

NATURAL

FOOD

COLORANTS

Edited by
G.A.F.Hendry & J.D.Houghton

B L A C K I E

Natural Food Colorants

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Preface

Natural food colorants have been widely used in the preparation of foods and beverages for centuries and, alongside artificial colorants, they continue to make a significant contribution to the preparation and processing of food. Recent years have seen an increased interest in natural colorants; in some cases the natural product can be priced competitively compared with the artificial alternative and, in others, the consumer has preferred not to use the artificial colorant. As yet, however, up-to-date and readily accessible information on natural pigments is not available, and several workers have commented on the errors and omissions that have become enshrined in the scientific and technological literature on food colorants. It is our aim to improve this situation by bringing together a full consideration of the biochemistry, chemistry and biology of natural pigments since these are the core areas on which the food chemist or technologist bases his or her creative skills.

The book has been written by experts on natural food colorants and the biochemistry of natural pigments, who have been drawn from both the academic and industrial spheres. We have allowed a certain amount of overlap between different chapters written with a different emphasis. Carotenoids, for example, are discussed as pigments in biology (chapter 1), as colorants with industrial applications (chapter 2) and as colorants of the future (chapter 7), as well as in the principal chapter devoted to them (chapter 5).

One of the aims in launching this book is to extend the horizons of those using natural colorants. Each contributor has considered pigments which are widely used today, and those of potential use in the near future. Natural pigments which are rare, of limited distribution in biology, or expensive to acquire, may soon become commonplace in the hands of the biotechnologist and offer considerable potential for exploitation. This is supported by the large increase in the number of patent applications for food colorants, covering a large number of unusual natural pigments. In the following chapters, over 400 natural pigments are described, with data on structure, stability and natural occurrence. We hope that our enthusiasm for exploring nature's rich box of colours will become apparent.

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1 Natural pigments in biology

G.A.F. HENDRY

1.1 Summary

This introductory chapter provides a working definition of 'natural' pigments. An outline is given of the way colours or hues are defined throughout the book, and of the areas of confusion surrounding colour description. The variation in colour perception by human eyes is also described. Chemical nomenclature and certain physical attributes common to pigmented compounds are provided, including a brief synopsis of the structural features needed to make an otherwise colourless molecule pigmented. The greater part of the chapter consists of a survey of pigmented compounds found in biology. Two systems of classification are adopted, one based on structural affinities, the second based on the natural occurrence of the pigment in biology. The latter system provides a resumé of almost all but the rarest pigments in higher and lower animals, plants including algae, fungi, lichens and bacteria. Where appropriate, the classifications are cross-referenced to the fuller treatment of certain pigments in the succeeding chapters.

1.2 Introduction

The immediate purpose of this chapter is to introduce the major and at least the most widespread of the minor pigments occurring naturally in plants, fungi, lichens, animals and bacteria. Whether or not these have previously been exploited as natural food colorants is not of immediate consideration. Neither is the availability of the pigment for commercial exploitation, particularly in view of the recent and promised advances in gene transfer and biotechnology. Theoretically, almost all reasonably stable biological pigments can now be considered for biotechnological production and, in future, may become available to the food and other industries as natural colorants.

1.3 Definitions

By definition, a natural pigment in biological systems is one that is synthesized and accumulated in, or excreted from living cells. In addition, certain

pigments, particularly the more simple phenolic derivatives such as the anti-coagulant coumarins, may be formed by the dying cell. What is *not* a natural pigment in biology is less easy to define. For the purposes of this chapter, inorganic pigments based on, for example, iron or titanium are not considered natural if only because they do not appear to play a significant or even minor role in biology. Nor are the several organic-iron complexes formed synthetically from naturally occurring organic complexes. That 'work-horse' of the food colorist's palette, caramel, is also not considered in depth in this chapter since it is not a pigment of living cells, even if its constituent parts (sugars and amino acids and amides) are.

