FOOD ANALYSIS BY FINGERPRINTING TECHNIQUES

TraceQ

This booklet summarises the output of Workpackage 2 (Fingerprinting and Profiling Techniques) of TRACE

(Tracing Food Commodities in Europe, FP6-2003-FOOD-2-A, contract number: 0060942)

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Dedicated to the memory of prof. Annalaura Segre, beloved friend and colleague

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Preface

This booklet has been written within the TRACE project (FP6-2003-FOOD-2-A, contract number: 0060942). The aim of this project was the development of reliable traceability methods and systems to establish the origin or mode of production of food products.

In particular, our workpackage has developed fingerprinting and profiling methods to characterise a number of foodstuffs
– chicken, honey, olive oil and beer.
A brief description of the fingerprinting techniques together with the most interesting results for these foodstuffs are reported. The possibilities for their deployment to other authenticity issues in a wide-range of foods are discussed.

Introduction

This booklet is intended to serve as a guide and help for companies, scientific professionals and consumers.
It contains results of the application of fingerprinting techniques to the analysis of foodstuffs in relation to identity or authenticity.

It should help to address some important issues concerning foodstuffs, such as:

- **Authenticity**
- **Identify confirmation**
- **Fingerprinting Control**
- **Product tracing**

GLOSSARY

Identity Confirmation
Methodology to confirm that a food is in compliance with claimed identity.

Food authenticity is a term which simply refers to whether the food purchased by a consumer matches its description.
Mis-descriptions can occur in many ways, from the undeclared addition of water or other cheaper materials, or the wrong declaration of the amount of a particular ingredient in the product, to making false statements about the source of ingredients i.e. their geographic, plant or animal origin.

Tracing Production
“Traceability” means the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution.

Fingerprinting Techniques
The term “fingerprinting techniques” describes a variety of analytical methods that can measure the composition of foodstuffs in a non-selective way such as by collecting a spectrum.
Mathematical processing of the information contained in such fingerprints may permit the characterisation of foodstuffs.

Fingerprinting Control
Methodology to control the food fingerprint in order to always have the same characteristics present in the same product. Fingerprinting techniques can help industry to have a standardised final product.

Protected Designation of Origin (PDO)

PDO is the name of a region, a specific place or, in exceptional cases, the name of a country, used to describe an agricultural product or a foodstuff

- which comes from such an area, place or country

- the quality or the characteristic of which are essentially or exclusively due to a particular geographical environment, including natural and human factors

- the production (all the stages from the production of raw materials until the preparation of the final product) must takes place in the defined geographical area

Protected Geographical Indication (PGI)

PGI is the name of a region, a specific place or, in exceptional cases, the name of a country, used to describe an agricultural product or a foodstuff

