Quality Assurance and Safety of
Crops & Foods

Quality Assurance and Safety of Crops & Foods 2011, **3**, 28–39

ORIGINAL ARTICLE

Emerging and persistent issues with artificial food colours: natural colour additives as alternatives to synthetic colours in food and drink

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Keywords
additives; analytical methods; colours; food legislation; food safety; natural.

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Received 13 October 2010; Revised 29 November 2010; Accepted 29 November 2010.

doi:10.1111/j.1757-837X.2010.00087.x

Abstract

Introduction Natural colour additives are increasingly used as alternatives to synthetic colours in food and drink. This is partly a reaction to concerns on the safety of certain synthetic food colours by consumers and as a result of safety evaluations and industry requirements. *Objectives* This article provides an overview of the key scientific, technical, regulatory and socio-economic aspects of replacing synthetic food colorants with natural alternatives. *Methods* The different types of food colour additives are discussed with respect to their derivation, source materials and stability as well as the range of formulations designed to meet the technical demands of food manufacturer's applications, regulatory compliance and safety evaluation. The socioeconomic impact of replacing synthetic colours with natural alternatives is discussed with reference to four case studies. An overview on available methods of analysis is also given. *Conclusions* Natural does not necessarily mean good or safe; hence natural colours have purity specifications, usage restrictions and maximum permitted levels in line with other food additives. Direct replacement of synthetic colours with natural alternatives is not always straightforward and requires development of technologies to cover the full range of colour/foodstuff combinations. As a result, a range of suitable extraction and analytical methods for determining added natural colours in food and drink are requisite. However, while methods are available for certain colours in source materials and specific foodstuffs, they are by no means comprehensive and further research is required to develop and validate suitable methods to cover the entire range of colour additives permitted in the EU.

Scotter MJ (2011). Emerging and persistent issues with artificial food colours: natural colour additives as alternatives to synthetic colours in food and drink. *Quality Assurance and Safety of Crops & Foods*, **3**, 28–39.

Introduction

The food additive industry is growing at a rapid pace. Consumers throughout the world have created an increased demand for processed foods requiring one or more additive ingredients and less calorific foods requiring substitutes for (principally) fats and sugars. According to one source, there are some 2500 chemicals that function as food additives, which constitute some 5000 trade name products on a worldwide basis. Specialized food additives are continually being developed to satisfy the demand for specific technical effects.

Colour has been used historically as an indicator of both the quality and safety of harvested fruits, vegetables and other foods, and nowadays consumers expect processed foods to be coloured attractively and with shades that are typical of their product variety. The use of colour additives in foods (including beverages) is therefore important to both the food manufacturer and the consumer in terms of determining the acceptability of processed foods. Added colouring materials have several functions in the finished product; they assist in assuring batch-to-batch uniformity and help to reinforce colourings that are naturally present but are less intense than the consumer would expect. They also help to restore the original appearance of foods whose natural colouring intensity has been reduced or altered in some way by processing, and to provide appeal to otherwise uncoloured foods such as soft drinks and confectionery.

Food colour additive types

Food colour additives are generally categorized as artificial, natural and nature-identical types. Artificial colours generally refer to synthetic organic dyestuffs, which do not occur naturally in foods. Artificial colouring materials otherwise referred to as 'dyes' have a long history of use principally in the dyeing of textiles. Their use in foods also has a long history.

Many more synthetic or 'coal-tar' dyes have been synthesized in a range of colours, some of which were eventually permitted for use in foodstuffs. They were much brighter than contemporary naturally derived colouring materials, cheaper to produce, offered a wide range of shades and were highly stable when added to foods. Interestingly, it was in the production of these dyes that the first apparent toxic effects were observed rather than as a result of their consumption via coloured foods, hence the need to regulate the use of food colouring materials was identified.

Nature-identical colours are essentially defined as synthetically produced analogues of naturally occurring compounds such as *trans*-β-carotene. Commercial synthesis provides a useful way of producing pure colourings of consistent quality with more precise specifications of purity than their naturally derived analogues, e.g. β-Apo-8′-carotenal, β-Apo-8′-carotenoic acid ethyl ester and riboflavin (vitamin B_2). Commercial exploitation of chemically synthesized nature-identical colourings is unlikely to develop further because (a) synthesis is relatively expensive, (b) yields are generally low and (c) the commercial development of alternative natural sources of colourings is growing rapidly. The development of chemically modified colours such as the green copper chlorophyllins does provide scope for colouring materials, which have improved functional qualities. However, some of these compounds are manufactured for uses other than as colouring materials, e.g. riboflavin and β-carotene. The latter is a precursor of vitamin A and is recognized as an important functional food due to its role as a dietary antioxidant through its ability to scavenge singlet oxygen.

