DETERMINATION OF AROMA COMPOUNDS USING CRYOFOCUSING

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Background

Aroma analysis is often done using either dynamic head space technology trapping compounds on tenax or examining extracts in organic solvents. Using extracts, one can benefit from the large volumes which can be injected using a cooled injector in the GC.

Objective

The study was done in order to examine the suitability of a cooled injector for determination of volatile aroma components. These were determined in cooked or fermented sausages, in cooked pork meat balls and in different types of soups.

The injection port can be used with electrical cooling down to approx. 17°C with GC oven temperature at 35°C, and down to -70 °C when cooled with with CO_2 (l). It can be used with extracts > 25 µl, further, as a conventional heated injection port (split/splitless) for liquid extracts or with volatiles carried through a heated liner situated in the cooled/heated injection port.

Experimental methods

Using dynamic head-space technology, aroma components were collected either on Tenax TA tubes or on charcoal tubes. The Tenax TA tubes were desorbed in a Perkin Elmer ATD400 Tenax tube autosampler. The sample was transfered by a heated liner (fused silica) which was connected with the column, a DB-1701 30m 1µ 0.25mm id (J & W Scientific), in the gas chromatograph, a HP 5890 Ser II equipped with a mass spectrometer HP 5972, through a specially designed Gerstel CIS 3 cooled injection system.

Alternatively aroma components were collected on charcoal tubes (150 mg charcoal tubes, SKC), which were subsequently extracted with diethylether. The ether extracts were injected into the Gerstel injector.

The injector was used both as a heated and a cooled injection port.

Aroma collection, using dynamic head-space technology, was done on 25 g sausage, 20 g minced meat ball or 5 ml soup using a 100 ml washing bottle connected to the Tenax tube or a charcoal tube. The bottle was placed at 50°C for 15 min equilibration without flow, followed by a flow of He at 60 ml/min for 15 min. Ether extracts were produced by eluting the charcoal tubes with 300 µl diethyl ether.

The Gerstel injection port was used for ether extracts either as a cooled port at -35°C/-40°C (one sample at -70°C) using CO_2 or at 50°C. Temperature program for the injector: initial temperature -40°C for 2 min with "stop flow" i.e. no flow/pressure on the injector during sample application, followed by 0.5 min with He flow at approx. 90 ml/min during which the solvent was purged off. Subsequently the injector was heated at a rate of 12°C/s untill 230°C with a half for 10 min followed by heating to 250°C and hold for 10 min.

With a liner connected to the Tenax-tube autosampler, it was used at -30°C og at 200°C.

Cryofocusing of ether extract allowed a sample of up to 25 µl to be injected.

Samples examined: beef and vegetable soup (Maggi cube, double strength), fermented sausage (Marburger, Vestjyske) and non-fermented sausage (Smicky), meat balls made from pork meat with approx. 15 % back fat.

4-methyl-2-pentanol (bp. 131°C) was used in all samples as an internal standard.

Results

Comparing cryofocusing (-30°C) with a temperature of 200°C on a liner placed in the injection port, higher concentrations of volatiles collected from Tenax tubes were observed using cooling. Fig. 1 shows total area of aroma components from two types of soups. In Fig.2, results with the liner is compared with ether extracts of a nonfermented sausage. Similar results as with the soups were observed using the liner. Fig. 2 also shows that injection of 5µl extract gave higher total area counts using a heated injection port compared with -40°C. However, injection of 10µl resulted in a higher concentration og aroma compounds both in the first (before the internal standard) and second part of the chromatogram.

In Fig.3. similar results regarding 5µl samples were observed using a fermented sausage. Even using 10µl extract and cooling gave a lower concentration of volatiles than using a heated injector, in the first part of the chromatogram; however, higher concentrations of higher boiling compounds were found using 10µl with cooling. The number of aroma compounds were 8, 36 and 53 for the 5µl cooling ("sm 5µl cryo"), 5µl no cooling and 10µl cooling samples respectively.

In Fig. 4. results from another type of fermented sausage are shown. The usual results were observed with the liner placed in the injector. Using -40°C, the results show higher concentrations with 10µl than with 5µl extract. Using 25µl gave even higher concentrations, while using an even lower temperature (-70°C, "10µlccryo") gave similar results.

