Macedonian pharmaceutical bulletin, **71** (2) 3 - 11 (2025)
ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2025.71.02.001
Review paper

Analytical methods for determination of sugar content in fruit juices: from classical methods to modern analytical techniques

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Received: February 2025; Accepted: April 2025

Abstract

The determination and quantification of sugars in fruit juices is especially important for quality control. In this article, the analytical methods for determination of sugars in fruit juices are presented and discussed. Available methods range from classical methods based on Fehling's and Luft-Shorl's methods, refractometric or enzymatic to liquid chromatography with different types of detectors. Although chromatography has been extensively used as a very accurate and sensitive technique, the sample preparation is time consuming and requires solvent extraction. Spectroscopic techniques (FTIR-ATR, MIR, Raman) have been recognized as potential and valuable alternative to chromatographic methods, since they are easy, simple and in many cases non-destructive. These methods that are fast, easy to use and non-destructive are in line with current analytical trends however they must be properly calibrated and validated.

Key words: sugars, fruit juices, classical methods, chromatography, spectroscopy

Introduction

Fruit juices are food products defined as fermentable but unfermented beverages obtained from the edible parts of the fruits and they are usually extracted or pressed out from the natural liquid contained in a fruit (Directive 2012/12/EU). They contain sugars, vitamins, minerals, antioxidants, and phytochemicals and are considered as one of the healthiest foods in human diet (Tyagi et al., 2014; Rajauria and Tiwari, 2018). Although sugars are dominant nutrients present naturally, they can also be added to juices to improve sweetness, or they can be used as preservatives (Tyagi et al., 2014). These components are commonly classified as simple sugars (monosaccharides or disaccharides, fruit juice concentrates, honey, and various syrups) that have been added to beverages or food products during their transformation or preparation by food industries or by the consumer per se (WHO, 2015).

Nowadays, people consume more refined sugar that is also present in fruit juices than their body weight allows. The average healthy digestive system can digest and eliminate two to four teaspoons of sugar daily, usually without noticeable side effects. An excess of sugar in the diet results in weight gain, thereby increasing the risk of heart disease, diabetes and high blood pressure, apart from dental caries. It is therefore important to know what amount of sugar is present in food and beverages that are

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commonly consumed. Recommended Dietary Allowance (RDA) of sugar is 25 g, based on the World Health Organization (Rambukwella et al., 2015; WHO, 2018). The determination of simple sugars in food and food products is an important step in the production and marketing of these products to consumers, as well as in the post market food quality control (Bicking, 2024; Dasenaki and Thomaidis, 2019).

Additionally, the globalization of the food trade and the world food markets over the last few decades have made an enormous variety of food products available, so the demand for high quality food products including fruit juices, is constantly increasing. In parallel with the increased demand, some manufacturers adulterate fruit juices by addition of cheap syrups or other sweet molecules to reach the declared sugar content (Dasenaki and Thomaidis, 2019). The most common types of juice fraud that are currently subject to legal sanctions include the addition of sugar and pulp wash solution without indicating this on product packaging (mislabeling), the addition of water, blending of juices, addition of colorants, organic acids, amino acids, and substitution with another juice that has a lower commercial value (Shokri et al., 2024). Fruit juices, and especially apple and orange juices, are among the most targeted items for fraud and adulteration (Dasenaki and Thomaidis, 2019; Shokri et al., 2024; Xu et al., 2021).

Since sugars show less susceptibility to changes during processing and storage compared to the other components present in food and beverages such as antioxidants, the sugar composition of fruit juices can be used as a reliable quality control marker for authenticity and adulteration control (BeMiller, 2017; Jalaludin and Kim, 2021). Considering that sugar analysis is standard practice in the food industry for products quality assurance and it is essential for proper labeling, suitable analytical methods are of an utmost importance. The sugar composition and content in fruit juices, as well as different food products can be successfully analyzed only if a sensitive and reliable analytical method is used (Magwaza and Opara, 2015; Shanmugavelan et al., 2013).

In this review, we will discuss the analytical methods used for determination of sugars in fruit juices. Besides the classical chemical methods as well as the commonly applied chromatographic techniques with various detectors, the modern non-destructive spectroscopic techniques used for sugar determination and food adulteration identification as modern alternative are also discussed.

