

Recent developments in instrumental analysis for food quality

André Müller, Hans Steinhart *

University of Hamburg, Institute of Biochemistry and Food Chemistry, Department of Food Chemistry, Grindelallee 117, D 20146 Hamburg, Germany

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Abstract

The definition of food quality has to be discussed in regard of changes of consumer expectations, legislative needs and new developments in instrumental analysis. The production process has to be controlled by fast new methods based on physical, chemical or increasingly biochemical/immunological principles. New chromatographic, immunological and mass-spectrometric methods allow the detection of trace amounts of residues/contaminants, allergens, *trans*-fatty acids and other undesired ingredients for food surveillance. Additionally, analysis of new metabolic pathways in nutritional research is of increasing interest, giving evidence for positive health effects of minor food ingredients such as omega-3 fatty acids, isoflavones or conjugated linoleic acids. Most recent efforts in mass-spectrometric, biochemical and online/at-line methods for analysis of allergenicity, residues/contaminants, *trans*-fatty acid and for determination of identity and geographic origin of food are presented.

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Keywords: Food quality; Mass-spectrometry; Allergens; Contaminants; Residues; *trans*-Fatty acids; Genetically modified organism

1. Introduction

The term “quality” is defined according to DIN ISO 9000 as the totality of features relevant to the ability of a product to fulfill its requirements (International Institute for Standardization, 2005). However, the concept of food quality should be considered on a much broader basis as the different demands of the manufacturer, the consumer, the surveillance and the legislative bodies must be taken into account in order to obtain healthy and safe products without neglecting the economic and ecological issues associated with food quality.

Therefore, food should not be evaluated solely on its safety and its nutritional value but also on its sensory, technological and even ideological (e.g., food of biodynamic origin) or religious value (e.g., Kosher food). The consumer wants “healthy” products with high nutritional value with regard to macronutrients like proteins, carbohydrates, fats

and fiber as well as minor nutrients as vitamins and trace elements. These products should also be superior in taste, flavor and texture. Thirdly, as consumer behavior is changing constantly just as society is, people also tend to favor prepackaged food for convenient preparation and food with a long shelf life. Allergens, contaminants and residues, *trans*-fatty acids, genetically modified organisms (GMO) are, for the consumer, catch words that are generally regarded as designating undesired components in food but unfortunately cannot be totally avoided by the manufacturers. Although these undesired components bear with them an underlying risk, avoiding microbial contamination is a far more important issue in ensuring food safety and food quality (WHO, 2002). All these factors add up to an ideal concept of food quality as presented in Fig. 1. At the very least, effort has to be expended on maximizing nutritional value and food safety but the economic success of a product is surely also affected by other factors like (irrational) consumer expectations. Additionally, as nutritional research is gaining in importance, new analytical methods enable on the one hand the analysis of biochemical pathways of minor food ingredients which can be considered to have positive effects for humans (e.g., isoflavones

* Corresponding author. Tel.: +49 40 42838 4356; fax: +49 40 42838 4342.

E-mail address: hans.steinhart@chemie.uni-hamburg.de (H. Steinhart).

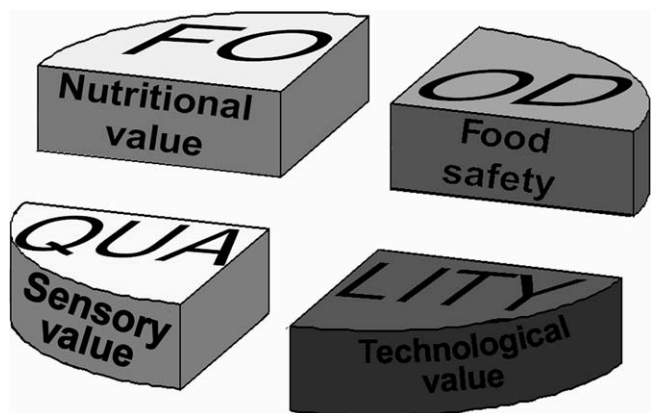


Fig. 1. Important factors influencing food quality.

or omega-3 fatty acids) and on the other hand the detection of trace amounts of potentially hazardous components like acrylamide, furan or *trans*-fatty acids. This means that the term food quality cannot be static and manufacturers and the legislative have to consider the most recent research results, mostly based on the progress in analytical methods.

