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Quantitative Analysis of Acetaldehyde in Foods Consumed by Children using SPME/GC-MS(Tof), On-fiber Derivatization and Deuterated Acetaldehyde as an Internal Standard

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Abstract. The aim of this study was to develop a precise quantitative method for acetaldehyde determination in solid food matrixes as, to the authors' best knowledge, no such method was available. The method was applied for quantification of acetaldehyde in various foods consumed by children such as yoghurt, purees, curd creams etc. On-fiber derivatization of acetaldehyde with PFBHA was used to increase the method sensitivity and deuterated acetaldehyde was used as an internal standard for exact quantification. The article is mostly focused on method development, including sample preparation. The amount of acetaldehyde in foods was found to be rather negligible, with the highest concentration (up to 31.5 ± 0.05 mg kg^{-1}) detected in yoghurts.

Key words: acetaldehyde, food products, PFBHA, SPME/GC-MS

INTRODUCTION

Acetaldehyde (AA) is a widely occurring compound in nature and is also industrially produced for various applications, including food processing. In nature, acetaldehyde is commonly found in fruits as an intermediate product in the respiration of higher plants and in alcoholic fermentation (Miyake & Shibamoto, 1993). AA is added to foods as a flavour enhancer (fruit flavour) but also as a preservative of fruits and fish products (IARC, 1985).

Exact quantification of acetaldehyde in food products has become of increased interest as the International Agency for Research on Cancer (IARC) has changed the cancer risk classification of AA from an agent possibly carcinogenic to humans (Group 2B) to an agent carcinogenic to humans (Group 1) (IARC Press release Nº196). The European Flavour and Fragrance Association (EFFA, 2010) has reported that the concerns raised for AA are mainly linked to the risk of potential cancer formation in the upper digestive tract after alcohol abuse and the combined effect of alcohol and smoking. Notably, there are no known safety concerns raised by EFFA so far related to the use of AA as a flavouring substance under normal conditions of use in food.

On the other hand, food products are the primary source of AA exposure to non-smokers and non-drinkers. The greatest concern regarding AA is focused on children and neonates, whose weight-adjusted AA exposure may be the highest. The Committee

of Experts on Flavouring substances from the Council of Europe (CoE) has approved AA concentration in beverages 23 mg kg^{-1} and in food 20 mg kg^{-1}. The FDA has reported in the Code of Federal Regulations, Title 21 (revised April 1, 2010) that AA is generally recognized as safe (GRAS), but that it should be used in the minimum quantity required to produce its intended effect, and otherwise in accordance with all the principles of good manufacturing practice. The Joint FAO/WHO Committee on Food Additives (JECFA) has reported no safety concern at the current level of intake (Burdock, 2001). Nevertheless, studies conducted in Finland show that the average AA concentration in yoghurts sold in Finland exceeds the level of mutagenicity eight-fold (Salaspuro, 2010). Therefore, foods typically consumed by small children in Estonia are under focus in this study. According to scientific literature, the dairy industry has engaged in an active years-long attempt to develop yeasts whose acetaldehyde-producing capacity is as high as possible (Salaspuro, 2010) to improve product quality by enhancing its flavour. Additionally, it is known that AA is lethal to yeasts and moulds (Barkai-Golan & Aharoni, 1976), and its use as a microbial inhibitor in foods is intriguing. Furthermore, Salaspuro (2009) has reported that AA appears to act as a cumulative carcinogen in the upper digestive tract of humans. This strongly suggests the importance of world-wide screening of AA levels in many beverages and foodstuffs, as well as an urgent need for regulatory measures and consumer guidance.

Even though AA has been found in many foods, the number of reports describing its quantification in food samples is rather scarce. Quantitative determination of AA is complicated because it is very volatile (T_{evap} = 20ºC), reactive and is present in very small concentrations. Thus, proper and sensitive methods need to be used in order to get reliable results. Basic methods relying on concentration of AA by distillation and following determination either by liquid chromatography (LC) or enzymatic methods are tedious and often difficult to apply on solid food matrices. To increase the sensitivity and selectivity of the method, derivatization of AA in combination with subsequent detection and quantification by mass spectrometry (MS) has been used. For example, solid phase micro extraction (SPME) combined with on-fiber derivatisation using O-2,2,4,5,6- (pentafluorobenzyl)-hydroxylamine hydrochloride (PFHBA) has been previously used to extract aldehydes from water (Tsai & Chang, 2003) and in exhaled breath (Poli et al., 2010). In light of these studies, but also those of others (Martos & Pawliszyn, 1998; Koziel et al. 2001; Wang et al. 2005), the method was modified in the present work. Solid-phase micro extraction (SPME) combined with GC/MS-Tof analysis and stable isotope dilution assay was used for quantification of AA in different food products.

