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Acrylamide Analysis in Food by Liquid Chromatographic and Gas Chromatographic Methods

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Acrylamide (AA) is a compound classified as carcinogenic to humans by the International Agency for Research on Cancer. It was first discovered to be present in certain heated processed food by the Swedish National Food Administration (SNFA) and University of Stockholm in early 2002. The major pathway for AA formation in food is the Maillard reaction between reducing sugar and the amino acid asparagine at high temperature. Since the discovery of AA's presence in food, many analytical methods have been developed for determination of AA contents in different food matrices. Also, several studies have been conducted to develop extraction procedures for AA from difficult food matrices. AA is a small, highly polar molecule, which makes its extraction and analysis challenging. Many articles and reviews have been published dealing with AA in food. The aim of the review is to discuss AA formation in food, the factors affecting AA formation and removal, AA exposure assessment, AA extraction and cleanup from food samples, and analytical methods used in AA determination, such as high-performance liquid chromatography (HPLC) and gas chromatography (GC). Special attention is given to sample extraction and cleanup procedures and analytical techniques used for AA determination.

Keywords  Acrylamide analysis in food samples, acrylamide formation, extraction procedures, GC, HPLC

INTRODUCTION

Acrylamide (2-propenamide) is an unsaturated amide that has been produced since the 1950s by hydration of acrylonitrile (Agency for Toxic Substances and Disease Registry [ATSDR], 2009; Castle and Eriksson, 2005; Friedman, 2003; Medeiros et al., 2012). Acrylamide (AA) (Figure 1) is an odorless, white solid, with a molecular mass of 71.08 g/mole and melting point of 84.5°C (Daughton, 1988; National Industrial Chemicals Notification and Assessment Scheme [NICNAS], 2002; Xia et al., 2012). AA is stable at room temperature but can violently polymerize at its melting point or under UV light (ATSDR, 2009). It dissolves readily in water and polar solvents such as acetone, methanol, and ethanol. It is, however, not soluble in nonpolar solvents (National Toxicology Program [NTP], 2011).

AA possesses two functional groups, an amide group and a reactive electron-deficient vinylic double bond. The limited conjugation involving π electrons means that AA has strong chromophore for UV detection and does not fluoresce (Castle and Eriksson, 2005; Daughton, 1988; Girma et al., 2005; Jezussek and Schieberle, 2003; Joint Institute for Food Safety and Applied Nutrition/National Center for Food Safety and Technology [JIFSAN/NCFST], 2002; Medeiros Vinci et al., 2012). AA exhibits both weakly acidic and basic properties. The electron-withdrawing carboxamide group activates the double bond, which reacts with nucleophilic regents by addition reaction mechanisms. Many of these reactions are reversible, and the rate of reaction depends on the strength of the nucleophile. Examples are Michael and Diel-Alder additions and radical reactions. These reactions are of importance in biological systems.

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Reactions of the amide residue include hydrolysis, dehydration, alcoholysis, and condensation with aldehydes, while the vinyl double bond reacts with ammonia, aliphatic amines, phosphines, chlorine, bromine, bisulphite, and dithiocarbamates, as well as proteins (Daughton, 1988; Friedman, 2003; Girma et al., 2005; Lignert et al., 2002; Medeiros Vinci et al., 2012).

AA is produced industrially mainly for the synthesis of polyacrylamide, which has several applications in the cosmetic and packaging industries, in soil and conditioning agents, in the treatment of sewage and wastewater, and in the purification of drinking water; it is also found in tobacco smoke (El-Kholy et al., 2005; Carere, 2006; Ragnar, 2003; Tareke et al., 2000).

AA is formed when foods rich in carbohydrates are subjected to high temperatures during cooking or other thermal processing (baking, frying, grilling, or microwave heating). Raw or boiled foodstuffs usually do not contain AA. The most important matrices are potato crisps, French fries, crisp bread, breakfast cereals, bakery products such as bread and biscuits, and coffee (Alves et al., 2010; Bent et al., 2012; Bortolomeazzi et al., 2012; Capuano and Fogliano, 2011; El-Ziney et al., 2009; Medeiros Vinci et al., 2012; Shepherd et al., 2010; U.S. Environmental Protection Agency [EPA], 2010; Zamora et al., 2010; Zhang et al., 2008).

The first studies on the mechanistic pathway for the formation of AA in food proposed the Maillard reaction (non-enzymatic browning reaction) as the major pathway, which occurs by a condensation of the amino group of the amino acid, asparagine (Asn), and the carbonyl group of reducing sugars (fructose and glucose) during high-temperature heating (Cesarová et al., 2006; De Vleeschouwer et al., 2008; Hidalgo et al., 2009; Mestdagh et al., 2008b; U.S. EPA, 2010; Zyzak et al., 2003).

Asn alone may release AA by thermally initiated decarboxylation and deadimation (Capuano and Fogliano, 2011). Some researchers have described an important role to oil hydrolysis products in the formation of AA, oxidative lipid degradation, when acrolein and acrylic acid, the precursors of AA, are formed and then react with ammonia formed from amino acids. Some studies indicate that AA formed from thermal degradation of 3-aminopropionamide (Granvogl and Schieberle, 2006; Granvogl et al., 2004; Guenther et al., 2007; Lignert et al., 2002; Marchetti et al., 2013; Yaylayan and Stadler, 2005).

An alternative route proposed is the formation of pyrolytic AA from wheat gluten, with protein-bound alanine as the key amino acid. In a model experiment, thermal treatment of gluten resulted in the formation of high amounts of AA. When gluten was added to the dough, the increase in AA content was in the order of 20%, with a high correlation to the amount of gluten added (Claus et al., 2006, 2008).

**FIG. 1. Chemical structural of AA.**

AA is known to be neurotoxic, and several toxicological studies have demonstrated its genotoxic carcinogenicity in animals, thus indicating potential human health risks (Park et al., 2002; Rice, 2005; Rudén, 2004). In 1994 the International Agency for Research on Cancer (IARC) classified AA as a possible carcinogen for humans (Group 2A), based on its carcinogenicity in rodents. AA’s electrophilic double bond can interacting in vivo with cellular nucleophiles such as the sulfhydryl groups in reduced glutathione and in proteins, and to a lesser extent protein amino groups (Environment Canada and HealthCanada, 2009; IARC, 1994; Medeiros Vinci et al., 2012; Pelucchi et al., 2011; Sanny et al., 2012).

In April 2002 the Swedish National Food Administration (SNFA) announced that, on the basis of the findings of researchers from Stockholm University, AA is formed in a large group of consumer food products (e.g., potato chips, French fries, processed cereals) when prepared/cooked at or above 120°C. Shortly after, the Swedish findings were confirmed in several other European countries as well as in the US. The levels found at that time were much higher than the levels recommended by the World Health Organization (WHO, 2011) for drinking water (0.5 μg/L) corresponding to 1 μg/day for a person who assumes 2 L per day (Anese et al., 2011; Bråthen and Knutsen, 2005; Carere, 2006; Ragnar, 2003; Tareke et al., 2000).

**AA FORMATION MECHANISM IN FOODS**

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**FACTORS AFFECTING AA FORMATION AND REMOVAL**

The pH, temperature, reaction time, moisture content, reactant concentration, and type of reactants are important determinants of the AA content in the food (Shaikh et al., 2009). Lowering the pH of food inhibits the formation of AA (Jung et al., 2003; Kita et al., 2004; Pedreschi et al., 2007). With addition of some acidifying compound (like citric acid) into the matrix, at a low pH (< 6), a decrease of AA formation was observed. In contrast, at high pH (~8) the highest content of AA is formed (Mestdagh et al., 2008a, 2008c; Rydberg et al., 2003).

Tareke et al. (2002) indicated that the formation of AA was temperature dependent, increasing with increasing temperatures. Similar results were reported in the literature (Bråthen and Knutsen, 2005; Koutsidis et al., 2007; Surdyk et al., 2004; Taeymans et al., 2004). Raw or unheated foods did not exhibit any AA formation.

In commercially processed foods as well as in home-cooked meals the AA content tends to increase with cooking time and temperature. The surface color of the products correlates highly with AA levels in food: the darker the surface, the more AA it contains (Castle and Eriksson, 2005; Tareke et al., 2000; U.S. EPA, 2010).
The free amino acid Asn is probably a major precursor of AA. The rate of formation of AA is proportional to the concentration of Asn; selecting cultivars for food use that contain low levels of Asn and/or devising conditions to hydrolyze Asn to aspartic acid chemically or enzymatically with asparaginase or other amidases prior to food processing may result in low-AA foods (Anese et al., 2011; Friedman, 2003; Pedreschi et al., 2008).

AA is formed in comparable amounts with several mono- or disaccharides. Even nonreducing sugars, such as sucrose, are efficient reactants, leading, after thermally induced hydrolysis, to the release of reducing sugars that are then available to react with the α-NH2 group of Asn via the Maillard route (Taeymans et al., 2004).

It is interesting to note that in most experiments mixtures of fructose with Asn were more efficient in AA formation than mixtures with glucose (Robert et al., 2004).

The baking agent NH4HCO3 has been found to enhance the formation of AA in bakery products in both model systems and practical production (Amrein et al., 2004, 2007).

Divalent cations, e.g., Ca2+ and Mg2+, were found to prevent AA formation completely, while monovalent cations, e.g., Na+ and K+, reduced AA formation by almost half (Gökmen and Şenyuva, 2007; Mestdagh et al., 2008a, 2008c).

In addition, antioxidants have been proposed as one highly possible mechanism to inhibit AA formation. In particular, antioxidants such as phenolic compounds, flavonoids, vitamins, and phenolic extracts from various spices have been reported to inhibit AA formation (Kotsiou et al., 2011). Yeast fermentation is reported to reduce AA formation in yeast-leavened wheat bread due to a consumption of the limiting precursor Asn by yeast (Bartkiene et al., 2013; Fredriksson et al., 2004; Mustafa et al., 2009).

SAMPLE PREPARATION

It became apparent that sample preparation and extraction had a great influence on the value of AA measured in food samples. Due to the diversity of sample matrices, various measurement methods and sample preparation procedures have been applied (Delatour et al., 2004).

For sample preparation, the routine procedure prior to instrumental analysis includes sample homogenization, addition of internal standard (IS), defatting, extraction, concentration, and cleanup. A critical factor for analyte recovery appears to be the extraction and cleanup of AA from different food matrices, since AA may be firmly enclosed and not homogeneously distributed (Biedermann et al., 2002; Rufán-Henares and Morales, 2006; Yusà et al., 2006).

Addition of Internal Standard

The commonly used methods for AA quantification include external and internal standard method. For determination of AA in foods, an external quantitative analysis revealed poor reproducibility and recovery yield (Kim et al., 2007; Wenzl et al., 2003; Zhu et al., 2008). To overcome these problems, an internal standard is added to the sample matrix after homogenization or to the food-extractant mixture, to keep control of the recoveries achieved and to keep track of possible losses occurring during the whole sample pretreatment (extraction and cleanup) (Zhang et al., 2005). Addition of an internal standard significantly improves the accuracy and precision of measurements, allowing repeatability and intermediate reproducibility relative standard deviations below 10%, even at low concentration levels. It is also helpful in overcoming the quantitative issues associated with ion suppression, although it is not a panacea for matrix effects (Aguas et al., 2006; Pittet et al., 2004; Zhu et al., 2008). Sample preparation would be ideal if the ratio of peak area of AA to that of internal standard obtained from the sample was identical to that in the control; this means the matrix in the sample had no effect on analysis (Liu et al., 2008). The internal standard data are mainly used for location of AA, verification of nondetection, quantitation, and confirmation of identity. Co-elution of the internal standard with the analyte identifies the location of the analyte in the ion profiles. The presence of an internal standard response and the absence of an AA response are used as evidence for the absence of AA in the test portion (Roach et al., 2003; Zhu et al., 2008). If the internal standard response in a sample set degrades or disappears, the data are demonstrating an analytical problem that must be corrected before the analyses can proceed. In contrast, the data would have been construed as non-detects or low results if the measurements were based on comparisons to external standard responses. Recovery of the internal standard is used to verify that the analyte can be recovered from the matrix and an internal correction for instrument performance or sample preparation effects (Roach et al., 2003).

The usually used internal standards for the determination of AA include isotopically labeled internal standards such as deuterium-labeled (D3-AA or d3-AA) or carbon-labeled (13C3-AA), and non-isotope-labeled internal standards such as methacrylamide, N,N-dimethylacrylamide, and acetamide (Jezussek and Schieberle, 2003; Kim et al., 2011; Sun et al., 2012; Tareke et al., 2000). The isotope-labeled internal standards are the most ideal internal standard and can be used only in MS-based analysis. It was reported that satisfactory repeatability of the results of the analysis could not be achieved until isotopically labeled AA was used. This could be due to the differing stability of the compounds. Another reason for variation might be the incomplete derivatization of structurally different internal standards. It was also reported that a large difference in the reaction kinetics of the bromination reaction of AA and methacrylamide existed. A long bromination reaction time was required when methacrylamide was used as internal standard (Kim et al., 2007; Wenzl et al., 2003).

Although d3-AA can be applied as internal standard, preference should be given to the 13C labeled analogue, since the deuterated compound would lose because the deuterium labeling is positioned at the double bond in [1H1]-AA, thereby
making a deuterium/protium exchange possible during workup, which would result in increased overlap of the mass spectra of the analyte and the internal standard (Castle and Eriksson, 2005; Jezussek and Schieberle, 2003; Wenzl et al., 2003).

Kim et al. (2011) used acetamide as internal standard for the quantitative determination of AA in fried potato matrices. Acetamide has a structure similar to that of AA and it fulfilled the requirements of an internal standard for quantitative analysis of AA due to its similar extraction and chromatographic properties.

Sun et al. (2012) used \(N,N\)-dimethylacrylamide (DMA) as internal standard for the following reasons: (i) DMA does not exist in the samples, (ii) its physical and chemical properties, such as volatility and the allotment between the eluting reagent and the sorbent, are similar to those of AA, and (iii) its retention time is different from that of AA. Some researchers have found that, reproducibility and accuracy was low when \(N,N\)-dimethylacrylamide was used as internal standard (Cheng et al., 2006; Tareke et al., 2002). Wenzl et al. (2003) reported that, the properties of \(N,N\)-dimethylacrylamide are obviously too different from those of AA.

Sample Extraction and Cleanup
All the extraction and cleanup steps of AA before sample injection into GC or LC column from many peer-reviewed articles are generally summarized and shown in Figure 2.

There is no extraction method that is fully accepted for the extraction of AA from various food samples (Başkan and Erim, 2007; Khoshnam, Zargar et al., 2010). Extraction conditions appear to be the most critical parameter in sample preparation for the detection of AA in a range of foods. To date, significant efforts have been directed to developing the best performing extract procedures with a focus on chromatographic separation and MS detection conditions (Gökmen et al., 2009). Many factors can affect AA extraction yield from food matrices such as sample particle size, defatting, extraction solvent, solvent-to-sample ratio, Ultra Turrax homogenization, the application of mechanical forces, extraction temperature, and extraction time (Gökmen et al., 2009; Pedersen and Olsson, 2003; Petersson et al., 2006; Wenzl et al., 2007).