Sample preparation for sugar analysis

One of the most critical parameters in the detection of sugars is the quantitative extraction due to low sugar content in natural juices, complex matrix, disproportion in content, large amount of isomer and chiral compounds. Because of the complexity of the fruit juice matrix, sugars, are often coexistent with many other substances, such as polyphenols, organic acids, etc. Since extraction process involves several factors, including material ratio, extraction solvent, extraction time and extraction temperature, an efficient approach is highly required. In the past, the extraction procedures included warm water to extract the soluble sugars followed by subsequent filtration of the sample. However, this was insufficient to remove other component interfering with the determination. Water extracts of many foods contain substances that are colored or produce turbidity, and thus interfere with spectroscopic analysis or endpoint determinations. For this reason, solutions are usually clarified prior to analysis. The most commonly used clarifying agents are heavy metal salts (such as lead acetate) which form insoluble complexes with interfering substances that can be removed by filtration or centrifugation. However, it is important that the clarifying agent does not precipitate any of the carbohydrates from solution as this would cause an underestimation of the carbohydrate content. Also, many monosaccharides and oligosaccharides are polar non-charged molecules and can therefore be separated from charged molecules by passing samples through ion-exchange columns. By using a combination of a positively and a negatively charged column it is possible to remove most charged contaminants. Non-polar molecules can be removed by passing a solution through a column with a non-polar stationary phase. Thus proteins, amino acids, organic acids, minerals and hydrophobic compounds can be separated from the sugars and sugar-like compounds prior to analysis (McClements, 2003).

Nowadays, more sophisticated approaches are applied, such as ultrasound assisted extraction (UAE). This technique has been successfully used, due to its advantages including lower temperature requirements, less use of solvents, improving the diffusion, solubility and extraction efficiency of the targeted compounds (Li et al., 2023; Abid et al., 2014; Sarvin et al., 2018).

Classical methods for sugar analysis

In classical methods based on sugar reducing properties, the amount of reagent reduced by the monosaccharides could be determined using spectrophotometry (e.g., dinitrosalicylic acid and Somogyi-Nelson methods (Başkan et al., 2016; Hossain et al., 2015; Shao et al., 2018), gravimetry (e.g., Munson-Walker method) (Silva et al., 2003) and titration (e.g., Lane-Eynon and Luff-Schoorl methods) (Kedokt et al., 2009; Komes et al., 2011; Lane and Eynon, 1923; Taufik and Guntarti, 2016). Despite the features as low complexity and the use of low-cost apparatus, these methods require excessive handling and a long time for analysis, impairing their use for continuum monitoring during processing. In addition, high amounts of reagents are consumed, and residue is generated (Sandra et al., 2022). Among the most commonly applied classical methods for determination of sugar content in fruit juices are the destructive chemical methods based on the reducing properties of monosaccharides. If the sample contains disaccharides or

other complex sugars, a hydrolysis of the sample is required. Destructive chemical, enzymatic, and instrumental techniques have been utilized in the analysis of sugars in foods. As an example of chemical analysis, the Luff−Schoorl method was used to determine the total content of reducing sugars by treating with excess CuSO4 in alkaline boiling solution, followed by the iodometric titration of the remaining Cu(II) ions. Methods such as Luff−Schoorl show high accuracy in sugar analysis but it cannot distinguish individual sugars. In contrast, enzymatic methods for sugar analysis are specific. The concentration of a specific sugar (e.g. glucose) is measured by stoichiometry of the reaction catalyzed a particular enzyme (e.g. glucose oxidase), and the change in the accompanied coenzyme system (e.g. NADH/NAD+) is often evaluated by photometric methods (e.g. the change of UV absorption at 340 nm for NADH). Calibration with standards is required for enzymatic assays because the enzyme activity may vary with reaction conditions (e.g. pH, temperature, and solvent) (Chen et al., 2017). The comparison of methods based on the reducing properties of monosaccharides are presented in Table 1.

Sometimes, the quantity of the glucose, fructose and sucrose is determined refractometrically with an Abbe refractometer, by which, through the refractive index, the percentage of dry matter is determined, or the degree of Brix. This term has been known in the food industry for years and represents the dry matter in solutions that mainly contain sucrose (1 Brix = 1g sugar/100mL solution) (Dongare et al., 2014; Echeverria and Ismail, 1978; Wardowsky et al., 1979). Numerous analytical approaches have been routinely used for the analysis of sugar compounds in food and supplement products. Each method has its advantages, analytical challenges and disadvantages depending on factors including matrix complexity, depth of information required, and the cost and complexity of the analysis. As instrument technologies have advanced, laboratories have striven to utilize the newest platforms for sugar testing to ensure quality control and to recognize potential adulterants (Vennard et al., 2020; Yaganeh-Zare et al., 2022).

