FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

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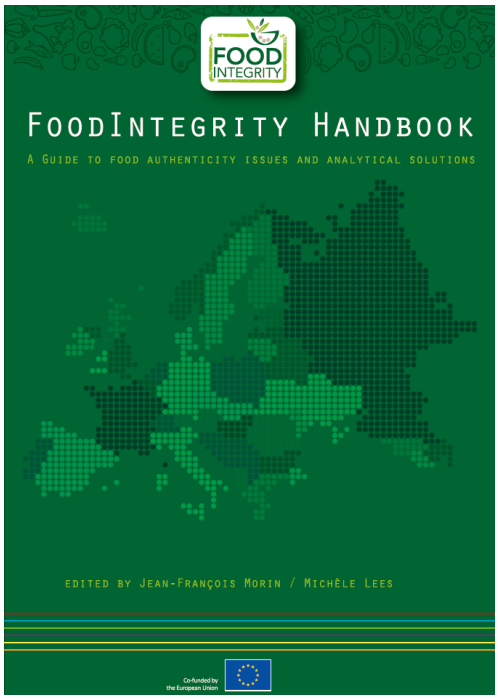

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Fruit juices

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General overview of the products

Fruit juices (100 % fruit) and nectars (25-99 % fruit) are an important sector of the food industry with a global consumption volume of fruit juice and nectars in 2017 of 36 247 million litres. The 28 countries of the European Union (EU-28) accounted for 9 187 million litres, with North America not far behind with 8 629 million litres followed by the Asia-Pacific region with 8 159 million litres [1].

In the EU, five countries account for over 70 % of the total fruit juice and nectar consumption, with Germany coming out on top (2 342 million litres), followed by France (1 406 million litres), the UK (1 079 million litres), Poland (820 million litres) and Spain (808 million litres). Total consumption of juices and nectars has been slowly declining over the last decade, particularly in the area of nectars, and ambient/from concentrate juices. One of the key drivers of fruit juice consumption has always been its nutritional image, as a natural product high in vitamins, anti-oxidants and other nutrients. However the juice sector has recently come under fire due to its relatively high content of sugar, one of the reasons responsible for the decline in juice, and particularly nectar, consumption.

Although orange and apple still top the popularity list, the number and diversity of types of fruit juice available in the market has changed considerably over the last few decades, with the regular appearance of novel exotic fruit types each claiming new health benefits. To counteract the downward trend in juice consumption, the industry is turning to new innovative and sophisticated flavours and mixes. Efforts have focused on the NFC (not from concentrate) and smoothies sectors with fruit and vegetable mixes that are lower in sugar, and the addition of functional ingredients such as proteins. Further insight into the juice market including an overview of the trends, opportunities, and threats facing the fruit juice industry is given in reference [2].

All these factors, in the changing landscape of the fruit juice sector, make ensuring the authenticity of what is available to the consumer an ongoing challenge for both the industry and the regulators.

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1. Product Identity

1.1. Definition of the product and manufacturing process

The type of fruit juice found on the market is generally conditioned by the processing method used to get the product from its growing region to the supermarket shelf and by the specific regulations in force in the country where it is sold. Fruit juice is traded either as natural strength juice or purée or as concentrated fruit juice or purée from which the water has been extracted. The latter greatly reduces storage space requirements and cuts transport costs considerably. The concentrate is stored at very low temperatures or in aseptic drums and transported in bulk from the production area to the main markets where it is reconstituted to single strength juice by adding water. The juice is then pasteurised, bottled and sold as “100 % fruit juice made from concentrate” or “100 % fruit juice made with concentrated fruit juice”.

Natural strength fruit juice is obtained directly from the fruit, pasteurised and bottled ready to be sold or kept in sterile tanks at low temperature for packing at a later date. It is sold as “100 % fruit juice” and sometimes as “direct juice”.

Chilled (refrigerated) short shelf life juices

- Chilled freshly squeezed juice: single strength juice not made from juice concentrate with a shelf life of between 3 days and 3 weeks depending on fruit type, storage temperature 0-5 °C
- Pasteurised 100 % (direct) juice: single strength juice not made from concentrate with shelf life of about 24 days
- Pasteurised juice made with concentrated fruit juice: reconstituted from juice concentrate (generally frozen), shelf life of about 24 days.

Pasteurised, ambient juices

- Pasteurised direct juice/freshly squeezed: long shelf life (6 to 12 months depending on fruit type)
- Pasteurised juice made with concentrated fruit juice: reconstituted from frozen concentrate with a long shelf life (6 to 12 months depending on fruit type).

Fruit nectars are also popular in Europe. These are blends of fruit juices (between 25 – 90 % juice content depending on the fruit type), water and sugar.

Fruit purées and pulps are ideal raw materials for soft fruits such as strawberry and raspberry that are prone to physical damage during transport. Such products are used in fruit juice blends, drinks and nectars as well as in jam and marmalade manufacture.

1.2. Current standards of identity or related legislation

1.2.1. Codex Alimentarius

The Codex Alimentarius (CODEX STAN 247-2005 [3]) gives the following definition for a fruit juice:

“Fruit juice is the unfermented but fermentable liquid obtained from the edible part of sound, appropriately mature and fresh fruit or of fruit maintained in sound condition by suitable means

including post-harvest surface treatments applied in accordance with the applicable provisions of the Codex Alimentarius Commission.
[….]

The juice is prepared by suitable processes, which maintain the essential physical, chemical, organoleptical and nutritional characteristics of the juices of the fruit from which it comes…"

Definitions given by the Codex Alimentarius can be taken as a general basis for export purposes. However these guidelines may differ from those of specific countries.

