

## **ADVANCED ANALYSIS OF CARBOHYDRATES IN FOODS.**

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In this chapter, modern analytical procedures used to study carbohydrates in foods and beverages are discussed. The main advanced analytical methodologies applied to determine the different carbohydrate families (monosaccharides, oligosaccharides, polysaccharides and macromolecules including different glycosylated compounds) are reviewed considering the sample preparation required and the type of technique (separation or spectroscopic) used, including their different couplings, multidimensional- approaches, modern glycomics strategies, etc. The goal of this contribution is, therefore, to provide the reader with a broad and critical view on the different analytical methods nowadays employed to analyze carbohydrates in foods and their foreseeable trends.

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## 1. Introduction.

Carbohydrates are the most abundant and diverse class of organic compounds occurring in nature. Chemically they are composed of carbon, hydrogen, and oxygen in the ratio  $C_n : H_{2n} : O_n$ . Food carbohydrates include a wide range of macromolecules that can be classified according to their chemical structure into three major groups: low molecular weight mono- and disaccharides, intermediate molecular weight oligosaccharides and high molecular weight polysaccharides. They can also be classified as simple or complex carbohydrates. Simple carbohydrates are monosaccharides and disaccharides while complex carbohydrates are made up of many monosaccharides such as starches and fiber (polysaccharides).

Carbohydrates are a major source of energy in the human diet with intakes ranging from 40 to 80% of total energy requirements (Muir et al. 2009). Carbohydrates constitute the main source of energy for all body functions, particularly brain functions, and are necessary for the metabolism of other nutrients. Other important effects of carbohydrates on human physiology are satiety and gastric emptying, control of blood glucose, insulin metabolism and serum cholesterol, and influencing colonic microflora and gastrointestinal processes such laxation and fermentation (Muir et al. 2009).

Cereals, vegetables, fruits, rice, potatoes, legumes, and flour products are the major sources of carbohydrates. Thus, naturally occurring sugars are consumed as part of a healthy diet. Monosaccharides, sucrose, and polysaccharides are present in all vegetables (Hounsome et al. 2008). Carbohydrates are synthesized by all green plants and in the body are either absorbed immediately or stored in the form of glycogen. They can also be manufactured in the body from some amino acids and the glycerol component of fats. Moreover, sugars can be added to foods during processing or preparation, mainly to enhance food sensorial quality (Murphy et al. 2003).

Nutritionists divide food carbohydrates into two classes: **a**, available or readily utilized and metabolized, including mono-, di-, or polysaccharides like glucose, fructose, lactose, dextrins and starch, and **b**, unavailable or carbohydrates not directly utilized but instead broken down by symbiotic bacteria yielding fatty acids, and thus not supplying the host with carbohydrates. The second class includes structural polysaccharides of plant cell walls and many complex polysaccharides like cellulose, pectins and  $\beta$ -glucans (Cui 2005). In other words, available carbohydrates are those that are hydrolyzed by enzymes of the human gastrointestinal system while the unavailable ones are not hydrolyzed by endogenous human enzymes (sugar alcohols, many oligosaccharides, and nonstarch polysaccharides) but they can be fermented by microorganisms in the large intestine to varying extents and then absorbed (Hounsome et al. 2008).

Sugars (glucose, sucrose, fructose, lactose and maltose), sugar polyols (sorbitol and mannitol), oligosaccharides (GOS, galactooligosaccharides and FOS, fructooligosaccharides) and polysaccharides (starch and non starch polysaccharides) have been described as the major classes of carbohydrates relevant for human nutrition. The fermentable-short-chain-carbohydrates-like oligo-, di- and monosaccharides and polyols that can be poorly absorbed by the small intestine are called FODMAPs and they provide different effects on gastrointestinal health. FODMAPs can be found in a wide variety of foods (Muir et al. 2009). Detailed information related to the chemistry of food carbohydrates can be found elsewhere (Cui 2005). In the next subsections, only key characteristic of the food carbohydrates relevant for human nutrition are provided together with the importance of developing powerful and informative techniques for their adequate analysis.

### 1.1. Simple carbohydrates: Monosaccharide and disaccharides.

Monosaccharides require no digestion and can be absorbed directly into the blood stream. All the monosaccharides can be synthesised by the body (Hounsome et al. 2008). The basic carbohydrates are the monosaccharide sugars, of which glucose, fructose, and galactose are the most important nutritionally. Fructose structure is shown in Figure 1. Fructose, a major component of fruits, fruit juices, honey and corn syrup (Park et al. 1993), is one of the principal FODMAPs of the Western diet (Gibson et al. 2007).

Disaccharides are composed of two monosaccharides. Nutritionally the important disaccharides are sucrose, a dimer of glucose and fructose; lactose, dimer of glucose and galactose; and maltose, a dimer of two glucose units. Lactose has also been classified as FODMAP (Muir et al. 2009).

INSERT FIGURE 1 HERE.

## 1.2. Sugar polyols.

Sugar alcohols, also called sugar polyols, are obtained by reduction of the corresponding aldoses and ketoses. Sugar polyols have generated much interest as food additives since they can be employed as low calorie sweeteners. Figure 2 shows mannitol and sorbitol structures, two sweeteners very frequently used in the food industry. Although few data on levels of polyols in foods have been published so far, it is known that mannitol is present in several vegetables such as celery, carrot, parsley, pumpkin, onion, endive and asparagus (Hounsome et al. 2008) while sorbitol is present in many fruits but it is also used as sweetener. Some authors have suggested their use as indicators of the authenticity or adulteration of foods (Martínez-Montero et al. 2004; Ruiz-Matute et al. 2007).

INSERT FIGURE 2 HERE

### 1.3. Oligosaccharides.

Numerous oligosaccharides, consisting of 3–5 monosaccharide units, occur in foods and they are in general non digestible. Oligosaccharides can also be defined as those carbohydrates formed by 2 to 10 basic sugar molecules. Fructans and fructooligosaccharides (FOS, as e.g., nystose, kestose, see Figure 3) and galactooligosaccharides (GOS, raffinose, stachyose) are considered FODMAPs and prebiotic carbohydrates (Miur et al. 2009). Fructooligosaccharides and non starch polysaccharides play an important role are dietary fiber (Hounsome et al. 2008).

INSERT FIGURE 3 HERE

Garlic, Jerusalem artichokes and onions contain high levels of fructans (Muir et al. 2007). The Jerusalem artichoke has been found to have the highest concentration of FOS in cultured plants. FOS have been used in dietary supplements in Japan since the 90's and they are now becoming increasingly popular in Western cultures for their prebiotic effects. FOS serves as a substrate for microflora in the large intestine, increasing the overall gastrointestinal tract health. Moreover, several studies have found that FOS and the polysaccharide inulin promote calcium absorption in both animal and human gut. The intestinal microflora in the lower gut can ferment FOS, resulting in the production of gases and in the reduction of pH that increases calcium solubility and bioavailability.

#### 1.4. Polysaccharides.

Glycans is a general term given to polysaccharides in which large numbers of monosaccharides are naturally joined by *O*-glycosidic linkages. Polysaccharides are condensation polymers in which glycosidic linkage is formed from the glycosyl moiety of hemiacetal, or hemiketal, and a hydroxyl group of another sugar unit, acting as an acceptor molecule or aglycone (see Figure 4). Polysaccharides may be linear or branched (see Figure 4). There are two types of polysaccharides: homo-polysaccharides and hetero-polysaccharides. A homo-polysaccharide has only one type of monosaccharide repeating in the chain; whereas, a hetero-polysaccharide is composed of two or more types of monosaccharides. Unbranched polysaccharides contain only alpha 1,4 linkages while some branched polysaccharides are linked to a molecule via alpha 1,4 and to another one via alpha 1,6 glycosidic bonds. These macromolecules have large physiological interest and they affect both food quality and nutrition.

INSERT HERE FIGURE 4.

#### 1.5. Conjugated carbohydrates.

Carbohydrates can also be attached to other different compounds as proteins, lipids, phenols. Thus, glycans covalently bound to these compounds (glycoproteins, glycolipids, glycophenols) play a pivotal role on their bioactivity and they are, for instance, involved in cell signaling or bioavailability. Glycation and deglycosylation of proteins, for instance, have

been proposed as strategies for modulating the immunoreactivity of key food allergens (van de Lagemant et al. 2007; Amigo-Benavent et al 2009) or the way to provide new technological attractive properties to proteins (Corzo-Martinez et al. 2010).

The development of new analytical approaches for separation, identification and quantification of carbohydrates is of huge importance in many research fields. Thus, knowledge on the quality and quantity of carbohydrates in fruits, vegetables, cereals and other natural food matrices is essential to determine food properties and other important characteristics like flavour, maturity, quality, authenticity, storage conditions, etc. A better knowledge on carbohydrates composition is mandatory in the new functional foods era, not only to produce healthier foods but also to be able to assess and understand their role in several functions in the human body.

## **2. Sample Preparation for Carbohydrates Analysis.**

A critical step when analyzing carbohydrates in food complex matrices is their separation from the rest of the main components, such as lipids and proteins, that can somehow interfere in their precise determination and quantification. Once isolated, carbohydrates can be either analyzed directly or subjected to some other additional treatments (such as hydrolysis and/or derivatization) to favour their subsequent analysis.

### **2.1. Extraction and fractionation.**

The separation of carbohydrates from other food components is generally carried out using some kind of extraction or clean-up step prior to their analysis. Nevertheless, in some cases

the sample obtained is still too complex to be easily analyzed and a further fractionation step is necessary to obtain a particular carbohydrate fraction. In this section, the methods used for extraction and fractionation of carbohydrates are described.

### *2.1.1. Filtration.*

Usually, when studying the carbohydrate fraction of a beverage, a simple filtration and dilution step could be enough as sample preparation. For instance, this has been the case of wines (La Torre et al. 2008), milk and whey (Mullin et al. 1997). Even ultrafiltration and filtration based on the use of membrane technologies have been employed to concentrate carbohydrates (Kamada et al. 2002). Nevertheless, when the original matrix is too complex or the amount of different class of carbohydrates is very different, more sophisticated sample preparation procedures are needed. It is interesting to mention that a filtration step can be employed as a clean-up step before using any of the procedures explained below.

