Aroma constituents in Swedish fermented sausage

II. Formation of neutral low molecular carbonyl compounds (C_1-C_8).

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Introduction

In part I (1) in this series a Swedish type of fermented sausage was presented. Mainly because these sausages are produced by different process techniques their aroma will be of variable quality.

To gain some understanding of the effect of these factors on the aroma forming reactions we are examining the development of certain aroma contributing substances during the natural ripening.

This paper deals with the qualitative and quantitative formation of neutral, volatile carbonyl compounds (C_1-C_8) in a smoked and a dried fermented sausage and possible explanations for their appearances.

MATERIALS AND METHODS

The production of the smoked and dried fermented sausage used in this investigation is described in part I (1) in this series. The cold smoking was carried out at $20-25^\circ C$ for two days after which the sausages were stored at $4-5^\circ C$ up to 3 weeks. The drying process took place at $11-13^\circ C$, 55-70% relative humidity up to 3 weeks.

Isolation of the volatile carbonyl compounds

The volatile carbonyl fraction was isolated by steam distillation in the following way. The distillation apparatus consisted of a 5 litre round flask, a condenser, a gas distribution tube and a receiver. The distillation flask was equipped with inlets for steam, nitrogen, a mechanical agitator and a distillation head. This system was, with the exception of the metallic agitator, entirely built of glass.

Before the steam distillation was started 500 ml carbonyl free 2 M HCl satured with 2,4-dinitrophenylhydrazine and 500 ml carbonyl free benzene was added to the receiver flask.

1000 g finely divided sausage, 150 g $MgSO_4 \cdot 7H_2O$ and 2000 ml double distilled (carbonyl free) water was mixed in the distillation flask. The distillation was then carried out by supplying steam from a steam generator under nitrogen atmosphere and continuous stirring until 2500 ml distillate was collected in the receiver.

The receiver flask was then shaken overnight at 37°C. After separation of the two phases the benzene layer was removed and evaporated in vacuum. The residue was dried over P_2O_5 in vacuum to constant weight. By using the benzene-aqueous system in the receiver and introducing the shaking step both water soluble ($C \leq 4$) and water insoluble ($C > 4$) carbonyl compounds were trapped as 2,4-dinitrophenylhydrazones (DNPH). The dried residue thus consisted of the DNPH of the volatile carbonyls plus the dinitrophenylhydrazine excess.

The efficiency of the dinitrophenylhydrazone formation was investigated for some typical carbonyls by addition of known quantities of model substances to 250 ml 2 M HCl saturated with 2,4-dinitrophenylhydrazine plus 50 ml benzene. This system was shaken and treated as described above. The composition of the residue was then evaluated by gas chromatography as described below. The results of these experiments are summarized in Table 1.

Table 1.   2,4-dinitrophenylhydrazone formation from some volatile carbonyl compounds.

Carbonyl compound	Added quantity (mg)	Found quantity (mg)	Yield (%)
CH_3CHO	0.78	0.65	85
C_2H_5CHO	0.78	0.69	89
$n\text{-}C_3H_7CHO$	0.80	0.80	100
$n\text{-}C_5H_{11}CHO$	0.85	0.73	86
$n\text{-}C_7H_{15}CHO$	0.83	0.38	46
$(CH_3)_2CO$	0.79	0.76	96
$C_3H_7CH=CHCHO$ (trans)	0.83	0.86	103

The yields given above are high and fairly constant for carbonyls up to 6 carbons and decrease with increasing molecular size. Correction factors compensating for non-quantitative DNPH formation can be introduced based on the results in Table 1.