Natural colours are defined as materials derived from natural (usually edible) sources, using recognized and specified preparation methods. Natural colour extracts are usually purified and concentrated before use, e.g. carotenoids and anthocyanins to remove unwanted substances. This definition would exclude caramels manufactured using ammonia and its salts, and copper chlorophyllins, because both of these products involve chemical modification during processing using methods not normally associated with food preparation. Nevertheless, they are classified as natural colours as are vegetable carbon and the permitted inorganic naturally occurring colouring materials titanium dioxide, calcium carbonate and iron oxides. The consumption of naturally occurring food ingredients such as colours as an integral part of the diet is far in excess of the quantities added as food colourings. The most widely used natural food colour additives in Europe and North America are caramel, annatto (bixin and norbixin), anthocyanins (from various sources), beetroot red (betanines and vulgaxanthines), curcumin (turmeric) and cochineal (carmine and carminic acid). Each type of colouring material has inherent advantages and disadvantages with respect to stability and scope of application. For example, while carmine (from cochineal) is more expensive to produce than beetroot red, this disadvantage is offset by its excellent stability and relatively higher tinctorial strength. Other natural colours such as natural β-carotene, chlorophyll (and chlorophyllins), lycopene and lutein have gained in popularity as alternative sources and associated extraction techniques have been developed. On a global scale, natural colours are generally more widely permitted in foodstuffs than artificial colours.

The number of permitted artificial colours has gradually reduced over the last 30 years as consumers have expressed a preference for food products and food components, especially additives, of natural origin. The range of natural colours available to the food, cosmetic and pharmaceutical industries has thus increased. This in turn has led to an increased awareness of the many attributes of natural colours; especially how these may be exploited to the advantage of both the consumer and the food manufacturer. The various sources of the major food colouring materials have remained largely unchanged. However, in recent years manufacturers have concentrated their efforts on maximizing yields from both conventional and novel sources, e.g. carotenes from *Dunaliella* algae and palm oil, lycopene from tomatoes and *Phycomyces* fungi, lutein from *Tagetes* (marigold) and alfalfa, and anthocyanins from various plants fruits and seeds such as chokeberry, red cabbage and radish. Several manufacturers are supporting research into *in vitro* production of natural colouring materials and in the selection of suitable plant cultivars, which offer improved yields, disease resistance and lower levels of odours. Moreover, research on the extraction of established and novel natural colouring materials from food and agricultural side products or wastes provides new opportunities, e.g. lycopene from corn fibre material (Food Navigator, 2004).

There is also an established interest in the role of natural food colours as functional foods. Several carotenoid compounds have been implicated in such roles, e.g. α- and β-carotenes (Britton *et al.*, 1995; Wargovitch, 1997) and lycopene (INFCOL, 1996). Curcumin (in turmeric) has been reported to exert an antitumorogenic effect in mice (INFCOL, 1996) and has also been reported to treat a variety of disorders, which has generated a great deal of scientific interest in their pharmacological properties and biological effects (Nurfina, Reksohadiprodjo, Timmerman, Jenie, Sugiyanto & Van der Goot, 1997; Majeed, Badmaev, Shivakumar & Rajendran, 2000). Various flavanoid compounds including the anthocyanin colours, have been identified as potent antioxidants and have been reported to have cardioprotective effects (Kinsella *et al.*, 1993; Cook & Samman, 1996).

Natural colour formulations

Food colour manufacturers are able to offer a complete spectrum of natural and naturally derived colours through expertise in formulation, and are able to provide easy to use and stable forms that are suitable for use in a wide range of applications. In addition, they offer colours that are free from other additives such as sulphur dioxide, as well as those that are acceptable to a wider range of communities and in accordance with specific dietary or ceremonial laws, e.g. kosher. Food colour manufacturers therefore continue to develop new technologies to meet customer needs and they are very proactive in offering technical and application support for the replacement of synthetic dyes with natural colour alternatives. Formulations can be produced using complex high pressure milling and processing which give enhanced light-stable colours (Overseal, 2010). Other formulations offer excellent dispersibility and stability to heat, light and oxidation and can be used in a variety of applications. For example, dispersible emulsions have been developed for carotenes, which overcome the oxidative colour fading which has previously limited their application. Micro-emulsions have been developed for clarity along with enhanced stability to heat, light and oxidation. Patented encapsulation technologies have been developed to meet the requirements of modern food processing, i.e. improved stability to light, pH and oxidation, reduced colour migration, extension of natural colour shades and increased colour intensity and brightness. These are available as water-dispersible forms of oil-soluble pigments (Hansen, 2010). Hydrocolloid complexation and cyclodextrin inclusion have all been used to promote stability and dispersibility of oil- and water-soluble colour formulations (Henry, 1992; INFCOL, 1996).

While the colouring of foodstuffs with natural products is usually viewed as a healthier option to synthetic dyes, the development of natural colour formulations may also require the use of other food additives such as antioxidants, emulsifiers and carriers, i.e. 'additives within additives'. It is arguable therefore that the removal of a synthetic dye (E-number) from a foodstuff ingredients list is not necessarily a healthier option if it is replaced by a natural colour along with one or more E-numbers to aid its application. Another issue that consumers have with natural colours is that in some cases, the natural colour source is not in itself regarded as a foodstuff. Cochineal, for example, is derived from an insect.

