

# Isolation and Identification of Pyrazines Present in Pressure-Cooked Beef

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Flavor concentrates were isolated from beef cooked superatmospherically at 162.7° by simultaneous steam distillation and continuous solvent extraction. Separation and identification were

accomplished by gas chromatography and coupled gc-mass spectrometry. Mass spectral identifications were confirmed by  $I_E$  values. A total of 33 pyrazines were identified.

The odor of pyrazines has been described as characteristically earthy, nutty, and roasted. Their presence in roasted foods is well documented. The earliest reference to pyrazines in food was British patent no. 260,960, issued in 1928, wherein Reichstein and Staudinger reported finding pyrazine, methylpyrazine, 2,5- and 2,6-dimethylpyrazine, and other higher homologs of pyrazine in coffee. Following this there are few references to these compounds in the literature until Dietrich *et al.* (1964) found 2,6-dimethyl and tetramethylpyrazine in a chocolate extract. Soon after, they were found in potato chips by Deck and Chang (1965), in peanuts by Mason *et al.* (1966), and in coffee by Viani *et al.* (1965) and Reymond *et al.* (1966). Through the efforts of many investigators since then, the literature has become replete with references to these compounds in roasted foods. For instance, Bondarovich *et al.* (1967) reported 22 pyrazines in coffee. Walradt *et al.* (1971) reported the identification of 36 pyrazines in roasted peanuts, and Kinlin *et al.* (1972) reported finding 42 pyrazines in roasted filberts. These are only a few of the many reports of pyrazines in roasted foods. There are, however, relatively few reports of their occurrence in meat. Flament and Ohloff (1971) reported the identification of 16 pyrazines in roast beef. Watanabe and Sato (1971) reported ten in fried beef, and Liebich *et al.* (1972) found three in boiled beef.

Pyrazines are known to arise from the condensation of sugars and amino acids. Hodge (1953) reviewed the literature published since 1940 pertaining to browning in nitrogenous model systems and reported that several substituted pyrazines had been detected and isolated from sugar-amine browning reactions in model systems. The necessary precursors, that is, amino acids and sugars, are available in beef and pyrazine formation, therefore, should occur readily upon heating.

The cooking temperature used in this study is one normally encountered during the roasting of beef. The sealed system was employed to attain this elevated temperature in an aqueous system.

## EXPERIMENTAL SECTION

Forty pounds of locally procured lean ground round steak was slurried with 5 gal of distilled water and placed in a 20-gal steam-jacketed doubly stirred stainless steel pressure reaction vessel (Groen Div., Dover Corp., Elk Grove, Ill.). The vessel was sealed, heated to 162.7°, and held at this temperature for 15 min. The maximum head pressure attained was 90 psi. The vessel was cooled by passing water through the jacket, and the contents were filtered through cheesecloth. The filtrate was placed in 5-gal plastic containers and stored at 6° for 12 hr.

The filtrate was simultaneously atmospherically steam distilled and the distillate was continuously extracted with distilled diethyl ether in an apparatus similar to that described by Williams (1969), which we modified to a 22-l. capacity. It was necessary to use approximately 2 ml of a 1:9 dilution in water of Dow Corning's antifoam emulsion FG-10 during the course of the 2-day distilla-

tion-extraction to prevent foaming. The quantity of antifoam used was not large enough to interfere with the analysis.

The extract was dried over anhydrous sodium sulfate and concentrated to approximately 8 ml by careful distillation in a Kuderna-Danish concentrator (Kontes Glass Co., Vineland, N. J.) equipped with a 300 mm × 13 mm i.d. Vigreux reflux column.

The concentrate was analyzed on a Hitachi model RMU-6E mass spectrometer coupled with a Hewlett-Packard model 5750 gas chromatograph using a Watson-Biemann helium separator (Watson and Biemann, 1965). The chromatographic columns used were a 1000 ft × 0.03 in. i.d. stainless steel open-tubular column coated with SF-96 and a 1000 ft × 0.03-in. i.d. stainless steel open-tubular column coated with Carbowax 20M. Further gc-ms analysis was preceded by area trapping using a Varian series 712 preparative gas chromatograph with a flame ionization detector and a 12 ft × 3/8 in. o.d. stainless steel column packed with 20% SE-52 on 45-60 mesh AW-DMCS Chromosorb W. The oven temperature was programmed from 100 to 225° at 2°/min. The injector and detector temperatures were 230° and the helium flow rate was approximately 300 ml/min. Seven traps were collected. Traps 1-5 were analyzed on 1000 ft × 0.03 in. stainless steel open-tubular SF-96 and CBW columns and traps 6 and 7 were analyzed on 500 ft × 0.03 in. open-tubular SF-96 and Carbowax 20M columns in the gc-ms system. Identifications were based on the comparison of known and unknown mass spectra and were confirmed wherever possible by determining the retention indices relative to ethyl esters, or  $I_E$  values, according to the method of van den Dool and Kratz (1963).

Additional confirmation was obtained by analyzing the total extract and each trap in a Tracor MT 200 gas chromatograph modified to detect nitrogen containing compounds (Craven, 1970). The columns and conditions employed were identical to those used in the mass spectral analysis.