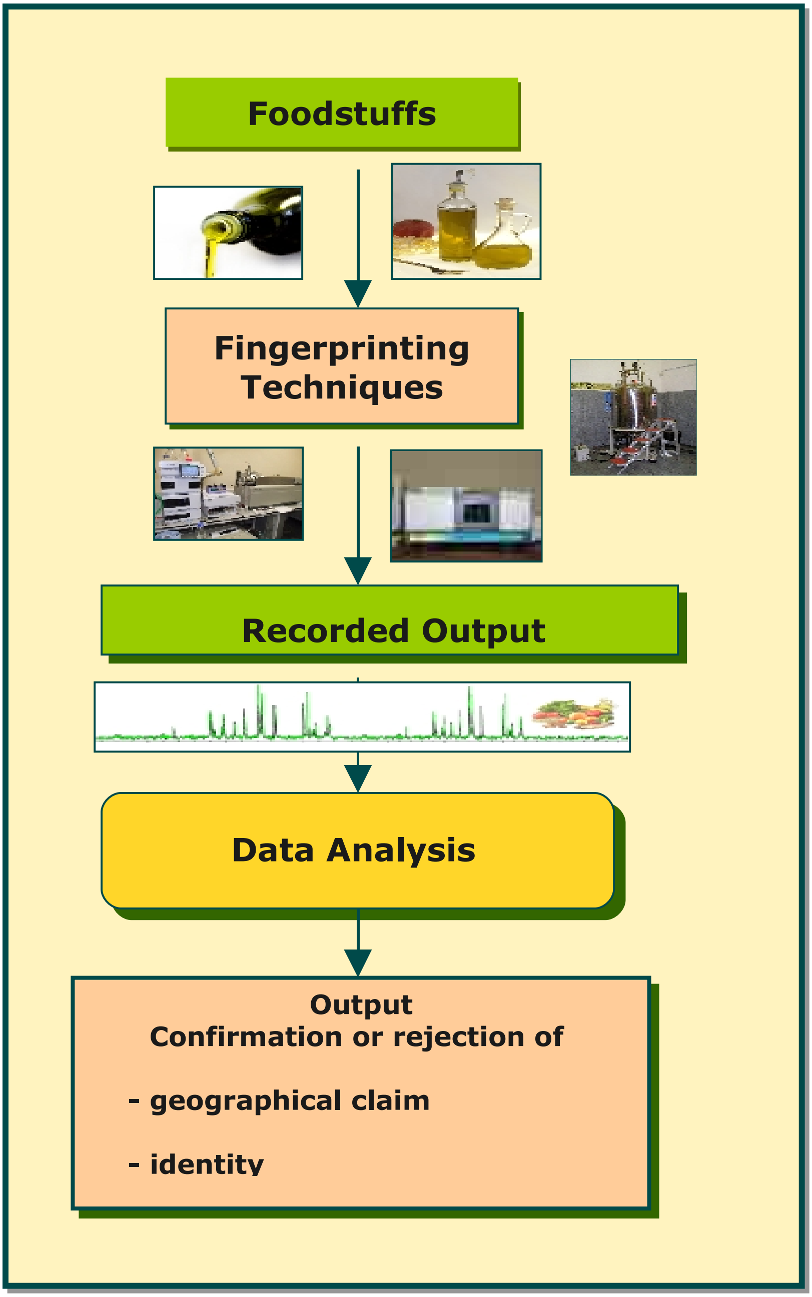
- which comes from such an area, place or country

- which has a specific quality, goodwill or other characteristic property, attributable to its geographical origin

- the production and/or processing and/or preparation of the product (only one of this three stages) take place in the defined geographical area

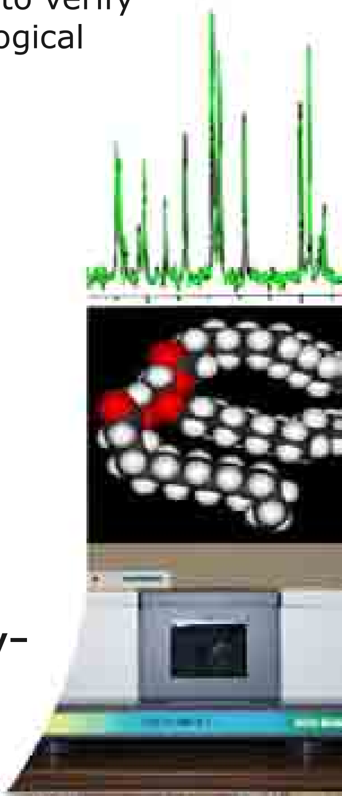
1 . Technical Overview

Fingerprinting techniques measure the composition of foodstuffs in a non-selective way, typically producing a spectrum as a fingerprint. Mathematical processing of such fingerprints may permit the characterisation of foodstuffs.
Foodstuff analysis by fingerprinting techniques is relatively simple and can be summarised in the following scheme.



1.1 Fingerprinting Techniques

Samples are analysed directly or after a simple extraction procedure. Analytical data collected are processed by suitable statistical methodologies which vary according to the specific problem. In many cases it is possible to obtain confirmation of the geographical claim of a given foodstuff as well as to verify the identity of a product produced by specific technological processes.



Within the TRACE project the following fingerprinting techniques were investigated:

- **Nuclear Magnetic Resonance Spectroscopy (NMR)**
- **Near and Mid-Infrared Spectroscopy (NIR, MIR)**
- **FT- RAMAN Spectroscopy**

Mass Spectrometry:

- **Ambient Mass Spectrometry, Direct Analysis in Real Time (DART)**
- **Solid-Phase Microextraction– Gas Chromatography–Mass Spectrometry (SPME–GC–MS)**
- **UPLC-QTof**
- **LC/MS/MS**

A brief description of each technique is reported.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy allows identification of the fingerprint of a sample and determination of the chemical structure of many of the compounds present in a sample.
It enables collection of a complete metabolic profile in a single experiment and generally requires little or no sample preparation.