2. Choosing the appropriate analytical procedure

To ensure food safety and that food will conform to consumer expectations, precautions have to be taken in terms of a total quality management system. Quality control must work on different levels and, therefore, different analytical procedures have to be established for different steps of production. One must distinguish between fast process analytics with simple (inexpensive) methods; surveillance methods with elaborated analysis methods for qualification and quantitation of residues and contaminants; and highly sophisticated methods for researchers as presented in Fig. 2. Nevertheless, all analytical methods suit their purpose of enhancing food quality and effort is expended on simplifying research methods to be applicable in the production process as fast methods or even online methods.

3. Recent analytical problems

Apart from microbial contamination, which can be controlled by meeting the requirements for heating and cooling procedures during production and storage to minimize microbial growth, other factors affecting food safety or consumer expectations are currently under discussion.

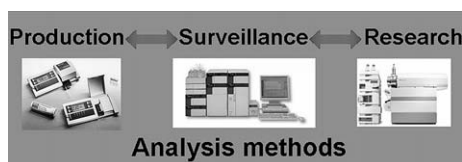


Fig. 2. Analysis methods for food quality at different stages of production.

Problems such as allergenicity, residues/contaminants, ensuring the identity and origin of food and *trans*-fatty acids are still under intensive research but preventive action has nevertheless been undertaken by the legislative for food safety.

3.1. Allergenicity

Allergenicity in terms of a specific change of the immunity situation of an organism in the sense of a hypersensitivity caused by proteins cannot be regarded as a typical disease of civilization and the often reported increase of allergic diseases during the last few years must be considered carefully, as physicians are not always adequately trained to distinguish between real food allergies and other intolerance reactions, thus compromising the quality of available statistical data (Vieths, Schöning, & Baltes, 1992).

Nevertheless, food allergies are a problem in some respects and contamination of non-allergenic products with allergenic factors can easily occur during transport and processing, due to incomplete cleaning of lines. Although some allergens can be reduced by heating, hydrolysis or enzymatic treatment, a few are resistant (Besler, Steinhart, & Paschke, 2001; Dube et al., 2004; Scheurer et al., 2004; Sell, Steinhart, & Paschke, 2005) and are dangerous to people with food allergies. To obtain qualitative and quantitative data on food allergens and information on how to reduce these compounds in foodstuffs, different analytical techniques have been developed to meet the requirements for food control. These methods are based on electrophoretic, chromatographic techniques with immunostaining or mass-spectrometric detection (Cramer, 2005; Fuchs et al., 2005; Natale et al., 2004; Oh-Ishi, 2005; Poznanovic, Schwall, Zengerling, & Cahill, 2005) as presented in Fig. 3. The allergic reaction occurs by binding of specific IgE antibodies, which are produced during first contact with an allergen, at a special location on the protein (epitope). This epitope can be “active” even if the protein has been modified or degraded during processing or other treatments. Structural elucidation of the epitope region of the allergen is an important step after characterization of the allergenic protein in terms of molecular weight, primary/secondary and tertiary structure and glycosylation (Meno et al., 2005; Pastorello et al., 2001). Allergens can be identified by immuno-blotting of HPLC pre-fractionated proteins after gel-electrophoresis or 2D-electrophoresis. After identification of the allergen, its primary structure can now be determined by using matrix assisted laser ionization/desorption time of flight (MALDI-TOF) analysis (Hoppe, Steinhart, & Paschke, 2006; Lee, Yoon, Kim, Choi, & Park, 2005; Westphal et al., 2003) or HPLC-ESI-TOF-MS (Wall et al., 2001), which allows the determination of the exact mass for database research and allows higher sample throughput than other sequencing methods such as the Edman-sequencing. X-ray and NMR-methods allow elucidating secondary, tertiary, and quaternary structures of proteins and epitope regions. These highly sophisticated

