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AROMA CONSTITUENTS IN SWEDISH FERMENTED SAUSAGE

I. Formation of lactic acid and short chain fatty acids (C_1-C_6).

By Hans Halvarson, Swedish Meat Research Centre, Kävlinge, Sweden

INTRODUCTION

In Sweden there exists a type of fermented sausage which is produced all over the country. The sausage can either be smoked or dried or treated by several combinations of these processes. As a consequence of mainly the process conditions and the fact that bacterial starter cultures are not used there are several possible ways for the aroma to develop during the ripening process.

To increase our knowledge about how these factors influence the development of volatile substances in the sausage we have begun to study two important classes of these compounds - volatile fatty acids and neutral, volatile carbonyl compounds.

This paper describes the formation of lactic acid and the short chain fatty acids (C_1-C_6) in two fermented sausages which were produced by two different process techniques.

MATERIALS AND METHODS

The sausages used in this investigation were made from the following raw materials.

Raw material	Quantity (kg)
Beef meat	4.0
Pork meat	6.6
Potatoes, cooked	2.6
Barley grain, precooked	9.4
Salt-nitrite mixture (0.6% $NaNO_2$)	0.4
Sugar	0.2
Spices	0.2

The pretreatment of the barley grains were done in two different ways. The grains to be used in the coldsmoked sausages were cooked in water in the barley grain - water ratio 1:3. The grains used for the dried sausages were cooked in the grain-water ratio 1:1.7.

The cold smoking was done at 20-25°C for two days after which the sausages were stored at 4-5°C up to 3 weeks. The drying process took place at 11-13°C, 55-70% relative humidity up to 3 weeks.

Determination of the C_1-C_6 fatty acids.

The isolation and the quantitative determination of the C_1-C_6 fatty acids was done according to the following procedure which is to be detailed described elsewhere (1).

In the isolation step the fatty acids were steam distilled in reproducible yields from the acidified sausage homogenate. By this treatment there was no contamination of lactic acid in the distillate.

The total amount of volatile fatty acids was determined by titrating the distillate with standard 0.1 M NaOH against phenolphthalein. The acids were then transformed to ethyl esters and as such quantitatively evaluated by gas chromatography relative to benzene as the internal standard.

The following conditions were valid during the gas chromatographic determination:

Instrument	Varian 1400 gas chromatograph equipped with a FID.
Recorder	Varian aerograph, model 20.
Columns	A 1/8" x 3.0 m. Porapak Q column preconditioned at 220°C for at least 24 h was used for determination of the ethyl esters of C_1 and C_2. Nitrogen flow 25 ml/min., hydrogen flow 25 ml/min. Injector temp. 200°C, column temp. 180°C, detector temp. 200°C. A 1 mm x 1.2 Porapak S, preconditioned as above was used for determination of the ethyl esters of C_3-C_6 under the same conditions as given above.

The resolution of these substances on the Porapak columns are demonstrated in the Figure 1 and 2.

