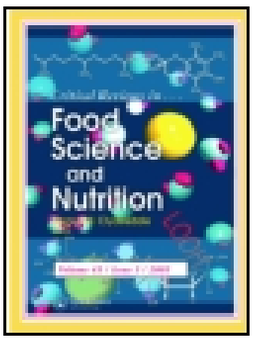

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Mass spectrometry–based analysis of whole grain phytochemicals

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Abstract: Whole grains are a rich source of several classes of phytochemicals, such as alkylresorcinols, benzoxazinoids, flavonoids, lignans, and phytosterols. A high intake of whole grains has been linked to a reduced risk of some major non-communicable diseases, and it has been postulated that a complex mixture of phytochemicals works in synergy to generate beneficial health effects. Mass spectrometry, especially when coupled with liquid chromatography, is a widely used method for the analysis of phytochemicals owing to its high sensitivity and dynamic range. In this review, the current knowledge of the mass spectral properties of the most important classes of phytochemicals found in cereals of common wheat, barley, oats, and rye, is discussed.

Keywords

barley, fragmentation, mass spectra, oats, rye, wheat

Introduction

Phytochemicals (from the Greek *phuton*, plant) are bioactive chemical compounds of plant origin that are present in all plant-based foods, including whole grains, fruits, and vegetables. In the fields of food science and clinical nutrition, the term is generally applied to non-nutrient compounds that are thought to have biological activity as part of the diet. The beneficial impact of a plant-based diet on human health, which has been indicated by epidemiological observations, has been suggested as potentially attributable to the composition of dietary phytochemicals (Slavin et al., 2001b, Liu, 2004, 2013). As discussed below, whole grains provide an important source of phytochemicals in the diet.

In the United States, whole grain has been defined as "the intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ, and bran – are present in the same relative proportions as they exist in the intact caryopsis" (AACC, 2014). However, in the European Union, the minimum requirement for the whole grain content of whole-grain flours and foods varies between 50 % and 90 % of their wet or dry weight (EFSA, 2010c). Currently, no universally accepted definition of whole grain exists.

Cereals are members of the monocot family Poaceae and are cultivated for their edible grains, also called caryopses in plant science. In terms of energy intake, cereals are by far the most important source of food worldwide, estimated to provide 53 % of the total calories consumed in 2015 and showing a slightly declining trend (Bruinsma, 2003). Therefore, they also make a major contribution to the total amounts of phytochemicals obtained from the diet, provided that the majority of the consumed cereals are whole grains. The four most abundantly produced

cereals in Northern Europe are wheat (*Triticum* sp.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and rye (*Secale cereale* L.), in decreasing order of production quantity in 2013 (FAO, 2014). Common wheat (*Triticum aestivum* L.), also known as bread wheat, accounts for over 95 % of worldwide wheat production (International Wheat Genome Sequencing Consortium, 2014). In addition to the common monocot cereals, seeds from various dicot species, termed pseudocereals, are also used in a similar manner to cereal grains. These species include amaranth and quinoa, both of which belong to Amaranthaceae, and buckwheat, a member of Polygonaceae.

This review focuses on the literature of identification of phytochemicals from whole grains and whole-grain breads obtained from common wheat, barley, oats, and rye and analyzed with methods that employ mass spectrometry (MS).

Whole grain phytochemicals and related health implications

Phytochemicals encompass many different metabolite classes and are the most diverse pool of chemicals in the biosphere. Phytochemicals are synthesized by plants as secondary metabolites that communicate with the environment; for example, they can act as defensive chemicals that are toxic to an attacking pathogen. Additionally, phytochemicals have a key role in the reproductive life cycle of plants. The major groups of phytochemicals present in whole-grain cereals include alkylresorcinols, avenanthramides, benzoxazinoids, carotenoids, flavonoids, lignans, phenolic acids, phytosterols, and tocols, all of which have been frequently reviewed and discussed in terms of their potential beneficial impacts on health (Slavin, 2003, Bondia-Pons et al., 2009, Okarter and Liu, 2010, Borneo and León, 2012, Bartłomiej et al., 2012, Andersson et

al., 2014). Whole grains also contain other lipophilic phytochemicals, such as linoleic acid (Prinsen et al., 2014) and sphingolipids (Zhu et al., 2013), which are known to have physiological functions in humans.

Some phytochemicals act as antinutrients that inhibit the absorption of nutrients. According to Slavin (2003), these include protease and amylase inhibitors, hemagglutinins, some phenolic compounds, and phytic acid. While such antinutrients can limit the nutritional value of food, they can also have beneficial health effects, such as reducing blood glucose and cholesterol levels and reducing the risk for certain types of cancer (Slavin, 2003). Various components of dietary fiber, including *beta*-glucan, inulin, and resistant starch, which are abundant in whole-grain cereals, have also been classified as phytochemicals because they resist digestion by human digestive enzymes (Liu, 2007).

Whole-grain cereals are one of the major food groups contributing to the intake of dietary phytochemicals, and frequent consumption of whole-grain products has been associated with a reduced risk of several chronic illnesses, including cardiovascular disease (Anderson, 2003, Jensen et al., 2004, 2006, Mellen et al., 2008), type 2 diabetes (de Munter et al., 2007, Aune et al., 2013), certain cancers (Jacobs Jr et al., 1998, Aune et al., 2011), as well as all-cause mortality (Jacobs Jr et al., 2000, Steffen et al., 2003, Olsen et al., 2011). Additionally, ferulic acid, the predominant phenolic acid in wheat (Zhou et al., 2004), has shown an ability in neuronal cell systems to greatly reduce free radical damage, which is involved in the etiology of neurodegenerative disorders such as Alzheimer's disease (Kanski et al., 2002). Despite convincing epidemiological evidence, little is known about the roles of specific components of

the cereal grain or the separate phytochemical groups that are responsible for the observed effects. Some specific phytochemicals that are present at appreciable concentrations in whole grains, such as phytosterols and lignans, have been reported to have beneficial health effects *in vivo* (Moghadasian and Frohlich, 1999, Buck et al., 2010) or have even been approved by authorities to be used in health claims (EFSA, 2010a, EFSA, 2010b). Nevertheless, the evidence suggests that a synergistic effect of the total mixture of bioactive chemicals may be behind the health benefits of whole-grain food consumption (Liu, 2004, Jacobs et al., 2009), as is the case for other plant-based foods.

An adequate amount of a phytochemical must be present in the gastrointestinal tract before a biological effect is manifested. The phytochemical concentration in the gastrointestinal tract is affected by its concentration in a given food item, the preparation process of the food, the amount of food consumed, and the bioavailability of the compound. For example, flax seeds and sesame seeds are rich in lignans, but their average consumption in the Western diet is low. Wheat and rye, although they each have a lower lignan content, are the major dietary sources of this compound group because they are consumed in vast amounts. Whole-grain rye is a rich source of alkylresorcinols (36–320 mg/100 g grain), phenolic acids (103–300 mg/100 g grain), and lignans (2 mg/100 g grain) (Bondia-Pons et al., 2009). The benzoxazinoid content in rye bread is in the range of 11 to 14 mg/100 g dry matter (DM) (Pedersen et al., 2011). Phytosterols have been detected in wheat grain in concentrations ranging from 67 to 96 mg/100 g DM (Nurmi et al., 2008), corresponding to 160–220 g of whole-grain wheat flour being required to deliver an adequate amount (150 mg) of phytosterols to induce a cholesterol-lowering effect (Ostlund et al., 2002). The amount of tocols in wheat grain has been determined to vary between 2.8 and 8.0

mg/100 g DM, of which 0.9–2.0 mg/100 g DM is *alpha*-tocopherol, the form that is mainly responsible for vitamin E activity (Lampi et al., 2008). A reasonable portion of the daily average requirement of *alpha*-tocopherol (5–6 mg) would be provided by 100 grams of whole-grain wheat, assuming an absorption rate of at least 55 % (Nordic Council of Ministers, 2012). Flavonoids, along with phenolic acids, tocols, and carotenoids, contribute to the antioxidant activity of a food product or its ability to inhibit the oxidation of other molecules (Velioglu et al., 1998). Wheat germ has a similar antioxidant activity to flaxseed and sunflower seed, but its antioxidant activity is lower than those of berries such as blueberries. The compounds responsible for the pigmentation of colored cereal varieties are anthocyanins, which are known for their strong antioxidative effects (Abdel-Aal et al., 2006). Some phytochemicals do not necessarily require absorption to function; these include phytosterols and *beta*-glucan, which act in the intestine to prevent the absorption of cholesterol (Ostlund et al., 2002, Liu, 2007). Additionally, several phytochemicals undergo microbiotic metabolism before absorption and might also affect the composition of the microbiota, as reviewed by Aura (2008) and Selma et al. (2009).

Effect of processing on the phytochemical profile of cereals

Because most phytochemicals are concentrated in the bran portion of cereals, it is assumed that the majority are lost during the milling process as a result of removal of the bran (Slavin et al., 2001a). Thermal treatment (oven baking) reduces the concentration of the natural antioxidants contained in unprocessed grain. However, it has been shown that heating induces the formation of new antioxidants, called Maillard reaction products (MRPs), which have a positive effect on

the overall antioxidant activity in the final bread product (Michalska et al., 2008). Michalska et al. also identified furosine, a common type of MRP, in rye bread by using HPLC-MS.

Bread-making modulates the phytochemical content of whole grains due to the hydrolysis of polysaccharides. For instance, arabinoxylan oligosaccharides (AXOS) are hydrolytic products of arabinoxylans, which are major constituents of cereal dietary fiber (Damen et al., 2012). Grain processing may alter the concentration and bioavailability of phytochemicals, while sourdough fermentation generally increases their ability to be absorbed, as reviewed by Poutanen et al. (2009). One exception is vitamin E activity, which is reduced by 20 to 60 percent after sourdough fermentation (Wennermark and Jagerstad, 1992). Overall, the bread-making process influences the concentrations of bioactive compounds in cereals, and to a lesser amount the qualitative phytochemical profile of the bread, as long as some of the bran portion is preserved.

Mass spectrometry in phytochemical research

Mass spectrometry (MS) is the prevailing method for the identification and quantification of phytochemicals (Allwood and Goodacre, 2010, Kaspar et al., 2011, Kueger et al., 2012, Wu et al., 2013). Applications of MS to the analysis of phytochemicals have been previously reviewed in more detail for flavonoids (Stobiecki, 2000, Cuyckens and Claeys, 2004, Fossen et al., 2006) and plant phenols (Ryan et al., 1999). MS detection is typically preceded by a chromatographic separation technique, such as high-performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UPLC or UHPLC), gas chromatography (GC), or thin-layer chromatography (TLC). Capillary electrophoresis (CE) may also be used. MS-based methods offer a higher sensitivity and selectivity compared to UV or diode array detectors (Andersson et

al., 2014); however, when coupled to MS, these detectors enhance metabolite identification capacity due to the particular absorption spectra related to different phytochemical classes. While NMR is useful for the structural elucidation of an unknown compound, it suffers from poor sensitivity compared to MS. It thus has limited applicability to non-targeted analysis of plant material, which often contains a wide range of compounds at low concentrations. In addition to the capability of highly accurate MS to detect substances at picomolar concentrations, tandem mass spectrometry (MS/MS or MS^n) is essential for providing structural information and for identifying compounds based on previously characterized fragmentation properties. With highly accurate MS instruments, it is possible to distinguish neutral losses of adducts which differ only slightly in mass, such as glucuronide (176.0321 Da) and feruloyl (176.0473 Da) moieties (Hanhineva et al., 2008).

The total number of metabolites produced in plants has been estimated to be as high as 1,060,000 (Afendi et al., 2012), although the estimates vary greatly. No single method is able to detect every compound present in a sample, but many important phytochemicals, such as flavonoids and phenolic acids, have semi-polar chemical characteristics and are therefore especially well-suited for separation and detection by reverse-phase LC-MS (De Vos et al., 2007). Indeed, all major groups of grain phytochemicals have been detected with LC-MS methods (Table 1). GC-MS is appropriate for volatile organic compounds (VOCs) and for a large variety of mainly primary metabolites, which must be derivatized via the addition of a trimethylsilyl (TMS) group to increase their volatility (Allwood and Goodacre, 2010). A clear advantage of GC-MS over LC-MS is its high reproducibility of both the retention time and the mass spectral fingerprint of an individual compound. These parameters have been collected in spectral libraries and greatly

aid compound identification, as reviewed by Scalbert et al. (2009). Even direct-injection approaches have been employed for crude plant extracts using MS instruments capable of high mass accuracy, but they suffer from adduct formation, ion suppression, and the inability to distinguish isomeric compounds (De Vos et al., 2007).

