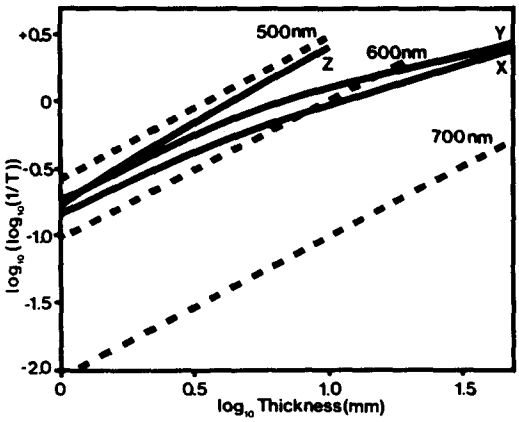

Fig. 2. Straight line relationship of log log reciprocal transmittance to log thickness (pathlength) of red wine with monochromatic illumination compared with curved line relationships of the tristimulus values.

What is not possible is to do the same from measured tristimulus values at only one concentration or pathlength. This is shown in Fig. 2 in the curved nature of X, Y, Z, because the relative weighting of the regions of the spectrum that generate X, Y, Z, when integrated with the standard observer by illuminant functions, change with pathlength. The CIELAB colour solid determined from the information in Figs 1 and 2 is shown in Fig. 3. Maximum chroma is at about 10 mm pathlength which is indicative of that observed visually: both longer and shorter pathlengths produce less intense dark and pale colours, respectively. In addition to relating chemically generated absorbance data to visual colour sensation, CIELAB can be used to estimate magnitude and direction of colour change with change in illuminant (Mori & Fuchida, 1982; MacDougall, 1984). The 2, 10 and 20 mm pathlength information, recalculated for three 'real' fluorescent lamps, is shown in Fig. 4. All three psychological colour concepts of lightness, hue and chroma are altered at each pathlength. The redder De Luxe Natural fluorescent tube, typical of that recommended by lamp manufacturers for displaying meat and foods in general, produces the most intense chroma, while Artificial Daylight, the lamp with the highest colour rendering index, moves all the colours in a blue direction hue shift towards purple. The White tube moves the colours towards a more desaturated yellow; that is, compared to De Luxe Natural or Artificial Daylight, the wine appears more brown. Figure 4 also clearly shows the dependence of hue change with

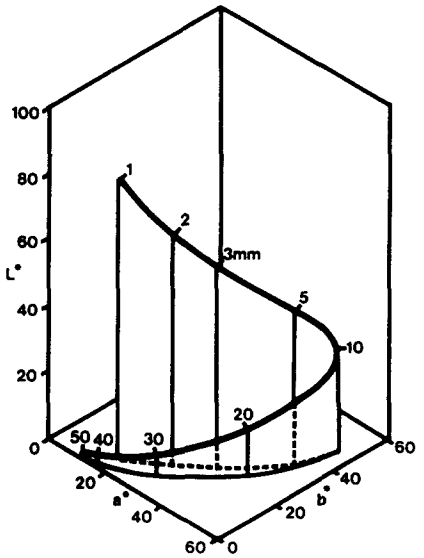

Fig. 3. CIELAB combined uniform lightness and chromaticness spacing of red wine from 1 to 50 mm pathlength.

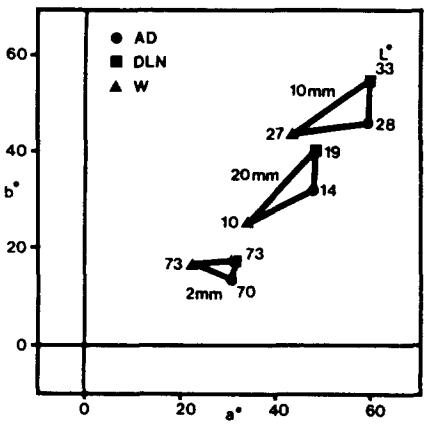

Fig. 4. CIELAB psychometric chroma diagram of the colour of red wine for Artificial Daylight (AD), De Luxe Natural (DLN) and White (W) fluorescent illumination at three pathlengths. The achromatic location of each lamp is at the origins.

pathlength; the 2 mm pathlength, with the proportionately greater transmittance at the blue end of the spectrum, is the most purple although it is twice as light as the 10 mm pathlength.

