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Review of the current methods of analytical traceability allowing determination of the origin of foodstuffs

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Abstract

Modern analytical techniques, in particular molecular biology techniques, can determine the plant or animal species present in a foodstuff. It is actually very difficult to determine the geographical origin of a food. The new European regulation 178/2002, applicable on January 1st, 2005 imposes this requirement for the traceability of food. This article gives a progress report on the physicochemical and microbiological analytical techniques available which make it possible to determine the origin of a food with some precision.

Keywords: Origin; Traceability; Food; Analytical methods

Introduction

This review is a progress report on the research techniques which would permit the determination of the geographical origin of foodstuff. It also presents a critical analysis of these methods and provides recommendations for the techniques best suited to different applications. The methods make possible the determination of the geographical origin based on the analysis of the product environment using discriminating criteria with reference to data banks of markers. The choice of a technique strongly depends on the substrate studied. The characterization of origin could ensue from coupled analytical techniques. The results are then analyzed by mathematical/statistical methods to process the data.

These methods can be categorized into two types: the physicochemical techniques, which use either the variation of the radioactive isotope content of the product, spectroscopy, pyrolysis or electronic nose, and the biological techniques which use the analysis of total bacterial flora through many techniques like the Denaturing Gradient Gel Electrophoresis (DGGE) and Denaturing High Performance Liquid Chromatography (DHPLC), the Polymorphism of Conformation of the Single Strand DNA (SSCP) or DNA chips. Each technique is illustrated by a published example. These studies will help in differentiating a milk produced on a mountain from that produced on the plains, of determining the origin of various cheeses or various wines, or of identifying the geographical origin of other foods like oysters, meats, fish, olive oils, teas or fruit juices.

1. Physicochemical techniques

1.1. Techniques using the variation of radioactive isotopes

1.1.1. Nuclear magnetic resonance coupled with mass spectrometry of isotopic ratio (NMR/MSIR)

Nuclear Magnetic Resonance (NMR) is a phenomenon which occurs when the nucleus of certain atoms, immersed in a uniform magnetic field, are exposed to another variable magnetic field. All of the nuclei are not affected in the same way to the signal and it is dependant on their

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nuclear spin. Mass Spectrometry of Isotopic Ratio (MSIR) is based on the principle that electrically charged particles, when they are subjected to a magnetic field, deviates according to their atomic mass. Besides hydrogen, it is thus possible to determine the isotopes of carbon (^{12}C and ^{13}C) and oxygen (^{16}O and ^{18}O). From the analyzed sample, the ratio of mass/charge (m/z) can be determined.

The advantage of coupling NMR to a MSIR is due to the fact that a NMR is not a very sensitive technique as it is related to a unique characteristic of the sample such as the spin. MSIR on the other hand is a technique which allows a fine analysis of each extracted element.

Renou et al. (2004) studied cow milks raised from two distinct sites, which differ in their geographical situations and their altitudes (mountain and plain). The researchers used NMR to determine the chemical characteristics of the poly-unsaturated fatty acids (PUFA), mono-unsaturated fatty acids (MUFA) and saturated fatty acids (SFA) in the lipid fraction of the milk and MSIR to measure the enrichment in ^{18}O of drinking water given to the ruminants which produced milk and dairy products.

The percentage of PUFA was significantly higher in the fatty acids of mountain milk ($4.3\% \pm 2.4\%$ to $6.2\% \pm 1.1\%$) that in the plain milk ($1.7\% \pm 0.4\%$ to $1.8\% \pm 1.5\%$), while the MUFA ($17.3\% \pm 2.4\%$ to $22\% \pm 3.5\%$ for the mountain milk and $19.7\% \pm 1.2\%$ to $26\% \pm 5.4\%$ for the plain milk) and the SFA ($73.3\% \pm 6\%$ to $76.5\% \pm 2.4\%$ for the mountain milk and $72.3\% \pm 6\%$ to $78.7\% \pm 1.4\%$ for the plain milk) did not differ significantly between the two locations. There is, thus, a geographical effect on the poly-unsaturated fatty acids. The microbial flora of the mountain pastures differed distinctly from that of the plains. The mountain pastures are very rich in dicotyledonous and herbaceous non-leguminous plants, while the plain pastures are mainly composed by graminaceous and leguminous plants. These two distinct botanical compositions generate a quantity of PUFA higher in mountain milk compared to that collected in the plains (Collomb, Bütikofer, Spahni, Jeangros, & Bosset, 1999).