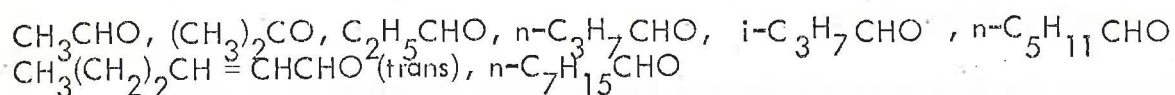Quantitative determination of the carbonyl compounds

The carbonyl composition of the isolated residue was evaluated by gas chromatography of the DNPH mixture. The carbonyls were regenerated from their DNPH prior to the GC analysis in a reactor described by Halvarson (2). The regeneration is based on the exchange reaction between the DNPH and α-ketoglutaric acid which is highly reproducible when a sufficiently large excess of the reagent is used. The carbonyls were chromatographed under the following conditions.

Instrument: Varian gas chromatograph 1400 equipped with a FID
Recorder: Varian Aerograph, model 20
The detector responses were calculated by a Varian 475 electronic integrator connected to a printer.
Column: Porapak S, 1.20 m x 1 mm preconditioned at 220°C
                               for at least 24 h.

Column temperature: 100-180°C, 10°/min., injector temp. 210°C, detector temp. 210°C.

Nitrogen flow: 25 ml/min.

The daily calibration of the chromatographic system was made by regeneration of a DNPH mixture of the following parent compounds:

CH_3CHO, $(CH_3)_2CO$, C_2H_5CHO, $n-C_3H_7CHO$, $i-C_3H_7CHO$, $n-C_5H_{11}CHO$
$CH_3(CH_2)_2CH = CHCHO$ (trans), $n-C_7H_{15}CHO$

The DNPH of these model compounds were synthesised from commercial products of highest purity. About 5.0 mg of each DNPH were brought together and mixed thoroughly. This DNPH mixture was then used as the calibration standard throughout this investigation. About 2.5 mg of the DNPH mixture was used at each calibration.

The quantitative determination of the carbonyls in the samples was made by addition of the DNPH of $i-C_3H_7CHO$ as the internal standard. After a first run of the sample a known quantity of the internal standard was added to a known quantity of the DNPH mixture. This mixture was then run again after which the components were quantitatively evaluated relative to the internal standard.

All of the components in the samples were not included in the DNPH mixture used as the calibration standard. These were treated as giving the same relative detector responses as related compounds. The amounts of the methyl ketones were calculated as acetone, the 2-enals as 2-hexen-1-al and the n-alkanals not included in the standard as the nearest lower homolog. These calculations were carried out on a Olivetti Programma 101 electronic desk-top computer.

HCHO cannot be detected by the FID and was therefore determined in the following way. The DNPH mixture from the sample (10 mg) was mixed with 30 mg α-ketoglutaric acid and put in a reactor similar to that used for the GC analysis related above. The carbonyls were then regenerated by heating the mixture at 250°C for 5 sec. During this time and for another 15 sec. a stream of nitrogen was swept through the reactor down into 1.0 ml carbonyl-free water. 1.5 ml of the specific formaldehyde reagent chromotropic acid (0.6 g $C_{10}H_6O_8S_2Na_2$ dissolved in 20 ml H_2O and diluted to 200 ml with conc. H_2SO_4) was then immediately added to the water solution which was then heated in a boiling water bath for 30 min. The absorption at 570 nm was then determined and the HCHO content evaluated by utilization of a calibration graph (Figure 1).
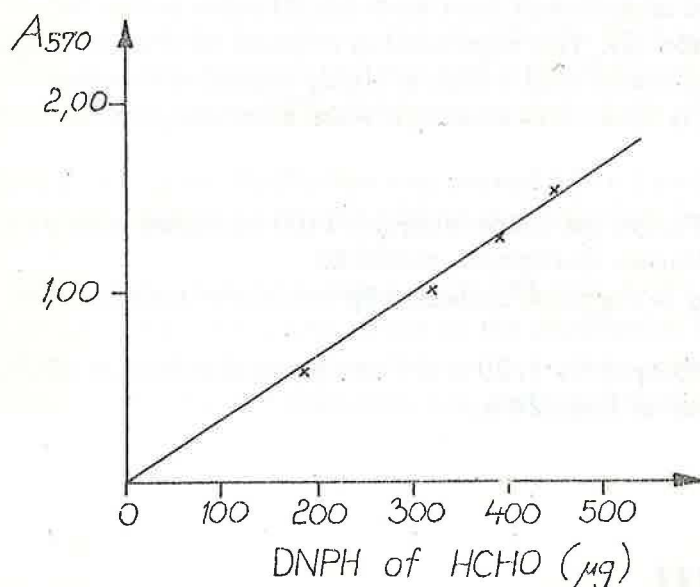