1.2.2. European Union

The EU "Fruit Juice Directive" 2001/112/EC [4] provides a slightly different definition to that given by Codex Alimentarius.

"The fermentable but unfermented product obtained from the edible part of fruit which is sound and ripe, fresh or preserved by chilling or freezing of one or more kinds mixed together having the characteristic colour, flavour and taste typical of the juice of the fruit from which it comes…"

Taking into account all other stipulations and paragraphs of both documents there is no relevant difference. However, according to the Codex Standard physical, chemical, organoleptic and nutritional characteristics of the fruit from which the juice comes should be maintained, whereas the EU Directive limits this aspect to colour, flavour and taste. This is somehow closer to industrial reality since a juice cannot have the same physical, chemical and nutritional characteristics as a fruit. A juice is liquid and a fruit is partly solid and has more fibre. In addition, the chemical structure cannot be the same because the use of authorised enzyme treatment changes the chemical composition and some substances are discarded with the solid fruit parts during the extraction process. However, fruit juice remains a healthy product with nutritional benefits through its constituents.

Fruit juices and related products like fruit juice concentrates are natural products. According to the applicable legislation only a very limited range of additives and further ingredients are allowed.

Similar properties are defined for fruit purees.

Any legal framework is national or regional specific. In Europe the above mentioned "Fruit Juice Directive" and other food related laws are applicable. Differences to other regional legislation exist and must be taken into consideration when interpreting analytical results. For example, it is not accepted in the EU to blend orange juice with juice from *Citrus reticulata* hybrids (mandarin and others). In most other parts of the world such blending is allowed up to a certain amount. Furthermore the use of conservation agents is regulated differently in some countries and differences exist in the expected minimum content of solid solids (density/Brix) that must be present in a juice or a juice reconstituted from juice concentrate.

For the EU an industrial Code of Practice has been developed by the European Juice Association or AIJN [5] which is regularly updated. This Code of Practice provides analytical reference data for specific fruit types and gives comments for interpretation. Some of the guide values listed are obligatory such as minimum Brix values, limits of heavy metals and spoilage parameters. On the other hand, analytical parameters which are used for authenticity assessment are generally indicative. Case specific interpretation through experts is always necessary.

A regional specific data base is accessible in the member portal of SGF International e.V. [6].

1.2.3. In the United States

The US Food and Drug Administration (FDA) regulations on foods are established in a Code of Federal Regulations (CFR) under Title 21. Under this, juices must conform to FDA standards of identity 21 CFR part 146 for fruit juices and to 21 CFR part 156 for vegetable juices [7]. In addition 21 CFR part 101.30 provides regulations for percentage juice declaration for beverages that contain fruit or vegetable juice [8].

The USA has always been primarily focused on food safety and in 2001, the FDA brought in a ruling requiring a mandatory HACCP (Hazard Analysis and Critical Control Points) plan for fruit juice (21 CFR part 120, the Juice HACCP regulation [9]). The regulation requires that processors apply HACCP principles if they make juice or juice concentrates for subsequent beverage use. In 2011, the FDA brought in its Food Safety Modernization Act (FSMA [10]), specific legislation that puts into place mandatory prevention-based controls across the food supply to protect public safety and prevent illness. And in 2013 it issued its final rule, as part of FSMA, for Mitigation Strategies to Protect Food Against Intentional Adulteration [11]. The FDA has recently issued guidance to address any discrepancies arising from the new FSMA regulations in relation to the juice HACCP regulation [12].

2. Authenticity issues

2.1. Identification of current authenticity issues

There are various potential frauds possible as regards fruit juices. The most important authenticity issues are listed below:

- **Water addition**: there is a natural variation in fruit juices for the ratio between soluble dry matter and water. However it is not allowed to add water, even if the fixed minimum density or Brix are lower than any naturally obtained product not from concentrate. Also, for reconstituted juice from concentrate, legislation stipulates that the quantity of water added to reconstitute the juice must be the same as that removed during concentration. As mentioned earlier, the AIJN, has laid down guidelines for minimum density and its corresponding Brix value, a measure of the soluble solids content. One of the simplest forms of adulteration is dilution of a concentrate to below the permitted minimum Brix.
- **Sugar addition**: As a commodity, sugar is much cheaper than fruit juice, and therefore its addition to the latter to increase Brix values can be an economic advantage. Sugar can be added as beet, cane or corn sugars, or as modified sugar syrups such HFCS (high fructose corn syrup) or BMIS (beet medium invert syrup).
- **Complete or partial replacement of juice by juices made from concentrate**: in some countries consumer preference has shifted in recent years to freshly squeezed or not from concentrate (NFC) juices, conferring a higher premium on these products. It is therefore not permitted to pass reconstituted juices off as NFC or to add a proportion of water or reconstituted juice to the direct product.
- **Added products from undeclared cheaper fruits**: the prices of certain fruit types can fluctuate widely from one season to another, affected by poor harvests, gluts, and trade regulations. The addition of a cheaper fruit alternative to stretch one in short supply and/or high demand is another fairly common form of adulteration. Examples include the addition of orange to passion fruit, apple to red fruit, grape to pomegranate