Near and Mid-Infrared Spectroscopy (NIR, MIR)

These techniques permit collection of a spectral fingerprint of a sample within seconds.
They generally involve no sample preparation and are non-destructive.
IR and NIR spectra can be used to identify compounds or investigate sample composition.
Information provided relates to the molecular structure of compounds present in a food.

FT-RAMAN Spectroscopy

The FT-Raman spectroscopy can be used for solids, liquids, powders, tablets, slurries, gels, suspensions.
The fact that glass, polymer films and water have relatively weak Raman spectra means that it is ideal for the analysis of samples in glass or polymer supports or in aqueous solutions.
It is a quick, non-destructive technique and does not require any sample preparation.

Ambient Mass Spectrometry. Direct Analysis in Real Time (DART).

The main advantages compared to conventional mass spectrometry techniques include direct sample examination in the open atmosphere, minimal or no sample preparation and remarkably high sample throughput. It allows rapid analysis of a wide range of small molecules (markers) occurring in food matrices representing either metabolomic profile or a chemical signature originating from processing practices.

Solid-Phase Microextraction –Gas Chromatography –Mass Spectrometry (SPME–GC–MS)

Examination of volatile profiles associated with a food can enable authentication/recognition of food (e.g. olive oil, honey) since its composition (including volatiles) is known to vary depending on various aspects such as floral origin, processing, the technology employed for particular production, the storage conditions, etc.

UPLC-QTof

The UPLC-QTof system employs time-of-flight mass spectrometry technology for the accurate mass determination of chemical compounds in a wide range of sample matrices.
Examples of sample matrices analysed are solutions in inorganic or organic solvents, biological matrices (blood, serum, plasma, urine, bile, tissue, etc.), nutrients (food, water, beverages, etc.), environmental samples (water, soil, agricultural products, plants, etc.), forensic samples.

LC/MS/MS

LC/MS/MS combines the use of liquid chromatography with multiple mass spectrometry steps.
This technique allows the separation of specific metabolites by isolating fragmentation transitions of parent to product ions.

1.2 Data Analysis

Fingerprinting techniques produce a large volume of information. Most of the information may not be useful for solving the problem of authentication or identity confirmation. Mathematical tools must be applied to this information to extract that which is helpful to solve the problem being investigated. These tools generate a model which, when applied to the fingerprint of a given sample, can for example answer the question "Is this food what it claims to be on the product label?".

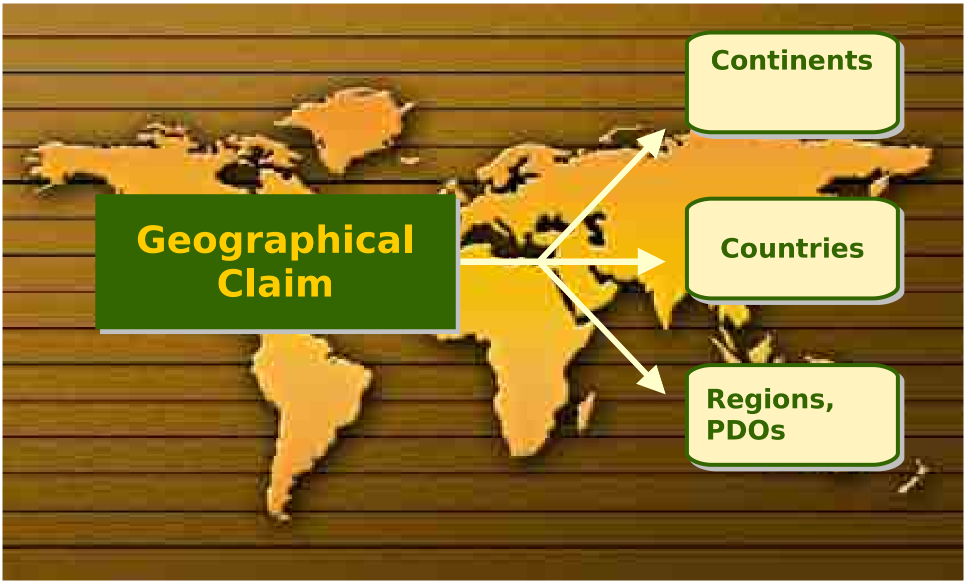
But what is a mathematical model?