To be detected, analytes must be positively or negatively charged. Depending on the ionization technique, protonated or deprotonated molecules ($[M + H]^+$ or $[M – H]^–$), molecules with another cation (e.g., $[M + Na]^+$ or $[M + K]^+$), or radical cations ($M^{+\bullet}$) are observed. Classical ionization techniques, including electron impact (EI), fast atom bombardment (FAB), and thermospray ionization (TSI), have gradually been replaced by newer soft ionization methods that better preserve the precursor ion (Ryan et al., 1999) and are able to ionize a wider range of compounds. Currently, atmospheric pressure ionization (API) is the most widely used technique; the absence of vacuum at the ion source simplifies coupling with HPLC, one of the most important chromatographic separation methods for phytochemicals (White et al., 2012). The main ionization sources in API are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), both of which can produce protonated (in positive mode) or deprotonated (in negative mode) ions. ESI is based on exposing the liquid sample ejected from the capillary end to a high voltage, which results in the formation of increasingly small droplets that eventually each contain only one charged molecule (Yamashita and Fenn, 1984). In APCI, ionization is conducted in the gaseous phase, which makes it suitable only for thermally stable volatile compounds (Ryan et al., 1999). The process also causes some fragmentation of the precursor ion, especially at high voltage (Aramendía et al., 1996), which can be considered an advantage if additional structural information is sought. A more recent development in APCI is atmospheric

pressure photoionization (APPI), which uses photons from a vacuum-ultraviolet lamp at an energy level that is sufficient to cause the ionization of organic molecules (Robb et al., 2000). APPI has a large range of ionizable compounds and is especially suitable for the detection of lipophilic compounds such as steroids (Cai and Syage, 2006). A completely different ionization technique, matrix-assisted laser desorption ionization (MALDI), uses a laser to irradiate analyte molecules embedded in a light-absorbing matrix to prevent them from excessive fragmentation (Fossen et al., 2006). MS using MALDI is capable of measuring masses reaching 10^6 Da from picomolar concentrations, but the mass accuracy is only 100 to 1,000 ppm. Therefore, it is mostly used for the characterization of large molecules, such as sorghum polyflavans with masses over 1 kDa (Krueger et al., 2003).

The other major component of a mass spectrometer is the mass analyzer, which measures the mass-to-charge ratio (*m/z*) of ions produced by the ionization source. The simplest mass analyzer is the single quadrupole, which allows access to the detector only to ions with a certain *m/z* (White et al., 2012). Although low in resolution, *m/z* values of up to 4,000 can be measured (Fossen et al., 2006), allowing the analysis of polymeric phytochemicals, such as tannins. An ion trap (IT) analyzer accumulates molecular ions into a chamber where they can be isolated or further fragmented. Tandem mass spectrometry (MS/MS) includes at least two stages in which ions are selected, fragmented, and then reselected and measured (White et al., 2012). The two selective stages may be separated in space, as in a triple-quadrupole (TQ) mass spectrometer, or in time, as in a Q-Trap or time-of-flight (TOF) instrument. A Q-Trap has been used by Pedersen et al. (2011) and Tanwir et al. (2013) to detect benzoxazinoids. Quadrupole time-of-flight (qTOF), a hybrid between in-space and in-time methods, has become common for the

characterization of phytochemicals in cereals (Liu et al., 2010, Inglett et al., 2011, Hanhineva et al., 2011, 2012). Its advantages include a theoretically unlimited mass range, high sensitivity, and high mass accuracy (Fossen et al., 2006). Fourier transform ion cyclotron resonance (FT-ICR) is another highly accurate mass analysis technique that can separate peaks with an *m/z* difference of 10^{-3} Da and measure masses with high accuracy (Matus-Cádiz et al., 2008). In a study conducted by Matus-Cádiz et al., it was used for the detection of phenolic compounds that caused the pigmentation of an Argent cultivar of hard white wheat. Beckmann et al. (2013) studied benzoxazinoids and their degradation products with the technique. Both qTOF and FT-ICR are capable of a mass accuracy better than 3 ppm, a suggested threshold for the reliable determination of molecular formulae (Kind and Fiehn, 2007). A third method especially suitable for untargeted metabolomics assays is the LTQ (linear trap quadrupole) Orbitrap instrument, which uses both a mass analyzer and a detector based on the ion trap method (Wu et al., 2013).

In the following section, the current knowledge about the mass spectral properties of major whole-grain phytochemicals is reviewed. The characteristic mass spectral properties of specific compounds are presented in Table 1 in ascending order based on calculated neutral mass.

Alk(en)ylresorcinols

Alk(en)ylresorcinols (ARs) are 1,3-dihydroxybenzene derivatives with a long alkyl or alkenyl chain (15 to 27 carbons, usually odd numbered, in cereals) in the *meta* position, which causes the compounds to be lipophilic. Oxygenated forms also exist, either as oxo- or hydroxyalk(en)ylresorcinols (Seitz, 1992). ARs can be found in barley, rye, and wheat (Knödler et al., 2008). The ratio of $C_{17:0}$ to $C_{21:0}$ homologues is approximately 0.1 in wheat and 1.0 in rye,

suggesting that ARs can be used as specific biomarkers of wheat or rye bran intake, as ARs are located exclusively in the bran portion of the cereal grain (Chen et al., 2004, Landberg et al., 2008). However, ARs have also been detected at low concentrations (at an average of 1/25 the concentration in whole-grain rye bread) in all of the five Latvian and Finnish white wheat breads analyzed by Meija et al. (2013); their presence may be due to grain processing techniques that result in a small amount of bran in the bread. A GC-MS method developed by Ross et al. (2001) has been used to analyze ARs in cereals and cereal products (Landberg et al., 2006, 2008, Meija et al., 2013). Suzuki et al. (1997) used TLC-FAB-MS/MS for the structural analysis of ARs in whole rye and wheat flour. Also HPLC-APCI-MS^n was used by Knödler et al. (2008) and Geerkens et al. (2015) to identify 29 and 39 AR homologues, respectively (Table 1). This method proved useful for compound characterization but was unable to indicate the positions of double bonds or keto/hydroxyl groups. Interestingly, an AR homologue with an even-numbered alkyl chain ($C_{18:0}$) was found at low concentrations in wheat and rye.

The fragmentation patterns of saturated and mono- or polyunsaturated AR ions differ significantly (Knödler et al., 2007, 2008). A typical positive fragment ion at *m/z* 111 is yielded from saturated $C_{15:0}$, $C_{17:0}$, $C_{18:0}$, and $C_{19:0}$ homologues, and it is suggested to arise from the heterolytic *alpha*-cleavage of the aromatic group (Figure 1 A). In other homologues, a positive product ion at *m/z* 123 is usually detected due to *beta*-fission of the alkyl chain, thus forming a 1,3-dihydroxytropylium ion. Ross et al. (2001) reported other typical positive fragments at *m/z* 124 that resulted from McLafferty rearrangement and at *m/z* 137 due to *gamma* cleavage. While Knödler et al. (2008) and Geerkens et al. (2015) found only a few minor peaks at *m/z* 124 for the identified compounds, Seitz (1992) reported *m/z* 124 as a major peak in all

oxoalk(en)ylresorcinols. Positive product ions at *m/z* 137, 149, and 163 have been identified as putative alkyl chains with one double bond conjugated with the aromatic group, thus indicating an unsaturated alkenyl structure (Knödler et al., 2008). A neutral loss of 18 Da (H_2O) is seen with oxidized ARs. The loss of water was assumed to occur from a hydroxyl group in the alk(en)yl chain, as dehydroxylation from a keto group is less common. Moreover, a product ion at *m/z* 123 was also observed with these compounds, indicating an intact dihydroxybenzene structure. However, when using HPLC-EI-MS, Iwatsuki et al. (2003) found only keto-substituted AR homologues and observed IR spectra to support the presence of a keto group. Seitz (1992) also identified keto groups when using HPLC and observed additional traces of hydroxyl groups by GC-MS. In FAB-MS/MS, saturated ARs produce an evenly spaced (14-Da) series of peaks originating from losses of C_nH_{2n+2} from the alkyl terminus (Suzuki et al., 1997). Unsaturated homologues give similar patterns but with a typical 'window' of 54 Da due to the presence of a double bond.

Avenanthramides

Avenanthramides are composed of hydroxycinnamic and anthranilic acids that form an amide. The most common forms are those derived from 5-hydroxyanthranilic acid, which is linked to *p*-coumaric (abbreviated as 2p), caffeic (2c), or ferulic (2f) acids (Bratt et al., 2003). The 5-hydroxyanthranilic acid may also be replaced by anthranilic acid, 5-hydroxy-4-methoxyanthranilic acid, or 4,5-dihydroxyanthranilic acid (Bratt et al., 2003, Ishihara et al., 2014). The name of the group was coined by Collins (1989) when he found them in groats and hulls of oats using EI-MS combined with thin-layer chromatography. Indeed, among cereals, this

compound group is found exclusively in oats (Bratt et al., 2003). In all five identified avenanthramides, the most abundant fragment ion was found at *m/z* 147, 163, or 177 (depending on the substituent groups), correlating with the cinnamoyl moiety (Collins, 1989). Similarly, ions at *m/z* 135 and 153 were identified as the protonated anthranoyl moiety. These fragments are easily detected, as the amide bond between them is usually cleaved (Ishihara et al., 2014). A loss of water was detected from the molecular and anthranoyl ions, while the cinnamoyl ion experienced a loss of CO (28 Da). Verardo et al. (2011) reported a neutral loss of 44 Da due to decarboxylation and a common fragment ion at *m/z* 178 in avenanthramides 2p, 2c, and 2f when using HLPC-ESI-MS. They also identified one avenanthramide dehydrodimer, namely bisavenanthramide B1. Recently, Ishihara et al. (2014) identified three avenanthramides containing 4,5-dihydroxyanthranilic acid; these avenanthramides showed stronger radical-scavenging activity *in vitro* than the most common forms.

Benzoxazinoids

Benzoxazinoids are heterocyclic compounds with an oxazine (a 5- or 6-carbon ring containing one oxygen and one nitrogen atom) connected to a benzene ring. Based on additional substituents, they are further divided into benzoxazolinones (e.g., benzoxazol-2-one or BOA, Table 1), lactams (e.g., 2-hydroxy-1,4-benzoxazin-3-one or HBOA), and hydroxamic acids (e.g., 2,4-dihydroxy-1,4-benzoxazin-3-one [DIBOA] and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one [DIMBOA]). Among cereals, benzoxazinoids have thus far been identified in corn, rye, and wheat (Pedersen et al., 2011). Benzoxazinoids can be found in plants as storage glycosides that are quickly and enzymatically hydrolyzed into the aglycone form when the plant is damaged

(Hietala and Virtanen, 1960). Increasing evidence suggests that dietary benzoxazinoids are converted into phenylacetamides that are detectable in urine and plasma after consumption of a meal rich in whole grains (Hanhineva et al., 2014). Mass spectrometric methods applied to the analysis of benzoxazinoids include UPLC-Q-TOF, which has been used to analyze whole-grain rye and wheat cereals (Hanhineva et al., 2011), FT-ICR (Beckmann et al., 2013), and LC-Q-Trap, which has been used for common wheat, durum wheat, and rye (Pedersen et al., 2011, Tanwir et al., 2013).

