

## Specific comments on the A.I.J.N. Reference Guidelines (RG)

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### 1. General

The following comments are only presented as a source of complementary information for the reader, **they cannot be considered as simple explanations for exceptions** from the guide values. They may, however, help the reader to understand specific outliers and instigate the procurement of additional information and/or confirmation by additional analysis.

The A.I.J.N. Reference Guidelines apply to both direct fruit juices and reconstituted fruit juices placed on the market as well as to their raw materials and are based on authentic juices without permitted ingredients and/or additives.

Fruit juices manufactured and sold in the EU have to comply with the provisions in the existing EU legislation applicable to foodstuffs in general and to fruit juices in particular.

### 2. Absolute Quality Requirements

The comments on the parameters mentioned below apply equally to all fruit juices.

#### a. Relative density

For those products, which are usually produced as a juice a minimum relative density is determined as such in relation to water at 20/20°C. The corresponding Brix value is directly obtained from the IFU table no. 8 .

For those products, which are usually produced as a purée, only a minimum uncorrected refractometric Brix reading (without correction of acid) is determined. In cases where a clarified purée/juice is used a minimum relative density is taken.

The vast majority of fruit juices marketed are reconstituted from concentrate.

The EU Directive requires reconstitution to the original value. The minimum values laid down in the guidelines are agreed upon by industry in order to meet this requirement of the Directive in the best possible way.

The values given are in line with Brix values commonly found in nature over the year.

Direct juices from defined origin and variety may have lower natural values as the respective minimum value indicated.

Nevertheless, setting the indicated minimum values is justified in order to meet justified consumer expectations.

Direct juices low in natural extract must be blended with direct juices of higher natural extract in order to come up to at least the minimum value. Correcting by adding concentrate is not permitted without the appropriate labelling (from concentrate). Otherwise it is not permitted to dilute direct juices higher in natural extract with water to a lower level.

#### b. Biogenic acids and ethanol

There are practically no volatile acids, lactic acid and ethanol in juices produced from suitable and properly stored fruit. Larger amounts than mentioned indicate a lack of hygiene in fruit handling or problems in plant sanitation.

As a rule, properly processed products should be clearly under the maximum values given. Usually contents over the maximum values given will be accompanied by a noticeable change in aroma.

Problems with fruit quality and/or sanitation may also result in undesired mycotoxins, for instance patulin should be kept under control for health reasons.

#### c. Arsenic and heavy metals

These substances considered as environmental contaminants are, as far as possible, to be avoided. The maximum values given in the reference guidelines comply with the current recommendations of the public health authorities. Products in metal containers may contain higher values for iron and tin, but should not exceed official limits.

#### d. Hydroxymethylfurfural (HMF)

In fruit juices processed according to "Good Manufacturing Practice" (GMP) only traces of HMF are found.

Higher values can be expected in cases of excessive thermal treatment during processing or filling as well as inappropriate storage conditions and/or times.

Packed juices should under normal storage conditions not exceed the maximum values indicated before the end of the indicated shelf life.

Usually, with increasing HMF values, a loss of L-ascorbic acid, colour and other sensorial characteristics may be observed.

Changes made in the A-criteria of a Reference Guideline become applicable 12 months after the date of publication of the adopted change(s) on the Code of Practice website.

### 3. **Specific Comments category B parameters**

#### a. Water for reconstitution

The new Fruit Juice Directive (2012/12) stipulates that the water used for the reconstitution of fruit juice concentrates has to comply with the EU Potable Water Directive (98/89). This is in-line with the Codex Standard 247 2005 for fruit juices. The EU Directive for potable water contains an extensive list of conformance parameters (given in Annex 1 parts A & B). The components include such substances as: - heavy metals, pesticides, halocarbons, acrylamide, halides, nitrate, nitrite, cyanide, benzo(a)pyrene, PAHs and pathogenic microorganisms. If using water from a commercial supply, conformance with the Directive should be guaranteed by the supplier.