- **Addition of undeclared ascorbic acid/vitamin C**: some fruit types are naturally high in vitamin C and use this as a selling point.
- **Addition of undeclared organic acids (e.g. citric acid, malic acid)**: in some cases the acidity of a juice can be corrected by the addition of organic acids within the limits tolerated by the legislation, and with suitable mention on the label. Specific practices such as addition of malic acid to apple juice is not permitted.
- **Addition of flavour compounds (natural or synthetic)**: authenticity issues arise when the product claiming to be "natural" contains flavours not from the named fruit or that have been chemically synthesised. Food fraud through addition of unauthorised flavour compounds is covered in the “flavourings” chapter.
- **Colourings (e.g. anthocyanin extracts, cochenille red, beetroot)**: the colour of fruit juices is an important part of the product's appeal. Colourings may be added to meet colour intensity specifications, to restore natural colour lost during processing conditions or storage, or to darken colour when the authentic product has been extended with a less pigmented fruit type.
- **Addition or over-proportional use of fruit extracts which were produced by non-authorised technology (water extractable solids)**: water extractable solids is the material obtained from washing the remaining pulp and cell membrane material after extraction of orange juice. Its overuse in juice is not permitted under the legal definition of the EU for citrus fruits.
- **Texture influencing agents (e.g. pectins)**: texturisers can enhance body and mouthfeel in juices that are less than 100 % fruit. This is authorised for specific fruit types only and provided that the compounds are mentioned on the product label.
- **Declaration of wrong origin**: the country of origin of a fruit product can become an authenticity issue if it is falsely declared on the juice label or in the product's trade specifications. This could be the concern of customs and excise authorities, if the fruit origin in question is subject to preferential import duties. Certain geographical origins may also carry a premium on the market
- **Declaration of wrong fruit variety**: this could be an issue where a specific variety is prized for its flavour or processing qualities

In particular if the fruit content in the falsified product is lower than in an authentic one the fraud could be covered up by adding other ingredients to adjust the analytical profile to the expected picture of an authentic product. Therefore minerals, organic acids, amino acids or a combination of different materials from other fruits can be added as part of the fraudulent practice.

2.2. Potential threat to public health

Most instances of fraud in fruit juices have no real food safety impact. However every food fraud is a potential health risk through the use of undeclared ingredients. Allergen issues or contamination with unexpected agrochemicals for wrongly declared origins or other contaminants present in an ingredient are possible. As analytical techniques become more and more sophisticated fraudsters are also pushed to better mask the analytical profile of the falsified product. This could lead to a vicious circle by increasing the risk of unexpected ingredients. An example is given in the following.

An orange juice concentrate is diluted with deionised well water and after respective flavour addition commercialised as juice not from concentrate. In this case an analysis of the isotope ratio $^{18}O/^{16}O$ of the water in the juice can detect the fraud. To avoid being discovered, the water used for dilution could be replaced by the dishonest juice producer by water obtained from the concentration process of grape juice. The $^{18}O/^{16}O$ isotope ratio – which is in many companies the routinely applied control parameter - would then be insufficient to detect the fraud. The risk increases that the fraud remains undiscovered. However [22]as most grape juices used for the production of concentrate are stabilised by sulfiting, the presence of sulphur dioxide (SO_2) in the final product is possible. SO_2 is listed as an allergen and represents a health risk for sensitive consumers.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. IFU Methods

The IFU (International Fruit and Vegetable Juice Association) has published a number of analytical methods to test for both juice authenticity and quality [13]. These methods include traditional wet chemistry methods such as refractometric index, density measurement, titratable acidity, formol index or photometric methods. In addition a list of enzymatic tests (e.g. organic acids, sugars), mineral determination (AAS, flame photometry) and different chromatography methods, e.g. for phenolic compounds, are suitable IFU methods to assess authenticity (cf. Table 1).

Methods are classified as IFU recommendation if no ring test results are available.

3.1.2. CEN Methods

The European Normalisation Committee, CEN, has also, through its technical committee CEN/TC 174, published a number of methods applied to the authenticity testing of fruit juice. A selection is listed in Table 2 below.

3.1.3. AOAC methods

The AOAC (Association of Official American Chemists) has also published a number of analytical methods for the authentication of fruit juices. These are listed in the Table 3.

Table 1: Available IFU methods and recommendations for fruit juice authenticity testing

IFU Method	
IFU 01	Determination of Relative Density (Pycnometer Method)
IFU 01A	Relative Density (Method using Density Meter)
IFU 02	Determination of Ethanol by Gas Chromatography
IFU 03	Determination of Titratable Acidity
IFU 05	Determination of Volatile Acids
IFU 07A	Determination of Total Sulphur Dioxide (SO2)
IFU 08	Determination of Soluble Solids (Indirect Method by Refractometry)
IFU 09	Determination of Ash
IFU 10	Determination of Ash Alkalinity
IFU 11	Determination of pH Value
IFU 12	Determination of Hydroxymethylfurfural (HMF)
IFU 12A	Microbiogical detection of taint alicyclobacillus
IFU 17A	Determination of ascorbic acid by HPLC
IFU 18	Fermentation Test (Screening Test for the Presence of Preservatives)
IFU 21	Determination of L-Malic Acid, Enzymatic
IFU 22	Determination of Citric Acid, (enzymatic)
IFU 24	Detection of Artificial Water-Soluble Artificial Colorants
IFU 25	Organoleptic Examination
IFU 26	Determination of Pectin
IFU 28	Determination of Total Nitrogen
IFU 30	Determination of Formol Number
IFU 33	Determination of Sodium, Potassium, Calcium and Magnesium
IFU 36	Determination of Sulphate
IFU 37	Determination of Chloride
IFU 45	Determination of Essential Oils (Bromate Method)
IFU 46	Determination of Pectin Esterase (PE) Activity in Citrus Juices and their Concentrates
IFU 49	Determination of Proline
IFU 50	Determination of Phosphate
IFU 52	Determination of Alcohol, Enzymatic
IFU 53	Determination of Lactic Acid, Enzymatic
IFU 54	Determination of D-Isocitric Acid, Enzymatic
IFU 55	Determination of Glucose and Fructose, Enzymatic
IFU 56	Determination of Sucrose, Enzymatic
IFU 57	Determination of Free Amino Acids
IFU 58	Determination of Hesperidin and Naringin, HPLC
IFU 59	Determination of Total Carotenoids and Individual Carotenoid Groups
IFU 60	Determination of Centrifugable Pulp
IFU 61	Determination of Total Dry Matter
IFU 62	D-Sorbitol (Enzymatic)
IFU 63	Preservatives (HPLC)
IFU 64	D-malic Acid (Enzymatic)