(b) Fresh meat

Myoglobin and residual haemoglobin are the pigments responsible for the colour of meat (MacDougall, 1982). A freshly cut beef surface rapidly changes in colour from the purple of ferrous myoglobin to the bright cherry red of oxymyoglobin as air penetrates to a depth of about 2 mm in <1 h. Obliteration of the interior purple by the semi-opaque red layer results in a massive increase in chroma (C^*) of about 10 units to a value approaching 30. If exposed to the $>60\%$ oxygen concentrations used in controlled atmosphere packing (Taylor, 1985), the thickness of the oxygenated layer approaches 10 mm in <1 day with an additional increase in C^* of >2 units. Oxymyoglobin is unstable at low partial pressure of oxygen (1 to 1·4 mm) and oxidises to ferric metmyoglobin. This layer forms preferentially at the limit of oxygen penetration and then progresses outwards to discolour the surface with

the unattractive grey to dull greenish brown colours which is the principal cause of consumer rejection in retail display. Meat packed in air discolours in 2 to 3 days but high oxygen concentrations delay discoloration by >1 week because the low partial pressure site of metmyoglobin formation at maximum rate is either deeply submerged, as in large roasting joints, or completely removed, as in steaks and chops which are <2 cm thick. A metmyoglobin concentration of 20% on the surface increases the hue angle (h^*) from oxymyoglobin in a yellow direction from 37° to 40° and decreases C^* by 3 units. This visual change is sufficiently large to be remembered and is the level that causes rejection in retail display (Hood & Riordan, 1973).

Metmyoglobin formation is not the only reason why h^* increases and C^* decreases. Oxymyoglobin is dichroic (Wright, 1980); that is, the colour changes with pigment concentration because of the differences in extinction of the several absorption bands. Reflectance spectra of oxygenated meat of normal pH (5·5 to 5·7) are shown in Fig. 5; they represent the range from veal to beef from mature animals. The difference in reflectance from 1 to 2 mg g^{-1} is similar to that from 2 to 5 mg g^{-1}. The straight line relationship of the Kubelka–Munk reflectance function (K/S) at 520 nm relative to pigment concentration is shown in Fig. 6. Figures 5 and 6 are based on data from a hundred samples with the scatter coefficients (S mm^{-1}) adjusted to 0·15 for tristimulus value Y

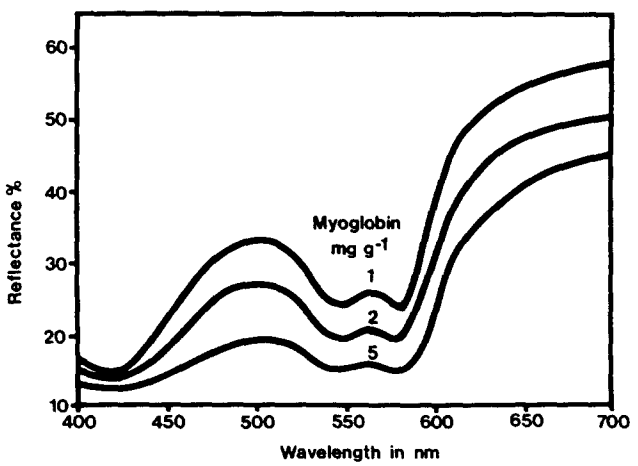

Fig. 5. Spectral reflectance of oxygenated bovine muscle at three levels of myoglobin pigmentation.

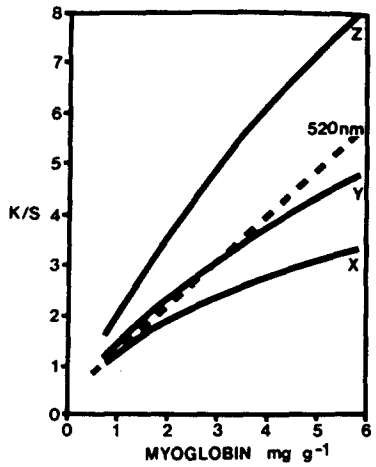

Fig. 6. Relationship of the Kubelka–Munk ratio of absorption and scattering coefficients (K/S) to pigmentation in beef for monochromatic illumination and tristimulus values.

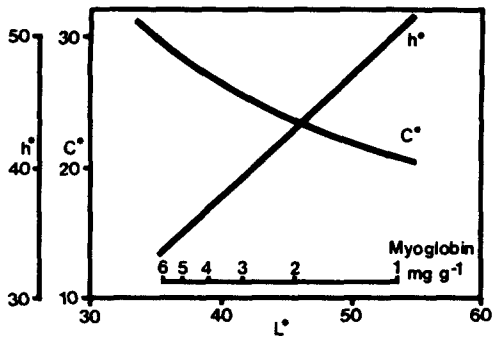

Fig. 7. Effect of pigment concentration in beef on lightness (L^*), hue angle (h^*) and chroma (C^*) at Kubelka–Munk scatter coefficients $S\,mm^{-1}$ of 0·15.