The results are expressed in a ratio of isotope (International Standard Vienna Mean Ocean Water) from where the presence of the negative values: $\partial = \left(\frac{R_S}{R_{SMOW}} - 1\right) \times 10^3$ with R_S and R_{SMOW} that are the respective ratios of the sample and the standard.

For drinking water, the values of ^{18}O were (-10.66 ± 0.08 to -10.50 ± 0.46 for the mountain water and -5.21 ± 0.30 for the plain water) and of ^{2}H (-50.4 ± 3.4 to -45.3 ± 6.1 for the mountain water and -21.8 ± 5.4 to -17.4 ± 5.6 for the plain water) differed between the sites but do not vary with season in the same site. There is thus a significant variation of these two isotopes according to the place of breeding.

Other examples include Pillonel, Collomb, Tabacchi, and Bosset (2003) who presented results obtained on the authenticity and the geographical traceability of the Emmental cheeses. The goal was to select the most discriminating methods and criteria. Martin and Martin (1995), Gremaud, Micaux, and Piantini (2002), Berdagué (2000), and Cuinier and Favarel (2000) all proposed to authenticate various wines by isotopic methods. Martin and Martin (1995) showed that the geographical origin of foodstuffs such as oils, fruit juices and wines could be determined successfully by using mass spectrometry of the stable isotopes. They studied deuterium enrichment on specific sites of molecules by NMR.

The NMR/MSIR produces significant results and a good differentiation of the products according to their geographical origin and diets. But it is not possible to automate this method which is limited by NMR sensitivity and is, moreover, an imposing and complex piece of equipment.

1.1.2. Ion exchange chromatography/atomic absorption spectrometry (AAS)

Ion exchangers are insoluble macromolecules carrying ionizable groups, which have the property to exchange in a reversible way some of their ions with other molecules. Atomic absorption spectrometry (AAS) permits the study of absorption of light by free atoms by the energy variation when one of the electrons passes from one electronic orbit to another. The absorption intensity depends directly of the number of particles that absorb the light. AAS is often used when elements are present in low concentrations. Ion exchange chromatography, which is a separative method, will be coupled with atomic absorption spectroscopy to quantify the elements isolated from samples.

Froidevaux, Geering, Pillonel, Bosset, and Valley (2004) quantified the natural or metabolized radioelements in Emmental cheese whose type and quantity varied according to the geographical location of the cows. Strontium ^{90}Sr is an artificial radioelement found everywhere in Europe whose presence is primarily due to the repercussions of the Chernobyl accident in 1986. ^{90}Sr passes into dairy products by the water consumed by animals and can be used to distinguish Emmental cheese type produced in Brittany and Finland from those produced in the Alp mountains (Switzerland, Savoy, Allgau and Vorarlberg). The observed differences of this radioelement are explained by the geographical protective barriers against radioactive fallout and by the weather conditions just after the Chernobyl accident. To a lesser extent, it is also possible to take into account the ratio of $^{234}U/^{238}U$ to find the authenticity of the product origin, because there is a variation of this ratio due to the natural phenomenon of ^{238}U elimination, which is characteristic of the pasture geology and the ground and underground water. It is thus interesting to seek particular geological or accidental events to trace a characteristic product from an area.

This method, based on radioisotope analysis, is simple, fast and sensitive but is limited by its non-universality, the resolution, the capacity and the high cost of the chromatography column. Moreover, AAS analysis could present some anomalies such as *spectral disturbances*, an

absorption line of a component of the matrix coincides with the emission line of the resonance source; *physical disturbances* due primarily to viscosity and surface tension, a low viscosity and a weak surface tension will, for the same concentration, lead to higher values of absorption, *chemical disturbances*: atoms present in the flame absorb only if they are in a fundamental state: if they are excited or ionized, they will not absorb. In addition, if they form oxides, hydroxides, hydrures with the atoms and radicals present in the flame, they do not contribute to absorption; *non-specific disturbances of absorption*: due to the presence in the flame of molecules which absorb the energy of the hollow cathode lamp. This molecular absorption is added to the atomic absorption and gives a response by excess.