Figure 1. Calibration graph for determination of HCHO from its 2,4-dinitrophenylhydrazone.

Identification of the carbonyls

The carbonyls were identified by combined gas chromatography - mass spectrometry of the regenerated samples. The obtained mass spectra were compared to spectra given in the literature (3,4) or to own reference spectra.

The instrument used for this was a LKB 9000 for combined GC-MS analysis. The temperature of the ion source was $290^\circ C$ and the effluents from the GC column were ionized at 70 eV.

Purification of the solvents

Water. Double distilled water was used throughout these experiments.
Benzene. Thiophene free benzene was made carbonyl free according to a method by A.M. Parsons (5).

RESULTS AND DISCUSSION

The content of volatile, neutral carbonyl compounds up to 8 carbons was evaluated from cold smoked and dried fermented sausage respectively. Samples were taken after the production of the emulsion and at the end of the ripening. For ripening conditions: see materials and methods. The results of the analysis are given in Table 2.

Table 2. The compositions of volatile carbonyls in dried and smoked fermented sausage after ripening for 0 and 21 days.

Compound	Smoked sausage carbonyl concentration (mg/kg) at		Dried sausage carbonyl concentration (mg/kg) at	
	0 days	21 days	0 days	21 days
HCHO	trace	trace	trace	trace
CH_3CHO	1.7	2.2	3.6	2.3
C_2H_5CHO	1.3	1.6	1.8	2.4
$(CH_3)_2CHCHO$	0.1	0.2	0.1	0.4
$n-C_4H_9CHO$	0.1	0.2	0.2	0.3
$(CH_3)_2CH\,CH_2CHO$	0.4	0.4	1.1	1.0
$C_2H_5CH(CH_3)CHO$	-	0.2	0.6	0.5
$n-C_5H_{11}CHO$	0.1	0.3	0.2	0.5
$n-C_6H_{13}CHO$	-	0.1	0.1	0.1
$n-C_7H_{15}CHO$	-	-	-	0.1
$(CH_3)_2CO$	2.4	1.3	1.5	0.6
$CH_3COC_2H_5$	trace	0.2	-	0.1
$CH_3COC_3H_7$	0.02	0.03	0.1	-
$CH_3COC_5H_{11}$	0.02	-	-	-

$(CH_3CO)_2$	+	+	+	–
$(C_2H_5)_2CO$	–	0.2	–	–
C_4H_7CHO (2-enal, branched)	0.5	0.5	0.8	1.9
$n\text{-}C_5H_9CHO$ (2-enal)	0.04	–	–	0.6
C_5H_9CHO (2-enal, branched)	–	–	–	0.05
$n\text{-}C_6H_{11}CHO$ (2-enal)	trace	–	trace	0.3
C_5H_7CHO (2,4-dienal)	–	+	+	–
C_6H_9CHO (2,4-dienal)	trace	–	+	–

– sign indicates that the substance could not be traced.
+ sign indicates that the substance was detected but the quantity could not be evaluated.
Trace indicates that the concentration is less than about 0.01 mg/kg.

The figures in Table 2 are not corrected for non-quantitative DNPH formation (see materials and methods).

