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L-malic acid in apple juice

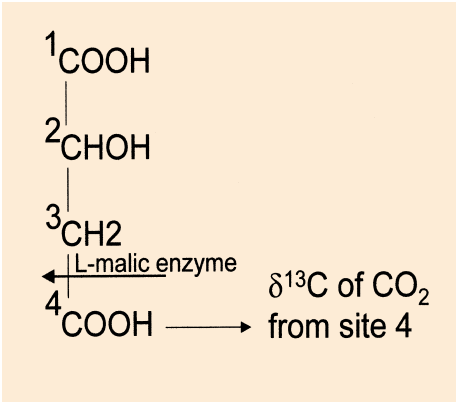
Detection of added L-malic acid in natural fruit juices

Malic acid takes its name from *Malus*, the Latin for apple, in which it is by far the major organic acid. The concentration of this acid is one of the key parameters that determines the organoleptic quality of apple juices and, in general, high acid apple juices are more commonly preferred to sweet apple juices by a majority of consumers. In times of shortage of such high acid apple juice, there is a tendency towards higher prices and an increased risk of finding apple juice concentrates on the market that contain undeclared added malic acid.

Several methods have been proposed to detect illegal addition of malic acid in fruit juices. The first consists in determining synthetic D-malic acid not naturally present in apple juice, which contains only L-malic acid (L stands for Levogyre and D for Dextrogyre). Synthetic malic acid is generally a racemic mixture of D and L forms. Unfortunately, reasonably priced L-malic acids can now be purchased on the market, thus decreasing the efficiency of this method. Analysing traces of fumaric acid, that may be an impurity in the synthetic malic acid, is not conclusive either since its presence could be due to microbial contamination.

Eurofins Scientific has recently set up, in collaboration with SGF[1], a new «site specific» isotopic technique detecting the addition of malic acid, both D and L forms, in apple juices. First of all, malic acid is extracted and purified and is selectively degraded using an enzymatic reaction and the $^{13}C/^{12}C$ ratio of the extracted CO_2 is measured by IRMS[2]. The isotopic deviation of this specific isolated carbon site provides a good means of discrimination between industrial and natural malic acids.

Eurofins Scientific has been tested by the SGF to determine the ability of this technique to differentiate authentic apple juices from adulterated ones. The results were presented at the EQCS[3] Experts Workshop in Frankfurt in March. The test was successful and clearly demonstrated the interest of this new isotopic technique to control the authenticity of fruit juices. Further developments are currently underway to apply the technique to other fruit with high malic acid content. ●



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1 SchutzGemeinschaft der Fruchtsaft-Industrie
2 Isotopic Ratio Mass Spectrometry
3 European Quality Control System

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An excursion into the tropical forests of Latin America...

...In pursuit of authenticity

Authenticating food products on the basis of analytical results generally requires careful comparison of the analytical parameters measured on the sample with chemical data obtained previously, using the same analytical methods, on the genuine product. However as anyone in the authenticity business knows, getting hold of guaranteed authentic samples for a reference data base is not always easy. Eurofins Scientific goes to considerable lengths to ensure that its fruit juice data base is kept up to date. It ensures the authenticity of its reference juices by taking samples directly from the growing areas, including whole fruit which is then transformed in the laboratory into juice or puree before being analysed. In late 1999, one of Eurofins staff, Frédérique Martin, braved Colombian bandits, a rumbling volcano in Ecuador and tropical storms in Costa Rica, to bring back samples of tropical fruit. In addition to collecting samples, Frédérique also visited the areas where the fruit is cultivated and processed. Her travel report included details on the length of the growing season for each fruit type, how they are harvested, local climatic conditions, specific processing treatment - all valuable information when it comes to making a judgement on the authenticity of a product based on analytical values. Here are some examples of the type of information that accompanies each sample collection.

Pineapples from Costa Rica

Costa Rica is one of the major producers of pineapple in Central America with 13 000 cultivated hectares and 400 000 metric tons produced in 1999 (FAO[1] source). The principal growing area is in the North West province of Alajuela. The main outlet for pineapple production is as whole table fruit for the North American market. Since not all the production meets the demanding requirements of either the importers or the American consumers, a minor proportion finds its way to the juice processors to be transformed into juice and juice concentrates. Choosing suitable table fruit is based both on appearance - size and shape and whether it is damaged or unripe - and on chemical characteristics - a minimum Brix/acid ratio of 20 is required. The selection procedure may also include the fruit's «floatability». This parameter, proportional to the weight and density of the pineapple, is judged by floating the fruit in a water tank as shown in the photograph ●

Mangoes from Colombia

There are two main mango growing regions in Colombia located along the Magdalena river between the central and western mountain ranges of the Andes. One is in the northern province of Magdalena where harvesting takes place from June to September. The other is in Tolima, in the centre of Colombia, with a production period of November to December.