1.1.3. Site-specific Natural Isotope Fractionation by Nuclear Magnetic Resonance (SNIF-NMR)

During food processing, isotopically accurate information is recorded not only about the nature of the precursors and the mechanisms of chemical or biochemical synthesis, but also about the environmental conditions. Study of specific natural isotopic fractionation (SNIF-NMR) permits the association of a pure product or a component of a complex product with a particularly reliable identity card and it is thus possible to know the geographical origin of a food.

The Swiss Federal Office of Public Health (Data bank of Swiss wines, 2002) discriminated the geographical origin of various wines coming from various sites by measuring the isotopic relationship between deuterium and hydrogen (D/H), contained in ethanol produced during alcoholic fermentation. The authors succeeded in discriminating the various Swiss wine areas by this method and obtained characteristic results for a given wine reproducible from one year to the other.

The SNIF-NMR is based on robust isotopic parameters, theoretically better controllable, allowing a discrimination of products having close geographical origins.

1.2. Spectroscopy techniques

1.2.1. Mid and Near Infrared Spectroscopy (MIRS–NIRS)

Bertrand (2002) gave an overview of the use of the Near Infrared Spectroscopy and its applications in the animal feed industries. It is a non-destructive analytical technique based on the principle of absorption of electromagnetic radiations by the matter. Infrared spectroscopy acts on energies of vibration of the molecular bonds. When the wavelength (energy) brought by the light is close to the energy of the molecular vibration, it will absorb the radiation and a reduction in the reflected or transmitted intensity will be recorded. Spectra are analyzed and calculated by statistical methods such as Discriminating Factorial Analysis (DFA) or by partial least squares (PLS).

In Germany, Wittkowski (2002) studied 25 wines from five countries of the New World, described by 77 analytical parameters and subjected them to a statistical multivariable calculation. It was found that 13 parameters were enough to differentiate wines according to their origin. Among these parameters, not only the content of inorganic molecules such as nitrates, magnesium and phosphate were studied but also the dry matter, the alcoholic degree, inverted sugars and anthocyanins.

In France, Picque, Cattenoz, Tréléa, Cuinier, and Corrieu (2002) studied 276 wines of the same variety of vine, the Gamay, which were harvested over 3 years (1998, 1999, 2000) and were analyzed by MIRS with Fourier transmission in order to authenticate the areas of origin as Gaillac, Beaujolais or Tourraine. The infrared analysis of the spectra by DFA and PLS appeared to be powerful enough to authenticate the classification of wine produced from the same type of vine but from three different French areas.

Other examples include Hall (1998) who showed that the MIRS technique is very powerful for the authentication of fruit juices because it is a non-destructive and fast analysis with a moderate cost and it does not need consumables for the equipment. But this method is not suitable to analyze traces. Furthermore, the size of the particles modifies the spectra and it is necessary to create a data bank of a great range of substrates which are susceptible to be analyzed.

1.2.2. Fourier Transform Mid-Infrared Spectroscopy (FT-MIRS)

This is based on the same principle as the previous technique mentioned. Vibrations conduct an absorption which will depend on the geometry of the molecule and, in particular, its symmetry. Consequently, a whole characteristic absorption bands will correspond to a substrate with a chemical composition and given structure that will permit the identification of the substrate. The advantage of this technique over MIRS is that the whole wavelengths are studied simultaneously, which leads to an important time saving and allows the acquisition of several spectra, increasing the signal/noise ratio and giving a better resolution.

Pillonel et al. (2002) authenticated 93 Emmental cheeses coming from five European countries by FT-MIRS (Germany, Austria, Finland, France, Switzerland). They were classified according to the geographical origin with a success rate higher than 80%.