Erroneous results in the analysis of AA might occur when the food is not sufficiently macerated or when a short extraction time or low extraction temperature is used (incomplete extraction), or consequent loss of analyte during the removal of fat or the evaporation of extractant, especially when these conditions are combined (Joint FAO/WHO Expert Committee on Food Additives [JECFA], 2005; Petersson et al., 2006; Zhang and Ren, 2009).

Certain pitfalls affecting the extraction of AA have been proposed, e.g., formation of AA during the extraction procedure, such as significant quantities of AA being generated during methanolic Soxhlet extraction of potatoes from AA precursors (DeVries and Post, 2004; Goldmann et al., 2006; Pedersen and Olsson, 2003). Other possible pitfalls include contamination of AA from lab-ware such as syringe- and ultra-filters and thermal degradation of AA. Some studies proposed that higher amounts of AA can be extracted from certain foods at strongly alkaline conditions (pH 12) than at more normal conditions (pH 6) (Goldmann et al., 2006; Petersson et al., 2006). Other works also suggested that AA can significantly co-evaporate with water (JECFA, 2011; Zhang and Ren, 2009).

A great disparity exists among different laboratories in the mechanical support of AA extraction from food samples. This could be, for example, ultrasonic bath or shaker (Bagdonaité et al., 2008; Geng et al., 2011; Mojska et al., 2012; Zhang et al., 2005), horizontal shaker (Cheng et al., 2009; Errola et al., 2007; Liu et al., 2008), rotating shaker (Teczan and Erim, 2008; Wang et al., 2008), Ultra Turrax homogenizer (Jezussek and Schieberle, 2003; Lasekan and Abbas, 2010), vortex oscillator or vibrator (Karasek et al., 2009; Shi et al., 2009), and thermostat water bath (Paleologos and Kontominas, 2005).

Different methods were used for the extraction of AA from food samples. These could be divided into two main categories: extraction with water or with an organic solvent (Khoshnam et al., 2010).

Water is highly efficient extraction solvent for AA from food matrices because AA is very hydrophilic and highly soluble in water (Rosén et al., 2007; Şenyuva and Gökmen, 2005, 2006). Water at room temperature has been frequently used to extract AA; this minimizes the dissolution of hydrophobic compounds in the food products, and also the co-extraction of other unwanted compounds in the matrix (e.g., salts, proteins, carbohydrates), which might interfere with the detection and degenerate the chromatographic system, if not removed by cleanup (Petersson et al., 2006; Wang et al., 2008).

Sometimes elevated temperature is used for swelling of the matrix and to achieve better penetration of the extraction solvent into the food matrix (Petersson et al., 2006).

Besides water as extractant, organic solvents such as n-propanol, methanol, acetone, and acetonitrile (ACN) also can be used for AA extraction from food samples (Başkan et al., 2007; Dunovská et al., 2006; Khoshnam et al., 2010; Palazoğlu and Gökmen, 2008; Tateo et al., 2007). The use of n-propanol as extractant followed by solvent exchange to ACN to avoid co-isolation of AA precursors and enable a simple cleanup by restricted solubility of the salts was reported (Dunovská et al., 2006). Compared to water as extraction solvent, n-propanol has several advantages: (i) it yields a clean solution mostly without centrifugation, (ii) it also extracts lipids, which might hinder extraction with water, (iii) it can be evaporated rather easily, and (iv) it azeotropically removes some 28% of water (Biedermann and Grob, 2002).

The use of methanol as extracting medium rather than water was investigated. Addition of water to a homogenized potato sample was found to result in a thick slurry, rendering extraction difficult (Palazoğlu and Gökmen, 2008; Şenyuva and Gökmen, 2006). Methanol is very compatible with food matrices.
FIG. 2. Representative steps of sample pretreatment and derivatization before the determination of acrylamide.

containing high amounts of fat such as potato chips. Since methanol does not extract starch and some other polysaccharides, it yields a much clearer extract than that with water even without centrifugation. In addition, it can be easily evaporated under a gentle stream of nitrogen to improve the limit of quantitation (LOQ) by concentration (Gökmen et al., 2005). Koshnam et al. (2010) proposed that acetone can be used for the extraction of AA from potato chips for its great range of ability to extract AA with minimum interferences. Takatsu et al. (2003) reported that when acetone is used for AA extraction, a sufficient amount of water should be used to extract AA entirely from samples. Although acetone extraction without water gave good recovery of AA from spiked potato chips and the extract was clean compared with the water extract, acetone without water failed to extract the AA contained originally in the sample. Some authors reported that high extraction recovery of AA can
be achieved by using concentrated sodium chloride aqueous solutions as extraction solvent in order to inhibit the emulsification process during sample pretreatment and partial denaturation of proteins (Zhang et al., 2005, 2006, 2009). Delatour et al. (2004) suggested liquid-liquid extraction of AA with ethyl acetate. The sample preparation is started by extracting the analyte from the ground sample with water or aqueous solution of sodium chloride (Kim et al., 2011; Wenzl et al., 2007). Ethyl acetate appeared to be a suitable solvent to “salt-out” AA from the aqueous mixture and could also be easily concentrated by rotary evaporation. Due to the hydrophilic nature of AA, the analyte was extracted three times with ethyl acetate to achieve a satisfactory extraction yield (Arissto et al., 2008; Delatour et al., 2004; Jiao et al., 2005; Zhang et al., 2005). Using ethyl acetate to extract AA from the aqueous phase enhanced the removal of interfering constituents such as salt, sugars, starches, and amino acids (Zhang et al., 2007b).

Processed potato and cereal-based food samples are usually composed of high amounts of colloids (starch and proteins) and fat, which should be separated after extraction with water (Şenyuva and Gökmen, 2006). Therefore, defatting has to be included in sample preparation because of the influence of high fat content on the analysis by giving peaks overlapping with the target analytes or blocking the analytical column. Some researchers have adopted a defatting step prior to or in combination with extraction (Geng et al., 2011; Keramat et al., 2011; Paleologos and Kontominas, 2005; Wenzl et al., 2003). Solvents such as hexane, dichloromethane, petroleum ether, and cyclohexane were used for fat extraction (Becalski et al., 2005; Olmez et al., 2008; Peterssson et al., 2006; Tateo et al., 2007; Zhang et al., 2007b). Wang et al. (2008) and Cheng et al. (2009) found that when the temperature was 0° or 5°C and the centrifugation speed was 13,000 × g, the oil layer was solidified and could be removed simply by filtration, thus greatly reducing the interference.

Fernandes and Soares (2007) and Hoenicke et al. (2004) concluded that the removal of the fats with n-hexane from, for instance, potato chips to approximately 40% in total weight improved the swelling properties and insured a more adequate contact of the water with the food matrices, improving the extraction yield of AA.

A deproteinizing or clarification step is needed for protein-rich sample matrices. Proteins are precipitated with ACN, methanol, ethanol, acetone, sodium chloride, or Carrez reagents (potassium ferricyanide [I], zinc sulfate [II]), and also with filtration through a cutoff filter (Geng et al., 2008; Lagalante and Felter, 2004; Oracz et al., 2011; Riediker and Studler, 2003; Schieberle et al., 2005; Zhang et al., 2006). Gökmen et al. (2005) reported that clarification not only purified the extract by precipitation of dissolved colloids, but also prevented the loss of AA during the evaporation to dryness under a gentle stream of nitrogen, which caused significant loss of AA (30%) from methanolic solution. Dispersive solid-phase extraction (dSPE), popularly known as, QuEChERS (quick, easy, cheap, effective, rugged, and safe), is a novel sample preparation method that offers advantages such as applicability to a wide variety of analyte chemistries, little use of glassware/plasticware, low solvent use, low cost, and automation (Anastassiades et al., 2003; Kole et al., 2011).

Mastovska and Lehotay (2006) developed a fast and easy dSPE method for AA extraction from different food matrices. This method is based on addition of an IS to a homogenized sample together with 5 mL of hexane, water, ACN, anhydrous magnesium sulfate, and sodium chloride. Water enhances the extraction of AA, hexane serves for sample defatting, and the addition of salt significantly reduced the mutual miscibility, even resulting in phase separation of ACN from aqueous phase and forcing the majority of AA into the ACN layer. An aliquot of 1 mL ACN extract is cleaned up by dSPE using a primary secondary amine sorbent (PSA) and anhydrous MgSO4. The final extract is analyzed by either liquid or gas chromatography (Mastovska and Lehotay, 2006; Zhang et al., 2009). This extraction method has several advantages, including higher sample throughput and lower costs. It avoids time- and labor-intensive steps such as evaporation/solvent exchange, filtration, quantitative transfers, and/or multiple SPE cleanups using traditional cartridges. Also, potential contamination by AA from labware is minimized due to the elimination of filters and the use of a fluorinated ethylene propylene (FEP) tube as the only reusable item (Mastovska and Lehotay, 2006; Zhang and Ren, 2009).

Liu et al. (2008) extracted AA from tea samples following the method described by Mastovska and Lehotay (2006) with some modification; AA was extracted by water followed by ACN, and then magnesium sulfate and sodium chloride were added. In order to increase the response of AA, a 9 mL ACN layer was taken and concentrated to 0.5 mL. Solid-phase extraction with an Oasis MCX cartridge was carried out for cleanup.

Matrix solid-phase dispersion (MSPD) is a particularly effective technique. It provides a porous structure to enable the solvent to penetrate the matrix and extract the analytes, but also has some functionality that can retain the fat/lipids. The sample is mixed with a matrix, such as C18 bonded silica, sodium sulfate, or HydroMatrix, followed by washing and elution with a small volume of solvent (Ridgway et al., 2007). MSPD was applied to the determination of AA in potato chips and variety of food matrices. In this method samples were ground and dispersed in C18 (2 g), transferred to an empty column, and after a previous cleanup with n-hexane (for potato chips only), AA was eluted with water (Fernandes and Soares, 2007; Soares and Fernandes, 2009). Soares et al. (2010) described MSPD for the extraction and purification of AA from coffee samples with a conditioned C18 phase followed by a custom-made bilayered (C18/Isolute Multimode) SPE column. MSPD increased the sensitivity of analysis, decreased consumption of organic solvents, and shortened the time of extraction. Furthermore, the problem caused by formation of emulsion, which is often encountered in liquid-liquid extraction, was avoided (Oracz et al., 2011). Recently, Xu et al. (2013) developed an MSPDE technique for AA
purification and extraction. The food samples were mixed with an aliquot of EXtrelut NT and then packed into a chromatographic glass column containing anhydrous sodium sulfate. The particle material of EXtrelut NT was used to adsorb the water and distribute the matrix in solid phase. The lipid-soluble matrix can be cleaned by a weak polar solvent mixture (70% hexane/diethyl ether (v/v)), and the analytes can be eluted by a strong polar solvent mixture (50% diethyl ether/ethanol acetate (v/v)). Extraction and purification can be finished by a single step of MSPDE.

Pressurized fluid extraction (PFE), sometimes referred to as pressurized liquid extraction (PLE), pressurized solvent extraction (PSE), or accelerated solvent extraction (ASE), is an innovative technique that has already been used for extraction of AA from foods. It involves extraction with liquid solvents, but at elevated temperatures and pressures (Carabias-Martínez et al., 2005). Cavalli et al. (2003) presented a fast, automated extraction method for AA from food using accelerated solvent extraction (ASE). Pure water and water with 10 mM formic acid were tested as the extraction solvent. Pure water extracts showed lower recoveries than the formic acid, but the formic acid extracts had lower stability. Hoenicke et al. (2004) investigated the applicability of ASE using both water and 1% formic acid as extraction solvent for AA from food matrices. However, in both cases a blockage of the cell followed by termination of the extraction process was observed. This problem was solved after the use of extraction thimbles, but the extracts remained opaque. Extraction with ethyl acetate at 80°C and 7 mPa pressure yielded clear extracts, but the extraction process was neither reproducible nor applicable to all kinds of matrices. A significantly lower yield of extraction was found for cacao and milk powder. On the other hand, extraction of raw sugar yielded higher amounts of AA than extraction with water. It is not clear why higher amounts were found in these cases. Since the concentration of AA analyzed in the sugar extract increased proportionally to the extraction temperature (50°C–80°C) it was assumed that the higher amounts might be attributable to a formation of AA during PLE. However, PLE at 80°C of raw mashed potatoes (which have a high potential of AA formation at temperatures above 120°C) did not result in the formation of AA. Yusa et al. (2006) described an ASE method for the determination of AA in different food products. The method involves PFE of foods with ACN and precipitation with Carrez reagents. The extraction of AA into a polar organic solvent (ACN) gives a clear extract and enables a simple concentration step that improves the LOQ. Dionex application note 358 describes a new ASE method that combines the extraction of low levels of AA from coffee and chocolate with an in-cell, solid-phase cleanup step. The samples were extracted by hot water, and Carrez solutions were added to precipitate the proteins. Florisil was added to the extraction cell to eliminate the need for an additional cleanup step of the extract (Dionex Corporation, 2012).

Solid-phase micro-extraction (SPME) technology is a recent advance in sample preparation for trace analysis. It is a solvent-free sample preparation technique that uses a fused silica fiber coated with an appropriate stationary phase attached to a modified microsyringe. SPME is essentially a two-step process, first the partitioning of analytes between the sample matrix and the fiber coating, and then desorption of the (concentrated) extract from the fiber into the analytical instrument, usually a GC, where the sample components are thermally desorbed. The fiber can also be extracted (desorbed) into an LC eluent using a static or dynamic mode, and several commercial interfaces are available. However, for LC automation, in-tube SPME devices are generally more suitable (Kataoka et al., 2000). The analytes can be extracted either by directly immersing the solid phase in aqueous solution, known as direct immersion (DI-SPME), or by immersing vapor phase above the aqueous solution, known as headspace (HS-SPME) (Kataoka et al., 2000; Vas and Vekey, 2004).

Lagalante and Felter (2004) developed a headspace solid-phase micro-extraction (HS-SPME) method for AA extraction from cereal matrices. In this method, AA undergos silylation with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) to form the volatile N,O-bis(trimethylsilyl)acrylamide (BTMSA). Once formed, BTMSA is readily extracted from the headspace over the silylation reaction using a 100 μm poly(dimethyldimethoxylane) SPME fiber.

The DI-SPME method has been developed to determine AA in aqueous matrices by Lee et al. (2007). This method involves the use of a carbowax/divinylbenzene (CW/DVB)-coated fiber at pH 7, extraction time of 20 min, and analyzer desorption at 210°C for 3 min. The proposed analytical method was successfully used for the quantification of trace AA in foodstuffs such as French fries and potato crisps. Motaghi et al. (2012) used DI-SPME for AA extraction from bread samples. They concluded that 65 μm CW (PEG) fiber was considered best for AA extraction. This fact is related to the polarity of AA. CW (PEG) is a polar coating, and its polarity is similar to that of AA. They also found that the sensitivity of DI-SPME is higher than that of HS-SPME for AA extraction. The absorption of HS-SPME is not suitable because AA is soluble in water and the volatility of AA is quite low. Therefore, AA in water is easily extracted by DI-SPME.

