ENZYMATIC AND COLORIMETRIC METHODS FOR JUICE ANALYSIS

Industrial production of packaged juices is a global type industry with a turnover of around USD 100 billion and an annual growth trend of over 6%, driven by the consumption of fresh, natural products that preserve the maximum of nutritional properties. It is a primary sector transformation industry with a presence in practically every country in the world, adapted to the characteristics of local products, that processes around 45 billion litres of juice. In many cases, production is local, which makes it a highly fragmented market, without the predominance of large multinationals, in which the price / quality ratio is decisive in consumer choice. Orange juice is the main product, but there is a growing consumer demand for experimenting with new juice combinations, with improved nutritional properties or new flavours.

Producing companies strive to maintain high production quality standards, which translate into better preservation of the nutritional properties and original flavours, additional contributions of vitamins and other supplements, and strict compliance with current legislation.

The manufacturing procedures, authorized additives, as well as the relevant parameters and methods in evaluating the quality of fruit juices, nectars, purees and concentrates are included in the National Technical Standards (in the European case, Directive 2012/12 / EU), which in turn include the provisions in CODEX STAN 247: 2005 (CXS-247), approved by FAO as a global reference framework to guarantee food safety to the consumer. In the CXS-247 the parameters to be evaluated are described, in order to determine the composition, quality criteria, possible adulteration and/or authenticity of the product, as well as the presence of additives and contaminants.

Enzymatic and colorimetric methods are widely used in the juice industry to evaluate specific components that have an impact both on the organoleptic characteristics of the product and on the detection of health risks or adulteration thereof. The vast majority of them are type II; that is, reference methods that meet all the metrological requirements required for use for control, inspection or regulatory purposes. Due to their simplicity of use, easy adaptation to automated systems, high specificity and the flexibility to adjust to different measurement conditions and matrices make its presence in the agri-food laboratory highly recommended.

These methods are based on the ability of enzymes to react specifically and quickly with certain compounds. This initial reaction can be combined with others until a product is produced that can be measured spectrophotometrically at a wavelength of the UV-Visible spectrum, usually between 340 and 800 nm. The measure of the absorbance change corresponding to the formation or disappearance of that product will be proportional to the concentration of the analyte.

Among the enzymatic methods collected in the CXS-247 are detailed, among others:

- **Glucose, Fructose and Sucrose** (EN 1140: 1995, EN 12146: 1997) for the measurement of reducing sugars contained in the juice and the relationship between them to detect adulterations due to the addition of non-natural sugars.

• **D-Sorbitol** (IFU nº 62: 1995) to determine natural sugars in apples, pears and red fruits, or adulteration (as an added sweetener) in others.

• **D- and L-Lactic Acids** (EN 12631: 2000) for the detection and measurement of fermentation products, since the legislation specifically prohibits fermentation in this type of products.

• **Citric and Isocitric Acids** (UNE-EN 1137: 1995, UNE-EN 1139: 1995) to determine both the quality of the product and the possible adulteration by the addition of water or citric acid.

• **D- and L-Malic Acids** (UNE-EN 12138: 2000, UNE-EN 1138: 1995) are present in practically all fruits and are one of the main causes of acidity in its L form, while the presence of D-isomer would be indicative of adulteration.

• **Acetic Acid, glycerol and ethanol** (UNE EN-12632: 2000, IFU nº 77: 2001, IFU nº 52: 1996) appear in fermentation processes; acetic can also be detected as a remnant of fruit and facility disinfection with peracetic acid.

• **Phosphorus** (EN 1136: 1994) is an essential component of the diet that participates in the physiological regulatory mechanisms; the juice content is directly related to the amount of phosphorous found.

In addition to these parameters in which the enzymatic method is the official one, in other cases enzymatic / colorimetric methods can be used to support process control as they are much faster and easier to implement than the reference methods. In these cases, the use of such methods is a viable alternative to obtain approximate results that allow decisions to be made during processing, even if they do not have sufficient metrological quality for use in labelling or certification requirements. Some of these methods would be available for measuring:

• **Ascorbic Acid** (reference methods IFU No. 17a and ISO 6557-1: 1986 use HPLC and fluorescence, respectively) can also be determined enzymatically by ascorbate oxidase / MTT with equivalent results in some fruits (orange juice) but not in other matrices .

• **Potassium, calcium and magnesium** (the reference method EN1134: 1994 is by atomic absorption) are essential ions present in large quantities in fruits and vegetables, which are the main contributors to the diet; its concentration is directly related to the fruit content.

Finally, specific parameters could be of interest on particular products, like measurement of **polyphenols** in red berries juices (with a high anti-oxidant properties), or **tartaric acid** for grape juice.

The Dionysos system offers producers of juices, nectars and packaged purees an optimal tool for the control of the production process capable of guaranteeing the quality and food safety requirements demanded by existing regulations.