

## 2nd International **MoniQA** Conference

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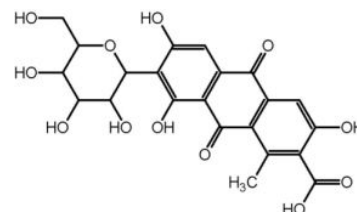
„Emerging and persisting food hazards:  
Analytical challenges and socio-economic impact“



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# Analytical Methods for Natural Colour Additives

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# Overview

- Background
  - Food colour additive types
  - Food colour formulations
  - Safety assessment - ADIs
  - Socioeconomic issues - 4 examples
- The review
  - Structure
  - Available literature
  - Outcomes



## Food colour additive types

- Synthetic (artificial)
- Azo dyes, triarymethane etc
  - e.g. Southampton 6
- Nature identical
  - e.g. *Trans*- $\beta$ -carotene, riboflavin
- Natural
  - Carotenoids, anthocyanins, chlorophylls, caramels, beet red, cochineal, others

## Food colour formulations: considerations

- Stability (heat, light, oxygen etc)
- Wide application range
- Free from other additives?
- Labelling issues (name or E number?)
- Dietary / religious acceptability
- Some patented technologies including nanotechnologies

# Safety assessment

- EFSA rolling programme
- JECFA
- ADIs
  - 14/16 artificials have numerical ADIs
  - 11/29 naturals have numerical ADIs
- Need reliable methods to monitor intake

## Socioeconomic aspects

- Move towards replacing synthetics with naturals
- Natural is better?
- Consumer acceptability
- Voluntary withdrawals (UK)
- 4 examples

# Comparison of attributes



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Artificial colours	Natural colours
Obtained by chemical synthesis. Year round availability.	Obtained from natural sources (few synthesized) but some chemically altered. Includes caramels and inorganic types. Seasonal availability but high potential for new sources including agricultural by products.
Low production cost.	Relatively high production cost.
Starting materials and side products present; few multiple colour principles. Purity specifications relatively simple.	Co-extractives present requiring purification; multiple colour principles; isomerism. Purity specifications often complex.
Generally good stability to light, oxygen, pH and heat; some technical formulation required including lakes. Problems include decolourization by ascorbic acid, microbial attack, precipitation by metal ions, reaction with proteins.	Wide variation in stability, require higher levels of technical formulation. Many prone to oxidation, isomerization and pH sensitive. Often require additional additives e.g. emulsifiers/stabilizers/antioxidants.
Water soluble or require dispersion technology.	Water and oil-soluble formulations/dispersions
Good colour uniformity and wide colour range.	Reasonably good colour uniformity; tend to be less intense and colour range not as wide but improving.
Virtually no microbiological contamination.	Some risk.
Consumer acceptability poor.	Consumer acceptability very good.
Labelling: E-No or chemical name.	Labelling: E number not often used as trivial names more acceptable.
Health concerns: perceived as risky. No added health benefits.	Health concerns: perceived as safe. Some clear benefits to health.
14/16 have numerical ADIs.	11/29 have numerical ADIs.
Analysis fairly straightforward, few dyes give problems. Some matrices difficult.	Requires different analytical methods depending upon chemical type; multianalyte methods required for many. Inorganic colours problematic. Some methods empirical.

## Example 1 – Red 2G

- Limited use in meat products prior to 2007
- Maximum level 20 mg/kg
- EFSA evaluation: genotoxic carcinogen → BAN
- Impact (costs incurred):
  - Food manufacturers/importers sausages and burgers
  - Companies manufacturing or importing Red 2G
  - No impact on social, economic or racial issues (UK FSA)
  - Natural alternative(s)?

## Example 2 – Southampton study 2007

- 6 synthetic dyes + benzoate; hyperactivity in children (2 different *mixtures*)
- EFSA evaluation: limited evidence therefore no basis for altering ADIs
- UK FSA Stakeholder meetings → Voluntary ban on 6 dyes with view to removal by 2009
- EP Ministers; Warning labels by July 2010
- Late 2009 EFSA lowered ADI for 3 of the dyes

## Example 3 - Lycopene

- 94/36/EC Annex III (jams, jellies and marmalades); Annex V part 2 50-500 mg/kg
- EFSA risk assessment 2010; intake high
- EC propose amendment to max levels
- Translational period to enable industry adaptation
- New levels significantly lower (up to 1/50)
- MoAs must be capable of e.g. 5mg/kg in processed cheese (was 100 mg/kg)

## Example 4 - Spirulina

- 2005 nestle-Rowntree remove all synthetic colourings → 9% increase in sales
- 2006 removed Brilliant Blue from Smarties®
- 17 000 consumed per minute in UK
- Following 3-year development period introduced Smatries® coloured with *Sprulina* extract
- Doubts over regulatory status ingredient/additive: UK FSA to examine on behalf of EC
- European trade associations views by end of May 2010