(MacDougall, 1982). The Kubelka–Munk reflectance functions, K/S, for X, Y, Z relative to concentration, are curved lines, which is similar to the relationship for log absorbance in Fig. 2 for the transparent wine. The relationship of CIELAB h^* to C^* for pigment concentration is shown in Fig. 7 where h^* decreases (becomes more purple–red rather than orange–red) as lightness (L^*) decreases and C^* increases with increasing pigmentation. This Figure also clearly illustrates the much larger colour changes that occur with small increases in pigmentation in a relatively low pigmented light-scattering material, e.g. veal, compared to the much smaller changes that occur at higher concentration.

Meat of normal pH, however, varies in S from $>0{\cdot}1$ to $<0{\cdot}3$ depending on such factors as chilling rate, degree of ageing and stress susceptibility (MacDougall, 1982) which results in highly light-scattering pale, soft and exudative (PSE) meat. Change in S affects L^*, h^* and C^* in a somewhat similar, but inverse, manner, as change in pigment concentration. An increase in $S\,mm^{-1}$ of 0·1 increases L^* by 6 to 7 units at all pigment concentrations and increases h^* by 5° at $1\,mg\,g^{-1}$ but only by 1–2° at 5 to $6\,mg\,g^{-1}$. There is negligible change in C^*; at $1\,mg\,g^{-1}$ it decreases by 1 unit and at $6\,mg\,g^{-1}$ it increases by 1 unit.

ILLUMINATION

The overall appearance of any visual scene is modulated by the intensity and spectral composition of the light while the colour–appearance characteristics of individual objects within that scene are modified by the light's colour-rendering properties (MacDougall, 1984). Different illuminants with different spectral power distributions, but the same location in CIELUV colour space, have the same colour when viewed directly. Any white object or background, which the visual system may use as reference in the process of chromatic adaptation, will appear white when viewed independently but coloured objects will be different. The size of this visual adjustment, accomplished automatically by visual adaptation on passing from one light to another, can readily be demonstrated by simultaneously viewing white in a side by side comparison of two lamps with different colour temperatures in adjacent booths; the obvious chromatic shift is indicative of the real difference between the colours of the lamps. Even more obvious is the chromatic shift of coloured objects if the observer is constrained to judge the objects in a comparative viewing arrangement. With the limitation of human colour memory eliminated, the magnitude of the colour shift caused by the difference in the spectral power distribution is strikingly evident. The two factors directly related to the emission spectrum of the lamp are colour temperature and colour rendering. The correlated colour temperature of a lamp gives no indication of its fidelity to render colours with their expected appearance but is only a guide to the position of the colour of the lamp itself on a colour chart relative to the temperature of the blackbody radiator; thus so-called warm-appearing lamps have correlated colour temperatures <3000 K, intermediate <3500 K, and cool >4000 K. The more important measure of colour fidelity is the colour-rendering index, Ra, which is the lamp's ability to render colours relative to a complete spectrum; thus, lamps with high values of Ra are more faithful in generating what would be expected as the true colours of coloured surfaces (Halstead, 1978).

Most food displays are illuminated by fluorescent, rather than incandescent, lamps because of their efficiency, low energy consumption and lower heat production. The range of lamps available is considerable, especially since the development of red-enhancing phosphors in the 1950s and the more recent concept of using triband emission at near 450, 540 and 610 nm (Thornton, 1973). The halophosphate used for

white fluorescent lamps emits a broad band spectrum peaking at 580 nm in the yellow part of the spectrum close to the eye's maximum sensitivity, but they are deficient in red energy. The incorporation of magnesium fluorogermanate, which emits at 630 and 660 nm, vastly improved the Ra from <60 to >90 and led to the development of special tubes for hospital illumination, for colour matching and for flattering food display. However, it is the new generation of triband lamps with their rare earth-activated aluminates for blue and green emission, and europium-activated yttrium oxide for red emission, that is likely to replace earlier types because of their distinct saving in energy and their capacity to make coloured materials appear maximally attractive. The effect of red enhancement and triband illumination on the appearance of meat colours has recently been shown to elicit a greater visual colour change in making brown appear red than in making red appear more red (MacDougall, 1985); for some people red enhancement could make meat appear too red. Broad band tubes with high Ra, such as 'Artificial Daylight', used for colour matching purposes, effectively separate food colours with maximum visual discrimination and should be used for accurate quality judgements. Red enhancing tubes, with their property to flatter appearance, reduce the observer's ability to assess differences in the colourfulness of bright colours in monadic presentation.

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