ORIGINAL ARTICLE

Assessing food additives: the good, the bad and the ugly

Vasso Oreopoulou[1], Vasso Psimouli[1], Dimitris Tsimogiannis[1], To Kim Anh[2], NguyenThi Minh Tu[2], Umran Uygun[3], Hamit Koksel[3], Vural Gokmen[3], Colin Crews[4], Sandor Tomoskozi[5], Lilla Domotor[5], Gabor Balazs[5], Lishi Zhang[6], Huanchen Liu[6], Yuanyuan Cui[6], Bin Liu[6], Ding Wenping[7], Wang Xingguo[7], Huang Weining[7], Hayrettin Ozer[8], Liu Zhongdong[9] & Mohamed El-Nawawy[10]

1 National Technical University of Athens, Athens, Greece
2 Hanoi University of Technology, Hanoi, Vietnam
3 Hacettepe University, Ankara, Turkey
4 Central Science Laboratory, York, United Kingdom
5 Budapest University of Technology Economics, Budapest, Hungary
6 Sichuan University, Sichuan, China
7 Chinese Cereals and Oils Association, Beijing, China
8 TUBITAK Marmara Research, Gebze, Turkey
9 Henan University of Technology, Henan, China
10 Ain Shams University, Cairo, Egypt

Keywords
analysis; food additives; validated methods.

Correspondence
Vasso Oreopoulou, National Technical University of Athens, Athens, Greece
Email: vasor@chemeng.ntua.gr

doi:10.1111/j.1757-837X.2009.00012.x

Abstract

Introduction The efficiency of analytical methods for the determination of food additives is of utmost importance since their content in food affects both quality and safety of foods. *Objective* The objective of the present article is to survey the current status of analytical methods for certain food additives (namely, colours, sweeteners, preservatives and antioxidants) and to point out the needs for the improvement of the existing, or the development of new, analytical methods. *Method* The criteria used to select the specific additives from among other categories of food additives, comprise broad use, legislative limits, and high frequency of notification through the Rapid Alert System for Food and Feed (RASFF). *Results* Certain needs were identified regarding the availability of rapid methods for the analysis of food additives. *Conclusion* Furthermore, there is need for the development of screening and high throughput methods for multi-component analysis.

Introduction

Food additives are defined according to Directive 89/107/EEC (European Regulation http://ec.europa.eu/food/food/chemicalsafety/additives/comm_legisl_en.htm) as 'any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose results in it or its by-products becoming directly or indirectly a component of such foods'. In the Annex of the same Directive, the categories of food additives are defined as: the four main categories of colours, sweeteners, preservatives and antioxidants, along with the miscellaneous additive categories of emulsifiers, emulsifying salts, thickeners, gelling agents, stabilizers, flavour enhancers, acids, acidity regulators, anti-caking agents, modified starches, raising agents, antifoaming agents, glazing agents, flour treatment agents, firming agents, humectants, sequestrants, enzymes, bulking agents, propellent gases and packaging gases.

The additives that are discussed in this article are limited to the four main categories of colours, sweeteners, preservatives (more specifically, benzoic and sorbic acids, sulphites, nitrates/nitrites) and antioxidants due to their broad use, the legislative limits set by EU, as well as the higher frequency of notification they presented recently through the Rapid Alert System for Food and Feed (RASFF), in comparison with other categories of food additives.

The scope of this paper is to reveal the current status of the analytical methods of the food additives selected and to identify the gaps and the needs for the development of new analytical tools. The standard and official methods of EU, international and national organizations, are presented. Additionally the paper provides information about the technological use, the frequency of notification and the European legislative status for the selected food additives. This work is based on the 6-month research of the Food Additives and Food Processing Toxicants Working Group of the European Union Network of Excellence Project, MoniQA, (2007).

Technological purpose of additives' use

The addition of food colourants contributes to the restoring of the lost or faded colour of the foodstuff (resulting from its process or storage), or can even function as a supplement to the natural colouring of foodstuff so as to avoid batch-to-batch colour variations (Smith, 1991). Food colourants can be natural or synthetic. Synthetic dyes show several advantages (high stability to light, oxygen and pH; colour uniformity; low microbiological contamination; low production cost) over natural ones (Alves *et al*., 2007). The most common synthetic food colourants are azo dyes, which are characterized by the presence of a chromophoric azo-group (Cornet *et al*., 2006; Mejia *et al*., 2007).