- The water used for reconstitution should be free from off tastes and aromas that could adversely affect the quality of the re-diluted concentrate.

- Fruit juices have, and should maintain, a healthy image as they are naturally almost free from sodium, nitrate and other negative substances. It is recommended that the sodium and nitrate values of the water used for reconstitution be kept as low as possible.

- The data presented in the various reference guidelines refers only to the natural concentrations seen in the juice/purée. It does not allow for the influence of the water used for the reconstitution of concentrate.

In the Potable Water Directive, as stated above, there is no limit set for sodium and the level permitted for nitrate (50 mg/l) is well above the values seen in most fruits. As a result of this the concentrations of these parameters, and to a lesser extent those of calcium and magnesium, can be significantly influenced by the composition of the water used for reconstitution. (See the individual commentary notes).

#### b. Water used for processing fruit juices

Process water e.g. that used for in line extraction of the pulp or for treating products during further processing should either be potable water or have the appropriate characteristics, particular from a chemical, microbiological and sensorial point of view, to maintain the requirements of the original juice as described in these reference guidelines.

#### c. Isotopic analysis

Oxygen isotopic analysis ( $\delta^{18}\text{O}$ ) can be used for the detection of water addition to direct juices.

The addition to juices of the so called  $\text{C}_4$  derived sugars, from cane or corn, acids and polymeric stabilisers can be detected by using carbon isotopic analysis. This can be on the bulk juice or, to get an enhanced level of sensitivity, on components isolated from it. These components would include the individual sugars and/or acids and the pectins (the alcohol insoluble materials). The carbon isotopes can be measured by isotope ratio mass spectrometry and in the case of sugars from pineapple by quantitative  $^{13}\text{C}$ -NMR after fermentation.

The addition of beet derived sugars can be detected using Deuterium-NMR  $\{(D/H)_1\}$ . However, the analyses and interpretation of the data requires specialised equipment and experience to obtain meaningful and reliable results.

#### d. Maltose in clear juices

Normal citrus juices or citrus juice prepared from concentrate do not contain any significant levels of maltose above the limit of quantification (LOQ) of the analytical procedures that is approximately 5mg/l. However, in clarified products low levels may be seen.

Some fruits naturally contain starch and others only if the fruit is unripe if this starch is not removed during processing into a clear juice, post processing hazes can result. Such juices are treated with an amylase preparation during processing. This action liberates small amounts of maltose that can be detected in finished product by Cap-GC (IFU recommendation 4), HPLC-linked with an electrochemical detector (e.g. HPAEC-PAD) or HPLC-linked with a light scattering detector.

Studies on clear apple and pear juices have shown that, in the majority of cases, samples prepared according to GMP contain less than 100 mg/l maltose (on a single strength basis). If considerably higher levels of maltose are found it can indicate the use of unripe fruits or the addition of sugar syrups derived from starch. This is not always the explanation but high levels require further investigation.

In some juices/purées maltose can be found as a natural component, without the action of an amylase. Typically guava purée may contain this compound but levels are normally less than 50 mg/kg and in mango purées the levels are normally less than 100 mg/kg.

Isomaltose is a natural component of pineapple juice but its presence in most juices is unusual. Apart from pineapple, isomaltose is normally associated with the presence of added sugar syrup derived from starch, such as HFCS. If both maltose and isomaltose are detected in a pineapple juice, the product should be examined very closely for the presence of syrup derived from starch, as maltose is not normally seen in this juice, quantitative  $^{13}\text{C}$ -NMR is a suitable procedure to use here.

The Cap-GC method, detailed in IFU recommendation 4 (IFU rec # 4) is a good procedure to detect both maltose and isomaltose in juices. If using this method in a quantitative manner it is critical to prepare your standards in the presence of another sugar, such as sucrose, as otherwise the derivatisation proceeds differently and false levels can result (see the IFU rec. # 4 for full details).

