

## MICROBIAL AROMA FORMATION IN PACKAGED MINCED BEEF MEAT AS INFLUENCED BY ATMOSPHERE AND STORAGE TEMPERATURE

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**Background:** The shelf life of fresh minced meat can be extended by using vacuum or modified atmosphere packaging. The drawbacks are the unattractive color of the product and the sometimes unpleasant odour produced from microbial activity during storage. The microbial flora composition differs according to atmosphere conditions. The aroma formation will reflect the composition of the microbial flora in relation to both growth, type of atmosphere and storage temperature.

**Objectives:** The aim of this project was to investigate the formation of microbial aroma components under three different packaging methods at two temperatures. The aroma production was correlated to microbial growth and flora composition as influenced by atmosphere and storage temperature.

**Methods:**

90 g fresh minced beef meat was packaged using three different conditions: vacuum, modified atmosphere of 80% O<sub>2</sub> / 20% CO<sub>2</sub> or 100% CO<sub>2</sub>. The packaging material were Combitherm XX80 (transmission rates N<sub>2</sub>: <0,1 cm<sup>3</sup>/(m<sup>2</sup>·24h·bar); O<sub>2</sub>: 0,5 cm<sup>3</sup>/(m<sup>2</sup>·24h·bar); CO<sub>2</sub>: 0,8 cm<sup>3</sup>/(m<sup>2</sup>·24h·bar)), SFK (Hvidovre, Denmark). Samples were stored in the dark at 5°C or 10°C. At intervals of 3-4 days samples were examined for the composition of microbial flora and aroma production.

Enumeration of microorganisms was carried out on Plate Count Agar for total count; De Man, Rogosa, Shape Agar for lactic acid bacteria; Violet Red Bile Agar for Gram negative strains and Streptomycine sulfate, Thallium acetat, Actidione Agar for *Brochothrix thermosphacta*.

Sampling of volatiles were done using a dynamic headspace methodology. 30 g minced beef meat were placed in a washing bottle and the headspace flushed with N<sub>2</sub> at 50°C. After 15 min's of calibration without flow, a total volume of 300 ml headspace was trapped on a tube with 230 mg Tenax TA 60-80 mesh. 4-methyl-2-pentanol was added as internal standard before purging of the sample.

Analysis of volatiles was done by a two stage thermal desorption followed by GC-MS.

The apparatus and conditions were: ATD400 tenax tube autosampler (Perkin Elmer) 1. desorption 250°C for 20 min's; cryofocusing on a tenax trap at 30°C; 2. desorption by flash heating to 300 °C in 2 min's. Flow He (N 6,0) with an outlet split flow at 22ml/min.

Separation and detection of volatiles using a HP 5890 Serie II gaschromatograph with HP 5972 (quadropole) mass selective detector. Gaschromatographic conditions were: carrier gas He (N 6,0) 0,68 ml/min; transfer line temperature 200°C; column: DB1701 (J&W Sci.), 30 m, 0,25 mm id, 1 µm; temperature program: 35°C for 10 min's, 1.rate 3°/min til 150°C, hold 5 min, 2.rate 30°/min till 250°C hold 5 min;

MS conditions were: interface temperature 280°C; emission current 70 eV, scan 35-250 m/z with 2,2 scan/s. Identification of compounds by comparison of retention times with known standards or by library search in NIST (NBS75K) library and by comparison of Kovat's retention indices.

**Results and discussion:** Figur 1 and 2 shows the development of the bacteria flora with time under the six different storage conditions. The influence of atmosphere is substantial. Vacuum packaging generally suppresses the growth especially of Gram negative strains and *B. thermosphacta* and the aroma production is very low. In a 100% CO<sub>2</sub> atmosphere aroma production is low at 5°C, but at 10°C more aroma is produced than under vacuum conditions. In the 80% O<sub>2</sub> / 20% CO<sub>2</sub> atmosphere only the Gram negative bacteria is suppressed and the lactic acid bacteria and *B. thermosphacta* produces a considerable amount of aroma. Generally the aroma production rises with time and temperature.

These results agree well with the observed change in flora composition for meat stored in CO<sub>2</sub> atmosphere. Lactic acid bacteria will develop and dominate the flora over a period of 21 days (Molin and Ternstrøm, 1979). Our results show a lag phase for growth of lactic acid bacteria of 7 days at 5°C and 3 days at 10°C.

Figures 3-4 show the production of major aroma compounds. Ethanol is the dominating compound related to bacterial activity and corresponding to 60 to 90% of the total aroma production for beef packed under anoxigenic conditions. Lactic acid bacteria produces most of the ethanol and acetone. As seen from fig. 3 the growth of lactic acid bacteria and production of ethanol and acetone are correlated. In the modified atmosphere with 80% O<sub>2</sub> / 20% CO<sub>2</sub>, acetoin (3-hydroxy-2-butanone) is a major compound. Acetoin and 2,3-butanedione are produced mostly by *B. thermosphacta*. Fig. 4 show the correlation between these aroma compounds and bacterial growth. The influence of temperature is less significant, although both number of bacteria and amount of aroma produced are higher at 10°C than at 5°C.

A total of about 40 aroma compounds were detected using a predefined detection limit. These included alcohols, aldehydes, ketones, alkanes and cyclic hydrocarbons, acids and a few esters including ethylacetate. The compounds produced agrees well with those cited in the literature (Bailey et al. (1993) and Stutz et al. (1991)).

**Conclusion:** The packaging conditions has a profound influence on the composition of the bacterial flora in minced beef during storage. The total bacterial flora will be dominated by lactic acid bacteria using packaging in vacuum or 100% CO<sub>2</sub>, leading to ethanol and acetone as major aroma compounds. In a modified atmosphere of 80% O<sub>2</sub> / 20% CO<sub>2</sub> growth of *Brochothrix thermosphacta* will be stimulated resulting in the production of acetoin and 2,3-butanedione.

**References:**

Bailey, M. E., Intarapichet, K.O., Gerhardt, K.O.; Gutheil, R.A. and Noland, L.A. (1993) p. 63-84 in Charalambous, G. (ed). Shelf life studies of foods and beverages. Elsevier Science.  
 Molin, D. and Ternström, A. (1979). Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4°C. J. App. Bact. 47, 197-208.  
 Stutz, H.K., Silverman, G.J.; Angelini, P. and Levin, R.E. (1991). Bacteria and volatile compounds associated with ground beef spoilage. J. Food Science. 56, 1147-53.