MATERIALS AND METHODS

Materials

Food products used in the study were as follows: i) purees: Hipp vegetable puree with rice and Hipp fruit puree (Pfaffenhofen, Germany), Milupa apple-pumpkin puree and Milupa chicken stew with potatoes and carrots (Dublin, Ireland), Põnn peach-yoghurt dessert (Salvest, Estonia), Gerber pear puree (Vevey, Switzerland), Bebivita apple puree with banana (München, Germany); ii) curd creams and desserts: Tere blueberry curd cream and Tere cherry dessert (Tallinn, Estonia), Valio Alma yoghurt

dessert with strawberries and blueberries (Laeva, Estonia); iii) yoghurts: Valio Alma wild-strawberry yoghurt (Laeva, Estonia), Tere Mumuu yoghurt with rye bread and hazelnuts and Tere Hellus wild berry yoghurt (Tallinn, Estonia), Valio Gefilus blueberry yoghurt (Laeva, Estonia), Farmi rhubarb yoghurt (Rakvere, Estonia), Nopri farm yoghurt with sea buckthorn (Võrumaa, Estonia); iv) puddings: Tere vanilla pudding (Tallinn, Estonia); v) yoghurt drinks: Activia raspberry and Actimel raspberry-cranberry semi-solid yoghurt (Paris, France), Valio Gefilus peach yoghurt drink (Laeva, Estonia), Tere Hellus tropic fruit yoghurt drink; vi) ready-to-drink baby milks: Semper baby milk (Sundbyberg, Sweden).

AA and O-2,2,4,5,6- (pentafluorobenzyl)-hydroxylamine hydrochloride (PFBHA, 98%) was purchased from Sigma-Aldrich, deuterated acetaldehyde (D_4-AA) from CIL (≥ 98%, M = 48.08 Da, Cambridge Isotope Laboratories Inc., MA), ethanol was from Rakvere Piiritusetehas (Rakvere, Estonia). Clear glass SPME crimp vials (20 ml) with the polytetrauoroethylene (PTFE)/silicone septa (20 mm) were purchased from Supelco and glass-covered magnetic stirrers (1 cm) from VWR Int. (PA, USA). MilliQ water (Millipore Corp., Molsheim, France) was used whenever samples were prepared.

Principle of the method

The PFBHA loaded on SPME fiber was used to absorb and derivatize AA from the headspace of the water extract of the samples. The reaction of derivatisation with PFBHA reagent gives two PFBHA-oxime isomers (cis and trans), which are desorbed and analysed by time-of-flight gas chromatography–mass spectrometry (Tof-GC/MS). D_4-AA, added before the extraction of the sample, was used as an internal standard for precise AA quantification in the sample extract. The exact amount of AA was calculated against internal standard integrating the peak area of the ions m/z = 209.08 (AA) and m/z = 213.08 (D_4-AA).

Stock solution preparation and determination of linear range of AA

All solutions and sample preparation were prepared in a cooling chamber at +4ºC. First, the D_4-AA stock solution was prepared by transferring 117 µl D_4-AA and 132 µl ethanol into a 100 ml volumetric flask and filled with cold oxygen-free water (MilliQ water treated with gaseous N_2), total concentration for both 1,000 µg ml^{-1}. An equal amount of ethanol was added to the stock in order to determine the exact concentration of D_4-AA by HPLC and the refractive index (RI) detection (considering that the RI values for ethanol and AA are very similar - 1.36 and 1.33, respectively). The concentration of internal standard was checked every time the standard was used.