Solid-phase extraction (SPE) involves a liquid-solid partition, where the extracting phase is a solid sorbent, and it has been used extensively to remove and concentrate trace organic materials from liquid samples or solutions. A choice of sorbents is available using different mechanisms for extraction/retention of analytes. The use of the optimum SPE cartridge can have significant effects on recoveries (Kole et al., 2011; Ridgway et al., 2007; Xu et al., 2012).

SPE is recognized as a very common sample pretreatment technique for its simple, stable, and easy automation, and has been extensively used to enhance the concentration of AA in sample analysis. Cleanup of AA crude extracts by means of SPE prior to chromatography and electrophoretic assay eliminates multiple interfering compounds and increases precision.
and accuracy of instrumental analyses (Oracz et al., 2011; Xu et al., 2012). Most cleanup procedures consist of the combination of several solid-phase extractions to reduce ion suppression or so-called matrix effects and achieve low limit of detection (LOD) (Aguas et al., 2006; Dunovská et al., 2006; Keramat et al., 2011).

Ahn et al. (2002) concluded that passage of the food sample extract through a mixed-mode SPE cartridge containing nonpolar (C18), strong cation exchanger (-SO\(_3^+\)), and strong anion exchanger (-NR\(_3^+\)) functional groups removed any entrained fats and largely eliminated the matrix effects in LC-MS/MS. Becalski et al. (2003) used a combination of three different cartridges: Oasis MAX (mixed-mode anion exchange), Oasis MCX (mixed-mode cation exchange), and ENVI-Carb (graphitized carbon) for cleanup of food sample extracts for AA analysis.

Zhang et al. (2005, 2007a) evaluated SPE cartridges such as nonpolar stationary phase (Varian Bond Elut-C18, 1 mL, 100 mg or 3 mL, 500 mg) and hydrophilic-lipophilic balanced copolymer (Oasis HLB, 3 mL, 60 mg or 6 mL, 200 mg). Results showed that AA was not completely adsorbed by cartridges of small size such as Varian Bond Elut-C18 (1 mL, 100 mg) and Oasis HLB (3 mL, 60 mg). Good adsorbability and recovery were found when using Oasis HLB (6 mL, 200 mg) as SPE cartridge. The Oasis HLB cartridge belongs to the main adsorbant type which is a hydrophilic-lipophilic balance and water-wettable reversed-phase sorbent for all compounds and all general SPE needs. Eerola et al. (2007) tested some column combinations for their ability to clean up problematic extracts (coffee and bread) and settled on a compromise of using the combination of the Oasis HLB/Accucat in further studies. This combination was able to overcome most of the sample matrix problems. Şenyuva and Gökmen (2006) identified the chemical nature of interfering co-extractives potato and cereal-based foods extract. In this study, the most interfering co-extractives present in the extract are amino acids. It is very well known that amino acids can be either positively or negatively charged according to the pH of the environment because of their amphoteric property. In their study the extraction was performed by acidified water, hence most of the amino acids present in the extract were positively charged. These amino acids, therefore, can be easily retained by passing through an Oasis MCX SPE.

Rufián-Henares and Morales (2006) assayed two different types of cartridges, Isolute Multimode and mixed Oasis HLB + MCX, to purify AA extracts from potato chips. The characteristic features of the Multimode sorbent are hydrophobic interaction (presence of C18 functional groups) and strong cationic (SCX) as well as anionic (SAX) exchange. The Oasis HLB is a C18 cartridge and Oasis MCX is a SCX cartridge. In this way, it minimized the interfering co-extractive load with both types of SPE cartridges, showing “cleaner” chromatograms and higher signal responses due to less ion suppression effects. Although Isolute Multimode and Oasis showed similar cleanup capacity, the Oasis cartridges were selected because of the lowest loss of AA during solvent removal and lowest rotary evaporation times. Because AA is eluted by methanol from Oasis cartridges the aqueous extract was collected after passing through Isolute Multimode. Aguas et al. (2006) reported a method that uses an aminopropyl cartridge to process extracts prior to LC-MS/MS analysis to quantify AA in coffee. Their initial attempts at using nonpolar multimode SPE cartridges to prepare the aqueous extracts of coffee samples for LC-MS/MS analysis were unsuccessful. The aminopropyl sorbent is of the normal-phase variety, which also has weak anion exchange properties. Thus, in addition to its absorption properties, it would also retain extraneous matrix components by an ionic mechanism. It retained AA from a relatively nonpolar solution. Bermudo et al. (2006) developed an improved purification procedure based on the coupling of two highly cross-linked polystyrene-divinylbenzene polymeric sorbents, Strata-X-C and ENV++, to obtain clean hydro-organic extracts of AA. Both cartridges are functionalized with polar and cation-exchange groups that exhibit numerous retention mechanisms including hydrophobic, hydrogen bonding, π–π, and cation exchange, making these columns ideal for the extraction of polar analytes.

Rosén et al. (2007) studied a wide variety of SPE columns for their capacity to retain AA from water. Silica-based phases (surface modified with C8, C18, benzene sulfonic acid, and/or quaternary amine groups) all gave poor retention. Losses of AA were detected at an elution volume of 2 mL/g solid phase, and more than 75% was lost with 4 mL/g. Higher retention was obtained with the polymeric and graphitized carbon phases. The strongest retention was obtained with the Isolute ENV+ column, comprising a highly cross-linked polystyrene divinylbenzene resin with a very high surface area modified with non-ionizable hydroxide groups. With this phase, 20 mL of water per gram solid phase could be passed with less than 5% loss of AA from the column. The retention of polar compounds on graphitized carbon has been attributed to charge-induced interactions with the polarizable surface of graphite. In addition, its flat crystalline surface is different from the brush-like surface of silica-bonded phases. Surface geometry and area might also contribute to the higher retention of AA on polymer than on silica-based phases. In addition, the phenolic groups on ENV+ might favor retention of AA through hydrogen bonding with the amide function, which can act as both a hydrogen bond donor and acceptor. Liu et al. (2008) used the Oasis MCX SPE cartridge for cleanup of ACN extract of a tea sample. This kind of SPE sorbent is effective for extraction of basic compounds from complex matrices, while AA exhibits weak basicity and does not interact strongly with the sorbent. The pass-through strategy for SPE cleanup was applied to retain the matrix interferences. Arisseto et al. (2008) used Oasis HLB and Bond Elut-Accucat cartridges in combination and also tested uses of Isolute Multimode cartridges separately for cleanup of cocoa and coffee sample extracts. The best cleaned-up extract and recovery yields were univocally obtained using the Isolute Multimode cartridges in combination with liquid-liquid extraction using ethyl acetate. The combination of Oasis HLB and Bond Elut-Accucat cartridges was used...
FIG. 3. Percentage usage of different types of SPE cartridges for all methods cited in this review article (color figure available online).

for purification of AA food sample extracts. The extracts were directly passed through the two SPE cartridges and all eluents were collected in order to obtain high recoveries (Wang et al., 2008). Some researcher indicated that when only Isolute Multimode cartridge was used, the AA signal was interfered by overlapped noises. The capacity of the column to remove all interferences simultaneously appeared to be limited (Cheng et al., 2009). Xu et al. (2012) investigated the potential applications of activated silica gel, carbon nanotubes, magnetic chitosan, modified chitosan, and reversed-phase C18 bonded silica gel in solid-phase extraction of AA. They indicated that C18 was more suitable as a SPE sorbent than the other four materials in the AA pretreatment procedure. They showed that C18 sorbent has good adsorption ability and rapid adsorption dynamic toward AA. Also they indicated that C18 had slower uptake kinetic than magnetic chitosan, which is the drawback of C18 for application as sorbent in SPE. Magnetic chitosan exhibited fast adsorption dynamics and had higher adsorption ability toward AA than that of activated silica gel, carbon nanotubes, or modified chitosan. Therefore, it will be a good potential sorbent in AA pretreatment after modification.

Bortolomeazzi et al. (2012) a developed rapid and reliable purification method based on a single mixed SPE column for the determination of AA in roasted coffee. This method was based on the retention of the matrix interferences and the analyte elution. It involved purification of water extract by a single SPE column consisting of 0.5 g of an in-house prepared mixture of C18, SCX, and SAX sorbents in the ratio 2/1.5/1.5 (w/w/w). The SPE column involved all three types of interactions (hydrophobic, strong anionic, and cationic exchange) in order to eliminate the majority of the interfering compounds in coffee extract. An activated carbon–packed extraction column coupled with GC to determine AA was demonstrated by Sun et al. (2012).

The percentages of the application of all types of SPE cartridges in cleanup of AA-containing extracts for all studies cited in this review are shown in Figure 3. It appears from this figure that the most frequently used cartridges are Oasis HLB and Isolute Multimode.

ANALYTICAL METHODS FOR AA DETERMINATION

An imperative requirement is the development and validation of sensitive, accurate, and inexpensive analytical methods that can determine AA in different food matrices down to the low μg/kg level (Dybing et al., 2005; Paleologos and Kontominas, 2005; Xu et al., 2012; Zhu et al., 2008). A great number of analytical methods have been developed for the determination of AA concentration in food samples since 2002. The majority of methods employed are based on a mass spectrometer as detection system coupled to liquid (LC-MS or LC-MS/MS) or gas (GC-MS) chromatography (Bagdonaite et al., 2008; Becalski et al., 2005; Bent et al., 2012; Daniali, 2010; Jezussek and Schieber, 2003; Kaplan et al., 2009; Lasekan and Abbas, 2010; Mizukami et al., 2006; Napolitano et al., 2008; Ölmmez et al., 2008; Sagratini et al., 2007; Soares and Fernandes, 2009; Tateo et al., 2007). Besides LC or GC combined with an MS technique, some researchers reported analytical methods for the determination of AA using simple LC or GC techniques and capillary electrophoresis (CE) methods based on ultraviolet (UV) (Bermudo et al., 2006; Geng et al., 2011; Shi et al., 2009; Tezcan and Erim, 2008; Wang et al., 2008; Xu et al., 2012), fluorescence
(Schieberle et al., 2005), flame ionization detection (FID) (Motaghi et al., 2012; Sun et al., 2012), nitrogen phosphorus (NPD) (Kim et al., 2011), and electron capture detection (ECD) (Zhang et al., 2006, 2007b; Zhu et al., 2008). However, classical methods based on LC with a UV detector or GC with ECD techniques lack selectivity and the additional degree of analyte certainty required to confirm the presence of a small molecule such as AA in a complex food matrix (Paleologos and Kontominas, 2005; Pittet et al., 2004; Wang et al., 2008; Zhang et al., 2005). Among the diversity of developed methods, good selectivity and sensitivity are reported to be achieved by chromatographic methods coupled with a mass technique (Khoshnam et al., 2010). To ameliorate selectivity and detection limits, tandem mass spectrometry has recently been used (Bermudo et al., 2006; Özer et al., 2012). To date, it can be concluded from recent methodological studies that GC-MS and high-performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) appears to be the most widely used and acknowledged as the most authoritative method for AA determination (Cheng et al., 2006; Kim et al., 2011; Mastovska and Sobotová, 2007; Pittet et al., 2004; Wenzl et al., 2003; Xu et al., 2012; Zhang et al., 2007b).

**HPLC-Based Methods**

The analysis of purified extracts of AA can be done directly using high-performance liquid chromatographic methods (HPLC). For detection, UV as well as MS is used (JECFA, 2011; Murvick, 2004). LC-UV has been used to determine AA contents in food products containing high levels of AA, so sensitivity is not a major issue (Castle and Eriksson, 2005). Because AA lacks a chromogenic group, UV measuring has to use a low wavelength (195–205 nm), which is quite universal for a whole range of unsaturated molecules. This means low selectivity for the analysis of AA in processed food at very low levels (Bermudo et al., 2006; Castle and Eriksson, 2005; Gökmen et al., 2005; Jezussek and Schieberle, 2003; Park et al., 2007). However, recent studies pay more attention to assays employing LC-MS/MS techniques for the routine analysis of AA because this chromatographic technique applied for the quantitative analysis of AA has high sensitivity, selectivity, and simplicity and avoids the derivatization step (Cheng et al., 2006, 2009; Kepekci Tekkeli et al., 2012; Murvick, 2004; Oracz et al., 2011; Wenzl et al., 2003; Zhang et al., 2005).

**HPLC Methods without Derivatization**

A simple and rapid method using HPLC coupled to diode array detection (LC-DAD) was developed for the determination of AA in potato-based foods at low levels by Gökmen et al. (2005). The chromatographic separations were performed on either Atlantis dC18 or HILIC columns (hydrophilic and hydrophobic interaction columns) with water as a mobile phase and detected at 226 nm. The LOQ was estimated to be 4.0 μg/kg. Recoveries of AA from potato chip samples ranged between 92.8 and 96.2% with relative standard deviations (RSD) of less than 5%. The result obtained by LC-DAD analyses was confirmed by LC-MS with atmospheric pressure chemical ionization (APCI). A normal phase HPLC with UV detection at 200 nm was proposed for detection and quantification of AA in food samples (Paleologos and Kontominas, 2005). The method is based on the separation of AA on a polar HPLC column (Aminex HPX-87H) using 0.01 M sulfuric acid as mobile phase. AA is converted into its cationic protonated ammonium product under these conditions. This product can interact with a polar column through acid-base interaction and be separated effectively and reproducibly. An LOD of 10 μg/L was obtained with the inter- and intra-day RSD for standard analysis lying below 1.0%. Use of ACN in the elution solvent lowers detection limits and retention times, without impairing resolution of peaks. The method was applied for the determination of AA in spiked food samples without native AA, yielding recoveries between 95 and 103%. AA can be determined directly by ion chromatography (IC) using an ion-exclusion column (Ion Pac ICE-AS1) and both UV and MS detection. With this column, AA is retained longer than on conventional reversed-phase columns, allowing separation from the many co-extractable compounds present in food samples. The detection is done by photodiode array (PDA) set at 202 nm. Formic acid in ACN/water 30% v/v was used as eluent. The amount of ACN was optimized to reduce the total run time and avoid interferences with matrix components (Cavalli et al., 2003). The AA level in some deep-fried flour-based Chinese foods commercially available in Hong Kong was investigated using reversed-phase HPLC with UV detection at 210 and 225 nm. A gradient elution program and a mobile phase of 4.0% v/v ACN in water allowed sufficient retention and well resolved AA from the food matrices in the sample extracts. The amounts of AA in food samples were 27–198 μg/kg. The recoveries of AA were greater than 78.0%. The LOD and LOQ were 6 and 23 μg/kg at a detection wavelength of 210 nm, respectively (Wang et al., 2008). Khoshnam et al. (2010) reported an HPLC-UV method for AA determination in potato chips. The method uses an ultrasonic reversed-phase C18-AQ column with water as eluent and UV detection at 202 nm. LOD and LOQ for the method were 2.46 and 3.14 ng/g respectively. A method using reversed-phase C18 column and UV detection was developed for analysis of AA in crust and potato chip samples. The mobile phase was methanol/water (15:85, v/v), and the detection was operated at 210 nm. The LOD of the method was 66.0 ng/L, and the recoveries ranged from 88.9 to 89.5% (Xu et al., 2012).