Sweeteners are added as sugar substitutes, in order to produce low calorie, dietetic products and to prolong the shelf life of the final product. Apart from the common (nutritive) sweeteners, known as polyols (e.g. sorbitol, mannitol), there are intense (non-nutritive) sweeteners such as sucralose and the salts of aspartame and acesulfame. The intense sweeteners however do not contribute to the viscosity or texture of the food or beverage to which they are added, and in case certain viscous or textural properties are required they should be combined with the nutritive sweeteners or other ingredients (e.g. bulking agents like polydextrose).

Preservatives are used in many foods to control microbial activity, though they may also present other functions. Sorbic and benzoic acids are two of the most important preservative organic acids in terms of antimicrobial effectiveness (Russell & Gould, 1991). They may be added to numerous foodstuffs, like non-alcoholic and alcoholic beverages, sugar-based confectionery, mayonnaise and other emulsified and non-emulsified sauces, mustard, salads and fat-based spreads. Sorbic acid may be used in processed fruit products and vegetables, in fruit- and dairy-based desserts while benzoic acid may be added to fruit products, vegetables and pickles. *Sulphite*, except for its antimicrobial activity, is also characterized by other functions, which include antioxidant activity, inhibition of chemical and enzymic reactions as well as of Maillard reactions. Sulphite is mostly used in fruit and vegetable products and in alcoholic and non-alcoholic drinks. *Nitrites* may be used in meat products and cured meat products, while *nitrates* may be used in non heat-treated meat products and cured meat products, cheese, pickled herrings and sprats (Directive 95/2/EC http://ec.europa.eu/food/food/chemicalsafety/additives/comm_legisl_en.htm).

Antioxidants act by delaying or inhibiting lipid oxidation, thus prevent undesirable changes that foodstuffs undergo during storage (e.g. flavour degradation, shelf life limitation, loss of nutritive value, even potential health risks). Examples include butylated hydroxyanisole, butylated hydroxytoluene (BHT) and propyl gallate (Madhavi *et al*., 1996).

The scope of this paper is to reveal the current status of the analytical methods of the food additives selected and to identify the gaps and the needs for the development of new analytical tools. This work is based on the 6-month research of the Food Additives and Food Processing Toxicants Working Group of the European Union Network of Excellence Project, MoniQA (MoniQA, 2007).

RASFF data

According to the data collected through RASFF (http://ec.europa.eu/food/food/rapidalert/index_en.htm) fraudulent use of dyes and too high content of sulphites in food have presented the highest occurrence throughout the recent years. However a sharp decrease in number of notifications, regarding illegal use of dyes in food, has been observed since 2003. In particular there were 390 notifications in the period 2003–2004, 213 in 2005 and 60 in 2006. In year 2004 presence of Sudan Dyes in ground and powdered chilly, curry, sumac and red palm oil was reported. Fraudulent use of Sudan Dyes continued in year 2005, while other illegal dyes were detected as well, the most important of which was Para Red.

Increased number of notifications in 2005 also concerned the detection of high levels of sulphites, mainly in shrimps. There was evidence that raw shrimps, respecting the limit of sulphite content, once cooked could exceed the limit of sulphite content for cooked shrimps. This evidence gave rise to a proposal amending Directive 95/2/EC, in terms of adjusting the limit of sulphite content of cooked shrimps to the one of raw shrimps. In 2006 fewer notifications regarding too high sulphite content were released – 80 notifications compared with 101 in year 2005 – a number of which is related to sulphite content in crustaceans (45 in 2006, 63 in 2005). This decrease can be attributed to the amendment of

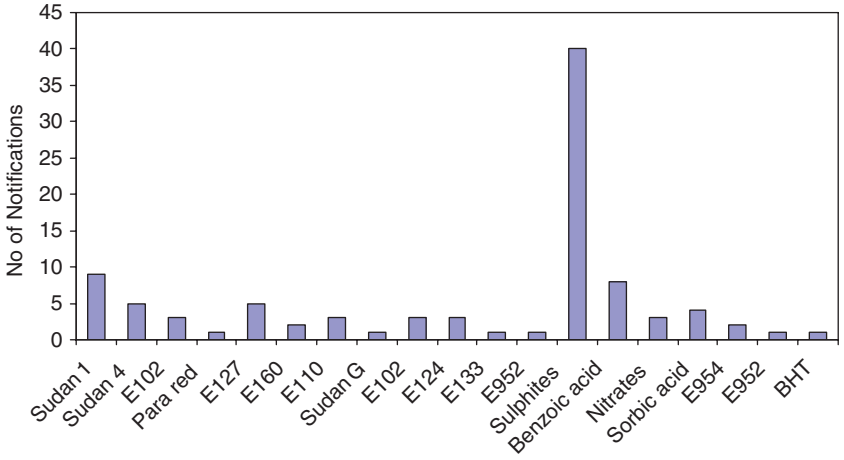

Figure 1 Notifications of additives during first semester of 2008.