Secondly, the calibration curve was prepared for determination of the linear range of AA extracted with SPME. Shortly thereafter, 100 µl of AA (99.9%, $\rho_{25ºC}$ = 0.785) was transferred into the 100 ml volumetric flask filled with cold oxygen-free water to produce an unlabeled AA stock solution with a concentration of 785 µg ml^{-1}. 6.37 ml of this unlabeled AA stock solution and 5 ml of D_4-AA stock solution (prepared previously) were transferred intoa 50 ml volumetric flask and filled with cold oxygen-free water to produce a solution with a concentration of 100 µg ml^{-1} for both standards. Then the dilutions (100, 80, 50, 20, 5 and 1 µg ml^{-1}) were transferred directly into the headspace vials, 4 ml in each vial. The linear range was determined with Targetlynx software (Waters Inc., Manchester, UK).

Sample preparation

For analysis of AA in food samples, 100 ± 2 g of baby puree, yoghurt, milk or curd cream was weighed into a Stomacher® bag with a filter containing a cooled (4ºC) mixture of MilliQ water (99 ml) and D_4-AA stock (1 ml, with conc. 1,000 μg ml^{-1}). The bag was quickly sealed with tape and placed into a Stomacher® blender. Homogenization was carried out at 4 ºC at 300 rpm for 1 min. Then 2 ml of the mixture was quickly transferred to a 20 ml headspace vial and capped (magnetic cap with PTFE septum). The vial with a sample was kept at +4ºC before sampling and brought to room temperature for 30 min using 250 rpm stirring the prior sampling with PFBHA-loaded SPME fiber.

Sample analysis

All analyses were carried out in triplicates. On-fiber derivatization with PFBHA reagent was carried out using polydimethylsiloxane/divinylbenzene (PDMS/DVB) SPME fiber (65 μm) from Supelco. Before each use, the fiber was cleaned in the CombiPAL (CTC Analytics, Zwingen, Switzerland) fiber conditioning station for 10 min at 250ºC in order to release any contaminants, and then exposed to the headspace of a PTFE-capped 20 ml vial containing 1 ml aqueous solution of PFBHA (17 mg ml^{-1}) for 10 seconds at room temperature under stirring conditions (250 rpm). After loading with PFBHA the fiber was placed directly into the headspace of the sample vial for 2 min at 22ºC (the on-fiber derivatisation phase) under stirring conditions (250 rpm). The sample had been stirred previously for 30 minutes at 22ºC for equilibrium. The fiber was then thermally desorbed in the GC injection port for 10 min at 250ºC and the absorbed compounds were separated using GC/MS-Tof (Agilent Technologies Inc., Santa Clara, CA and Waters, Manchester, UK).

GC/MS-Tof conditions and data analysis

Agilent 6890 gas chromatograph was equipped with a DB-5MS column (J&W Scientific, Folsom, CA) with a 1.0 μm film thickness, 30 m length and 0.25 mm inner diameter (i.d.). Helium (purity 5.5, 0.9 ml min^{-1}) obtained from AGA Eesti AS (Estonia) was used as the carrier gas and the injector was in splitless mode for 10 minutes using an inlet liner of 0.75 mm i.d. The detector temperature was 200ºC and for ionization, electron energy of 70 eV was used. The column temperature was initially maintained at 60ºC and instantly increased to 95ºC at a rate of 10ºC min^{-1} and held for 25.50 min, then increased to 280ºC at a rate of 40ºC min^{-1} and held for 1.38 min with a total run time of 35.01 minutes. Data were analysed using Targetlynx software (Waters Inc., Manchester, UK). Concentrations of AA (C_{AA}) in the samples were calculated based on the ratio of the integrated area of the Tof chromatogram of D_4-AA internal standard (m/z=213.08) and that of unlabeled AA (m/z=209.08) containing the sample (Eq 1).

$$\frac{(Area\ ion\ 209.08\ (cis) + area\ ion\ 209.08\ (trans)) \times conc\ D_4\text{-}AA\ in\ stock \times RF}{(Area\ ion\ 213.08\ (cis) + area\ ion\ 213.08\ (trans)) \times 100} \quad (1)$$

RESULTS AND DISCUSSION

The calibration curve prepared with 1, 5, 20, 50, 80 and 100 μg ml^{-1} of AA and D_4-AA demonstrated that under described conditions the linear relation of AA concentration and the respective MS response was up to a concentration of at least 20 μg ml^{-1}. At concentrations higher than 50 μg ml^{-1} the standard curve flattened, which suggests the saturation of the PFBHA-loaded fiber or the TOF detector. Thus, if the determined concentration of AA in the sample exceeded 20 μg ml^{-1}, dilutions of the extract were made in order to remain in the linear range of MS response. The response factor (RF) calculated for unlabeled and D_4-AA was 1.32 ± 0.06 (Fig. 1).