Published data on the MS-based identification of benzoxazinoids from cereals is scarce. In benzoxazinoid glycosides, a neutral loss of one or two hexose units (nominal masses of 162 or 324 Da, respectively) is typically observed (Hanhineva et al., 2011). Further fragmentation of the aglycone form produces ions that have lost a water molecule (18 Da) or 1–2 CO moieties (28 Da each). In benzoxazinoids containing a methoxy group, such as DIMBOA, the loss of a methoxy radical (15 Da) may be observed. In Figure 1 B, the fragmentation pattern of DIBOA hexoside is presented.

Betaine

Betaine (more accurately glycine betaine or *N*,*N*,*N*-trimethylglycine) is a trimethyl derivative of the amino acid glycine that holds a quaternary ammonium ion and exists as a zwitterion at physiological pH (Craig, 2004). It is an important osmolyte in plants and animals that protects cells against changes in salinity. In humans it also acts as a methyl donor and takes part in the conversion of homocysteine into methionine. Because it is highly polar, betaine is poorly separated from other polar compounds by traditional reverse-phase LC methods, and it requires

chemical derivatization for GC-MS. Hydrophilic interaction chromatography (HILIC) more strongly retains polar compounds such as quaternary amines, and it is therefore a suitable method for the detection of betaine (Guo, 2005). Ross et al. (2014) used LC-ESI-MS/MS coupled with an HILIC column for the detection of betaine from refined and whole-grain wheat. The typical fragments at *m/z* 58 and 59 were used as identifier ions. The method was developed by Bruce et al. (2010), who detected betaine in barley, oats, rye, and wheat.

Carotenoids

The basic structure of carotenoids consists of eight isoprene units and contains a total of 40 carbon atoms. Oxygenated carotenoids such as lutein are called xanthophylls, while pure hydrocarbons are carotenes. ESI-IT-MS coupled with liquid chromatography was used by Abdel-Aal et al. (2007) for the identification and quantification of carotenoids in various wheat species and cultivars. In the ESI(+) ionization mode, lutein produces ions at *m/z* 551 and 533 that correspond to a sequential loss of two water molecules (Table 1). Zeaxanthin, an isomer of lutein, shows similar fragmentation but with a different relative abundance; while lutein shows a dominant ion at *m/z* 551, the protonated zeaxanthin ion at *m/z* 569 is more abundant than its product ions, indicating that lutein is more prone to lose water. For *beta*-carotene, the main fragment is the protonated molecular ion at *m/z* 537. While zeaxanthin was detected in common wheat in only trace amounts, it was present in einkorn (*Triticum monococcum* L.), Khorasan (*Triticum turanicum* Jakubz.), and durum wheat (*Triticum durum* Desf.). Using HPLC-ESI-MS, Moore et al. (2005) reported that zeaxanthin was present in common wheat at a higher concentration than *beta*-carotene.

Flavonoids

Flavonoids are a large group of phenolic compounds, all of which consist of a six-membered ring condensed to a phenyl ring carrying a second phenyl ring in the 2' position (Havsteen, 1983). They are further divided into anthocyanidins, flavanols (flavan-3-ols), flavanones, flavones, flavonols, isoflavones, and several other minor classes according to their chemical structures and characteristics. Flavonoids exist as monomeric aglycones, oligomers, and forms with one or several saccharide units (Fossen et al., 2006). Proanthocyanidins, also referred to as condensed tannins, are oligomers composed of flavanol units (Dykes and Rooney, 2007). Flavonoids are produced by all cereals, with inter-species variation in their composition. According to Dykes and Rooney, anthocyanins have been reported in pigmented varieties of barley, rye, and wheat, flavanols in barley, flavanones in barley and oats, and flavones in oats and wheat. Additionally, flavonols are known to exist in barley (Bollina et al., 2010) and colored wheat (Liu et al., 2010). HPLC coupled with MS using different atmospheric pressure ionization techniques has been used for the detection of wheat anthocyanins (Abdel-Aal et al., 2006, Hosseinian et al., 2008) and wheat flavones (Asenstorfer et al., 2006, Ioset et al., 2007). Dinelli et al. (2011) used HPLC-ESI-TOF-MS in the analysis of flavonoids and other phenolic compounds in modern and ancient wheat varieties. Verardo et al. (2008a) analyzed free flavan-3-ols in barley using a similar technique but without time-of-flight detection.

According to Abdel-Aal et al. (2006), anthocyanins have been observed in the ESI(+) mode as molecular ions $[M]^+$ and fragment ions $[M - X]^+$ due to their inherent positive charge. Using both ESI and APCI, Hosseinian et al. (2008) reported only protonated ions in the positive mode,

although the reported masses correspond to unprotonated molecular ions. The typical fragment ion is the anthocyanin aglycone that is deprived of the saccharide moiety (Abdel-Aal et al., 2006). The main aglycones are pelargonidin (*m/*z 271), cyanidin (*m/*z 287), peonidin (*m/*z 301), delphinidin (*m/*z 303), petunidin (*m/*z 317), and malvidin (*m/*z 331), all of which are present in some colored cereal varieties (Hosseinian et al., 2008). The saccharide unit may be arabinose (132 Da), galactose (162 Da), or glucose (162 Da). Flavones often exist in cereals as diglycosides, which can be esterified to a hydroxycinnamic acid, namely ferulic or sinapic acid (Asenstorfer et al., 2006). Some anthocyanin or flavone aglycone pairs, such as pelargonidin and apigenin or cyanidin and luteolin, respectively, share the same mass as positive ions and require an examination of the fragmentation pattern to distinguish them. Proanthocyanidins are fragmented by three mechanisms: retro-Diels–Alder cleavage that results in the elimination of H_2O, a quinone–methine mechanism that leads to interflavanic bond cleavage between (epi)catechin and (epi)gallocatechin units, and heterocyclic ring fission (Verardo et al., 2008a). Catechin may be observed as a neutral loss of 290 Da or as a charged fragment ion in both positive and negative mode. Notable product ions yielded by catechin are at *m/z* 245 in the ESI(–) mode, suggested to result from the loss of a CH_2CHOH group, and at *m/z* 139 in the ESI(+) mode, resulting from retro-Diels–Alder cleavage.

Lignans

Lignans are polyphenolic compounds consisting of a dibenzylbutane skeleton (Axelson et al., 1982). They are further divided into dibenzylburytolactones (e.g., matairesinol), butanediols (secoisolariciresinol), aryltetralines, furanolignans, and furofuranolignans (pinoresinol and

syringaresinol) (Eklund et al., 2008). Lignans that contain three or four phenylpropanoid units are called sesquilignans or dilignans, respectively (Willför et al., 2006). Axelson et al. first reported evidence that plant lignans are converted into the mammalian lignans enterodiol and enterolactone by the human microbiota. Lignans have been detected in several higher plants, including barley, oats, rye, triticale, and wheat (Dykes and Rooney, 2007). Hanhineva et al. (2012) used UPLC-ESI-QTOF-MS to identify previously undetected lignans, including buddlenols, in rye bran. CE-ESI-IT-MS has been used for profiling lignans in common wheat cultivars (Dinelli et al., 2007).

Only lignans with one or more phenolic groups can be deprotonated and become visible in negative ion mode, unless they are analyzed as their acetate adducts (Eklund et al., 2008). The presence of a hydroxymethyl group, as in lariciresinol, typically leads to the neutral loss of formaldehyde (30 Da) in MS/MS fragmentation (Hanhineva et al., 2012). Dibenzylbutyrolactones exhibit a neutral loss of 15 Da, which results from the cleavage of a methyl radical ion from a methoxy group (Hanhineva et al., 2012), and a 44-Da loss of CO_2, presumably from the lactone ring (Eklund et al., 2008). Among butanediols, a loss of 48 Da is observed, which is likely from simultaneous cleavage of formaldehyde and water. Furofuranolignans, such as pinoresinol and syringaresinol, are cleaved inside the tetrahydrofuran ring, thus producing negative fragment ions at *m/z* 151 and 181, respectively (Hanhineva et al., 2012). In addition, these fragments may each lose a methyl radical, resulting in ions at *m/z* 136 and 166, respectively. In a study by Hanhineva et al., all sesquilignans and dilignans produced a fragment ion at *m/z* 195 that originated from the additionally cleaved guaiacylglycerol moiety.

The fragmentation pattern of buddlenol C, another furofuranolignan abundant in rye, is shown in Figure 1 C.

Phenolic acids

Phenolic acids, which are present in all cereals, can be divided into two major groups, namely hydroxybenzoic acids, with a C6–C1 skeleton, and hydroxycinnamic acids, with a C6–C3 skeleton (Liu, 2007). Hydroxybenzoic acids, including protocatechuic, *p*-hydroxybenzoic, vanillic, and syringic acid, as well as ferulic, caffeic, *p*-coumaric, and sinapic acid, which are representative hydroxycinnamic acids, have been identified in most of the common cereals (Dykes and Rooney, 2007). Ferulic acid may be dimerized through photochemical or radical coupling reactions (Bunzel et al., 2001). According to Bunzel et al., the oligomerized forms are suggested to cross-link cell wall polysaccharides. Verardo et al. (2008b) analyzed bound hydroxycinnamic acids in barley using HPLC-ESI-MS, while Bauer et al. (2012) used a similar method to identify hydroxycinnamic acids in wheat bran. Among other phenolic compounds, Dinelli et al. (2011) found phenolic acids in wheat using HPLC-ESI-TOF-MS. Bunzel et al. (2001) identified eight different diferulates from barley, oats, rye, and wheat using gas liquid chromatography (GLC) coupled with IT-MS.

Ferulic acid produces typical negative fragment ions at *m/z* 178 (from demethylation) and 134 (from demethylation and decarboxylation) (Figure 1 D). Meanwhile, *p*-coumaric acid produces a negative fragment ion at *m/z* 119, possibly due to decarboxylation. Several phenolic acids were found to be esterified to sugars, either pentose (neutral loss of 132 Da) or hexose (neutral loss of 162 Da). Diferulic acids produced a major fragment ion at *m/z* 341 (Bauer et al., 2012), which

likely corresponds to the decarboxylation of the other carboxyl group, thus releasing CO_2 (neutral loss 44 Da). However, no such fragment was detected by Bunzel et al. (2001) from TMS derivatives of diferulates.

Phytosterols

Phytosterols or plant sterols encompass plant-derived sterols and stanols. Both share a steroid structure, but stanols have a fully saturated sterol ring skeleton. In addition to their free forms, phytosterols also exist in plants as esters that are bonded with fatty acids or phenolic acids (Table 1) and with sugars (Nyström et al., 2007). Mass spectrometric data have been obtained from GC-EI-MS analysis of free and esterified phytosterols in corn, rye, spelt and wheat (Esche et al., 2012). Steryl fatty acid esters showed a concomitant neutral loss of the fatty acid moiety and water, and they displayed a fragment ion at *m/z* 81. For *p*-coumarate esters, a fragment $[M - 15]^+$ was detected instead of the molecular ion. Typical fragments for sterols and their fatty acid esters were observed at *m/z* 255 and 213, respectively, and fragments for stanols and their fatty acid esters were observed at *m/z* 257 and 215, respectively.

Sphingolipids and other lipids

Sphingolipids are complex compounds that consist of a fatty acid residue and a variable polar group (e.g., phosphocholine, sugar, or complex carbohydrates) that is bonded to a sphingosine backbone. They represent major components of the eukaryotic cell membrane, and a large variety of sphingolipids is also present in cereal grains (Table 1). There are some challenges in the analysis of sphingolipids that are related to sample preparation (because of their varied lipophilicity) and the identification of individual components in MS/MS due to structural

complexity (Merrill Jr et al., 2005). According to Merrill Jr. et al., a wider range of sphingolipids is readily ionized in positive mode than in negative mode. In a study published by Zhu et al. (2013), sphingolipids were analyzed in wheat bran using LC-ESI-IT-MS. All of the identified sphingolipids were also found in the ESI(–) mode as acetate adducts $[M + 60 – H]^-$, which were used by the authors as selective markers for the compound class. The acetate adducts have been previously reported under both positive and negative ionization modes (Kerwin et al., 1994). The cleavage of carboxylic acid led to a neutral loss of a particular mass, which was indicative of the fatty acyl chain length. All cerebrosides (which contain sugar as the polar group) showed neutral losses of 162 and 180 Da.