### *2.1.2. Traditional extraction methods (LLE, SPE).*

Nowadays, the so-called traditional extraction techniques, such as liquid-liquid extraction (LLE) and solid phase extraction (SPE), have been widely applied to the extraction of carbohydrates from food matrices prior analysis. SPE has been used to extract carbohydrates from wine (Castellari et al. 2000), beer (Lehtonen et al. 1994) or honey (Weston et al. 1999), among others. The nature of the solid column packing as well as the solvent employed for elution is selected according to the nature of the sample to be analyzed. C<sub>18</sub> cartridges are probably the most used (Megherbi et al. 2008), although others such as C<sub>8</sub> (Kitahara et al. 2004), porous graphitic carbon (Arias et al. 2003), or even cartridges based on strong cation exchange (Castellari et al. 2000) are also employed. SPE has shown its capability in fractionating complex samples; for instance, active carbon and celite columns were used to

fractionate monosaccharides and oligosaccharides from honeys using different ethanol/water ratios to obtain a selective elution (Weston et al. 1999). Although the results that can be obtained using these techniques might be satisfactory, some important shortcomings inherent to their experimental principles, mainly related to the great amounts of organic solvents needed, have brought about an increase in the use of other extraction techniques.

### *2.1.3. Supercritical fluid extraction (SFE).*

SFE is based on the use of fluids above their critical pressure and temperature. Under these conditions, supercritical fluids acquire properties between liquids (similar solubility) and gases (similar diffusivity). CO<sub>2</sub> is the most commonly used fluid, thanks to its moderate critical temperature and pressure, along with other important advantages such as the easiness to modify the selectivity of the extraction by tuning the operating pressure, the possibility to obtain fractions by carrying out different depressurization steps and the attainment of an extract free of solvent, since CO<sub>2</sub> is released as a gas when pressure is decreased under ambient conditions. However, its use in carbohydrate analysis has been partly hampered by its low polarity, which makes difficult the solubilisation of these compounds. In order to overcome this problem different amounts of organic solvents employed as modifiers are used. SFE has been employed several times to fractionate different carbohydrates that could be used in the food industry as prebiotics (Montañés et al. 2007; Montañés et al. 2006). In these works, the authors demonstrated that the selectivity was mainly influenced by the co-solvent composition while other parameters such as temperature, pressure, and amount of polar co-solvent had an effect on carbohydrates recovery. It is expected that other applications of this technique to the extraction and fractionation of carbohydrates can be developed following similar methodology.

#### *2.1.4. Pressurized Liquid Extraction (PLE).*

PLE is based on the use of solvents at high temperatures and pressures enough to maintain their liquid state during the whole extraction process. As a result of these experimental conditions, the extraction processes are faster, produce higher yields and consume fewer amounts of solvents compared to the more traditional extraction techniques. Due to these advantages, PLE is being widely employed to extract sample components from food and natural products (Mendiola et al. 2007). Different solvents including water, organic solvents and their mixtures can be used. As for carbohydrates fractionation, few works have been published dealing with the selective extraction of lactulose from its mixtures with lactose using a 70:30 ethanol/water mixture at 40°C for 30 minutes (Ruiz-Matute et al. 2007). Also, enriched fractions of di- and trisaccharides were obtained from honey by using PLE combined with the use of activated charcoal (Ruiz-Matute et al. 2008). In this case, honey was absorbed onto activated charcoal and packed into a PLE extraction cell that was submitted to two different extraction cycles. The first one using 1:99 ethanol/water for 5 minutes, and a second using 50:50 ethanol/water for 10 minutes. This PLE procedure can easily be adapted for carbohydrates fractionation from other sources.

#### *2.1.5. Field Flow Fractionation (FFF).*

This technique is based on the separation of sample components being pumped by a laminar flow inside an open channel in which a perpendicular (electric, thermal, magnetic or gravitational) field is applied. The separation or fractionation takes place according to the differences on the mobility of the components as a result of the applied field. For carbohydrates analysis, this technique is often employed to fractionate large polysaccharides that could not be otherwise fractionated, mainly cellulose, starch, pullulan, among others.

#### *2.1.6. Chromatography-based methods.*

Different techniques based on the use of chromatographic procedures have been employed to fractionate and separate carbohydrates. Thus, size exclusion chromatography (SEC) is one of the most popular techniques for determination of molecular mass distributions of (relatively large) carbohydrates (Eremeeva 2003). Due to these characteristics, SEC has been widely employed to fractionate and separate oligo- and polysaccharides, including the analysis of prebiotic galactosaccharides (Hernandez et al. 2009), the analysis of carbohydrates in rice (Ward et al. 2006), sorghum (Aboubacar et al. 2000), wheat (Hartmann et al. 2005) or coffee (Arya et al. 2007).

Another chromatography-based technique broadly applied to fractionate carbohydrates is Ion Exchange Chromatography (IEC). IEC is mostly suited for oligo- and monosaccharides fractionation. Both anion and cation exchange resins can be used depending on the particular application. The normal approach is to use IEC as analytical step rather than for fractionation and, therefore, IEC will be discussed in more detail below in section 3.3.

The last technique included in this group is simulated moving bed chromatography (SMBC). This technique has been widely employed in the sugar industry for large-scale palatinose-trehaulose fractionation. Two streams of products (raffinate and extract) are obtained from the continuous counter-current separation of a sample, eluted from a series of different adsorbents. These adsorbents can be based on ligand-exchange chromatography or on size-exclusion chromatography. This technique has also been employed to fractionate lactose from a complex mixture of human milk oligosaccharides (Geisser et al. 2005).

#### *2.1.7. Membranes and other methods.*

The use of membranes is not new in carbohydrates sample preparation. In fact, the fractionation of sugars contained in sugar-cane has been carried out by dialysis, while the use of membranes combined with ultrafiltration and nanofiltration systems have also allowed the

separation and fractionation of carbohydrates according to their molecular mass (Godshall et al. 2001).

Another technique worth to mention is carbon fractionation. This technique has been extensively used for many years in official methods and it consists in the adsorption of the carbohydrates in an activated charcoal column and a later elution with different proportions of ethanol (1% for monosaccharides, 5% for disaccharides and 50% for oligosaccharides (AOAC Official method of analysis n954.11) to fractionate them selectively according to their degree of polymerization. The fractionation with active charcoal has been shown to be particularly useful to obtain monosaccharides from very complex food samples (Morales et al. 2006). This technique may also be used in combination with other sample preparation processes or fractionation steps (e.g., PLE) in order to allow a more convenient characterization of carbohydrates in a subsequent analytical phase (Ruiz-Matute et al. 2008; Wei et al. 1997).

Other methods employed in carbohydrates sample preparation include the use of molecularly imprinted polymers (MIPs). MIPs have been proven to be useful for the recognition of different saccharides or for the epimeric differentiation of disaccharides. Although the potential of this technology is remarkable, its use in food science is still not very extended due to the difficulty of obtaining selective and robust MIPs in a reproducible way.

## 2.2. Chemical treatments.

Different chemical procedures are commonly employed in the sample preparation step of food carbohydrates in order to facilitate their subsequent analysis.

### 2.2.1 Hydrolysis

Frequently, prior to a chromatographic analysis, a hydrolysis step is carried out of large carbohydrates to obtain fractions of low molecular mono- or oligosaccharides that can be analyzed more easily. Under heating and in the presence of a strong acid, the glycosidic bonds joining the different monosaccharides forming a polysaccharide can be cleaved. The most commonly employed acids are trifluoroacetic acid, hydrochloric acid or even sulphuric acid. The treatment conditions, mainly acid concentration, temperature and time, have to be optimized to achieve a total hydrolysis of the polysaccharides contained in the sample. At the same time, these conditions should not be too strong because they can produce the sample degradation, since it is well known that during this procedure, the released monosaccharides are prone to degradation. As an example, typical conditions applied to process wheat flour include the use of 2 M HCl at 100°C for 90 min. Polysaccharides can be also submitted to partial hydrolysis before analysis. Thanks to the differential susceptibility to hydrolysis of the different glycosidic linkages, partial degradations can be obtained at controlled conditions in order to produce mixtures of different mono- and oligosaccharides whose analysis could be useful to determine the polysaccharide structure. Recently, microwave irradiation has been employed as a tool to reduce the process time to hydrolyzate carbohydrates (Corsaro et al. 2004).

### *2.2.2 Derivatization*

Given their intrinsic properties, carbohydrates can be difficult to analyze by different separation techniques due to their low volatility, lack of chromophore or very similar polarity. Thus, if analyzed by GC, carbohydrates have to be derivatized so that their volatility is increased. Monosaccharides can be analyzed by GC as alditol acetates obtained by reduction and acetylation, while oligosaccharides can be analyzed by GC as trimethylsilyl or trifluoroacetyl ethers.

If CE or HPLC are the techniques of choice, care must be taken about the sensitivity required, since the vast majority of intact carbohydrates can only absorb UV light below 195 nm, or can be detected using amperometric or refractive index detectors. Therefore, to increase the sensitivity of their detection, UV-absorbing or fluorescent labels are employed in different derivatization processes. The reaction may take place before or during the analysis, being the more common procedure pre-column derivatization (Lamari et al. 2003; Molnar-Perl 2000). The most employed approach implies the use of reductive amination. These procedures are based on the condensation of a carbonyl group with primary amines to produce a Schiff base that is later on reduced to give a N-substituted glycosil amine. 2-Aminopyridine or trisulfonates are among the reagents more commonly employed. Besides, considering that most carbohydrates are neutral and hydrophilic compounds, derivatization produces not only a sensitivity enhancement, but also polarity and electrical charge variations that can make easier their separation by CE or HPLC. Extensive reviews about this topic can be found in the literature (Lamari et al. 2003; Molnar-Perl 2000; Suzuki et al. 2003).

### **3. Advanced Analysis of Carbohydrates in Food.**

Spectroscopic techniques, separation techniques and their multiple combinations are the main tools used to analyze carbohydrates in foods, although some other approaches based on the use of sensors or the new glycomics platforms are also of importance in this field. The selection of the papers cited in this section has been performed based on their novelty and relevance, trying to provide an updated overview to the reader on food carbohydrate analysis.

#### **3.1. Spectroscopic techniques.**

For years, spectroscopic techniques have been employed for qualitative and quantitative analysis of foods and food ingredients, using mainly ultraviolet (UV), visible (Vis), fluorescence, infrared (IR), raman, atomic absorption, atomic emission, electron spin resonance (ESR), nuclear magnetic resonance (NMR) and mass spectrometry (MS) (Ibáñez et al. 2003).

Nowadays, there is a clear trend to use high resolution NMR and MS instruments for the development of different foodomics approaches (Cifuentes 2009) including metabolomics. Metabolomics attempts to detect and identify compounds (metabolites) such as carbohydrates, amino acids, vitamins, hormones, flavonoids, phenolics and glucosinolates, which are essential for plant and animal growth, development, stress adaptation and defense and for determining the nutritional and sensorial quality of foods. Therefore, NMR and MS techniques provide impressive possibilities in food carbohydrates analysis and many structural studies involve the application of both NMR and MS for identification and characterization of food components.

