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Measurement of volatile oxidation products from milk using solvent-assisted flavour evaporation and solid phase microextraction

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Abstract

A method for direct distillation of milk was developed using a high-vacuum distillation unit: solvent-assisted flavour evaporation unit (SAFE unit). Distillation of flavour compounds was carried out at low temperature, reducing the risk of artefact formation during the distillation process. After distillation, volatiles were extracted into dichloromethane and concentrated before separation on a gas chromatograph coupled with mass spectrometer detection (GCMS). Reproducibility of the SAFE method was determined by analysing the volatiles in 6 milk samples from 3 different cartons of milk. For 20 out of 27 volatile compounds, coefficients of variation below 40% were found. The method proved applicable to measure accumulation of volatile oxidation products in raw milk with 25 μM copper(II)sulfate added, and stored in the dark for 3 days at 4 °C. A simpler solid phase microextraction (SPME) method was used for the same milk, and oxidation products could only be identified after 3 days of storage at 4 °C. The SPME method can be used to describe the ongoing oxidation and the oxidative capacity of milk. Because of the high sensitivity of the SAFE method it was possible to identify compounds present in low concentrations, meaning that compounds with low-flavour thresholds and potentially high impact on the flavour may be identified.

Keywords: High-vacuum distillation; SAFE; SPME; Volatiles; Oxidation; Milk

1. Introduction

Analysis of flavour compounds in milk is complicated due to low concentrations, and extraction is difficult because of the complexity of the milk emulsion. Several methods for analysis of flavour compounds in milk have been described based on dynamic headspace analysis (Marsili, 1999; Toso, Procida, & Stefanon, 2002; Urbach, 1990), purge and trap (Contarini & Povolo, 2002; Larráyoz, Addis, Gauch, & Bosset, 2001; Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001) or vacuum distillation (Moio, Dekimpe, Etievant, & Addeo, 1993). One of the challenges in analysing flavour compounds from milk is to isolate flavour compounds with higher boiling points from the milk matrix. Engel, Bahr, and Schieberle (1999) have introduced a high-vacuum distillation unit called solvent-assisted flavour evaporation unit (SAFE unit), which is made from one piece of glassware, minimizing the risk of losing flavour compounds during extraction. Furthermore, the glassware of the SAFE unit is designed to reduce the risk of condensation of the higher boiling compounds inside the unit. The method is gentle due to the low temperature during the extraction. Engel et al. (1999) proved the efficiency of the SAFE unit by distillation of high-boiling *n*-alkanes (C10–C26) from diethyl ether. Direct distillation of milk using the SAFE unit has been carried out before for identification of volatiles (Bendall, 2001), where 2 × 2 L of milk was distilled under a pressure of 1.5×10^{-2} mbar.

In the present study, the direct distillation of flavour compounds with the SAFE unit was done to determine the reproducibility of the method. To illustrate the applicability of the SAFE method, milk was stored in the dark and oxidized with copper(II)sulfate for 3 days at 4 °C, and the accumulation of volatiles was analysed using both the

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SAFE method and a more simple solid-phase microextraction (SPME) method.

