Identification of Some Previously Unknown Aldehydes in Cooked Chicken

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ABSTRACT

Aldehydes present in a flavor concentrate, obtained from cooked chicken were separated and isolated by means of gas liquid chromatography. Subsequently, they were converted into their 2,4-dinitrophenylhydrazones and identified by thin layer chromatography on Kieselguhr G-Carbowax 750 and Silica Gel G-$AgNO_3$ and by analysis after partial hydrogenation. Finally, they were compared with model substances. Besides the aldehydes which had been found earlier in cooked chicken the following new aldehydes were identified: 3-c-nonenal; 4-c-decenal; 2-t,4-c,7-c-decatrienal; 2-t,5-c-undecadienal; 2-t-dodecenal; 2-t,4-c-dodecadienal; 2-t,6-c- and 2-t,6-t-dodecadienal; 2-t-tridecenal; 2-t,4-c-tridecadienal; 2-t,4-c,7-c-tridecatrienal; and 2-t,4-c-tetradecadienal. Three of them, 4-c-decenal; 2-t,6-c-dodecadienal; and 2-t,4-c,7-c-tridecatrienal are typical breakdown products of arachidonic acid, and to a major extent also 2-t,5-c-undecadienal.

INTRODUCTION

During the last two decades many articles on chicken flavor have been published, and the compounds described have been summarized by Wilson and Katz (1). As to aldehydes, octadecanal was the longest chain found in the saturated group, 2-t-undecenal in the alkenals and 2-t,4-t-decadienal in the alkadienals. Many of the aldehydes present also have been found in beef fats (2,3), milk fat (4), and peanut oil (5).

About the origin of the chicken flavor and its possible precursors, e.g. the following statements can be made. Chicken flavor is produced during cooking, whereas leaf fat contributes very little to the chicken flavor (6,7). Chicken flavor precursors are extracted readily from raw meat by cold water (8). Slightly autoxidized arachidonic acid has a flavor resembling cooked chicken meat (9). The percentage of arachidonic acid in the phospholipid fraction is highest in the low fat tissues (white and dark meat) and lowest in the high fat tissues (depot fat) (10). Removal of the carbonyls from a chicken flavor concentrate results in a loss of chickeny flavor and intensification of the meaty or beef-like odor (11).

These statements suggest that the breakdown products of arachidonic acid play an important role in the development of the cooked chicken flavor. If this is so, only aldehydes with a long chain length and polyunsaturated should be considered, as many of the unsaturated aldehydes already found also can be derived from oleic and linoleic acid which are the main unsaturated fatty acids of the chicken lipid fraction.

EXPERIMENTAL PROCEDURES

Preparation of Chicken Flavor Concentrate

Starting from five freshly slaughtered chickens, the flavor concentrate was obtained as shown in Figure 1. The volatiles, obtained after degassing and collected in cooled U-tubes (12), were taken up in purified pentane and combined.

Isolation of Carbonyls

A chicken flavor concentrate is a very complex mixture which was confirmed by Nonaka, et al., (13) who counted ca. 227 peaks on the gas chromatogram. Moreover, he noted that many peaks could not be identified due to incomplete separation of the peaks and a continuous bleeding of high boiling material from the chromatographic column, by which the mass spectra could not be interpreted. Therefore, the flavor concentrate was separated into 27 fractions by gas liquid chromatography (GLC), as indicated in Figure 2. On the column 40 μliter concentrate was injected, and each fraction was trapped in a small U-tube which was cooled in a mixture of solid carbon dioxide and ethanol. This operation was repeated five times and each fraction always was trapped in the same U-tube. By rinsing with carbonyl free light petroleum, the contents of each tube were brought onto a small 2,4-dinotrophenylhydrazone (DNPH) reaction column (14). The DNPHs thus formed were eluted with light petroleum and further analyzed.

Identification of Carbonyls

As the total amount of the DNPH of each fraction was very small and as we wanted to get as much information as possible, the following sequence of analyses was used.