### *3.1.1. Nuclear Magnetic Resonance (NMR).*

Since the structure of the carbohydrate will determine its function, the use of NMR is becoming crucial for an accurate characterization of both the newly designed and natural carbohydrates. The application of NMR may provide information related to the structure, purity and safety of carbohydrate samples. NMR has been extensively employed for identification of food carbohydrates and related compounds. This analytical approach has shown its feasibility for the analysis of simple and complex carbohydrates either isolated or incorporated to food matrices (Hounsome et al. 2008). Currently, oligosaccharides naturally composing the food matrices and those newly designed are of great interest because their

numerous applications in the food industry (sweetener, health promoting, etc.). As a consequence of the proved influence of nutrition in health and the demand of food consumers, there is an increasing interest for the search of natural, low cost, bioactive compounds to be used as health promoting food ingredients, also called functional foods. FOS are the most popular oligosaccharides worldwide, however, there is a lack of information related to the properties of non inulin-type fructans. Recently, the application of the  $^1\text{H}$  NMR along with enzymatic hydrolysis allowed identify fructans in milling fractions of two wheat cultivars (Haska et al. 2008). Arabinoxylan, an important indigestible component in cereal grains with beneficial health effects, was found as a main component of the dietary fibre of all fractions. Complementary characterization of the fructans, such as determination of molecular weight, involved the use of other techniques for structural analysis such as high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) and MALDI-TOF-MS (Haska et al. 2008).

Synthetic carbohydrates can also be used in the formulation of specialty foods for people with particular health concerns. The presence of glucose and lactose, for instance, may be risky for diabetics and lactose intolerants. As an example, new FOS prepared from sucrose using fungal fructosyl transferase have been identified using NMR spectroscopy and LC-MS (Mabel et al. 2008). In this work, the detection and quantification of monosaccharides and disaccharides in the FOS preparations to be employed as non-nutrients sweeteners was also undertaken. In addition, the spectral data obtained by application of these two analytical approaches proved the absence in the carbohydrate preparation of any toxic microbial metabolites certifying its safety as non-nutrient sweetener with potential benefits to diabetics (Mabel et al. 2008).

NMR has been also successfully employed for structural characterization of trisaccharides obtained by enzymatic transglycosylation of lactulose. Authors use either 1D ( $^1\text{H}$  and  $^{13}\text{C}$

TOCSY) and 2D (gCOSY, TOCSY, ROESY, gHSQC and gHMBC) NMR techniques. The structure of the two trisaccharides obtained by enzymatic treatment of lactulose was completely elucidated and full proton and carbon assignment were carried out. Based on the structural data derived of NMR analysis, authors proposed the enzymatic transgalactosylation as a new route for prebiotic synthesis (Martínez-Villaguenga et al. 2008).

NMR has been also employed as a tool for searching economically viable sources for mass production of pinitol, a sugar alcohol that possesses hypoglycaemic effect. The study involved the analysis of 115 natural and food materials. Based on the NMR results, authors suggested soy whey and carob pod as economically viable sources of pinitol (Kim et al. 2005).

Characterization of very complex molecules containing carbohydrates like coffee melanoidins has been also undertaken by application of NMR. Melanoidins contribute to the colour and flavour of coffee and are believed to have physiological activity. The application of 2D-NMR technique permitted to obtain novel information supporting the idea that coffee melanoidins are based condensed polyphenols. Data is in opposition to those previously published indicating that they can be formed by low-molecular weight chromophores bound to polysaccharides or proteins (Gniechwitz et al. 2008). It is believed that the spectrometric approaches, and particularly NMR, are very important for the analysis of carbohydrates linked to proteins and other physiological relevant food components.

Other complex carbohydrates like polysaccharides have been identified by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. For instance, major polysaccharides of hazelnut cell walls, have been determined by NMR (Dourado et al. 2003); also, polysaccharides composition of unripe tomato and a medicinal mushroom has been also recently characterized by using different NMR experiments (Chandra et al. 2009).

Various papers related to carbohydrate metabolomic studies in vegetable and animal foods have been also reported (Karakach et al. 2009; Hounsome et al. 2008). It is expected an increase of the number of applications of NMR in the complex food carbohydrates field.

### *3.1.2. Mass Spectrometry (MS).*

MS is a powerful analytical technique used to identify chemical compounds based on their different mass-charge ratio ( $m/z$ ). In this section only the application of direct mass spectrometry analysis to food carbohydrates is described. The use of the mass detectors coupled to different separation techniques will be discussed in following sections.

The chemical compound has to be ionized before entering the MS analyzer; electron impact ionization (EI), chemical ionization (CI), electrospray ionization (ESI), plasma desorption ionization (PDI) and matrix laser desorption ionization (MALDI) are the most frequently used ionization techniques for analysis of organic compounds such as food carbohydrates. High quality reviews providing huge and detailed information related to the state of the art of mass spectrometry analysis of simple, complex and conjugated carbohydrates have been published (Zaia, 2004; Harvey, 2008).

Historically, the structural analysis of carbohydrates has been a difficult task to be undertaken due to their multiple combinations, complex nature and low volatility; consequently, the functional implications of the carbohydrates are poorly known. At present, mass spectrometry is starting to be considered crucial for a fast characterization of structures and functions of these macromolecules under the so-called field of glycomics.

The selection of the right matrix and ionization technique are critical for obtaining good structural results by mass spectrometry (Lastovickova et al. 2009; Cmelik et al. 2004). For instance, MALDI-TOF-MS is considered to be more sensitive for the detection of larger

oligosaccharides, while ESI-MS seems to be more useful for structural studies (Cmelik et al. 2004). MS techniques have been used for the analysis of isolated carbohydrates important in food chemistry. Applications of MS to the analysis of oligosaccharides in various foods were revised by Careri et al. in 2002. More recently, MALDI-TOF-MS has been applied for characterization of oligosaccharides in some vegetable samples (Jerusalem artichoke, red onion and glucose syrup from potatoes); once the spectrometric conditions were optimized, the degree of polymerization of the oligosaccharides in each food was established. Authors found MALDI-TOF-MS more sensitive for detection of oligosaccharides than the chromatographic methods used for the same purpose (Stikarovska et al. 2004), underlying the importance of MS for food carbohydrate characterization. According to the tremendous impact of MS on glycomics and food chemistry, it is expected a larger number of MS application for analysis of food carbohydrates.

### 3.2. Gas Chromatography (GC).

GC has been extensively applied to carbohydrates determination in foods. Considering the high diversity of carbohydrates in terms of structure, size and functionality and their low volatility, GC has been mainly applied to study mono-, di- and tri-saccharides composition of foods. However, oligosaccharides with polymerization degree up to 7 have been successfully determined by GC using a temperature programming reaching 360°C as maximum temperature (Montilla et al. 2006). Trimethylsilyl oximes and ethers are possibly the most used derivatives nowadays for the GC analysis of carbohydrates (Rojas-Escudero et al. 2004). Nevertheless, acylated derivatives are preferred to detect closely related species, such as aldohexoses (mannose, galactose, glucose) or sugar alcohols (mannitol, galactitol, sorbitol) which only differ in one or more hydroxyl groups.

Generally, for the separation of sugars by GC, dimethyl polysiloxane stationary phases with a given percentage of polar phenyl groups are employed, with final programmed temperatures reaching typically 300-320 °C. Concerning the detection methods, flame ionization detector (FID) is the most commonly used. More recently, the use of mass spectrometers as detectors has increased, providing several advantages over the more traditional FIDs, mainly, the possibility of attaining a higher degree of certainty in the identification of carbohydrates thanks to the determination of the molecular mass and on the reproducible fragmentation patterns. A common approach consists of using both, a GC-MS method to identify the carbohydrates and a GC-FID to carry out the quantification. This approach was used to identify and quantify minor carbohydrates in carrots (Soria et al. 2009) and coffee (Ruiz-Matute et al. 2007). In fact, thanks to the MS detector employed, it was possible to identify for the first time the trimethylsilyl oximes of scyllo-inositol and sedoheptulose in the carrot chemical composition, together with other two known minor carbohydrates, mannitol and myo-inositol.

Honey is a carbohydrate rich food whose composition depends on its floral origin. The study of carbohydrates contained in honeys as well as their relationships have been used to assess the particular composition of unifloral honeys as well as their origin (de la Fuente et al. 2007). In this sense, statistical analyses such as principal component analysis (PCA) have demonstrated its usefulness for correct sample assignment (Terrab et al. 2001; Cotte et al. 2004).

The use of GC-MS has been also shown to be valuable to identify unknown carbohydrates. The disaccharides composition of several honeys was determined in different analytical columns. The use of two different columns allowed studying the elution behaviour of an unknown disaccharide. This was then compared to other commercial standards. Combining

all this information and its fragmentation in the MS, the presence of inulobiose could be confirmed (Ruiz-Matute et al. 2007b).

The carbohydrates composition of fruits and fruit-related products have also been widely studied by GC. Mono-, di- and trisaccharides from fruits have been analyzed together with sugar alcohols, flavonoids and carboxylic acids by GC using FID (Bartolozzi et al., 1997) and MS (Fuzfai et al. 2004) detectors. Different flavonoids together with mono- and disaccharides, sugar alcohols, carboxylic and amino acids could be directly identified and quantified in several citrus juices after sample hydrolysis (Fuzfai et al. 2007).

Another interesting example is the determination of lactulose; this disaccharide is formed by lactose isomerisation under heat treatment in commercial milk. Several studies demonstrated its different concentration depending on the type and intensity of heat treatment; therefore, this compound can be considered as a marker of heat treatment processes in sterilized and UHT commercial milks. To determine lactulose, different methods have been described; among them, the GC analysis of their corresponding trimethylsilyl ethers separated on a 50% diphenyl / 50% dimethylsiloxane capillary column. Using this protocol, it was possible to quantify lactulose on different treated milks (UHT, sterilized, pasteurized, condensed and powder milk) (Montilla et al. 2005). Authors demonstrated that the amount of lactulose quantified on UHT milks was significantly higher than those determined in pasteurized samples, thus, demonstrating that this compound could serve as heat treatment marker in commercial samples (Montilla et al. 2005). In this regard, carbohydrate determination can also be used for detecting food adulterations (Cotte et al. 2003).