The insolubility of some natural colours in water, moderate solubility in fats and oils and susceptibility to oxidation impede the direct use of the relatively coarse particles, which also limits their colouring ability. Processes have been described for the production of nanoparticulate active substance dispersions to overcome these limitations (BASF, 2010). The technological requirements for these formulations are particularly high, for example carotenoid use in the colouring of aqueous media. However, the nanoparticulate nature of the products are stated to realize a wide diversity of colouring properties associated with improved bioavailability. A molecular-disperse solution of a carotenoid is prepared with or without an emulsifier and/or edible oil, in a volatile, water-miscible organic solvent at elevated temperature, with the addition of an aqueous solution of a protective colloid. The hydrophilic component is then transferred into the aqueous phase leaving the hydrophobic phase of the carotenoid as a nanodisperse phase. Examples of carotenoid permitted food colourings which can be used in this type of product are well characterized, widely available and occur in both natural or synthetic forms, e.g. β-carotene, bixin, β-apo-8′-carotenal, the ethyl ester of β-apo-8′-carotenoic acid and lycopene.

The applications of nanotechnology in the food sector are only recently emergent, but they are predicted to grow rapidly in the coming years. According to Chaudry *et al.* (2007), many of the world's largest food companies are reported to have been actively exploring the potential of

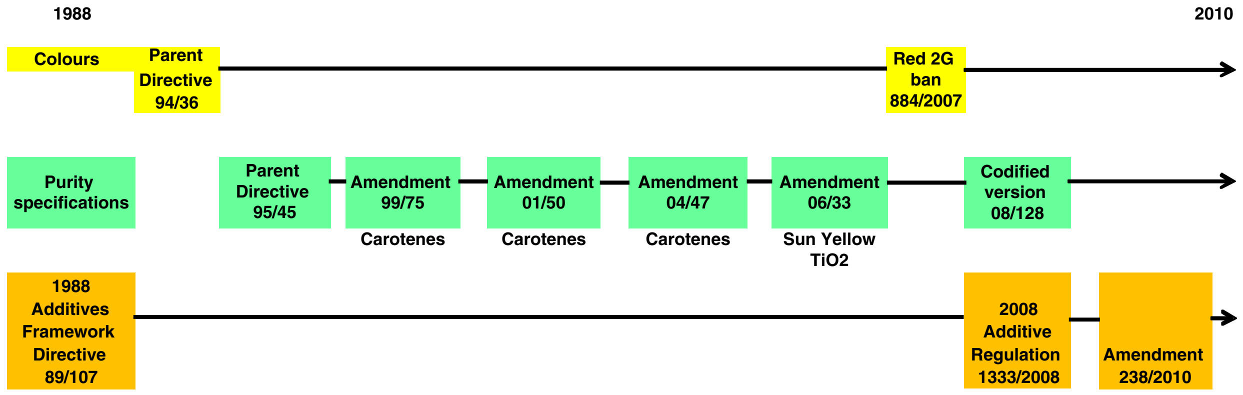

Figure 1 Chronology of EU food colours legislation.

nanotechnology for use in food or food packaging. Among many other food additive functionalities, applications in this area already span development of improved colour. However, the rapid proliferation of nanotechnologies in a wide range of consumer products has also raised a number of safety, environmental, ethical, policy and regulatory issues. The interactions of nanosized materials at the molecular or physiological levels and their potential effects and impacts on consumer's health and the environment are the main concerns, which arise from the lack of knowledge. The nanotechnology-derived foods are also new to consumers and it remains unclear how public perception, attitudes, choice and acceptance will impact the future of such applications in the food sector.

Colour legislation and purity specifications

Over the last 100 years there have been several key changes to national and international legislature covering food and drugs, where those parts dealing with legislation of food colours have been essentially prescriptive. These controls have been mirrored by an increasing interest in the toxicity of synthetic dyes. Different countries within and outside Europe have therefore evolved their own lists of dyes that are permitted for use in foodstuffs. Within the EU, before 2008 food additives legislation was complex and amendments were by co-decision of the European Council and Parliament. Three separate Directives were controlled food colours (EU, 1994a), sweeteners (EU, 1994b) and additives other than colours and sweeteners (EU, 1995). These Directives prescribed positive lists of approved additives along with limitations on their use. Regulation 1333/2008 revoked and re-enacted on a traditional basis certain (but not all) provisions of the three separate Directives and introduced a comitology route for amendments to the Annexes of those Directives (EU, 2008a). By June 2011, additives currently approved under the three earlier Directives will be transferred to the relevant Annexes of Regulation 1333/2008, at which point compliance with the provisions of the Regulation will be required instead of compliance with the surviving provisions of the Directives. Regulation 1333/2008 refers to general purity criteria for colours as laid down in Commission Directive 95/45/EC, which has been amended several times and eventually superceded by a codified Commission Directive 2008/128/EC (EU, 2008b). The specifications do not include any supporting qualitative or quantitative methods of analysis. The current EU list comprises 42 different colouring materials permitted for use in food, of which 15 are synthetic organic dyes, the remainder designated as natural or nature-identical colours. Figure 1 summarizes the development of EU legislation on food colours.