Partition thin layer chromatography (TLC) on Kieselguhr G-Carbowax 750: The DNPH of each tube was brought as a band on a Kieselguhr G chromatoplate impregnated with 33% Carbowax 750 analogous to the method used by Badings (15). Eluant was light petroleum. Using this system, DNPHs of saturated aldehydes with a chain length from C_1 to C_{16} can be separated. Therefore, these DNPHs were used as model substances to mark the place of the migration of the unknown DNPHs. There also is a separation into classes as the migration rate of the

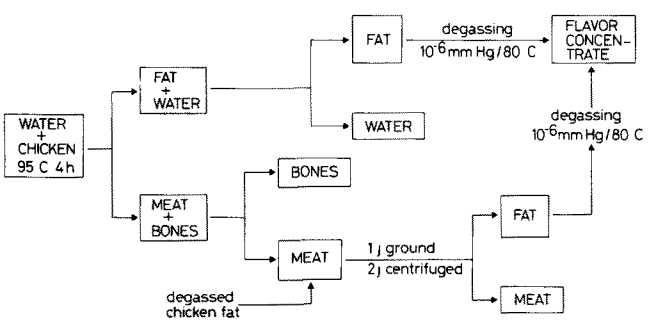

FIG. 1. Preparation of a chicken flavor concentrate.

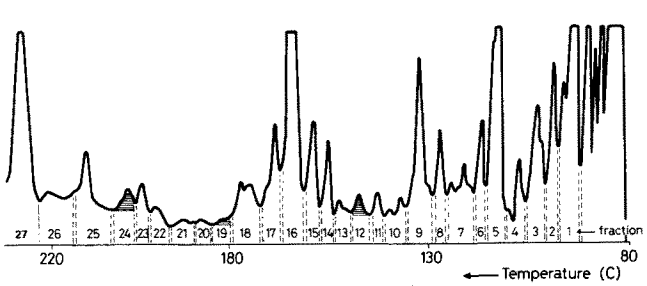

FIG. 2. Gas chromatogram of a chicken flavor concentrate. Column length: 2 m; internal diameter: 4 mm; stationary phase: silicon oil 20%; temperature: 1 C/min; nitrogen flow: 15 ml/min; support: Chromosorb W 60-100 mesh.

TABLE I

Identification of Aldehydes Present in Chicken Flavor Fractions Isolated by Gas Liquid Chromatography (GLC)

GLC fraction no.	Carbowax 750 Band no.	R_f	γ_{max} nm	$AgNO_3$ (R_f acetone = 1)	Carbowax R_f after partial hydrogenation[a]	Conclusion aldehyde	Identical with aldehyde
1	1	C_6	358	1.6		C_6	C6
4	1	C_7	358	1.6		C_7	C_7
7	1	$C_{5.5}$	375	1.5	C_7	2 C_7	2t C_7
8	1	$C_{3.5}$	387	1.5	$C_{5.5}$ C_7	2,4 C_7	2t,4c C_7
9	1	$C_{6.5}$	374	1.5	C_8	2 C_8	2t C_8
10	1	$C_{7.5}$	358	0.95	C_9	p[b] C_9[c]	3c C_9
	2	C_9	358	1.6		C_9	C_9
11	1	$C_{7.5}$	374	1.5	C_9	2 C_9	2t C_9
12	1	$C_{8.5}$	358	0.7	C_{10}	p[b] C_{10}[c]	4c C_{10}
	2	C_{10}	358	1.6		C_{10}	C_{10}
13	1	$C_{5.5}$	387	1.5	$C_{7.5}$ C_9	2,4 C_9	2t,4c C_9
14	1	$C_{8.5}$	374	1.5	C_{10}	2 C_{10}	2t C_{10}
16	1	$C_{6.5}$	391	1.5	$C_{8.5}$ C_{10}	2,4 C_{10}	2t,4c C_{10}
17	1	$C_{4.5}$	390	1.0	$C_{6.5}$ $C_{8.5}$ C_{10}	2,4,p[b] C_{10}[c]	2t,4c,7c C_{10}
	2	$C_{6.5}$	390	1.5	$C_{8.5}$ C_{10}	2,4 C_{10}	2t,4c C_{10}
	3	$C_{7.5}$	374	1.0	$C_{9.5}$ C_{11}	2,p[b] C_{11}[c]	2t,5c C_{11}
	4	$C_{9.5}$	375	1.5	C_{11}	2 C_{11}	2t C_{11}
19	1	C_8	375	0.8	C_{10} C_{12}	2,p[b] C_{12}[c]	2t,6c C_{12}
	2	$C_{8.5}$	374	1.2	C_{10} C_{12}	2,p[b] C_{12}[c]	2t,6t C_{12}
	3	C_{10}	374	1.5	C_{12}	2 C_{12}[c]	2t C_{12}
20	1	C_{13}	358	1.6		C_{13}	C_{13}
21	1	$C_{8.5}$	390	1.5	C_{10} C_{12}	2,4 C_{12}[c]	2t,4c C_{12}
22	1	$C_{11.5}$	374	1.5	C_{13}	2 C_{13}[c]	2t C_{13}
23	1	C_{14}	358	1.6		C_{14}	C_{14}
24	1	C_7	387	1.0	C_9 $C_{11.5}$ C_{13}	2,4,p[b] C_{13}[c]	2t,4c,7c C_{13}
	2	C_9	388	1.5	$C_{11.5}$ C_{13}	2,4 C_{13}[c]	2t,4c C_{13}
25	1	C_{15}	358	1.5		C_{15}	C_{15}
26	1	$C_{10.5}$	388	1.5	$C_{12.5}$ C_{14}	2,4 C_{14}[c]	2t,4c C_{14}
	2	C_{16}	359	1.5		C_{16}	C_{16}