Maltulose, another disaccharide resulting from maltose isomerisation during heat treatments has been also suggested as a possible marker of heat treatment. In fact, the maltose/maltulose ratio measured after GC analysis was suggested as a possible indicator to assess heat treatment during manufacture and storage of infant and enteral formulas (Morales et al. 2004;

García-Baños et al. 2002). Difruuctose anhydrides have been also employed as quality markers in sugar-rich foods such as honey and coffee (Montilla et al. 2006b). These compounds are produced by condensation of two fructose molecules during caramelization reactions that take place under heating. These compounds were analyzed by GC being possible to statistically differentiate between samples treated under different heating treatments (Montilla et al. 2006b)

In conclusion, although GC provides excellent results, the adequate application of this technique is limited by the required sample preparation steps and its application to relatively small carbohydrates. In fact, there is a fair chance of sample losses during all the procedure, mainly due to derivatization reactions and/or hydrolysis not completely finished. Other techniques, such as CE or HPLC have been developed trying to overcome these limitations.

### 3.3. High Performance Liquid Chromatography (HPLC).

HPLC is at present the main analytical technique used for carbohydrates analysis. Compared to the other analytical techniques, HPLC is able to provide some additional advantages. For instance, although less sensitive than GC, HPLC is faster, does not necessarily require sample derivatization, allows the analysis of larger carbohydrates and it is more versatile thanks to the different separation mechanisms and detectors that can be employed.

#### *3.3.1 HPLC separation mechanisms.*

The separation mechanism can be selected depending on the sample to be analyzed, the particular carbohydrates of interest and the instrumental availability. The most employed HPLC modes for carbohydrate analysis are ion-exchange chromatography, size exclusion chromatography and partition (normal or reversed phase) chromatography.

Ion-exchange chromatography can be applied using anionic or cationic exchange resins. Particularly, high performance anion exchange chromatography (HPAEC) has found great success in carbohydrates analysis (Cataldi et al. 2000). To carry out the separation, quaternary ammonium resins are commonly used under highly alkaline conditions to allow carbohydrates ionization, although different types of commercial columns are available nowadays. Mobile phase has to be carefully selected since it will strongly influence not only the selectivity of the separation but also the total analysis time. Besides mono- and disaccharides, oligosaccharides can also be analyzed by HPAEC. Different methods have been developed to separate oligosaccharides according to their polymerization degree. For instance, oligosaccharides from honey samples and corn syrups have been studied using a gradient from 1 M sodium acetate to deionized water, maintaining 10% 1 M sodium hydroxide constant during the whole analysis (Morales et al. 2008). Using this method different malto-oligosaccharides (from polymerization degree 1 to 16) were adequately separated, as it can be appreciated in Figure 5. Oligo- and polysaccharides can also be analyzed by HPAEC after sample hydrolysis in order to simplify the complex carbohydrates profile to a more simple mono- and disaccharides composition.

INSERT FIGURE 5 HERE.

Cationic metal ions, such as  $\text{Ca}^{2+}$  or  $\text{Ag}^+$ , have been also employed to separate mono- and oligosaccharides from different food matrices. The retention mechanism in this case relies on the formation of weak complexes between the metal ions and the carbohydrates hydroxyl groups. This particular separation method, also called ligand exchange chromatography, allows the complete separation of monosaccharide enantiomers, such as glucose, mannose or

galactose. Besides, sugar alcohol can be also correctly separated. However, this technique has several shortcomings: columns are not very robust as the metals can be eluted from the resins when analyzing complex samples and the resolution of oligosaccharides is frequently poor due to the very weak interaction with the metal ions.

Another separation mechanism widely employed for food carbohydrates separation is size exclusion chromatography (SEC). This technique has been already mentioned in the sample preparation section, since it is also used to fractionate carbohydrates. Moreover, technological developments have allowed manufacturing analytical columns that have enabled its use as a high performance liquid chromatography-based procedure. SEC is based on the permeation of substances through resins with a particular pore size and structure. Polysaccharides, being large molecules, cannot penetrate into the three-dimensional network inside the resins, and consequently are eluted first. The rest of the components will elute according to a decreasing molecular size. Thus, monosaccharides with small size, will interact more with the resins and will take longer to elute. This technique has been applied for instance to the chemical characterization of arabinoxylans from cereals (Hartmann et al. 2005). Arabinoxylans are related with different organoleptic parameters of bread as well as with several technological aspects in breadmaking. SEC was employed together with a UV detector to determine the wheat and rye arabinoxylans mass distribution during the breadmaking process.

The last separation mechanism that has to be mentioned is partition chromatography, either normal or reversed phase separations. The notorious improvements and developments made in the field of column technology are increasing the number of applications based on the use of this HPLC mechanism. In this regard, the new stationary phases composed of spherical silica particles bonded with trifunctional amino propylsilane are finding many applications in carbohydrate analysis. This special case of normal-phase separations is called hydrophilic interaction liquid chromatography (HILIC). These functionalized amino columns have shown

to be more resistant than the first commercial amino-bonded silica gel columns that were used to separate mono- and disaccharides. This column was eluted with acetonitrile/water (75:25, v/v) for the analysis of fermentable oligo-, di- and monosaccharides and polyols, including glucose, fructans, fructooligosaccharides, galactooligosaccharides and different sugar polyols in several vegetables and fruits (Muir et al. 2009). The same mobile phase was employed with other column type, based on secondary-tertiary amines, for the analysis of oligosaccharides from chickpeas (Xiaoli et al. 2008). More standard C<sub>18</sub> RP-HPLC columns have been also used although the results obtained are not, in general, as satisfactory as with the amino columns. Besides, in this latter case, derivatization of the carbohydrates may be necessary.

### *3.3.2 HPLC detectors.*

When analyzing carbohydrates by HPLC, one of the parameters to be considered is the detection mode since its choice will greatly influence the sample preparation to be performed. In fact, if a photometric detection is going to be used (UV-Vis or fluorescence), it will be necessary to submit the carbohydrates to a derivatization step in order to enable their detection and/or to increase sensitivity. Carbohydrates derivatization reactions can be divided into two main groups; the first consist on the introduction of chromophores reacting with hydroxyl groups and the second involves the reaction with amines at the carbonyl group. One of the most utilized derivatizing reagents to confer UV absorbance is 1-phenyl-3-ethyl-5-pyrazolone. This compound can react with carbohydrates under mild conditions without the common requirements of an acidic catalyst, which avoids degradation and unwanted isomerization providing a sensitive UV detection at 245 nm (Zhang et al. 2003). Other reagents that are also used are p-nitrobenzoyl chloride (absorbance at 260 nm) or phenylisocyanate (detection at 240 nm).

Although pre-column derivatization reactions are the most commonly employed, post-column derivatization reactions have also been described. Benzamidine was used as derivatizing agent to produce a fluorescent complex in the presence of reducing carbohydrates in alkaline medium and high temperature. This reaction was implemented for the simultaneous determination of mono- and oligosaccharides in different food related materials by HPLC (Kakita et al. 2002). The fluorescence detection was carried out using 288 nm as excitation wavelength and 470 nm for emission, resulting in LOD of 1.78 and 2.59 pmol for D-glucose and maltohexaose, respectively. A similar strategy was followed to detect reducing sugars as well as phosphorylated sugars in chicken meat. In this case tetrazolium blue was the chosen reagent, which conferred absorbance at 550 nm (Aliani et al. 2002).

Considering the problematic photometric detection and in order to be able to avoid laborious derivatization steps, other detection systems have been traditionally employed in food carbohydrate analysis. In fact, refractive index (RI) detection has been widely employed; this detector is not selective, incompatible with gradient elution, gives low sensitivity and is quite susceptible to flow rate and temperature changes. Still, it has been broadly applied to different matrices, such as powder milk (Chavez-Servin et al. 2006), cider (Picinelli et al. 2000) or meat products (Vendrell-Pascuas et al. 2000), among many others.

Evaporative-light scattering detectors (ELSD) have been also utilized in order to improve sensitivity. This detector is for non-volatile components and provides approximately the same response for every component. Moreover, ELSD allows gradient elution since the solvent is evaporated just before detection. This kind of detector has been employed together with HPLC and a silica gel column for the direct analysis of carbohydrates in food (Wei et al. 2002). The presence of soluble sugars in onion was also confirmed by ELSD detection coupled to size exclusion and amino columns (Davis et al. 2007). Moreover, ELSD has

proven to be also useful as detector in combination with HPAEC separations of carbohydrates (Muir et al. 2009).

Although these two detectors have found many applications in food carbohydrate analysis, the most widely employed detection method has been electrochemical detection. Namely, pulsed amperometric detection (PAD) has been considered the ideal detector for HPLC separations of carbohydrates. PAD is suitable for those compounds that contain functional groups that are oxidizable at the detection voltage employed. Basically, PAD uses a gold electrode and works better in alkaline medium, which make it perfectly suitable to be coupled to HPAEC carbohydrates separations. In the analysis of underivatized carbohydrates, compared to the above mentioned RI and ELSD detectors, PAD provides with better sensitivities that can reach LODs below 10 µg/L (Cataldi et al. 2000). When using a PAD the potential waveform has to be adequately optimized for carbohydrates detection. The most frequently used waveform implies three steps, each of one lasting a given time (60-300 ms): detection, that involves the direct oxidation of the electrode surface; cleaning, using higher potential in order to fully oxidize the electrode surface and regeneration, by means of a very low potential to eliminate the previously gold-oxide film and leave the electrode ready for the next detection step. Both, potentials and times intervals, have to be set to attain the best possible results in each application.

In spite of the good results that have been obtained using PAD detection in food carbohydrates analysis, the use of MS detectors together with HPLC has also seen an important increasing. The use of HPLC-MS in carbohydrate analysis has several advantages because it can corroborate saccharides identification and help in the characterization of unknown carbohydrates reducing the necessary time for isolation and purification. HPAEC has been successfully coupled to MS detection for the analysis of food carbohydrates. This coupling is not easy, given the technological incompatibility that poses the typical alkaline

conditions of HPAEC with volatilization and ionization prior to MS typically using an electrospray interface (ESI). The salts inhibit the correct formation of the electrospray due to their low volatility. Therefore, when operating at these conditions, a desalting device has to be employed between the HPAEC column and the ESI-MS detector. Moreover, to further increase carbohydrates signal in the MS analyzer, a solution of lithium chloride is added to the eluent in order to produce  $\text{Li}^+$  (or chloride) adducts. Using this approach several carbohydrates of different nature were correctly detected in diverse food and beverages allowing the determination of high molecular mass carbohydrates (>2000 Da) with a single quadrupole analyzer (Bruggink et al. 2005).

HPLC can also be combined with isotope ratio mass spectrometry (IRMS) for the analysis of sugars. This technique is able to measure the abundance of  $^{13}\text{C}$  in different compounds. It was found that high fructose corn syrup and honeys had significantly different natural abundance of  $^{13}\text{C}$ . Therefore, this technique was proven to be useful to detect adulteration of honey with low amounts of corn syrup (Abramson et al. 2001). The same approach was also successfully tested in order to reveal adulterations in fruit juices by studying the  $^{13}\text{C}$  ratios of different sugars (Guillou et al. 1999).