A mathematical model is simply a mathematical equation which can convert measurements, perhaps many hundreds or more, made by a fingerprinting technique into a single indicator, or number. The value of this number may be used in food authentication applications to decide if the sample being tested conforms with labeling claims relating to origin or method of production for example.
A wide range of mathematical methods may be used for this purpose and they include:

PLS-DA (Partial Least Squares – Discriminant Analysis)
NOPLS (Non-Orthogonalised Partial Least Squares)
SIMCA (Soft Independent Modeling of Class Analogy)
SVM (Support Vector Machines)
ANN (Artificial Neural Networks)

2 . Geographical Claim

Consumers are becoming increasingly interested in the geographical origin of their foods. Geographic origin may be an essential element of a foodstuff's authenticity.
In particular, a geographical claim may be essential to protect the reputation of national or regional foods and to confirm that any given food meets the necessary conditions relating to geographic origin.



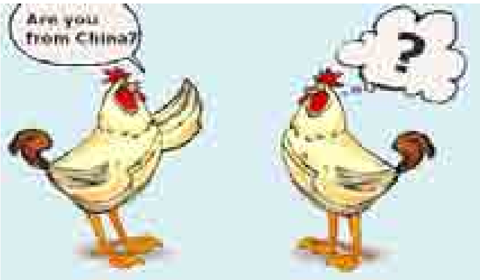
Methods that can provide a characteristic metabolomic profile can be used for the determination of geographical origin and can therefore provide a valuable tool for the authentication and traceability of foods.
Within TRACE, the aim was to create, using a variety of techniques, mathematical models able to confirm or refute any claimed geographical origin of a food.
The confirmation of geographical claim can be performed at different geographical scales (continent, country, region, PDO) according to the specific problem.

2.1 The example of chicken

Question 1:

Can the discrimination of chicken by geographical origin be achieved using metabolomic profiling?

A comparison between the fingerprint of Chinese chicken and chicken from other continents and countries was performed.



A total of 383 chicken breast meat samples obtained directly from China, Europe, South America and Thailand were analysed by NMR spectroscopy.

Results generated by the best mathematical model are reported in Figure 1.
These results show that out of 100 samples labeled as originating in China, 99 were identified as Chinese (green bar) by the model. The same consideration can be performed for the Thailand, Europe and South America samples labeled as Rest.

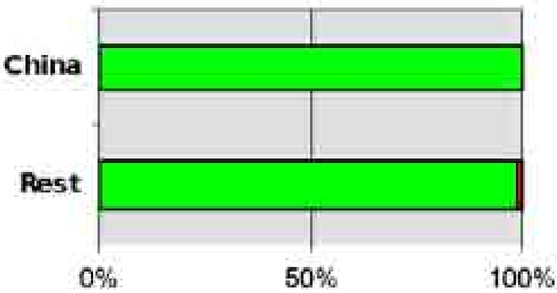
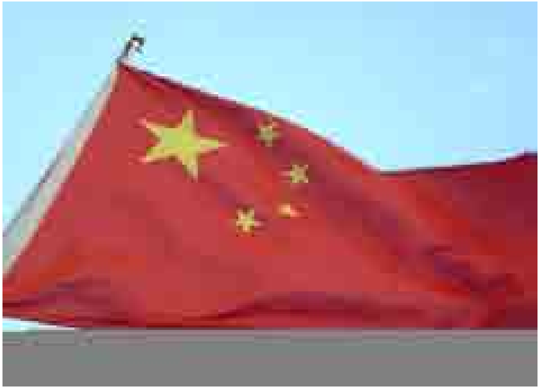


Figure 1: Green = % Success

Question 2:

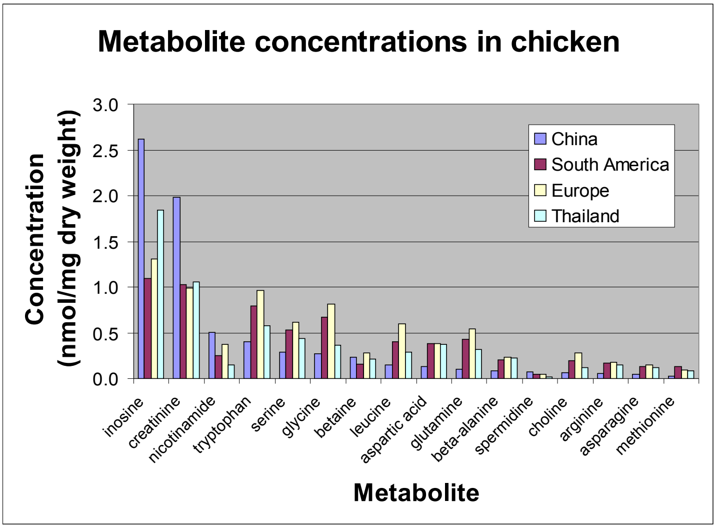
Why do Chinese samples differ from the others?