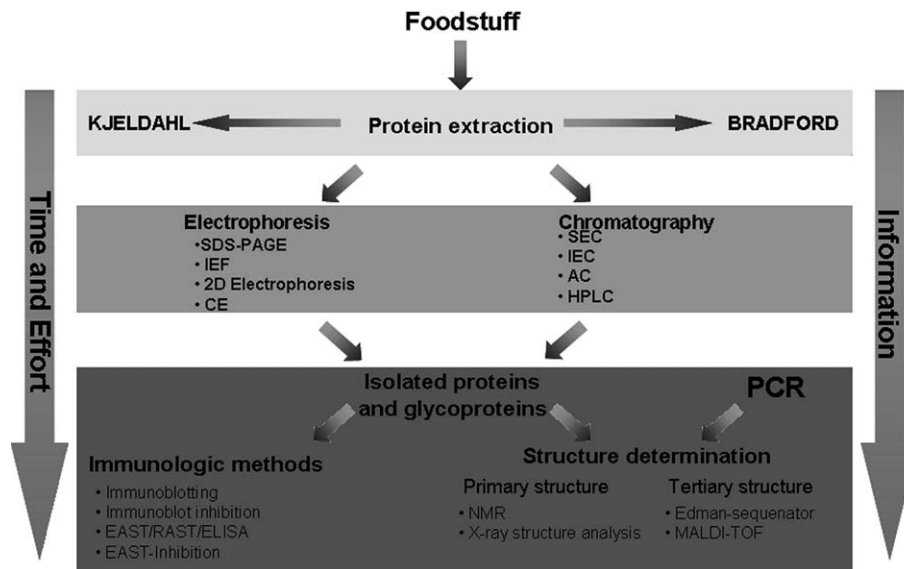


Fig. 3. Analysis scheme for elucidating the allergenicity of proteins from simple chromatographic/electrophoretic to modern biochemical/spectrometric methods (PCR, ELISA, NMR, MS, X-ray).

research methods are necessary to understand allergen reactions. With this information it may be possible to modify the production process to reduce allergenicity by altering the epitopes.

Alternatively, immunological methods based on specific antigen–antibody reaction (enzyme linked immunosorbent assay, ELISA) or polymerase chain reaction (PCR) based methods have been developed and are sensitive enough to identify and quantify allergens in protein extracts (at 10 mg/kg level) as demonstrated for peanuts and hazelnuts, respectively (Holzhauser, Stephan, & Vieths, 2002; Stephan, Möller, Lehmann, Holzhauser, & Vieths, 2002). An example of detection of peanuts in chocolate is presented in Fig. 4. With an ELISA based dip stick test it is possible to detect 0.001% peanut in a protein extract of chocolate. These fast and sensitive methods will facilitate the control of the risk to the consumer at production or surveillance stage.

3.2. Contaminants/residues

One of the most important aims in obtaining high quality food is controlling for contaminants and residues (Dabrowski & Sikorski, 2004). These compounds can either be brought in during production (e.g., pesticide residues, monomers from coatings) or can be formed during storage or processing of food (e.g., mycotoxins, aflatoxin, botulinum toxins, acrylamide, and furan) (Oguma, 2004). These compounds have been shown to have toxic, genotoxic, teratogenic or carcinogenic effects in animal models and humans and maximum limits have been set by the legislative. Maximum limits for zearalenone, an important toxin produced by fusarium species, are presented in Table 1 (EC directive 856/2005, 2005).

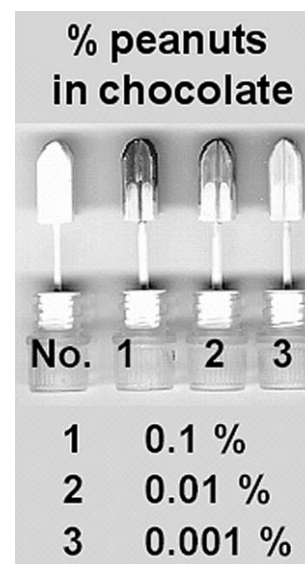


Fig. 4. ELISA for determination of trace amounts of peanuts in protein extracts in a dip stick format suitable for production control. (1) 0.1% peanuts in chocolate, (2) 0.01% peanuts in chocolate, (3) 0.001% peanuts in chocolate (Stephan et al., 2002).