IFU Method	
IFU 65	Tartaric Acid in Grape Juice (HPLC)
IFU 66	Acetic Acid (Enzymatic Method)
IFU 67	Determination of Sugars and Sorbitol (HPLC)
IFU 68	Test for Pectin (Turbidimetric)
IFU 69	Determination of Hydroxymethylfurfural (HPLC)
IFU 70	Cell Content of Pulps and Juices
IFU 71	Anthocyanins by HPLC
IFU 72	Fumaric Acid (HPLC)
IFU 73	Detection of Starch in Fruit Juices
IFU 74	Determination of Nitrate by Ion Chromatography
IFU 76	Determination of D-Gluconic Acid in Grape Juice (Enzymatic)
IFU 77	Determination of Glycerol in Grape Juice (Enzymatic)
IFU 78	Determination of Galacturonic Acid using High Performance Anion Exchange Chromatography
IFU 79	Measurement of Polyols in Fruit and Vegetable Juices using Electrochemical detection
IFU 80	Measurement of the Colour of Clear and Hazy Juices (Spectrophotometric Method)
IFU 81	Determination of Ergosterol by HPLC (Provisional)
IFU 82	Determination of Nitrate (Provisional)
IFU 83	Colour measurement of blood orange juices
IFU 84	Stability test for clarified juices

IFU Recommendations	
IFU R01	Detection of Invert Syrup Addition by Oligosaccharide Analysis
IFU R02	Recommendation for the Determination of Patulin
IFU R03	The Use of Isotopic Procedures in the Analysis of Fruit Juices
IFU R04	Detection of Syrup Addition to Juices by Capillary Gas Chromatography
IFU R05	Recommendation for Vitamin C Analysis
IFU R06	Determination of Heavy Metals in Fruit Juices
IFU R07	Recommendations for Turbidity Measurements
IFU R08	Recommendations for Analysis of High Intensity Sweeteners
IFU R09	Recommendation for Colour Measurements in Cloudy Juices
IFU R10	Recommendations for Analysis of Ochratoxin in Fruit Juices
IFU R12	Methods for the Conformation of Country of Origin
IFU R13	The use of DNA Methods in the analysis of fruit juices, purees and concentrates
IFU R14	Recommendation. Methods to assess the organic or bio nature of juices
IFU R15	Recommendations, Basic quality systems for juice laboratories

Table 2: CEN methods for the authenticity testing of fruit juices

Reference	Application
EN 1131:1994	Determination of relative density
EN 1132:1994	Determination of the pH-value
EN 1133:1994	Determination of the formol number
EN 1134:1994	Determination of sodium, potassium, calcium and magnesium content by atomic absorption spectrometry (AAS)
EN 1135:1994	Determination of ash
EN 1136:1994	Determination of phosphorus content - Spectrometric method
EN 1137:1994	Enzymatic determination of citric acid (citrate) content - NADH spectrometric method
EN 1138:1994	Enzymatic determination of L-malic acid (L-malate) content - NADH spectrometric method
EN 1139:1994	Enzymatic determination of D-isocitric acid content - NADPH spectrometric method
EN 1140:1994	Enzymatic determination of D-glucose and D-fructose content - NADPH spectrometric method
EN 1141:1994	Spectrometric determination of proline content
EN 1142:1994	Determination of the sulphate content
EN 12133:1997	Determination of chloride content - Potentiometric titration method
EN 12134:1997	Determination of centrifugable pulp content
EN 12136:1997	Determination of total carotenoid content and individual carotenoid fractions
EN 12137:1997	Determination of tartaric acid in grape juices - Method by high performance liquid chromatography
EN 12138:1997	Enzymatic determination of D-malic acid content - NAD spectrometric method
EN 12143:1996	Estimation of soluble solids content - Refractometric method
EN 12144:1996	Determination of total alkalinity of ash - Titrimetric method
EN 12145:1996	Determination of total dry matter - Gravimetric method with loss of mass on drying
EN 12146:1996	Enzymatic determination of sucrose content - NADP spectrometric method
EN 12147:1996	Determination of titratable acidity
EN 12148:1996	Determination of hesperidin and naringin in citrus juices - Method using high performance liquid chromatography
EN 12630:1999	Determination of glucose, fructose, sorbitol and sucrose contents - Method using high performance liquid chromatography
EN 12631:1999	Enzymatic determination of D- and L-lactic acid (lactate) content - NAD spectrometric method
EN 12632:1999	Enzymatic determination of acetic acid (acetate) content - NAD Spectrometric method
EN 12742:1999	Determination of the free amino acids content - Liquid chromatographic method
ENV 12140:1999	Determination of the stable carbon isotope ratio ($^{13}C/^{12}C$) of sugars from fruits juices - Method using isotope ratio mass spectrometry
ENV 12141:1996	Determination of the stable oxygen isotope ($^{18}O/^{16}O$) of water from fruit juices - Method using isotope ratio mass spectrometry
ENV 12142:1996	Determination of the stable hydrogen isotope ratio ($^{2}H/^{1}H$) of water from fruit juices - Method using isotope ratio mass spectrometry
ENV 13070:1998	Determination of the stable carbon isotope ratio ($^{13}C/^{12}C$) in the pulp of fruit juices - Method using isotope ratio mass spectrometry

Table 3: Available AOAC methods and recommendations for fruit juice authenticity testing