An LC-MS method using column switching was developed for the determination of AA in processed or cooked foods (Takatsuki et al., 2003). AA was detected by LC-MS using the selected ion recording (SIR) mode. Monitoring ions for AA were m/z 72 and 55, and those for internal standard [13C3]-AA were m/z 73 and 56. The recoveries of AA from spiked food samples were in the range of 99.5–101%. The LOD and LOQ of the method were 9 and 30 ng/g respectively.
A reversed-phase HPLC-MS method based on a stable isotope dilution assay was used for AA analysis in potato chips. Different columns were investigated for AA analysis. Classical ODS-2 analytical columns showed poor separation since AA co-eluted with the chromatographic front. In contrast, the ODS-2 column Synergi Hydro-RP showed an excessive retention for AA (12 min). Because of that, different ODS-1 columns (partially deactivated) were assayed, finding excellent AA separation. Acetic acid was selected as acidic modifier in the mobile phase because it significantly improved the response of AA compared to formic acid, and with ammonium acetate. Electrospray ionization in the positive ionization mode was used. The MS detector operated in selected ion monitoring (SIM) mode at $m/z$ ratios of 72.1 and 75.1 for AA and labeled [13C3]-AA, respectively. The LOD was 23.2 μg/kg and the LOQ 91.8 μg/kg. The linear range was 25–1000 μg/kg and recovery was 98.8% (Rufián-Henares et al., 2006). A simple and rapid interference-free method was developed and validated for the determination of AA in potato and cereal-based foods by using a single quadrupole HPLC-MS interfaced with positive atmospheric pressure chemical ionization (APCI+) by Şenyuva and Gökmen (2006). The analytical separation was performed on an Inertsil ODS-3 column using the isocratic mixture of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid and 0.2% acetic acid in ACN (98.2, v/v). The ions monitored were $m/z$ 72 and 55 for AA and $m/z$ 75 and 58 for [13C3]-labeled AA. The major interfering co-extractive was identified as valine, which yields characteristic [M + H]+ and compound-specific product ions having $m/z$ of 118 and 72, respectively. The adverse effect of valine could be limited by instrumentally adjusted delay time or by solid-phase extraction with strong cation-exchanger sorbent. The mean recovery was found to be 99.7% and LOD and LOQ were determined to be 6 and 18 ng/g. Kaplan et al. (2009) optimized HPLC-MS for the determination of AA levels in Turkish foodstuffs, including potatoes, potatoes chips, coffee, and cocoa. The purified samples extracts were chromatographed on a Hypercarb column, the effluent of which was monitored for AA were $m/z$ 72 > 72 and 72 > 55 and for labeled AA were 75 > 75 and 75 > 58. The analytical method in the present study was well validated, and good results were obtained with respect to repeatability (RSD < 6.5%) and recovery (87–96%), which fulfilled the requirements defined by European Union (EU) legislation for AA detection methods. The AA levels in infant rice cereals and other cereal-based foods were 3.3–37.1 μg/kg and 10.9–1568.9 μg/kg respectively.

An accurate and precise method for the quantification of AA using stable isotope-dilution HPLC-MS/MS was developed and used to measure AA in coffee and cocoa samples (Aguas et al., 2006). The purified samples extracts were chromatographed on a Hypercarb column, the effluent of which was monitored for AA at 12.3 μg/kg. An automated and rapid method for the determination of AA in different food products is presented by Yus¸a et al. (2006). The method involves pressurized fluid extraction (PFE) of foods with ACN, precipitation with Carrez reagents, and analysis by LC-MS/MS. Analytical separation was carried out on the porous graphitic carbon column Hypercarb. AA was detected in the positive ESI mode. The transitions monitored for AA were $m/z$ 72 > 55 at 12 V and 72 > 44 at 43 V, corresponding to C2H5CO+ and CONH2+, respectively. For [13C3]-AA the transition monitored was 75 > 58 at 22 V. The LOQ of the method was 5 μg/kg, and recoveries from samples ranged between 93 and 101%. A European inter-laboratory study was conducted to validate two analytical procedures, HPLC-MS/MS and GC/MS, for the determination of AA in baked goods (crisp
breads, biscuits) and potato products (Wenzl et al., 2006). The authors claimed that the HPLC-MS/MS method studied was superior to the GC-MS method investigated. HPLC-MS/MS was described for the determination of AA in foodstuffs; APCI as ionization source and an ion-trap (LCQ) analyzer were used (Bermudo et al., 2006). In this study APCI was used because the gas-phase ionization mechanism of APCI is less influenced by the mobile-phase composition. In contrast, it is well known that ESI-MS is problematic when highly aqueous solutions, such as those required for the reversed-phase LC separation of AA, are used (Bermudo et al., 2006). The validation of the method was carried out using HPLC-MS/MS with a triple quadruple as mass analyzer. The detection limits was found to be 250 pg and 45 ng/g for the standard and samples, respectively. Different levels of AA were obtained; pastry and dried fruits showed lower levels (<20 ng/g), and potato chips and French fries gave values of the order of 500–9250 ng/g.

An improved method was validated for the determination of AA in foods using ultra-performance liquid chromatography (UPLC) coupled to electrospray ionization tandem mass spectrometry (MS/MS) by Zhang et al. (2007a). The performance of this method was compared to that of a previously validated HPLC-MS/MS analytical method for the quantification of AA in various foods (Zhang et al., 2005, 2006). The UPLC-M/MS-based method could offer significant improvements in sensitivity, analytical speed, and resolution. Kim et al. (2007) developed an LC-MS/MS method for the analysis of AA in processed foods. The AA extract was directly analyzed using LC-MS/MS without derivatization. In this method [13C3]-AA was used as an internal standard and d5-3-chloropropanediol as a recovery standard. The d5-3-chloropropanediol standard was found to be the most reliable for the identification and quantification of AA in multiple reaction monitoring. The limit of quantification for AA was 2 μg/kg. Another LC-MS/MS method was validated for the routine analysis of AA in various foodstuffs (Eerola et al., 2007). The AA was separated on a graphite carbon analytical column (Hypercarb). The MRM mode was used for ion detection, and the transitions from 72 to 55 m/z of AA and from 75 to 58 m/z for the deuterated analogue internal standard were monitored. AA was detected at levels, ranging from nondetectable to 1480 μg/kg in solid food, with crisp bread exhibiting the highest levels. A method for determination of AA in Chinese traditional carbohydrate-rich foods using HPLC coupled to quadrupole tandem mass spectrometry (MS/MS) in positive electrospray ionization mode was developed (Zhang et al., 2007b). Chromatographic separation was carried out on an Atlantis dC18 column. Recoveries were in the range 84–97% for AA spiked at 50, 500, and 1000 μg/kg, and the LOQ was 4 μg/kg. AA contaminant was found in all samples at concentrations up to 771.1 and 734.5 μg/kg.

A robust LC-MS/MS method was designed for validation by a multi-center collaborative validation trial (Rosén et al., 2007). The study provided a base for developments of new methods for cleanup, enrichment, and chromatography of AA. A wide range of solid phases for SPE and HPLC were compared for the chromatographic retention of AA. For SPE, a hydroxylated polystyrene-divinylbenzene copolymer phase (ENV+) gave the strongest retention. For HPLC, columns were evaluated in both reversed-phase and aqueous normal-phase (hydrophilic interaction chromatography) modes. The best retention was obtained with a phase comprising porous graphitic carbon (Hypercarb). LOQ was 5 μg/kg. The method was applied for determination of AA level in different spiked food matrices (e.g., mashed potato, coffee, and cereals). A reliable and efficient method for analysis of AA in cocoa powder, chocolate, and coffee by HPLC-MS/MS was presented (Ariseto et al., 2008), and a modified sample preparation for AA determination in difficult matrices was described. LOD and LOQ of 10 and 20 μg/kg and mean recoveries ranging from 93 to 99% were obtained during a laboratory validation procedure. An effective sample preparation procedure was optimized and an LC-MS/MS method was developed for the quantitative analysis of AA in tea (Liu et al., 2008). A reversed-phase ODS-C18 column was used for AA separation. AA was detected using electrospray ionization in the positive ion mode. The LOD and LOQ were found to be 1 and 5 ng/mL, respectively. A recovery efficiency of the extraction procedure ranging between 74 and 79% was obtained. The levels of AA in 30 tea samples were found to be less than 100 ng/g. Cheng et al. (2009) validated an LC-MS/MS method for analyzing AA in foods including fried potato snack food, baked breakfast food, bread, coffee, and tea drinks in Taiwan. AA was analyzed using electrospray ionization in positive ion mode, and MRM was acquired. The elution of AA was done using two columns, Atlantics dC18 and AQUALSIL C18. The AQUALSIL C18 column yielded better separation efficiency for obtaining AA from the sample matrix and for higher resolution. The LOD was 3 μg/kg and mean recoveries ranged from 95 to 113%. Brown sugar and asparagus juice were found to carry high AA content and thus increased AA contents in related products.

A collaborative trial tested an isotope-dilution HPLC method with positive electrospray MS/MS for the detection of AA in bakery goods and potato products, which was extended to the determination of AA in roasted chestnuts and chestnut-based foods (Karasek et al., 2009). The analytical separation was performed on a Hypercarb graphitized carbon HPLC column. The method was validated in-house for AA analysis in various chestnut products. AA contents were measured in 31 different chestnut samples (fresh, roasted, flour, cooked, and glazed). AA concentrations in purchased roasted chestnuts were in the range of 8–1278 μg/kg, whereas only low amounts (4–159 μg/kg) were found in chestnut products. A study on the occurrence of AA in traditional foodstuffs consumed in Zimbabwe was carried out using HPLC-MS/MS operated in positive electrospray mode (Sanganyado et al., 2011). The LOD of this method was 0.02 μg/kg. AA was not detected in boiled maize. Roasted maize contained 450 μg/kg AA. Roasted groundnuts had a concentration of 140 μg/kg and roasted soybeans 70 μg/kg AA. A rapid and reliable purification method based on a single
custom-made mixed SPE column for the determination of AA in roasted coffee by HPLC-ESI-MS/MS in positive mode was developed (Bortolomeazzi et al., 2012). The Synergi Hydro analytical column was used for AA separation. The recovery of AA at three spiked levels ranged from 92% to 95%. LOD and LOQ were 5 and 16 μg/kg respectively. The method was considered to be suitable for routine analysis of AA in roasted coffee. AA content in baby food products was analyzed by LC-MS/MS methods (Mojska et al., 2012). For AA separation a Hypercarb analytical column was used. LOQ was calculated as 2.5 μg/kg for water, 10 μg/kg for cereal matrix, and 25 μg/kg for potato matrix. The study provides information on AA levels in commercially produced baby food in Poland. The AA content in the 111 tested samples of baby food products ranged widely, from 2 to 516 μg/kg of commercial product.

An LC-MS/MS-based method was used for investigation of AA contents of some Turkish desserts and their raw materials (Ozer et al., 2012). Triple-quadrupole API LC-MSMS was used to determine AA. LOD and LOQ were 3 and 5 ng/g, respectively. An isotopic-dilution HPLC-MS/MS was developed to analyze AA in a variety of Belgin food samples such as potatoes, French fries, crisp bread, coffee, and corn flakes (Douny et al., 2012). For analytical separation, an Alltima HP C18 amide column and Alltima HP C18HL were tested. The mass spectrometer was operated in positive-ion mode. LC analysis realized with the amide column gave better results than with the HL column, as well as for the area of the peaks than for their shape. The retention times increased to 4.85 minutes with the amide column, compared to 1.85 minutes with the HL column. LOQ of 50 ppb was achieved using this method. AA content in instant noodles from supermarkets in the Hebei province of China was investigated using HPLC-MS/MS (Yamazaki et al., 2012). Chromatographic separation was performed on an Atlantis dC18 column. AA concentrations in instant noodles (90 samples, covering 10 different brands) were determined by LC-MS/MS. The average content of AA for all 10 instant noodle brands was 6–145 μg/kg.

HPLC Methods with Derivatization

Because AA is a very polar molecule and highly soluble in water, its retention on conventional reversed-phase sorbent is poor, which results in an unsatisfactory separation between AA and the co-extracts of the matrix in LC (Geng et al., 2011; Jezussek and Schieberle, 2003). Then, generally, high water content mobile phases are used, which assumes poor ionization efficiencies when ESI is employed as an ionization source in LC-MS (Bermudo et al., 2006). Also, AA lacks a chromogenic group, hence its detection using UV or DAD on conventional reversed-phase sorbent columns does not have the desired selectivity and could be applied only to those foods with high levels of AA, such as potato-based foods (Bermudo et al., 2006; Geng et al., 2011; Jezussek and Schieberle, 2003). Derivatization of AA solves the problem of its poor retention on RP-HPLC column and avoids the interference of co-extracts. Also, the derivatization process increases the selectivity and sensitivity in instrumental analysis (Jezussek and Schieberle, 2003; Shi et al., 2009). AA was found to form a stable thioether in reasonable yields (45–50%) when reacted with 2-mercaptobenzoic acid at 20°C for 3 hours. On the basis of this finding and using [13C3]-AA as the internal standard, a sensitive and selective new stable isotope-dilution analysis for AA quantitation in food samples was developed based on single-stage LC/MS (Jezussek and Schieberle, 2003). This method is base on derivatization of AA using 2-mercaptobenzoic acid at pH 8. The excess of the reagent was removed by treatment with lead (II) acetate, followed by liquid-liquid extraction with ethyl acetate. The AA derivatized molecule was separated in a Luna Phenyl-Hexyl HPLC column. The mass spectrometer was operated in the positive electrospray mode. For quantitation, the mass traces m/z 226 and m/z 229 for the unlabeled and labeled thioethers formed in the derivatization procedure were monitored. LOD of 6.6 μg/kg was obtained by this method, lower by a factor of 1000 than that of direct LC/MS measurement using LC/MS in single-stage mode. An HPLC/fluorescence method for AA quantitation after derivatization with 5-(4,6-dichloro-striazine-2-ylamino)-fluoresceine hydrochloride (DTAF) was presented (Schieberle et al., 2005).

Chromatographic separation of AA derivative was done with a Luna Phenyl-Hexyl 100 A HPLC column. The fluorescence detector was operating at an excitation wavelength of 480 nm and an emission wavelength of 520 nm. LOQ of 0.3 μg/L was obtained by this method. Due to the complex matrix of coffee, AA was determined in coffee samples with HPLC-MS after derivatization with 2-mercaptopentobenzoic acid to increase selectivity (Bagdonaite et al., 2008). Detection was performed at a MS positive (API-ES) mode, SIM at m/z 226 (for protonated AA derivative) and 248 (for Na+ adducts of derivatized AA), and at m/z 229 and 251 for labeled derivatives, respectively. The LOD in coffee was determined as 157 ng/g and the LOQ as 270 ng/g.

An ultrasonic-assisted precolumn derivatization-HPLC method was established and validated for the determination of AA formed in the traditional Chinese herb Radix Asparagi during a heating process (Shi et al., 2009). This method entails extraction with water and ultrasonic-assisted derivatization with 2-mercaptopentobenzoic acid. The final extracted AA derivative was separated on a C18 column and the detection wavelength was set at 238 nm. The LOD was estimated to be 25 μg/kg based on the signal-to-noise ratio of 3 recorded at 238 nm. Recovery of AA from the sample was 106.6%. A method for the determination of AA in starch-based food was developed (Geng et al., 2011). The method is based on derivatization of AA with potassium bromate (KBrO3) and potassium bromide (KBr). The final analyte (2-bromopropenamide, 2-BPA) was analyzed by HPLC-DAD. The chromatographic analysis was performed on an ODS-3 C18 column, and good retention and peak response of AA were achieved under the optimal conditions. LOD and LOQ were estimated to be 15 and 50 μg/kg, respectively. The recoveries of AA from commercial samples spiked at levels of 50–1000 μg/kg range between 89.6 and 102.0%.
The authors claim that the method should be considered a new, low-cost, and robust alternative for conventional investigation of AA.