Other detection methods have been also applied to a less extent combined with HPLC to determine carbohydrates. For instance, HPLC has been coupled to Fourier transform infrared spectrometry (FTIR) to detect different carbohydrates in food samples. Fructose, glucose, maltose and sucrose were determined and quantified in beverages using this procedure (Kuligowski et al. 2008). Inductively coupled plasma-atomic emission spectrometry (ICP-AES) has been also coupled to HPLC to be used as a carbon detector. The use of organic compounds in the mobile phases has to be avoided to prevent interferences in the detection. Under these conditions, glucose, fructose, sucrose, sorbitol and lactose, together with other

carboxylic acids and alcohols, were detected and quantified. Results were comparable, in terms of LODs, to those obtained with RI and UV-Vis detectors (Paredes et al. 2006).

In summary, HPLC has been widely employed to analyze carbohydrates in foods combining different mechanisms and detectors (Martinez-Montero et al. 2004; Soga 2002; Peris-Tortajada 2000). These applications include: control of manufacturing processes, control of products, detection of adulterations, authenticity determination, etc (Cataldi et al. 1999; Megherbi et al. 2009).

#### 3.4. Capillary Electrophoresis (CE).

CE has already demonstrated to be a useful analytical technique in food analysis (Herrero et al. 2009; García-Cañas et al. 2008). Under the conditions typically employed in CE, charged compounds are separated in dissolution according to their effective electrophoretic mobilities under the influence of an electric field. Regarding carbohydrates analysis by CE, the main drawbacks to be solved are similar to those mentioned for HPLC, i.e., most of these compounds are neutral, polar and lack of chromophores. In spite of these limitations, carbohydrates have directly been separated without derivatization using UV detection at 195 nm (Xu et al. 1996) or indirect UV detection. By using indirect detection it was possible to improve the low response of these compounds at 195 nm, detecting fructose, glucose, maltose, maltotriose, galactose, lactose and sucrose in cereal (after ultrasonication) and dairy (after dilution) samples with relatively low LODs (Jager et al. 2007).

Modification of the carbohydrate structure through derivatization procedures can improve both sensitivity and selectivity of the CE separation. Among the derivatizing compounds used in carbohydrate analysis to allow UV detection, phenylethylamine (200 nm), 4-amino-benzoic acid ethyl ester (306 nm) or 6-aminoquinolone (245 nm) can be cited as examples. *p*-Nitroaniline was used to allow UV detection of different hexoses in powdered milk and rice

syrup, using a blue light-emitting diode as light source and detecting at 406 nm. The use of this reagent was justified considering its high solubility in water and molar absorptivity. The detection limits could be lowered down to 1.1  $\mu\text{M}$  using a boric acid buffer at pH 9.7 (Momenbeik et al. 2006). Analysis of carbohydrates in beverages has also been described using *p*-aminobenzoic acid as derivatizing agent; the reaction took place at 40 °C for 1 hour in the presence of the derivatizing agent and 20% acetic acid. The products of this reaction were separated with borate buffer (pH 10.2) in less than 12 minutes. Glucose, maltose and maltotriose were identified in different beverages (Cortacero-Ramirez et al. 2004). Fluorescence labels that allow using laser-induced fluorescence detectors have been also employed for carbohydrate analysis. 9-aminopyrene-1,4,6-trisulfonic acid can be employed to detect oligosaccharides with different polymerization degrees (Khandurina et al. 2005).

All the above mentioned protocols are based on pre-column derivatization, however, other alternative approach as on-capillary reaction are also available in CE (Taga et al. 2001). Neutral mono- and disaccharides can form enediolate anions thanks to a cascade reaction comprising ionization, mutarotation, enolization and isomerisation in aqueous alkaline solution (Rovio et al. 2007). Using this approach, eleven different neutral carbohydrates were detected at 270 nm, after reaction in an alkaline electrolyte composed of 130 mM sodium hydroxide and 36 mM disodium hydrogen phosphate hydrate. Detection limits as low as 0.02 mM could be obtained. The methodology was successfully applied to the determination of target carbohydrates in fruit juices and cognac, with only a dilution step as sample preparation (Rovio et al. 2007).

The interest of on-column reaction derives from the search of more straightforward methods that do not imply extensive and labour intensive sample preparation procedures. An on-capillary clean-up has been also proposed for carbohydrate analysis from juices. This procedure was based on the partial filling of the capillary with carboxylated single-walled

carbon nanotubes that are able to efficiently retain interferences from the matrix allowing a more precise identification and quantification of the compounds of (Morales-Cid et al. 2007). At it has been already mentioned, the derivatizing agents may not only improve the detection of carbohydrates but can also effectively influence the separation itself. In this sense, a different kind of derivatization procedure has been also developed including the reductive amination of aldoses with phenylethylamine; giving rise to carbohydrates labelled with a chiral UV tag. Then, the separation of these carbohydrates took place according to the formation of charged complexes between the sugar hydroxyl groups and the tetrahydroxyborate contained in the separation electrolyte (Noe et al. 1999). Using this method, the presence of different enantiomeric aldoses in several juices and wines could be confirmed.

Although difficult to implement, CE coupled to MS has been used to analyze carbohydrates in red and white wines (Klampfl et al. 2001). In this case, volatile organic bases, such as diethylamine, were combined with ESI negative ionization mode. These conditions allowed the detection of deprotonated carbohydrates. 15 different carbohydrates could be separated and quantified, obtaining LODs ranging 0.5-3.0 mg/L. Results were in agreement with those previously published using other analytical techniques. Other detection methods that have been used together with CE to detect underivatized carbohydrates are electrochemical detectors or even Fourier transform infrared (FTIR) detection. In the latter case, an original approach was followed to couple these two techniques (Kolhed et al. 2005). The separation took place in high alkaline conditions in order to have the carbohydrates deprotonized, and a FTIR spectrometer was adapted as detector on-line. Under these conditions sucrose, galactose, glucose and fructose were detected and quantified in orange juices. Besides, their spectra could be recorded and the compounds accordingly identified. The LODs obtained

ranged between 0.7 and 1.9 mM for the studied carbohydrates, more sensitive than those from a more common UV detector (Kolhed et al. 2005).

There is a clear trend in CE towards the development of CE analysis in microchips. In this case, the separation is performed in a miniaturized device that allows the separation in a narrow channel typically manufactured on a glass, poly(dimethylsiloxane) or polymeric plate. The separations on these chips have several advantages, such as low reagents and sample consumption and high speed of analysis, i.e., high-throughput. A silicone/quartz chip was employed combined with an amperometric detector to analyze carbohydrates in acacia honey samples, with LOD as low as 90 amol (Zhai et al. 2007). A typical electropherogram obtained using this device under optimized conditions can be observed in Figure 6. An increase of the number of applications of chips for the detection of carbohydrates in food analysis can be expected in a near future.

INSERT HERE FIGURE 6.

### 3.5. Multidimensional techniques, glycomics and others.

In this section, multidimensional techniques along with hyphenated techniques for carbohydrates analysis together with new approaches for glycomics are presented. Sample complexity has led to the development of new methods involving, in some cases, on-line sample preparation through microdialysis coupled to HPAEC and integrated pulsed electrochemical detection-mass spectrometry (IPED-MS) to solve in a fast and automatic way the complete analysis of carbohydrates. HPAEC-IPED-MS was used to determine oligosaccharides during the on-line monitoring of wheat starch hydrolysis (Torto et al. 1998). One of the key steps was the selection of a desalting device based in a single cation-exchange

membrane able to exchange sodium ions by hydronium ions, allowing an easy coupling to a MS detector. Several food applications have already been described using the same approach (Okatch et al. 2003; Torto et al. 2000).

Although in a less extent, multidimensional techniques have also been developed for carbohydrates analysis. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS) has been used to analyze metabolites in plant samples such as basil, peppermint and sweet herb stevia. Results showed that relative abundances of amino acids, carboxylic acids, and carbohydrates were responsible for differentiating the three plants (Pierce et al. 2006). As for HPLC, the use of an hyphenated RP-HPLC/HILIC approach has been proposed to analyze different model samples, including sugars. Even if the application is not focused on carbohydrates separation in food samples, the set-up might be suitable for other complex samples (Louw et al. 2008). In this contribution, authors described a simple approach to couple HILIC to RP-HPLC through the use of a 2 mm I.D. column in the first dimension and a 4.6 mm I.D. in the second dimension combined with the addition of an excess of mobile phase containing high acetonitrile content (i.e.  $\geq 80\%$ ) to the mobile phase eluting from the RP-HPLC column via a T-piece. Combination of both mechanisms allowed the elution of compounds widely different in their structure and properties.

Glycomics, that is, the systematic study of glycans, is rapidly gaining attention. Until recently, the complexity of glycan structures was a barrier in understanding the relationship between glycan structure and function. The development of new analytical tools based on the use of micro- and nanochips coupled to biosensors and/or mass spectrometry along with HPLC mapping methods have increased the knowledge on the role played by glycans in different biological functions and on their mechanisms of action. Takahashi et al. started developing a 3-D sugar mapping technique using 3 different HPLC columns (diethylaminoethyl, DEAE;

octadecylsilica, ODS; and amide) to separate pyridyl-2-aminated derivatives of oligosaccharides (Takahashi et al. 1995; Tomiya et al. 1988). Data in three dimensions, corresponding to the elution in the three HPLC columns was used to map the position of several N-glycans and thus to identify their structure. Data on more than 500 different glycans, mainly those derived from glycoproteins, is available in the web application GALAXY (Takahashi et al 2003), including MS data. Recently, data on anionic oligosaccharides (Yagi et al. 2009) and on sulphated N-glycans (Yagi et al. 2005) has also been reported, widening the applicability of the technique.

Microarray technologies have emerged as an important analytical tool in glycobiology, complementing other techniques such as mass spectrometry (Haslam et al. 2006). One approach is the use of lectin-based microarrays; lectins are carbohydrate binding proteins that can help to efficiently profile glycan variations in a wide range of biological systems (Sharon 2008; Gemeiner et al. 2009; Zhao et al. 2007). More information on microarray-based analysis of glycans can be found in this review paper (Hsu et al. 2009). Other approaches have been used, for instance, to analyze the degree of glycosylation of proteins using a chip-based method based on the energy transfer between quantum dots (QDs) and gold nanoparticles (AuNPs). Basic principle relies on modulation in the energy transfer efficiency between the lectin-modified QDs and carbohydrate-conjugated AuNPs by glycoproteins. The chip-based format enables more reliable analyses with no aggregation of the nanoparticles, requiring smaller amount of reagents. Moreover, the energy transfer mechanism allows the development of a simple and highly sensitive method for the detection of glycoproteins, in contrast with the chip-based systems employing a single fluorophore (Kim et al. 2009a). In terms of improving detection of glycosylated proteins, a system based on the use of a protein chip together with a plasmon resonance imaging (SPRI) biosensor has been developed to

determine the presence of advanced glycation end products, implicated in diabetic complications, in serum samples from diabetic rats (Kim et al. 2009b).