Reference	Application
2004.01-2004	Carbon stable isotope ratio of ethanol derived from Fruit Juices and Maple Syrups
2005.02-2005	Total Monometric Anthocyanin Pigment Content
969.30-1980(1998)	Organic acids (foreign) in fruit juices.
971.18-1980	Carbohydrates in fruit juices. Gas chromatography
981.09-1983(1997)	Carbon stable isotope ratio of apple juice
982.21-1983(1997)	Carbon stable isotope ratio of orange juice
986.13-1989(1996)	Quinic, Malic and Citric acids in cranberry juice cocktail and apple juice. Liquid chromatography
986.14-2008	Orange Pulpwash and/or Added H_2O in Processed Florida Orange Juice Spectral Characterization
991.30-1994	Polydimethylsiloxane in pineapple juice. Atomic Absorption
991.46-2008	Glycerol in wine and grape juice. Liquid chromatography
992.09-1997	Sugar-Beet-Derived Syrups Frozen Concentrated Orange Juice $\delta^{18}O$ Measurements in Water Stable Isotope Ratio Mass Spectrometric Method
993.05-1997	L-malic/total malic acid ratio in apple juice.
995.06-1998	D-malic acid in apple juice. Liquid chromatography
995.17-1998	Beet Sugar In Fruit Juices Site Specific Natural Isotope Fractionation– Nuclear Magnetic Resonance Method
999.05-1999(2002)	Naringin and neohesperidin in orange juice. Liquid chromatography

3.2. Strategy for the authenticity assessment of fruit juices

The authenticity assessment of fruit juices is complex and does not follow the same strategy for all types of fruits and products. As shown above, a considerable number of analytical methods and associated parameters make up the official methods for fruit juice analysis. It is not realistic to assume that all possible analytical checks to cover all aspects of food fraud can be applied for each individual sample. A typical authenticity assessment of a fruit juice sample without former information about possible fraud consists of two steps. First the analyst tries to get an overview of the overall analytical profile and decides in a second step which aspect should be checked by specific analyses. Ideally a vulnerability assessment on the selected product should be carried out first, in order to better orientate the analytical focus.

The analytical strategy for the authenticity assessment of fruit juice can be classified into different groups of analyses:

- Metabolomic fingerprinting, e.g. proton-NMR Juice Screening (SGF-Profiling^{TM})
- Parameters obtained via chromatographic techniques, enzymatic tests, atomic absorption spectrometry, Inductively Coupled Plasma (ICP)-spectrometry, classical wet chemistry methods; named here "**conventional methods**".
- Detection of marker substances for specific adulterants
- Stable Isotope analyses
- Chromatographic fingerprinting

For all analyses the quality and specificity of reference databases is important. A critical consideration is recommended in this regard. In many cases region-specific reference data help to better recognise food fraud or to avoid false positive interpretation. Regional differences are mainly due to weather conditions, cultivated varieties and process techniques. If one of these parameters changes, then an incorrect analytical evaluation could result. However experience has shown that such changes are relatively seldom, and when they do, the reference databases must be adjusted by addition of appropriate authentic reference samples when they do occur.

To obtain an overall profile of the fruit juice, the best choice today would be, where available, an untargeted proton NMR juice screening combined with selected conventional parameters. If the NMR screening is not possible, a larger set of conventional analyses is needed to cover a maximum of fraud possibilities. The comparison in table 4 below shows how conventional parameters can be replaced by the NMR screening for a minimum scope to check the authenticity for apple and orange juice. Some conventional parameters are not necessary because the NMR screening gives the same information as these parameters.

Wherever possible, positive fraud detection should be confirmed by at least a second analytical approach.

Table 4: Extract from the SGF-Conformity matrix for apple and orange juice/-concentrate. The columns indicate the analytical order with or without SGF-Profiling™

Analysis	Apple		Orange	
	Without SGF Profiling™	With SGF Profiling™	Without SGF Profiling™	With SGF Profiling™
SGF-Profiling ™		x		x
Relative density 20/20	x	x	x	x
Brix (table)	x	x	x	x
Soluble solids	x		x	
Glucose	x		x	
Fructose	x		x	
Sucrose	x		x	
Titrat. acidity expr. as tart. acid pH 7.0	x	x	x	x
Titrat. acidity expr. as citric acid pH 8.1	x	x	x	x
L-malic acid	x		x	
Citric acid	x	x	x	
Isocitric acid			x	x
L-ascorbic acid			x	x
Sodium	x	x	x	x
Potassium	x		x	
Calcium	x	x	x	x
Magnesium	x		x	
Nitrate	x	x	x	x
Phosphate	x		x	
Sorbitol	x	x		
Formol number	x		x	
Proline			x	
Water-soluble pectins			x	
Lactic acid	x		x	
HMF (5-hydroxmethylfurfural)	x		x	

3.2.1. SGF-Profiling™ ^{1}H-NMR-Juice Screening

As a powerful fingerprint method, proton-NMR juice screening SGF-Profiling™ [14–16], which stands for Spin Generated Fingerprint Profiling, is used for authenticity control enabling the screening of a large range of potential adulterations. It is a non-targeted metabolomics application and quantification is performed with a high throughput. The presence and quantity of natural fruit compounds for which the chemical structure is unknown are used in the same way as signals from those compounds that can be identified. The statistical evaluation and quantitative determination of several parameters is done with one single 400 MHz NMR experiment. Analysis time is about 15 minutes per sample and the process can be automated to facilitate a large sample throughput.

Sample preparation consists of centrifugation and diluting a juice or a concentrate with a buffer containing TSP (sodium salt of 3-(trimethylsilyl)-propionate acid-d_4) as an internal standard and sodium azide for preservation. The pH of the buffer solution is 3.1 or 3.4 depending on fruit type. Use of these specific buffer solutions is essential so that the required reproducibility is achieved due to the pH sensitivity of the chemical shifts of some of the polar compounds (e.g. organic acids).

