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Note

Detection and quantitation of pork in unheated pork–beef blends by high-performance liquid chromatography

SAMY H. ASHOOR*,* and MAGDI A. OSMAN
Division of Agriculture, Arizona State University, Tempe, AZ 85287 (U.S.A.)
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Pork and beef have different quality attributes and they are sold for different prices. Consumers, therefore, need to be assured that the identity of the meat species they bought is right and that the price they paid is fair. Moreover, some consumers do not eat pork because of religious convictions, *i.e.* Moslems and Jews, and they need to be absolutely sure that meats or meat products they buy do not contain pork. Because of these reasons and others, *i.e.* government laws and quality control regulations, there is a need for a sensitive and reliable method for the quantitative identification of pork and other meat species in meats and meat products.

The present methods for identification and quantitation of meat species[1–7] are simply inadequate. Electrophoretic methods[1–3] involve many preparation steps and do not yield reproducible quantitative results. Immunological methods[4,5] are only qualitative and require the production of specific antisera with high titers. Chemical methods[6,7] involve several steps also and are in general time consuming.

The main objectives of the present study were to detect and quantitate pork in unheated pork–beef blends by a simple and rapid high-performance liquid chromatographic (HPLC) method reported by us[8] earlier, and to determine the sensitivity and reliability of the method in prepared blends with 0–100% pork in beef.

EXPERIMENTAL

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph with Model 6000A pump, Model U6K injector, and Model 730 data module was used for HPLC analysis. An Autochrom (Milford, MA, U.S.A.) Model 111-2 gradient controller was used for gradient elution. A variable-wavelength Model 222 detector (Gilson Medical Electronics, Middleton, WI, U.S.A.) was used for detection at 280 nm and 0.02 a.u.f.s.

A 250 × 4.6 mm I.D. Hi-Pore reversed-phase column RP-304 with a Microguard Hi-Pore cartridge (Bio-Rad Labs., Richmond, CA, U.S.A.) was used for HPLC analysis.

* Address for correspondence: 2715 E. Leonora St., Mesa, AZ 85203, U.S.A.

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The meat samples were blended in a Waring blender with a variable transformer (VWR Scientific, Norwalk, CA, U.S.A.) to regulate speed and prevent excessive foaming and overheating.

Reagents

HPLC mobile phase. Double-distilled water filtered through a 0.45-μm membrane filter (Gilman Sciences, Ann Arbor, MI, U.S.A.) was used for preparation of HPLC solvents and will be referred to as HPLC-grade water. HPLC solvent A was 0.1 trifluoracetic acid (TFA), and HPLC solvent B was acetonitrile–water–TFA (95:5:0.1). A linear gradient from 37 to 60% solvent B in 55 min with a flow-rate of 1.5 ml/min was used.

Bovine serum albumin (BSA) standard solution. A standard solution with a concentration of 4 mg/ml was prepared from analytical-grade BSA (Sigma, St. Louis, MO, U.S.A.) and HPLC-grade water.

Sodium azide solution. A 1% sodium azide (J. T. Baker, Phillipsburg, NJ, U.S.A.) solution in HPLC-grade water was prepared for preservation of meat extracts.

Sample preparation

Pork retail cuts (shoulder, rib, loin and ham), and beef retail cuts (chuck, rib, loin, round and flank) were purchased from local stores. Each meat cut was deboned, fat-trimmed then cut into small pieces. Pork sample was made by mixing equal weights of prepared pork cuts, and beef sample was made by mixing equal weights of prepared beef cuts. These samples were used in making pork–beef blends.

Eleven pork–beef standard blends, 30 g each, with pork content of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% were prepared for the construction of pork–beef standard curves.

Each pork–beef sample was blended with twice the weight of distilled water for 5 min in a Waring blender with a variable transformer (set at 30 units). Blended meat sample was filtered through Whatman No. 4 filter paper, and a volume of sodium azide solution was added to the filtrate to give a final sodium azide concentration of 0.01%. An aliquot of the filtrate was filtered again through a 0.45-μm membrane filter before it was injected into the HPLC system.

HPLC analysis

BSA was injected separately to avoid interference with sample peaks. Three injections of BSA solution, each 5 μl, were made to establish the average retention time of BSA peak. A volume of 10–25 μl of each meat filtrate was then injected, and relative retention time (relative to BSA peak) and % area of each peak in the resulting chromatogram were calculated.

Pork–beef standard curves

Percent area of the specific peak of pork (relative retention time of 1.72) or of beef (relative retention time of 1.45) was plotted against % composition of the pork–beef standard blends. The standard curves were used to determine the % composition of unknown pork–beef blends.