Chromatographic techniques for sugar analysis

Amongst the modern techniques of separating naturally occurring compounds, HPLC has been widely accepted to be the most effective and easily available method for sugar analysis (Ma et al., 2014). As a result, numerous procedures have been described using various chromatographic columns and detectors. Extensive literature research into various HPLC methods for sugar analysis are available in several review articles (Raessler, 2011; Stefansson and Westerlund, 1996). These sources show that HPLC has been extensively used for the determination of sugar compounds in foodstuffs.

The compounds such as sugars do not have UV chromophores in their structure, so other types of detectors such as refractive index detector (RID) and evaporative light scattering detector (ELSD) are typically used. The most extensively used detector in sugar analysis in food samples is RID. This detector works as a differential refractometer that measures changes in the deflection of a light beam due to the difference in the index of refraction of the eluent induced by the solute, exploiting the improbability that solvents and solutes would share the identical refractive index (Raessler, 2011). The drawback of RID is that its signal is highly dependent on wavelength and density of the solute. Furthermore, RID is very sensitive to eluent composition and precludes gradient elution because of baseline drift when using gradient. However, RID is not as sensitive as ELSD (Cools and Terry, 2012). Apart from its sensitivity, the wide acceptance of ELSD is based on the fact that it does not suffer from limitations such as sample composition, flow rate and temperature of the column compartment. Therefore, baseline drift caused by mobile phase and temperature effects can be avoided (Ma et al., 2014). Unlike RID, ELSD does not rely on optical properties of the analytes, but nebulizes the sample and evaporates the mobile phase. Due to their size, sugar molecules have good light scattering properties, which form the basis of ELSD. The amount of scattered light as a result of dried analyte particles in the nebulized sample is then detected and presented as a peak (Cools and Terry, 2012). As an additional advantage, ELSD can detect most compounds less volatile than the mobile phase (Ma et al., 2014; Nogueira et al., 2005). Therefore, the use of this detector type represents a practical solution for sugar analysis in real samples including various sugars and sugar-like compounds (sucrose, fructose, glucose, mannitol, sorbitol, ketose, xylose, and fructans) (Downes and Terry, 2010).

Table 1. Characteristics of methods for sugar analysis based on reducing properties of monosaccharides

Method	**Reaction type**	**Titrant**	**Application**
Luff-Schoorl	Iodometric (back titration)	$Na_2S_2O_3$	Food analysis
Bertrand's	Redox ($KMnO_4$ titration)	$KMnO_4$	Food & industrial analysis*
Benedict's	Iodometric	$Na_2S_2O_3$	Clinical & food analyses
Munson & Walker	Gravimetric + Titration	$Na_2S_2O_3$	Industrial analysis*

*A control process used in the sugar industry at continuous and semi-batch level.

Table 2. Examples of the application of chromatographic and spectroscopic techniques in the analysis of sugars in fruit juices

Analytical technique/ detector type	Reference	Sample type
	Chromatography	
HPLC-RID	Zielinski et al., 2014	Apple juice
HPLC-RID	Rokai and Sana, 2020	Apple juice, apple pulped juice, carrot juice, cabbage juice
HPLC-RID	Tyagi et al., 2014	Tropicana apple, pineapple, Appy classic apple, Frooti mango, Real guava, litchi, pineapple, mixed fruit, mango, mausami, orange, Onjus mixed fruit, apple juices
HPLC-ELSD	Ma et al., 2014	Peach, watermelon, apple and cherry
HPLC-RID	Kelebek et al., 2009	Orange juice
GC-MS	Cerdán-Calero et al., 2012	Orange juice
HPAE/PAD	Zhang and Ritenour, 2016	Commercially citrus fruit juices
HILIC-MS	Stander et al., 2013	South African fruit juices
LC-MS/MS	Thacker and Shug, 2020	Apple juice
LC-MS/MS	Thacker and Shug, 2020	Apple juice
GC and GC-MS	Sanz et al., 2004	Different fresh juices
	Spectroscopy	
FT-NIR spectroscopy	Rodriguez-Saona et al., 2001	Orange juice
NIR	Xie et al., 2009	Bayberry juice
Handheld short-wavelength NIR spectroscopy	Chen et al., 2021	Fresh apple, banana, cucumber, celery, orange and melon mixtures
NIR	Wlodarska et al., 2021	Apple juice
FTIR-ATR	Nair et al., 2016	Mango, pineapple and grapes juice
FTIR	Ellis et al., 2016	Freshly prepared orange juice
FTIR	Yajima et al., 2024	Citrus fruit juices
FTIR	Duarte et al., 2002	Mango juices
Raman spectroscopy	Camerlingo et al., 2007	Clarified apple and apricot juices
Raman spectroscopy	González-Viveros et al., 2021	Watery sugar solutions
Raman spectroscopy	Gao et al., 2023	Pomegranate juice