This working definition and the delimitation of natural pigments inevitably highlight certain 'grey' areas, and so common sense and a little pragmatism have been employed to keep within the spirit of the above definitions while remaining relevant to the particular interests of the food colorant user. For example, green chlorophylls are truly natural compounds: the grey-green phaeophytin pigments extracted from green plants are natural in that they may be formed from chlorophyll during the natural senescence of the dying leaf and incidentally during industrial extraction of chlorophylls from leaves. The bright green Cu-chlorophyllins (see chapter 3) are not found in nature but represent man's close attempt to restore the original colour of true chlorophyll from these phaeophytins. All of these green pigments are considered here as 'natural'. Indigo, a well-known plant dye, is present in living tissues but as the colourless glycoside. Only on extraction, hydrolysis and oxidation does this pigment acquire its blue colour. For this reason, an expanded definition of a natural colorant as a 'pigment formed in living or dead cells of plants, animals, fungi or micro-organisms, including organic compounds isolated from cells and structurally modified to alter stability, solubility or colour intensity' is offered. The definition must also allow for pigments whose synthesis is engineered in genetically transformed organisms. For pragmatic reasons, structural colours due to diffraction, interference or Tyndall scattering of light are not discussed in depth; nevertheless, many play a significant role in the coloration of animals.

1.4 Colour and colour description

Throughout this book and most of the literature colours are described by the wavelength (λ) of the maximum absorption (A_{\max}) in the visible part of the electromagnetic spectrum, expressed in nanometres (nm). White light is seen as the simultaneous incidence of the full range of the visible spectrum (wavelengths between 380 and 730 nm approximately) at the *same relative intensities*. Any object that lessens the intensity of one part of the spectrum of white light by absorption (as in a monochromatic filter) will bring about the perception of colour in the residual transmitted light, as well as a reduction

in irradiance (measured in Watts per m^2 , Einsteins or moles of photons) or more simply a darkening. As more of the spectrum is filtered out the perception of colour will be increasingly monochromatic (shades of grey) darkening ultimately to black.

To those familiar with quantifying and qualifying light, a compound with an absorption maximum of, for example 600 nm, in the yellow part of the spectrum, will be thought of as deep blue in perceived colour. One absorbing light at say 470 nm, the blue-green part of the spectrum, will be predictably a yellow, leaving the unfamiliar reader thoroughly confused. The reason for the confusion is readily explained: light, or more specifically visible radiation, when perceived simultaneously over the complete range of the spectrum as in unfiltered sunlight, is seen as white light by the human eye. When separated by passing the light through a prism, the refracted beams of light are displayed as the sequence of colours familiar in a rainbow. The human eye 'senses' six hues:

1. Red light at around 700 nm;
2. Orange at 625 nm;
3. Yellow at about 600 nm;
4. Green at 525 nm;
5. Blue at around 450 nm; and
6. Violet at and below 400 nm.

Hence the casual laboratory description of a compound as having a 'strong red absorbance' instead of the more precise 'absorbance maximum at 680 nm.' Such a compound would probably appear to most human eyes to have a deep blue or green-blue colour! The confusion arises because the pigment in solution with say a strong red *absorbance* when placed in front of a beam of light, would allow only the passage, that is *transmission*, of the remaining non-red part of the spectrum—the broad area yellow-green-blue-violet. In white light, such a compound would appear to be blue (or green-blue). For a workable translation of description based on absorption maxima into hue perceived, see Table 1.1. The first line of Table 1.1 represents the colours absorbed by a range of pigments (dyes and colorants) that absorb light at

Table 1.1 Perception of colours according to the wavelength of light absorbed

Colour absorbed	Red	Orange	Yellow	Yellow-green	Green	Green-blue	Blue	Violet
Absorption wavelength (maximum) nm	675	600	585	570	540 525	490	460	410
Colour perceived	Blue-green	Blue	Violet	Mauve Red	Orange	Yellow	Yellow-green	

various wavelengths (second line) leaving the remaining colours (third line) to be transmitted and so perceived by the human eye as a distinct colour. Thus a pigment such as anthocyanin under white light will absorb the green portion (first line) of the visible light spectrum at around 520 to 550 nm (second line). Under white light the pigment will be perceived as mauve (third line). Green light has been 'removed' or absorbed by the anthocyanin leaving purple-blue, orange-yellow and red, which, as a mixture, appears as mauve.

In the succeeding chapters data will be presented with the absorption maxima of several hundred different natural pigments. By reference to Table 1.1 the portion of the spectrum of white light absorbed by the pigment can be predicted as well as the colour of the compound as seen by the human eye. Table 1.1 can also be used in the reverse order to predict the part of the spectrum that any coloured compound might absorb when viewed under white light. Thus from the previous example, grape juice anthocyanins that appear to be mauve (line 3) under white light are likely to absorb light at around 510 to 540 nm (line 2), that is the green part of the spectrum (line 1). There is then little point in observing the colour of a treasured glass of wine under the soft green lights sometimes favoured by restaurateurs for the wine will appear as black as ink!