The acquired analysis methods must be sensitive enough to detect these trace amounts and on the other hand must be selective enough to clearly identify these compounds free of matrix interferences. Although GC-FID and HPLC-UVD/FLD methods are very sensitive they often lack in selectivity. Selective sample preparation by liquid–liquid-extraction or solid-phase extraction is advantageous but also tedious and expensive. That is why most common analysis methods for residues/contaminants tend to utilize GC–MS- and/or HPLC–MS-methods with fast sample preparation techniques (mostly automated solid phase extraction) based on single-quadrupol or, for better selec-

Table 1
Maximum limits for zearalenone in food (EC Regulation No. 856/2005)

	Maximum limit µg/kg
Unprocessed cereals other than maize	100
Unprocessed maize	–(200) ^a
Cereal flour except maize flour	75
Maize flour, maize meal, maize grits, and refined maize oil	–(200) ^a
Bread, pastries, biscuits	50
Processed maize-based foods for infants and young children	–(20) ^a
Other processed cereal-based foods for infants and young children and baby food	20

^a If no other limits will be pronounced, limits in brackets will apply from July 1, 2007.

tivity and sensitivity, triple-quadrupol devices (Biselli, Hartig, Wegner, & Hummert, 2004; Nuñez, Moyano, & Galceran, 2005; Plattner, 1999). The selectivity of the triple-quadrupol devices allows the analysis of compounds on their specific mass fragments with different polarities (positive/negative) even if co-eluting compounds are present as presented for fusarium toxins in Fig. 5. In multiple reaction monitoring (MRM) mode, the mass-spectrometer scans specifically for one daughter ion resulting from fragmentation of one parent ion. Selectivity is sufficient to quantify chromatographically co-eluting ochratoxin A (OTA), 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON). Simultaneous determination of most relevant compounds can be easily performed by HPLC-MS/MS or alternatively GC-MS/MS methods, though the latter are limited to volatile compounds. As quality control is also an important issue in the surveillance laboratories, mass-spectrometric methods offer a higher reliability, especially when it comes to legal hassles. Contaminated food is

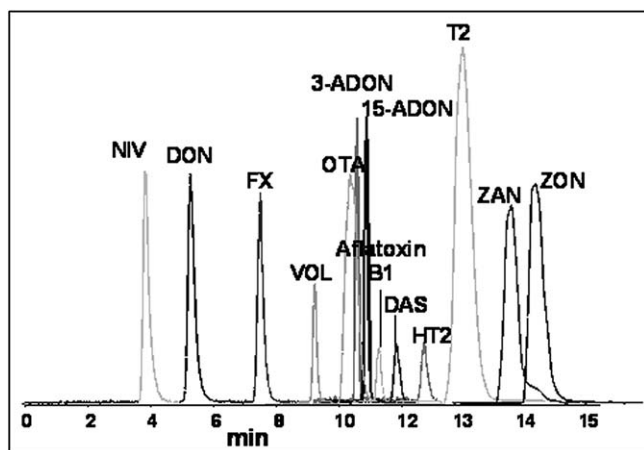


Fig. 5. HPLC-MS/MS chromatogram of different MRM traces for fusarium toxin analysis. NIV nivalenol, DON desoxynivalenol, FX fusarenon X, VOL verrucarol, OTA ochratoxin A, 3-ADON 3-acetyldeoxynivalenol, 15-ADON 15-acetyldeoxynivalenol, Aflatoxin B1, DAS diacetoxyscirpenol, HT2 HT-2 toxin, T2 T-2 toxin, ZAN zearalenone, ZON zearalenone (Biselli et al., 2004).

not allowed to be marketed and has to be destroyed, resulting in huge economic losses for the producer. Therefore, surveillance has to accommodate manufacturers' needs by auditing the production facilities and using the most sensitive analysis methods for their work.

3.3. Identity

Labeling of ingredients is of particular importance for consumer protection. European legislation demands the correct labeling of ingredients, possible allergens and nutritional facts (Humieres & Wal, 2004). For the consumer labeling is very important in terms of the identity of food, for two reasons. Firstly, if products from different animal or plant sources were used for production (e.g., poultry and pig meat) the ingredients must be qualitatively and quantitatively analyzable in food. Secondly, the consumers are very concerned about GMO in food. Even if an ingredient like soya is correctly labeled it must be possible to trace back whether its origin is from GMO plants.