Directive 95/2/EC and the adoption of European Parliament and Council Directive 2006/52/EC. In the following year notifications concerning too high sulphite content (60 of which 37 were detected in crustaceans) reduced and a similar trend is observed throughout the first semester of 2008.

Furthermore in the same year significant number of notifications were released regarding the carbon monoxide treatment of tuna fish. Carbon monoxide treatment of fish and meat aims at maintaining the fresh red colour of the product and is not authorized by Directive 95/2/EC. By maintaining the fresh red colour, the use of carbon monoxide may disguise the spoilage of the product and can therefore lead to the risk of microbiological contamination not being visually detected.

During the first semester of 2008, 96 notifications of unauthorised use of high levels of food additives were reported, as illustrated in Figure 1. Most notifications (55) concerned preservatives, with 40 referring to sulphites mainly in chilled and frozen shrimp and prawns. High levels of benzoic acid were detected in sauces, syrups, jams and tomato paste. Nitrates and sorbic acid were found in high levels in fresh lettuce and spinach, chilly sauce, smoked squid, mustard, dried prunes and non-alcoholic wine. Colours followed in number of notifications (37), including illegal dyes, which were detected in curry powder, chilly flakes, pepper sauce, ground chilly and seasonings. Fewer notifications concerned sweeteners (E962, E954) and referred to pickled vegetable and fruit drinking powders, while high levels of the antioxidant BHT was detected in fresh meat.

Legislation

The criteria for the use of food additives are provided by Directive 89/107/EEC (Report from the Commission on Dietary Food Additive Intake in the European Union http://ec.europa.eu/food/food/chemicalsafety/additives/comm_legisl_en.htm), according to which food additives can be approved provided that there is a technological need for their use, they do not present a risk to the health of the consumer and do not mislead the consumer. In Annex II it is also pointed out that the food additive should be subjected to toxicological testing in order to evaluate the potential harmful effects.

All food additives are subjected to extensive toxicological evaluation before they are permitted for use. Also, as our knowledge of toxicology and especially of potentially sensitive or vulnerable groups in the population increases, food additives should be kept under a constant process of re-evaluation. The purpose of this evaluation is to establish any hazard that the chemical may present to human health. This is most commonly expressed as an acceptable daily intake (ADI) value, which is the maximum quantity that can be consumed each day for a lifetime without any appreciable risk to health. The ADI value is normally expressed as an intake value in units of mg additive per kg bodyweight per day (mg/kg bw/day).

Taking the maximum risk that is a function of hazard combined with exposure ('the dose makes the poison') the ADI value is then used along with other considerations by the risk manager to decide to which foods the chemical can be added and at what levels. These then are the three reasons that analysis of foods for additives is important. First, to ensure that they are added only to those foods for which they are permitted and are not added to any other foods. Second, to ensure that the maximum addition level set in legislation for safety reasons is not exceeded. Third, to allow consumer exposure to be monitored so that, as eating habits

change and the frequency and level of additive usage changes over time, consumer exposure (including high consumers) does not exceed safe levels of the ADI.

Colours are defined in Directive 94/36/EC (http://ec.europa.eu/food/food/chemicalsafety/additives/comm_legisl_en.htm) as 'substances which add or restore colour in a food and include natural constituents of foodstuffs and natural sources which are normally not consumed as foodstuffs as such and not normally used as characteristic ingredients of food'. The substances that can be used as colours in foods are listed in Annex I of this Directive. Annexes III, IV and V specify the conditions and the foodstuffs in which colours can be used and the permissible limits of each colour, while in Annex II are listed the foodstuffs, which shall not contain added colours. Regarding the colours that fall into the 'quantum satis' principle and therefore no maximum level is specified, they should be used according to good manufacturing practice at the quantity necessary for the intended purpose provided that their addition does not mislead the consumer.

Directive 94/35/EC (http://ec.europa.eu/food/food/chemicalsafety/additives/comm_legisl_en.htm) applies to the food additives used to impart a sweet taste to the foodstuff, together with those for a particular nutritional use or for use as table-top sweeteners. In the Annex of this Directive are listed the sweeteners that may be placed in the market, as well as the foodstuffs in which they can be added and the permissible dose. The sweeteners that follow the 'quantum satis' principle should be added according to good manufacturing practice, at a level not higher than necessary to achieve the intended purpose.