A full proton-NMR spectrum is recorded at 300K. A NOESYPRID™ pulse sequence with continuous wave pre-saturation of the water resonance is used. Baseline and phase corrections can be included in an automatic data treatment. The whole instrument configuration can be designed as a push button system with a flow cell or individual tubes.

A standard routine includes J-resolved NMR spectroscopy (JRES) which allows better signal assignment to molecular structures which show interferences with other compounds in the one-dimensional spectrum along the chemical shift axis [14,17]. JRES spectra are obtained by suppressing J-coupling to separate chemical shifts and J-coupling; both are projected on two different axes which results in a two-dimensional spectrum.

For those products for which SGF-Profiling™ models exist it is recommended to base further analytical choice for juice authentication on the outcome of this screening. It uses a spectral data base of authentic reference material. Different independent verification and classification models are applied. A number of analytical aspects such as type of product, origin or mixture with other varieties are checked in parallel. For some fruits a check of fruit content in a sample is possible too. However, not all products have the same possibility to be analysed. Depending on fruit type, the analytical possibilities and available models may differ. With increasing amount of collected reference samples a development of new and improved models is permanently ongoing.

Table 5 summarises the currently available classification models, and Table 6 lists the quantitative parameters provided. Depending on the type of fruit more or less of the listed substances are determined by automatic quantification routines.

Despite its growing importance, this proton-NMR screening technique is not recognised as an official method yet. However, all measurements and the statistical treatments are accredited according to ISO 17025 in certain laboratories.

Table 5: Classification models by SGF-Profiling™

Analytical aspect	Classes to differentiate
Origin Apple Juice	Poland/Germany, Turkey, China, Hungary, Spain/Italy
Origin Orange Juice	Brazil, Spain, Greece, Belize/Costa Rica/Cuba, Mexico, USA, Argentina/Paraguay/Uruguay
Origin Lemon Juice	Spain/Italy, Argentina
Origin Pineapple juice	Asia, Middle America, Brazil
Origin Sour Cherry Juice	Poland, Turkey
Origin Mango puree	Mexico, India
Product Type Apple Juice	Direct juice, Juice made from Concentrate
Product Type Orange Juice	Direct juice, Juice made from Concentrate
Product Type Lemon Juice	Direct juice, Juice made from Concentrate
Product Type Pineapple Juice	Direct juice, Juice made from Concentrate
Citrus type	Orange, Blood Orange, C. reticulata

Table 6: Parameters which can be automatically quantified from proton-NMR spectrum of SGF Profiling™

4-aminobutanoic acid	chlorogenic acid	gluconic acid	proline
acetaldehyde	citramalic acid	glucose	pyruvic acid
acetoine	citric acid	HMF	quinic acid
Alanine	ethanol	isocitric acid	shikimic acid
Arbutin	formic acid	lactic acid	sorbic acid
arginine	fructose	malic acid	succinic acid
benzaldehyde	fumaric acid	methanol	sucrose
benzoic acid	galacturonic acid	phlorin	xylose

Calculated values

glucose/fructose	% sucrose	total sugar	malic acid/quinic acid

3.2.2. Authenticity strategy using conventional methods

Authenticity patterns for fruit juices are generally composed of a considerable number of analytical figures, many of which have guidance ranges in the AIJN Code of Practice (3). For a number of types of fraud, the overall profile obtained by different conventional analyses provides a first indication and sometimes even clear evidence of adulteration. In any case, if there is no particular authenticity indicator or suspicious parameter, an analytical pre-screening is recommended to provide some idea of suitable follow-up specialised analyses. This can consist of a compilation of typical analytical data, sometime also called “full analyses” combined with an expert interpretation. The value of the analyses depends strongly on available reference databases and the ability to interpret the analytical data. In fact, producing correct analytical results is often not the main challenge in fruit juice authenticity control, but the interpretation of obtained data.

Every laboratory and its analysts have to develop a way of judging the whole analytical pattern as such and not only value by value. Therefore no standardised procedure exists, however hereafter follows an attempt by the chapter's authors try to give a rough description of one way of proceeding.

1. Check the Brix value or density and Brix/acidity ratio.
   - High values of Brix or density in single strength products stand for high sugar content and high degree of ripeness. Ripe products would probably tend to have a low acid content and high Brix/acidity ratio. Particularities of certain varieties should be taken into account.
   - Single strength products have natural variations, official reference values are generally set at the lower limit. If products from one source are regularly close to this minimum level without variation, a systematic standardization through water addition is to be considered.
2. Check the sugar profile.
   - The glucose/fructose ratio is specific for different fruit types.
   - The glucose/fructose ratio generally decreases with microbiological spoilage. Check consistency with other metabolites indicative of spoilage (e.g. lactic acid, ethanol, volatiles acids)
   - Sucrose content is typical for different fruit types, some fruits do not have any. The step of invertase deactivation in the process flow plays an important role.
   - A product which has undergone heat stress, a long storage time or inappropriate transport conditions can show sucrose inversion to glucose and sucrose in equal quantities. Inversion is also favoured by high acid content.
3. Check the sugar free extract and its relation to the total amount of sugar.
   - The sugar free extract contains all soluble compounds which are not the main sugar compounds glucose, fructose and sucrose. This would decrease if external sugar is added.
   - The relation between total sugar and the sugar free extract would shift towards the sugar content for products which are supposed to derive from very ripe fruits.
4. Try to explain the sugar free extract with available data.
   - An analytical profile will never cover the total soluble extract. Depending on the type of product and the chosen analytical parameters the gap between measurable compounds of the sugar free extract and the total sugar free extract is more or less great. However, the sum of measured concentrations for organic acids, minerals, sorbitol (if present) could show experienced analysts whether the usually expected gap is in the natural range. If the gap is too small, an adjustment of the analytical profile to mask a low fruit content would be expected.
   - If the gap is too big unexpected compounds must be present (e.g. sorbitol, solubles from mash extraction, starch or other polysaccharides)
5. Check relation of compounds inside the sugar free extract.
   - Group of organic acids: the ratio of citric acid and D-isocitric acid is typical for every fruit type. High values indicate the addition of citric acid.
   - Group of minerals: higher sodium and nitrate values can indicate the presence of minerals from process water or process agents. Regional exceptions are possible.