Detailed information of HPLC analytical methods for AA determination with and without derivatization in various food matrices are summarized in Table 1.

**GC-Based Methods**

The gas chromatography techniques for AA analysis in food matrices are based on derivatization of the analyte or on direct analysis without derivatization (Kepekci Tekkeli et al., 2012; Linebuck et al., 2012; Oracz et al., 2011; Soares et al., 2006; Wenzl et al., 2003, 2007; Zhang et al., 2005, 2006). Most of the GC methods are based on derivatization, because of the low volatility and polarity of AA (Dunovská et al., 2006; JECFA, 2011; Zhang and Ren, 2009). These problems can be solved by (i) application of GC-MS/MS, and interference caused by the matrix, which increases weight) and interference caused by the matrix, which increases background noise, and hence a low limit of detection is impossible to obtain (Mastovska and Lehotay, 2006; Oracz et al., 2011); (ii) potential generation of AA in situ in the heated GC injector from co-extracted precursors (asparagine and reducing sugars) when using water as extractant solvent (Dunovská et al., 2006; Weisshaar, 2004); (iii) the high solubility of AA in water in comparison with organic solvents complicates sample preparation for GC (Castle and Eriksson, 2005; Mastovska and Lehotay, 2006); (iv) owing to the higher polarity of underivatized AA, it has poor retention on conventional GC capillary columns (Castle and Eriksson, 2005; Zhang et al., 2006); and (v) the compound 3-hydroxypropionitrile may be co-eluted with AA, causing falsely high AA (Biedermann and Grob, 2008; JECFA, 2011; Zhang and Ren, 2009). These problems can be solved by (i) application of GC-MS/MS, allowing a decrease in interference (Lee et al., 2007; Oracz et al., 2011); (ii) extraction with n-propanol followed by solvent exchange ACN, avoiding co-isolation of AA precursors that could yield additional analyte in the hot splitless GC injector (Dunovská et al., 2006; Kepekci Tekkeli et al., 2012); (iii) derivatization of AA followed by extraction from the aqueous phase into ethyl acetate (Wenzl et al., 2006); and (iv) use of columns with polar phases, e.g., polyethylene glycol (carbowax) for separation (Castle and Eriksson, 2005; Zhang et al., 2005).

An ion-trap GC-MS was developed for direct determination of AA in various food samples (Mastovska and Lehotay, 2006). Methanol was used as a liquid chemical ionization reagent. Direct sample introduction (DSI) was used as an injection technique to prevent contamination of the GC system by nonvolatile matrix co-extractives. Full-scan mode was used for data acquisition in (m/z 50–85). In this method dSPE using PSA sorbent was used for AA extraction and cleanup. This extraction procedure is very effective in terms of removal of polar co-extractives, thus avoiding AA formation in the GC injector. A simple and rapid GC method employing a high-resolution time-of-flight mass (GC-HR-TOF-MS) analyzer that enables direct analysis of AA in various heat-processed foodstuffs was developed and validated (Dunovská et al., 2006). GC-HR-TOF-MS analyzer enables partial or complete resolution of interfering matrix components, yielding ions with nominal masses close to those of the target analyte. LOQs obtained were in the range between 15 and 40 µg/kg and recoveries were between 97 and 108%, depending on the examined food matrix. A gas chromatography-positive chemical ionization tandem mass spectrometry (GC-PCI-MS/MS) in the SRM mode was developed to quantify AA in aqueous matrices using DI-SPME (Lee et al., 2007). A fused-silica capillary column, DB-WAX, was used for chromatographic separation. LOD of 0.1 µg/L in water was achieved. The concentrations of AA detected in French fries and potato crisps were found to be 1.2 and 2.2 µg/g, respectively. A GC-MS analytical method was used to examine the AA levels in three foods commonly found in the Italian national diet (Tateo et al., 2007). Analysis was performed using EI (70 eV) and SIM mode. The ions monitored for identification of the analyte were m/z 55, 71, and 72, using m/z 71 for quantification. LOD was found to be 25 µg/kg. An improved sensitive and selective GC-NPD method was developed for AA determination in fried potato matrices (Kim et al., 2011). A DB-WAX fused-silica capillary column was used for AA separation. The LOQ was 0.5 ppm, and the recovery rate was 106 ± 8%. The AA level of fried potato sold in a local restaurant was 2.7 ± 0.3 ppm. AA levels in selected breads commonly consumed in Iran were evaluated using the GC-FID method (Motaghi et al., 2012). AA extraction from bread samples was carried out using DI-SPME. LOD was calculated to be 25 µg/kg. The AA contents in the breads were found to be in range between 26.43 and 145.87 µg/kg. A convenient method for detecting AA content in microwaved and conventional heated popcorn and rice was developed using an activated carbon packed extraction column coupled to GC-FID (Sun et al., 2012). The LOD and LOQ of AA were 3 ng/mL and 10 ng/mL, respectively.
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<th>Matrix</th>
<th>Method and internal standard</th>
<th>Sample preparation</th>
<th>LC column</th>
<th>UV-vis or MS or MS/MS parameters</th>
<th>LOD/LOQ, WR, and recovery</th>
<th>Ref.</th>
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<tr>
<td>Food samples</td>
<td>LC/MS; IS: $^{[13]C_3}$-AA</td>
<td>Extraction with water, defatting with hexane, derivatization with 2-mercapto</td>
<td>Luna Pheny-Hexyl (250 ×</td>
<td>ESI, capillary temp.: 200°C, capillary voltage: 16 V. For quantitation AA, the mass traces $m/z$ 226 and $m/z$ 229 $m/z$ 72 → 55 as quantifier and $m/z$ 72 → 54 and $m/z$ 72 → 27 as qualifier ions</td>
<td>LOD: 6.6 μg/kg; WR: 10.4–104.0 μg/kg Recoveries: 96–112%</td>
<td>Jezussek and Schieberle, 2003</td>
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<td></td>
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<td>2-mercaptobenzoic acid at basic media, addition of (CH$_3$COO)$_2$Pb, acidification by HCl, LLE extraction with EtAc</td>
<td>4.6 mm i.d., 5 μm</td>
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<td>Chocolate powder, cocoa, and coffee</td>
<td>LC-MS/MS; IS: d$_3$-AA</td>
<td>Extraction with water, deproteintion with Carrez I and II solutions, derivatization with dichloromethane, LLE with EtAc using NaCl for salting out, SPE with Isolute Multimode cartridge</td>
<td>Shodex RSpak DE-413L (250 × 4.6 mm i.d.)</td>
<td>LOQ: 0.3 μg/L</td>
<td>CC$\alpha$: 9.2 μg/kg  CCF: 12.5 μg/kg WR: 10.0–2500 μg/kg</td>
<td>Delatour et al., 2004</td>
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<td>Rusk</td>
<td>HPLC/fluorescence analysis; IS: $N,N$-dimethylacrylamide</td>
<td>Extraction with water, defatting with hexane and addition of acetone, evaporation, derivatization at basic media with DTAF-Cysa</td>
<td>Luna Pheny-Hexyl 100 Å (250 × 4.6 mm i.d., 5 μm)</td>
<td>Fluorescence: at an excitation wavelength of 480 nm and an emission wavelength of 520 nm</td>
<td>LOQ: 1 μg/kg; WR: 0.1–5 ng/mL and 5–200 ng/mL; Recoveries: 87–96%</td>
<td>Schieberle et al., 2005</td>
</tr>
<tr>
<td>Infant cereal-based foods</td>
<td>LC-MS/MS; IS: $^{[13]C_3}$-AA</td>
<td>Defatting with petroleum ether, extraction with water, LLE with EtAc, evaporation by N$_2$, SPE with Oasis HLB SPE cartridge</td>
<td>Atlantis dC18 (210 × 1.5 mm, 5 μm)</td>
<td>LOD: 1 μg/kg; WR: 45–2000 μg/L; Recoveries: 95–105%</td>
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<td>Zhang et al., 2005</td>
</tr>
<tr>
<td>Commercial samples of French and roasted fries, cookies, cocoa, and coffee</td>
<td>Normal phase HPLC-UV</td>
<td>Extraction with water, defatting with hexane</td>
<td>Aminex HPX-87H (300 × 7.8 mm)</td>
<td>For MP: H$_2$SO$_4$ LOD: 15 μg/L; WR: 45–2000 μg/L For MP H$_2$SO$_4$ and ACN LOD: 1.5 μg/L; WR: 4.0–2000 μg/L; Recoveries: 95–105%</td>
<td></td>
<td>Paleologos and Kontominas, 2005</td>
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<tr>
<td>Coffee and cocoa</td>
<td>LC-MS/MS; IS: $^{[13]C_3}$-AA</td>
<td>Extraction with water, defatting with dichloromethane, LLE with 1:3 EtAc/ACN, addition of isoctane, concentrate, dilution with EtAc, addition of cyclohexane, SPE with aminopropyl cartridge</td>
<td>Hypercarb (2.1 × 150 mm)</td>
<td>Column: APCI+, $m/z$ for AA: 55, 44 and for $^{[13]C_3}$-AA: 58, 45</td>
<td>LOD: 9.2 μg/kg WR: 0–2.500 ng</td>
<td>Aguas et al., 2006</td>
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<th>Matrix</th>
<th>Method and internal standard</th>
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<th>LOD/LOQ, WR, and recovery</th>
<th>Ref.</th>
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<tr>
<td>Potato chips</td>
<td>LC-MS; IS: [13C₃]-AA</td>
<td>Extraction with 2 M NaCl, deproteintion with Carrez I and II solutions, SPE with Isolute Multimode, or by Oasis HLB and MCX cartridges coupled together</td>
<td>Extrasyl ODS1 (20 × 0.3 cm, 5 μm)</td>
<td>ESI+, SIM at m/z: 72.1 for AA and 75.1 for [13C₃]-AA</td>
<td>LOD: 23.2 μg/kg WR: 25–1000 μg/kg Recovery: 98.8%</td>
<td>Rufián-Henares and Morales, 2006</td>
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<tr>
<td>Spanish cereal-based foods</td>
<td>LC-MS/MS; IS: [13C₃]-AA</td>
<td>PFE: Extraction with PFE system, accelerated solvent extraction using ACN, deproteintion with Carrez I and II solutions, evaporation and redissolving</td>
<td>Hypercarb, (50 × 2.1 mm i.d., 5 μm)</td>
<td>ESI+, m/z 72 &gt; 55 at 12 V for AA and m/z 72 &gt; 44 at 43 V, and for [13C₃]-AA m/z 75 &gt; 58 at 22 V</td>
<td>LOQ: 5 μg/kg; WR: 1–2000 ng/mL Recoveries: 93 and 101%</td>
<td>Yusa et al., 2006</td>
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<tr>
<td>Potato and cereal-based foods</td>
<td>LC-MS; IS: [13C₃]-AA</td>
<td>Deproteintion with Carrez I and II solutions, extraction with 0.01 mM acetic acid, SPE with Oasis MCX cartridge</td>
<td>Column: Inertsil ODS-3 column (250 × 4.6 mm, 5 μm)</td>
<td>m/z 72 and 55 for AA and m/z 75 and 58 for [13C₃]-AA</td>
<td>LOD: 6 ng/g; WR: 1–2000 ng/mL Recoveries: 98.5–100.4%</td>
<td>Şenyüva and Gökmen, 2006</td>
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<tr>
<td>Bakery and potato products</td>
<td>HPLC-MS/MS; IS: d₃-AA</td>
<td>Extraction with water, SPE with Isolute Multimode cartridge and then with Isolute ENV+ cartridge</td>
<td>Hypercarb column (50 × 2.1 mm)</td>
<td>m/z 72 &gt; 55, 72 &gt; 54 and 72 &gt; 44 for AA, m/z 75 &gt; 58 for d₃-AA</td>
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<td>Wenzl et al., 2006</td>
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<tr>
<td>Potato crisps</td>
<td>UPLC-MS/MS; IS: d₃-AA</td>
<td>Defatting with petroleum ether, extraction with 2 M NaCl, and LLE with EtAc, SPE with OASIS HLB cartridge</td>
<td>UPLC BEH C18 (50 mm length, 2.1 mm i.d., 1.7 μm)</td>
<td>ESI+, MRM</td>
<td>LOD: 1 μg/kg; WR: 1–200 ng/mL Recoveries: 81.6–99.0%</td>
<td>Zhang et al., 2007a</td>
</tr>
<tr>
<td>Processed foods</td>
<td>LC-MS/MS; IS: [13C₃]-AA</td>
<td>Extraction with water, SPE with C18 Sep-Pak Plus cartridge</td>
<td>Aqua C18 (250 × 2.1 mm, 5 μm)</td>
<td>ESI+, MRM; 72- to 55- m/z for AA, for [13C₃]-AA 75- to 58- m/z, and for d₃-3-chloropropanediol 116- to 98-m/z</td>
<td>LOD: 0.1 μg/kg; WR: 0.01–50 μg/mL Recoveries: 97–102%</td>
<td>Kim et al., 2007</td>
</tr>
<tr>
<td>Finish foodstuffs</td>
<td>HPLC/MS/MS; IS: d&lt;sub&gt;3&lt;/sub&gt;-AA</td>
<td>Extraction with water, SPE with combination of Isolute Multimode, Oasis HLB 200, and Bond Elute Accucat cartridges</td>
<td>Hypercarb (100 x 2.1 mm id, 5 μm)</td>
<td>ESI+, MRM; m/z 72 to 55 for AA; m/z 75 to 58 m/z for d&lt;sub&gt;3&lt;/sub&gt;-AA</td>
<td>LOD: 34 μg/kg for solid samples, 150 μg/kg for bacon, and 5 μg/L for liquid sample; WR: 34–3750 μg/kg; Recoveries: 98–109%</td>
<td>Eerola et al., 2007</td>
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<td>Chinese traditional carbohydrate-rich foods</td>
<td>HPLC-ESI-MS/MS; IS: [13C&lt;sub&gt;3&lt;/sub&gt;] AA</td>
<td>Defatting with petroleum ether, extraction with 2 M NaCl, LLE with EtAc, evaporation then drying by N&lt;sub&gt;2&lt;/sub&gt;, redissolving, SPE with Oasis HLB SPE cartridge</td>
<td>Atlantis dC18 column (210 mm length, 1.5 mm i.d., 5 μm)</td>
<td>ESI+, m/z 72 &gt; 72 at 1 eV, 72 &gt; 55 at 6 eV, 72 &gt; 44 at 9 eV, and 72 &gt; 27 at 15 eV for AA; and m/z 75 &gt; 75 at 1 eV, 75 &gt; 58 at 6 eV, and 75 &gt; 30 at 15 eV for [13C&lt;sub&gt;3&lt;/sub&gt;]-AA</td>
<td>LOQ: 4 μg/kg; WR: 1–200 ng/mL; Recoveries: 84–97%</td>
<td>Zhang et al., 2007b</td>
</tr>
<tr>
<td>Italian coffee</td>
<td>LC/MS</td>
<td>SPE with STRATA-X-C cartridge</td>
<td>Synergi 41 Polar-RP 80 A (150 x 4.6 mm i.d., 4 μm)</td>
<td>ESI+, m/z 72</td>
<td>LOQ: 0.025 mg/kg; WR: 0.025–1 mg/kg; Recoveries: 52–78%</td>
<td>Sagratini et al., 2007</td>
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<tr>
<td>Food samples</td>
<td>LC-MS/MS; IS: d&lt;sub&gt;3&lt;/sub&gt;-AA</td>
<td>Extraction with water, SPE with Multimode solid-phase cartridge and then with ENV&lt;sup&gt;+&lt;/sup&gt; cartridge</td>
<td>Hypercarb (50 mm x 2.1 mm, 5 μm)</td>
<td>m/z 72 &gt; 55</td>
<td>LOQ: 5 μg/kg; Recovery: 103%</td>
<td>Rosén et al., 2007</td>
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<tr>
<td>Cocoa and coffee products</td>
<td>LC-MS/MS; IS: d&lt;sub&gt;3&lt;/sub&gt;-AA</td>
<td>Extraction with water, deproteination with Carrez I and II solutions, LLE with EtAc, addition of water, evaporation with N&lt;sub&gt;2&lt;/sub&gt;, SPE with Isolute Multimode cartridges</td>
<td>μ-Bondapak C18 (300 x 3.9 mm i.d., 10 μm)</td>
<td>ESI+, SIM at m/z 72 &gt; 71.99 and m/z 72 &gt; 55 for AA and m/z 75 &gt; 58 for d&lt;sub&gt;3&lt;/sub&gt;-AA</td>
<td>LOD: 5.5 μg/kg; WR: 0–1,000 μg/kg; Recoveries: 93–99%</td>
<td>Arisseto et al., 2008</td>
</tr>
<tr>
<td>Tea</td>
<td>LC–MS/MS; IS:[13C&lt;sub&gt;3&lt;/sub&gt;]-AA</td>
<td>Extraction with water, LLE extraction with ACN with MgSO&lt;sub&gt;4&lt;/sub&gt; and NaCl, evaporation with N&lt;sub&gt;2&lt;/sub&gt;, redissolving, SPE with Oasis MCX SPE cartridge</td>
<td>ODS-C18 column (250 x 4.6 mm, 5 μm), Hypersil</td>
<td>ESI+, MRM m/z 72 → 55 for AA and m/z 75 → 58 for [13C&lt;sub&gt;3&lt;/sub&gt;]-023AA</td>
<td>LOD: 1 ng/mL; WR: 1–20 ng/mL; Recoveries: 74–79%</td>
<td>Liu et al., 2008</td>
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<td>Fried crisps</td>
<td>HPLC-MS; IS: $^{13}$C$_3$-AA</td>
<td>Extraction with MeOH, deproteination with Carrez I and II solutions, evaporation, SPE with Oasis-HLB</td>
<td>ODS-C18 column (250 x 4.6 mm, 5 μm)</td>
<td>ESI+, MRM $m/z$ 72 → 55 for AA and $m/z$ 75 → 58 $^{13}$C$_3$-AA</td>
<td>LOD: 1 ng/mL; WR: 1–20 ng/mL; Recoveries: 74–79%</td>
<td>Napolitano et al., 2008</td>
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<tr>
<td>Deep-fried flour-based leavened dough</td>
<td>HPLC-UV</td>
<td>Extraction with water, filtration with PVDF syringe filter, SPE with combined Oasis HLB and Bond Elut-Accucat cartridges</td>
<td>Alltima C18 LC-MS (150 x 2.1 mm i.d., 3 μm)</td>
<td>UV at 210 and 225 nm</td>
<td>LOD: 6 μg/kg; WR: 50–200 μg/L; Recoveries: 78–107%</td>
<td>Wang et al., 2008</td>
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<tr>
<td>Arabica and robusta coffee beans</td>
<td>LC-MS; IS: $^{13}$C$_3$-AA</td>
<td>Defatting with hexane, drying with N$_2$, extraction with water, SPE with Bond Elut Accucat, derivatization with 2-mercaptobenzoic acid at basic media</td>
<td>Phenyl-hexyl (150 x 3 mm i.d., 3 μm)</td>
<td>API-ES+, SIM at $m/z$ 226 (for protonated AA derivative) and 248 (for Na$^+$ adducts of derivatized AA) and at $m/z$ 229 and 251 for labeled derivatives</td>
<td>LOD: 157 ng/g; Recovery: 45%</td>
<td>Bagdonaite et al., 2008</td>
</tr>
<tr>
<td>Foods</td>
<td>LC/MS/MS; IS: $^{13}$C$_3$-AA</td>
<td>Extraction with water, filtration with syringe filter, SPE with Oasis HLB cartridge connected in tandem to an Oasis MCX cartridge</td>
<td>AQUA SIL C18 column (2.1 x 250 mm, 5 μm)</td>
<td>ESI+: for AA $m/z$ 72 &gt; 72 at 5 V, 72 &gt; 55 at 13 V, 72 &gt; 54 at 11 V, 72 &gt; 44 at 10 V, and 72 &gt; 27 at 25 V. For $^{13}$C$_3$-AA $m/z$ 75 &gt; 75 at 5 V, 75 &gt; 58 at 13 V, and 75 &gt; 29 at 25 V</td>
<td>LOD: 3 μg/kg; WR: 5 and 500 ng/mL; Recoveries: 86–113%</td>
<td>Cheng et al., 2009</td>
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<td>Radix Asparagi</td>
<td>HPLC-UV/vis</td>
<td>Extraction with water, derivatization with 2-mercaptobenzoic acid at basic media, addition of (CH$_3$COO)$_2$Pb, acidification by HCl, LLE extraction with EtAc</td>
<td>Diamonsil C18 (150 x 4.6 mm i.d., 5 μm)</td>
<td>UV at 238 nm</td>
<td>LOD: 25 μg/kg; WR: 015–4.5 μg/mL; Recovery: 106%</td>
<td>Shi et al., 2009</td>
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<td>Roasted chestnuts and chestnut-based foods</td>
<td>HPLC-MS/MS; IS: $^{13}$C$_3$-AA</td>
<td>Extraction with water, SPE with Isolute Multimode cartridge and then with Isolute ENV$^+$ cartridge</td>
<td>Zorbax SB-C 18 (4.6 x 250 mm, 5 μm)</td>
<td>$m/z$ 72 &gt; 55, 72 &gt; 54, 72 &gt; 44 and 75 &gt; 58</td>
<td>LOD: 9 μg/kg for roasted chestnuts and 4 μg/kg for chestnut puree</td>
<td>Karasek et al., 2009</td>
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<td>Grilled foodstuffs</td>
<td>HPLC-MS</td>
<td>Defatting with hexane, deproteination with Carrez I and II solutions, extraction with 0.2 mM acetic acid</td>
<td>Zorbax SB-C 18 (4.6 × 250 mm, 5 μm)</td>
<td>Drying gas temp.: 300°C, vaporizer temp.: 325°C, capillary voltage: 3000 V</td>
<td>LOD: 0.5 μg/L; WR: 5–100 μg/L; Recovery: 90%</td>
<td>Kaplan et al., 2009</td>
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<td>Roasted Malaysian tropical almond nuts</td>
<td>LC-MS; IS: d3-AA</td>
<td>Extraction with water, freezing at −30°C, thawing in water bath, SPE with Isolute Multimode cartridge</td>
<td>Symmetry C 18 Column (150 × 2 mm i.d., 4 μm)</td>
<td>Ion spray voltage: 5200 V, turbo gas temp.: 450°C</td>
<td>LOD: 0.1 ng/mL</td>
<td>Lasekan and Abbas, 2010</td>
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<td>Potato chips</td>
<td>HPLC-UV</td>
<td>Defatting with hexane, drying, extraction with acetone and water, filtration, evaporation to dryness, re-dissolved</td>
<td>C18-AQ (2 × 250 mm)</td>
<td>UV at 202 nm</td>
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<td>Starch-based foods</td>
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<td>Extraction with water, deproteination with Carrez I and II solutions, defatting with hexane, derivatization with 0.1 M KBrO3 and KBr powder at acidic media, LLE with EtAc–hexane (4:1, v/v), Filtered the organic phase through calcinated Na2SO4, evaporate to dryness, re-dissolve</td>
<td>ODS-3 C18 column (250 × 4.6 mm, Intersil)</td>
<td>UV at 215 nm</td>
<td>LOD: 15 μg/kg; WR: 50–2000 μg/L; Recoveries: 84.53–98.37%</td>
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<td>Traditional foodstuffs in Zimbabwe</td>
<td>LC-MS/MS; IS: [13C3]-AA</td>
<td>Extraction with water, filtration with syringe filter, SPE with Oasis HLB cartridge follow by Isolute cartridge</td>
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<td>ESI+, m/z 72 &gt; 55, 72 &gt; 54 for AA and m/z 75 &gt; 58 for [13C3]-AA</td>
<td>LOD: 0.02 μg/kg</td>
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<td>Roasted coffee</td>
<td>HPLC-MS/MS; IS: d3-AA</td>
<td>Extraction with water, filtration with membrane filter, SPE with C18, SCX and SAX sorbents mixture</td>
<td>Synergi Hydro (250 × 2.0 mm, 4 μm)</td>
<td>ESI+, full MS2 monitoring of the protonated adducts of AA and d3-AA at m/z 72 and m/z 75, respectively</td>
<td>LOD: 5 μg/kg; WR: 2–104.3 μg/L; Recoveries: 92–95%</td>
<td>Bortolomeazzi et al., 2012</td>
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<td>Commercial baby foods</td>
<td>LC-MS/MS; IS: d₃-AA</td>
<td>Extraction with water, defatting with hexane, deproteination with Carrez I and II solutions, SPE with Bakerbond Carbon column</td>
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<td>ESI+, multiple degradation patterns m/z 72.1→55.2 (AA) and m/z 75.1→58.1 (d₃-AA) for quantification. For verification, the AA degradation pattern m/z 72.1→44.1 and m/z 75.1→47.1 for d₃-AA</td>
<td>Water matrix: LOQ: 2.5 μg/kg; WR: 2.5–500 μg/L; cereal matrix: LOQ: 10 μg/kg; WR: 10–1500 μg/L; potato matrix: LOQ: 25 μg/kg; WR: 25–3000 μg/L</td>
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<td>Potato chip and bread crust</td>
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<td>Turkish traditional desserts</td>
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<td>ESI+, MRM, m/z 72 &gt; 55 at 10 eV, 72 &gt; 44 at 30 eV, 72 &gt; 27 at 35 eV for AA and 75 &gt; 58 for [¹³C₃]-AA</td>
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<td>Instant noodles from China</td>
<td>LC-MS/MS; IS: [¹³C₃]-AA</td>
<td>Defatting with petroleum ether, extraction with water, SPE with Oasis HLB columns</td>
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<td>LOD: 5 mg/L LOQ: 15 mg/L</td>
<td>Yang et al., 2012</td>
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PFE: pressurized fluid extraction; DE: diatomaceous earth; LLE: Liquid-Liquid extraction; MRM: multiple reaction monitoring.
GC Methods with Derivatization