[a]The R_f of the original 2,4-dinitrophenylhydrazone has been omitted.

[b]An isolated double bond is present but the position is unknown.

[c]Isolated for the first time.

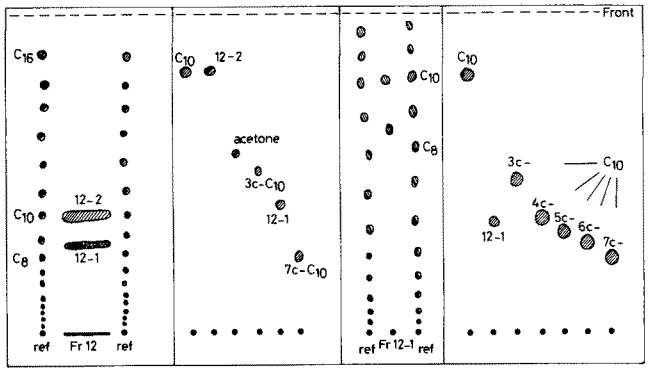

FIG. 3. Thin layer chromatograms of the 2,4-dinitrophenylhydrazones (DNPHs) of fraction 12. I, adsorbent: 33.3% Carbowax 750 on Kieselguhr G; mobile phase: light petroleum. Ref: DNPHs of C_1-C_{16} alkanals; II, adsorbent: 25% $AgNO_3$ on Silica Gel G; mobile phase: benzene; III, after partial hydrogenation; conditions as in I. Ref: DNPHs of C_1-C_{12} alkanals; and IV, conditions as in II.

DNPH decreases with increasing number of unsaturated double bonds in the aldehyde chain (16).

UV measurement: Each separated band was scraped off and eluted with chloroform. The wavelength of maximum absorption was measured to determine whether a saturated or a conjugated unsaturated aldehyde with one or two double bonds was present.

Argentation TLC on Silica Gel G-$AgNO_3$: A small amount of each band was further analyzed on a Silica Gel G plate impregnated with 25% $AgNO_3$ and using benzene as eluant (17). With this system, DNPHs containing one or more isolated double bonds can be separated from the saturated and conjugated unsaturated ones. The relative migration of the DNPHs was measured with respect to that of acetone-DNPH, as values of ca. one or less are an indication that the DNPH possesses a *cis*-isolated double bond, whereas values between 1 and 1.5 point to presence of a *trans*-isolated double bond.

