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AN OVERVIEW OF THE PROGRESS IN THE CHARACTERIZATION OF VOLATILE CONSTITUENTS OF MEAT FLAVOUR USING THE NITROGEN-PURGE-AND-TRAP METHOD*

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INTRODUCTION

Present day meat-curing practice involves the addition of sodium nitrite and salt along with other additives such as sugar, antioxidants, phosphates and, where appropriate, seasonings to impart characteristic properties to the end product. Nitrite imparts the typical pink colour to cured meat and provides oxidative stability to meat by preventing lipid oxidation. Its key function is its antimicrobial effect which prevents the outgrowth of *Clostridium botulinum* and the formation of a deadly toxin.

Raw meat has little odour and only a blood-like taste, and cooking develops its flavour (Bender and Ballance, 1961). Although nitrite is closely associated with cured-meat flavour, the chemistry behind the formation and composition of this unique flavour is not clearly understood (Gray and Pearson, 1984). In our attempts to unravel the complex nature of cured-meat flavour, we have adopted a stepwise approach. As a first step, we isolated the volatile components from cured and uncured pork using the conventional steam-distillation and continuous steam-distillation-extraction (SDE) methods (Ramarathnam *et al.*, 1991a). Using the SDE technique, we have also isolated, identified and quantified the individual components of the aroma concentrates prepared from uncured and nitrite-cured beef and chicken, and provided a summary of those carbonyl compounds that may be responsible for the species differences (Ramarathnam *et al.*, 1991b).

In continuation of our attempts in identifying the key-components that are responsible for the "cured-meat" aroma or the basic "meaty aroma" of cooked meat, we isolated the volatiles from cured and uncured pork, beef and chicken, using the nitrogen purge-and-trap (NPT) method. We reported the identification of 32 new compounds of which 8 were heterocyclic in nature (Ramarathnam *et al.*, 1992). though we had successfully demonstrated the extraction and identification of heterocyclic compounds in our previous investigation, we observed that the flavour spectrum still consisted of carbonyls and hydrocarbons as the major constituents. In order that the heterocyclic constituents be extracted quantitatively, we suppressed the formation of carbonlyls in the present investigation by the use of defatted meat samples. In addition, we also wish to investigate the effect of removal of carbonyl components, by the use of carbonyl-specific reagent, on the quality of meat-flavour compositions.

MATERIAL AND METHODS

Meat

Fresh pork loin, ground beef (lean meat from shoulder), and chicken breasts (with skin-on) were purchased from a local market and used immediately. The skin in chicken and excess fat in chicken and pork were removed. The meat was deboned manually, cut into small pieces, ground twice using an Oster meat grinder (0.476-cm grind plate, Model 990-68), and then freeze-dried using a Labconco freeze-dryer at -50°C for about 48 hours. The dried meat was defatted in two steps. The nonpolar lipids were removed by the Soxhlet extraction method (AOAC, 1984) using hexane as the solvent. After the removal of hexane, the polar lipids were extracted by the use of a mixture of $CHCl_3$-MeOH (2:1 v/v) (AOAC, 1984).

Reagents

Anhydrous sodium sulphate, sodium chloride, sodium nitrite, methanol and chloroform (all of analytical grade), and sodium ascorbate (USP grade) were purchased from BDH Chemicals. Sodium tripolyphosphate (food grade) was obtained from ERCO Industries Ltd., while *n*-pentane (spectral grade) was purchased from Caledon Laboratories Ltd. 2,4-Dinitrophenylhydrazine was obtained from J. T. Baker Chemical Co., and the hydrocarbons used for the determination of Kovats indices were obtained from Nu Check Prep.

Cooking

The dried and defatted meat was rehydrated to its initial water content with distilled water. The rehydrated meat (250-300g) was placed in a 2-L beaker. Cooked uncured and nitrite-cured pork, beef and chicken were prepared by our previously published technique (Ramarathnam *et al.*, 1991a,b).