As is apparent from Table 2 10 alkanals, 5 saturated ketones 4 2-alkenals and 2 2,4-alkadienals were totally indicated in the volatile carbonyl fractions. Figure 2 and figure 3 illustrate the actual carbonyl compositions of the smoked and dried sausage respectively after 21 days ripening. At 0 days the carbonyl composition of the sausage to be dried is for natural reasons about the same as that to be smoked. The levels of the carbonyls are slightly higher in the "dried" sausage which merely reflects the slightly different composition of the sausage, especially the lower water content compared to the "smoked" sausage. Ethanal, propanal, propanone and in the "dried" sausage also i-pentanal are the quantitatively dominating carbonyls in concentrations of 1.1–3.6 mg/kg. The emulsions also contain comparatively high levels of a branched 2-pentenal, 0.5 and 0.8 mg/kg in the "smoked" and "dried" sausage respectively. The concentrations of carbonyls with more than 5 carbons are relatively low.

After 21 days the levels of the lower carbonyls are remarkably similar to those at 0 days. However, the concentrations of the higher molecular compounds have risen, particularly in the dried sausage, where the most pronounced increase is attributed to n-hexanal, n-oktanal, a branched 2-pentenal, n-hex-2-enal and n-hept-2-enal.

These substances can have different origins. Thus, most of the n-alkanals have been obtained from fat. Hornstein and Crowe (6,7) found e.g. ethanal, propanal, n-hexanal, n-oktanal, 2,4-heptadienal and propanone from beef fat and pig fat. The increase of the higher carbonyls in the dried sausage reflects probably the oxidation of unsaturated fatty acids such as palmitoleic acid, linoleic acid and arachidonic acid. The well-known anti-oxidative effect of the smoking process or (and) the lower storing temperature can account for the lower concentrations of these carbonyls in the smoked sausage after 21 days storing.

The lower aldehyds can also be oxidation products from amino acids. Thus the following amino acids can by Strecker degradation be precursors to this group.
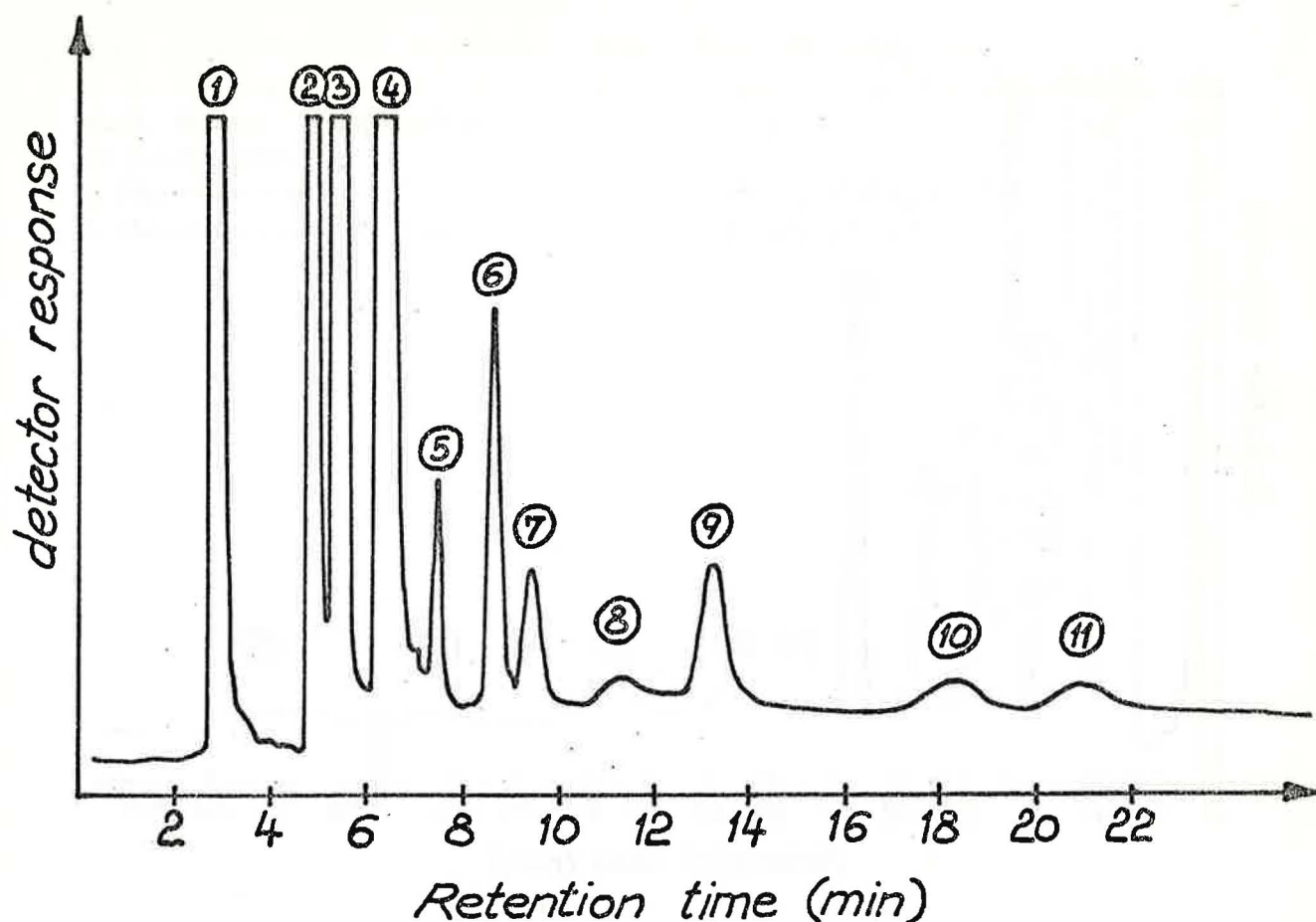
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Figure 2. Gas chromatogram showing the carbonyl composition in smoked fermented sausage after storing for 21 days after addition of the internal standard. For conditions: see text.