Food additives derived from natural sources are by their nature, less easy to define than synthetic additives and therefore present a number of problems in the establishment of meaningful specifications. The specifications in 2008/128/EC comprise listings of names and synonyms, followed by

- a definition of the product (i.e. pure compound, oleoresin, aqueous solution, etc.), chemical name(s) and formulae, total assay limit;
- a description and functional use;
- a listing of characteristics such as solubility, melting range, colour reactions and chromatographic identification tests;
- listings of purity and identification tests and criteria *e.g.* residual solvents and heavy metals, and assay tests generally based on spectrophotometric methods.

Safety evaluation

All countries need to have access to reliable risk assessment of chemicals in food, but not all have the expertise and funds available to carry out separate risk assessments on large numbers of chemicals. Under the auspices of the European Commission (EC), this responsibility lies with the European Food Safety Authority (EFSA), whose role is to assess and communicate on all risks associated with the food chain including food additives. The EFSA Panel on food additives and nutrient sources added to food deals with questions of safety in the use of food additives, nutrient sources and other substances deliberately added to food, excluding flavourings and enzymes. EFSA are responsible for evaluating the various data in order to calculate acceptable daily intake (ADI) values for all additives (Table 1), which is the amount of an additive that can be taken in daily over a lifetime without damaging health. It is expressed in relation to body weight (bw) in order to allow for different body size, such as for children of different ages. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) performs a similarly vital role in providing a reliable and independent source of expert advice in the international setting, thus contributing to the setting of standards on a global scale. To date, JECFA has evaluated more than 1500 food additives. The ADI values are then used to calculate the maximum permitted levels of additives in specific foodstuffs.

In line with all food additives, food colours colour manufacturers and the food industry have to demonstrate not only a technological case for need for the colour (or a particular formulation) but it must also undergo stringent toxicity testing before consideration for inclusion on the permitted list. However, the degree of safety evaluation required of a synthetic colouring materials designed for food use is currently prohibitively expensive and the less stringent testing designated for natural compounds *per se* has obvious economical attractions. As part of the substantial data package required for the approval of a new additive, industry must provide sufficient information on the potential uses and levels of use for their respective competent authorities. As part of the assessment of the continuing acceptability of individual additives, estimates are made of their toxicology and potential intakes, and EFSA then asked to advise the EC whether the use of any particular additive needs to be restricted.

The interpretation of the test results and formal safety assessment carried out by EFSA ensures that all the tests have been carried out in accordance with the published guidelines. It also makes sure that the results and conclusions of the studies are scientifically valid. It is not unusual for a complex case that requires new trials and additional data to take several years, which has obvious economic implications for the food industry. Moreover, the safety of all additives will be reviewed every 10 years as a matter of routine and the safety of any additive can be reviewed again in the light of new toxicological data that might have become available. EC law also requires additive manufacturers to demonstrate that there is a genuine need for their product. In the 2010 EFSA Management Plan Activity 2 (evaluation of products, substances and claims subject to authorization), the planned actions include 25 opinions on applications with respect to the re-evaluation of food colours.

Article 12 of EC/1333/2008 states that:

> When a food additive is already included in a Community list and there is a significant change in its production methods or in the starting materials used, or there is a change in particle size, for example through nanotechnology, the food additive prepared by those new methods or materials shall be considered as a different additive and a new entry in the Community lists or a change in the specifications shall be required before it can be placed on the market.

The most significantly aspect related to the use of nanoscale food additives may be perhaps in the re-evaluation of safety assessment. Whether or not developments in nanotechnology constitute new scientific information may be for EFSA to assess in the first instance. The current EU purity specification for TiO_2, for example, does not prescribe criteria related to particle size, which clearly is a principal issue with nanotechnology. This additive was last evaluated in 1977 but is scheduled for re-assessment in 2010.

In cases where EFSA (or JECFA) consider that the use of an additive is safe for use over the period of time required to generate and evaluate further safety data, they will assign a temporary ADI. There is also a category of ADI 'Not Specified', which is applied to additives generally of very low toxicity, where the maximum possible dietary intake of the additive arising from its use at levels necessary to achieve the desired effect is not considered to represent a hazard to health. In some cases the ADI's allocated by EFSA and JECFA may differ. This can be because expert groups differ on judging how each toxic effect should be weighted and in deciding which no-effect levels and safety factors to apply, but often it is simply due to evaluations being carried out at different times and hence are based on different data sets. At present, all ADI's used by national and international