1.5 Colour perception

Numerous definitions of colour are available, the most useful one in this context being that part of the electromagnetic spectrum visible to the human eye and generally regarded as lying between 380 and 730 nm. The key element in the special context of food colorants, however, is the hue *perceived* by the eye of the normal, healthy and average adult. Such an eye is less well-defined.

One problem with defining pigments by their apparent or perceived hue is that one man's mauve is another man's purple or red (and here male gender *is* meant). Precisely where red moves to the description mauve is less a matter of definition but more a subjective decision dependent on variations in colour reception and processing that exist in the eyes of different humans—particularly males. While the human eye can detect up to six major colours and many more intermediate blends, just three predominate—red, yellow and blue. These 'primary' colours (nearly) coincide with the three types of eye pigments.

The retina at the back of the human eye is made up of some 3 million colour-perceiving cone cells and about 100 million rod cells involved in providing monochromatic vision under low light. Each cell contains one of several types of visual pigment, which are structurally based on the β -carotene and vitamin A derivative 11,*cis*-retinol and bound to a particular class of proteins, the opsins. 11,*cis*-retinol itself, without modification, absorbs light maximally at around 500 nm, coinciding conveniently with the spectral maximum of sunlight. The cone-cell pigment proteins are sensitive to different

ranges of predominantly blue, green and red light. Colour in this sense is the sum of excitation of red-sensitive, green-sensitive and blue-sensitive cones. The difference between the 'red' and 'green' protein lies in just 15 out of 348 amino-acid residues, and there is evidence from studies of higher mammals that the evolution of the 15-residue difference may have occurred relatively recently in humans. Perhaps because of this recent evolution, considerable variation appears to persist within the human genetic make-up in these 15 or so residues. Small variations between two individuals in the DNA coding for the protein can lead to quite distinct differences in distinguishing the colour perceived by the different viewers. Disagreements over the matching or separation of colours lie particularly at the junctions of what are described as blue/green, red/orange and mauve/violet hues. These differences are not classed in normal parlance as colour blindness since what is green, blue or blue/green is a matter of unspoken consensus. The degree of variation in colour perception and its prevalence is unknown but is believed to be considerable.

Clinically defined 'colour blindness' is, in most cases, the result of an X chromosome-linked recessive mutation occurring in approximately 1 in 12 males. The incidence in females is about 1 in 170. As a brief description of what is a complex and fascinating subject in its own right, the following is offered as an introductory guide. An individual classed as 'colour blind' and lacking say the 575 nm red-light cones will 'see' only green and blue, or if lacking the 540 nm green-light cones will 'see' green as red, the latter condition being the more common. Other individuals carry cone cells with a maximum absorbance lying between green and red, resembling the cone cells of the more primitive apes. In most cases of colour-blindness, the individual is well able to distinguish the colour between blue and yellow but distinctions between green and red are possible often only as differences in brightness. In the most common forms of colour blindness, a red and green mixture of light is seen as either too 'red' or too 'green' by the standards prevailing among the non colour-blind majority. Given the immense effort to achieve food colorants that are perceived by the food chemist as a reproducibly distinct green or red hue, it is worth noting that the endeavour will never be appreciated by one in twelve male consumers!

1.6 Colour and chemical name

One way of pinpointing coloured compounds from a list of organic chemicals is through the name of the substance. There has been a long tradition particularly among German chemists of the last century of giving coloured compounds a name derived from a Greek or Latin pigment. These names have persisted in the trivial (and familiar) names of many chemicals. A short list is given in Table 1.2. A more comprehensive treatment of the subject is provided by Stearn [1] and Flood [2].