Finally, micro and nanochips have been used, coupled to mass spectrometry, as a tool for structural analysis of carbohydrates in biological samples. Advantages of the chip-based MS over the conventional MS include high sensitivity for minute samples, prevention of sample loss and extensive sample handling, increased ionization efficiency, high reproducibility, high quality spectra, superior stability of the electrospray over extended analysis time and possibility of high-throughput MS screening and sequencing (Zamfir et al. 2005; Lion et al. 2006). Recently, the integration of various analytical steps onto chips and the direct hyphenation to mass spectrometry has given rise to a new tool for glycan studies: the lab-on-a-chip MS glycomics. The use of chip devices for mass spectrometric studies of glycans follows basically two different trends: the direct infusion of the sample mixture for ionization through the chip-emitters, and the use of integrated chips able to deliver in a single device various sample treatment procedures, chromatographic or electrophoretic separation, purification, enzymatic reactions, derivatization and sample ionization (Bindila et al. 2009). One interesting application is the analysis of human milk oligosaccharides mixture by chip-LC in direct conjunction to orthogonal TOF mass analyzer (Ninonuevo et al. 2006). In Figure 7, typical spectra of oligosaccharides used for identification are shown. The chromatographic separation was conducted on a HPLC-chip consisting of an integrated sample loading structure, an LC column packed with graphitized carbon media and nanoelectrospray tip (Ninonuevo et al. 2005). A total of 150 distinct neutral and acidic species expressing a high structural variety were found, including novel glycan structures. The potential of the HPLCchip/MS approach was further explored for differential profiling of human milk oligosaccharides (HMO) in five different randomly selected individuals to study person-to-person variability of the oligosaccharide expression.

INSERT HERE FIGURE 7.

These technologies (microarrays, chips, and their couplings with biosensors and MS) are expected to find interesting applications in the Food Science and Technology domain, more probably within the glycomics area, although some other interesting applications within the foodomics field can not be discarded.

#### 4. References.

- Aboubacar, A., Hamaker, B. R. 2000. Low molecular weight soluble starch and its relationship with sorghum couscous stickiness. *J. Cereal Sci.* 31:119-126.
- Abramson, F. P., Black, G. E., Lecchi, P. 2001. Application of high-performance liquid chromatography with isotope-ratio mass spectrometry for measuring low levels of enrichment of underivatized materials. *J. Chromatogr. A* 913:269-273.
- Aliani, M., Farmer, L. J. 2002. Postcolumn derivatization method for determination of reducing and phosphorylated sugars in chicken by high performance liquid chromatography. *J. Agric. Food Chem.* 50:2760-2766.
- Amigo-Benavent, M., Athanasopoulos, V. I., Ferranti, P., Villamiel, M., del Castillo, M. D. 2009. Carbohydrate moieties on the in vitro immunoreactivity of soy beta-conglycinin. *Food Res. Int.* 42:819-825.
- Arias, V. C., Castells, R. C., Malacalza, N., Lupano, C. E., Castells, C. B. 2003. Determination of oligosaccharide patterns in honey by solid-phase extraction and high-performance liquid chromatography. *Chromatographia* 58:797-801.
- Arya, M., Mohan Rao, L. J. 2007. An impression of coffee carbohydrates. *Crit. Rev. Food Sci. Nutr.* 67:47-51.
- Bartolozzi, F., Bertazza, G., Bassi, D., Cristoferi, G. 1997. Simultaneous determination of soluble sugars and organic acids as their trimethylsilyl derivatives in apricot fruits by gas-liquid chromatography. *J. Chromatogr. A* 758:99-107.
- Bindila, L., Peter-Katalinic, J. 2009. Chip-Mass spectrometry for glycomic studies. *Mass Spectrom. Rev.* 28:223-253.

- Bruggink, C., Maurer, R., Herrmann, H., Cavalli, S., Hoefler, F. 2005. Analysis of carbohydrates by anion exchange chromatography and mass spectrometry. *J. Chromatogr. A* 1085:104-109.
- Careri., M. Bianchi, F., Corradini C. 2002. Recent advances in the application of mass spectrometry in food-related analysis. *J. Chromatogr. A* 970:3-64.
- Castellari, M., Versari, A., Spinabelli, U., Galassi, S., Amati, A. 2000. An improved HPLC method for the analysis of organic acids, carbohydrates, and alcohols in grape musts and wines. *J. Liq. Chromatogr. Related Technol.* 23:2047–2056.
- Cataldi, T. R. I., Angelotti, M., Bufo, S. A. 1999. Method development for the quantitative determination of lactulose in heat-treated milks by HPAEC with pulsed amperometric detection. *Anal. Chem.* 71:4919-4925.
- Cataldi, T. R. I., Campa, C., De Benedetto, G. E. 2000. Carbohydrate analysis by high-performance anion-exchange chromatography with pulsed amperometric detection: the potential is still growing. *Fresenius J. Anal. Chem.* 368:739-758.
- Chandra, K., Ghosh, K., Ojha, A. K., Islam, S. S. 2009. A protein containing glucan from an edible mushroom. *Termitomyces microcarpus*. *Nat. Prod. Commun.* 4:553-556.
- Chavez-Servin, J. L., Romeu-Nadal, M., Castellote, A. I., Lopez-Sabater, M. C. 2006. Evolution of free mono- and di-saccharide content of milk-based formula powder during storage. *Food Chem.* 97:103-108.
- Cifuentes, A. 2009. Food Analysis and Foodomics. *J. Chromatogr. A* 1216:7109.
- Cmelik, R., Stikarovska, M., Chmelik, J. 2004. Different behavior of dextrans in positive-ion and negative-ion mass spectrometry. *J. Mass Spectrom.* 39:1467-1473.
- Corsaro, A., Chiacchio, U., Pistara, P., Romeo, G. 2004. Microwave-assisted chemistry of carbohydrates. *Current Org. Chem.* 8:511-538.

- Cortacero-Ramirez, S., Segura-Carretero, A., Cruces-Blanco, C., Hernainz-Bermudez, M., Fernandez-Gutierrez, A. 2004. Analysis of carbohydrates in beverages by capillary electrophoresis with precolumn derivatization and UV detection. *Food Chem.* 87:471-476.
- Corzo-Martinez, M., Moreno, F. J., Villamiel M., Harte, F. M. 2010. Characterization and improvement of rheological properties of sodium caseinate glycated with galactose, lactose and dextran. *Food Hydrocolloids* 24:88-97.
- Cotte, J. F., Casabianca, H., Chardon, S., Lheritier, J., Grenier-Loustalot, M. F. 2004. Chromatographic analysis of sugars applied to the characterization of monofloral honey. *Anal. Bioanal. Chem.* 380:698-705.
- Cotte, J. F., Casabianca, H., Chardon, S., Lheritier, J., Grenier-Loustalot, M. F. 2003. Application of carbohydrate analysis to verify honey authenticity. *J. Chromatogr. A* 1021:145-155.
- Cui, Steve W. 2005. *Food carbohydrates: Chemistry, physical properties and applications*. Boca Raton, CRC Press.
- Davis, F., Terry, L. A., Chope, G. A., Faul, C. F. J. 2007. Effect of extraction procedure on measured sugar concentrations in Onion (*Allium cepa* L.) bulbs. *J. Agric Food Chem.* 55:4299-4306.
- de la Fuente, E., Sanz, M. L., Martinez-Castro, I., Sanz, J., Ruiz-Matute, A. I. 2007. Volatile and carbohydrate composition of rare unifloral honeys from Spain. *Food Chem.* 105:84-93.
- Dourado, F., Vasco, P., Barros, A., Mota, M., Coimbra, M. A., Gama, F. M. 2003. Characterisation of Chilean hazelnut (*Gevuina avellana*) tissues: light microscopy and cell wall polysaccharides. *J. Sci. Food Agric.* 83:158-165.
- Eremeeva, T. 2003. Size-Exclusion chromatography of enzymatically treated cellulose and related polysaccharides: a review. *J. Biochem. Biophys. Methods* 56:253-264.

- Fuzfai, Z., Katona, Z. F., Kovacs, E., Molnar-Perl, I. 2004. Simultaneous identification and quantification of the sugar, sugar alcohol and carboxylic acid contents of sour cherry, apple and ber fruits as their trimethylsilyl derivatives by gas chromatography-mass spectrometry. *J. Agric. Food Chem.* 52:7444-7452.
- Fuzfai, Z., Molnar-Perl, I. 2007. Gas chromatographic-mass spectrometric fragmentation study of flavonoids as their trimethylsilyl derivatives: analysis of flavonoids, sugars, carboxylic and amino acids in model systems and in citrus juices. *J. Chromatogr. A* 1149:88-101.
- García-Baños, J. L., Olano, A., Corzo, N. 2002. Changes in the carbohydrate fraction during manufacture and storage of enteral formulas. *J Food Sci.* 67:3232-3235.
- García-Cañas, V., Cifuentes, A. 2008. Recent advances in the application of capillary electromigration methods for food analysis. *Electrophoresis* 29:294-309.
- Geisser, A., Hendrich, T., Boehm, G., Stahl, B. Separation of lactose from human milk oligosaccharides with simulated moving bed chromatography. *J. Chromatogr. A* 1092:17-23.
- Gemeiner, P., Mislovicova, D., Tkac, J., et al. 2009. Lectinomics II. A highway to biomedical/clinical diagnostics. *Biotechnol. Adv.* 27:1-15.
- Gibson, P. R., Newham, E., Barrett, J. S., Shepherd, S. J., Muir, J. G. 2007. Fructose malabsorption and the bigger picture. *Aliment. Pharmaciol. Ther.* 25:349-363.
- Gniechwitz, D., Reichardt, N., Ralph, J., Blaut, M., Steinhart, H., Bunzel, M. 2008. Isolation and characterisation of a coffee melanodin fraction. *J. Sci. Food Agric.* 88:2153-2160.
- Godshall, M. A., Roberts, E. J., Miranda, X. M. 2001. Composition of the soluble, nondialyzable components in raw cane sugar. *J. Food Process. Preservation* 25:323-335.
- Guillou, C., Koziat, J., Rossmann, A., Martin, G. J. 1999. Determination of the <sup>13</sup>C contents of organic acids and sugars in fruit juices: an inter-comparison study. *Anal. Chim. Acta* 388:137-143.