Food additives other than colours and sweeteners are specified in Directive 95/2/EC (http://ec.europa.eu/food/food/chemicalsafety/additives/comm_legisl_en.htm). The permitted preservatives, antioxidants and other additives are listed in the Annexes of this Directive, followed by the foodstuffs in which they can be added and the maximum dose.

All additives must comply with the purity criteria of three other Directives; Directive 95/45/EC for colours, Directive 2008/60/EC for sweeteners and Directive 2008/84/EC concerning additives other than colours and sweeteners (http://ec.europa.eu/food/food/chemicalsafety/additives/comm_legisl_en.htm).

According to Directives 94/36/EC, 94/35/EC and 95/2/EC, a monitoring system should be established by the Member States in order to ensure that the consumption of food additives does not exceed the ADI. The results of the first attempt of an overview about the daily food additive intake are presented in the Commission Report regarding Dietary Food Additive Intake in the European Union (http://ec.europa.eu/food/food/chemicalsafety/additives/intake_en.htm), which also lays down the approach and methodologies for intake estimation that could be followed by the Member States for harmonized intake studies of food additives in the European Union. The objective was to gather information from as many Member States as possible. According to the 'preliminary' data that has been gathered so far, the dietary intake for the majority of food additives is below ADI. Currently the European Food Safety Authority (EFSA) and the European Commission are dealing with a legislation package consisting of four new regulations concerning food additives, flavourings and enzymes. The first of the new regulations will introduce an EU-level 'common authorization procedure' for additives, enzymes and flavourings while the other three will deal with each of these categories separately (http://www.europarl.europa.eu/news/expert/infopress_page).

The first regulation of 'common authorization procedure' will contribute to the free movement of food within the community. The second regulation concerning additives, will list the safe to use additives. It will also, introduce food additives, which might have been changed by nanotechnologies. The third upcoming regulation will give stricter conditions on the use of term 'natural flavouring' emphasizing on the effect on vulnerable groups. It will also set new maximum levels for 'undesirable substances' that naturally occur in this kind of additives. Finally, the fourth regulation will cover the issue of food enzymes for which nowadays there is no legislation within the European Union and their use only depends on national guidelines. Due to the new legislation, harmonized EU rules will be laid down for the evaluation, approval and control of enzymes used in food processes.

Status of analytical methods

According to a survey conducted within the members of MoniQA Food Additives and Processing Contaminants Working Group, several standard and official methods are available for the analysis of certain food additives. Most of these methods refer to specific foods on which they can be applied. In-house methods have also been developed, while rapid methods or test kits have been scarcely adopted for the determination of additives. The results of this survey are summarised below.

Colours

A limited number of the permitted food colourants can be determined by official methods (AOAC methods based

Table 1 Standard and official methods for the analysis of Food Colours

Food colourant	Principle of analysis	Food matrix	Method/standardization status
E102 – tartrazine, E 110 – sunset yellow FCF, E 123 – Amaranth, E 124 – ponceau 4R/cochineal red A, E 127 – erythrosine, E 133 – brilliant blue FCF	CC		AOAC 930.38/official
Synthetic colour (new red, lemon yellow, amaranth, sun set yellow, erythrosine, brilliant blue)	HPLC		GB/T5009.35-2003/standard
Sudan dyes (I–IV)	HPLC-UV	Chilli products	FSA Method 145A
	HPLC-UV/ViS	Chilli powder and products containing chilli powder	FSA Method 145B
	RP - HPLC	Chilli sauce	GB/T 19681—2005/standard
	HPLC	All kinds of food	
	HPLC-DN-TRAP-MS/MS	Hot pot dish	

Table 2 Standard and official methods for the analysis of Sweeteners

Sweetener	Principle of analysis	Food matrix	Method/standardization status
Acesulfame K	Spectroscopy	Table top sweetener preparations	EN 1377: 1996/standard
Aspartame	HPLC	Table top sweetener preparations	EN 1378: 1996/standard
Cyclamate	HPLC/UVD	Food raw material, beverages, sweets, dairy – baking – and preserved products	EN12857: 1999/standard
	Sodium nitrate test		AOAC 957.09/official
	Colourimetry		AOAC 969.28/official
Saccharin	Spectroscopy	Table top sweetener preparations	EN 1376: 1996/standard
	Phenol–sulfuric acid test		AOAC 941.10/official
	Sublimation method		AOAC 947.10/official
	LC		AOAC 979.08/official
	Differential pulse polarography		AOAC 980.18/official
Acesulfame-K, aspartame and saccharin	HPLC/UVD	Raw materials, beverages, sweets, dairy Baking – and preserved products	EN12856: 1999/standard
Saccharin and cyclamate	HPLC/UVD	Raw material, beverages, sweets, dairy – baking – and preserved products	EN 1379:1998/standard