Distinguishing between different animals and plants can be done by electrophoretic separation of protein but this has proven to be insufficiently sensitive and selective. Immunological methods such as the determination of different milk types by the method of Ouchterlony are simply applicable for this specific purpose but are time consuming and lack in sensitivity (Günther, 1991; Ouchterlony, 1958; Ouchterlony, 1962). Current methods are based on the polymerase chain reaction method (PCR), which is being constantly improved to identify the smallest amount (<0.1%) of different meat species or GMO ingredients in food products. Three different PCR-techniques are currently utilized for species identification. Normal PCR needs specific primers for DNA to be amplified, such as primers for the pig or cattle growth hormone sequence (Meyer, Candrian, & Lüthy, 1994). As this single PCR may lack in selectivity due to cross-reactivity, alternative methods such as the restriction fragment length polymorphism PCR (RFLP-PCR) (Meyer, Höfelein, Lüthy, & Candrian, 1995) or the terminal restriction fragment length polymorphism PCR (t-RFLP-PCR) have been developed recently (Seidel, 2005; Seidel, Böhle, & Kördel, 2004). These methods amplify mitochondrial cytochrome b gene or other suitable genes but unless separating these PCR products by electrophoresis as in normal PCR, amplicates are cut by specific restriction enzymes (e.g., HAE III endonuclease) inside the molecule (RFLP-PCR) or from the terminal end (t-RFLP-PCR). The obtained fragment pattern is characteristic for different species as these genes differ slightly (polymorphism), as presented in Fig. 6. Especially the t-RFLP-PCR with its fluorescence labeled amplicates is a promising technique capable of distinguishing between 40 different species in low amounts (detection limit 1–5%).

GMO can be identified and quantified by analog PCR methods. Real time PCR allows quantitation of GMO content of 0.1% sufficient for most applications. This method was used to detect GMO pollen in rape honey (Fig. 7) or

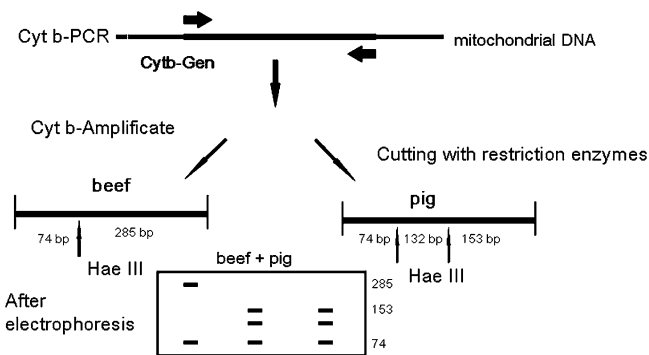


Fig. 6. Schematic presentation of RFLP-PCR analysis (Meyer et al., 1995).

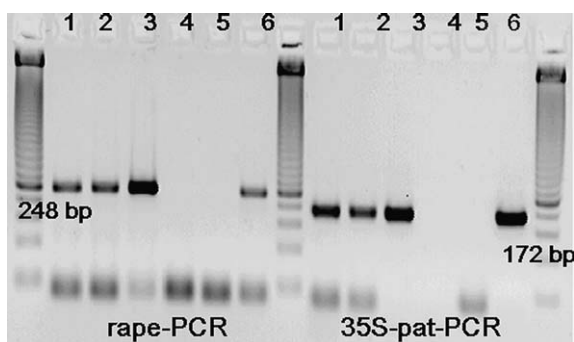


Fig. 7. Electrophoresis of PCR-amplificates from pollen DNA of rape for 248 bp and 172 bp constructs of glufosinolate resistance genetically modified rape in honey. (1) Rape-clover honey, Canada, (2) rape honey, Canada 1, (3) rape honey, Canada 2, (4) "Waldhonig", Germany, (5) negative control, (6) positive control glufosinate resistant rape (Waiblinger et al., 1999).

GMO soya DNA in lecithin (Waiblinger, Wurz, Freyer, & Pietsch, 1999; Zeitler, Pietsch, & Waiblinger, 2002).

Unfortunately, cross-contamination is not always avoidable and in that sense zero tolerance for GMO, as demanded by certain organizations, will not be accomplishable in the future and has to be discussed.

3.4. *trans*-Fatty acids

A special topic is the *trans*-fatty acid (TFA) content of food stuff as currently discussed in the United States, Canada, and the EU (EFSA, 2004). TFA occur naturally in food due to biohydrogenation reactions of microorganisms (e.g., butyrivibrio fibrisolvens in rumen of cattle) producing *trans*-monoenoic, dienoic, and trienoic fatty acids. Secondly, these fatty acids are formed during hardening of fats and during processing of food (Hunter, 2005). Although mechanisms of action are still under research, studies have shown that *trans*-monoenoic fatty acids decreases high density lipoprotein cholesterol and increases low density lipoprotein cholesterol, which are both associated with higher risk of cardiovascular disease (Mensink & Vermunt, 2005). In contrast to these studies, other TFA like the con-