By FT-MIRS, it is possible to differentiate *cis* and *trans* unsaturated fatty acids. It is reliable and more rapid than MIRS and traditional infrared. This method could be coupled with other techniques to increase the accuracy of the results. For example, coupling FT-MIRS and Gas chromatography (GC) made it possible to analyze directly complex mixtures such as flavours and fatty acids isomers in methyl ester form.

1.2.3. Fluorescence spectroscopy

This technique gives information on the presence of fluorophores and their environment in the sample. Using fluorescence properties of certain amino acids or extrinsic probes added to the medium, the structure of proteins

alone or interacting with small hydrophobic molecules can be characterized. The emission spectra of fluorescence is acquired after excitation with a certain wavelength. The data are analyzed by Principal Components Analysis and Discriminating Factorial Analysis. By those method it is, however, very difficult to discriminate geographically close regions.

Dufour, Karoui, and Bosset (2002) used fluorescence spectroscopy to ensure the quality control of Gruyere cheeses under Protected Designation of Origin (PDO). The authors produced by fluorescence analysis the emission spectra of aromatic amino acids and nucleic acids of PDO Gruyeres for their geographical origin. The 25 hard cheeses analyzed, Etivaz or Gruyere types, were manufactured in three places of different altitudes: Etivaz is produced at 1500 m and Gruyeres are manufactured either in a mountain zone (1100–1500 m) or in a plain zone (800 m). The analysis of the spectra discriminated the three groups of cheeses with 100% of the individuals allotted to the correct group. The cheese characteristics strongly depended on the manufacture but also on the conditions of milk production.

Other example include Dufour and Frencia (2001) who used frontal fluorescence spectroscopy to obtain a characteristic spectrum of meat and to measure the lipid oxidation in fish. They were correlated with the state of degradation of the product because it is fast (time of analysis <1 s) and it is 100–1000 times more sensitive than other spectroscopic techniques. It is also non-destructive and inexpensive.

1.3. Other techniques

1.3.1. Curie point pyrolysis coupled to mass spectrometry (Cp–PyMS)

Molecules resulting from a thermal degradation under vacuum are analyzed by mass spectrometry. The mass spectrometer records the kinetics of molecule's degradation and generates a print which is compared, by using a recognition algorithm, to a collection of prints of known systems indexed in a computerized data bank of spectra.

In order to solve the problem of determining the origin of oysters Cardinal, Viallon, Thonat, and Berdagué (2000) proposed the use of Cp–PyMS to study 180 samples harvested during the four seasons of the year, on nine different sectors of breeding and in 4–8 different sites per sector. The results showed that it is possible to quickly identify a sector of production but it is not yet possible to differentiate the oyster species because this team worked only on a unique species. This fast technique provided the global prints for the product matrix (carbohydrates, lipids and proteins). Eighty nine percent of the samples could be classified successfully, independently of the season. The errors undoubtedly come from the very similar composition of certain oysters making their discrimination difficult. Their planktonic food should be different according to the breeding area, thereby improving their discrimination. Moreover, the physiological state and the period of production of oysters were different according to sectors. It would thus be necessary to take into account these parameters, as well as the environment for future analyses.

Others examples include Salter et al. (1997) determined the geographical origin of extra virgin oil from Italy by PyMS by the use of Artificial Neural Networks Technology. Berdagué, Rabot, and Bonneau (1996) classified porcine carcasses with different contents in androstenone and scatol and characterized dry hams or cheeses resulting from different technologies, processes of refrigeration/freezing, oysters from the same species but from different sites of production, milks from distinct production area (plain or mountain) and followed microbial interactions in yogurt by PyMS. Dalgaard, Manfio, and Goodfellow (1997) classified bacteria responsible for fish rotting by taxonomic studies and PyMS. Within the framework of its research programmes, the company Bio-Sens (France) setup protocols for the characterization of the geographical source of raw material such as milk, tea, wine, cheese and the determination of their methods of manufacture.