on column chromatography) or standard methods (GB/T-national standard method issued by the National Standardisation Authority of China), as presented in Table 1. For the determination of the non-authorized Sudan Dyes FSA (Food Standard Agency, UK) Method 145A and Method 145B are the only official and validated methods. On the other hand, in-house methods are available for the determination of numerous food colourants (including Sudan Dyes). However, issues regarding the interpretation and the comparability of the analytical result arise. Therefore, action should be taken in order to establish official methods of international magnitude, which will ensure a harmonized approach of analysis. The development of these new official methods can be based on the most promising – in terms of fulfilling the method performance criteria – of the available in-house developed methods.

Sweeteners

The validation status of analytical methods appears to be sufficient, since standard (EN) and official (AOAC) methods are available (Table 2) for the determination of all intense sweeteners for which limits are set by legislation. Methods for multi-components analysis have also been standardized (Table 2). There is, however, need for rapid analytical methods, which will prove valuable for screening purposes (e.g. in industry).

Table 3 Standard and official methods for the analysis of Preservatives

Preservatives	Principle of analysis	Food matrix	Method/standardization status
Sorbic acid	GC, TLC or HPLC		GB/T5009.29-2003/standard
Benzoic acid	Spectroscopy	Non-solid foods	AOAC 960.38/official
	Titrimetry – modified Mohler test chloride		AOAC 963.19/official
	TLC		AOAC 967.15/official
	GC	Fish	AOAC 983.16/official
	HPLC	Orange juice	AOAC 994.11/official
	HPLC	Moon cake, beverages	GB/T5009.29-2003/*standard*
	GC	Soy	
	LC	Royal jelly	SN/T 1303- 2003
	LC	Chinese cheese	SN/T1548-2005
			NMKL 124-2 1997
Sorbic acid and	Oxidation/spectroscopy	Cottage cheese	AOAC 971.15/official
benzoic acid	Spectroscopy	Wines	AOAC 974.08/official
	Spectroscopy	Dairy	AOAC 974.10/official
	Colourimetry		AOAC 975.10/official
	GC		AOAC 983.16/official
	HPLC	Milk and dairy products, baked food, meat products, cooked meat products, dried sleeve-fish products	GB/T5009.29-2003/standard
	LC	Royal jelly	SN/T 1303- 2003
	LC	Chinese cheese	SN/T1548-2005
			NMKL 124-2 1997
Sulphites	Optimized Monier–Williams method	Foodstuffs	EN ISO 1988-1: 1998/standard
	Enzymatic method	Foodstuffs	EN ISO 1988-2: 1998/standard
	Modified Monier–Williams Method		AOAC 962.16/official
	Spectroscopy	Meat	AOAC 980.17/official
	Differential pulse polarography	Apricots, peas	AOAC 987.04/official
	Optimized Monier–Williams		AOAC 990.28/official
	FIA	Foods, Beverages	AOAC 990.29/official
	FIA	Wines	AOAC 990.30/official
	Ion exclusion chromatography	Beverages and beverage materials, foods	AOAC 990.31/official
	Gravimetry	Wine	EC 2676/90/12/2/official
	Distillation	Wine	EC 2676/90/25/2.2/official
	Iodometry	Wine	EC 2676/90/25/2.3/official
	FIA	Wine	OIV F.V. 823
	Iodometry	Brandy	MSZ 9589/10:1985
	pararosaniline hydrochloride method–colourimetry	Succade, dry fruit, dry vegetable, white fungus, bean vermicelli, biscuit, granulated sugar, crystal sugar, candy, bamboo shoot, mushroom, yam starch, beer	China food industry standard compilation
	Distillation iodometric method	Glucose, cider, wine, amino acid infusion fluid, dexamethasone infusion fluid	China food industry standard compilation
Nitrites and Nitrates	Cadmium reduction and spectrometric determination	Milk and milk products	EN ISO 14673-1. 2001/standard
	Segmented flow analysis method	Milk and milk products	EN ISO 14673-2. 2001/standard
	FIA	Milk and milk products	EN ISO 14673-3. 2001/standard
	Spectrometric determination	Meat products	BS EN 12014-3: 1998/standard
	IC	Meat products	BS EN 12014-4: 1998/standard
	Colourimetry	Cured meat	AOAC 973.31/official
	Modified Jones reduction method	Cheese	AOAC 976.14/official
	Xylenol method	Meat	AOAC 935.48/official
	Spectroscopy	Meat products	MSZ 6905:1981/official
	Spectroscopy	Preserved food, quick frozen foods	MSZ 3615:1983
	Spectroscopy	Cheese, milk and whey powder	MSZ 12058:1987
	Spectroscopy	Cheese, milk and whey powder	MSZ 12058:1987
	HPLC/UVD	Vegetables	MSZ ENV 12014-2:1999
	HPLC/conductivity	Food of plant origin	MSZ ENV 12014-2:1999
	HPLC/conductivity	Meat	MSZ ENV 12014-4:2000
	HPLC/UVD	Meat products	MSZ ENV 12014-4:2005
Nitrite	Spectroscopy	Meat products	MSZ ENV 12014-3:2005