Nitrogen Purge-and-Trap (NPT) Technique

The assembly used in the NPT technique is essentially the same as that described earlier (Ramarathnam *et al.*, 1992). The cooked meat (250-400g) was homogenized with 500mL distilled water using a Polytron homogenizer (Brimkmann Instruments, Model PT 10/35) until a free flowing meat slurry was obtained. The slurry was placed in the extraction jar, where it was constantly maintained at 65±5°C and stirred with the help of a magnetic hot plate/stirrer. A slow stream of oxygen-free nitrogen gas was passed through the meat slurry so as to purge the volatiles from the headspace. The effluent stream was passed through a solution of 2,4-dinitrophenylhydrazine (30mL; 10g/L in 2N HCl), held at room temperature, to remove the carbonyl components. The effluent from this treatment was subsequently absorbed into *n*-pentane maintained at -60°C by dry ice. This cold trap was further connected to an aspirator. The volatiles were collected over a 10-hour purging period. At the end of the experiment the pentane extract was dried over anhydrous sodium sulphate, and concentrated using a slow stream of nitrogen gas, at room temperature, to a final volume of around 250µL.

Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

A Hewlett-Packard Model HP 5880A gas chromatograph equipped with a capillary column [0.13mm(i.d.)x 30m] and coupled to a Hewlett-Packard Model HP 5987A mass spectrometer was used. Analytical conditions were presented by Ramarathnam *et al.* (1991b). Kovats retention indices were calculated against C_7-C_{25} *n*-paraffins as references (Jennings and Shibamoto, 1980). The identification of the individual constituents was based on the Kovats retention indices and the MS data.

Quantitation of the Individual Components

Quantitative analysis of the individual constituents identified in uncured- and cured-pork, beef and chicken aroma concentrates was carried out by the method described by Ramarathnam *et al.* (1991b).

RESULTS AND DISCUSSION

Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

The components identified in the aroma concentrates of defatted uncured and cured pork, beef and chicken prepared by the NPT method are listed in Table 1. A total of 78 compounds were detected in the three meat species. Of these 38 were hydrocarbons, 10 carbonyls, 6 alcohols, 6 phenols, 5 esters, 8 heterocyclic compounds and 5 remained unidentified. Aroma concentrates isolated from pork had 21 hydrocarbons, 6 carbonyls, 4 alcohols, 5 phenols, 3 esters, and 8 heterocylic compounds. Of the total number of compounds identified in uncured and cured beef, 32 were hydrocarbons, 3 carbonyls, 4 alcohols, 5 phenols, 4 esters and 6 were heterocyclic compounds. The corresponding figures for uncured and cured chicken were 21 hydrocarbons, 3 carbonyls, 4 alcohols, 6 phenols, 5 esters and 5 heterocyclic compounds. In comparison with these results, irrespective of the species, the number of carbonyl compounds identified in the aroma concentrates prepared by the continuous steam-distillation-extraction (SDE) method was nearly three times higher (Ramarathnam *et al.*, 1991a; 1991b). This is clearly indicative of the mild nature of the NPT method that limits the breakdown of the volatile components and protects them from undergoing further oxidation by exclusion of air. Further, the use of defatted meat and the carbonyl-specific reagent also drastically reduced the formation of carbonyls in the meat-flavour concentrates prepared for GC-MS analysis.

Forty-five compounds not perviously reported in the literature have been identified in the present investigation. Of the various heterocyclic components identified, terahydro-cis-2,4-dimethylfuran (RT, 8.62 min), 3-propyl, 1H-1,2,4-triazole (RT, 11.32 min) and 2,4,6-trimethylpyridine (RT, 19.08 min) were not previously identified in the aroma concentrates of pork. Hydrocarbons such as 1,1-dimethylcyclopentane (RT, 11.74 min) and the newly identified compounds 2-methylundecane (RT, 11.94 min) and 4-methyl-1-decene (RT, 13.00 min) were found to be present only in the pork aroma concentrates. In addition, certain carbonyls such as (E,E)-2,4-nonadienal (RT, 8.41 min), (E)-2-undecanone (RT, 12.93), (E,E)-2,4-decadienal (RT, 13.76 min) and 4-

pentylbenzaldehyde (RT, 15.79 min) were also found only in the aroma concentrates of pork. 4-Ethylbenzaldehyde (RT, 11.46 min), 1,12-dodecanediol (RT, 18.89 min) and the phenolic component 2,6-bis(1,1-dimethylethyl)-4-methylphenonl (RT, 16.49 min) were unique to chicken.