jugated linoleic acids (CLA) are supposed to have protective effects in terms of atherosclerosis, carcinogenesis, and inflammation (Belury, 2002). TFA, excepting conjugated fatty acids, have to be labeled from January 1, 2006 in the US. Analysis of these fatty acids requires highly sophisticated methods, as simple methods such as FTIR-spectroscopy or UV-spectroscopy are not sufficient to comply with labeling demands. Separation of TFA requires highly polar GC-columns (e.g., 100 m CP Sil 88 or SP2560 or BPX70) but for complete baseline separation pre-fractionation by Ag^+ -TLC or Ag^+ -HPLC for *cis*- and *trans*-monoenoic fatty acid is necessary (Fritsche et al., 1999; Juaneda & Sebedio, 1999; Mossoba et al., 2001). These methods are tedious and unsuitable for routine analysis, let alone production control. Additionally, artifact formation due to lipid oxidation and false quantitation due to matrix interferences are crucial. Ag^+ -HPLC has been shown to be a suitable alternative to GC-FID or GC-MS/MS, avoiding artifact formation by low analysis temperatures. TFA are separated by interaction with silver-ions, bound by means of a strong-cationic exchange resin. Silver ions can form charge-transfer-complexes with the double bond and TFA are separated depending on the configuration (*cis/trans*) and the position of the double bond (Fig. 8). In combination with atmospheric pressure photo ionization interface (APPI) (Robb, Covey, & Bruins, 2001), which ionizes compounds by irradiation of a strong UV-lamp (10 eV) and triple-quadrupole MS detection, it is possible to analyze *trans*-monoenoic fatty acids and CLAs free of interferences of matrix compounds such as lipid oxidation products, as demonstrated in Fig. 9 for analysis of human coronary artery smooth muscle cell. Ag^+ -HPLC-APPI-MS/MS is sensitive as the eluent cannot be ionized by the UV-lamp, resulting in low background. Nevertheless, analysis of TFA requires very sophisticated equipment and there are as yet no sufficiently sensitive methods for production control.

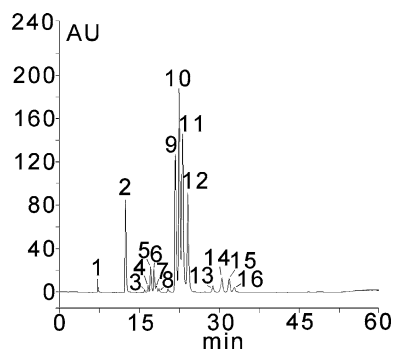


Fig. 8. Ag^+ -HPLC chromatogram of CLA methyl esters (2 ChromSpher 5 Lipids columns, Varian, Darmstadt, Germany) using *n*-hexane/acetonitrile 0.15% as eluent. (1) toluene, (2) SAHE, (3) t12t14-CLA, (4) t11t13-CLA, (5) t10t12-CLA, (6) t9t11-CLA, (7) t8t10-CLA, (8) t7t9-CLA, (9) c11t13-CLA, (10) t10c12-CLA, (11) c9t11-CLA, (12) t8c10-CLA, (13) c11c13-CLA, (14) c12c14-CLA, (15) c9c11-CLA, (16) c8c10-CLA.

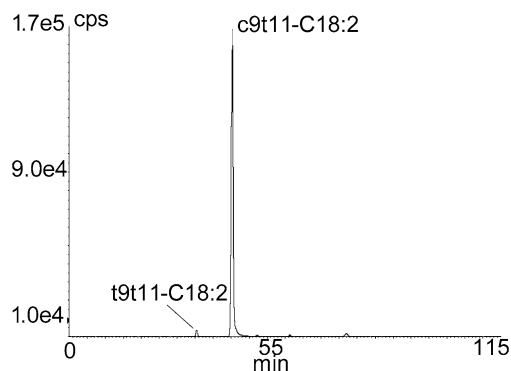


Fig. 9. Selective analysis of CLA isomers in human coronary artery smooth muscle cells by Ag^+ -HPLC-APPI-MS/MS.