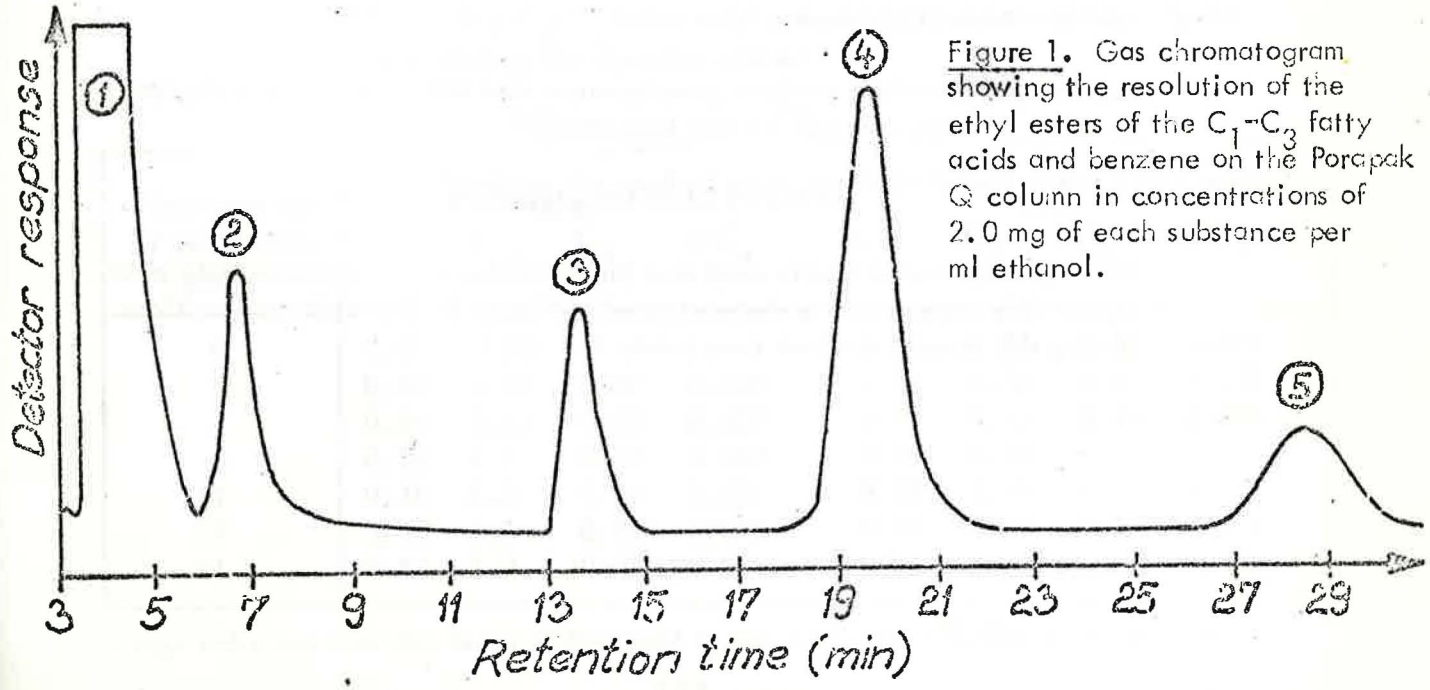


Figure 1. Gas chromatogram showing the resolution of the ethyl esters of the C_1-C_3 fatty acids and benzene on the Porapak Q column in concentrations of 2.0 mg of each substance per ml ethanol.

