Aroma detection using supercritical fluid extraction compared with diffusion sampling.

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Background

Supercritical fluid extraction (SFE) has been used for extraction of a number of different matrices.The supercritical fluid used most frequently is carbondioxid and different combinations of extraction modes, static and dynamic, of temperature - time combination and flow rates have been used. King *et al.* (1993) extracted raw beef using SFE and collected 59 volatiles in head space. Snyder and King (1994) identified volatiles collected on a trap by either SFE or thermal desorption, where SFE was superior in the less volatile components.

Aroma formation from meat starter cultures like *Lactobacillus plantarum* and *Staphylocococcus carnosus* in meat products has not been reported using SFE.

Objective

This work was undertaken in order to compare SFE with subsequent injection of extracts using a cooled injection port and diffusion sampling, trapping on Tenax TA tubes on a minced meat model system.

Methods

A meat matrix of minced pork loin was used, with 0.5 % glucose and 4 % NaCl added.

The pork loin was sterilized on the surface by submersing the meat in boiling water for 10 min; subsequently 1 cm of the surface was removed, all visible fat removed and the meat minced. Meat samples of 10 g was put into 100 ml Blue cap bottles and inoculated with the bacterial cultures. The cultures used were *Lactobacillus plantarum* and *Staphylocococcus carnosus* (Chr. Hansen, Denmark). *L. plantarum* was cultured in All Purpose Medium with Tween (APT, Oxoid) and *S.carnosus* in Nutrient Broth (Oxoid). The bacteria were grown in the respective broths for 1 day at 30°C, centrifuged at 15000 rpm for 5 min and used for inoculation. Inoculation levels were approx. 10^7/g. Samples were made with pure cultures, mixtures and controls without inoculation.

Fermentation of the meat was done for 3 or 6 days at 25°C or 7 days at 18°C with/without 3 days at 25°C. Following fermentation the meat samples were stored at -20°C until extraction. pH was measured in the meat samples.

Diffusion sampling was done for 3 days at 25°C this corresponds to a total of 6 days at 25°C or 7 days at 18°C followed by 3 days at 25°C. Sampling was done using tubes with Tenax TA.

Supercritical fluid extraction was done using a HP 7680 T extraction unit, on a 2 g sample, and the meat was mixed with 1 g hydromatrix (Varian), 50 µl 4-methyl-2-pentanol was added. Extraction was done using thimbles of 7 ml, carbondioxide (99.9992 %) density 0.65 g/ml was used, extraction conditions were: static at 41°C for 10 min followed by dynamic extraction for 10 min at 41°Cand 1 ml/min, nozzle temperature 45°C, and trap (Tenax TA) temperature -20°C. Extraction of the trap was done using 1 ml chloroform at 50°C, resulting in approx. 0.66 ml being collected in the vial. This amount was added 5 µl heptan as an internal standard for volume correction. Thermal desorption of Tenax tubes was done using a Perkin Elmer ATD 400 autosampler coupled via a heated liner (200°C) to a Hewlet Packard 5890Ser II GC with a 5972 MS detector. Perkin Elmer ATD 400 run at oven temperature 200°C , desorption time 20 min, trap low/high -30°C/240°C. GC parameters 35°C 10 min, 3°C/min until 150°C, hold for 5 min, 30°C/min until 250°C with hold 5 min. MS scanning 35-250 m/z 2.2 scan/s and identification using a NIST library.

SFE extracts (10 µl) were injected on a Gerstel Cooled Injection Port at -50°C followed by time temperature programing (12°C/s until 350°C).

Results and discussion

Inoculation levels were approx. 1 x 10^7 /g for *L. plantarum* and 1.5 x 10^7 /g for *S. carnosus*. pH levels decreased during incubation of samples in the order *L. plantarum* > mixture > *S. carnosus* > uninoculated controls (which did not decrease).

Using SFE in combination with GS-MS a total of 45 components were detected and 31 of these identified. Using diffusion sampling 62 komponents were detected with 13 unknown. The aroma components are seen in Table 1.

A number of compounds were unique for a given culture, however, in a mixture of the two an increased concentration of hexan, c-1,2-dimethylcyclopentan, 2 (3)- methyl-1-butanol, 2,4-decadienal, acetic acid and 2-butyl-furan was observed. Others like 1-hexanol, 1-octanol, butyric acid and diacetyl decreased compared with pure *L.plantarum* culture. Methylbranched acids were especially produced by *S.carnosus*, the levels decreased in mixed cultures. Some differences could be seen in the two different incubation procedures, thus increasing levels of the branched acids was produced at the higher incubation temperature.

Fig. 1 and Fig. 2 shows differences between SFE and diffusion sampling. When the aroma components are looked upon as groups (Fig. 1) it is observed that acids, alcohols and alkanes are 4.2; 1.4 and 1.4 times higher using SFE compared with diffusion sampling.

On the other hand ketones, benzenederivatives and aldehydes are 5.4 ; 2.4 and 1.6 times higher with diffusion sampling.

Looking at the peak areas of aroma components, the acids are by far the most important group using SFE (6.8 times higher). Aldehydes were by far the most important using diffusion sampling. Alcohols, aldehydes and ketones were 14.4; 3.9 and 3.1 times higher respectively using diffusion sampling. Problems arose when it was not possible to selectively desorbe the solvent chloroform using the cooled injection port. This resulted in a number of components in the first part of the chromatogram being hidden by the solvent peak and not being detected.