The most used derivatization procedure for AA prior to GC analysis is its bromination (Oracz et al., 2011; Zhang et al., 2005). A few studies have been carried out using other derivative methods (Dunovská et al., 2006; Lagalante and Felter, 2004; Yamazaki et al., 2012; Zhang et al., 2005).

AA undergoes ionic bromination by addition of bromine to the acrylyl double bond (at low pH) to form the 2,3-dibromopropionamide derivative (Daughton, 1988; Lineback et al., 2012; Tareke et al., 2002). The bromination process was performed in many laboratories by addition of a prepared bromination reagent consisting of saturated bromine (Br_2) solution, potassium bromide (KBr), and hydrobromic acid (HBr) to an aqueous extract of AA from food matrices (Castle, 1993; Pittet et al., 2004; Tareke et al., 2002; Zhu et al., 2008). It also can be carried out by an alternative bromination recipe based on the use of a potassium bromide-potassium bromate (KBrO_3) mixture in acidic medium, in which bromine is formed in situ by an oxidation-reduction reaction (Bent et al., 2012; JECFA, 2011; Keramat et al., 2011; Nemoto et al., 2002; Zhang et al., 2006, 2007b). This approach is more convenient and safer than the use of hazardous saturated Br_2 water with HBr; besides that, the reaction is performed in about 30 minutes at cold storage temperature with excellent reproducibility (Castle and Eriksson, 2005; Nemoto et al., 2002; Pittet et al., 2004; Wenzl et al., 2003; Zhang et al., 2006). The bromination process is conducted in a refrigerator or on ice in a dark place (Castle and Eriksson, 2005; Mikuliková and Sobotová, 2007). After derivatization with bromine, a thiosulfate solution is added to remove the excess bromine. The bromination derivative is extracted by liquid-liquid extraction using organic solvents such as ethyl acetate or ethyl acetate/n-hexane mixture. The obtained organic phase is mostly dried over sodium sulfate anhydrous (Becalski et al., 2005; Daniali, 2010; Mizukami et al., 2006; Pittet et al., 2004; Soares et al., 2006, 2010). The organic extract may be injected directly into the GC instrument or subjected to future cleanup, which is achieved by fractionation of the organic extract on a Florisil adsorbent, gel permeation chromatography (GPC), and Bond Elut silica cleanup cartridge (Castle, 1993; Ölmel et al., 2008; Pittet et al., 2004; Tareke et al., 2002). The bromination product 2,3-dibromopropionamide is thermally unstable and may decompose (dehydrobromination) in the GC injector or at the front end of the packed column during analysis to the more stable derivative 2-bromopropenamide (2-BPA) (Andrawes et al., 1987; Nemoto et al., 2002; Pittet et al., 2004; Yamazaki et al., 2012). This decomposition is partial and not constant and may yield poor repeatability and accuracy; it is preferable to convert 2,3-dibromopropionamide to the stable 2-BPA by addition of 10% triethylamine (TEA) to the final extract prior to GC analysis (Andrawes et al., 1987; Cheng et al., 2006; Dunovská et al., 2006; Nemoto et al., 2002; Pittet et al., 2004; Wenzl et al., 2007; Zhang et al., 2006). The bromination of AA has several advantages: (i) in extraction with water, bromination produces fewer polar molecules than the native AA, which is easily extracted by ethyl acetate or hexane, eliminating many water-soluble components; (ii) bromination enlarges AA molecular weight, which leads to improved MS characteristics, giving several ions to monitor and confirm; and (iii) the relatively more volatile derivatization product improves GC characteristics. All of these advantages enhance the selectivity of determination, which compensates for a difficult and time-consuming derivatization process (Keramat et al., 2011; Oracz et al., 2011; Pittet et al., 2004; Wenzl et al., 2003, 2007; Zhu et al., 2008).