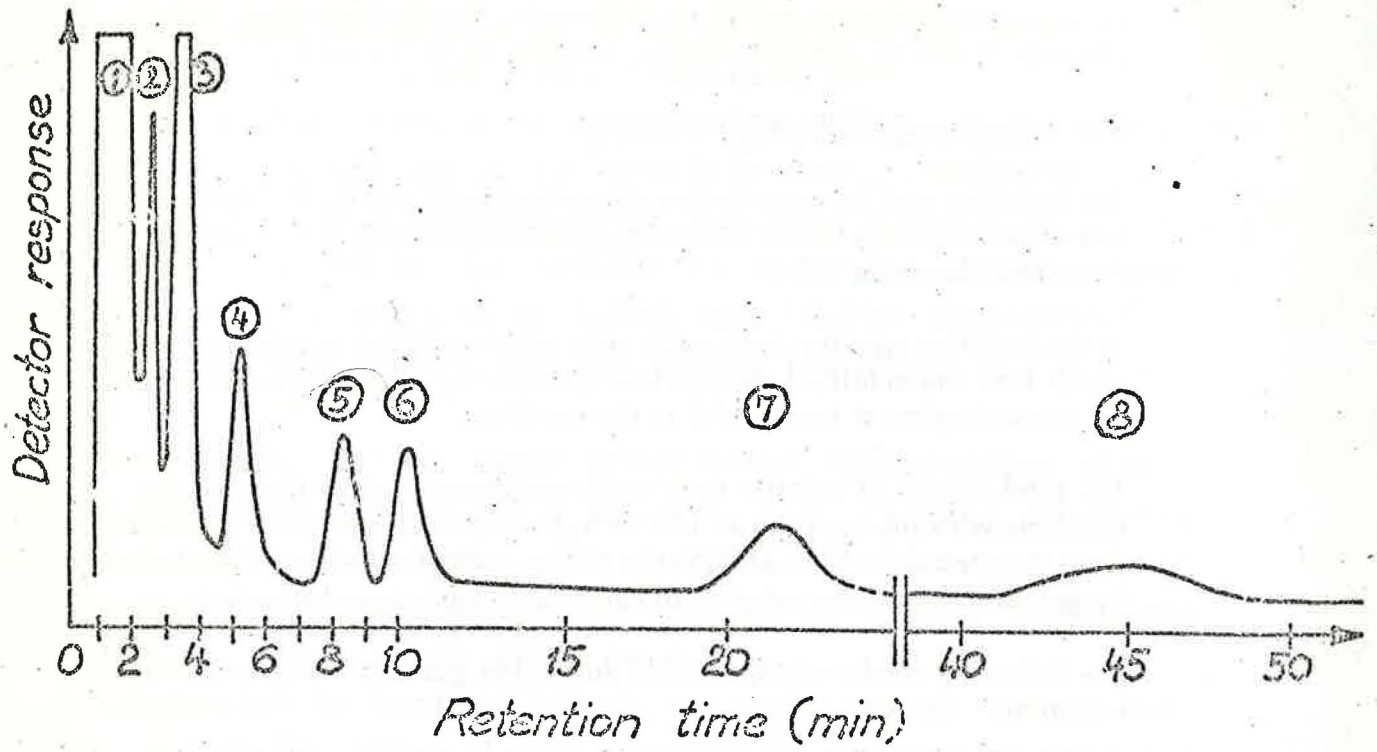


Figure 2. Gas chromatogram showing the resolution of some ethyl esters of the C_2-C_6 fatty acids and benzene on the Porapak S column in concentrations of 2.0 mg of each substance per ml ethanol.

The calibration of the GC system was done with standard solutions containing the internal standard and the ethyl esters to be determined in appropriate concentrations.