The components uniquely identified in beef aroma concentrates were mainly terpene hydrocarbons such as the newly identified β-pinene (RT, 7.40 min) and camphene (RT, 11.85). Other components that were present only in the volatile mixtures of beef aroma were 1,3,5-trimethylbenzene (RT, 8.50 min), 4-ethyl-1,2-dimethylbenzene (RT, 10.13 min) and 3,6-dimethylundecane (RT, 11.91 min). Nonylcyclopropane (RT, 13.11 min) has been found in the aroma concentrates of all uncured meat samples, while 2-methylcyclopentanol (RT, 12.02 min) was unique to the cured-meat aroma.

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*This paper is dedicated to the late Prof. LEON J. RUBIN who initiated the meat-flavour research program at the University of Toronto, Canada.

Table 1. Oxidative stability and psychrotrophic plate counts.

Beef type/trait	Distrib. Treatment[a]	Days after initial packaging 1	14	16	18	20
Steaks/TBARS[b]	4.4°C/N_2	0.47^{eg}	0.30^{g}	0.27^{g}	1.02^{ef}	0.75^{ef}
	-3.8°C/N_2	0.27^{fg}	0.14^{g}	0.43^{fg}	0.80^{ef}	1.18^{e}
	-12.2°C/N_2	0.47^{fg}	0.17^{fg}	0.25^{fg}	0.44^{df}	0.38^{f}
	4.4°C/0_2	0.54^{ef}	0.99^{e}	1.05^{e}	0.70^{ef}	2.28^{d}
Steaks/PPC[c]	4.4°C/N_2	2.89^{h}	4.65^{egh}	5.65^{eg}	4.83^{dg}	5.67^{eg}
	-3.8°C/N_2	0.93^{i}	2.81^{f}	1.97^{f}	3.21^{fg}	4.24^{e}
	-12.2°C/N_2	0.69^{i}	2.94^{f}	2.23^{f}	2.81^{f}	2.68^{f}
	4.4°C/0_2	0.69^{i}	5.65^{e}	5.30^{e}	6.23^{de}	7.23^{d}
Ground beef/ TBARS[b]	4.4°C/N_2	0.54^{g}	0.62^{g}	1.79^{g}	1.87^{g}	2.39^{g}
	-3.8°C/N_2	0.48^{g}	0.39^{g}	2.21	1.93^{g}	2.49^{g}
	-12.2°C/N_2	0.46^{g}	1.33^{g}	1.88^{g}	2.09^{g}	2.28^{g}
	4.4°C/0_2	0.76^{g}	6.37^{de}	7.21^{d}	3.30^{f}	4.22^{ef}
Ground beef/ PPC[c]	4.4°C/N_2	3.06^{gh}	5.32^{f}	6.57^{e}	6.62^{e}	7.16^{de}
	-3.8°C/N_2	2.78^{eh}	3.28^{hi}	2.76^{h}	4.30^{g}	5.39^{f}
	-12.2°C/N_2	2.19^{e}	3.32^{gi}	3.44^{gh}	3.72^{g}	4.86^{fg}
	4.4°C/0_2	2.45^{e}	7.37^{d}	7.81^{d}	7.30^{d}	7.48^{d}

N2 = 80% N2; 20% CO2
O2 = 80% O2; 20% CO2 for distribution storage

[b] TBARS = thiobarbituric acid reactive substances as mg/kg sample
SEM = 0.20 for steaks; 0.83 for ground beef

[c] PPC = psychotrophic plate counts as log colony forming units/g
SEM = 0.48 for steaks; 0.22 for ground beef

[d,e,f,g,h,i] Least squares means for each trait in same row or column with same superscript are not different ($P<0.05$).