Results from extracts from meat balls were similar (not shown). The number of components were 30 using cooling and 19 without cooling.

Conclusion

Using a cooled liner placed in the injector or concentrating ether extracts "on line" by injection of large volumes and venting the solvent before injection of the sample onto the column is a promising technique for the determination of aroma components. The effect using the liner was obvious; using extracts, it was shown that large volumes could be injected and larger concentrations and numbers of volatiles especially of the higher boiling compounds could be detected.

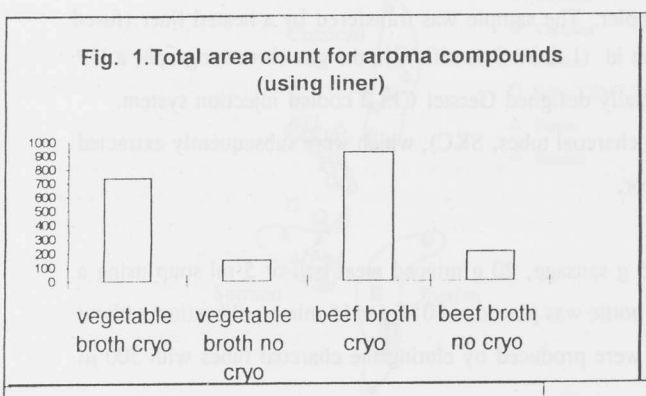

Fig. 1.Total area count for aroma compounds (using liner)

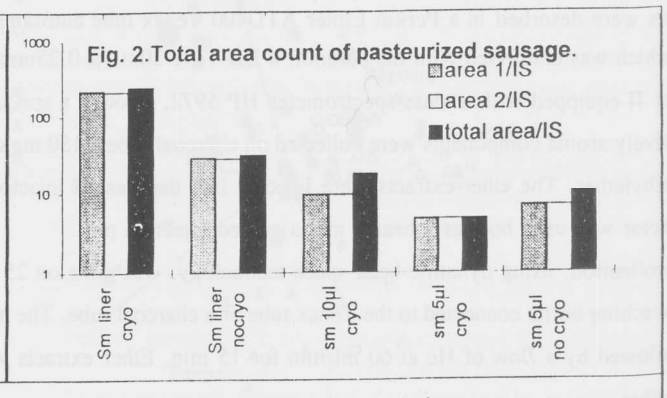

Fig. 2.Total area count of pasteurized sausage.

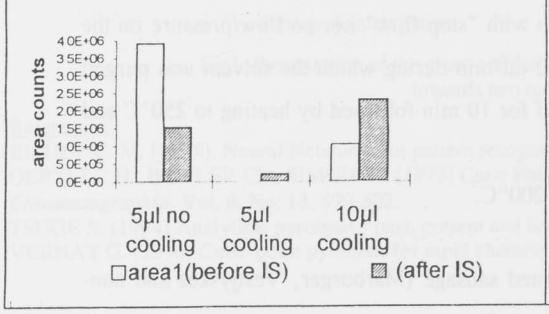

Fig. 3. Total area count of fermented sausage

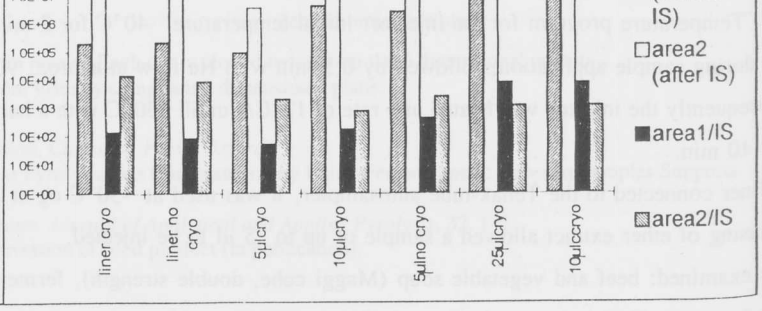

Fig. 4. Total area counts for fermented sausage (Marburger)