Table 4 Standard and official methods for the analysis of Antioxidants

Antioxidant	Principle of analysis	Food matrix	Method/standardization status
BHA BHT PG THBP NDGA Ionox-100	Extraction-HPLC	Animal and vegetable fats and oils and shortenings	AOCS Ce 6-86/official
BHA, BHT	Steam distillation and colourimetry GC	Animal and vegetable fats and oils	IUPAC 2.622/official GB/T5009.30-2003/standard

Preservatives

Standard and official methods for the analysis of food preservatives are presented in Table 3.

Sorbic and Benzoic acid

There are official AOAC methods, for the determination of sorbic acid and benzoic acid, which are based on spectrophotometry, gas chromatography and thin layer chromatography. A limited number of standard methods are available, and these are of national level, as can be seen from Table 3. However, there is a need for improvement of the existing methods. Even the official methods present problems, for example the pretreatment is complicated and the recovery of elements may be too low (Zhongdong, 2001, 2006).

Sulphites

There are numerous standard (EN) and official (AOAC, EC) methods – such as Flow Injection Analysis – available for the analysis of sulphites in foods. The selection of the method depends on the predictable level of sulphite and the matrix to be analyzed. However further method development is required in order to determine sulphite in food matrixes (e.g. cabbage, dried garlic, dried onions, leeks and soy proteins) to which the standard methods cannot be applied (Wood *et al*., 2004).

Nitrites and nitrates

International and European standard methods are available for the determination of nitrite in milk products and in meat products. In addition Hungarian standard (MSZ ENV) methods can be applied in food matrices of plant origin, as presented in Table 3.

Rapid methods, more specifically test kits, have been developed and are generally used to determine the nitrate/nitrite content of the water. The test kit provides semi-quantitative estimates of nitrate-N content. However, interference of the presence of high levels of iron, NaCl, sulphate and acid leads to overestimation of nitrate levels. Since these interference effects cannot be quantified, they affect negatively the accuracy of the method. There are also colorimetry-based test strips and a reflectrometric method, which is simple fast and cost effective. However it provides poorer results than the standardized methods (the RSD was 7% during the reproducibility test) (Novič *et al*., 1996; Nidal *et al*., 1999; Ximenes *et al*., 2000; Wood *et al*., 2004; Kmecl *et al*., 2005; Erkekoglu & Baydar, 2006).

Antioxidants

Few official methods, presented in Table 4, are available for the determination of antioxidants, and they refer to oils and fats. Therefore, there is a need for the development of official-validated methods, especially for other food matrixes that contain oils and fats, like fried products, mayonnaise, salad sauce, etc. Also there is a need for standardization of the extraction/pretreatment of the sample, since the recovery values – which are defined by the extraction procedures – present high variation.

Towards harmonization

General issues

The selection of a reliable method, appropriate for the food matrix to be analyzed, is of utmost importance in terms of quality control. In general the selection of the analytical method should be based on the method performance criteria, i.e. accuracy, applicability, detection limit, quantification limit, precision (repeatability and reproducibility), recovery, selectivity, sensitivity and linearity as well as to compliance with the legal requirements.

Standard and/or official methods include some but not all information about performance criteria (e.g. most include precision data, but few recovery data). However,

laboratories usually select use of non-official methods, based on their experience or infrastructure. In such a case, (inter- and intra-laboratory) studies for validation of the method are required to define the method performance criteria. Moreover, since most of the official methods for the analysis of food additives have been developed for specific food products (Tables 1–4), modification or development of a new method is necessary if the analysis has to be applied to another food material.

On the other hand, the development of reliable, rapid methods is becoming a necessity nowadays as they can be used for screening purposes or for high throughput, quick controls of raw materials and food products. Additionally rapid methods offer an easy handling solution for industry that can be also used on-site.