Table 1 Numerical ADIs for permitted food colours

Name	E-number	EFSA ADI[1] (mg kg^{-1} bw)	JECFA ADI[3] (mg kg^{-1} bw)
Synthetic colours			
Tartrazine	102	7.5	0.7.5
Quinoline yellow	104	0.5	0–10
Sunset yellow FCF	110	1[T]	0–2.5
Carmoisine, azorubine	122	4.0	0–4
Amaranth	123	0.015	0–0.5
Ponceau 4R	124	0.7	0–4
Erythrosine	127	0.1	0–0.1
Allura red AC	129	7.0	0–7
Patent blue V	131	15.0	NA
Indigo carmine, indigotine	132	5.0	0–5
Brilliant blue FCF	133	10.0	0–12.5
Green S	142	5.0	NA
Black PN, brilliant black BN	151	5.0	0–1
Brown FK	154	NA	NA
Brown HT	155	1.5	0–1.5
Litholrubine BK	180	NA	NA
Natural colours			
Curcumin	100	3	0–3
Riboflavin	101(i)	Acc	0–0.5
Riboflavin-5-phosphate	101(ii)	Acc	0–0.5
Cochineal	120	5[6]	NA
Carminic acid			0–5
Chlorophylls	140(i)	Acc	NL
Chlorophyllins	140(ii)	Acc	[7]
Copper complexes of chlorophylls	141(i)	15	0–15
Copper complexes of chlorophyllins	141(ii)	15	0–15
Caramel class I	150a	Acc	NS
Caramel class II	150b	200	0–160
Caramel class III	150c	200	0–200
Caramel class IV	150d	200	0–200
Vegetable carbon	153	Acc	DP
Mixed carotenes from plants[2]	160a(i)1	Acc	Acc
Mixed carotenes from algae[2]	160a(i)2	Acc	NA
β-Carotene synthetic[2]	160a(ii)1	Acc	0–5
β-Carotene from *B. trispora*[2]	160a(ii)2	Acc	0–5
Annatto (bixin/norbixin)	160b	0.065	0–12[4] 0–0.6[5]
Paprika extract (capsanthin/capsorubin)	160c	NA	Acc
Lycopene	160d	0.5	0–0.5
β-Apo-8′-carotenal	160e	5	0–5
β-Apo-8′-carotenoic acid ethyl ester	160f	5	0–5
Lutein	161b	1	0–2
Canthaxanthin	161g	0.03	0–0.03
Beetroot red	162	Acc	NS
Anthocyanins	163	Acc	0–2.5[8]
Calcium carbonate	170	Acc	NL
Titanium dioxide	171	Acc	NL
Iron oxides and hydroxides	172	Acc	0–0.5

1. Most originally from SCF-COM(2001)542; 2. prescriptive differences cf. JECFA; 3.taken from JECFA monographs; 4. bixin; 5. norbixin; 6. no distinction made between cochineal and carmines; 7. no distinction for chlorophyllins; 8. grape skin extract.

Acc, acceptable; DP, decision postponed; NA, not allocated; NL, not limited; NS, not specified; T, temporary; EFSA, European Food Safety Authority; JECFA, Joint FAO/WHO Expert Committee on Food Additives.

authorities are based on the highest intake in mg kg^{-1} bw day^{-1}, which does not give rise to observable adverse effects. The fact that an ADI can be developed for a substance does not, however, mean that its use in food will be automatically permitted. It is a matter for EFSA to decide firstly whether there is a demonstrable need for the additive, and secondly whether it is necessary to place restrictions on the use of an additive to ensure that dietary exposure to it remains within acceptable limits as defined by the ADI. In some cases, such restrictions may make it impractical to use the additive at all. Interestingly, and perhaps due to market forces, there is an increasing tendency to categorize additives as natural or artificial and to make assumptions about their safety accordingly. However, there is no inherent reason why chemicals present in nature or derived thereof should be safer than any others. EFSA and JECFA advise that all additives, whatever their origin, need to be examined for both need and safety-in-use. Table 1 summarizes the EFSA and JECFA ADIs for permitted food colours.

The EC therefore has a continuing need for reliable, up-to-date information on food additive intake to:

- determine whether the dietary intakes of additives remain within safe limits;
- identify the size and nature of population groups that may have high intakes of specific additives or groups of additives;
- monitor the effects of economic, technological or other developments on the use of additives and hence on intakes; and
- aid the development of sound food policies which enable the maximum to be derived from the use of food additives without prejudice to the health of the consumer.

Food colours from natural sources tend to exhibit variable composition because of the inherent variability of their source materials and their different methods of extraction. There is therefore a requirement to continually improve specifications and to have available suitable analytical methods for natural food colour additives because their consumption is both widespread and increasing. Specific dietary advice and other strategies to ensure that consumers can maintain a safe and adequate diet in terms of additive intake may only be established using relevant scientific knowledge. Consequently, there is a clear need for analytical methods to support the purity specifications, provide intake data on additives and enforce EU Regulations.

Many of the ADI's for natural and nature-identical colourings (Table 1) are designated as 'acceptable' due to their historical use as food ingredients. However, once they have been isolated from their source materials, many natural colouring materials are particularly susceptible to oxidation, photo-induced degradation and isomerization, and may be exposed to any number of agents, which may affect their stability. The processing of natural products may give rise to various artefacts as well as degradation products, which may be carried through to the final colour formulation and thence into a foodstuff where they may be considered undesirable. Moreover, once added to a foodstuff, a colouring material may be further 'processed', e.g. by cooking or by mixing with other foods which may affect its stability depending upon the stabilizing effects of the food matrix. It is therefore necessary to:

- investigate the various manufacturing processes where degradation may occur;
- understand the underlying bases of stability, how and when they occur and to what extent;
- determine the consequences of degradation product contamination of foodstuffs.