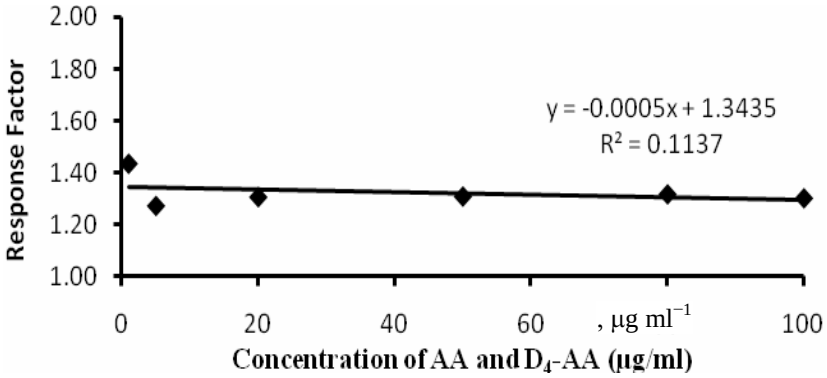

Figure 1. Response factor for unlabeled and D_4-AA is 1.32 ± 0.06.

Quantification of AA in food products

Results of AA concentration determined in different food products are shown in Fig. 2. The highest concentration of AA was determined in yoghurts. This is not surprising because AA is formed during lactic acid fermentation. Nevertheless, the concentration of AA in all studied yoghurts was in the range of the limits set by the Council of Europe (23 mg kg^{-1} in beverages and 20 mg kg^{-1} in food). Therefore, the concern that AA is considerably higher than the mutagenicity level in foods for children could not be shown in this study. The concentration of AA in all baby purees was remarkably lower (up to 6.4 ± 0.02 mg kg^{-1}) than the limits suggested by the Council of Europe.

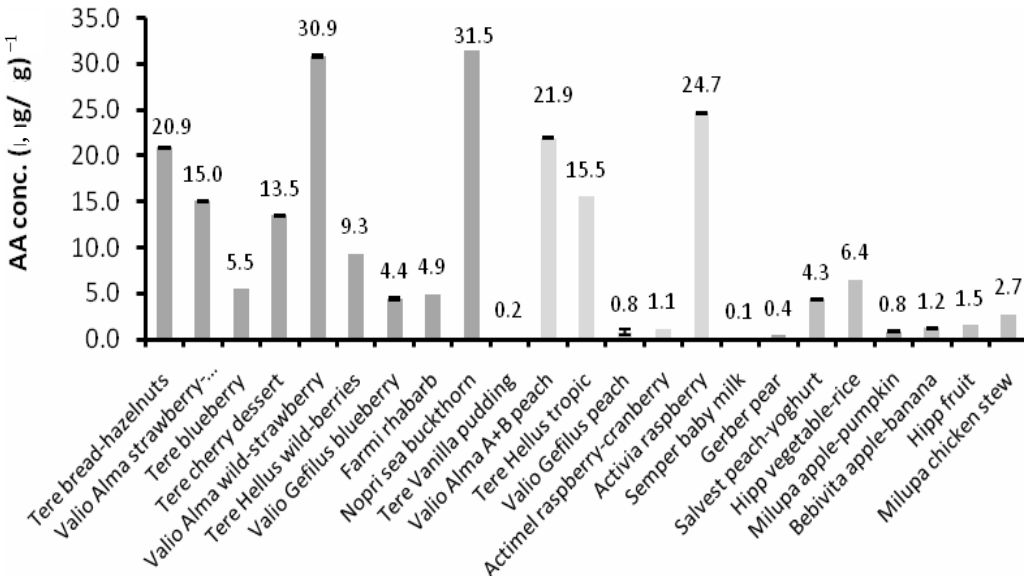

Figure 2. AA concentration in yoghurts and other milk desserts (dark gray), in yoghurt and milk drinks (light gray) and in baby purees (middle gray).

CONCLUSIONS

The method developed in the present study was shown to be adequate to analyse acetaldehyde (or other aldehydes) in solid and half-solid food matrixes. The concentration of AA in different food products, mostly consumed by small children, is not a considerable health risk.

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