Cp–PyMS is automatizable, fast, applicable to several substrates and allows the identification of bacteria, but the equipment is expensive and there are a few applications. Moreover, it presents a gradual loss of sensitivity and it is thus necessary to limit the quantity of material used for the pyrolysis to obtain good food spectra. A source of ionization by bombardment of metastable atoms considerably improved the characterization of the complex biological systems.

1.3.2. Electronic nose coupled with mass spectrometry (Smartnose.com, 2004)

The electronic nose analyzes the global intensity and objectively recognizes volatile compounds of a sample at very low concentrations (ppb) by comparison with a data bank acquired by training. Like the human sense of smell, it progressively improves its capacities during its use. The users will gauge the apparatus and fix the thresholds of acceptance of the analyzed product. The electronic nose correlates human perception (data bank) and analyzes the chromatogram by mathematical systems. Broadly, this technology is based on the absorption and the desorption of volatile chemical substances. It is generally composed of a system of detection of the odorous molecules released by the sample after heating. The system of detection could be a network of gas sensors or a mass spectrography, coupled with a system of statistical processing that lead to a classification of the odours and generate a particular finger-print.

The electronic nose is used in many fields in quality control such as the acceptance or the refusal of raw materials such as coffee (Gretsch, Toury, Estebaranz, & Liardon, 1998) and tea (Dutta, Kashwon, Bhuyan, Hines, & Garner, 2003), determination of manufacturing defects during cheese processing (Decker, Trihaas, & Nielsen, 2003) or a finished cheddar cheese (Chung, Partridge, & Harte,

2003), appreciation of fruit maturity (Supriyadi et al., 2004), differentiating fruit juices (Goodner & Baldwin, 2001), wine quality control (Chauvet, Tan, Schmitt, & Yoshida, 2000), beef freshness control (Suwansri, Pohlman, Meullenet, & Mc Eylea, 2001). It finds other applications in the determination of the origin of products such as olive oil (Garcia & Gonzalez, 2002; Gonzalez, Perez, Moreno, & Garcia, 1999) and orange (Goodner, Baldwin, Jordán, & Shaw, 2000; Steine, Beaucousin, Siv, & Peiffer, 2001), etc. The electronic nose can recognize the unpleasant odour of two hormones which affect pig meat and permit the differentiation of the origin of the meat and its process of transformation (Agroscope, Switzerland). The European project "Fishnose (2004)" consists of developing an electronic nose for the determination of fish quality.

By characterizing a product by its odour, electronic noses can help to decide on its nature, origin or its quality. This technique is reproducible, selective, general–purpose, sensitive and fast. Moreover, remedial measures are possible during the transformation of the product but this equipment is expensive and it is necessary to create an large data bank.

2. Techniques using the analysis of microbial flora

Methods which use the analysis of microbial flora are based on the principle that the environment has an effect on the bacterial ecology of food. The bacteria can indeed differ by their quantity but especially by their species and characteristics (e.g. resistance to certain antibiotics). The variation of these parameters can be used to discriminate the places of production.

2.1. Traditional biochemical techniques

API galleries (Narayanan, Nagaraja, Staats, Chengappa, & Oberst, 1998). Traditional techniques of identification by multiple biochemical tests using the inoculation of a biochemical gallery such as API gallery 20 E (Mérieux, France) can be used to determine the microbial flora present in the substrate. But these methods are indirect and require the isolation of viable bacterial strains which diminishes their use as fast tests for the determination origin.

Analysis of antibiotic resistance. The recent analysis of antibiotic resistance of bacteria conducted by Agui et al. (2004) is an interesting approach. The massive use of antibiotics in intensive aquaculture causes an important modification of the bacterial biodiversity of both the fish and the aquatic environment. The analyses carried out from farmed fish in Vietnam and Thailand have shown that both locations represent potential reservoirs for antibiotic resistance. The bacterial resistance and multi-resistance profiles could be markers for intensive farming but this assumption needs to be confirmed through a large scale survey in both countries from distinct production areas. The type of flora found in a specific location varies and can be used to discriminate between samples.