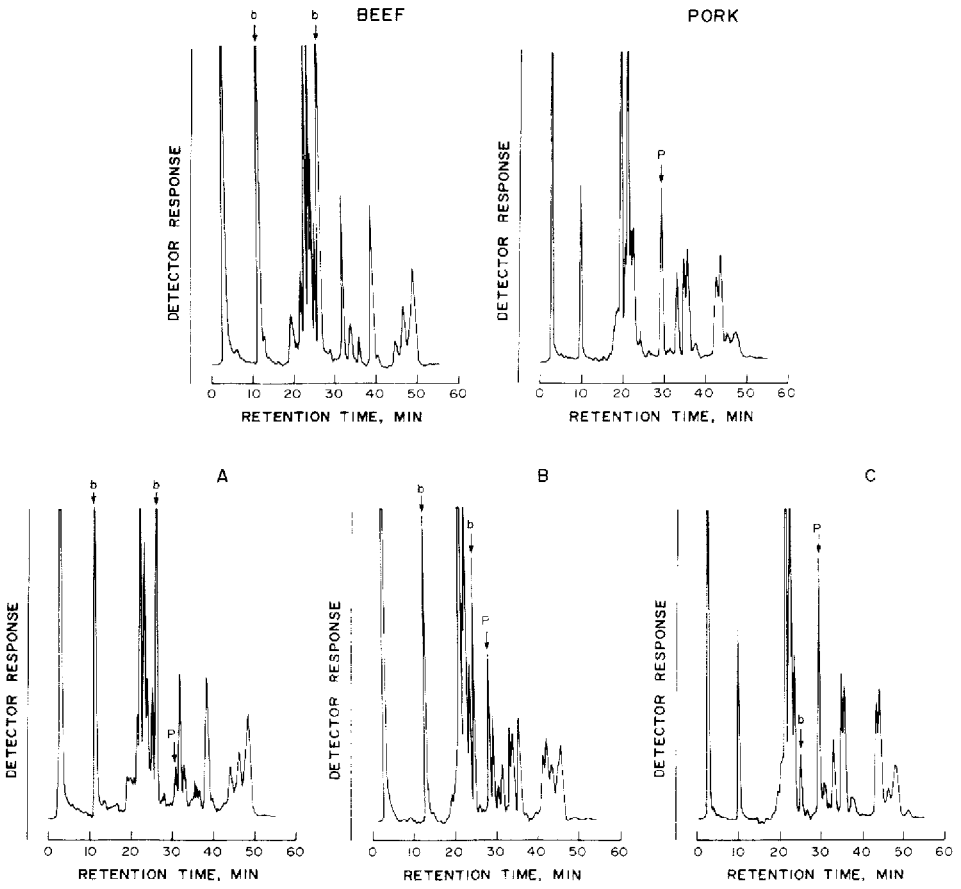

Fig. 1. Chromatograms of beef, pork, 10% pork in beef (A), 50% pork in beef (B), and 90% pork in beef (C). Arrows indicate specific peaks to beef (b) and pork (P).

Pork–beef unknown blends

Twenty pork–beef blends, each 30 g, with pork content of 0–100% were prepared in our laboratory, coded properly then made unknowns. The unknown blends were then analyzed by the HPLC method to test its sensivity and reliability.

RESULTS AND DISCUSSION

Results obtained from our previous study[8] indicated that major cuts from a meat species differ significantly in their water-soluble proteins content. For this reason, pork and beef samples used in this study were made by mixing as many major cuts as available. This way the composite pork or beef sample would be as representative to the species as possible.

Analysis of pork and beef samples by the HPLC method revealed that each species has more than one specific peak (Fig. 1 and Table I). However, only one well resolved specific peak with a relative retention time of 1.45 (beef) or 1.72 (pork) was

TABLE I

HPLC ANALYSIS OF PORK AND BEEF BLENDS

Species-specific peaks are italicized. – = Peak is absent or with area of less than 1%; PO = peak overlapped with another.

*Relative retention time**	*Percent of total area***				
	Pork	*Beef*	*10% Pork in beef*	*50% Pork in beef*	*90% Pork in beef*
0.61	6.5	16.4	14.5	8.4	5.5
1.16	2.0	11.9	12.1	PO	PO
1.21	21.6	9.6	9.7	18.2	21.1
1.26	*19.6*	–	4.7	12.8	21.5
1.45	–	*15.2*	*14.0*	*6.9*	*1.6*
1.72	*9.0*	–	*1.2*	*4.3*	*8.4*
1.79	–	*5.5*	5.2	2.9	1.3
2.00	3.8	–	1.1	1.8	3.1
2.10	4.2	–	–	2.3	3.6
2.22	6.4	–	–	3.4	5.5
2.27	–	*7.5*	6.5	4.0	1.5
2.59	*5.5*	–	–	2.8	4.5
2.65	*6.5*	–	2.7	3.9	6.3
2.77	–	4.4	4.2	3.2	1.8
2.86	–	*8.5*	8.1	6.4	4.0

* Relative to retention time of BSA peak.
** Average of two determinations.