A quantitative GC-MS method has been developed for the determination of trace levels (< 50 μg/kg) of AA in cereal-based foods (Pittet et al., 2004). The AA derivatization product 2-BPA was separated on a ZB-WAX capillary column. The LOD and LOQ were estimated at 2 and 5 μg/kg, respectively, and recoveries of AA from samples spiked at levels of 5–500 μg/kg ranged between 93 and 104% after correction of analyte loss by the internal standard. A GC-MS method for AA determination in various food samples was presented (Becalski, 2005). The capillary column Supelcowax-10 was used for separation of the 2-BPA derivative. The LOD was uniform for all sample extracts, and was calculated as 15–20 ppb (injected), which is approximately equivalent to 5 ng/g in the samples. A GC/ion-trap MS method has been employed to determine the AA content in some Chinese foods (Cheng et al., 2006). A HP-5MS column was used to separate the brominated derivative, 2-BPA. The analysis was performed using EI ionization and SIM. The recoveries were between 102 and 110% when wheat flour dough was spiked with AA at 10–50 μg/kg, with an LOD of 5 μg/kg. Among the Chinese foods tested (fried gluten, instant noodles, and twisted cruller), old twisted cruller had the most AA (890–900 μg/kg), whereas fried gluten yielded the least AA (less than 20 μg/kg), probably due to the absence of starch. A GC-MS method to determine AA in coffee and coffee products was developed (Soares et al., 2006). A layered SPE column was used for elimination of the main chromatographic interferences in the coffee extract. The analytical separation was performed in the capillary column DB 1301. The method is applicable to a wide range of coffee products. Twenty-six samples of different coffee products were analyzed. The levels of AA were in the range 11.4–36.2 μg/L for “espresso coffee” and 200.8–229.4 μg/L for coffee blends with cereals. The results indicate that the presence of cereals significantly increased the levels of AA. A rapid GC-ECD method was developed and applied for determination of AA in conventional fried foods, such as potato crisps, potato chips, and fried chicken wings (Zhang et al., 2006). AA was derivatized with KBrO_3 and KBr. The chromatographic analysis for AA derivative was performed on the HP-INNOWax capillary column. The LOD was estimated to be 0.1 μg/kg. Recoveries of AA from conventional samples ranged between 87 and 97%. A GC-MS method was optimized for analysis of AA in tea samples without the interference of bromination by tea catechins (Mizukami et al., 2006). An ADB-17 MS capillary column was used for separation of 2,3-DBPA derivative. The mass spectrometer was operated in EI ionization and SIM mode. For AA-derivative characterization,
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<td>Cereal-based foods</td>
<td>GC-MS; IS: $^{[13}C_3$]-AA</td>
<td>Extraction with water, acidified with glacial acetic acid, deproteination with Carrez I and II solutions, derivation with bromination reagent (KBr, HBr, and Br$_2$ water), addition of Na$_2$S$_2$O$_3$, LLE with EtAc/n-hexane 4:1 (v/v), filtered through Na$_2$SO$_4$, drying with N$_2$, SPE with calcinated Na$_2$SO$_4$ activated Florisil column, drying under N$_2$, redissolving, addition of TEA</td>
<td>ZB-WAX (30 m x 0.25 mm i.d., 0.25 μm)</td>
<td>El$^+$; SIM, m/z 70, 149, and 151 for AA derivative and m/z 110 and 154 for $^{[13}C_3$]-AA derivative</td>
<td>LOD: 2 μg/kg; Recoveries: 93–104%</td>
<td>Pittet et al., 2004</td>
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<td>Various food matrices</td>
<td>GC-MS and GC-HR-MS and GC-LR-MS; IS: $^{[13}C_3$]-AA</td>
<td>Extraction with water, defatting with CH$_2$Cl$_2$, SPE with Oasis HLB cartridge followed by Accucat cartridge or Isolute Multimode cartridges, derivatization with bromination reagent (KBr, HBr, and Br$_2$ water), addition of Na$_2$S$_2$O$_3$, LLE with EtAc, drying over Na$_2$SO$_4$, spiked with TEA</td>
<td>Supelcowax-10, (30 m x 0.25 mm, 0.25 μm)</td>
<td>El$^+$; SIM, m/z 55, 71, 74 for underivatized (water) samples and m/z 70, 106, 110, 149, 151, 154 for derivatized (EtAc) samples; HR MS: 70 eV El$^+$; SIM; ions (m/z): 148.9476, 150.9456, 151.9577, 153.9556</td>
<td>GC/MS underivatized AA: LOD: 40 ng/g; WR: 100–1000 ng/mL; GC/LR-MS derivatized AA: LOD: 10 ng/g; WR: 25–1000 ng/mL; GC-HR-MS derivatized: LOD: 5 ng/g; WR: 25–1000 ng/mL</td>
<td>Becalski et al., 2005</td>
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<td>Chinese foods</td>
<td>GC-Ion-trap MS; IS: $^{[13}C_3$]-AA</td>
<td>Extraction with water, SPE with Oasis HLB cartridge connected in tandem to an Oasis MCX cartridge, derivatization with bromination reagent (KBr, HBr, and Br$_2$ water), addition of 1 M Na$_2$S$_2$O$_3$, LLE with EtAc, drying over Na$_2$SO$_4$, evaporation, redissolving, addition of TEA</td>
<td>HP-5MS column (30 m length x 0.25 mm i.d., 0.25 μm)</td>
<td>El, SIM, m/z 152, 150, 106, and 70 AA derivative and 154, 152, 110, 108, and 73 $^{[13}C_3$]-AA derivative</td>
<td>LOD: 5 μg/kg; WR: 5–80 μg/kg; Recoveries: 102–110%</td>
<td>Cheng et al., 2006</td>
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<td>Food products</td>
<td>GC-MS; IS: d$_{3}$-AA</td>
<td>Extraction with water and n-propanol, addition of drops of olive, evaporation, redissolving in ACN, defatting with hexane, dSPE with PSA</td>
<td>Innowax capillary column (30 m × 0.25 mm i.d., 0.25 μm)</td>
<td>m/z 45–500</td>
<td>LOD: 15–40 μg/kg</td>
<td>WR: 50–1500 μg/kg</td>
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<td>Fried foods</td>
<td>GC-ECD</td>
<td>Defatting with hexane, extraction with NaCl, derivatization with KBrO$_3$ and KBr at acidic media, addition of Na$_2$S$_2$O$_3$, LLE with EtAc, drying over Na$_2$SO$_4$</td>
<td>i9091N-113 HP-INNOWax capillary column (30 m length, 0.32 mm i.d., 25 μm)</td>
<td>ECD at 250°C</td>
<td>LOD: 0.1 μg/kg; WR: 0.5–125 ng/mL; Recoveries: 87–97%</td>
<td>Zhang et al., 2006</td>
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<td>Various food matrices</td>
<td>GC-MS; IS: d$_{3}$-AA</td>
<td>dSPE: defatting with hexane, extraction with water, LLE with ACN with MgSO$_4$ and NaCl, dSPE with PSA</td>
<td>Stabilwax-DB (Restek) capillary column (20 m × 0.32 mm i.d., 1 μm)</td>
<td>Cl$^+$, full scan mode (m/z 50–85)</td>
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<td>Green tea</td>
<td>GC/MS; IS: [13C$_3$]-AA</td>
<td>Extraction with water, SPE with Isolute Multimode cartridge, derivatization with bromination reagent (KBr, HBr, and Br$_2$ water), addition of Na$_2$S$_2$O$_3$, LLE with EtAc, drying over Na$_2$SO$_4$, evaporation</td>
<td>DB-17 MS capillary column (30 m × 0.25 mm i.d., 0.15 μm)</td>
<td>EI, SIM, m/z 150 and 152 AA derivative and m/z 153 and 155 [13C$_3$]-AA derivative</td>
<td>LOD: 0.2 ng/mL; WR: 1.6–1280 ng/mL; Recoveries: 94 to 108%</td>
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<td>Coffee and coffee products</td>
<td>GC-MS; IS: [13C$_3$]-AA</td>
<td>Extraction with EtOH, acidified with CH$_3$COOH, deproteination with Carrez I and Carrez II solutions, reduction of volume, SPE with C18 sorbent added to the Isolute Multimode column, derivatization with bromination reagent (KBr, HBr, and Br$_2$ water), addition of Na$_2$S$_2$O$_3$, saturation with NaCl, LLE with EtAc/n-hexane 4:1 (v/v), filtered through Na$_2$SO$_4$, drying with N$_2$</td>
<td>DB 1301 capillary column (30 m × 0.25 mm i.d., 0.25 μm)</td>
<td>EI, SIM, m/z 106, 108, 150, and 152 AA derivative and m/z 110, 153, 155 for [13C$_3$]-AA derivative</td>
<td>WR: 0–300 μg/L</td>
<td>Recoveries: 97.4–108.4%</td>
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<td>Matrix</td>
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<td>Detector parameters</td>
<td>LOD/LOQ, WR and recovery</td>
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<td>Food</td>
<td>GC-PCI/MS/MS</td>
<td>SPME: extraction with water by supersonic nebulizer, dilution with water, mixing with buffer solution (pH 7), immersing the SPME (CW/DVB)-coated fiber in the mixed solution</td>
<td>DB-WAX fused-silica capillary column (30 m x 0.25 mm i.d.; 0.25 μm)</td>
<td>MS, EI and PCI with ACN as reagent gas; m/z scan range from 40 to 100 u.</td>
<td>LOD: 0.1 μg/L in water; WR: 1–1000 μg/L</td>
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<td>Chinese traditional carbohydrate-rich foods</td>
<td>GC-MECD; IS: [13C3]-AA</td>
<td>Defatting with petroleum ether, extraction with 2 M NaCl, derivatization with KBrO3 and KBr at acidic media, addition of Na2S2O4, LLE with EtAc, drying over Na2SO4</td>
<td>19091N-113 HP INNOWax capillary column (30 m length, 0.32 mm i.d., 0.25 μm)</td>
<td>MECD at 250°C</td>
<td>LOD: 0.15 μg/kg (instrumental); 4 μg/kg (spiked food); WR: 0.5–125 ng/mL</td>
<td>Zhang et al., 2007b</td>
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<tr>
<td>Cooked rice, tomato sauces, and some fast food on Italian market</td>
<td>GC-MS</td>
<td>Drying with Na2SO4, defatting with hexane, extraction with 2-propanol</td>
<td>Supelcowax-10 fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μm)</td>
<td>EI, SIM, m/z 55, 71, and 72</td>
<td>LOD: 25 μg/kg WR: 150–1000 μg/kg Recoveries: 79–85%</td>
<td>Tateo et al., 2007</td>
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<td>Malt</td>
<td>GC-MSD; IS: [13C3]-AA</td>
<td>Extraction with water, derivatization with bromination reagent (KBr, HBr, and Br2 water), addition of Na2S2O3, LLE with EtAc, addition of TEA</td>
<td>DB-WAX capillary column (30 m x 0.25 mm i.d., 0.25 μm)</td>
<td>SIM with EI+, m/z 149 and 151 for AA derivative and m/z 152.154 for [13C3]-AA derivative</td>
<td>LOQ: 25 μg/kg; WR: 30–620 μg/kg; Recoveries: 72–86%</td>
<td>Mikulíková and Sobotová, 2007</td>
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<tr>
<td>Commercial frying oil</td>
<td>GC-MS; IS: d3-AA</td>
<td>Extraction with water, defatting with hexane, SPE with Sep-Pak, xanthydrol at acidic media, addition of water and NaCl, LLE with EtAc</td>
<td>DB-5 MS (0.25 mm x 30 m, 0.25 μm)</td>
<td>EI, SIM: m/z 251 and 234 for AA derivative, and m/z 254 and 210 for d3-AA derivative</td>
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<td>Sample Type</td>
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<td>Extraction Method</td>
<td>Derivatization Method</td>
<td>Instrument Details</td>
<td>LOD/WR/Recovery</td>
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<tr>
<td>Potato chips</td>
<td>GC-MS</td>
<td>$^{[13C_3]}$-AA</td>
<td>MSPD: blended sample and C$_{18}$ sorbent, packed into column, defatting with hexane, elution, derivation with bromination reagent (KBr, HBr, and Br$_2$ water), addition of Na$_2$S$_2$O$_3$, saturation with NaCl, LLE with EtAc/hexane 4:1 (v/v), filtered through Na$_2$SO$_4$, drying with N$_2$.</td>
<td>El, SIM, m/z 106, 150 and 152 for AA derivative and m/z 110, 153, 155 for $^{[13C_3]}$-AA derivative.</td>
<td>MDN-12 capillary column (30 m x 0.25 mm, 0.25 μm i.d.)</td>
<td>12.8 μg/kg WR: 0–1500 μg/L Recovery: 98–111%</td>
</tr>
<tr>
<td>Black ripe olives</td>
<td>GC-MS</td>
<td>$^{[13C_3]}$-AA</td>
<td>Extraction with water, SPE with Discovery DSC-MCAX, derivation with bromination reagent (KBr, HBr, and Br$_2$ water), addition of Na$_2$S$_2$O$_3$, LLE with EtAc, drying over Na$_2$SO$_4$, evaporation, redissolving.</td>
<td>El+, SIM, at m/z 70, 149, and 151 for AA derivative and m/z 110 and 154 for $^{[13C_3]}$-AA derivative.</td>
<td>BPX70 capillary column (30 m x 0.22 mm i.d., 0.25 μm)</td>
<td>6 ng/mL WR: 5–200 ng/mL Recovery: 94–105%</td>
</tr>
<tr>
<td>French fries</td>
<td>GC-MS</td>
<td>$^{[13C_3]}$-AA</td>
<td>Extraction with water, derivatization with brominating reagent (KBr, HBr, and Br$_2$ water)</td>
<td>El, SIM, at m/z 106, 108, 150, and 152 for AA derivative and m/z 108, 110, 153, and 155 for $^{[13C_3]}$-AA derivative</td>
<td>InnoWax capillary column (30 m x 0.25 mm, 0.15 μm)</td>
<td>15 ng/g WR: 0.01–1.0 μg/mL</td>
</tr>
<tr>
<td>Foods</td>
<td>GC-MS</td>
<td>d$_3$-AA</td>
<td>Extraction with water, defatting with hexane, derivation with bromination reagent (KBr, HBr, and Br$_2$ water), addition of Na$_2$S$_2$O$_3$, LLE with EtAc, drying over Na$_2$SO$_4$, evaporation, SPE with Bond-Elut silica gel column</td>
<td>El, SIM, at m/z 150 and 152 for AA derivative and m/z 153 and 155 for d$_3$-AA derivative</td>
<td>HP 50C capillary column (30 m x 0.25 mm i.d., 0.25 μm)</td>
<td>10 μg/kg WR: 120–1200 μg/kg Recovery: 107%</td>
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<tr>
<td>Heat-processed foods</td>
<td>GC-ECD</td>
<td></td>
<td>Extraction with water, defatting with hexane, derivation with bromination reagent (KBr, HBr, and Br$_2$ water), addition of Na$_2$S$_2$O$_3$, LLE with EtAc, drying over Na$_2$SO$_4$, filtration, addition of TEA</td>
<td>ECD at 260°C</td>
<td>Suplecowax-10 capillary system (30 m x 0.25 mm i.d., 0.25 μm)</td>
<td>0.6 μg/kg WR: 0–0.2 μg/mL Recoveries: 92.5–101.5%</td>
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<tr>
<td>Food</td>
<td>GC-MS; IS: ([^{13}C_3])-AA</td>
<td>MSPD: blended sample and C(_{18}) sorbent, packed into column, defatting with hexane, elution, derivation with bromination reagent (KBr, HBr, and Br(_2) water), addition of Na(_2)S(_2)O(_3), saturation with NaCl, LLE with EtAc/hexane 4:1 (v/v), filtered through Na(_2)SO(_4), drying with N(_2)</td>
<td>MDN-12 capillary column (30 m × 0.25 mm i.d., 0.25 μm)</td>
<td>SIM, (m/z) 106, 150, and 152 for AA derivative and (m/z) 110, 153, and 155 for ([^{13}C_3])-AA derivative</td>
<td>LOD: 5.2 μg/kg WR: 0–1.500 μg/L</td>
<td>Soares and Fernandes, 2009</td>
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<tr>
<td>Coffee and Coffee Substitutes</td>
<td>GC-MS; IS: ([^{13}C_3])-AA</td>
<td>Blended sample and C(_{18}) sorbent, SPE with ISOLUTE C18/Multimode (1 g + 1 g) bilayered cartridge, derivatization with bromination as previously reported (Soares et al., 2006)</td>
<td>MDN-12 (30 m × 0.25 μm 0.25 mm i.d.) capillary column</td>
<td>SIM, (m/z) 106, 150, and 152 for AA derivative and (m/z) 110, 153, and 155 for ([^{13}C_3])-AA derivative</td>
<td>LOD: 5 μg/kg WR: 0–1500 μg/kg Recoveries: 84–97%</td>
<td>Soares et al., 2010</td>
</tr>
<tr>
<td>Banana-based snacks</td>
<td>GC-MS; IS: ([^{13}C_3])-AA</td>
<td>Extraction with water, addition of 2 M NaCl, filtration through glass wool, SPE with HLB, MCX column, derivatization with bromination reagent (KBr, HBr, and Br(_2) water), addition of Na(_2)S(_2)O(_3), addition of Na(_2)SO(_4), LLE with EtAc/hexane4:1 (v/v), filtration, evaporation, addition of TEA</td>
<td>Innowax capillary column (30 m × 0.25 mm i.d., 0.25 μm)</td>
<td>EI, (m/z) 149 for AA derivative and (m/z) 154 for ([^{13}C_3])-AA derivative</td>
<td>LOD: 5 μg/g; Recoveries: 84–110%</td>
<td>Daniali, 2010</td>
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<td>Fried potato chips</td>
<td>GC-NPD; IS: acetamide</td>
<td>Extraction with water, filtration, saturation with NaCl, LLE with EtAc, drying over Na(_2)SO(_4), evaporation</td>
<td>DB-WAX fused silica capillary column (30 × 0.32 mm i.d., 0.25 μm)</td>
<td>NPD at 280°C</td>
<td>LOD: 0.15 μg/g; WR: 1–100 ppm; Recovery: 106%</td>
<td>Kim et al., 2011</td>
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<tr>
<td>Food Type</td>
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<td>MS and FID at Temperature</td>
<td>LOD</td>
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<td>Authors</td>
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<td>Iranian breads</td>
<td>GC-MS and GC-FID</td>
<td>SPME: defatting with hexane, filtering and drying, extraction with water,</td>
<td>Fused-silica capillary</td>
<td>LOD: 25 μg/kg</td>
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<td>Motaghi et al., 2012</td>
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<td></td>
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<td>deproteination with Carrez I and II solutions, SPE with Oasis HLB, extraction</td>
<td>column Bpx70 (50 m</td>
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<td>through PEG, PDMS/DVB, and PA fibers by DI-SPME</td>
<td>× 0.25 mm i.d., 0.25 μm)</td>
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<td>MS and FID at 260°C</td>
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<td>Caribbean foods</td>
<td>GC-MS; IS: [13C₃]-AA</td>
<td>Defatting by Soxhlet, extraction with water, deproteination with Carrez I and II</td>
<td>DB-1701 capillary column</td>
<td>LOD: 20 μg/kg for DB-1701 and 4 μg/kg for DB-VRX columns</td>
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<td>solutions, filtration, derivatization with bromination by BrO₃/Br in acidified</td>
<td>column (30 m × 0.53 mm i.d., 250 μm) and DB-VRX capillary column (20 m × 0.18 mm i.d., 1 μm)</td>
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<td>media, addition of Na₂S₂O₅, LLE with EtAc, drying over Na₂SO₄, SPE with packed</td>
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<td>silica column followed by diatomaceous earth, addition of TEA, evaporation,</td>
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<td>redissolving</td>
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<td>Starchy food</td>
<td>GC-FID; IS: DMA</td>
<td>Extraction with water, freezing, thawing, SPE with activated carbon packed</td>
<td>PEG-20M capillary column</td>
<td>LOD: 10 ng/mL; Recovery: 100.60%</td>
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<td>Sun et al., 2012</td>
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<td></td>
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<td>column (30 m × 0.32 mm i.d., 0.4 μm)</td>
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<td>Thai conventional snacks</td>
<td>GC-MS; IS: [13C₃]-AA</td>
<td>Defatting with isohexane/t-butyl methyl ether (95:5), drying, extraction with</td>
<td>HP5-MS capillary column</td>
<td>LOD: 4 μg/kg; LOQ: 15 μg/kg; WR: 5–50 μg</td>
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<td>water, deproteination with Carrez I and II solutions, filtration, SPE with</td>
<td>(30 m × 0.25 mm i.d., 0.25 μm)</td>
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<td>Chromabond ABC18 cartridge, derivatization with bromination reagent (KBr, HBr, and Br₂ water), addition of Na₂S₂O₅, LLE with EtAc, drying over Na₂SO₄</td>
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<tr>
<td>Processed foods</td>
<td>GC-MS; IS: d$_3$-AA</td>
<td>Extraction with water, defatting with hexane, SPE with Sep-Pak C18 (upper) connected in series with Sep-Pak AC-2 cartridge (lower) or SPE with EXtrelut column for amino acid-rich samples, derivatization with xanthodrol in acidic media, LLE with EtAc</td>
<td>DB-5MS (30 m length, 0.25 mm i.d., 0.25 mm)</td>
<td>EI, SIM, m/z 251, 234, 206 for AA derivative and m/z 254, 210 for d$_3$-AA derivative</td>
<td>LOD: 0.5–5 µg/kg; LOQ: 5–20 µg/kg; WR: 0.01–5 µg/mL Recoveries: 85–110%</td>
<td>Yamazaki et al., 2012</td>
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TEA: triethylamine; PSA: Primary secondary amine; CW/DVB: carbowax/divinylbenzene; dSPE: Dispersive solid phase extraction; SPME: Solid phase microextraction; MSPD: matrix solid-phase dispersion.
two ions \((m/z \ 150 \text{ and } 152)\) were used. LOD was calculated to be 0.2 ng/mL. An analytical method based on derivatization with KBrO\(_3\) and KBr without cleanup prior to GC-MECD for quantification of AA in complex food matrices, such as Chinese traditional carbohydrate-rich foods, was developed (Zhang et al., 2007b). The analytical column used for 2-BPA derivative separation was HP-INNOWax capillary column. LOQ of 10 \(\mu g/kg\) in Chinese style foods was achieved. Spiked recovery ranged from 79.1 to 92.7%. AA contaminant was found in all of samples at concentrations up to 771.1 \(\mu g/kg\). AA contents in malt samples were determined with GC-MS after bromination (Mikulíková and Sobotová, 2007). LOQ was 25 \(\mu g/kg\) and recovery ranged from 72 to 86%. Higher AA contents were found in special and colored malts. An MSPD-GC-MS method was developed for determination of AA content in potato chips. AA was determined after derivatization to 2,3-DHPA (Fernandes and Soares, 2007). The analytical separation was performed in the capillary column MDN-12. The MS detector was operated in EI mode. The LOD and LOQ were estimated to be 12.8 and 38.8 \(\mu g/kg\), respectively. The AA recovery from the spiked solutions varied between 98 and 111%. AA contents in potato chips samples ranged from 186.42 to 1828.75 \(\mu g/kg\). The presence of AA was investigated in different commercial black ripe olives (Casado and Montaño, 2008). The analysis was done by GC-MS after bromination. The chromatographic separation of 2-BPA was done on a BPX70 capillary column. LOD and LOQ were calculated to be 6 and 20 ng/mL, respectively. The AA level in olive pulp ranged from 176 to 1578 \(\mu g/kg\) of fresh weight. A GC-MS method was used for determination of the AA level in French fries (Palazoğlu and Gökmen, 2008). The MS detector was operated in SIM mode with EI ionization. Four ions were used to characterize brominated \(^{[\text{13C3}]\text{AA, m/z} 108, 110, 153, \text{and} 155}\), and another four ions were used to characterize brominated AA, \(m/z\ 106, 108, 150, \text{and} 152\). AA levels in foods obtained from the Turkish market were determined using a GC-MS method with bromine derivatization (Ölmez et al., 2008). Separation was performed by an HP 50C capillary column. LOD and the LOQ were found to be 10 and 30 \(\mu g/kg\), respectively. GC-ECD using the standard addition method was developed for the determination of AA in heat-processed foods (Zhu et al., 2008). The chromatographic analysis was performed on polar columns, e.g., a Supelcowax-10 capillary column. The qualification of the analyte was by identifying the peak with same retention time as the standard compound 2,3-DHPA and confirmed by GC-MS; the GC-MS analysis showed that 2,3-DHPA was converted to 2-BPA nearly completely on the polar capillary column, whether or not treated with TEA. LOD was estimated to be 0.6 \(\mu g/kg\). Soares and Fernandes (2009) developed and optimized MSPD-GC-MS for the analysis of AA in a variety of food matrices. The analytical separation was performed in the capillary column MDN-12. MSPD was used in the SIM mode, using six selected fragments characteristic of derivatized AA \((m/z\ 106, 150, \text{and} 152)\) and derivatized IS \((m/z\ 110, 153, \text{and} 155)\). The MSPD-GC-MS method presented LOD of 5.2 \(\mu g/kg\) and LOQ of 15.7 \(\mu g/kg\). An MSPD procedure combined with an SPE cleanup followed by GC-MS analysis was developed for determination of AA levels in coffee and coffee substitute samples (Soares et al., 2010). The brominated AA derivative was separated on an MDN-12 capillary column. LOD obtained for this method was 5 \(\mu g/kg\) and LOQ was 15 \(\mu g/kg\). The amounts of AA found varied widely in the range of 14 to 762 \(\mu g/kg\) for ground coffee and 6 to 229 \(\mu g/L\) for brewed coffee. AA concentration in popular banana-based snacks in Malaysia was determined using a modified GC-MS method (Daniali, 2010). AA was determined as a 2-BPA derivative. An Innowax capillary column was used for analytical separation. The ions monitored were \(m/z\ 149\) for 2-BPA, and \(m/z\ 154\) for \(2-^{13}\text{C3}\) BPA by EI mode. LOD and LOQ of this method were 5 and 15 \(\mu g/g\), respectively. GC-MS detection was used to determine AA levels in commercial and homemade Caribbean foods (Bent et al., 2012). AA was pre-derivatized to 2-BPA. Two capillary columns were tested for chromatographic separation, DB-1701 capillary column and DB-VRX column. The MS detector was operated in EI ionization with SIM mode. Ions used for 2-BPA monitoring were \(m/z\ 70, 106, 133, \text{and} 149\) and for \(^{13}\text{C3} \ 2\text{-BPA m/z\ 73, 108, 136, 152}\). LOD for the GC-MS method was found to be dependent on the type of column used for the GC/MS analysis. The DB-1701 and the DB-VRX columns gave LODs of 20 and 4 \(\mu g/kg\), respectively. The AA contents in food samples had concentrations in the range 65–3640 \(\mu g/kg\).