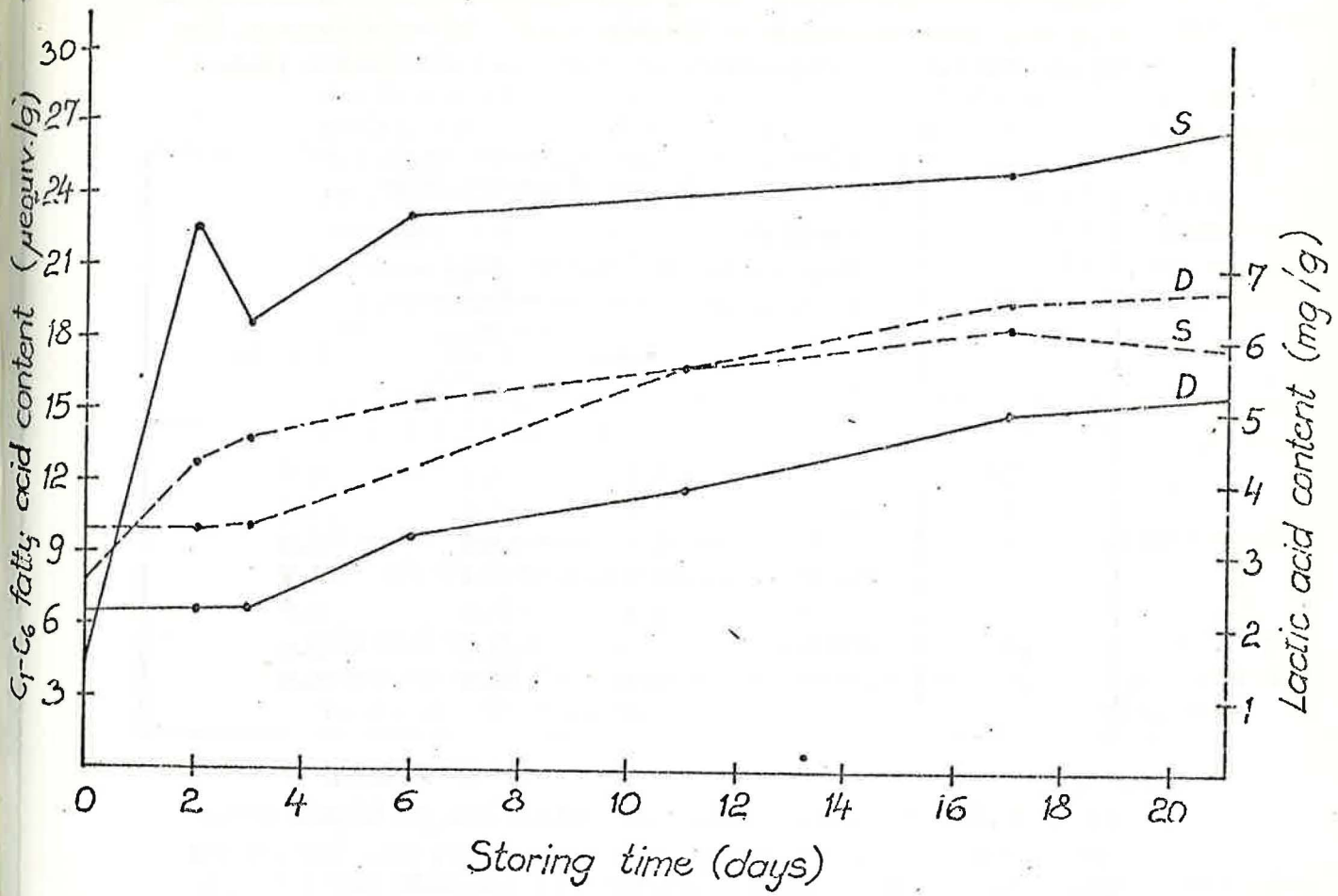
The detector responses were determined as the product of the peak height and the width at half height.

The lactic acid determination was done according to a method by Harper and Randolph (2).

Besides the chemical analysis samples were also taken for microbiological analysis of Coliforms (37°C) and Lactobacilli.

RESULTS AND DISCUSSION

The development of lactic acid and the total amount of volatile fatty acids during this experiment is demonstrated in Figure 3. The acid compositions during this time is evident from Table 1.



The dashed lines denote lactic acid content. The heavy lines denote C_1-C_6 fatty acid content.

Figure 3. The development of lactic acid and volatile fatty acids up to C_6 in the smoked (S) and the dried (D) sausage during the ripening process.

Table 1. The C_1-C_6 fatty acid composition in the experimenterial material during the ripening process.

Time from day of production (days)	Acid levels in the smoked sausage mg/g				Acid levels in the dried sausage mg/g			
	C_1	C_2	C_3	n-C_4	C_1	C_2	C_3	n-C_4
0	0.09	0.20	-	-	0.14	0.23	0.002	0.006
2	0.40	0.94	0.02	0.005	0.12	0.28	0.004	0.002
3	0.26	0.86	0.03	0.007	0.14	0.23	0.002	0.003
6	0.28	1.1	0.03	0.004	0.18	0.40	-	-
11	0.31	1.2	0.03	0.005	0.22	0.49	-	-
17	0.40	1.1	0.03	0.003	0.31	0.58	0.005	0.004
21	0.42	1.2	0.03	0.007	0.25	0.70	0.004	0.004

- sign indicates that the level of the acid in question was <0.001 mg/g sausage.

No other fatty acid up to C_6 could be detected on the gas chromatograms, e.g. their concentrations were less than about 0.001 mg/g sausage. The results of the microbiological determinations are summarized in Table 2.