The difference between samples of different origin is due to the different composition of the samples.



Over 50 metabolites were assayed and their levels calculated. Figure below shows a comparison of the content of some metabolites which enables discrimination between chicken samples coming from different geographical areas.

It is important to note that Chinese samples have a higher content of inosine and creatinine than the others. On the other hand, the other metabolites are present in Chinese samples in a lower concentration with respect to the other ones.



Insights and curiosity

In the case of chicken meat, public awareness has been increased by recent food scares such as Avian Flu and the malpractice of some food producers (e.g. the use of banned nitrofurans in chicken production). The validity of the declared quality, safety and origin of the chicken meat is in question.

Question 3

Can the claimed geographical origin of honey be confirmed using fingerprinting techniques?

The case of Corsican honey

Corsica was chosen as a good PDO region to study honey because of its island nature. The specific task investigated was to confirm that honey labelled as Corsican was actually from Corsica. To do this, a comparison between Corsican and non-Corsican honey samples was performed.

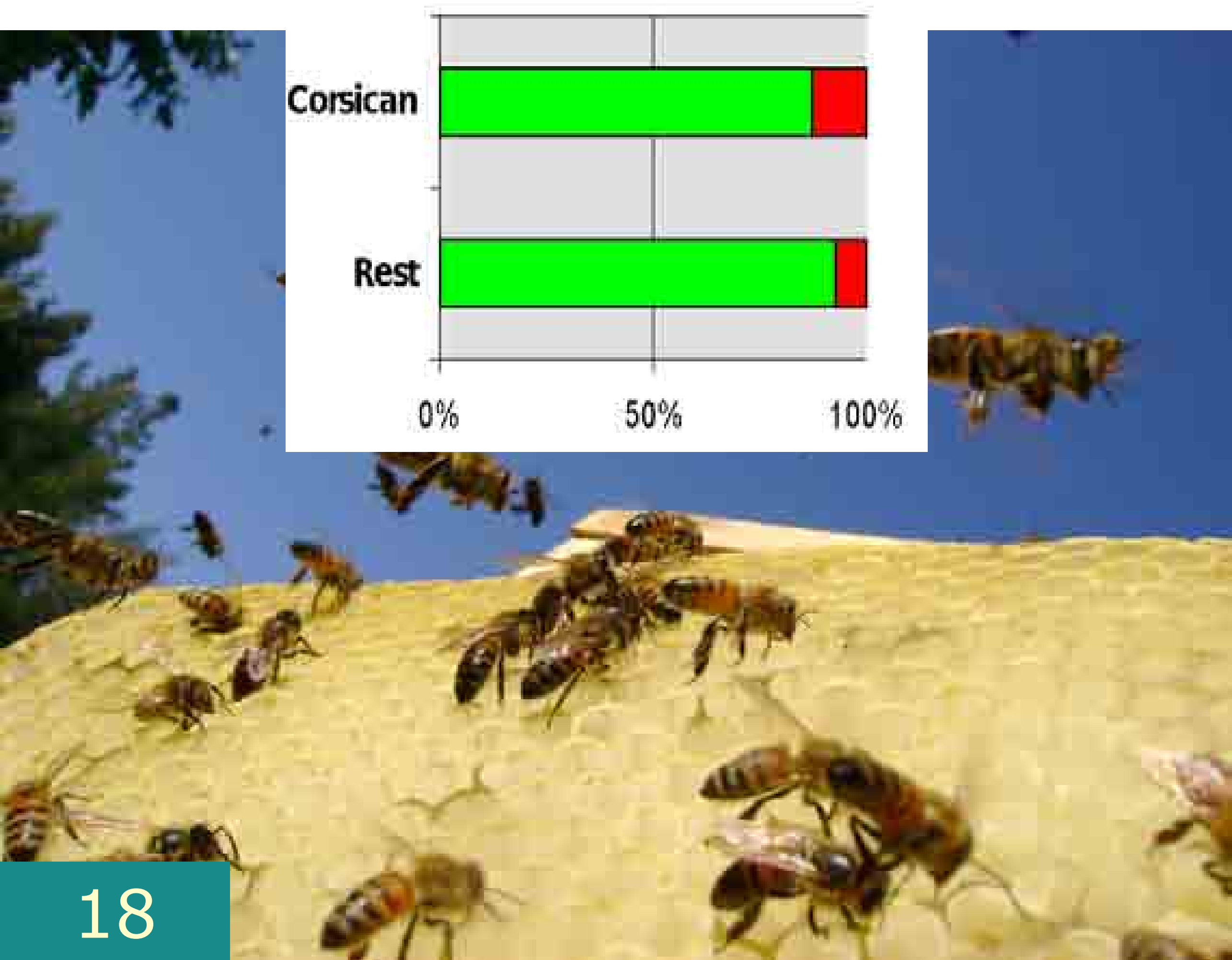
Samples of authentic Corsican honey were collected directly from suppliers on the island; honeys from a number of nearby countries (France, Germany, Italy, Austria) and regions were collected indirectly from beekeepers in those regions.