- Hartmann, G., Piber, M., Koehler, P. 2005. Isolation and chemical characterisation of water-extractable arabinoxylans from wheat and rye during breadmaking. *Eur. Food Res. Technol.* 221:487-492.
- Harvey, D. J. 2008. Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: An update covering the period 2001-2002. *Mass Spectrom. Rev.* 27:125-201.
- Haska, L., Nyman, M., Andersson, R. 2008. Distribution and characterisation of fructan in wheat milling fractions. *J. Cereal Sci.* 48:768-774.
- Haslam, S. M., North, S. J., Dell, A. 2006. Mass spectrometric analysis of N and O-glycosylation of tissues and cells. *Current Opin. Struct. Biol.* 16:584-591.
- Hernandez, O., Ruiz-Matute, A. I., Olano, A., Moreno, F. J., Sanz, M. L. 2009. Comparison of fractionation techniques to obtain prebiotic galactooligosaccharides. *Int. Dairy J.* 19:531-536.
- Herrero, M., García-Cañas, V., Simo, C., Cifuentes, A. 2009. Recent advances in the application of capillary electromigration methods for food analysis and foodomics. *Electrophoresis* in press.
- Hounsome, N., Hounsome, B., Tomos, D., Edwards-Jones, G. 2008. Plant metabolites and nutritional quality of vegetables. *J. Food Sci.* 73:R48-R65.
- Hsu, K. L., Mahal, L. K. 2009. Sweet tasting chips: microarray-based analysis of glycans. *Current Opin. Chem. Biol.* 13:427-432.
- Ibañez, E., Cifuentes, A. 2003. New analytical techniques in food science. *Crit. Rev. Food Sci.* 41:413-450.
- Jager, A. V., Tonin, F. G., Tavares, M. F. M. 2007. Comparative evaluation of extraction procedures and method validation for determination of carbohydrates in cereals and dairy products by capillary electrophoresis. *J. Sep. Sci.* 30 :586-594.

- Kakita, H., Kamishima, H., Komiya, K., Kato, Y. 2002. Simultaneous analysis of monosaccharides and oligosaccharides by high-performance liquid chromatography with postcolumn fluorescence derivatization. *J. Chromatogr. A* 961:77-82.
- Kamada, T., Nakajima, M., Nabetani, H., Iwamoto, S. 2002. Pilot-scale study of the purification and concentration of non-digestible saccharides from Yacon Rootstock using membrane technology. *Food Sci. Technol. Res.* 8:172–177.
- Khandurina, J., Guttman, A. 2005. High resolution capillary electrophoresis of oligosaccharide structural isomers. *Chromatographia* 62:S37-S41.
- Kim D., Park C. S., Park K. H. 2003. Preparation and characterization of maltosyl-sucrose isomers produced by transglycosylation of maltogenic amylase from *Bacillus stearothermophilus*. *J. Mol. Catal. B-Enzymatic.* 26:293-305.
- Kim, H. S., Yi, S. Y., Kim, J., et al. 2009. Novel application of surface plasmon resonance biosensor chips for measurement of advanced glycation end products in serum of Zucker diabetic fatty rats. *Biosens. Bioelectron.* 25:248–252.
- Kim, Y. P., Park, S., Oh, E., Oh, Y. H., Kim, H. S. 2009a. On-chip detection of protein glycosylation based on energy transfer between nanoparticles. *Biosens. Bioelectron.* 24:1189–1194.
- Kitahara, K., Copeland, L. 2004. A simple method for fractionating debranched starch using a solid reversed-phase cartridge. *J. Cereal Sci.* 39:91-98.
- Klampfl, C. W., Buchberger, W. 2001. Determination of carbohydrates by capillary electrophoresis with electrospray-mass spectrometric detection. *Electrophoresis* 22:2737-2742.
- Kolhed, M., Larlberg, B. 2005. Capillary electrophoretic separation of sugars in fruit juices using on-line mid infrared Fourier transform detection. *Analyst* 130:772-778.

- Kuligowski, J., Quintas, C., Garrigues, S., de la Guardia, M. 2008. On-line gradient liquid chromatography-Fourier transform infrared spectrometry determination of sugars in beverages using chemometric background correction. *Talanta* 77:779-785.
- La Torre, G., La Pera, L., Rando, R. et al. 2008. Classification of Marsala wines according to their polyphenol, carbohydrate and heavy metal levels using canonical discriminant analysis. *Food Chem.* 110:729-734.
- Lamari, F. N., Kuhn, R., Karamanos, N. K. 2003. Derivatization of carbohydrates for chromatographic, electrophoretic and mass spectrometric structure analysis. *J. Chromatogr. B* 793:15-36.
- Lastovickova, M., Chmelik, J., Bobalova, J. 2009. The combination of simple MALDI matrices for the improvement of intact glycoproteins and glycans analysis. *Int. J. Mass Spectrom.* 281:82-88.
- Lehtonen, P., Hurme, R. 1994. Liquid-chromatographic determination of sugars in beer by evaporative light-scattering detection. *J. Inst. Brew.* 100:343-346.
- Lion, N., Rossier, J. S., Girault, H. H. 2006. Microfluidic applications in biology: From technologies to systems biology. Weinheim, Wiley-VCH.
- Louw, S., Pereira, A. S., Lynen, F., Hanna-Brown, M., Sandra, P. 2008. Serial coupling of reversed-phase and hydrophilic interaction liquid chromatography to broaden the elution window for the analysis of pharmaceutical compounds. *J. Chromatogr. A* 1208:90-94.
- Mabel, M. J., Sangeetha, P. T., Platel, K., Srinivasan, K., Prapulla, S. G. 2008. Physicochemical characterization of fructooligosaccharides and evaluation of their suitability as a potential sweetener for diabetics. *Carbohydrate Res.* 343:55-66.
- Martínez Montero C., Rodríguez Doderó, M. C., Guillén Sánchez, D. A. , Barroso, C. G. 2004. Analysis of low molecular weight carbohydrates in food and beverages: a review. *Chromatographia* 59:15-30.

- Martínez-Villaluenga, C., Cardelle-Cobas, A., Olano, A., Corzo, N., Villamiel, M., Jimeno, M. L. 2008. Enzymatic synthesis and identification of two trisaccharides produced from lactulose by transgalactosylation. *J. Agric. Food Chem.* 56:557-563.
- Megherbi, M., Herbretau, B., Dessalces, G., Grenier-Loustalot, M. F. 2008. Solid phase extraction of oligo- and polysaccharides; application to malodextrins and honey qualitative analysis. *J. Liq. Chromatogr. Rel. Technol.* 31:1033-1046.
- Megherbi, M., Herbretau, B., Faure, R., Salvador, A. 2009. Polysaccharides as a marker for detection of corn sugar syrup addition in honey. *J. Agric. Food Chem.* 57:2105-2111.
- Mendiola, J. A., Herrero, M., Cifuentes, A., Ibáñez, E. 2007. Use of compressed fluids for sample preparation: food applications. *J. Chromatogr. A* 1152:234-246.
- Molnar-Perl, I. 2000. Role of chromatography in the analysis of sugars, carboxylic acids and amino acids in food. *J. Chromatogr. A* 891:1-32.
- Momenbeik, F., Johns, C., Breadmore, M. C., Hilder, E. F., Macka, M., Haddad, P. R. 2006. Sensitive determination of carbohydrates labelled with p-nitroaniline by capillary electrophoresis with photometric detection using a 406 nm light-emitting diode. *Electrophoresis* 27:4039-4046.
- Montañes, F., Fornari, T., Martin-Alvarez, P. J., Corzo, N., Olano, A., Ibañez, E. 2006. Selective recovery of tagatose from mixtures with galactose by direct extraction with supercritical CO<sub>2</sub> and different cosolvents. *J. Agric. Food Chem.* 54:8340-8345.
- Montañes, F., Fornari, T., Martin-Alvarez, P. J., et al. 2007. Selective fractionation of disaccharide mixtures by supercritical CO<sub>2</sub> with ethanol as co-solvent. *J. Supercrit. Fluids* 41:61-67.
- Montilla, A., Moreno, F. J., Olano, A. 2005. A reliable gas capillary chromatographic determination of lactulose in dairy samples. *Chromatographia*, 62:311-314.

- Montilla, A., Ruiz-Matute, A. I., Sanz, M. L., Martinez-Castro, I., del Castillo, M. D. 2006b. Difuctose anhydrides as quality markers of honey and coffee. *Food Res. Int.* 39:801-806.
- Montilla, A., van de Langemaat, J., Olano, A., del Castillo, M. D. 2006. Determination of oligosaccharides by conventional high resolution gas chromatography. *Chromatographia* 63:453-458.
- Morales, V. Olano, A., Corzo, N. 2004. Ratio of maltose to maltulose and furosine as quality parameters for infant formula. *J. Agric. Food Chem.* 52:6732-6736.
- Morales, V., Corzo, N., Sanz, M. L. 2008. HPAEC-PAD oligosaccharide analysis to detect adulterations of honey with sugar syrups. *Food Chem.* 107:922-928.
- Morales, V., Sanz, M. L., Olano, A., Corzo, N. 2006. Rapid separation on activated charcoal of high oligosaccharides in honey. *Chromatographia* 64:233-238.
- Morales-Cid, G., Simonet, B. M., Cardenas, S., Valvarcel, M. 2007. On-capillary sample cleanup method for the electrophoretic determination of carbohydrates in juice samples. *Electrophoresis* 28:1557-1563.
- Muir, J. G., Rose, R., Rosella, O. et al. 2009. Measurement of short-chain carbohydrates in common australian vegetables and fruits by high-performance liquid chromatography (HPLC). *J. Agric. Food Chem.* 57:554-565.
- Muir, J. G., Shepherd, S. J., Rosella, O., Rose, R., Barrett, J. S., Gibson, P. R. 2007. Fructan and free fructose content of common Australian vegetables and fruit. *J. Agric. Food Chem.* 55:6619-6627.
- Mullin, W. J., Emmons, D. B. 1997. Determination of organic acids and sugars in cheese, milk and whey by high performance liquid chromatography. *Food Res. Int.* 30:147-151.
- Murphy, S. P., Johnson, R. 2003. The scientific basis of recent US guidance on sugars intake. *Am. J. Clin. Nutr.* 78:827S-833S.