3.5. Origin

The geographic origin is also an important factor for evaluating the quality of food, not because of changes in nutritional value but in terms of consumer deception, selling cheap foreign products as high-price regional food. Criminal mislabeling of vegetables (e.g., asparagus, strawberries) or wine may increase a distributor's profits significantly. Alternatively, adulteration of low quality juices with exogenic sucrose or organic acids or selling fruit juices from concentrates as fresh juices or the origin of aroma compounds are also topics for food surveillance (Parker, Kelly, Sharman, Dennis, & Howie, 1998; Spangenberg & Ogrinc, 2001; Tamura, Appel, Richling, & Schreier, 2005). These changes can be detected by analyzing isotope mass ratio of different elements (e.g., ^2H , ^{18}O , ^{13}C , ^{15}N) by high resolution mass-spectrometry (HRMS) (Calderone, Naulet, Guillou, Reniero, & Cortes, 2005; Christoph et al., 2004; Naulet, 1996) or ^2H NMR (Kocjancic, Kosir, Kidric, & Ogrinc, 2002; Martin, Martin, Mabon, & Michon, 1983). A schematic presentation of a sector field mass spectrometer for analysis of gases after combustion of the sample is shown in Fig. 10. These methods are based on the different regional isotope distribution, which allows identification of the geographic origin of a product if sufficient data of original samples can be utilized to produce a database for comparison. $\delta^2\text{H}$ - and $\delta^{18}\text{O}$ -values differ in samples depending on the distance to the sea as deuterated water is discriminated during evaporation of sea water, which will result in changes in isotope ratio in the rain on the mainland. Changes in $\delta^2\text{H}$ - and $\delta^{18}\text{O}$ -values for asparagus from different origins are shown in Fig. 11 (Schlicht, personal communication). Although deviation within one sample is high, comparison of samples of different geographic origin by principle component analysis (PCA) is possible. For organic compounds like sugars it is possible to distinguish from C_4 - or C_3 -sources (cane or beet sugar) by isotope ratio mass-spectrometry and measuring simple gases such as CO_2 , N_2 , CO but especially the $^{13}\text{C}/^{12}\text{C}$ -ratio.

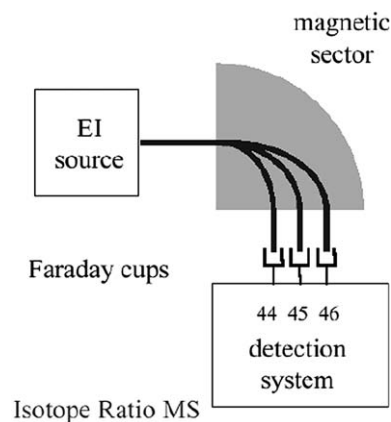


Fig. 10. Schematic presentation of a high resolution-(sector field)-mass spectrometer.

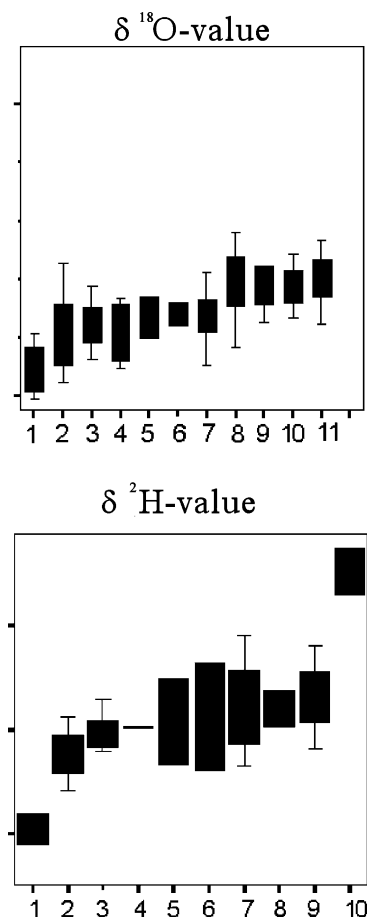


Fig. 11. $\delta^{18}\text{O}$ - and $\delta^2\text{H}$ -value obtained from C-IRMS for determination of geographic origin of asparagus. Top $\delta^{18}\text{O}$ -value of asparagus from (1) Ainach, (2) Schrobenshausen, (3) Nürnberg, (4) Ungarn, (5) Italien, (6) Klingenhain, (7) Kelheim, (8) Bamberg, (9) Griechenland, (10) Kitzingen, (11) Fürth. Bottom $\delta^2\text{H}$ -values of asparagus from (1) Schrobenshausen, (2) Komitat Csongrad, (3) Böhmerwald Meinik, (4) Kelheim, (5) Pfaffenhofen, (6) Groß-Gerau, (7) Bamberg, (8) Knoblauchsland, (9) Kitzingen, (10) Bergstraße (Schlicht, personal communication).