According to the above, harmonization of the analytical methods for food additives is necessary. Harmonization should not be considered as a guideline for laboratories, industry and surveillance authorities to use common analytical methods. It is related to method characteristics (like detection and quantification limit, recovery and measurement uncertainty) so as to ensure that a 'harmonized' approach is followed in terms of method performance criteria. In particular the recovery level and the measurement uncertainty affect the reporting of the analytical result and the interpretation of the result. A harmonized approach appears to be needed so that the results are comparable.

Measurement uncertainty and recovery

In the year 2005, the European Commission issued a guidance report, entitled 'Report on the Relationship between Analytical Results, Measurement Uncertainty, Recovery Factors and the provisions of EU Food and Feed Legislation' related to how the Member States that apply the EU legislation on food and feed contaminants could express whether a sample is in compliance with the EU specifications. This guidance document focuses on problems that occur when a Member State need to report an analytical result, such as the number of significant figures taken into account, the treatment of measurement uncertainty and the use of recovery correction in the interpretation of a result. This report is only concerned with quantitative analytical results and may be applied to the 'contaminants' as well as the additives, composition and microbiological aspects of food and feed analysis.

During the development of legislation containing maximum levels, attention should be paid to the number of significant figures into which the specification is laid down. The following should be stated or considered: the units in which the results are to be expressed; the number of significant figures to be included in the reported result; the interpretation of any analytical result in relation to a statutory limit; and the expressed precision of the method of analysis likely to be used for the determination, and thus, whether the number of significant figures being specified in legislation is 'realistic'. In cases where the legislation does not provide information on the significant figures of the result, it is recommended that the analyst should report to one significant figure more than is laid down in the specification. Most additives are used in foods in appreciable amounts; therefore, significant figures are not specified in legislation. However, significant figures may be taken into account in some cases, e.g. for intense sweeteners content, or for specific claims like free from preservatives.

The measurement uncertainty has a significant role in the interpretation of the analytical results – in terms of compliance to EU specification of the maximum or minimum limit of the substance. Thus a result of an analysis conducted with the same method at the same sample may be in accordance with legislation requirements if the analytical result is not adjusted with uncertainty, whereas the analytical result with uncertainty of measurement may not be in compliance with EU specification. It is therefore recommended that the measurement uncertainty is used when assessing compliance. In food additives analyses a lot of sources of uncertainty are usually implicated. The main ones are related to sample effects, instrument effects and measurement conditions. A pre-treatment of a complex sample, which is often used, may lead to partial loss of the analyte or to interference of other components of the matrix. Instrument response may be also affected by other components of the matrix.

The recovery of the analyte plays an important role in measurement uncertainty and sample's compliance with legislative requirements. For example, the determination of several additives, like antioxidants, involves an extraction procedure and possibly other pre-treatment of the sample. The recovery depends on the procedure followed and presents high variations among procedures. Hence the analytical results should be adjusted for recovery when a compliance with specifications is checked. The guidance report recommends that the food and feed legislative requirements should specify whether an analytical result needs to be reported according to recovery-corrected basis or not, if this recovery is needed to be indicated and whether any minimum or maximum recovery is acceptable or not. A number of procedures for the estimation of measurement

uncertainty are given in the report. All of these procedures can be considered as equally valid but any procedure that a laboratory is using should be considered appropriate as part of the 17025 accreditation.

Sampling

The sampling procedure is an important factor in the analysis of any component, additive or contaminant. Additives are generally homogeneously distributed in food materials, resulting in minor random variations between different samples. However, variations may become significant in non-liquid raw material, unprocessed food or processed food products, like fruits, grains, meat, fish, etc. and in that case the sampling procedure should be defined and the effect on measurement uncertainty should be considered.

Challenges in food additives analysis

From the issues examined so far, certain specific actions for improvement of the current situation are emerging:

- Overview of the method performance criteria of the standard and official methods.
- Identification of promising methods where a lack of official method is encountered.
- Development of:
  - rapid, screening and/or high throughput methods
  - methods for multi-component analysis
  - methods using state of the art equipment that can provide higher accuracy and selectivity
- Development of harmonization guidelines to validate the above methods.

The Food Additives and Food Processing Toxicants Working Group of MoniQA will proceed with the identification of needs and gaps in official and non-official analytical methods, with the implication and input of a wider range of stakeholders, including industry, analytical laboratories and legislative authorities. More specifically MoniQA partners will collect information about the method performance criteria of the national standards used in their country, contact state laboratories or accredited laboratories in order to collect data about the methods they use to determine food additives and about their validation status and performance criteria. Future work will also focus on new rapid methods, through a literature survey and personal contact with stakeholders and on development of guidelines for harmonization of these methods.