- In particular, for products not from concentrate high values of sodium or nitrate – even below the AIJN-Code of Practice maximum guide values – could hint at added water or the use of reconstituted concentrates.
- Group of flavonoids, anthocyanins: different fruit types have different patterns.

6. Check individual marker substances. Examples:
   - Some substances are untypical for certain fruit types, e.g. sorbitol, sucrose,
   - Some substances can indicate the presence of another fruit type, e.g tartaric acid indicates grape, phloridzin indicates apple, arbutin indicates pear, naringin indicates grapefruit.
   - Lactic acid and ethanol indicate fermentation.
   - 5-hydroxy-methylfurfural (HMF) is a typical Maillard product and indicates heat stress and/or long-term storage in inappropriate conditions.

There is no obligation to follow this list in this order or limited to the afore-mentioned examples. However it represents a useful approach to interpretation for the less experienced analyst, while building up his/her own referential of importance for any parameter. Table 7 provides an idea of which authenticity aspect a parameter could contribute to.

An authenticity check based on the overall analytical profile is particularly efficient if the analyst has a good idea about the product: its ripeness, applied process, microbiological status, etc. Meta data information with influence to the analytical profile can be counter checked.

Table 7: Choice of conventional parameters and their use for interpretation (frequent analytical targets are indicated; fruit and product type specific deviations are possible)

Parameter	Fruit content	Sugar addition	Organic acid addition	Foreign fruit	Water addition	Technology (citrus juice)
Brix / Density					X	
Total titratable acidity			X			
Potassium	X					
Formol number	X					
L-Malic acid	X		X			
D-Malic acid			X			
Magnesium	X					
Calcium	X					X
Phosphorus	X					
D-isocitric acid	X		X			
Proline	X			X		
Citric acid			X	X		
Ratio citric / isocitric acid			X			
pH			X			
Maltose/Isomaltose		X				
Chloride					X	
Sulphate					X	
Glucose		X				
Fructose		X				
Ratio Glucose/Fructose		X		X		
Sucrose		X		X		
Total sugar (glucose, fructose, sucrose)		X				
Sucrose versus total sugar		X				
Sodium					X	
Nitrate					X	
Carotenoid profile (diff. fractions)				X		
Total Carotenoids, β-Carotene				X		
Sorbitol	X	X		X		
Water soluble pectins						X
Centrifugeable pulp						X
Phlorin						X
Ascorbic acid			X			

3.2.3. Detection of marker substances for specific adulterants

A specific marker substance for an adulterant can be used to highlight the fraud. Methods for the detection of these markers can be selective or specific, and the limit of detection of any adulteration depends on the sensitivity of the method being used. A list of marker substances for undeclared fruit types is given in Table 8.

In most cases marker substances are used to identify so called “foreign fruits”, or a cheaper fruit type added to the declared one. It is therefore necessary to know the concentration of a certain marker substance in both the declared fruit juice and in the adulterant.

Table 8: Marker substances of undeclared fruit types

Adulterant	Marker substance	Suitable for	Method
Pear	Arbutin	Most other juices	HPLC/UV phenolic compounds, proton-NMR
Apple	Phloridzin	Most other juices	HPLC/UV phenolic compounds
Pome and stone fruits	Sorbitol	Citrus, most berry fruits, tropical	HPLC/IC, enzyme test
Lime	Iso-pimpinellin, Bergapten 7-methoxy- coumarin	Lemon juice	HPLC/UV(DAD) and or MS
Lemon	Eriocitrin	Citrus juices	HPLC/UV Flavonoid glycoside (IFU 58)
Grapefruit	Naringin	Citrus juices	HPLC/UV Flavonoid glycoside (IFU 58)
Orange	Hesperidin	Passion fruit juice	HPLC/UV Flavonoid glycoside (IFU 58)
Grape	Tartaric acid	Other juices	HPLC/IC (IFU 65)

The example of sorbitol as a marker substance for the presence of undeclared fruit types is discussed here. It can be used to detect apple, pear, aronia and certain other fruits which might be added to blackcurrant juice. Apples, pears and aronia naturally contain sorbitol, whereas blackcurrant does not. Therefore positive detection of sorbitol in blackcurrant juice would clearly show that the juice is not authentic, but it would not differentiate which type of fruit is the adulterant. A more fruit specific indicator for added apple juice would be the phenolic compound phloridzin, which is a typical marker for apple and not present in pears or aronia.

If the marker substances are present in low concentrations, the possibility of unintentional product cross-contamination must be considered and manufacturing practices should be investigated. Furthermore, the natural occurrence of traces of sorbitol through naturally present micro flora should be looked at. A reference value to which a measured concentration remains acceptable is complex and needs more investigation. Official guidelines such as the AIJN Code of Practice have to apply relative high uncertainty margins in the benefit of the doubt, whereas individual company policies can be different.