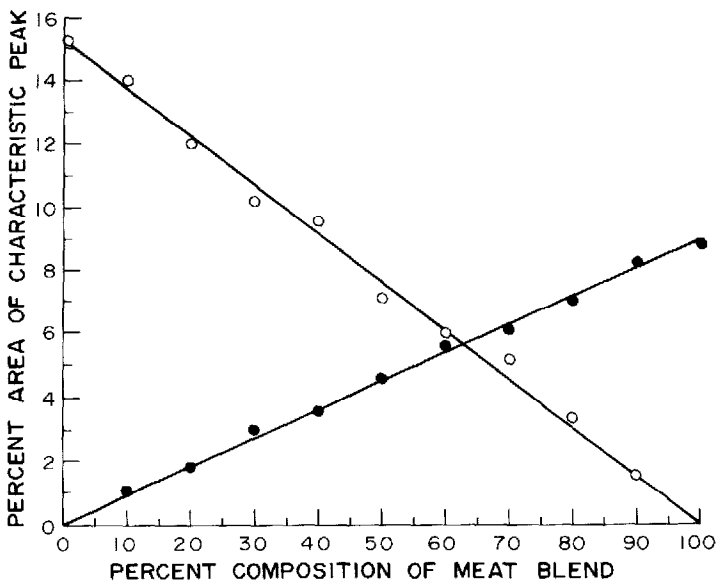

Fig. 2. Standard curves for pork–beef blends using peak specific to beef with relative retention time of 1.45 (○), and peak specific to pork with relative retention time of 1.72 (●). Horizontal axis indicates % pork in beef.

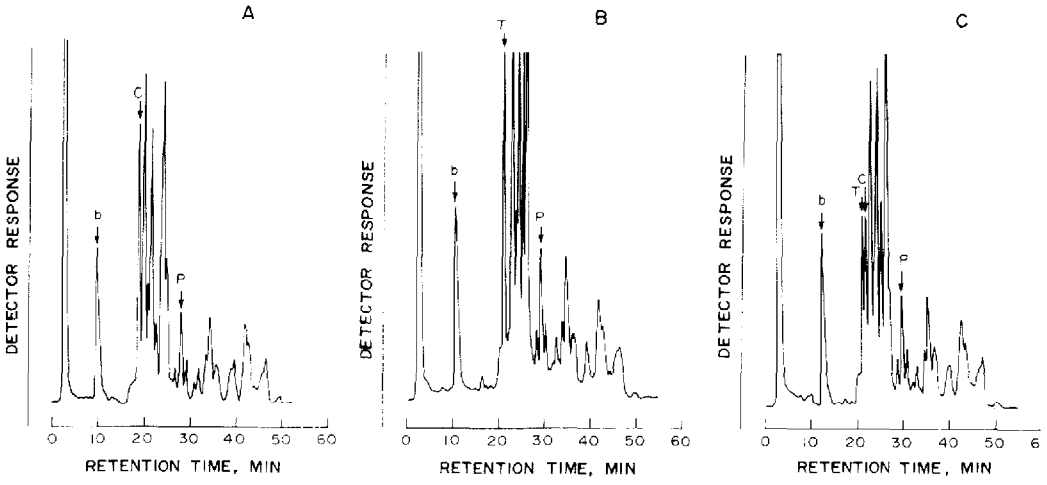

Fig. 3. Chromatograms of pork–beef blends containing chicken (A), turkey (B) and both chicken and turkey (C). Arrows indicate specific peaks to beef (b), pork (P), chicken (C) and turkey (T).

used for detection and determination of either beef or pork in blends. Using these two specific peaks only simplified the detection procedure and resulted in more accurate quantitative data. When the area of either peak was plotted against pork % or beef % in the standard pork–beef blends, a linear relationship was obtained over a composition range of 0–100% (Fig. 2). The results obtained from the analysis of three standard pork–beef blends, 10%, 50% and 90% pork in beef, were included in Fig. 1 and Table I to show the actual increase or decrease of specific peaks to pork or beef as a function of blend composition.

The sensitivitivity and reliability of HPLC method were tested by analyzing the unknown blends with a composition range of 0–100% pork in beef. The results indicated that the method can detect as low as 1% pork in beef, and that pork can be quantitated accurately and reliably (coefficient of variation of less than 5%) at levels of 5% and higher. However, at pork levels lower than 5%, the quantitative data were not as accurate (coefficient of variation of higher than 10%).

The versatility of the method was also tested by analyzing pork in pork–beef blends containing poultry meat. The results indicated that the method is applicable to pork–beef blends containing chicken and/or turkey as shown in Fig. 3.

Frozen pork and beef samples stored at −40°C for 3 months yielded similar results to fresh meat samples when analyzed by the HPLC method. Therefore, it was concluded that freezing in general does not affect detection and quantitation of pork in meat blends by the method. However, when pork and beef samples were exposed to heat, even at mild conditions such as boiling in water for 5 min then analyzed by the method, chromatograms with specific peaks absent, and, in general, different than those of fresh or frozen meats were obtained. Therefore, the method is not applicable to heated pork, beef or blends of both meats.

The present HPLC method was shown suitable for detection and quantitation of pork in unheated pork–beef blends. It is simple and rapid since it involves few preparation steps and requires a total analysis time of about 1 h. The method is also

sensitive and versatile since it detects down to 1% pork in unheated meat or poultry blends. It yields reliable quantitative data over a range of 5–100% pork in blends. Means to increase reliability of the method at levels below 5% pork in unheated meat and poultry blends will be investigated in the future.

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