Authentic, unfiltered honeys (219 Corsican and 154 non-Corsican) were collected over 2 production seasons (2006 and 2007).

All samples were analysed using the fingerprinting techniques and the analytical data were analysed by statistical analysis. The results are reported in Figure 2. The results show that out of 100 samples labeled as Corsican, 89 are well classified (green bar) as being from Corsican origin.

In this way their claimed identity was confirmed.
On the other hand, the other 11 samples (red-bar) can not classified as Corsican honey samples.
The same consideration can be performed for non Corsican honey samples reported as Rest.

Figure 2: Green= % Success



Insights and curiosity

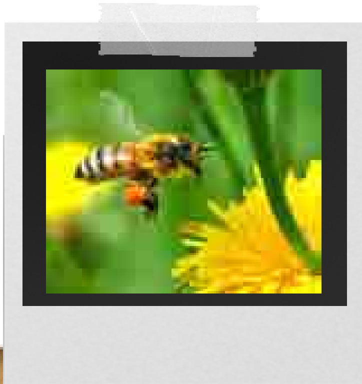
> *Honey is defined as the natural sweet substance produced by Apis mellifera bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store, and leave in honey combs to ripen and mature.*

Honey is an extremely popular product with high glucose and fructose contents. It is widely appreciated as the only concentrated form of sugar available worldwide and is also used as a food preservative.

As defined above, honey is a pure natural product with no substances added. There is a strong relationship between the origin of honey and its quality; therefore, in the European Union, it is necessary to include the origin of honey on the associated label. Honey may also merit a protected designation of origin (PDO) or protected geographical indication (PGI). Honeys so labeled can command a price premium, opening the way for economic fraud.

Such fraud may involve (1) the deliberate addition of an adulterating sugar or syrup or (2) non-compliance with an origin name; this may involve substitution or mixing of honeys from several locations.

Therefore, honey quality must be controlled analytically to guarantee authenticity and protect consumers from fraudulent commercial exploitation.

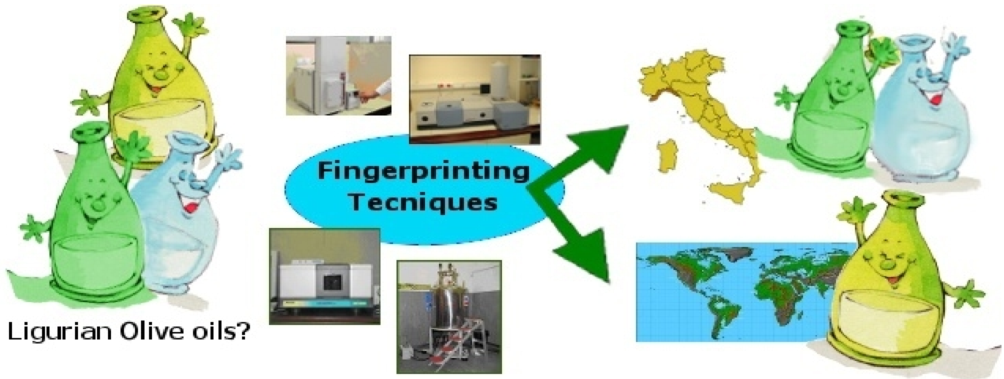


Question 4

Can the claimed geographical origin of olive oils be confirmed using fingerprinting techniques?

The case of Ligurian olive oil

Ligurian olive oils, characterised by a particular sensory quality, were chosen as a study case since they also have an important commercial value. The specific task was to confirm that olive oils labeled as originating in Liguria actually did so.