Conclusions

For the majority of the food additives studied there are standard and/or official analytical methods available. Most of these methods, as indicated in Tables 1–4, have been developed for application to a certain food. The main gaps in official methods are encountered in the field of colourants, followed by antioxidants. Additionally, for some additives (e.g. Sudan Dyes) only national standards for their determination are available, thus act needs to be taken in order to establish standardized, validated methods for the European or the International Community. In the cases where there are only non-official methods available there is need to proceed to the validation of the most promising ones.

Rapid methods for the analysis of food additives are very limited. Although legislative authorities enforce the development and application of high-accuracy methods, the development of reliable, rapid methods is beneficial for both industry and authorities. Also there is need for the development of screening and high throughput methods for multi-component analysis (e.g. for sweeteners or antioxidants).

There is a challenge for MoniQA Noe to work towards the solution of existing problems in food additives analysis, to face gaps and needs and to contribute with harmonization guidelines for method validation and standardization.

Acknowledgement

The funds for this research were provided by the EU 6th Framework Network of Excellence Project 'MoniQA' (Contract Number Food-CT-2006-036337).

References

Alves S.P., Brum D.M., Andrade E.C.B., Netto A.D.P. (2007) Determination of synthetic dyes in selected foodstuffs by high performance liquid chromatography with UV-DAD detection. *Food Chemistry*, **107**, 489–496.

Cornet V., Yasmine G., Goedele M., Loco J.V., Degroodt J.M. (2006) Development of a fast analytical method for the determination of sudan dyes in chilli- and curry-containing foodstuffs by high performance liquid chromatography-photodiode array detection. *Journal of Agricultural and Food Chemistry*, **54**, 639–644.

Erkekoglu P., Baydar T. (2006) Assessment of nitrite contamination in baby foods, marketed in Turkey. *Toxicology Letters*, **164**, S272–S273.

European Food Safety Authority (EFSA). Available at http://www.efsa.eu.int. Last accessed 6 February 2009.

European Parliament Press Service. Available at http://www.europarl.europa.eu/news/expert. Last accessed 8 July 2008.

European Regulation. Available at http://ec.europa.eu/food/food/chemicalsafety/additives/comm_legisl_en.htm

Food Standards Agency. Available at http://www.food.gov.uk

Kmecl V., Sušin J., Zupančič-Kralj L. (2005) Validation of analytical methods used for determination of nitrate in soil. Accreditation and Quality Assurance. *Journal for Quality, Comparability and Reliability in Chemical Measurement*, **10**, 172–176.

Madhavi D.L., Deshpande S.S., Salunkhe D.K., Mekker M. (1996) Food antioxidants. In: *Technological, Toxicological and Health Perspectives* eds Marcel Dekker. D.L., Madhavi S.S., Deshpande D.K.Salunkhe, Marcel Dekker, Inc., New York.

Mejia E., Ding Y., Mora M.F., Garcia C.D. (2007) Determination of banned sudan dyes in chili powder by capillary electrophoresis. *Food Chemistry*, **102**, 1027–1033.

MoniQA (2007) Available at http://www.moniqa.org. Last accessed 6 February 2009.

Nidal A., Zatar N.A., Maher A., Abu-Eid M.A., Abdullah A.F. (1999) Spectrophotometric determination of nitrite and nitrate using phosphomolybdenum blue complex. *Talanta*, **50**, 819–826.

Novič M., Divjak B., Pihla B., Hudnik V. (1996) Influence of the sample matrix composition on the accuracy of the ion chromatographic determination. of anions. *Journal of Chromatography*, **739**, 35–42.

Report from the Commission on Dietary Food Additive Intake in the European Union. Available at http://ec.europa.eu/food/food/chemicalsafety/additives/intake_en.htm

Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU Food and Feed Legislation, with particular reference to Community Legislation concerning contaminants in food (Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food) and undesirable substances in feed (Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in feed).

Russell N.J., Gould G.W. (1991) Food Preservatives. In: Food Preservatives eds Russel N.J., Gould G.W.Blackie, Glasgow.

Smith J. (1991). *Food Additive User's Handbook*. Blackie Academic & Professional, London.

Wood R., Foster L., Damant A., Key P. (2004). *Analytical Methods for Food Additives*. First published 2004, Woodhead Publishing Ltd and CRC Press LLC, Cambridge, UK.

Ximenes M.I.N., Rath S., Reyes F.G.R. (2000) Polarographic determination of nitrate in vegetables. *Talanta*, **551**, 49–56.

Zhongdong L. (2001). *Principle & Application of Food Additives*. China Light Industry Publishing Company, Beijing, China.

Zhongdong L. (2006). *Food Additives*. South and East University Publishing Company, China.