Suitable analytical methods are required in order to carry out surveillance for additives in food, especially those for which no suitable methods of analysis currently exist, and to ensure the ADIs are not exceeded, especially by young children. In order to build on the systems already being used, related research work on the fate of colour additives in food must also be carried out. This is usually achieved through the development and application of analytical methods to the measurement of additives and their degradation products in foods.

Socioeconomic issues

The socioeconomic impact of replacing synthetic dyes with natural colour alternatives is discussed below with reference to four examples.

Red 2G

Before 2007, the synthetic dye Red 2G (E128) was only permitted to be used in specific meat products, namely breakfast sausages with a minimum cereal content of 6% and burger meat with a minimum vegetable and/or cereal content of 4%. Following an EFSA evaluation of Red 2G, legislation is now in force which bans its use in food in the EU (EU, 2007). EFSA's evaluation showed that in laboratory tests, Red 2G may have the potential to damage the genetic material in cells and cause cancer in animals. The UK industry confirmed that Red 2G was used in a small percentage of burgers and sausages on sale. They also noted that some companies manufactured seasonings to be sold to producers of sausages and burgers who then used this as one of their ingredients, because Red 2G was included in the seasoning mixture. During a stakeholder consultation, the UK Food Standards Agency (FSA) issued an explanatory memorandum including a regulatory impact assessment detailing the likely costs and benefits of the ban. Among those most likely to be affected were food producers manufacturing or importing those specific categories of sausages and burgers, and companies manufacturing or importing seasonings containing Red 2G to be used for the manufacture of these products (as seasonings with Red 2G have no alternative use). Companies that manufactured or imported Red 2G were also likely to be affected, though there are alternative industrial uses for this colour. The UK FSA considered the ban would have no impact on racial equality, social or environmental issues.

The UK FSA stated that to have done nothing would have provided no incremental benefit and no consumer protection because the requirements of the Commission Regulation could not have been enforced. The United Kingdom would not have been able to fulfill its Community obligation to enforce the Commission Regulation, which would have left the United Kingdom open to infraction proceedings by the Commission for failing to comply with these requirements. By banning the use of Red 2G, consumer protection was maintained by allowing for enforcement and sanctions if Red 2G was continued to be used in food. However, there were costs incurred by producers of the affected types of sausages and burgers who continued to hold stock of seasoning containing Red 2G, as well as by seasoning manufacturers. The producers were not permitted to use any remaining stock of seasonings containing Red 2G, or any stock of packaging/labelling referring to Red 2G. The consequence of this was a short period of disruption to production as companies arranged for replacement seasoning mixes. Similarly, seasoning manufacturers effectively lost the value of any stocks held.

Other costs were incurred by industry because many consumers wished to avoid food products containing Red 2G. Retailers had the option of returning products already supplied and requesting alternatives and some companies may have been able to offset some of these costs through insurance. Seasoning mixes were also used by some independent butchers to produce the specific sausages and burgers affected by this legislation. Because the Regulation was applied uniformly across all food producers, the FSA did not envisage any impact on competition and understood that alternative red food colouring agents exist for those food producers to whom this colour is important. It is likely that alternative natural red colours such as carminic acid and paprika extract (capsanthin and capsorubin) will be used as alternatives to Red 2G. Mercadante *et al.* (2010) has recently used the natural pigments norbixin (from annatto), lycopene and β-carotene to replace sodium erythorbate as antioxidant in sausages, which have an inevitable secondary colouring effect.

The Southampton study

Since the publication of the Southampton study (McCann *et al.*, 2007), the issue of synthetic colours and the possible link to hyperactivity in children has remained in the media spotlight. Following a request from the EC, the EFSA Panel on Food Additives, Processing Aids and Food Contact Materials was asked to assess the results of the study and provide an opinion on the findings, taking into account other available scientific literature in the related area. The Panel concluded that the Southampton study provides limited evidence that the two different mixtures of the colours (and sodium benzoate) tested had a small and statistically significant effect on the test subjects. Moreover, because mixtures and not individual additives were tested, it was not possible to ascribe the observed effects to any of the individual compounds. The Panel therefore concluded that the findings of the study could not be used as a basis for altering the ADI of the respective food colours.

Following the Southampton study, the UK FSA held a series of meetings with stakeholders and in the light of the EFSA opinion, called for a voluntary ban of the 'Southampton six' artificial colours in 2008, with a view to their removal by 2009. This was in line with parallel action in the EU to phase the colours out. European Parliament ministers voted subsequently that products containing the six synthetic colours in question should carry a warning label by July 2010. However, a warning label stating 'may cause hyperactivity' on their products is not an attractive prospect for manufacturers. It was therefore predicted that many manufactures will voluntarily remove synthetic colours, and switch to natural alternatives. In late 2009, EFSA lowered the ADI for three of the Southampton colours; however, none of the scientific reasons given are associated with hyperactivity. Nevertheless, this could possibly lead to future restrictions on the range of foods in which they are permitted and reduction in the maximum permitted levels.