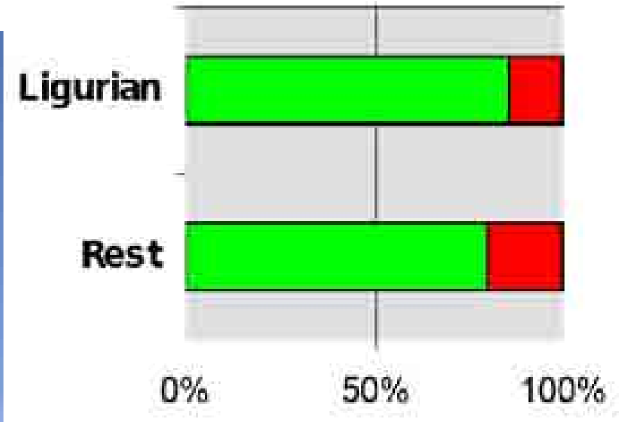
A comparison between Ligurian and non-Ligurian olive oil samples was performed.

Olive oil sampling took place over three harvest years (2005, 2006 and 2007) and involved 913 oil samples from different Mediterranean areas. In particular, Ligurian olive oils (210 samples) were collected together with olive oils (703 samples) coming from other Italian areas and from Spain, Greece, France and Turkey.

All samples were analysed using the fingerprinting techniques and the resulting data were statistically analysed.
The results can be reported in Figure 3. The results show that out of 100 samples labeled as Ligurian, 90 are well classified (green bar) resulting from Liguria.
In this way the claimed identity was confirmed.

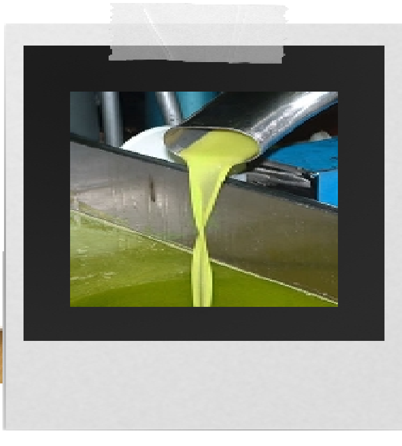
On the other hand, the other 10 samples (red-bar) can not classified as Ligurian olive oils. The same consideration can be performed for non Ligurian olive oils reported as Rest.

Figure 3: Green= % Success



Insights and curiosity

Olive oil is obtained from the fruit of the olive tree (Olea europaea L.) and is a genuine fruit juice with excellent nutritional and functional qualities. Olive oil is produced by mechanical means and requires no refinement prior to consumption; thus, it retains its characteristic aroma.
This aroma arises from the content of phenolic compounds and other volatile constituents.
The specific volatile compounds present in an olive oil depend on different factors such as fruit genotype, processing equipment used, and geographical origin. The influence of climate and soil type are fundamental for olive oil volatile profiles. Consumers have great interest in olive oil, particularly for its sensory and nutritional properties. Extra virgin oil is a high quality virgin olive oil; it has a free acidity, expressed as oleic acid, of not more than 0.8g/100g. This and other specific characteristics are defined by the European Regulation. Because this olive oil type is viewed as high quality, it is one of the more expensive vegetable oils. To clearly label high quality food products, the European Union has created the PDO (Declared Geographical Origin) designation.
The PDO allows the labelling of some European extra virgin olive oils with the names of the areas where they are produced. This certification improves the commercial value of the product. However, the development of accurate fingerprinting methods for the authentication of specific PDO is a real issue.



3. Identity Confirmation

An important aspect of food production is to produce a product which always has the same characteristics and therefore, by extension, with the same fingerprinting.
The food industry can verify the consistency of their product using fingerprinting techniques.

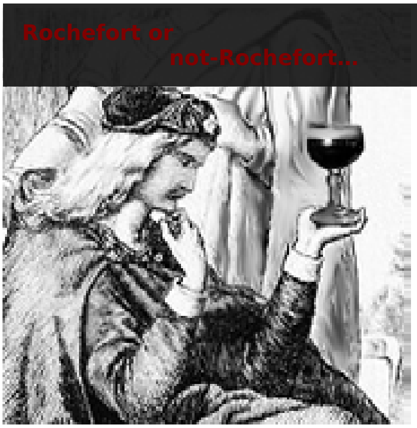
3.1 The example of beer

Question 4:

Can fingerprinting techniques confirm that the production process always gives the same product?