## Synopsis

- Issues add weight to increased consumer demand for natural additives especially in children's food
- Naturals can be named rather than by E-number → 'clean label' declaration
- Challenge to colour manufacturers for applications → new technologies
- Colours market expected to grow inline with increase in processed foodstuffs particularly in Asia/China
- Natural colours vulnerable to supply issues
- Additive safety / intake continually assessed
- **Need for reliable methods of analysis**

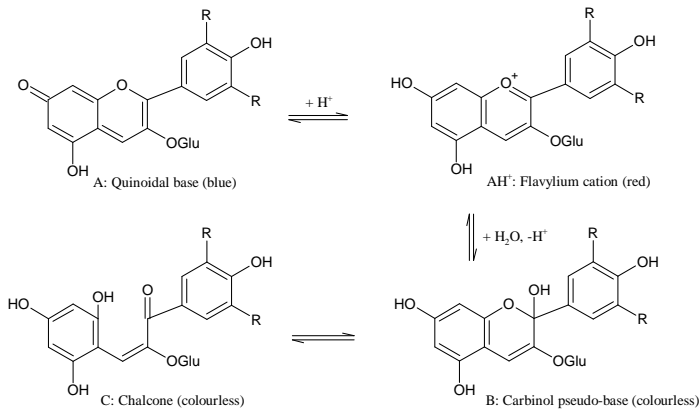
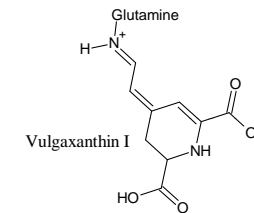
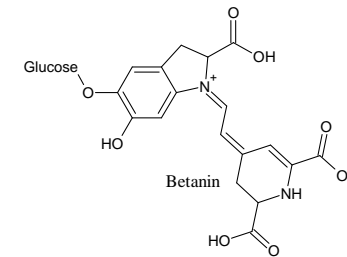
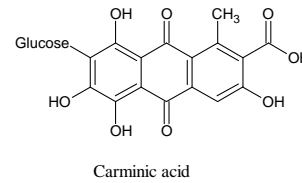
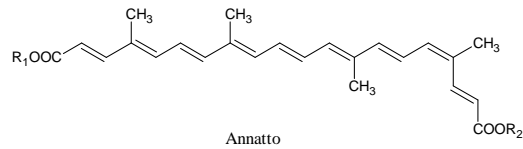
## Review structure (1)

- Nine groups of colours
  - **Group 1** - Curcumin E100
  - **Group 2** - Riboflavin E101(i) and E101(ii) Riboflavin-5'-phosphate
  - **Group 3** - Cochineal, carminic acid and carmines E120
  - **Group 4** - Chlorophylls E140(i) and Chlorophyllins E140(ii), and Copper chlorophylls E141(i) and Copper chlorophyllins E141(ii)
  - **Group 5** - Caramels E150a-E150d

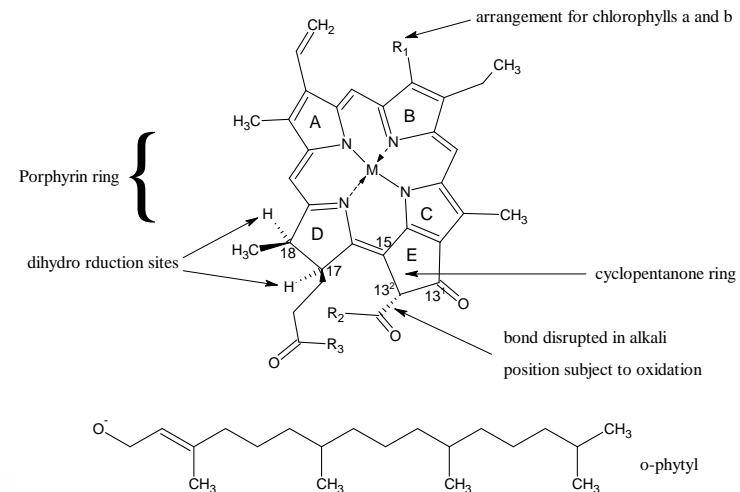
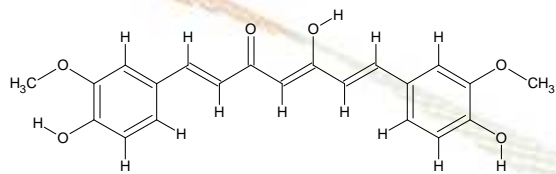
## Review structure (2)

- **Group 6** - Carotenoids other than annatto E160a, E160c, E160d, E160e, E160f, E161b, E161g; and Annatto E160b
- **Group 7** - Beet red E162
- **Group 8** - Anthocyanins E163
- **Group 9** – Other colours
  - Vegetable carbon E153
  - Calcium carbonate E170
  - Titanium dioxide E171
  - Iron oxides and hydroxides E172