Pulsed amperometric detector (PAD) is another commonly used detector in sugar analysis, particullarly fructans in fruit and vegetable extracts. PAD is considered as the most highly sensitive and reliable detection method for all compounds whose chemical structure is similar to sugars. Because of affinity between ionized groups of sugars at alkaline pH and a pellicular quaternary amine stationary phase, PAD has the advantage over ELSD as a high resolution, highly selective and very sensitive technique for determining both simple and complex sugars in different samples (LaCourse, 2002)). Because PAD requires alkaline conditions, it is often coupled with anion exchange chromatography (AEC) which also requires high alkalinity. High-performance anion-exchange chromatography with PAD is suitable to analyze original carbohydrates without derivatization, however the instrument is expensive and the decay of the column and electrode is a concern in the system with strong alkaline elution (Zook and LaCourse, 1995).

Ligand-exchange chromatography (LEC) based on cation exchangers in metal ion forms has also been extensively used in the separation and quantification of sugars. One of the main advantages of LEC is that it offers reasonable resolution of most sugar analytes. However, this

advantage is overshadowed by the fact that analyses are time-consuming. Other disadvantages of the system are its lack of sensitivity depending on temperature and flow rate and its incompatibility with gradient elution when monitored by RID (Ma et al., 2014). In addition to these disadvantages, the combination of LEC and RID escalates these problems because RID is also susceptible to interference from co-eluting sample components (Ma et al., 2014; Magwaza and Opara, 2015).

Another chromatography mode which has been used in the analysis of sugars is hydrophilic interaction liquid chromatography (HILIC). Over the past two decades HILIC has gained in popularity for the separation and analysis of many compounds in foods, including organic nutrients (carbohydrates, phospholipids, amino acids, peptides, and proteins), contaminants, and toxic compounds (Marrubini et al., 2018). HILIC coupled to MS has gained popularity in the analysis of complex mixtures of monosaccharides, disaccharides, and oligosaccharides in liquid samples such as wines, beers, milks, fruit juices or plant extracts (Kotoni et al., 2014; Liu et al., 2014). Despite obvious advantages, HILIC-MS is an expensive technique which is not readily available for routine laboratory use.

Spectroscopic techniques for sugar analysis

Infrared spectroscopy (IR) is one of the most often used spectroscopic tools for the analysis of numerous molecules. It has gained a lot of attention in the past years, especially because of its easy handling and simple sample preparation before analysis. IR analysis is rapid and sensitive, with a great variety of sampling techniques, and yet the instrumentation can still be considered inexpensive (Dasenaki and Thomaidis, 2019; de Oliveira et al., 2013). The infrared absorption spectrum of a compound is probably its most unique physical property and the spectrum is often called the fingerprint of a molecule. Although IR was first used as an identification tool for relatively pure compounds, new spectroscopic techniques have allowed more detailed structural analysis of pure molecules, analysis of polymer mixtures and crude samples, and even the investigation of interactions of particular macromolecules (Chen et al., 2021; Coelho et al., 2020; Ramasami et al., 2004). Additionally, spectroscopic techniques produce large data sets which are handled and interpreted by chemometric algorithms for multivariate data analysis (Cozzolino et al., 2011; Dhaulaniya et al., 2024).