#### e. Interpretation of data from profiling procedures

Over the last 10 years a number of profiling or “fingerprinting” methods have been developed that are very useful in assessing the quality and authenticity of fruit juices/purées. In most of these methods the pattern of peaks is often more important than their actual size. The fingerprinting methods include oligosaccharide, anthocyanin and polyphenol profiles and the profile of volatile flavour compounds. The pattern of isotopic data in a sample can also be useful to enhance the detection of added sugars and/or acids or in the assessment of country of origin.

The interpretation of the data from these profiling methods is often complicated as the pattern can be dependant on season, variety and processing conditions etc. This means that to produce an accurate and reliable assessment significant experience is required and so is best left to experts.

Below is a section, on each of the profiling procedures, which gives an indication of their importance and use. In time it is highly likely that new profiling methods will be added to this list.

#### **Oligosaccharide profile**

The pattern of peaks in the Capillary Gas Chromatography (Cap-GC) profile is determined, after derivatisation, using the procedure given in IFU recommendation number 4.

If the sample has been adulterated with a sugar derived from a starch or inulin source, characteristic peaks will often be present. These peaks should not be confused with the two maltose peaks that can arise by amylase treatment of fruits that naturally contain low levels of starch.

Samples adulterated with partial or total invert syrups can also be identified by the presence of specific “invert marker peaks”. However in high acid juices in particular,

heating also influences the size of these peaks and caution should be exercised as false results can be produced. A full description of how to interpret the results from this method are detailed the IFU recommendation.

The method can also be used to detect the presence of cellobiose, which is used as a marker for the action of a cellulase. This enzyme is prohibited by the EU fruit juice directive for fruit juice. It is also prohibited in the US if the enzyme causes a significant hydrolysis of the cell wall which would significantly alter the composition of the juice.

### **Anthocyanin profile**

This approach is particularly useful for the evaluation of red/black fruit juices and purées, such as raspberry. The sample is examined by a reverse phase HPLC method, such as IFU No. 71 or similar procedure. The pattern of peaks is then compared with a reference sample of the fruit or with the profiles given in the IFU method.

The pattern is characteristic of the fruit and can sometimes even differentiate between different varieties of the same fruit. When assessing the HPLC profiles the following points should be taken into account:

1. A particular fruit or fruit variety will contain a number of anthocyanins that are specific to that fruit.
2. The presence of extra or unusual peaks should be examined critically as this can be indicative of the addition of undeclared fruit and/or colour.
3. The enzymes used during processing can remove or distort the relative sizes of some of these peaks
4. A reduction in the size of all the peaks can be indicative of dilution of the product or poor and/or extended storage.
5. If the analysis is correctly performed the baseline of the profile is normally flat. If a pronounced hump is present this is also an indication of poor and /or extended storage that has caused the anthocyanin materials to degrade (polymerise).

### **Polyphenol profiles and polymethoxyflavones**

These methods have particular use in the detection of the presence of one type of fruit in another. They again involve the separation of the compounds by reverse phase HPLC.

The methods produce a pattern of peaks that can be compared with a reference sample of fruit. These patterns are generally very complex. However, it is often only particular regions of the profile that are of specific interest in detecting the addition of one fruit to another.

Typical examples of the use of these methods are: -

Differentiation of citrus juices

The detection of mixtures of citrus juices such as sweet (*C. sinensis*) and sour (*C. aurantium*) orange

The presence of grapefruit in orange

Differentiation of lime from lemon.

Differentiation of apple and pear or mixtures thereof

Utilisation of these methods should be left to experts who are familiar with the use of the HPLC procedures and the interpretation of the profiles that they produce, as they are very complex

### **Profile of volatile flavour compounds**

The pattern of volatile compounds seen in the Cap-GC profile of a sample should be characteristic of that fruit. The presence of small unusual peaks can arise from "carry-over" of one product to another due to incomplete CIP of bottling systems etc. Further guidance on this subject can be found in the "AIJN position paper on carry-over aroma in the fruit juice industry" published in 2005 (see Annex 9 of the COP). The quantitative evaluation of the volatile flavour compounds in a product can be useful to ensure that the aroma in a "from concentrate juice" has been restored.