Derivatization with other reagents has also been carried out. AA content of commercial frying oil was determined by a GC-MS method after derivatization with xanthyl (Totani et al., 2007). The AA derivative was separated on a DB-5 MS capillary column. EI ionization detected mass numbers of \(m/z\ 251\) and 234 for AA derivative and \(m/z\ 254\) and 210 for d$_1$-AA derivative. AA was not detected in oil that has been used for deep-frying at food manufacturing companies. A novel GC-MS method was developed for the determination of AA, which is applicable to a variety of processed foods (Yamazaki et al., 2012). The method involves the derivatization of AA with xanthyl at acidic medium, and the resultant N-xanthyl AA was determined by GC-MS. A DB-5MS analytical column was used for derivative separation. MS was operated in EI mode. The AA derivative (xanthyl AA) was detected using SIM with the quantification ion at \(m/z\ 251\) and confirmation ions at \(m/z\ 234\) and 206. The internal standard (d$_3$-AA) derivative was detected by monitoring the equivalent ion at \(m/z\ 254\). LOD and LOQ ranged between 0.5 and 5 and 20 \(\mu g/kg\), respectively.

Detailed information on GC analytical methods with and without derivatization for AA determination in various food matrices are summarized in Table 2.

**CONCLUSIONS**

From data reviewed at this article it appears that AA formed in carbohydrate-rich food processed by frying, roasting, grilling, and baking and that boiled processed food do not contain AA. The most used extraction and cleanup procedure for AA extraction from food samples is aqueous extraction, followed by solid-phase extraction. From our view of point, AA extraction...
and cleanup procedures are still complicated and time consuming and need more development, except for some extraction procedures such as DSPC and SPME, toward simpler and more rapid methods. To date, there is no universal procedure for AA extraction from different food matrices; there are differences for sample cleanup procedures applied for GC and HPLC.

The most used analytical methods for AA analysis are HPLC-MS/MS and GC-MS. Determination of AA by HPLC-MS(IMS) is simpler because AA is determined directly without derivatization, but it requires more sample cleanup prior to analysis to prevent the interference and enhance sensitivity. The challenge in AA determination using HPLC is the analytical column selection, because the AA molecule is polar and difficult to retain at a conventional reversed-phase column. HPLC-TOF-MS is a promising technique for AA determination because TOF-MS is very efficient detector for resolving the AA peak from interfering peaks and hence improving the sensitivity. For analysis of AA by GC-MS there is a need for derivatization, mostly bromination process is time consuming and includes handling of hazardous compounds such as bromine, but recently there is an improvement in the bromination process by using a mixture of KBrO3 and KBr for bromine in situ. Determination of native AA directly with GC-MS is simpler, but it lacks sensitivity and selectivity because AA is a simple molecule and lacks characteristic ions in the mass spectrum; this causes interference by the matrix, which increases background noise, and hence a low limit of detection is impossible to obtain. The other drawback of direct analysis of AA by GC-MS is potential formation of AA in situ in the heated GC injector from co-extracted precursors (asparagine and reducing sugars) when using water as extractant solvent. To solve these problems an efficient extraction procedure is needed to remove AA precursors before analysis.

MS is chosen as the main technique for GC- and HPLC-based methods of AA analysis. However, the instrument coupled to MS is very expensive and not available in many laboratories, especially in developing countries. There is still a need to a develop rapid, sensitive, and inexpensive technique for routine analysis of AA in food such as determination with HPLC or CE coupled with a fluorescence detector, which is less expensive and very sensitive and selective and may be available in many laboratories.

REFERENCES