Lycopene

Directive 94/36/EC on colours for use in foodstuffs authorizes in Annex V, Part 2 the use of the colour E160d lycopene from red tomatoes in certain foods singly or in combination with some other colours up to the maximum levels specified, and in Annex III in jams, jellies and marmalades (EU, 1994a). Following a risk assessment carried out by the EFSA on lycopene, the EC proposes to amend currently permitted levels for lycopene.

Before an amended Directive comes into force there will be a transitional period, the length of which is yet to be agreed, to enable manufacturers to adapt to the new levels. While the proposed changes to two foodstuff categorizations, the proposed maximum levels of lycopene in foodstuffs are significantly lower than the currently permitted levels; in one case 50 times lower. Analytical methods for lycopene must therefore be able to quantify at or below these levels, e.g. 5 mg kg^{-1} in flavoured processed cheese (previously 100 mg kg^{-1}).

Spirulina

During 2005, in response to concerns over artificial additives Nestlé-Rowntree made a strategic decision to remove all synthetic colourings from its entire range. As an indication of the response by consumers, the company reported an increase in sales of ca. 9%. During 2006, the company removed the permitted colourant brilliant blue (E133) from Smarties® (Nestlé UK Ltd, Croydon, UK) replacing this with a white one while a suitable alternative blue colour was sought. It has been estimated that 17 000 Smarties® are consumed every minute in the United Kingdom. Incidentally, Brilliant Blue was not included in the Southampton study. Following a 3-year period of development, the company reintroduced the blue Smarties®, coloured with an extract of the blue-green algae *Spirulina*, a permitted health food ingredient. However, *Spirulina* is among 10 food colouring ingredients that have recently come under legal scrutiny. The UK FSA has been asked by the EC to examine the 10 substances with a view to establishing their regulatory status. To this end UK stakeholders were consulted and feedback has now been received. The FSA has also contacted European trade associations for their views, to be submitted by the end of May 2010. A report will then be submitted in the autumn before a meeting of the Commission's working group of food additive experts from Member States, to decide how to proceed. The overriding issue for the working group to consider is how the substances in question should be defined, i.e. should they be defined as naturally coloured legal food ingredients (which do not require E-numbers), or as food colour additives, which do require E-numbering and must therefore be pre-approved. This is an important distinction because the blue colour has been selectively extracted from its natural source, but the definition of selective extraction has not itself been agreed; hence legal clarity is sought by food and drink manufacturers. The other colouring materials to be examined are beetroot, black carrot, orange carrot, elderberry, gardenia, hibiscus, nettle extract, paprika, pumpkin, red cabbage, safflower and turmeric. Those deemed to be food additives will require approval as set out in EC Regulation 1331/2008. *Spirulina* is currently used in several own-label bakery and confectionery products, while many of the other substances on the list are widely used in food and drink products sold in the United Kingdom.

Outcomes and Consequences

These issues add more weight to the already existing trend of increased consumer demand for natural alternatives to synthetic additives. Consequently, food manufacturers are moving towards increased usage of approved natural colours, especially in children's food. Furthermore, natural colours may be listed under ingredients by name rather than by E-number, making them ideal for products with a 'clean label' declaration. The downside to this practice is that it can lead to consumer confusion. Nevertheless, even before the publication of the Southampton study two major UK retailers decided to ban artificial colours from their own-label ranges, including ready meals. To date, a significant number of UK manufacturers, retailers, caterers and restaurants have declared voluntary withdrawal of the six colours from their product ranges. The UK FSA provides a regularly updated list of these product ranges on their website (FSA, 2010). The challenge this presents to the natural colour industry, is to produce natural colour alternatives to synthetic dyes that give manufacturers the required processing and shelf-life stability. They also require as an extensive range of shades that depict the flavours that the consumer desires, and that are as good as the standards set by traditional colouring systems in terms of depth and vibrancy of colour. Validated analytical methods are therefore needed to fulfill the EU obligation to monitor the expected increase in usage and intakes of natural colours that will result.

Even so, it is arguable that there will always be a market for synthetic colours, mainly because the market is so diverse and there is a constant need for cost effective, readily available foods such as 'value' brands coupled with a major increase in restaurants, where ingredients are not declared (Downham & Collins, 2000). According to one source

(Crowley, 2008), the value of the international colours market was estimated to be ca. US$1.15 b (€731 m) in 2007, the most important single colour variety being caramel with sales worth over US$353 m (€224 m). Natural colours accounted for 31% of the market share but it is predicted that they will overtake synthetic colours in the medium term. According to NATCOL (2010) the European market contributes 40% of the global colours revenue.