## RESULTS AND DISCUSSION

Table I shows the 33 pyrazines that were identified. The occurrence of these compounds in three other roasted products—peanuts, filberts or hazelnuts, and coffee—is also given in this table. All of the compounds which we found in beef except for 1-pyrazinyl-2-propanone have been previously identified in at least one of these products. The latter compound, however, is mentioned by Firmenich in a French patent (Winter *et al.*, 1968).

The  $I_E$  values given were obtained on a Carbowax capillary column unless otherwise indicated. We wish to stress the usefulness of reliable  $I_E$  values in assigning specific structures to compounds for which a number of positional isomers are possible. For instance the  $I_E$  for 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine are significantly different although their mass spectra are virtually identical.

## SUMMARY

A total of 33 pyrazines have been isolated and identified, 19 of which are being reported for the first time as

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Table I. Pyrazines Identified in Cooked Beef

Compound* <sup>a</sup>	First report in beef	<i>I<sub>E</sub></i> known	<i>I<sub>E</sub></i> unknown	Occurrence in roasted foods		
				Peanut	Filbert	Coffee
Pyrazine	X	5.87	5.83	g	b	c
Methylpyrazine		6.42	6.37	g	b	c
2,5-Dimethylpyrazine		6.92	6.94	g	b	c
2,6-Dimethylpyrazine		6.98	7.00	g	b	c
2,3-Dimethylpyrazine		7.15	7.19	g	b	c
Ethylpyrazine		7.11	7.10	g	b	d
2-Ethyl-6-methylpyrazine		5.91 <sup>s</sup>	5.86 <sup>s</sup>	g	b	c
2-Ethyl-5-methylpyrazine		7.62	7.64	g	b	c
Trimethylpyrazine		7.72	7.73	g	b	c
Tetramethylpyrazine		8.41	8.42	g	b	c
* 5-Ethyl-2,3-dimethylpyrazine			8.28			e
2-Ethyl-3,5-dimethylpyrazine		8.27	8.18	g	b	c
2-Ethyl-3,6-dimethylpyrazine		8.14	8.13	g	b	c
* 2,6-Diethylpyrazine	X	8.14	8.28	g	b	d
* Methylpropylpyrazine	X	8.39	8.45	g		e
2-Methyl-3,5-diethylpyrazine		8.60	8.46	g	b	d
2-Methyl-3,6-diethylpyrazine		8.70	8.61		b	
5-Methyl-2,3-diethylpyrazine	X	7.63 <sup>s</sup>	7.59 <sup>s</sup>	g	b	c
3,6-Diethyl-2,5-dimethylpyrazine	X	9.10	9.00	g	b	
Triethylpyrazine	X	9.06	9.00		b	
6,7-Dihydro-5(H)-cyclopentapyrazine	X	10.25	10.31	g	b	
* 5-Methyl-6,7-dihydro-5(H)-cyclopentapyrazine	X	7.47 <sup>s</sup>	7.40 <sup>s</sup>	g	b	
2-Methyl-6,7-dihydro-5(H)-cyclopentapyrazine	X	10.26	10.65	g	b	
* 2(3),5-Dimethyl-6,7-dihydro-5(H)-cyclopentapyrazine	X	10.39	10.19	g	b	
* 5,6,7,8-Tetrahydroquinoxaline	X	8.05 <sup>s</sup>	8.06 <sup>s</sup>	g	b	
* 2-Methyl-5,6,7,8-tetrahydroquinoxaline	X	9.00 <sup>s</sup>	8.82 <sup>s</sup>		b	
* Vinylpyrazine	X	8.06	7.98	g	b	d
* 2-Methyl-6-vinylpyrazine	X	8.58	8.35	g	b	d
* Isopropenylpyrazine	X	8.67	8.60			d
Acetylpyrazine	X	9.92	10.00	g	b	
2-Methyl-5-acetylpyrazine	X	7.27 <sup>s</sup>	7.36 <sup>s</sup>		b	
2-Ethyl-5-acetylpyrazine	X	11.15	11.10		b (isomer not specified)	
1-Pyrazinyl-2-propanone	X	10.46	10.37		French Patent no. 1,530,436 <sup>f</sup>	

<sup>a</sup> An asterisk preceding the compound name indicates that the mass spectral identification is only tentative. *I<sub>E</sub>* values followed by a superscript "S" were determined on an SF-96 column. <sup>b</sup> Kinlin *et al.* (1972). <sup>c</sup> Stoffelsma *et al.* (1968). <sup>d</sup> Bondarovich *et al.* (1967). <sup>e</sup> Friedel *et al.* (1971). <sup>f</sup> Winter *et al.* (1968). <sup>g</sup> Walradt *et al.* (1971).

constituents of beef volatiles. The presence of these compounds is logical since the mechanisms which have been proposed for their formation involve precursors which are readily available in beef. In addition, based on their fundamental roasted aroma, it is believed that pyrazines contribute to the characteristic aroma of cooked beef.

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