# Chemical structures (1)



Anthocyanins



## Chemical structures (2)

$\text{CaCO}_3$

$\text{TiO}_2$

$\text{Fe}_2\text{O}_3$

C

Caramel = ? C,H,O,N,S



## Available literature (1)

- Large numbers of analytical methods for natural colours with widespread use
- Most concerned with identification and measurement in source materials / nutritional purposes or colouring effect
- Not all permitted colours in a given group covered to the same extent but some methods multianalyte
- Reference standards and CRMs important

## Available literature (2)

- Information on certain colours mirrors research effort e.g. annatto and lycopene intake concerns
- Very few methods available for ‘foodstuffs’ especially processed foods; very few official methods
- Many methods require broadening in scope to cover permitted uses and improved sensitivity
- Technological input required for some methods development
- Very few ‘official’ methods and degree of validation very variable

## Group 1: Curcumin

- Three main colouring principles
- Relatively few methods for foods, mostly other biomatrices (health aspects)
- HPLC-based methods (PDA, FLU or MS detection)
- Reasonably well validated
- Require broadening of analytical scope
- Sensitivity should not be an issue

## Group 2: Riboflavins

- Nutritional importance reflects large amount of methods available
- HPLC based
- Good level of validation
- Some broadening of analytical scope required



## Group 3: Cochineal

- Carminic acid and aluminium lakes (carmines)
- Relatively few methods available HPLC based
- Some methodologies well established for source materials
- Significant method development required for foods
- Validation poor

## Group 4: Chlorophylls

- Complex chemistry; includes native and coppered analogues
- HPLC based (PDA/FLU/MS)
- Several methods developed in recent years but applications to processed foods limited
- Some validation data
- Many analogues to measure – sensitivity an issue?

## Group 5: Caramels

- Most widely used natural food colouring (and additive)
- Chemistry still not well understood – 4 classes
- Methods are empirical – rely on marker peaks
- HPLC and CZE-based methods well defined but limited
- Application of new technologies?
- Scope then needs widening and full validation required

## Group 6: Carotenoids

- Carotenoids: many methods available but not all carotenoids covered to the same extent
- Nutritionally important
- Many for carotenes, lycopene, lutein, paprika but few for  $\beta$ -apo-8'-carotenal, canthaxanthin
- Several methods multianalyte
- *Cis-trans* isomers complicate analysis
- Several require only refinement prior to collaborative trial whereas others require development and single-lab validation
- All need widening in scope to some degree

## Group 7: Beet red

- Betacyanins and vulgaxanthins
- Relatively few methods available
- Some methodologies established for source materials
- Significant method development required for foods
- Validation poor
- Degradation products important

## Group 8: Anthocyanins

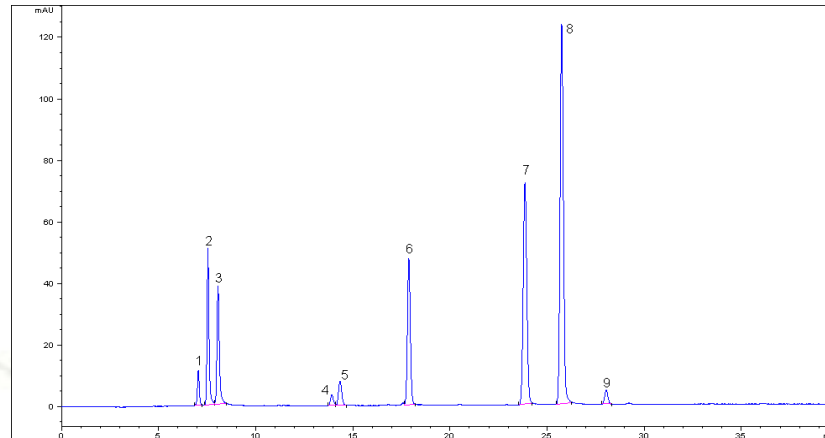
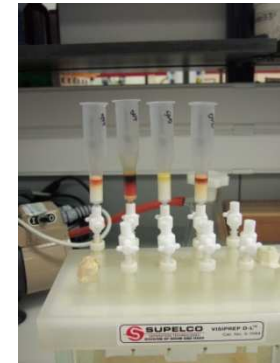
- Complex chemistry: based on 6 aglycones but many analogues
- Many literature methods available but few for processed foods – requires broadening in scope
- Two possible analytical approaches: parent aglycones or intact anthocyanins
- HPLC based (PDA/MS)
- Variable levels of validation

## Group 9: Other colours

- Vegetable carbon
  - No methods available for food or biomatrices only few for industrial purposes
- $\text{CaCO}_3$ 
  - Number of methods for Ca (elemental) but few for carbonate (IEC,  $\text{CO}_2$  generation)
- $\text{TiO}_2$ 
  - Number of methods for Ti (elemental and colorimetric) but none for intact  $\text{TiO}_2$
- $\text{Fe}_2\text{O}_3$ 
  - Number of validated methods for Fe (elemental and colorimetric) but none for intact  $\text{Fe}_2\text{O}_3$
- All require method development and validation



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 \*DAD1, 17.956 (80.2 mAU, -) Ref=14.3  
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