Partial hydrogenation of the DNPH: If there are indications of an unsaturated DNPH, a small amount was hydrogenated partially with palladium on calcium carbonate. This technique (18), offers two data, viz the chain length of the straight chain aldehyde and the number of double bonds. The chain length follows from the R_f of that spot, which, after hydrogenation, is the most mobile and which corresponds with that of one of the reference DNPHs of the saturated aldehydes on a Kieselguhr G-Carbowax 750 plate.

By partial hydrogenation, a number of DNPHs is obtained, the unsaturation of which in the aliphatic chain decreases. The number of spots minus the original spot corresponds with the number of double bonds present in the original aliphatic chain of the DNPH.

RESULTS AND DISCUSSION

The amount of DNPHs was too small to carry out a double bond location analysis by oxidative degradation (19). Therefore, we could only locate the position of the isolated double bond by comparing the unknown DNPH with a model substance, but, on account of the various data already obtained, the choice of the model substance was much easier.

In the last column of Table I, the aldehydes are given which are equal to the isolated ones with respect to their retention time on GLC and their DNPH behavior on TLC. In fractions 2, 3, 5, 6, 15, 18, 27 no DNPHs could be detected. The aldehydes (Table I) discussed below were isolated from cooked chicken for the first time.

3-c-Nonenal (fraction [Fr] 10, band [b] 1): As linoleic acid is present in the neutral lipids and phospholipids and arachidonic acid in the phospholipids, the presence of 3-c-nonenal is understandable.

4-c-Decenal (Fr 12, b1): In the first instance, it was not obvious to predict the position of the isolated *cis*-double bond as, according to the generally accepted route proposed by Farmer, et al. (20), this aldehyde could not be derived from the polyunsaturated fatty acids normally occurring. In Figure 3, the identification procedure is shown. However, later on, Badings (21) detected 4-c-decenal as one of the autoxidation products of arachidonic acid. So it is most likely that 4-c-decenal, as found in cooked chicken, has been derived from arachidonic acid.

2-t,4-c,7-c-Decatrienal (Fr 17, b1): This aldehyde can be derived from linolenic acid which occurs in small amounts in the depot fat. It also has been identified in other ω3,6,9 unsaturated fatty acids by Meyboom and Stroink (22).

2-t,5-c-Undecadienal (Fr 17, b3): This aldehyde can be a breakdown product of linoleic acid, as well as of arachidonic acid. As an α-methylene group is known to be more reactive when it is flanked on either side with a double bond (arachidonic acid) than on one side (linoleic acid), it is more obvious that this aldehyde comes from arachidonic acid.

2-t-6-c-Dodecadienal (Fr 19, b1): One of the autoxidation products of arachidonic acid is 3-c,6-c-dodecadienal. Aldehydes with a 3-*cis*-double bond isomerize easily to the 2-*trans*-configuration, as shown by Grosch and Schwartz (23) who isolated 2-t,6-c-nonadienal from cucumber homogenates incubated with ($U^{14}C$)-linolenic and ($U^{14}C$)-linoleic acid. They did not find 3-c,6-c-nonadienal which would be formed from linolenic acid but 2-t,6-c-nonadienal instead.

2-t,6-t-Dodecadienal (Fr 19, b2): We have no explanation for its formation.

2-t,4-c,7-c-Tridecatrienal (Fr 24, b1): This aldehyde is most likely a breakdown product of arachidonic acid.

The other aldehydes isolated for the first time from cooked chicken are 2-t-dodecenal (Fr 19, b3); 2-t,4-c-dodecadienal (Fr 21, b1), already tentatively identified (24); 2-t-tridecenal (Fr 22, b1); 2-t,4-c-tridecadienal (Fr 24, b2); and 2-t,4-c-tetradecadienal (Fr 26, b1). All these aldehydes cannot be predicted on theoretical grounds to be derived from the common polyunsaturated fatty acids present in chicken meat and fat.

Although, according to the GLC separation on silicone oil, we might expect two other breakdown products typical of arachidonic acid viz 2-t,5-c,8-c-tetradecatrienal in fraction 24 or 25 and 2-t,6-c,9-c-pentadecatrienal in fraction 26 or 27, we could not detect them in these fractions.

Some examples about the effect of these aldehydes upon chicken flavor are given in two patents (9,25).

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