The bran and germ portions of a grain contain a diverse mixture of other lipophilic compounds, such as *alpha*-linolenic acid (Fardet, 2010). Lipids are easily ionized, resulting in their dominance in total ion chromatograms taken from cereal or bread samples (Figure 2). LC-MS is currently the preferred method for lipid analysis, as lipids are generally too non-volatile and thermolabile for gas chromatography, and UV detection lacks sensitivity and specificity (Cai and Syage, 2006). Among ionization techniques, atmospheric pressure photoionization (APPI) has shown the best performance when compared with APCI and ESI. Nevertheless, Prinsen et al. (2014) successfully conducted a thorough analysis of lipophilic compounds in wheat bran using GC-IT-MS. Among the identified compounds were fatty acids and their mono-, di-, and triglycerides; phytosterols and their glycosides, esters, and ferulates; steroid hydrocarbons and ketones; and alkylresorcinols, for a total of 114 compounds.

Tocols

Tocols include four tocopherols (*alpha*, *beta*, *gamma*, and *delta*-T) and four tocotrienols (*alpha*, *beta*, *gamma*, and *delta*-T3), all of which are also known as vitamin E (Bustamante-Rangel et al., 2007). They consist of a polar chromanol ring substituted with a 16-carbon side chain. While tocopherols have a completely saturated side chain, in tocotrienols the side chain has three double bonds (Lampi et al., 2008). Because they are highly lipophilic, they are not readily ionizable by standard ESI-MS and thus require the addition of a base (e.g., ammonia) into the mobile phase (Bustamante-Rangel et al., 2007). Using this method, tocols in wheat and rye cereals were analyzed by Bustamante-Rangel et al. The tocol profile of several wheat varieties was determined by Lampi et al. using LC-ESI-IT-MS. *Alpha*-tocopherol and -tocotrienol produced typical fragment ions at *m/z* 205 and 165, respectively, while *beta*-tocopherol and -tocotrienol produced ions at *m/z* 191 and 151, respectively, and *gamma*-tocopherol and -tocotrienol produced ions at *m/z* 177 and 137, respectively (Lampi et al., 2008).

Other phytochemicals

Beta-glucan, more specifically (1→3),(1→4)-*beta*-D-glucan, is a linear polysaccharide composed of D-glucose units that are mainly linked together by *beta*-(1→4) glycosidic bonds (Jiang and Vasanthan, 2000). Owing to the long and variable polymer chains of *beta*-glucan, it is more feasible to analyze shorter oligosaccharides that have been hydrolyzed by the lichenase enzyme. Such oligosaccharides, composed of 3 to 12 glucose units (DP3–DP12), have been extensively studied in barley using MALDI-MS (Jiang and Vasanthan, 2000, Yoo et al., 2007, Ghotra et al., 2008). The dominant hydrolysates, DP3 and DP4, produced sodium adducts at *m/z* 526 and 688, respectively (Ghotra et al., 2008). Potassium adducts were also detected.

Dicarboxylic acids are organic acids with the general formula $HO_2C–(CH_2)_n–CO_2H$. Compounds in this group include oxalic acid (n = 0), malonic acid (n = 1), succinic acid (n = 2), and azelaic acid (n = 7). Azelaic acid has bactericidal properties, and it has been used for the topical treatment of acne vulgaris (Charnock et al., 2004). It has also been reported to possess antitumor and radical-scavenging activity (Nazzaro-Porro et al., 1980, Passi et al., 1991). Dicarboxylic acids, such as malonic acid from barley, have been identified in cereals by using a metabolomics-based approach with LC-ESI-LTQ-Orbitrap (Bollina et al., 2010), while succinic acid has been identified in wheat using GC-IT-MS (Zörb et al., 2009, Högy et al., 2010). Azelaic acid has been detected in oats by HPLC-ESI-MS (Verardo et al., 2011). In the ESI(–) mode, it produces a main fragment ion at *m/z* 125, which has been proposed to result from a loss of water and CO_2 (Yasmeen et al., 2011).

Inositols are polyols of cyclohexane. The most abundant form in nature among the nine possible stereoisomers is *myo*-inositol, which may in turn be phosphorylated to form 63 different inositol phosphate isomers (Liu et al., 2009). In plants, the primary phosphates are the completely phosphorylated phytic acid (inositol hexakisphosphate, or $InsP_6$) and its lower-phosphate derivatives ($InsP_1$–$InsP_5$). *Myo*-inositol and its phosphates have shown chemopreventive and chemotherapeutic effects in cancer cells and in animal cancer models (Liu et al., 2009). Using ESI-MS/MS coupled with anion-exchange HPLC, Liu et al. were the first to simultaneously detect *myo*-inositol and the phosphates $InsP_1$–$InsP_6$ in oat grains. In the negative ion mode, *myo*-inositol produced a negative fragment at *m/z* 161 due to the loss of a water molecule. $InsP_1$ exhibited a neutral loss of 180 Da, indicative of the loss of the inositol ring. Other InsP forms showed a neutral loss of 98 Da, which is characteristic of the cleavage of one phosphate group.

Inositols have also been detected in wheat and barley using a metabolomics approach and by HPLC-MS or GC-MS (Zörb et al., 2009, Högy et al., 2010, Gorzolka et al., 2012).

Policosanols is the name assigned to long-chain aliphatic primary alcohols, primarily docosanol (C_{22}), tetracosanol (C_{24}), hexacosanol (C_{26}), octacosanol (C_{28}), and triacontanol (C_{30}) (Irmak et al., 2006). Although their nutritional value has not been widely studied, they have been shown to reduce total and LDL cholesterol and to increase HDL cholesterol levels in humans, as reviewed by Fardet (2010). Irmak et al. (2006) used GC-MS for the detection of policosanols in wheat extract. Because of their simple structure, mainly positive fragments from $[M - 15]^+$ and from the terminal ether group of TMS at *m/z* 103 are expected for all of these compounds, as presented in Table 1 (Harrabi et al., 2009).

Polyamines are compounds containing two or more primary amino groups. They occur in plants as free bases, as peptide or amino acid derivatives (Guggisberg and Hesse, 1984), and as phenolic acid conjugates (Savolainen et al., 2014). The most-studied compounds in this group are putrescine, spermidine, and spermine. They are also biosynthesized in animal cells, where they act as modulators of DNA, RNA, proteins, and nucleoside triphosphates, such as ATP (Igarashi and Kashiwagi, 2000). Spermidine and spermine were detected from wheat grains by Zörb et al. (2009) after metabolic profiling with GC-IT-MS. Both compounds produced a fragment ion at *m/z* 144, which corresponds to a deprotonated spermidine ion. Högy et al. (2010) also found putrescine in wheat grain using a similar method. Savolainen et al. (2014) used UPLC-qTOF for the analysis of phenolic acid derivatives of polyamines and other metabolites in fermented wheat bran.

Saponins contain 1–3 sugar chains linked to an aglycone that has either a steroid or triterpene structure (Güçlü-Üstündağ and Mazza, 2007). Steroidal saponins are referred to as avenacosides and triterpene saponins as avenacins, both of which are found in oats but with mutually exclusive distributions inside the plant (Osbourn, 2003). Several biological activities have been reported for saponins, including antithrombotic and immunostimulatory effects, but the clinical significance of such findings is unclear, as saponins show low bioavailability (Güçlü-Üstündağ and Mazza, 2007). Pecio et al. (2013) analyzed avenacosides from the grains of 16 oat cultivars using UPLC-ESI-TQ-MS. Molecular ions of three avenacosides (avenacosides A and B and 26-desglucoavenacoside A) were detected in the ESI(–) mode (Table 1).

Conclusion

Various analytical methods have been developed for the identification of particular phytochemical groups. The metabolomics approach with high mass accuracy LC-MS systems has been used for profiling the overall phytochemical content of cereals and cereal products. As reviewed in this article, the MS-based knowledge of phytochemicals in the four principal Northern European cereals includes compounds from a variety of chemical classes, and altogether more than 200 individual metabolites with their fragmentation data are reported in the literature. However, it is clear that the list is still far from complete because numerous compounds detectable in MS still lack structure elucidation, available reference compounds, or published MS/MS data. Further examination of the chemical properties of these unknown compounds is necessary in order to identify them and take them into account when addressing the research question of how whole grain phytochemicals affect health.

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of normal skin (ID 164), maintenance of normal nails (ID 164), maintenance of normal cardiac function (ID 166), maintenance of normal vision by protection of the lens of the eye (ID 167), contribution to normal cognitive function (ID 182, 183), regeneration of the reduced form of vitamin C (ID 203), maintenance of normal blood circulation (ID 216) and maintenance of normal a scalp (ID 2873) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J.* **8 (10)**: 1816.

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Table 1. Phytochemicals detected in the four main Northern European cereals using MS-based methods, with fragmentation data and the method used for obtaining the data. Species: b = barley, o = oats, r = rye, w = common wheat. Orb. = Orbitrap. References: (1) Högy et al., 2010; (2) Bruce et al., 2010; (3) Dinelli et al., 2011; (4) Hanhineva et al., 2011; (5) Zörb et al., 2009; (6) Verardo et al., 2008b; (7) Bauer et al., 2012; (8) Liu et al., 2009; (9) Verardo et al., 2011; (10) Geerkens et al., 2015; (11) Collins, 1989; (12) Verardo et al., 2008a; (13) Ishihara et al., 2014; (14) Zarnowski and Suzuki Y, 2004; (15) Knödler et al., 2008; (16) Irmak et al., 2006; (17) Bollina et al., 2010; (18) Hanhineva et al., 2012; (19) Esche et al., 2012; (20) Seitz, 1992; (21) Hosseinian et al., 2008; (22) Bustamante-Rangel et al., 2007; (23) Lampi et al., 2008; (24) Iwatsuki et al., 2003; (25) Savolainen et al., 2014; (26) Liu et al., 2010; (27) Ioset et al., 2007; (28) Abdel-Aal et al., 2007; (29) Zhu et al., 2013; (30) Asenstorfer et al., 2006; (31) Pecio et al., 2013. Fragments reported in italic are provided by the authors (using UPLC-ESI-qTOF-MS) from unpublished data.

neutral mass	molecular formula	putative name	class	fragments (−)	fragments (+)	species	method	ref.
88.100	$C_4H_{12}N_2$	putrescine	polyamine	174		w	GC-IT	(1)
117.079	$C_5H_{11}NO_2$	trimethylglycine	betaine		58, 59	r, w	UHPLC-MS/MS	(2)
122.037	$C_7H_6O_2$	*p*-hydroxybenzaldehyde	phenolic acid			w	HPLC-ESI-TOF	(3)
135.032	$C_7H_5NO_2$	benzoxazol-2-one (BOA)	benzoxazinoid	112, 91, 78	136,04	r, w	UPLC-PDA-qTOF	(4)
145.158	$C_7H_{19}N_3$	spermidine	polyamine	144		w	GC-IT	(5)