Marker substances are also used in chromatographic fingerprint methods such as anthocyanin or flavonoid profiles. The occurrence of fruit specific substances allows the differentiation and identification of undeclared fruit types present in a sample. Important methods are shown in Table 9.

Furthermore there are also methods which use typical by-products from the production of sugar syrups as marker substance for added sugar. Such marker substances could be oligosaccharides. Maltose, maltotriose and higher starch degradation products can be detected with ion HPLC. Typical by-products of inversion or degradation of polysaccharides give characteristic peak patterns in GC chromatograms obtained after silylation [18].

Table 9: Fingerprint methods for fruit juice authentication

Fingerprint markers	Sugar addition	Acid addition	Foreign fruit	Applicable in juices/purees of…
Amino acid - HPLC or Amino Acid Analyser (IFU 57)			X	All fruits
Flavonoids - HPLC (IFU 58)			X	Citrus fruits
Anthocyanins - HPLC (IFU 71)			X	Red-coloured fruits
Polymethoxylated flavanones – HPLC			X	Citrus fruits
Oligosaccharides – HPLC / IC	X			Most fruits
Oligosaccharides – GC	X			Most fruits
Phenolic Fingerprint - HPLC			X	All fruits
Carotenoid profile - HPLC			X	Yellow/orange coloured fruits
Organic acids – HPLC		X	X	All fruits

3.2.4. Isotopic methods

The isotopic profiles of juice constituents are often “the ultimate weapon” for confirmation of a supposed adulteration. In some cases they are the only means of identifying a certain type of food fraud, especially when it is masked by “cocktails” of typical components (e.g. minerals, organic/amino acids, etc.)

A recent revision of the IFU recommendation explains the use of isotopic procedures in the analysis of fruit juices [19]. The possibilities offered by these parameters are summarised in Table 10.

Besides the determination of the isotopic ratios of juice water or sugars, refined approaches have been developed during the last three decades to enhance the sensitivity of isotopic methods, using:

- Multi-component approaches (looking at inner correlations between sugars, acids, etc.): [20–26],
- Multi-element approaches (combining several isotopes) [21],
- Site-specific approaches [27–29].

Stable isotopes can also help for origin confirmation [19]. In particular the use of strontium (^{87}Sr & ^{86}Sr) has been shown to be quite useful as it can be a very good marker for the “age”, in the geological sense, of rocks, which can also be used for origin assessment. However, it is much harder to use them in a predictive sense, that is to infer an origin of a product purely from analytical data. This is due in part to the overlapping of ratios seen for many geographical regions around the world.

Table 10: Available stable isotope analyses for the authentication of fruit juices

Parameter	Water addition	Sugar addition	Organic acid addition	Origin	Fertilisation regime
Oxygen or hydrogen isotope ratios of water (and ethanol from fermentation)	X				
Carbon and hydrogen isotope ratios of sugars or ethanol from fermentation		X			
Carbon and hydrogen isotope ratios of citric acid			X		
Global & positional carbon isotope ratios of malic, tartaric and ascorbic acids			X		
Carbon, hydrogen, oxygen, nitrogen, sulphur and strontium isotope ratios of bulk juice / juice components				X	
Nitrogen isotope ratio					X

3.3. Other commonly used methods

Not all aspects of fruit juice authenticity are controlled with required precision with methods described above. The identification of different fruit types and varieties is one of them. There DNA analyses are used successfully in a few cases. The analytical challenge is the low amount of DNA in the product and its degradation due to low pH and processing conditions. If a juice is clarified there is even no chance to obtain any exploitable DNA information. Nevertheless with advanced techniques the following differentiations have been validated successfully [30]:

- *Citrus sinensis* (orange) and *Citrus reticulata* (mandarin and other hybrids),
- Different varieties of mango.

The quantification of the adulterant is difficult because the available amount of DNA differs significantly from one sample to another. Results are semi-quantitative or qualitative only.

New DNA approaches will certainly increase the range of applications in the near future.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address. More details are given in the tables in the relevant sections.

Analytical Technique	Indicative data or Analyte or Parameter	Authenticity issue or information
Conventional methods	Main compositional parameters (Tables 1,2,3,7)	Compositional parameters are out of set specifications or AIJN limits
Metabolomic fingerprinting using ^{1}H NMR (SGF-Profiling^{TM})	Overall profile from 1H NMR spectrum + selection of compositional parameters (Tables 4,5,6)	Fruit type, geographical origin, addition of other fruits
Chromatography (HPLC/UV, HPLC/IC, HPLC/UV(DAD) or MS)	Marker substance (Table 8)	Undeclared fruit types
Chromatographic techniques (GC, HPLC) as fingerprint methods	Fingerprint markers (Table 9)	Addition of sugar, acids, foreign fruit
Stable isotope analyses	Isotope ratios of carbon, hydrogen, oxygen, nitrogen, sulphur, strontium (Table 10)	Various authenticity issues

5. Conclusion

Due to the complexity of authenticity control in fruit juices an analytical authenticity check is always a more or less tailor-made combination of different methods. A first establishment of an overview profile can be followed by analyses with a more precise focus and a lower limit of detection.

The high number of individual analyses to establish a meaningful overall profile is an economic and time-consuming handicap. If available, the SGF NMR-Profiling^{TM} is part of a better alternative. For the future it can be expected that this technique using modified sample preparation (e.g. fractionation, concentration) or other techniques with large data treatment like LC/HRMS [31] will successively enhance fruit juice integrity analyses.

However there will remain the necessity to confirm analytical observation by targeted methods. Isotopic techniques are likely to fulfil a major part of this need. Internal referencing methods will play an important role there.

Due to the growing number of production regions for semi-finished goods, agricultural development and the changing climate the interpretation of analytical results will become more difficult and specific reference data bases will be increasingly required in the future.

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