ELISA Tests (Enzyme Linked Immuno Sorbent Assay). This immuno-enzymatic technique visualizes the specific antigen/antibody bounding by the production of a coloured compound by an enzyme linked beforehand to the substrate specific antibody. For bacteria, different antigens used are mainly somatic (ag O), capsular (ag K) or toxins. It is then very easy to determine quickly and with great specificity and speed all of the bacteria present in a sample. These tests are rather specific to the search for a type of bacteria like RapidChek™ Salmonella Test (Guide of Strategic diagnoses Inc.).

2.2. Molecular biological techniques

2.2.1. Denaturant Gel Gradient Electrophoresis (DGGE) and Single Strand Conformation of Polymorphism of DNA (SSCP)

DGGE (CMGS, 2005) and SSCP are based on the electrophoretic analysis of PCR products. SSCP is based on the electrophoretic analysis of PCR products in the form of single strand fragments. A mono-strand DNA can form secondary structures due to pairing of bases within the molecule. The studied area is amplified by PCR by using a marked primer. The mobility of the denatured DNA which carries a modification is compared with those of a reference fragment including a normal sequence. A specific change within a sequence modifies sufficiently the secondary structure of the single strand DNA so that it results in a change in the migration.

The SSCP can be carried out on polyacrylamide gel or can be coupled with an automated system of Capillary Electrophoresis (CE-SSCP) which seems at the present time to be the most reliable technique to characterize bacterial populations (Delbès, Moletta, & Godon, 2000).

The double strand amplicon is denatured by heating at 94 °C, then quickly cooled in ice to become a stable secondary structure. These fragments are then separated by migration on polyacrylamide gel. This technique is fast and leads to a profile of the microbial population. But the electrophoretic behaviour of the single strand fragments is very dependent on the temperature and the electrophoresis conditions. For fragments larger than 200 bp, all of the changes do not seem to be detectable. Using this technique Delbès et al. (2000) followed the dynamic of the bacterial populations in the environment.

DGGE uses the melting point of a PCR product (double strand DNA). It can separate two strands of the same size but with different sequences. A specific mutation which thus changes the sequence involves a modification of its annealing temperature. This modification is emphasised by electrophoresis on a polyacrylamide gel in the presence of a gradient of denaturing agent (urea + formamide) whose role is to imitate a linear increase in the temperature from the top to the bottom of the gel, that is to say, an increasing of the temperature during the electrophoresis (Temperature Gradient Gel Electrophoresis: TGGE).

The dissociation transforms the fragment of DNA into a partially open structure and creates a significant reduction in its mobility, which leads the DNA to be concentrated in a point of the gel, so that its final position in gel depends exclusively on its melting point (T_m) of the least stable area. The migration of the molecules is then very dependent on the sequence. It is estimated that approximately 95% of the sequence variations of the least stable fusion area can be detected by differences in electrophoretic migration.

The complete separation of the strands is prevented by the presence of a fusion area which is artificially created at the end of a molecule by the incorporation of a GC link. It is fixed during the PCR with a primer whose 5' end is composed of a sequence of 40 GC repeats.

DGGE is very sensitive and simple and it allows a high rate of detection. PCR fragments can be isolated from a gel and be used in reactions of sequencing. But the analysis of the 400 bp PCR fragments of is less satisfactory and the genes which are exceptionally rich in GC are not easily analyzed by DGGE.

DGGE and SSCP techniques are rivalled by the technique of high performance liquid chromatography with variable temperatures of elution (DHPLC: Denaturing High Pressure Liquid Chromatography). Van den bossche et al. (2000) recently used this technique to characterize the microbial flora in pigs. Montet (2004) proposed to use DGGE to analyze the totality of the microbial flora present in fresh water fish to create a biological bar code (DNA) determining the origin of the foodstuff. In order to validate this method, it is necessary to create a data bank representative of the various origins.

3. Other techniques that could be used in the determination of origin

There are some other techniques, which have not yet had an application in the field of the geographical traceability of the foodstuffs. We present two, particularly promising ones: A more rapid competitor of the DGGE is the Denaturing Chromatography in High Performance Liquid Phase (DHPLC). The second is a very powerful technique of molecular biology combined with data processing, DNA chips.