Mid-infrared spectroscopy (MIR) has revolutionized the approach to food integrity analysis as a non-destructive and non-invasive technique (Kačuráková and Wilson, 2001). In terms of instrumentation, it can be found in desktop or portable forms and it is quick, highly sensitive and robust technique, providing a comprehensive array of spectral data that encode the molecular structure of the food sample. MIR can rapidly determine the molecular fingerprint of foods from various industry sectors, including dairy, meat, vegetable oil, wine, and beverages with applications ranging from compositional to authenticity analysis. Moreover, combining MIR with multivariate chemometric data analysis has been successfully applied for prediction of the chemical composition of various food products including fruit juices, such as mango juice and apple juice (Duarte et al., 2002; Leopold, 2010; Vlaic et al., 2018).

One of the primary limitations of FT-MIR spectroscopy is its sensitivity to water. Since water has strong absorption in the mid-infrared region, samples with high water content can pose challenges in obtaining accurate spectra. Another limitation is the complexity of the spectra obtained from FT-MIR spectroscopy. If the sample has a complex composition, the spectra often contain overlapping absorption bands, which can make it difficult to identify specific compounds or contaminants without the use of complex data analysis techniques (Smith, 2011). Fourier transform infrared spectroscopy (FTIR) is a well-established technique that is widely employed analysis of different components in food samples (Beullens et al., 2006; Jha and Gunasekaran, 2010; Vermeir et al., 2009). It is non-destructive, requires minimal amounts of sample and little or no sample preparation, provides good reproducibility, and enables rapid analyses at high spectral resolution (Griffiths and De Haseth, 2007). FTIR with attenuated total reflection (FTIR-ATR) combined with principal component analysis (PCA) has been employed for the classifications of beers according to sugar type dominantly present in the investigated sample (Duarte et al., 2004). Fifty different beers were differentiated according to dextrine, maltose and glucose content. Grassi et al. (2014) using FTIR monitored fermentable sugars and ethanol during six different fermentation processes, with differentiation based on the compounds maltose, maltotriose, and ethanol. The use of ATR-FTIR measurements ameliorates the use of mid-infrared region (MIR) by avoiding common problems experienced in transmission cells like filling, cleaning, and variation of the path length due to window wear (Almeida et al., 2018).

Raman spectroscopy, a branch of vibrational spectroscopy, has risen in importance as a powerful tool for investigating the structure of mono- and disaccharides and is increasingly used in biology (Das and Agraval, 2011; Ilaslan et al., 2015). Water has a minor scattering effect and does not interfere with the Raman scattering from solutes in aqueous solutions such as fruit juices or other soft drinks (Cammerlingo et al., 2007, 2018; Ilaslan et al, 2015). Some examples of chromatographic and spectroscopic techniques for sugar analysis in fruit juices are presented in Table 2. The available data on application of the spectroscopic techniques in the analysis of sugars in fruit juices and other food samples are scarce in literature. However, they are promising due to their simplicity, speed, cost, and because they are friendly for the environment.

Conclusion

During the past ten years, considerable research has been made for implementing advanced analytical methodologies for identification and quantification of sugars in fruit juices. According to the available data discussed in our review, there is a substantial progress in analytical instrumentation development and data analysis techniques. In terms of chromatographic separation, HILIC potential is under intensive study due to the possibility to provide chromatographic fingerprints of polar fruit juices components. Also, spectroscopic techniques have attracted serious attention to be implemented by industry and inspection agencies as food fingerprinting methodologies to ensure fruit juices authenticity and overall quality.

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Резиме

Методи за определување на содржина на шеќери во овошни сокови: од класични методи до модерни аналитички техники

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Клучни зборови: шеќери, овошни сокови, класични техники, хроматографија, спектроскопија

Идентификацијата и квантитативното определување на шеќерите во овошни сокови е особено важно при контролата на квалитет на овие производи. Во овој прегледен труд, се претствени најзначајните аналитички техники за определување на содржината на шеќери во овошни сокови. Обработени се класичните методи како што се методите по Feling и Luft-Schorl за определување на редуцирачки моносахариди, рефрактометриските и ензимските техники. Од посовремените техники опишана е примената на хроматографијата со различни типови детектори што се од избор при анализата на шеќери во овошни сокови. Иако хроматографијата се одликува со голема точност и осетливост, подготовката на примерокот бара многу време и голема количина органски растворувачи за екстракција. Спектроскопските техники (MIR, FTIR-ATR, Raman), чија употреба е во подем заради нивната едноставност, брзина и недеструктивност, се исто така анализирани од аспект на нивна идна примена во анализата на шеќери. Сепак, се наметнува потреба од соодветна калибрација и валидација на овие современи техники.