The overall colour market is expected to grow in line with technological and sociological changes that will lead to an overall increase in processed foodstuffs. The population of the world is increasing quickly along with the global marketplace, especially in Asia. European and American colour manufacturers have invested significantly in the Chinese and Indian economies, not just as a means of securing raw materials but also by investing in the construction of colour production plants in order to meet local demands. Because natural colours are derived from fruit, vegetables and other organic sources, they are more vulnerable to supply issues that synthetic dyes. Supply of natural colour source materials is susceptible to weather-related issues hence climate change may have a significant future impact on natural colour availability. There is no doubt that the rapidly increasing demand for natural colours is prompting manufacturers to invest continually in research and development to broaden the scope of application of their products.

Replacing a synthetic colour with a natural alternative is, however, not straightforward, largely because natural colours are usually less vivid than synthetics and an exact colour/hue match may not always be possible, although the range of shades available can be widened considerably by blending (NATCOL, 2010). Natural colours are generally less stable than their synthetic counterparts and can often interact with other food ingredients. Anthocyanins, for example fade rapidly at neutral pH values. They are more stable under acid conditions but appear increasingly red, rather than blue or purple at low pH. One of the main issues in overcoming stability and thereby application problems is that of cost. This applies not only to the cost of the colours themselves, but also to the costs associated with product development and shelf-life testing. The cost of natural colours is usually higher than that of synthetic dyes and may fluctuate depending upon the availability of source material(s) from year to year, but suppliers argue that in terms of overall product costs the difference is negligible (NATCOL, 2010). Nevertheless, the benefits of a 'no artificial colours' statement on the ingredients list is thought likely to outweigh development costs in the longer term. Thus, in this context the notion of colouring food with food without compromising on taste and appearance is appealing to all stakeholders.

Analytical methods for natural colour additives

The available literature on methods of extraction and analysis for approved natural colours in food and drink has been reviewed recently in order to inform and direct future research in this area with special consideration given to selectivity, sensitivity and validation, as well as their applicability for use in surveillance and in an enforcement role (Scotter, 2010). Nine natural colour classes are covered: 1. curcumin (E100), 2. riboflavins (E101i–ii), 3. cochineal (E120), 4. chlorophylls – including chlorophyllins and copper analogues (E140–141), 5. caramel classes I–IV (E150a–d), 6. carotenoids (E160a–f, E161b, E161g), 7. beetroot red (E162), 8. anthocyanins (E163) and 9. other colours—vegetable carbon (E153), calcium carbonate (E170), titanium dioxide (E171) and iron oxides and hydroxides (E172).

A large number of analytical methods are available for colouring materials with relatively widespread occurrence and use, e.g. carotenoids and anthocyanins, which are concerned either with the analysis of source materials for colour component profiling and/or strength, e.g. anthocyanins or for measurement of levels for nutritional purposes, e.g. β-carotene which has vitamin A activity and riboflavin (vitamin B2). Furthermore, there are a relatively large number of methods available on the analysis of carotenes, lycopene, lutein and paprika extract (capsanthin and capsorubin), compared with relatively very few for β-apo-8′-carotenal, ethyl ester of β-apo-8′-carotenoic acid and canthaxanthin. However, many carotenoid methods possess sufficient scope for the simultaneous analysis of several carotenoids. The identification and measurement of all carotenoid geometric isomers is also very important and many suitable methods for their detection and separation are available.

The analysis of anthocyanins and of chlorophylls/chlorophyllins is complicated by the complexity of their chemistries. There are many literature methods available for the identification and measurement of anthocyanins based either on the analysis of the intact anthocyanins, or on the production and measurement of the parent aglycones. However, very few have been developed specifically for processed foodstuffs. A number of methods have been developed for chlorophylls/chlorophyllins analysis in recent years, which require broadening in scope to cover all foodstuffs permitted under the Regulations. Very few methods have been suitably validated.

Methods for the analysis of curcumin are largely restricted to biomatrices other than food, which reflects the interest in its nutritional properties. Some of these have been reasonably well validated but along with a small number of methods for food analysis, require adaptation and must encompass all three curcuminoid analogues. Methods for the determination of cochineal in source materials are reasonably well established whereas methods for foods are sparse. Further development of methods is required in order to modernize and broaden the scope. A similar situation exists for beet red but methods for its analysis are poorly established and should include not only the main beet colour principles but also degradation products.

Caramels have the greatest use by far as added food colours (and flavours) but their chemistry is not well understood, complicated further by their classification into four types. As a consequence methods for the determination of caramels are empirically based, i.e. are reliant on the measurement of unidentified but characteristic marker peak. While a reasonable number of well defined but limited methods are available, there is a clear need for the application of new analytical technologies to the development of methods for caramel.

There are very few methods available on the determination of titanium oxide in foods, and none for the direct determination of calcium carbonate and iron oxides in foods. No methods are available for the determination of vegetable carbon in foods. Methods based on elemental analysis are likely to be the best approach for these colours but they will require development and validation.

Acknowledgement

This work was funded by the MoniQA Network of Excellence (contract no. FOOD-CT-2006-36337) within the sixth framework, Work Package 4: Harmonization and standardization of analytical methods, Food Additives and Processing Toxicants Working Group.

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