4. New analytical tool

4.1. Sensors

The need of manufacturers to efficiently control the production processes prompts the development of online and at-line methods for quality control. Product quality cannot be sufficiently controlled by recipes because it is not possible to check every single unit of a batch. Electronic sensors for moisture, pH value, and sugar content can now be easily installed on the basis of conductivity, refractometric or polarimetric measurements in homogenous samples such as liquid or dough for the beverage or roasting/bakery industry. The use of electronic noses is convenient and capable of measuring changes in air composition for detection of mould infected stocks. These electronic sensors consist of metal oxide semiconductors, conducting polymer sensors or quartz crystal microbalance sensors (Holm, 2003), the latter, being the most versatile sensors, are based on the piezoelectric effect. When molecules are absorbed on the coated layer on the quartz crystal surface, the frequency of oscillation changes in proportion to the amount of mass absorbed and the resulting change in current can be measured. A schematic presentation of an electronic sensor is shown in Fig. 12. These sensors can, however, be easily contaminated by adsorbed molecules, which is especially unfavorable with the metal oxide sensors. Sensors have to be calibrated for specific aim and lack robustness.

Current applications are limited to detection of volatile compounds (Dello Monaco et al., 2005; Garcia et al., 2006; Nimmermark, 2004) and include tracking of aroma evolution of ice-stored fish or meat and cheese aroma during aging, testing of coffee, whiskey and soft drinks and classification of wines (Holm, 2003; Patel, 2002).

4.2. NIR methods

Other fast methods have been developed for at-line analysis/production control such as NIR-spectroscopy. Although these methods have to be extensively calibrated they are suitable for simple matrices such as yoghurt or beverages to determine the content of macronutrient (protein, fat, carbohydrates). At least it is possible to control for specific production standards, so tedious reference methods need only be used if deviations from these quality

standards occur during production (Azizian et al., 2004; Büning-Pfaue, Hartmann, Kehraus, & Urban, 1998; Endo, Tagiri-Endo, & Kimura, 2005; Prevolnik, Candek-Potokar, & Skorjanc, 2004).

4.3. Multi-methods/fast methods

Not only for pesticide or residues will development of multi-methods help to save time and costs, as laboratories are always in need of developing faster methods. For determination of alcohol content a new method based on an oscillation measurement in a U-tube is gaining popularity. As oscillation is influenced by the density of the liquid inside the U-tube density and/alcohol content can be measured in a few minutes. Pycnometric determination of the alcohol content is therefore only used as reference method for confirmation of results (Lachenmeier, Sviridov, Frank, & Athanasakis, 2003).

Fatty acid determination according to new labeling demands can be easily performed within 30 min by the Caviezel method. Fats are saponified and instead of analyzing the fatty acid methyl esters, free fatty acids are analyzed using a packed column with automated quantitation of results. This method is suitable for determination of butyric acid and total fat content but lacks resolution for TFA (Gertz & Fiebig, 2000; Pendl, Bauer, Caviezel, & Schultheis, 1998).

Trace elements can be determined by “continuum sources”, covering wide spectral ranges, for atom absorption spectroscopy with no need of changing the lamp. Alternatively, element determination based on inductively coupled plasma–mass-spectrometry will increase as nutritional research results will show the necessity to control for composition and amount of trace element (Gleisner, Liebmann, & Furche, 2005).

5. Conclusion

Methods for quality control will change as demands of the consumers, manufacturers, researchers and the legislative change. Simple methods based on chemical, physical and increasingly biochemical/immunological principles are commonly used for routine analysis, but in case of doubt sophisticated reference methods will be the methods of choice.

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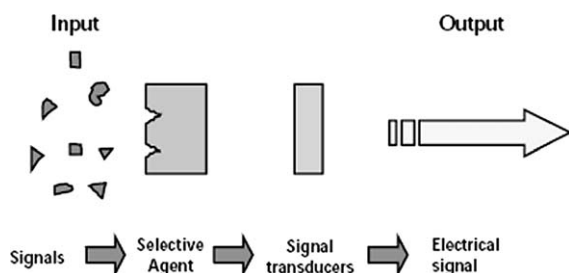


Fig. 12. Schematic presentation of electronic sensor (Holm, 2003).

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