146.037	$C_9H_6O_2$	coumarin	phenolic acid			w	HPLC-ESI-TOF	(3)
152.047	$C_8H_8O_3$	vanillin	phenolic acid			w	HPLC-ESI-TOF	(3)
164.047	$C_9H_8O_3$	*p*-coumaric acid	phenolic acid	119	187, 165	b, w	HPLC-ESI	(3,6,7)
168.042	$C_8H_8O_4$	vanillic acid	phenolic acid			w	HPLC-ESI-TOF	(3)
180.042	$C_9H_8O_4$	caffeic acid	phenolic acid	135		b	HPLC-ESI	(6)
180.063	$C_6H_{12}O_6$	*myo*-inositol	alcohol	161		o	HPLC-ESI	(8)
182.058	$C_9H_{10}O_4$	syringaldehyde	phenolic acid			w	HPLC-ESI-TOF	(3)
188.105	$C_9H_{16}O_4$	azelaic acid	dicarboxylic acid			o	HPLC-ESI	(9)
194.058	$C_{10}H_{10}O_4$	ferulic acid	phenolic acid	*133, 134, 178*	217, 195	b, w	HPLC-ESI	(3,7)
198.053	$C_9H_{10}O_5$	syringic acid	phenolic acid			w	HPLC-ESI-TOF	(3)
202.216	$C_{10}H_{26}N_4$	spermine	polyamine	144		w	GC-IT	(5)
224.069	$C_{11}H_{12}O_5$	sinapic acid	phenolic acid	*193, 93, 121, 149, 164*		w	HPLC-ESI-TOF	(3)
258.014	$C_6H_{11}O_9P$	inositol monophosphate ($InsP_1$)	alcohol	79		o	HPLC-ESI	(8)
270.053	$C_{15}H_{10}O_5$	apigenin	flavonoid			w	HPLC-ESI-TOF	(3)
278	*unknown*	*unknown*	alkylresorcino		*see*	r	HPLC-	(10)

			l		*referenc e*		APcI	
283.08 5	$C_{16}H_{13}NO_4$	avenanthramide D (1p)	avenanthrami de		147, 119, 117, 135, 265	o	TLC-EI	(11)
290.07 9	$C_{15}H_{14}O_6$	(+)-catechin	flavonoid	245, 221, 203, 137	329, 313, 255, 237, 139	b	HPLC-ESI	(12)
296.09 0	$C_{14}H_{16}O_7$	*p*-coumaroyl-pentose	phenolic acid	235, 163	335, 319	b	HPLC-ESI	(6)
299.07 9	$C_{16}H_{13}NO_5$	avenanthramide A (2p)	avenanthrami de		147, 153, 119, 135, 281	o	TLC-EI	(11)
312.08 5	$C_{14}H_{16}O_8$	caffeoyl-pentose	phenolic acid	179	351, 335, 181	b	HPLC-ESI	(6)
313.09 5	$C_{17}H_{15}NO_5$	avenanthramide E (1f)	avenanthrami de		177, 149, 135, 117, 295	o	TLC-EI	(11)
315.07 4	$C_{16}H_{13}NO_6$	avenanthramide C (2c)	avenanthrami de		163, 153, 135, 297	o	TLC-EI	(11)
315.07 4	$C_{16}H_{13}NO_6$	*N*-*p*-coumaroyl-4,5-dihydroxyanthranilic acid	avenanthrami de	314, 315	147, 316, 158, 317	o	HPLC-ESI-Orb.	(13)
320.27	$C_{21}H_{36}O_2$	pentadecylresorcinol ($C_{15:0}$)	alkylresorcino	*81, 277, 319*	111, 99,	b, r, w	HPLC-	(14,15

2			l		123, 110		APcI	)
322.024	$C_{15}H_{11}ClO_6$	cyanidin chloride	flavonoid			w	HPLC-ESI-TOF	(3)
326.100	$C_{15}H_{18}O_8$	feruloyl-pentose	phenolic acid	193, 175	365, 349	b	HPLC-ESI	(6)
326.100	$C_{15}H_{18}O_8$	*p*-coumaroyl-hexose	phenolic acid	163, 145	365, 349	b	HPLC-ESI	(6)
326.355	$C_{22}H_{46}O$	1-docosanol	alcohol		384, 103, 385	w	GC-MS	(16)
327.095	$C_{14}H_{17}NO_8$	HBOA-hexose	benzoxazinoid	164, 136, 118, 108	328, 166, 148, 120	r, w	UPLC-PDA-qTOF	(4)
329.090	$C_{17}H_{15}NO_6$	avenanthramide B (2f)	avenanthramide		177, 153, 135, 149, 311	o	TLC-EI	(11)
329.090	$C_{17}H_{15}NO_6$	*N*-feruloyl-4,5-dihydroxyanthranilic acid	avenanthramide	344, 187, 113, 345	346, 177, 110, 171, 347	o	HPLC-ESI-Orb.	(13)
330.074	$C_{17}H_{14}O_7$	5,7,4′-trihydroxy-3′,5′-dimethoxy-flavone (tricin)	flavonoid			w	HPLC-ESI-TOF	(3)
331.069	$C_{16}H_{13}NO_7$	*N*-caffeoyl-4,5-dihydroxyanthranilic acid	avenanthramide	330, 113, 331	332, 163	o	HPLC-ESI-Orb.	(13)
339.996	$C_6H_{14}O_{12}P_2$	inositol 1,3-bisphosphate ($InsP_2$)	alcohol	241		o	HPLC-ESI	(8)
340.131	$C_{20}H_{20}O_5$	6-prenylnaringenin	flavonoid	289, 183, 307, 321		b	LC-ESI-LTQ-Orb.	(17)
342.09	$C_{15}H_{18}O_9$	caffeoyl-hexose	phenolic acid	179, 161	365	b	HPLC-ESI	(6)

5								
342.110	$C_{19}H_{18}O_6$	diferulic acid	phenolic acid	325		w	HPLC-ESI	(7)
342.256	$C_{23}H_{34}O_2$	heptadecatrienylresorcinol ($C_{17:3}$)	alkylresorcinol		123, 163, 177, 137, 109, 191, 149	r	HPLC-APcI	(10)
343.090	$C_{14}H_{17}NO_9$	DIBOA-hexose	benzoxazinoid	180, 162, 134, 118, 108	344, 182, 164, 136	r, w	UPLC-PDA-qTOF	(4)
344.272	$C_{23}H_{36}O_2$	heptadecadienylresorcinol ($C_{17:2}$)	alkylresorcinol		123, 163, 149	r, w	HPLC-APcI	(15)
346.287	$C_{23}H_{38}O_2$	heptadecenylresorcinol ($C_{17:1}$)	alkylresorcinol		123	r, w	HPLC-APcI	(15)
348.303	$C_{23}H_{40}O_2$	heptadecylresorcinol ($C_{17:0}$)	alkylresorcinol	*305, 81, 122, 79, 347*	111, 113, 123	b, r, w	HPLC-APcI	(14,15)
354.110	$C_{20}H_{18}O_6$	licoisoflavone A	flavonoid	235, 320, 255		b	LC-ESI-LTQ-Orb.	(17)
354.110	$C_{20}H_{18}O_6$	hinokinin	lignan			w	HPLC-ESI-TOF	(3)
354.386	$C_{24}H_{50}O$	1-tetracosanol	alcohol		412, 103, 413	w	GC-MS	(16)
358.105	$C_{19}H_{18}O_7$	5-hydroxy-3,6,7,4′-tetramethoxyflavone	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
358.142	$C_{20}H_{22}O_6$	(–)-dihydrocubebin	lignan			b	LC-ESI-LTQ-Orb.	(17)
358.14	$C_{20}H_{22}O_6$	pinoresinol	lignan	342, 311, 175, 151,		r	UPLC-	(18)

2				136			qTOF	
358.142	$C_{20}H_{22}O_6$	matairesinol	lignan	342, 313, 298, 221, 161, 147, 137, 122		r	UPLC-qTOF	(18)
360.157	$C_{20}H_{24}O_6$	lariciresinol	lignan	329, 192, 178, 175, 160, 116		r	UPLC-qTOF	(18)
362.282	$C_{23}H_{38}O_3$	hydroxyheptadecenylresorcinol	alkylresorcinol		123, 345, 163, 125, 149; [345]: 345, 123	r	HPLC-APcI	(15)
362.319	$C_{24}H_{42}O_2$	octadecylresorcinol ($C_{18:0}$)	alkylresorcinol		111, 343	r, w	HPLC-APcI	(15)
370.287	$C_{25}H_{38}O_2$	nonadecatrienylresorcinol ($C_{19:3}$)	alkylresorcinol		123, 163, 137, 177	w	HPLC-APcI	(15)
372.303	$C_{25}H_{40}O_2$	nonadecadienylresorcinol ($C_{19:2}$)	alkylresorcinol		123, 137, 163, 251	r, w	HPLC-APcI	(15)
374.137	$C_{20}H_{22}O_7$	hydroxymatairesinol	lignan	355, 340, 329, 311, 299, 296, 284, 178, 160, 161, 148		r	UPLC-qTOF	(18)
374.319	$C_{25}H_{42}O_2$	nonadecenylresorcinol ($C_{19:1}$)	alkylresorcinol		123, 248, 197, 124	b, r, w	HPLC-APcI	(14,15)
376.334	$C_{25}H_{44}O_2$	nonadecylresorcinol ($C_{19:0}$)	alkylresorcinol	*333, 375, 81, 122, 123*	111	b, r, w	HPLC-APcI	(15)
378	*unknown*	*unknown*	phenolic acid	341, 215; [341]: 179,		w	HPLC-ESI	(7)

				161, 143				
382.418	$C_{26}H_{54}O$	1-hexacosanol	alcohol		440, 103, 441	w	GC-MS	(16)
386.100	$C_{20}H_{18}O_8$	5,5′-diferulic acid	phenolic acid	341, 297		w	HPLC-ESI	(7)
386.100	$C_{20}H_{18}O_8$	8,5′-diferulic acid	phenolic acid	341, 326, 370, 283		w	HPLC-ESI	(7)
386.355	$C_{27}H_{46}O$	cholesterol	phytosterol		368, 353, 213, 371, 255, 129	r, w	GC-MS	(19)
390.313	$C_{25}H_{42}O_3$	hydroxynonadecenylresorcinol	alkylresorcinol		123, 149, 163, 125, 373; [373]: 149, 123, 163	r, w	HPLC-APcI	(15)
390.313	$C_{25}H_{42}O_3$	5-(2-oxononadecanyl)resorcinol	alkylresorcinol		43, 57, 123, 267, 124	r, w	HPLC-MS	(20)
398.319	$C_{27}H_{42}O_2$	heneicosatrienylresorcinol ($C_{21:3}$)	alkylresorcinol		123, 163, 137, 329	w	HPLC-APcI	(15)
400.334	$C_{27}H_{44}O_2$	heneicosadienylresorcinol ($C_{21:2}$)	alkylresorcinol		123, 137, 289, 177	r, w	HPLC-APcI	(15)

400.371	$C_{28}H_{48}O$	campesterol	phytosterol		382, 385, 367, 213, 255, 129	r, w	GC-MS	(19)
402.132	$C_{21}H_{22}O_8$	pinosylvin glycolysate	other phenolic			w	HPLC-ESI-TOF	(3)
402.350	$C_{27}H_{46}O_2$	heneicosenylresorcinol ($C_{21:1}$)	alkylresorcinol		123, 163, 124, 137	b, r, w	HPLC-APcI	(14,15)
402.386	$C_{28}H_{50}O$	campestanol	phytosterol		387, 369, 215, 129, 384, 257	r, w	GC-MS	(19)
403.103	$C_{20}H_{19}O_9^+$	pelargonidin 3-arabinoside	flavonoid		271	w	UPLC-API	(21)
404.147	$C_{21}H_{24}O_8$	hydroxymedioresinol	lignan	388, 373, 343, 221, 181, 166, 151		r	UPLC-qTOF	(18)
404.365	$C_{27}H_{48}O_2$	heneicosylresorcinol ($C_{21:0}$)	alkylresorcinol	*403, 361, 81*	123	b, r, w	HPLC-APcI	(15)
410.319	$C_{28}H_{42}O_2$	*gamma*-tocotrienol	tocol		177, 137	r, w	HPLC-ESI	(22)
410.319	$C_{28}H_{42}O_2$	*beta*-tocotrienol	tocol		191, 151	w	HPLC-ESI	(23)
410.449	$C_{28}H_{58}O$	1-octacosanol	alcohol		468, 103, 469	w	GC-MS	(16)
412.371	$C_{29}H_{48}O$	stigmasterol	phytosterol		255, 213,	r, w	GC-MS	(19)

					394, 129, 379, 397			
414.386	$C_{29}H_{50}O$	sitosterol	phytosterol		396, 213, 381, 255, 399, 129	r, w	GC-MS	(19)
416.329	$C_{27}H_{44}O_3$	5-*n*-(2′-*oxo*-14′-(*Z*)-heneicosenyl)resorcinol	alkylresorcinol		124, 267, 123, 293, 166, 137, 294	r, w	HPLC-EI	(24)
416.365	$C_{28}H_{48}O_2$	*gamma*-tocopherol	tocol		177, 137	r, w	HPLC-ESI	(22)
416.365	$C_{28}H_{48}O_2$	*beta*-tocopherol	tocol		191, 151	w	HPLC-ESI	(23)
416.402	$C_{29}H_{52}O$	sitostanol	phytosterol		215, 401, 398, 257, 383, 129	r, w	GC-MS	(19)
418.345	$C_{27}H_{46}O_3$	5-*n*-(2′-oxoheneicosyl)resorcinol	alkylresorcinol		295, 124, 123, 321, 166, 137	r, w	HPLC-EI	(24)
418.34	$C_{27}H_{46}O_3$	hydroxyheneicosenylresorcinol	alkylresorcino		123,	r, w	HPLC-	(15)