The higher concentrations of acids using SFE compared to hexane extraction was also observed by Snyder and King (1994) in their study of soy beans; however, extraction of aldehydes were more or less the same. The results in the present study concerning the superior detection of very volatile components in diffusion sampling and the less volatiles using SFE was also observed by Snyder and King (1994) comparing thermal desorption and SFE.

Conclusion

A minced meat fermentation model was used for detecting aroma components from growth of two starter cultures *L.plantarum* and *S. carnosus*. Using SFE combined with a cooled injection port and GC-MS many of the same chemical components could be detected as in diffusion sampling using Tenax TA tubes coupled with thermal desorption and GC-MS. However som marked differences could also been, SFE was especially good in detecting acids but inferior for aldehydes/ketones, furans and esters. Speciel problems arose from the solvent used in SFE as a number of compounds in the first part of the chromatogram could not been detected. However, marked differences between the components detected by the two methodologies was evident.

King, M. F., Hamilton, B.L., Mathews, M.A., Rule, D.C. and Field, R.A. (1993) J.Agric. Food Chem. 41(11), 1974-81.
Snyder, J.M. and King, J.W. (1994) J.Sci.Food Agric. 64,257-63.
Snyder, J.M. and King. J.W. (1996) JAOCS 71(3), 261-65.

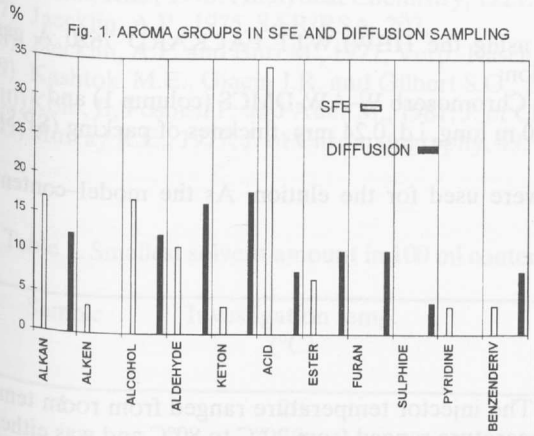

Fig. 1. AROMA GROUPS IN SFE AND DIFFUSION SAMPLING

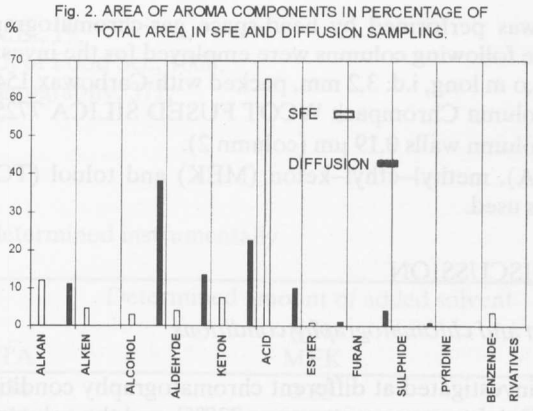

Fig. 2. AREA OF AROMA COMPONENTS IN PERCENTAGE OF TOTAL AREA IN SFE AND DIFFUSION SAMPLING.

Table 1. Aroma components identified with diffusion sampling and SFE.

<u>Alkanes</u>	<u>Ketones</u>	<u>Esters</u>	<u>Benzenederivatives</u>
hexane*	acetone*	ethylacetate*	toluene
heptane*	2,3-butadione*	ethylhexanoate	benzene*
octane**	2-me-2-cyclopentane-1-on*	2,4-hexandien-diacidethylester	3-methyltriophene*
nonane*	2-pentanone*	ethyl-2-me-butanoate*	p-xylene*
c-1,2-dimethylcyclopentane	3-pentanone*	ethyl-3-me-butanoate*	1-ethyl-2-methylbenzene*
2,2-diethoxypropane	methylisobutylketone*	3-me-1-butylacetate*	
1,1,2,trichlorethane	3-hydroxy-2-butanone**	pentylacetate*	<u>Furanes</u>
1,1,2,2-tetrachlorethane	2-heptanone*		tetrahydrofuran*
methylcyclopentane*	2,3-octadione*	<u>Acids</u>	2-methylfuran*
pentadecane		acetic acid**	2-ethylfuran*
<u>Alkenes</u>	<u>Aldehydes</u>	2-me-propanic acid	2-n-butylfuran*
trichlorethethylene	propanal*	butanoic acid**	2-pentylfuran*
<u>Alcohols</u>	2-methylpropanal*	dimethylpropanoic acid*	<u>Sulphides</u>
ethanol*	butanal*	2-me-butanoic acid	dimethyldisulphides*
1-pentanol**	3-methyl-1-butanal*	3-me-butanoic acid**	dimethyltrisulphides*
1-hexanol**	pentanal*	haxanoic acid	
1-octanol	hexanal**	heptanoic acid	<u>Pyridines</u>
1-octen-3-ol*	heptanal*	2,3-hexadienoic acid	2,4,6-trimethylpyridine
nonanol	octanal*	octanoic acid	
1-(2-butoxyethoxy) ethanol	2,4-decadienal	nonanoic acid	
3-buten-2-ol*	2-butyl-2-octenal		
2(3)-methyl-1-butanol*	2-undecanal		

*=found with diffusion sampling, ** both diffusion and SFE, no indices = SFE only