The case of Rochefort 8° beers

The fingerprint of Rochefort 8° beers is always the same, see Figure 4, showing always the same metabolites with the almost the same concentrations. This means that the Trappist industry can easily monitor the product and therefore the functionality of production cycle.



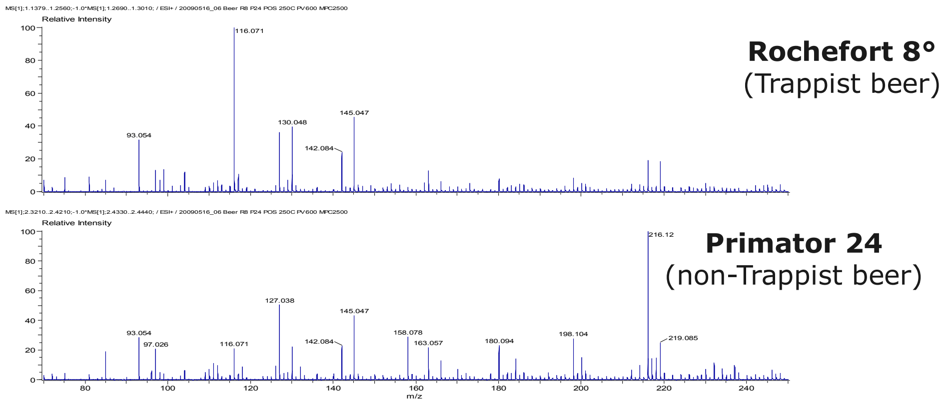


Figure 4: Fingerprint of Rochefort 8° beer and Primator 24 beer.

In order to confirm the identity of Rochefort 8° beers a comparison between Rochefort 8° beers and other Trappist and non-Trappist beers were performed.

All samples were analysed using the fingerprinting techniques and the analytical data were analysed by statistical analysis. The results can be reported in Figure 5. The results show that out of 100 samples labeled as Rochefort 8°, 95 are well-classified (green bar) as Rochefort 8°. In this way the claimed identity was confirmed.

On the other hand, the other 5 samples (red-bar) can not be classified as Rochefort 8°. The same consideration can be performed for the other Rochefort and Trappist beers.

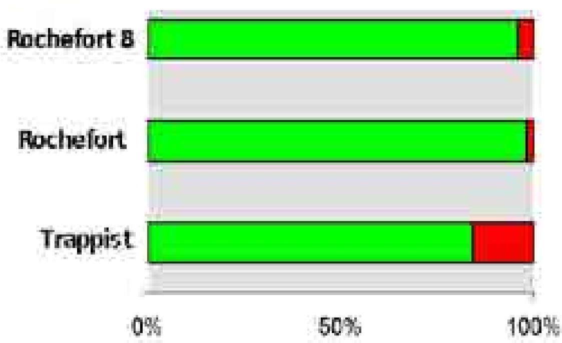


Figure 5: Green= % Success

Insights and curiosity

TRAPPIST BEER is a beer brewed by or under control of Trappist monks. Of the world's 171 Trappist monasteries (as of April 2005), seven produce beer, six in Belgium (Orval, Chimay, Westvleteren, Rochefort, Westmalle and Achel) and one in The Netherlands (La Trappe).

Only these seven breweries are authorized to label their beers with the Authentic Trappist Product logo that indicates a compliance to various rules edicted by the International Trappist Association. The aim of this association is to prevent non-Trappist commercial companies from abusing the Trappist name. The criteria to be a trappist beer are the following:

The beer must be brewed within the walls of a Trappist abbey, by or under control of Trappist monks.

The brewery, the choices of brewing, and the commercial orientations must obviously depend on the monastic community.

The economic purpose of the brewery must be directed toward assistance and not toward financial profit.

Among the Trappist beers, due to its growing success the Rochefort 8° beer accounts for most production. It is a yellowish-brown beer with a pronounced fruity aroma. Known since 1954, initially it was called Spécial".

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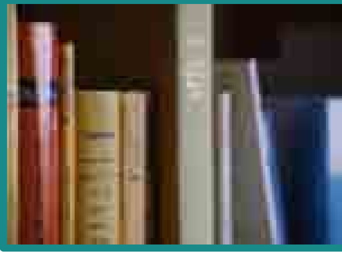

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