5			l		149, 163, 401; [401]: 149, 123, 163		APcI	
419.098	$C_{20}H_{19}O_{10}^{+}$	cyanidin 3-arabinoside	flavonoid		287	w	UPLC-API	(21)
419.171	$C_{22}H_{27}O_8$	syringaresinol	lignan	402, 387, 372, 355, 205, 181, 166, 151		r	UPLC-qTOF	(18)
419.962	$C_6H_{15}O_{15}P_3$	inositol 1,4,5-trisphosphate ($InsP_3$)	alcohol	321		o	HPLC-ESI	(8)
424.334	$C_{29}H_{44}O_2$	*alpha*-tocotrienol	tocol		205, 165	r, w	HPLC-ESI	(22)
428.168	$C_{20}H_{28}O_{10}$	trans-p-ferulyl alcohol 4-O-[6-(2-methyl-3-hydroxypropionyl)] glucopyranoside	phenolic acid			b	LC-ESI-LTQ-Orb.	(17)
428.365	$C_{29}H_{48}O_2$	tricosadienylresorcinol ($C_{23:2}$)	alkylresorcinol		123, 137, 163, 317	r, w	HPLC-APcI	(15)
430.381	$C_{29}H_{50}O_2$	*alpha*-tocopherol	tocol		205, 165	r, w	HPLC-ESI	(22)
430.381	$C_{29}H_{50}O_2$	tricosenylresorcinol ($C_{23:1}$)	alkylresorcinol		123, 144, 149, 124	r, w	HPLC-APcI	(15)
432.106	$C_{21}H_{20}O_{10}$	kaempferol 3-O-rhamnoside	flavonoid	153, 171, 399, 385		b	LC-ESI-LTQ-Orb.	(17)
432.106	$C_{21}H_{20}O_{10}$	vitexin/isovitexin	flavonoid			w	HPLC-ESI-TOF	(3)

432.397	$C_{29}H_{52}O_2$	tricosylresorcinol ($C_{23:0}$)	alkylresorcinol	*431, 389, 81, 122*	123, 391, 199, 141	b, r, w	HPLC-APcI	(15)
433.114	$C_{21}H_{21}O_{10}^+$	pelargonidin 3-glucoside	flavonoid		271	w	UPLC-API	(21)
433.114	$C_{21}H_{21}O_{10}^+$	pelargonidin 3-galactoside	flavonoid		287	w	UPLC-API	(21)
434.121	$C_{21}H_{22}O_{10}$	naringenin 7-glucoside	flavonoid	313, 253, 310, 231		b	LC-ESI-LTQ-Orb.	(17)
434.158	$C_{22}H_{26}O_9$	hydroxysyringaresinol	lignan	418, 403, 385, 373, 358, 181, 166, 138		r	UPLC-qTOF	(18)
435.093	$C_{20}H_{19}O_{11}^+$	delphinidin 3-arabinoside	flavonoid		303	w	UPLC-API	(21)
437.231	$C_{25}H_{31}N_3O_4$	dicoumaroylspermidine	polyamine		204, 147	w	UPLC-qTOF	(25)
438.480	$C_{30}H_{62}O$	1-triacontanol	alcohol		496, 103, 497	w	GC-MS	(16)
444.142	$C_{23}H_{24}O_9$	formononetin (glycosylated and methylated)	flavonoid			w	HPLC-ESI-TOF	(3)
444.360	$C_{29}H_{48}O_3$	5-(2-oxotricosenyl)resorcinol	alkylresorcinol		55, 321, 43, 123, 124	r, w	HPLC	(20)
446.376	$C_{29}H_{50}O_3$	hydroxytricosenylresorcinol	alkylresorcinol		149, 163, 429, 123; [429]: 149, 163, 123	b, r, w	HPLC-APcI	(15)

446.376	$C_{29}H_{50}O_3$	5-(2-oxotricosanyl)resorcinol	alkylresorcinol		43, 57, 123, 124, 323	r, w	HPLC	(20)
448.101	$C_{21}H_{20}O_{11}$	orientin/isoorientin	flavonoid			w	HPLC-ESI-TOF	(3)
448.101	$C_{21}H_{20}O_{11}$	luteolin-7-*O*-hexoside (cynaroside)	flavonoid	285		w	HPLC-qTOF	(26)
449.108	$C_{21}H_{21}O_{11}^+$	cyanidin 3-galactoside	flavonoid		287	w	UPLC-API	(21)
449.108	$C_{21}H_{21}O_{11}^+$	cyanidin 3-glucoside	flavonoid		287	w	UPLC-API	(21)
453.226	$C_{25}H_{31}N_3O_5$	coumaroyl-caffeoylspermidine	polyamine		308, 204, 163, 147	w	UPLC-qTOF	(25)
456.397	$C_{31}H_{52}O_2$	pentacosadienylresorcinol ($C_{25:2}$)	alkylresorcinol		137, 163, 177, 359, 149, 439, 373, 345, 331	r	HPLC-APcI	(10)
458.412	$C_{31}H_{54}O_2$	pentacosenylresorcinol ($C_{25:1}$)	alkylresorcinol		334, 375, 180, 403; [334]: 123	r, w	HPLC-APcI	(15)
460.42	$C_{31}H_{56}O_2$	pentacosylresorcinol ($C_{25:0}$)	alkylresorcino	*459, 417, 81, 415,*	419	b, r, w	HPLC-	(15)

8			l	*122, 123*			APcI	
462.116	$C_{22}H_{22}O_{11}$	6-hexosyl chrysoeriol	flavonoid		445, 397, 427, 367, 343, 409, 313	w	HPLC-API-IT	(27)
463.124	$C_{22}H_{23}O_{11}^{+}$	peonidin 3-glucoside	flavonoid		301	w	UPLC-API	(21)
465.103	$C_{21}H_{21}O_{12}^{+}$	delphinidin 3-galactoside	flavonoid		303	w	UPLC-API	(21)
467.242	$C_{26}H_{33}N_3O_5$	coumaroyl-feruloylspermidine	polyamine		322, 204, 177, 147	w	UPLC-qTOF	(25)
469.221	$C_{25}H_{31}N_3O_6$	dicaffeoylspermidine	polyamine		308, 220, 163	w	UPLC-qTOF	(25)
479.119	$C_{22}H_{23}O_{12}^{+}$	petunidin 3-glucoside	flavonoid		317	w	UPLC-API	(21)
483.237	$C_{26}H_{33}N_3O_6$	caffeoyl-feruloylspermidine	polyamine		322, 220, 177, 163	w	UPLC-qTOF	(25)
484.428	$C_{33}H_{56}O_2$	heptacosadienylresorcinol ($C_{27:2}$)	alkylresorcinol		467, 373, 468, 345, 163, 137, 177, 149	r	HPLC-APcI	(10)
486.44	$C_{33}H_{58}O_2$	heptacosenylresorcinol ($C_{27:1}$)	alkylresorcino		467,	r	HPLC-	(10)

4			l		310, 217, 165, 373, 137		APcI	
488.459	$C_{33}H_{60}O_2$	heptacosylresorcinol ($C_{27:0}$)	alkylresorcinol		459	b, r, w	HPLC-APcI	(15)
489.148	$C_{20}H_{27}NO_{13}$	HBOA-dihexose	benzoxazinoid	221, 164, 136, 118, 108	166, 148	r, w	UPLC-PDA-qTOF	(4)
492.127	$C_{23}H_{24}O_{12}$	3′,4′,5′-trihydroxy-3,7-dimethylflavone glycolysate	flavonoid			w	HPLC-ESI-TOF	(3)
492.127	$C_{23}H_{24}O_{12}$	*O*-hexosyl tricin	flavonoid		331	w	HPLC-API-IT	(27)
493.135	$C_{23}H_{25}O_{12}^{+}$	malvidin 3-glucoside	flavonoid		331	w	UPLC-API	(21)
499.929	$C_6H_{16}O_{18}P_4$	inositol tetrakisphosphate ($InsP_4$)	alcohol	401		o	HPLC-ESI	(8)
505.143	$C_{20}H_{27}NO_{14}$	DIBOA-dihexose	benzoxazinoid	180, 162, 134, 118, 108, 101, 85, 78, 59	506, 344, 325, 182, 164, 136, 80	r, w	UPLC-PDA-qTOF	(4)
534.137	$C_{25}H_{26}O_{13}$	glycosylated and acetylated 3′,4′,5′-trihydroxy-3,7-dimethylflavone	flavonoid			w	HPLC-ESI-TOF	(3)
534.137	$C_{25}H_{26}O_{13}$	apigenin-6,8-di-*C*-pentoside	flavonoid	353, 383, 443, 413, 473, 515		w	HPLC-qTOF	(26)
535.154	$C_{21}H_{29}NO_{15}$	DIMBOA-dihexose	benzoxazinoid	221, 192, 164, 149, 133, 121, 101, 98	536, 374,	r, w	UPLC-PDA-qTOF	(4)

					212, 194, 166			
536.189	$C_{26}H_{32}O_{12}$	pinosylvin (double glycosylation)	other phenolic			w	HPLC-ESI-TOF	(3)
536.438	$C_{40}H_{56}$	*beta*-carotene	carotenoid			w	HPLC-ESI	(28)
548.153	$C_{26}H_{28}O_{13}$	hemsleyanoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
548.423	$C_{37}H_{56}O_3$	*trans*-campestanyl-*p*-coumarate	phytosterol		236, 219, 191, 384, 369, 164, 605	r, w	GC-MS	(19)
562.439	$C_{38}H_{58}O_3$	*trans*-sitostanyl-*p*-coumarate	phytosterol		236, 219, 398, 383, 191, 619, 164	r, w	GC-MS	(19)
564.148	$C_{26}H_{28}O_{14}$	apigenin-6-*C*-arabinoside-8-*C*-hexoside (schaftoside/isoschaftoside)	flavonoid			w	HPLC-ESI-TOF	(3)
564.148	$C_{26}H_{28}O_{14}$	apigenin-6-*C*-pentoside-8-*C*-hexoside	flavonoid	353, 383, 443, 473, 413, 503, 545		w	HPLC-qTOF	(26)
568.428	$C_{40}H_{56}O_2$	lutein	carotenoid		551, 533	w	HPLC-ESI	(28)
576.418	$C_{38}H_{56}O_4$	campesteryl ferulate	phytosterol		249, 633,	r, w	GC-MS	(19)

					382, 266, 367, 221, 194			
576.418	$C_{38}H_{56}O_4$	24-methyllathosterol ferulate	phytosterol		576,42	r, w	HPLC-EI	(24)
576.418	$C_{38}H_{56}O_4$	24-methylenecholestanol ferulate	phytosterol		194, 177, 394, 298, 256, 449, 367, 561	r, w	HPLC-EI	(24)
578.142	$C_{30}H_{26}O_{12}$	procyanidin B3	flavonoid			w	HPLC-ESI-TOF	(3)
578.142	$C_{30}H_{26}O_{12}$	procyanidin B2	flavonoid	425, 407, 289	617, 601, 579, 427, 291	b	HPLC-ESI	(12)
578.164	$C_{27}H_{30}O_{14}$	isovitexin-2″-*O*-rhamnoside	flavonoid			w	HPLC-ESI-TOF	(3)
578.164	$C_{27}H_{30}O_{14}$	apigenin-7-*O*-neohesperidoside	flavonoid	269		w	HPLC-qTOF	(26)
578.434	$C_{38}H_{58}O_4$	campestanyl ferulate	phytosterol		249, 266, 221, 384, 369, 635, 194	r, w	GC-MS	(19)