Time from day of production (days)	Degree of contamination			
	Smoked sausage (log. number of bacteria/g)		Dried sausage (log. number of bacteria/g)	
	Colif. (37°C)	Lactob.	Colif. (37°C)	Lactob.
0	2.1	<3.0	1.6	<3.0
2	4.9	5.3	2.0	3.4
3	4.3	5.4	3.4	4.6
6	>5.0	5.1	1.4	4.9
9	<2.0	5.6	<2.0	5.9
17	<2.0	5.9	<2.0	5.6
21	<2.0	5.6	<2.0	5.0

The formation of lactic acid was similar in the sausages in spite of the different process techniques and the different storing conditions. In the smoked sausage the lactic acid concentration increased from 2.7 mg/g at 0 days to 5.9 mg/g after 3 weeks storing with a slightly accelerated rise during the smoking process. The Lactobacilli content increased more than 100 fold during this treatment but was almost constant throughout the experiment. The pH in the smoked sausage fell from 5.8 to its final pH 5.3 in 3 days. Normally the ultimate pH of this type of sausage lies just below 5. The lactic acid level in the dried sausage was constant (3.3 - 3.4 mg/g) during the first 3-6 days of the storing time but rose to 6.7 mg/g after 3 weeks. This sausage reached its final pH 5.4 in 6 days.

The smoking process had a stimulating effect on the development of the volatile fatty acids. As is demonstrated in Figure 3 the total amount of these acids increased from 4.7 μeq/g at 0 days to 22.6 μeq/g after the smoking (at 2 days) to reach 27.1 μeq/g after 3 weeks. As is clear from Table 1 the rise concerning total volatile acid content was mainly due to formic acid and acetic acid and to a lesser degree to propionic acid. The formic acid level did not change significantly from this concentration (0.40 mg/g) throughout the experiment. The further rise after 2 days to 3 weeks storing could be attributed entirely to acetic acid which increased from 0.9 mg/g sausage to 1.2 mg/g during this period. The concentrations of propionic acid and n-butyric acid in the smoked sausage remained at 0.03 mg/g from the 3rd day and at about 0.005 mg/g respectively from the 2nd day throughout the experiment. The rapid increase in volatile fatty acid content during the smoking was probably related to the growth of Coliform (37°C) bacteria which increased about 1000 fold during this process. It is known that certain coli cultures actually produce formic acid and acetic acid as break down products.

The formation of the C_1-C_6 fatty acids in the dried sausage was rather different from the development in the smoked sausage. The total amount of volatile acids was constant at about 6.5 µeq/g between the first 3-6 days and then increased almost linearly to 15,8 µeq/g after 3 weeks storing. The levels of the acids changed during this time, for formic acid from 0.1 mg/g to 0.3 mg/g, for acetic acid from 0.25 mg/g to 0.7 mg/g, and for propionic acid from 0.002 mg/g to 0.004 mg/g. The concentration of n-butyric acid changed irregularly between 0.002-0.006 mg/g sausage. These changes can hardly becaused by coli bacteria since they occurred in relatively low concentrations in the dried sausage. It is more probable that the acids were produced by some Lactobacilli strains.

The effect of the process technique and the storing conditions on the development of lactic acid in the sausages was very small although the temperature difference was 7-8°C during the maturing for 3 weeks.

However, the process technique had a definite effect on the formation of the volatile fatty acids in this experiment.

The smoking process accelerated the formation of formic acid, acetic acid and propionic acid probably due to the rapid coli growth bringing them to a final higher level compared to the concentrations in the dried sausage.

In the dried sausage it took 3-6 days to obtain a stable increase in the volatile fatty acids.

These differences related to the final treatment of the sausages were verified in another experiment and therefore seem to be conclusive. The effect of these substances on the aroma is yet uncertain. At the moment we have a test panel examining the aromas obtained when the appropriate acids are added to deodorised sausage homogenates.

Acknowledgment

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References

1. H. Halvarson. A procedure for isolation and quantitative determination of volatile fatty acids from meat products. To be published.
2. N.J. Harper and H.E. Randolph, American milk review 22:6 (1960), 43.

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