The mango tree is hardy and thrives in most conditions including the hot, humid tropical climate of these regions. Surprisingly, the relative dryness of the last three years attributed to El Niño has actually favoured mango cultivation, with a two to three-fold increase in production, whereas these same climatic conditions have had the opposite effect on guavas and passion fruit. In 1999 production of mangoes reached 98 000 metric tons from only 8 000 cultivated hectares (FAO source).

One of the most commonly-used varieties for mango purees and puree concentrates is known as Hilaza, Brechoso, Del Rio Magdalena or Pig Mango. Table fruit varieties include Tomy, Manzano and Kent ●

Passion fruit from Ecuador

There are three main regions in Ecuador : the Amazonian region in the East, the Andes in the centre and the western coastal region, Costeña, bordered by the Pacific Ocean. It is in this region, along either side of the river Daule, that the main passion fruit or maracuya plantations are located.

The region's tropical climate, with its rainy season from June to September, is ideally suited to passion fruit cultivation. In the last 4 years, passion fruit exports have increased by 158% and now represent 93% of total fruit export from Ecuador well in front of mangoes and pineapples (from the local newspaper, El Commercio).

Plants produce fruit after 10 months and last for 5 years, after which the trees are cut down, burned and new ones planted. In the larger plantations, the trees are permanently irrigated and fruit is harvested all year round, although only fruit that has fallen on the ground is collected.

The same maracuya species can produce both yellow (90%) and red (10%) fruit ●

With special thanks to all those in the countries visited for their help and hospitality.

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1 Food & Agriculture Organisation

The new regulations for labelling of GMOs

In force on April 10th 2000

The essential points of the new regulations 49/2000/EC and 50/2000/EC are outlined below:

1. Compulsory indication on the labelling of certain foodstuffs produced from genetically modified organisms (49/2000/EC)

* The regulation sets a threshold of 1% at the individual ingredient level
* The threshold relates to the above-mentioned products and products approved or notified under the Novel Foods regulation 278/97/EC.
* The operator must prove that he has taken «appropriate steps» to avoid GMOs (e.g. obtain a certificate for the supplier that the product originates from conventional crops)
* A negative list of products which are exempt from this regulation should be drawn up

2. Labelling of foodstuffs and food ingredients containing additives and flavourings that have been genetically modified or have been produced from genetically modified organisms (50/2000/EC)

* Applies to additives regulated under 89/107/EEC and flavourings regulated under 88/388/EEC

* Products have to be labelled if they are «no longer equivalent». The presence of recombinant DNA or protein makes an additive or flavour «no longer equivalent».

An important modification is the onus placed on the producer or retailer to prove that their product originates from non-GMO sources. To find out whether or how much GM DNA is present in a sample, two analytical methods are mainly used :

* Protein detection (ELISA)

The target protein is detected by an antibody and by adding a chemical substance a colour is produced which is approximately proportional to the amount of target protein present, e.g. Roundup Ready® protein. The advantage is that the test is relatively cheap and quick. The disadvantage is that it does not work for most processed material, e.g. heat treated protein extract etc.

* Real-time PCR

The second option is real-time PCR that uses laser technology to measure the exact amount of fluorescence produced during the PCR reaction (over approximately 2 hours). At the end of the reaction the amount of target DNA (e.g. 35S promoter) can be calculated exactly from the fluorescence produced.

Eurofins Scientific use the ABI Prism 7700 and the LightCycler. Both machines measure at several different wavelengths which allows to simultaneously measure an Internal Positive Control (IPC). The internal positive control is essential to detect inhibition of PCR (i.e. to exclude false-negative results). Machines that only measure at one wavelength (e.g. ABI Prism 5700) cannot use IPC and are less reliable. The choice of a laboratory using state-of-the-art equipment and technology and having up-to-date knowledge of regulations is therefore important ●

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UK Food Standards Agency comes into being

Salmonella in raw eggs, Listeria in soft cheese, E. coli O157 in meat, pesticide residues, dioxins, GM foods ... the continuous series of microbiological and chemical food safety scares in recent years has meant that it is hardly surprising that in some quarters food is regarded as a key factor in the major causes of death! Moreover there has been a growing public perception in the UK that the dual role of their Ministry of Agriculture, MAFF, in promoting the interests of the agricultural sector, while at the same time ensuring food safety, was undesirable.

The BSE crisis in the UK gave the final impetus which led to the commissioning of Professor James to report on the feasibility of establishing a Food Standards Agency, the primary objective of which was to be the protection of public health in relation to food. It was envisaged that the FSA would be independent of any sector and at arm's length from government. In this way the FSA would be able to command the respect and trust of both the public and the industry. The proposals met with widespread support and the Food Safety Act 1999 received royal assent in November 1999, establishing the Food Standards Agency. The FSA aims to protect public health and also other food-related interests of consumers. Government departments must take the FSA's advice into account when formulating any legislation or policy impacting on food safety. On the other hand, the FSA must consult with all bodies likely to be affected by its advice and must take due consideration of issues of risk, cost and benefit.