578.434	$C_{38}H_{58}O_4$	24-methylcholestanol ferulate	phytosterol		578	r, w	HPLC-EI	(24)
579.895	$C_6H_{17}O_{21}P_5$	inositol pentakisphosphate ($InsP_5$)	alcohol	481		o	HPLC-ESI	(8)
580.143	$C_{26}H_{28}O_{15}$	lucenin-1/3 (luteolin-6/8-*C*-xyloside-8/6-*C*-glucoside)	flavonoid			w	HPLC-ESI-TOF	(3)
582.210	$C_{31}H_{34}O_{11}$	auriculatin 4′-*O*-glucoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
584.226	$C_{31}H_{36}O_{11}$	buddlenol E	lignan	535, 505, 387, 372, 357, 329, 195, 165, 150		r	UPLC-qTOF	(18)
590.434	$C_{39}H_{58}O_4$	sitosteryl ferulate	phytosterol		396, 249, 266, 381, 647, 221, 194	r	GC-MS	(19)
590.434	$C_{39}H_{58}O_4$	schottenol ferulate	phytosterol		590,43	r, w	HPLC-EI	(24)
592.449	$C_{39}H_{60}O_4$	sitostanyl ferulate	phytosterol		249, 649, 398, 266, 383, 221, 194	r, w	GC-MS	(19)
594.137	$C_{30}H_{26}O_{13}$	prodelphinidin B3	flavonoid	467, 303, 289	617, 443, 427, 289	b	HPLC-ESI	(12)
594.13	$C_{30}H_{26}O_{13}$	prodelphinidin dimer (GC-C)	flavonoid	467, 289	633,	b	HPLC-ESI	(12)

7					617, 443, 427, 291			
594.158	$C_{27}H_{30}O_{15}$	kaempferol 3-rhamnoside-7-glucoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
594.159	$C_{27}H_{30}O_{15}$	apigenin-6,8-di-C-hexoside (vicenin 2)	flavonoid	353, 383, 473, 503, 443, 575		w	HPLC-qTOF	(26)
594.159	$C_{27}H_{30}O_{15}$	chrysoeriol-6-*C*-pentoside-8-*C*-hexoside	flavonoid	383, 413, 473, 503, 575, 443, 533		w	HPLC-qTOF	(26)
594.159	$C_{27}H_{30}O_{15}$	kaempferol-7-*O*-neohesperidoside	flavonoid	285		w	HPLC-qTOF	(26)
594.159	$C_{27}H_{30}O_{15}$	luteolin-7-*O*-neohesperidoside	flavonoid	285		w	HPLC-qTOF	(26)
608.174	$C_{28}H_{32}O_{15}$	kaempferide 3-glucoside-7-rhamnoside	flavonoid	299, 284, 300, 269		b	LC-ESI-LTQ-Orb.	(17)
608.174	$C_{28}H_{32}O_{15}$	methylisoorientin-2″-*O*-rhamnoside	flavonoid			w	HPLC-ESI-TOF	(3)
608.174	$C_{28}H_{32}O_{15}$	chrysoeriol-7-*O*-neohesperidoside	flavonoid	299		w	HPLC-qTOF	(26)
610.153	$C_{27}H_{30}O_{16}$	6-*O*″-hexosyl isoorientin	flavonoid		431, 449, 329, 383, 299	w	HPLC-API-IT	(27)
610.153	$C_{27}H_{30}O_{16}$	7-*O*-hexosyl isoorientin	flavonoid		449, 431, 383, 353, 413, 329	w	HPLC-API-IT	(27)
610.15	$C_{27}H_{30}O_{16}$	kaempferol-7-*O*-sophoroside	flavonoid	285, 447		w	HPLC-	(26)

3							qTOF	
610.153	$C_{27}H_{30}O_{16}$	quercetin-3-*O*-rutinose	flavonoid	301, 447		w	HPLC-qTOF	(26)
614.236	$C_{32}H_{38}O_{12}$	buddlenol C	lignan	565, 535, 417, 387, 225, 221, 195, 165, 150		r	UPLC-qTOF	(18)
616.449	$C_{41}H_{60}O_4$	24-methylenecycloartanyl ferulate	phytosterol		249, 407, 422, 673, 266, 221, 194	r	GC-MS	(19)
624	*unknown*	methoxylated *O*-hexosyl isoorientin	flavonoid		463, 445, 397, 427, 367	w	HPLC-API-IT	(27)
626	*unknown*	*unknown*	*unknown*	485, 383, 221, 179; [485]: 467, 383, 341		w	HPLC-ESI	(7)
638	*unknown*	*O*-hexosyl-*O*-pentosyl tricin	flavonoid		493, 331	w	HPLC-API-IT	(27)
638.600	$C_{44}H_{78}O_2$	campesteryl-16:0	phytosterol		382, 383, 367, 81, 255, 213	r, w	GC-MS	(19)
640.616	$C_{44}H_{80}O_2$	campestanyl-16:0	phytosterol		384, 215, 385, 369, 257, 81	r, w	GC-MS	(19)

644.247	$C_{33}H_{40}O_{13}$	buddlenol D	lignan	595, 565, 417, 387, 357, 255, 195, 180, 165, 137		r	UPLC-qTOF	(18)
650.600	$C_{45}H_{78}O_2$	stigmasteryl-16:0	phytosterol		394, 395, 379, 255, 81, 213	r, w	GC-MS	(19)
652.616	$C_{45}H_{80}O_2$	sitosteryl-16:0	phytosterol		255, 396, 213, 381, 397, 81	r, w	GC-MS	(19)
654.180	$C_{29}H_{34}O_{17}$	syringetin 3-rutinoside	phenolic acid			b	LC-ESI-LTQ-Orb.	(17)
654.632	$C_{45}H_{82}O_2$	sitostanyl-16:0	phytosterol		215, 398, 383, 81, 399, 257	r, w	GC-MS	(19)
655.612	$C_{40}H_{81}NO_5$	(2*S*,3*S*,4*R*)-2-*N*-[(2′*R*)-hydroxy-docosanoyl]-4-hydroxysphinganine	sphingolipid	410, 355, 309; [410]: 309; [355]: 309		w	HPLC-ESI	(29)
656.164	$C_{34}H_{28}N_2O_{12}$	bisavenanthramide B1	avenanthramide			o	HPLC-ESI	(9)
659.861	$C_6H_{18}O_{24}P_6$	phytic acid ($InsP_6$)	alcohol	561		o	HPLC-ESI	(8)
662.600	$C_{46}H_{78}O_2$	campesteryl-18:2	phytosterol		382, 383, 81, 367,	r, w	GC-MS	(19)

					255, 213			
664.616	$C_{46}H_{80}O_2$	campestanyl-18:2	phytosterol		385, 384, 81, 369, 215, 257	r, w	GC-MS	(19)
664.616	$C_{46}H_{80}O_2$	campesteryl-18:1	phytosterol		382, 383, 367, 213, 81, 255	r, w	GC-MS	(19)
666.632	$C_{46}H_{82}O_2$	campestanyl-18:1	phytosterol		385, 384, 81, 215, 257, 369	r, w	GC-MS	(19)
667.612	$C_{41}H_{81}NO_5$	2-*N*-(2′-hydroxy-15′-tricosenoyl)-4-hydroxysphinganine	sphingolipid	584, 422, 410, 367; [422]: 404		w	HPLC-ESI	(29)
674.600	$C_{47}H_{78}O_2$	sitosteryl-18:3	phytosterol		396, 397, 81, 255, 381, 213	r, w	GC-MS	(19)
674.600	$C_{47}H_{78}O_2$	stigmasteryl-18:2	phytosterol		394, 395, 255, 81, 379, 213	r, w	GC-MS	(19)
676.616	$C_{47}H_{80}O_2$	sitosteryl-18:2	phytosterol		397, 81, 396, 213, 255, 381	r, w	GC-MS	(19)

676.616	$C_{47}H_{80}O_2$	stigmasteryl-18:1	phytosterol		394, 395, 255, 81, 379, 213	r, w	GC-MS	(19)
678.632	$C_{47}H_{82}O_2$	sitostanyl-18:2	phytosterol		399, 81, 398, 215, 257, 383	r, w	GC-MS	(19)
678.632	$C_{47}H_{82}O_2$	sitosteryl-18:1	phytosterol		396, 397, 81, 381, 255, 213	r, w	GC-MS	(19)
680.647	$C_{47}H_{84}O_2$	sitostanyl-18:1	phytosterol		399, 398, 81, 215, 257, 383	w	GC-MS	(19)
681.627	$C_{42}H_{83}NO_5$	(2*S*,3*S*,4*R*,8*Z*)-2-*N*-[(2′*R*)-hydroxytetracosanoyl]-4-hydroxy-8-sphingenine	sphingolipid	662, 438, 426, 383; [662]: 408, 382; [426]: 408, 378, 337; [383]: 337		w	HPLC-ESI	(29)
681.627	$C_{42}H_{83}NO_5$	2-*N*-(2′-hydroxy-15′-tetracosenoyl)-4-hydroxysphinganine	sphingolipid	662, 644, 436, 381; [436]: 418, 394		w	HPLC-ESI	(29)
683.643	$C_{42}H_{85}NO_5$	(2*S*,3*S*,4*R*)-2-*N*-[(2′*R*)-hydroxytetra-cosanoyl]-4-hydroxysphinganine	sphingolipid	438, 426, 383; [438]: 410, 365, 337; [426]: 365, 337; [383]: 337		w	HPLC-ESI	(29)
686.279	$C_{32}H_{46}O_{16}$	secoisolariciresinol diglucoside	lignan	685, 361		w	UPLC-API	(21)
697.65	$C_{43}H_{87}NO_5$	(2*S*,3*S*,4*R*)-2-*N*-[(2′*R*)-	sphingolipid	452, 440, 397; [452]:		w	HPLC-ESI	(29)

8		hydroxypenta-cosanoyl]-4-hydroxysphinganine		434, 396, 351; [440]: 351; [397]: 351				
710.206	$C_{32}H_{38}O_{18}$	kaempferol 3-apiosyl-(1→4)-rhamnoside-7-rhamnoside	flavonoid	401, 311, 283, 341		b	LC-ESI-LTQ-Orb.	(17)
711.674	$C_{44}H_{89}NO_5$	2-*N*-(2-hydroxyhexacosanoyl)-4-hydroxysphinganine	sphingolipid	626, 466, 454, 411, 365; [411]: 365		w	HPLC-ESI	(29)
713.544	$C_{40}H_{75}NO_9$	1-*O*-glucopyranosyl-2-*N*-(2′-hydroxy-hexadecanoyl)-4,8-sphingadienine	sphingolipid	550, 532; [550]: 532, 271, 261, 225; [532]: 340, 324, 322, 306		w	HPLC-ESI	(29)
715.560	$C_{40}H_{77}NO_9$	1-*O*-β-glucopyranosyl-(2*S*,3*R*,8*Z*)-2-*N*-[(2′*R*)-hydroxypalmitoyl]-8-sphingenine	sphingolipid	552, 534; [552]: 534, 297, 271; [534]: 281, 237, 225		w	HPLC-ESI	(29)
740	unknown	procyanidin dimer monoglycoside	flavonoid	451, 425, 407, 289	453, 291	b	HPLC-ESI	(12)
740.216	$C_{33}H_{40}O_{19}$	kaempferol 3-rhamninoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
740.216	$C_{33}H_{40}O_{19}$	kaempferol 3-rhamnoside-7-glucosyl-(1→2)-rhamnoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
741.576	$C_{42}H_{79}NO_9$	1-*O*-β-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-*N*-[(2′*R*)-hydroxyoctadecanoyl]-4,8-sphingadienine	sphingolipid	578, 560; [578]: 548, 299; [560]: 519, 352, 306		w	HPLC-ESI	(29)
743.591	$C_{42}H_{81}NO_9$	1-*O*-β-glucopyranosyl-(2*S*,3*R*,8*Z*)-2-*N*-[(2′*R*)-hydroxyoctadecanoyl]-8-sphingenine	sphingolipid	580, 562; [580]: 299		w	HPLC-ESI	(29)