3.1. Denaturing chromatography in high performance Liquid Phase (DHPLC)

DHPLC is based on the principle of the HPLC. This method underlines the presence of "variation" in a DNA fragment by locating the areas of the double helix of DNA that are not linked together (Troesch et al., 1999). These regions that are not complementary are an indicator of a mutation. The DHPLC System of fragment analysis (DNA Wave®, Transgenomics, USA) could sift through the nucleotide variations. This technique detects all of the mutations in a DNA fragment amplified by PCR. This amplicon analysis is not expensive by itself if the DHPLC material which is an expensive apparatus is acquired.

The DHPLC is based on the possibility of separating heteroduplex DNA (two different strands) and homoduplex DNA (two identical strands). In practice, the fragment of studied DNA is amplified by PCR under partially denaturing conditions. Strands carrying a heterozygote mutation give homoduplexes (two identical strands) and heteroduplexes (two different strands) of double strands of DNA, whereas the homozygotes samples give only homoduplexes. The fragments of DNA are brought through the system by a plug and constitute the mobile phase (liquid). Size separation of DNA fragments is carried out by differential adsorption between the liquid and the matrix in the DNASep® column (Transgenomic™). The resolution is dependent upon the position of the mutation, the length of the fragment as well as its sequence. In the chromatography column, the heteroduplexes elutes first (acetonitrile gradient) and it is possible to analyze the associated mutation. This method is fast, economical, sensitive and automatizable but the study on DNA fragments is limited at 150–450 base pairs.

3.1.1. DNA chips

Born from the marriage of micro-electronics, biochemistry, combinative chemistry, molecular biology, data processing and image analysis, DNA chips or biochips permit the analysis of several thousands of different pieces of genetic information simultaneously without using a gel. It is based on the reaction of hybridization and can identify and quantify a considerable number of sequences of nucleic acid contained in a biological sample.

GeneChip® (Affymetrix Inc., USA) consists of a surface of glass of approximately 1 cm^2, on which could be deposited up to 400,000 DNA strands (markers = probes). The place where these probes were positioned is known exactly. Thus, among the sequences positioned on the GeneChip®, two markers are represented: a marker of species (ribosomal 16S RNA area), and a marker of rifampicin resistance (*rpoB* gene area). To have sufficient material, the nucleic acid is amplified, marked by fluorescence then deposited on the chip. If the target DNA is present in the sample, it will be hybridized on the chip with its complementary probe. By measuring the fluorescence, the probes which reacted can be located. Reading requires sophisticated optical methods (laser scanning, camera, etc.) coupled to a data-processing image treatment.

DNA chip have the advantage that their data-processing results are easily exchangeable within a international network of laboratories. Compared to current technologies, it is an accurate and a fast technology (results given in less than 4 h) making it possible to act more quickly when an incident occurs such as a food crisis and to reduce its consequences. Moreover, the support is universal because it uses nucleic acids and the analysis could cost 10 times less than a spectrometric analysis. On the other hand, it is a new method and its development could be lengthy and

the data banks could be non-existent. Moreover, it is a method which is not quantitative in the absolute and the extraction of the bacterial material can be complicated by the structure of the substrate.

4. Conclusions

The new regulations and in particular the new European regulation 178/2002, applicable on January 1st, 2005, impose the requirement for the traceability of food. But in the event of frauds or commercial disputes, what will be the types of analyses which could be used to solve these problems? This article is a tool to help professionals to make the best technical choice according to their objectives.

Universal scientific methods for the determination of the geographical origin of a foodstuff do not really exist. There are only indirect methods which often have to be coupled to increase their accuracy.

The methods which permit the analysis of the micro-environment of food are very promising and have to be better studied by research teams in the world. The main problem will be the construction of data banks which are very necessary for all of these techniques. Some others techniques will be developed in the near future taking into account, for example, the micro-constitution of food. One could consider the micro-components of the lipids like tocopherols, phospholipids or sterols or other molecules brought by the environment like the pesticides, traces of insects, heavy metals, radioactives isotopes, etc.

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