2. Materials and methods

2.1. Direct distillation of flavour compounds in milk using the SAFE unit

The head and legs of the SAFE unit (Glasbläserei Bahr, Manching, Germany) were thermostated at 45 °C and a 1000 mL distillation flask including a volume of approximately 200 mL of Raschig rings (glass, 8 × 8 mm; VWR International, Albertslund, Denmark) was surrounded by water (62 °C) in a water bath. Before pouring 150 mL of milk into the dropping funnel, the stopcock was carefully closed and vacuum applied to the system by a diffusion/turbomolecular vacuum pump system (Leybold Vakuum GmbH, Cologne, Germany). Liquid nitrogen was poured into the cooling trap and also into a beaker surrounding a 500 mL receiver flask. A vacuum in the range of 10^{-4}–10^{-5} mbar was achieved, and the milk was slowly dropped from the funnel into the distillation flask. Because of the high vacuum, the milk droplets flashed out and formed a thin film at the surface of the Raschig rings in the distillation flask. Fats, sugars, proteins and other non-volatile compounds were left in the distillation flask, while the volatiles condensed in the body of the SAFE unit were collected in the receiver flask. After the milk was distilled, the system was left for 30 min to evaporate remaining volatiles; subsequently, the pump was turned off, vacuum released and the cooled receiver flask removed. The distillates were stored at −80 °C until extraction and concentration. Distillates were thawed and extracted into 3 × 20 mL freshly redistilled dichloromethane (Rathburn, Walkerburn, Scotland). The three extracts were collected and concentrated to a final volume of approximately 300 μL using a 10 cm long and 2 cm in diameter vigreux column (Buch & Holm, Herlev, Denmark). Aliquots of 1 μL of the concentrated extract were analysed on a gas chromatograph coupled with mass spectrometer detection (GCMS) for compound identification. Fig. 1 illustrates the 3 main steps of which the SAFE method is comprised.

2.2. Solid-phase microextraction of flavour compounds in milk

A total of 0.25 g of sodium chloride was weighed into an annealed 4 mL vial with screw cap including a small glass covered magnet and 2 mL of milk. An SPME fibre (StableFlex DVB/CAR/PDMS, 50/30 μm, part no. 57348-U, Supelco, Bellefonte, PA, USA) was preconditioned by placing it in the inlet of the GC injector port for 2–4 h. The conditioned fibre was manually placed in the headspace of the vial approximately 5 mm above the milk surface, and the milk was stirred for 30 min at 45 °C at approximately 500 rpm using a magnetic stirrer (RCT basic, IKA, Staufen, Germany) to allow adsorption of volatiles to the fibre before introduction to the GC injector port. The fibre was left in the injector port for desorption for a minimum of 30 min before introducing the fibre to the headspace of a new sample.

DISTILLATION → EXTRACTION → CONCENTRATION

Fig. 1. The three main steps in the method for measuring volatiles using the solvent-assisted flavour evaporation unit.

2.3. Analysis of volatiles in milk by GCMS

GCMS analyses were performed on a Varian 3400CX GC coupled with a Saturn 3D ion trap mass spectrometer (Varian Inc., Walnut Creek, CA, USA). The volatiles were separated on a DB-FFAP column, P/N 122-3232 (30 m length, 0.25 mm id and 0.25 μm film thickness: Agilent Technologies, Palo Alto, CA, USA). Helium was the carrier gas with a constant inlet pressure of 1034 hPa to the column. The injector with splitless injection (splitless for 0.6 min) was kept at a temperature of 250 °C. The temperature for the column was programmed at 35 °C for 1 min, 3 °C min^{-1} to 225 °C with a holdtime of 1 min, 10 °C min^{-1} to 250 °C with a holdtime of 5 min. The mass spectrophotometer was operated in the electron impact mode with electron energy of 70 eV, and spectral data from mass range m/z 35–300 were obtained. The GCMS transfer line temperature was 275 °C, the temperature of the trap was 200 °C and the manifold temperature was 50 °C.

2.4. Identification of volatile compounds

A software method including 74 different authentic aroma compounds was set up in SaturnView™ version 5.40 (Varian Inc., Walnut Creek, CA, USA). All 74 compounds listed in the method were analysed on the system, and retention time and spectral data were used for identifying unknown volatiles in the milk samples analysed on the same GCMS system. The retention time, a characteristic target ion and two or three qualifier ions for each of the compounds in the method were used to confirm and support the identification of every compound found in the milk sample.

2.5. Reproducibility of the SAFE method

Commercially available organic unhomogenized and pasteurized whole bovine milk (ARLA Foods, Denmark) was used for the study. The cartons were stored at 4 °C and used before the date of expiration as labelled on the cartons. The study was carried out as 6 replicates originating from 3 different cartons, and milk from different cartons was not pooled. The milk cartons were opened on the day on which direct distillation was done using the SAFE unit.