If abnormal peaks are detected in a sample or if an adulteration is suspected, then chiral-GC and or GC linked with isotope ratio mass spectrometry (IRMS) can be used.

### **Chiral-GC analysis**

Natural flavour compounds often exist in specific spatial forms known as enantiomers, D & L or (+) & (-) forms. Chiral analysis is able to separate these 2 forms and thereby provide valuable evidence of the presence of synthetic flavour compounds. For example, if a particular flavour compound is known only to occur naturally in the "L" form then the presence of the "D" form in a juice indicates adulteration.

The situation is more difficult to interpret if the compound occurs naturally in both forms but often the "normal/expected ratio" of the "L" and the "D" are known. A marked deviation from this expected ratio should be judged as suspicious and investigated closely.

The most difficult situation arises when the natural ratio is close to 1:1 because this is the ratio that is also often found in synthetic flavours. Here mixtures of natural and synthetic flavours are not detectable. Under this circumstance resort has to be made to isotopic techniques.

### **GC-IRMS**

Isotopic analysis of flavour compounds can also be useful for detecting adulterations. This type of approach can be particularly useful if the flavour of interest does not occur in (D/L) forms. The compounds of interest are first separated on a GC and then individually analysed by isotope ratio mass spectrometer (IRMS). Here the carbon isotope ratios of the individual flavour compounds are measured. (This type of analysis is generally carried out in association with normal GC but can in special cases be linked with chiral GC as well.)

The carbon isotopic ratios of naturally occurring flavour compounds are known. If synthetic flavour compounds are present the carbon isotopic ratios will be altered and hence the adulteration can be identified

As these two approaches require very specialised and expensive equipment and as the interpretation of these results can be very complex it is critical that this type of analysis is only carried out by experts.

## General isotopic analysis

The use of hydrogen, carbon and oxygen isotopes, have been used for many years to detect adulteration of juices. Values for these isotope ratios are included in section B for a number of the individual fruits.

Recently the detection limits for the isotopic methods have been improved by the use of internal isotopic rationing. Individual compounds from the same sample e.g. the glucose, fructose and sucrose are separated and the carbon isotope ratios are measured. Authentic samples have ratios, for the individual compounds, that fall within known limits. Similarly it is possible to measure the carbon isotope ratios of different sites within the same molecule e.g. malic acid. Natural malic acid has differing ratios than malic acid from a synthetic source which allows its detection.

Under favourable circumstances the use of these improvements can enhance the detection limit by about a factor of 2.

It has been known for some time that the proportions of the metal ions in a sample could be extremely useful at detection the origin of juices. A good example here would be the use of Ba and Rb to confirm a juice to be Floridian rather than one from Brazil for instance. More recently the use of some of the heavy isotopes, such as <sup>87</sup>Sr, has been found to be extremely useful at confirming the country of origin. This element is found at relatively higher levels in samples grown in areas where the rocks are old such as in Brazil whereas in other regions where the rocks are much younger the level of this element is lower.

Again the use of light and heavy isotopes requires expensive equipment and the interpretation of this type of data should be left to experts.

### f. Handling normalised data

The values contained within the AIJN code are derived from data from both not from concentrate juices (NFC) and juices from concentrate. When/If evaluating analytical data of ready to drink fruit juices, be it NFC juices or juices reconstituted from concentrate, the values should be compared directly, i.e. without normalisation. When evaluating data obtained from NFC juices and juices from concentrate with Brix values well away from the AIJN COP min. reference guidelines values it should be realised that some of the data for the product may well fall outside the values given in the code.