756.211	$C_{33}H_{40}O_{20}$	kaempferol 3-gentiobioside-7-rhamnoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
756.211	$C_{33}H_{40}O_{20}$	kaempferol 3-sophoroside-7-rhamnoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
769.607	$C_{44}H_{83}NO_9$	1-*O*-β-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-*N*-[(2′*R*)-hydroxyicosanoyl]-4,8-sphingadienine	sphingolipid	606, 588; [606]: 576, 558, 327; [588]: 560, 380, 288		w	HPLC-ESI	(29)
770.191	$C_{33}H_{38}O_{21}$	apigenin-6-*C*-β-galactosyl-8-*C*-β-glucosyl-*O*-glucuronopyranoside	flavonoid			w	HPLC-ESI-TOF	(3)
770.227	$C_{34}H_{42}O_{20}$	rhamnetin 3-rhamninoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
770.4	unknown	apigenin-*C*-diglycoside sinapoate	flavonoid		705, 681, 207, 651, 735, 753	w	HPLC-APPI	(30)
771.622	$C_{44}H_{85}NO_9$	1-*O*-β-glucopyranosyl-(2*S*,3*R*,8*Z*)-2-*N*-[(2′*R*)-hydroxyicosanoyl]-8-sphingenine	sphingolipid	608, 590; [608]: 590, 334, 327, 309, 298, 281; [590]: 352, 281		w	HPLC-ESI	(29)
786.222	$C_{34}H_{42}O_{21}$	isorhamnetin 3-rutinoside-7-glucoside	flavonoid			b	LC-ESI-LTQ-Orb.	(17)
810.310	$C_{42}H_{50}O_{16}$	hedyotisol A	lignan	743, 713, 667, 613, 595, 565, 535, 417, 387, 195, 165, 150		r	UPLC-qTOF	(18)
823.654	$C_{48}H_{89}NO_9$	1-*O*-β-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-	sphingolipid	660, 642; [642]: 434, 381, 363, 306		w	HPLC-ESI	(29)

		2-*N*-[(2′*R*,15′*Z*)-2′-hydroxy-15′-tetracosenoyl]-4,8-sphingadienine						
825.669	$C_{48}H_{91}NO_9$	1-*O*-β-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-*N*-[(2′*R*)-hydroxytetracosanoyl]-4,8-sphingadienine	sphingolipid	662, 644, 382; [662]: 630, 424, 383, 351, 337; [644]: 603, 464, 452, 436, 383, 337, 278		w	HPLC-ESI	(29)
827.685	$C_{48}H_{93}NO_9$	1-*O*-glucopyranosyl-2-*N*-(2′-hydroxy-tetracosanoyl)-8-sphingenine	sphingolipid	664, 646, 448, 429, 383, 309; [664]: 298; [383]: 337		w	HPLC-ESI	(29)
840.321	$C_{43}H_{52}O_{17}$	methoxyhedyotisol A	lignan	821, 791, 645, 643, 613, 595, 417, 376, 225, 195, 165		r	UPLC-qTOF	(18)
841.664	$C_{48}H_{91}NO_{10}$	1-*O*-β-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*)-2-*N*-[(2′*R*,15′*Z*)-2′-hydroxy-15′-tetra-cosenoyl]-4-hydroxy-8-sphingenine	sphingolipid	678, 660, 436, 418, 381; [678]: 381; [436]: 408, 406, 363, 335; [381]: 335		w	HPLC-ESI	(29)
843.680	$C_{48}H_{93}NO_{10}$	1-*O*-β-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*)-2-*N*-[(2′*R*)-hydroxytetracosanoyl]-4-hydroxy-8-sphingenine	sphingolipid	680, 662, 438; [680]: 383, 365; [662]: 420; [438]: 420, 410, 393, 365, 337		w	HPLC-ESI	(29)
866.206	$C_{45}H_{38}O_{18}$	procyanidin trimer (C-C-C)	flavonoid	713, 739, 577, 289	579, 291	b	HPLC-ESI	(12)
871.711	$C_{50}H_{97}NO_{10}$	1-*O*-β-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*)-2-*N*-[(2′*R*)-2′-	sphingolipid	690, 466, 445, 411; [466]: 365		w	HPLC-ESI	(29)

		hydroxyhexaconasoyl]-4-hydroxy-8-sphingenine						
882.201	$C_{45}H_{38}O_{19}$	prodelphinidin trimer (GC-C-C)	flavonoid	751, 729, 713, 577	595	b	HPLC-ESI	(12)
882.201	$C_{45}H_{38}O_{19}$	prodelphinidin trimer (C-GC-C)	flavonoid	751, 729, 713, 593, 467, 289	731, 595, 443, 425, 289	b	HPLC-ESI	(12)
898.196	$C_{45}H_{38}O_{20}$	prodelphinidin trimer (GC-GC-C)	flavonoid	729, 711, 593, 289	731, 595, 443, 425, 305, 291	b	HPLC-ESI	(12)
900.472	$C_{45}H_{72}O_{18}$	26-desglucoavenacoside A	saponin			o	HPLC-ESI-TQ	(31)
1062.53	$C_{51}H_{82}O_{23}$	avenacoside A	saponin			o	HPLC-ESI-TQ	(31)
1224.58	$C_{57}H_{92}O_{28}$	avenacoside B	saponin			o	HPLC-ESI-TQ	(31)

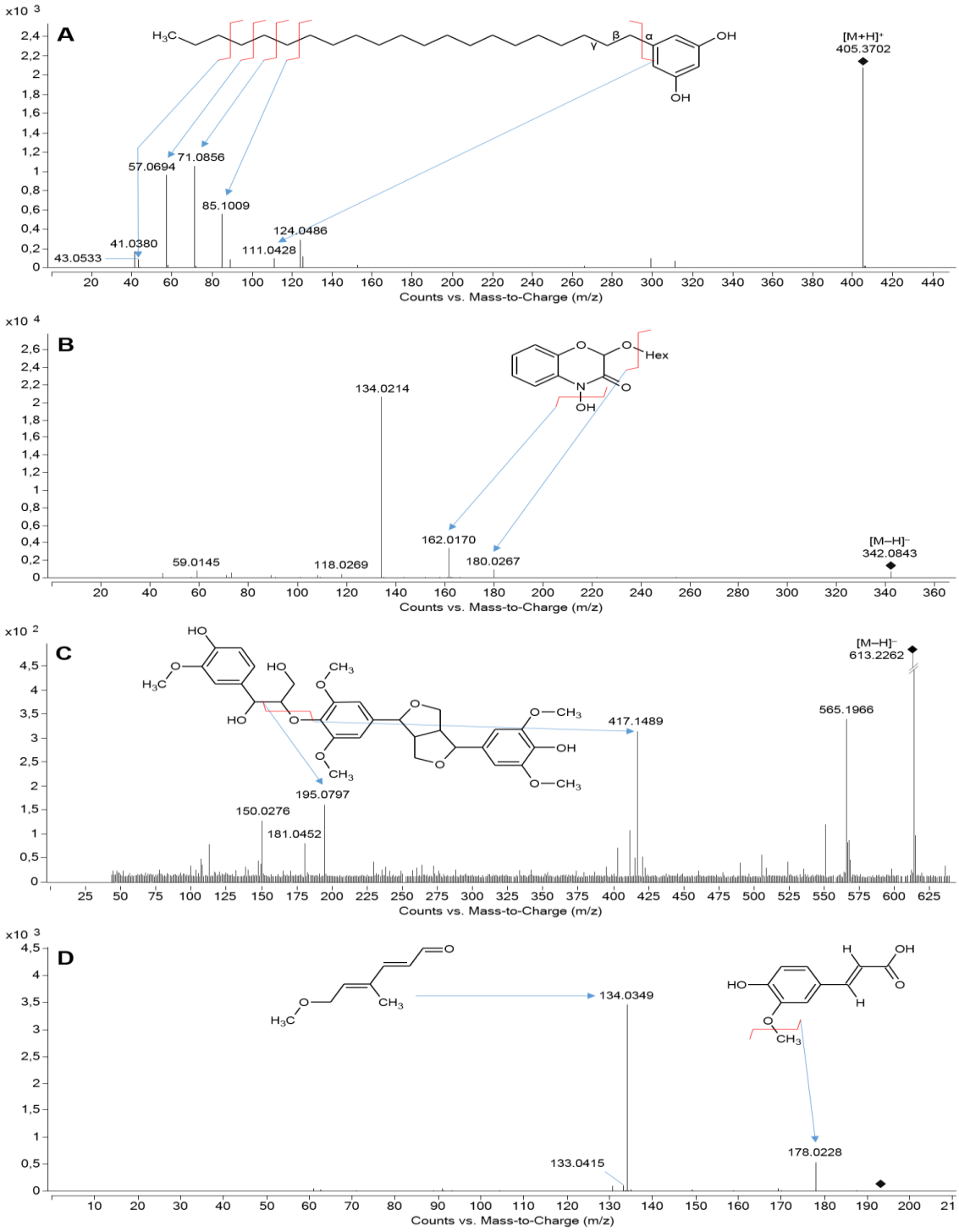

Figure 1. Tandem mass spectra of selected phytochemicals in ESI–qTOF–MS. **A** Heneicosylresorcinol (alkylresorcinol $C_{21:0}$) in the ESI(+) mode and 20 V source voltage with typical cleavage sites of the aromatic group marked with Greek letters. **B** DIBOA hexoside (benzoxazinoid) in ESI(–) and 10 V; Hex = hexose group. **C** Buddlenol C (lignan) in ESI(–) and 10 V. **D** Ferulic acid in ESI(–) and 20 V.

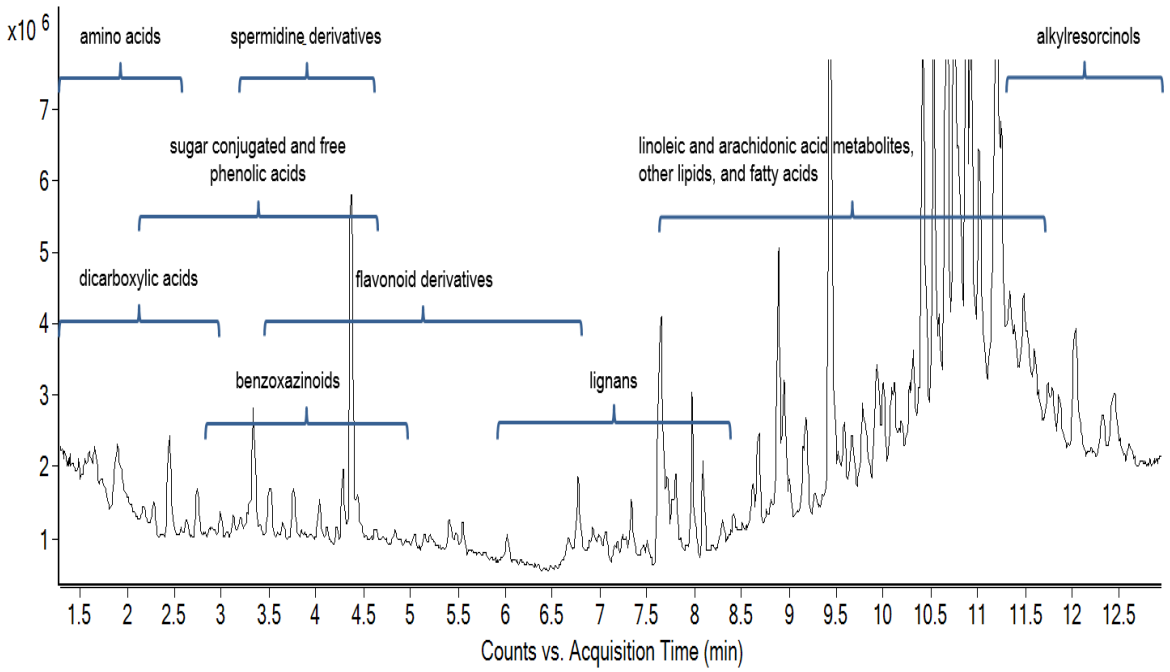

Figure 2. ESI(–) total ion reversed phase chromatogram of rye bread sample marked with elution regions of major phytochemical groups.