Table 1.2 Chemical names implying colour^a

Chemical prefix or suffix	Classical derivation or root	Implied colour	Chemical example
anil-	al-nil (Arab)	Deep blue	Aniline
arg-	argentum (L)	Silver	Arginine
aur-	aurum (L)	Gold	Aureomycin
-azur	azul (OSp)	Sky blue	Aplysioazurin
chloro-	chloros (Gk)	Yellow-green	Chlorophyll
chrom- or -chrome	chroma (Gk)	Coloured	Chromotropic acid
chryso-	chrysos (Gk)	Gold	Chrysin
citr-	citrus (L)	Lemon-coloured	Citruline
-cyanin	kyanos (Gk)	Blue	Anthocyanin
erythro-	erythros (Gk)	Red	Phycoerythrin
-flavin	flavus (L)	Pale yellow	Riboflavin
fulv-	fulvus (L)	Red-yellow	Fulvine
fusc-	fuscus (L)	Brown	Fuscin
haem- or heme-	haima (Gk)	Blood red	Haematin
indigo-	indikon (Gk)	Deep blue	Indigo
leuco-	leukos (L)	Colourless/white	Leucoprotein
lute-	luteus (Gk)	Yellow	Lutein
mela-	melas (Gk)	Black	Melanin
phaeo-	phaios (Gk)	Dark-coloured	Phaeophytin
porph(o)-	porphyros (Gk)	Purple	Porphyrin
purpur-	purpura (L)	Purple	Purpurin
-pyrrole	pyrros (Gk)	Fiery-red	Pyrrole
rhodo-	rhodon (Gk)	Rose-coloured	Rhodophyllin
rub-	ruber (L)	Red	Bilirubin
ruf-	rufus (L)	Red-brown	Anthrarufin
sepia-	sepia (L)	Brown-pink	Sepiapterin
stilb-	stilbein (Gk)	Glitter	Stilbene
verd- or virid-	viridis (L)	Bright green	Biliverdin
viol-	viola (L)	Purple-blue	Aplysioviolin
xanth-	xanthos (Gk)	Yellow	Xanthophyll

^a Arab = Arabic; Gk = Ancient Greek; L = Latin; OSp = Old Spanish.

Caution is needed, however, in using these names as indicators of *particular* colours. For example, porphyra of the Greek aristocratic toga gives rise in translation to purple; such a toga seen today would be described by most observers as crimson. Similarly, not all melanins are black as implied by the Greek melas. Given these limitations, however, the terminology is a fast way to recognize a coloured chemical.

1.7 Electronic structure of pigments

Whether or not a biological molecule is coloured, is determined by its structure, particular electronic, size of the molecule, solubility and elemental composition. Fortunately most natural pigments have several common features that immediately distinguish them from the larger number of colourless

compounds found in biological material. All biological material is composed of a limited range of elements within the periodic table. Within this narrow selection, pigmented organic compounds generally possess three features:

1. Almost all biological molecules are composed of no more than 17 elements within the periodic table. Of these, just four elements, H, C, N and O predominate.
2. Most pigmented compounds, particularly those other than yellow, contain either N or O, often both.
3. Most are relatively large molecules. Among the more common pigmented compounds, molecular weights range from about 200 (anthraquinones), 300 (anthocyanidins), 400 (betalaines), 500 (carotenoids) to 800 (chlorophylls).

Much greater molecular weights are of course found in pigment polymers such as melanins.

With this limited range of attributes, it is possible to restrict a discussion on the electronic structure of biological pigments to bare essentials (see Brown [3] for a more full explanation). The electrons in the outer shells over the surface of biological molecules, coloured or not, exist in distinct orbits. Each shell holds a finite number of electrons arranged to occupy the least energy-demanding orbits possible. Such electrons are described as being in a resting state. When irradiated with light of sufficient energy, the electrons in the outermost orbitals oscillate in resonance with light of sufficient energy, and are raised from the resting state to a higher excited state. After the excitation, as the electrons decay (relax) to the original resting state so the energy is released in a less energetic form, most commonly as heat. As biological molecules are composed of generally no more than four, perhaps five out of seventeen elements, almost all of the electronic orbitals participating in colour are from just three orbitals designated d, p and n. On excitation, one or more electrons within these orbitals may be raised to respectively d^* and p^* orbitals.

Transitions from d to d^* , which are characteristic of saturated hydrocarbons (with no double bonds), are the most energy demanding, requiring more energy than that provided by the visible part of the spectrum and so appearing colourless. Transitions from p to p^* as in unsaturated hydrocarbons (which contain double bonds) are somewhat less demanding energetically but are usually still beyond the energy levels provided by the visible part of the spectrum beyond the ultra-violet. The larger molecules at least may appear as pale yellow, a characteristic of many vegetable oils. The third transition n to d^* , characteristic of saturated hydrocarbons particularly with nitrogen (N) or oxygen (O) substitutions may also just appear in the visible part of the spectrum as pale yellow compounds, as in many fungal pigments. However, the least energy demanding are the n to p^* transitions found in compounds that are essentially unsaturated hydrocarbons with N or O substitutions (Table 1.3) and it is this group that provide the greatest range of pigmented organic molecules.