The FSA is also to have a role in educating the public on food related matters, so that the consumer will be able to make purchasing choices based on scientifically sound and impartial information. The intention is to make public as much as possible of the basis of any advice but the FSA is required to balance the public interest against any confidentiality issues. In this way it will counterbalance sensational and ill-informed media reports.

Except for specific areas such as Meat Hygiene, the Agency does not have powers of enforcement of Food Law - these are retained by the local authorities. Ultimately the Agency may be given the power to undertake enforcement action on behalf of a local authority. It is hoped that by this means the FSA will address the issue of uneven enforcement across the country but as there is no indication that additional resources are to be made available to local authorities nor that there will be any ring-fencing of existing resources, it remains to be seen to what extent this will be successful. The FSA will also represent the UK in the development of harmonised European Food Law.

Eurofins Scientific Ltd is an official laboratory for the purpose of food law enforcement in the UK, employing at its New Cross site six Public Analysts who are appointed to approximately 40 local authorities. As such Eurofins is well placed to help meet the challenge of providing the scientific services required by enforcement authorities in the 21st century. Our wide range of expertise amongst the group laboratories could well be of use to the Food Standards Agency in surveillance exercises and research.

For further information on the role of Public Analysts in UK Food Law enforcement see www.the-apa.org.uk/home.htm ●

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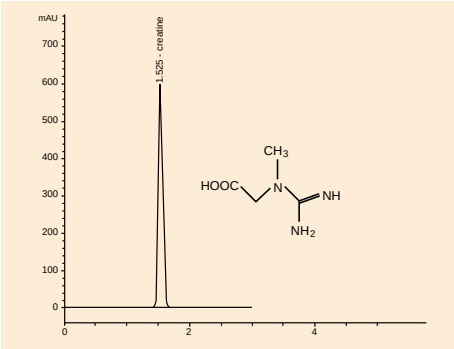
Capillary Electrophoresis

Migrating from Obscure to Popular

Over the last 10 years Capillary Electrophoresis (CE) has «migrated» from an academic interest to a widely applied, analytical technique. A common measure of this progression, number of publications, has just recently leveled off. But development activity in the field continues high as indicated by the interest in CEC (Capillary Electrochromatography, an HPLC-CE hybrid) and CE-Mass Spectrometry.

For those that have not participated in this «migration», CE is a separation technique that is based upon the differential migration of solutes in an electric field. In CE, this electrophoresis is done in a narrow-bore, buffer filled, capillary column using voltages up to 30 kV. This reduces migration times and produces very efficient separations. Several detection schemes are available but UV-Vis is the most widely used. Different modes of CE (e.g., capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC)) provide a high degree of flexibility in developing the required separation.



At Alpha, one of the North American Laboratories of the Eurofins Scientific Group, we have been using CE since 1993 to help clients solve their separation problems. Like many users, we have found its speed, simplicity, and rapid method development capabilities to be invaluable. The result at Alpha, and therefore for our clients, is a very impressive list of analyses or applications. If you have not looked at it lately, visit our web site and peruse the list. You will see that it spans quite a variety, from simple anions and cations to more complex phytochemicals and biomolecules.

One area where CE has found a special application niche at Alpha is in support of the sports nutrition industry. These products used by serious body builders, Olympic athletes, as well as the weekend warriors are complex mixes of ingredients including such things as Creatine Monohydrate, Calcium Pyruvate, amino acids, and vitamins. Verifying label claims on complex products like these can be quite a challenge for a technique such as HPLC due to long run times, coelutions, column fouling, etc. But CE has the capability of producing an electropherogram like the one shown here for a commercial product containing Creatine Monohydrate - a single peak in less than 5 minutes. Now that's impressive! ●

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The EuroConference on Modern Analytical Methods for Food and Beverage Authentication

Lednice, Czech Republic, August 31 to September 3, 2000

Dr. Milan Hajék, from Eurofins Scientific Czech Division, is organising with the Mendel University of Agriculture and Forestry in Brno and the Horticultural Faculty of Lednice, a conference on Modern Analytical Techniques for Foods and Beverages. The event is supported by DGXII of the European Commission, within the **Human Potential Programme** (High-Level Scientific Conferences) of the 5th Framework Programme. The conference will combine a presentation of existing and potential methods for the authentication of foods and beverages with a forum for discussions and exchange of knowledge.

Oral and poster presentations are welcome.
Grants for participants from EU and Associated Countries will be available.
For further information you can visit the conference website at http://www.zf.mendelu.cz/authenticity or contact the Organising Committee by e-mail : authent@mendelu.cz or by fax : + 420 - 627 340 159

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