1/ CH_3CHO, 2/ C_2H_5CHO, 3/ $(CH_3)_2CO$, 4/ $(CH_3)_2CHCHO$, 5/ $CH_3COC_2H_5$ + $(CH_3CO)_2$, 6/ $(CH_3)_2CH\,CH_2CHO$ + $C_2H_5CH(CH_3)\,CHO$, 7/ $n-C_4H_9CHO$ + $CH_3CO\,C_3H_7$ + $(C_2H_5)_2CO$, 8/ C_4H_7CHO (2-enal, branched), 9/ $n-C_5H_{11}CHO$, 10/ $n-C_5H_7CHO$ (2,4-dienal), 11/ $n-C_6H_{13}CHO$.

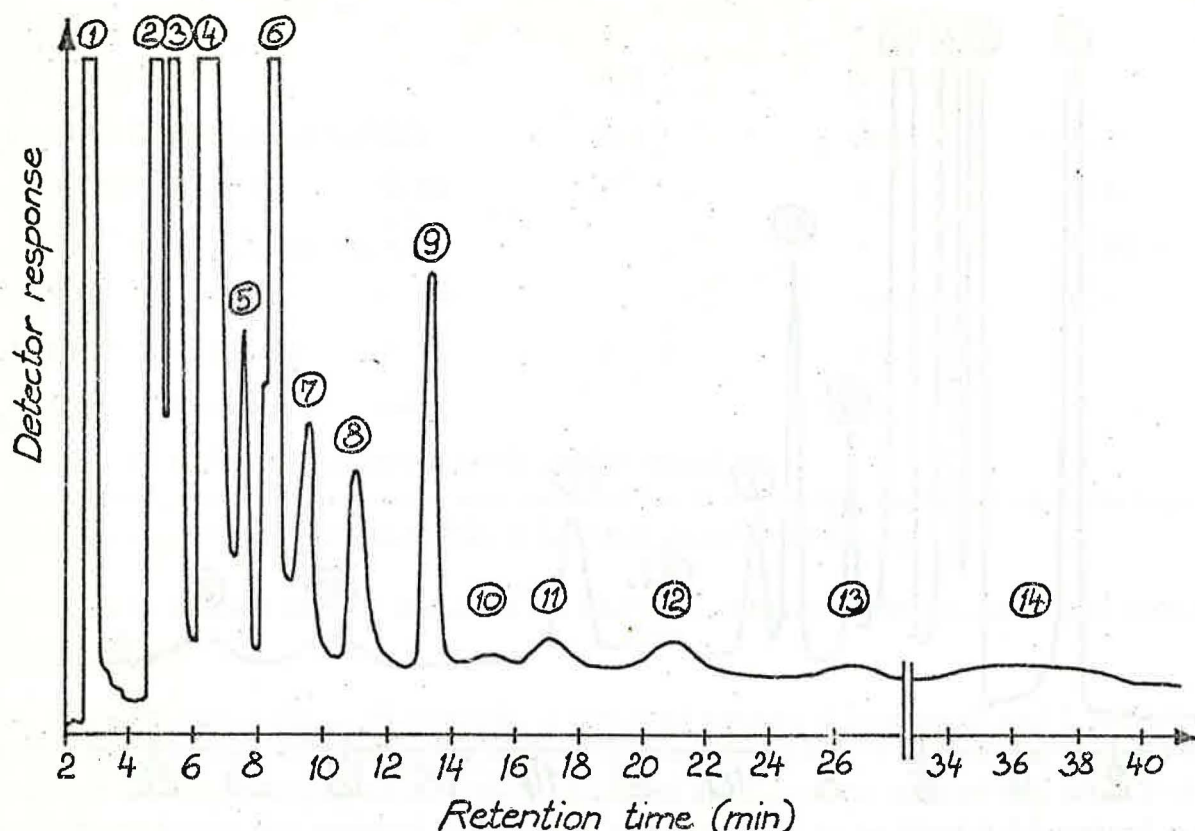| Precursor	Carbonyl compound
$CH_2(NH_2)COOH$	$HCHO$
$CH_3CH(NH_2)COOH$	CH_3CHO
$C_2H_5CH(NH_2)COOH$	C_2H_5CHO
$(CH_3)_2CHCH(NH_2)COOH$	$(CH_3)_2CHCHO$
$(CH_3)_2CHCH_2CH(NH_2)COOH$	$(CH_3)_2CHCH_2CHO$
$C_2H_5CH(CH_3)CH(NH_2)COOH$	$C_2H_5CH(CH_3)CHO$

Figure 3. Gas chromatogram showing the carbonyl composition in dried fermented sausage after storing for 21 days after addition of the internal standard. For conditions: see text.

1/ CH_3CHO, 2/ C_2H_5CHO, 3/ $(CH_3)_2CO$, 4/ $(CH_3)_2CHCHO$, 5/ $CH_3COC_2H_5$ + artifact, 6/ $(CH_3)_2CHCH_2CHO + C_2H_5CH(CH_3)CHO$, 7/ $n-C_4H_9CHO$, 8/ C_4H_7CHO (2-enal, branched), 9/ $n-C_5H_{11}CHO$, 10/ C_5H_9CHO (2-enal, branched), 11/ $n-C_5H_9CHO$ (2-enal), 12/ $n-C_6H_{13}CHO$, 13/ $n-C_6H_{11}CHO$ (2-enal), 14/ $n-C_7H_{15}CHO$

The results of this investigation can be summed up by stating that under the conditions used the composition of the volatile carbonyl compounds up to C_8 are almost the same in the smoked fermented sausage after smoking and storing for 3 weeks while where the dried sausage is concerned there is mainly an increase in the concentrations of carbonyls with higher molecular weight than C_5. The effect these substances may have on the aroma are at present not clear. We are going to do more work in this field which, among other things, will include panel tests for aroma description.

Acknowledgement

References

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