- Ninonuevo, M. R., Park, Y., Yin, H., et al. 2006. A strategy for annotating the human milk glycome. *J. Agric. Food Chem.* 54:7471-7477.
- Ninonuevo, M., An, H., Yin, H., et al. 2005. Nanoliquid chromatography-mass spectrometry of oligosaccharides employing graphitized carbon chromatography on microchip with a high-accuracy mass analyzer. *Electrophoresis* 26:3641-3646.
- Noe, C. R., Lachmann, B., Mollenbeck, S., Richter, P. 1999. Determination of reducing sugars in selected beverages by capillary electrophoresis. *Z. Lebensm. Unters. Forsch A* 208:148-152.
- Okatch, H., Torto, N., Armateifio, J. 2003. Characterisation of legumes by enzymatic hydrolysis, microdialysis sampling, and micro-high-performance anion-exchange chromatography with electrospray ionisation mass spectrometry. *J. Chromatogr. A* 992:67-74.
- Paredes, E., Maestre, S. E., Prats, S., Todoli, J. L. 2006. Simultaneous determination of carbohydrates, carboxylic acids, alcohols and metals in foods by high-performance liquid chromatography inductively coupled plasma atomic emission spectrometry. *Anal. Chem.* 78:6774-6782.
- Park, Y. K., Yetley, E. A. 1993. Intakes and food sources of fructose in the United States. *Am. J. Clin. Nutr.* 58:737S-747S.
- Peris-Tortajada, M. 2000. HPLC determination of carbohydrates in foods. In: *Food analysis by HPLC*, ed. L. M. L. Nollet, New York, Marcel Dekker.
- Picinelli, A., Suarez, B., Moreno, J., Rodriguez, R., Caso-García, L. M., Mangas, J. J. 2000. Chemical characterization of asturian cider. *J. Agric. Food Chem.* 48:3997-4002.
- Pierce, K. M., Hope, J. M., Hoggard, J. C., Synovec, R. E. 2006. A principal component analysis based method to discover chemical differences in comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) separations of metabolites in plant samples. *Talanta* 70:797-804.

- Rojas-Escudero, E., Alarcon-Jimenez, A. L., Elizalde-Galvan, P., Rojo-Callejas, F. 2004. Optimization of carbohydrate silylation for gas chromatography. *J. Chromatogr. A*. 1027:117-120.
- Rovio, S., Yli-Kauhaluoma, J., Siren, H. 2007. Determination of neutral carbohydrates by CZE with direct UV detection. *Electrophoresis* 28:3129-3135.
- Ruiz-Matute A. I., Montilla A., del Castillo M. D., Martinez-Castro, I., Sanz, M. L. 2007. A GC method for simultaneous analysis of bornesitol, other polyalcohols and sugars in coffee and its substitutes. *J. Sep. Sci.* 30:557-562.
- Ruiz-Matute, A. I., Ramos, L., Martinez-Castro, I., Sanz, M. L. 2008. Fractionation of honey carbohydrates using pressurized liquid extraction with activated charcoal. *J. Agric. Food Chem.* 56:8309-8313.
- Ruiz-Matute, A. I., Sanz, M. L., Corzo, N., et al. 2007. Purification of lactulose from mixtures with lactose using pressurized liquid extraction with ethanol-water at different temperatures. *J. Agric. Food Chem.* 55:3346-3350.
- Ruiz-Matute, A. I., Sanz, M. L., Martinez-Castro, I. 2007b. Use of gas chromatography-mass spectrometry for identification of a new disaccharide in honey. *J. Chromatogr. A* 1157:480-483.
- Sharon, N. 2008. Lectins: past, present and future. *Biochem. Soc. Trans.* 36:1457-1460.
- Soga, T. 2002. Analysis of carbohydrates in food and beverages by HPLC and CE. In: *Carbohydrate analysis by modern chromatography and electrophoresis*, ed. Z. El Rassi. 483-503. Amsterdam, Elsevier Science.
- Soria, A. C., Sanz, M. L., Villamiel, M. 2009. Determination of minor carbohydrates in carrot (*Daucus carota L.*) by GC-MS. *Food Chem.* 114:758-762.
- Stikarovska, M., Chmelik, J. 2004. Determination of neutral oligosaccharides in vegetables by matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chim. Acta*, 520:47-55.

Suzuki, S., Kelly, J. F., Locke, S. J., Thibault, P., Honda, S. 2003. Derivatization of Carbohydrates. In: *Capillary electrophoresis of carbohydrate*, ed. P. Thibault, S. Honda. New York, Humana Press.

Taga, A., Suzuki, S., Honda, S. 2001. Capillary electrophoretic analysis of carbohydrates derivatized by in-capillary condensation with 1-phenyl-3-methyl-5-pyrazolone. *J. Chromatogr. A* 911:259-267.

Takahashi, N., Kato, K. 2003. GALAXY (Glycoanalysis by the three axes of MS and chromatography): a web application that assists structural analyses of N-glycans. *Trends Glycosci. Glycotech.* 15:235–251.

Takahashi, N., Nakagawa, H., Fujikawa, K., Kawamura, Y., Tomiya, N. 1995. Three-dimensional elution mapping of pyridylaminated N-linked neutral and sialyl oligosaccharides. *Anal. Biochem.* 226:139–146.

Terrab, A., Vega-Perez, J. M., Diez, M. J., Heredia, F. J. 2001. Characterization of northwest Moroccan honeys by gas chromatographic-mass spectrometric analysis of their sugar components. *J. Sci. Food Agric.* 82:179-185.

Tomiya, N., Awaya, J., Kurono, M., Endo, S., Arata, Y., Takahashi, N. 1988. Analyses of N-linked oligosaccharides using a two dimensional mapping technique. *Anal. Biochem.* 171:73–90.

Torto, N., Hofte, A., Van Der Hoeven, R., et al. 1998. Microdialysis introduction high-performance anion-exchange chromatography/ion-spray mass spectrometry for monitoring of on-line desalted carbohydrate hydrolysates. *J. Mass Spectrom.* 33:334-341.

Torto, N., Lobelo, B., Gorton, L. 2000. Determination of saccharides in wastewater from the beverage industry by microdialysis sampling, microbore high performance anion exchange chromatography and integrated pulsed electrochemical detection. *Analyst* 125:1379-1381.

Van de Lagemaat, J., Silvan, J. M., Moreno, F. J., Olano, A., del Castillo, M. D. 2007. In vitro glycation and antigenicity of soy proteins. *Food Res. Int.* 40:153-160.

Vendrell-Pascuas, S., Castellote-Bargallo, A. I., Lopez-Sabater, M. C. 2000. Determination of inulin in meat products by high performance liquid chromatography with refractive index detection. *J. Chromatogr. A* 881:591-597.

Ward, R. M., Gao, Q., de Bruyn, H., Gilbert, R. G., Fitzgerald, M. A. 2006. Improved methods for the structural analysis of the amylase-rich fraction from rice flour. *Biomacromolecules* 7:866-876.

Wei, Y., Ding, M. Y. 2002. Ethanolamine as modifier for analysis of carbohydrates in foods by HPLC and evaporative light scattering detection. *J. Liq. Chromatogr. Rel. Technol.* 25:1769-1778.

Wei, Y., Hendrix, D. L., Nieman, R. 1997. Diglucomelezitose, a novel pentasaccharide in silverleaf whitefly honeydew. *J. Agric. Food Chem.* 45:3481-3486.

Weston, R. J., Brocklebank, L. K. 1999. The oligosaccharide composition of some New Zealand honeys. *Food Chem.* 64:33-37.

Xiaoli, X., Liyi, Y., Shuang, H., et al. 2008. Determination of oligosaccharide contents in 19 cultivars of chickpea (*Cicer arietinum* L) seeds by high performance liquid chromatography. *Food Chem.* 111:215-219.

Xu, X., Kok, W. T., Poppe, H. *J. Chromatogr. A* 716:231-240.

Yagi, H., Kato, K. 2009. Multidimensional HPLC mapping method for the structural analysis of anionic N-glycans. *Trends Glycosci. Glycotech.* 21:95-104.

Yagi, H., Takahashi, N., Yamaguchi, Y., et al. 2005. Development of structural analysis of sulfated N-glycans by multidimensional high performance liquid chromatography mapping methods. *Glycobiology* 15:1051-1060.

Zaia, J. 2004. Mass spectrometry of oligosaccharides. *Mass Spectrom. Rev.* 23:161-227.

Zamfir, A. D., Bindila, L., Lion, N., Allen, M., Girault, H. H., Peter-Katalinic, J. 2005. Chip electrospray mass spectrometry for carbohydrate analysis. *Electrophoresis* 26:3650-3657.

Zhai, C., Li, C., Qiang, W., Xei, J., Yu, X., Ju, H. 2007. Amperometric detection of carbohydrates with a portable silicone/quartz capillary microchip by designed fracture sampling. *Anal. Chem.* 79:9427-9432.

Zhang, L., Xu, J., Zhang, L., Zhang, W., Zhang, Y. 2003. Determination of 1-phenyl-3-methyl-5-pyrazolone-labeled carbohydrates by liquid chromatography and micellar electrokinetic chromatography. *J. Chromatogr. B* 793:159-165.

Zhao, J., Patwa, T. H., Qiu, W., et al. 2007. Glycoprotein microarrays with multi-lectin detection: unique lectin binding patterns as a tool for classifying normal, chronic pancreatitis and pancreatic cancer sera. *J. Proteome Res.* 6:1864-1874.

## FIGURE CAPTIONS.

**Figure 1.** Example of monosaccharide and disaccharide structures.

**Figure 2.** Example of the chemical structure of two sugar polyols.

**Figure 3.** Example of the chemical structure of two fructooligosaccharides.

**Figure 4.** Typical polysaccharide basic structure.

**Figure 5.** HPAEC-PAD chromatographic profiles of oligosaccharide fraction of a honey sample H1 and H1 adulterated with 20% of (a) corn syrup (CS); (b) 20 high fructose corn syrup (HFCS) and (c) 40HFCS. Reprinted with permission from Morales et al. 2008.

**Figure 6.** Separation of carbohydrates (125  $\mu$ M each) under optimized conditions in a miniaturized device with amperometric detection. Tre, trehalose; Suc, sucrose; Lac, lactose; Gal, galactose; Glu, glucose; Man, mannose; Fru, fructose; Xyl, xylose. Reprinted with permission from Zhai et al. 2007.

**Figure 7.** IRMPD (Infrared multiphoton dissociation) spectrum of  $m/z$  1244 from a HPLC fraction in positive mode  $[M + Na]^+$  and (B) MALDI-FTMS spectrum of  $m/z$  1244 in digestion mixture with recombinant  $\beta(1-3)$ -galactosidase. The loss of a  $\beta(1-3)$ -galactose during digestion is consistent with the structure of 3'-FLNH (fucosyllacto-N-hexaose). Reprinted with permission from Ninonuevo et al. 2006.