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Note

Determination of soy protein, whey protein, and casein in unheated meats by high-performance liquid chromatography

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The use of non-meat proteins, especially soy protein, in meat products has been increasing markedly during recent years. Government regulations specify maximum allowable amount of non-meat proteins added to meats. Consequently, a simple and reliable analytical method for determination of non-meat proteins in meat products should be available.

Presently, there are several methods for detection and quantitation of non-meat proteins, mainly soy protein, in meat products. These methods have been reviewed previously[1–3]. Among these methods, electrophoretic[4–6] and immunochemical methods[7,8] are used commonly for quantitative determination of non-meat proteins in meat products. However, these quantative methods have serious limitations. Electrophoretic methods lack reproducibility needed for quantitative results, involve too many preparation steps, and are time consuming. On the other hand, immunochemical methods require specific antisera for every non-meat protein added to meats, and may not yield constant quantitative responses with various commercial forms of non-meat proteins[8]. In a recent collaborative study[9] involving quantitative analysis of soy protein in meat products by an electrophoretic method[6] and an immunochemical method[8], it was concluded that both methods require more refinements to be acceptable for control purposes.

The main objective of the present study was to develop a simple high-performance liquid chromatographic (HPLC) method for detection and determination of soy protein, whey protein and caseinate added to raw beef, pork, chicken and turkey.

EXPERIMENTAL

Apparatus

The liquid chromatograph used in this study was Waters Assoc. equipped with Model 6000A pump, Model U6K injector (Waters Assoc., Milford, MA, U.S.A.), and Model 111-2 one pump gradient controller (Autochrom, Milford, MA, U.S.A.).

The data module was Waters Assoc. Model 730 with chart speed set at 0.2 cm/min and run stop at 60 min.

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The HPLC column was a 250 × 4.6 mm Hi-Pore RP-304 column with a Microguard Hi-Pore cartridge (Bio-Rad Labs., Richmond, CA, U.S.A.).

The UV detector was Gilson Model 222 (Gilson Medical Electronics, Middleton, WI, U.S.A.) set at 280 nm and sensitivity of 0.02 a.u.f.s.

A Waring blender connected to a variable transformer (VWR Scientific, Norwalk, CA, U.S.A.) was used for blending and a Sorvall Model RC-5 centrifuge with rotor No. SS-34 (DuPont, Newton, CT, U.S.A.) was used for centrifugation.

Reagents

The following reagents were used: HPLC-grade water (double-distilled water filtered through a 0.45-μm membrane filter), acetonitrile (analytical grade), and trifluoroacetic acid (TFA) (anhydrous, analytical grade).

The HPLC mobile phases were: solvent A, 0.1% TFA in HPLC-grade water and solvent B, acetonitrile–HPLC-grade water–TFA (95:5:0.1). A linear gradient from 30 to 75% B in 70 min with a flow-rate of 1.5 ml/min was used.

The extracting solution was 0.05 *M* phosphate buffer of pH 7.0 with 0.5% sodium dodecyl sulphate (SDS) (Sigma, St. Louis, MO, U.S.A.) and 0.1% 2-mercaptoethanol (J. T. Baker, Phillipsburg, NJ, U.S.A.). Further, a bovine serum albumin (BSA) solution of 4 mg/ml in HPLC-grade water and a sodium azide solution of 1% in HPLC-grade water were used.

Non-meat proteins standards

Isolated soy protein (Supro 620) was donated by Ralston Purina (St. Louis, MO, U.S.A.), whey protein concentrate (Savorpro 50) was donated by Express Foods (Louisville, KY, U.S.A.), and caseinate (Ultra Supreme Sodium Caseinate) was donated by Erie Casein (Erie, IL, U.S.A.).

Non-meat protein standard solutions

Four standard solutions of each non-meat protein standard were prepared in HPLC-grade water with concentration ranges of 10–30 mg/ml for isolated soy protein, and 5–15 mg/ml for both whey protein concentrate and caseinate. These standard solutions were used for constructing non-meat protein standard curves.

Protein determination

Protein content of non-meat protein standards was determined by the AOAC method[10].

Meat samples preparation

Lean beef, pork chops, chicken breast and turkey breast were purchased from local stores. Meat samples were deboned, fat-trimmed then ground. Poultry samples were deboned, skinned, fat trimmed then ground. Ground meat was used in preparation of unspiked and spiked meat samples.

Unspiked meat samples were prepared by blending 10 g of meat or poultry sample with 40 ml of extracting solution for 5 min at low speed to avoid excessive foaming. The blend was transferred quantitatively to a 250-ml beaker with additional 10 ml of extracting solution and stirred on a heater–magnetic stirrer for 30 min at 35–45°C. The blend was then centrifuged at 5000 *g* for 10 min and sodium azide

solution was added to the supernatant with known volume to a final concentration of 0.01% for preservation. An aliquot of supernatant was filtered through a 0.45-μm membrane filter before injection into HPLC.

Spiked meat samples were prepared by spiking 10 g of meat or poultry sample with each of the non-meat proteins at levels of 1%, 3% and 5% of meat fresh weight. After non-meat proteins were mixed thoroughly with meat samples, the spiked samples were blended and prepared as mentioned above.

HPLC analysis

BSA. A 5-μl volume of BSA solution was injected three times and the average retention time of BSA was calculated. Retention time of BSA was checked periodically.

Non-meat proteins. Volumes of 5–25 μl of non-meat protein standard solutions were injected, and relative retention times (relative to BSA peak) and areas for all peaks of each non-meat protein were determined. A standard curve for each non-meat protein was constructed by plotting area of major peak against amount of non-meat protein injected.

Unspiked and spiked meat samples. Volumes of 10–30 μl of filtered supernatant of unspiked or spiked meat samples were injected and relative retention times (relative to BSA peak) and areas of all peak were determined. Major peak of non-meat protein in spiked meat samples was identified, and amount of non-meat protein added to meat sample was determined using corresponding standard curve.

RESULTS AND DISCUSSION

In order to avoid effects of protein-dissociating reagents on HPLC system in general and HPLC column in particular, attempts were made to extract meat proteins with phosphate buffer containing no dissociating reagents. Whey protein and casein-

TABLE I

ANALYSIS OF NON-MEAT PROTEINS BY HPLC

Major peaks underlined are italicized. – = Peak is absent or with area less than 2%.

*Relative retention time***	*Percent of total area**		
	Soy protein	*Whey protein*	*Caseinate*
0.76	9.4	–	–
0.78	17.2	–	–
0.91	–	–	4.5
0.98	3.7	–	5.4
1.02	–	–	5.4
1.07	*36.9*	–	*36.5*
1.16	10.5	–	–
1.19	19.2	–	36.0
1.26	–	*100*	–
1.28	3.0	–	14.9

* Average of two determinations.

** Relative to BSA peak.

ate were soluble in 0.05 *M* phosphate of pH 7.0 at all spiking levels used. However, soy protein was not readily soluble, especially at high spiking levels. When phosphate buffer with 6 *M* urea, 1% SDS and 1% 2-mercaptoethanol was used for extraction, all non-meat proteins were soluble at all spiking levels used. The high concentration of urea, however, caused a sharp increase in back pressure of HPLC column which interfered with HPLC analysis. Urea, therefore, was omitted from extracting solution. Several extracting solutions with varying concentrations of SDS and 2-mercaptoethanol were tried during early phases of this study. The reported extraction conditions resulted in high recoveries of non-meat proteins from spiked meat samples (85.0–91.2%) and normal HPLC column back pressure.

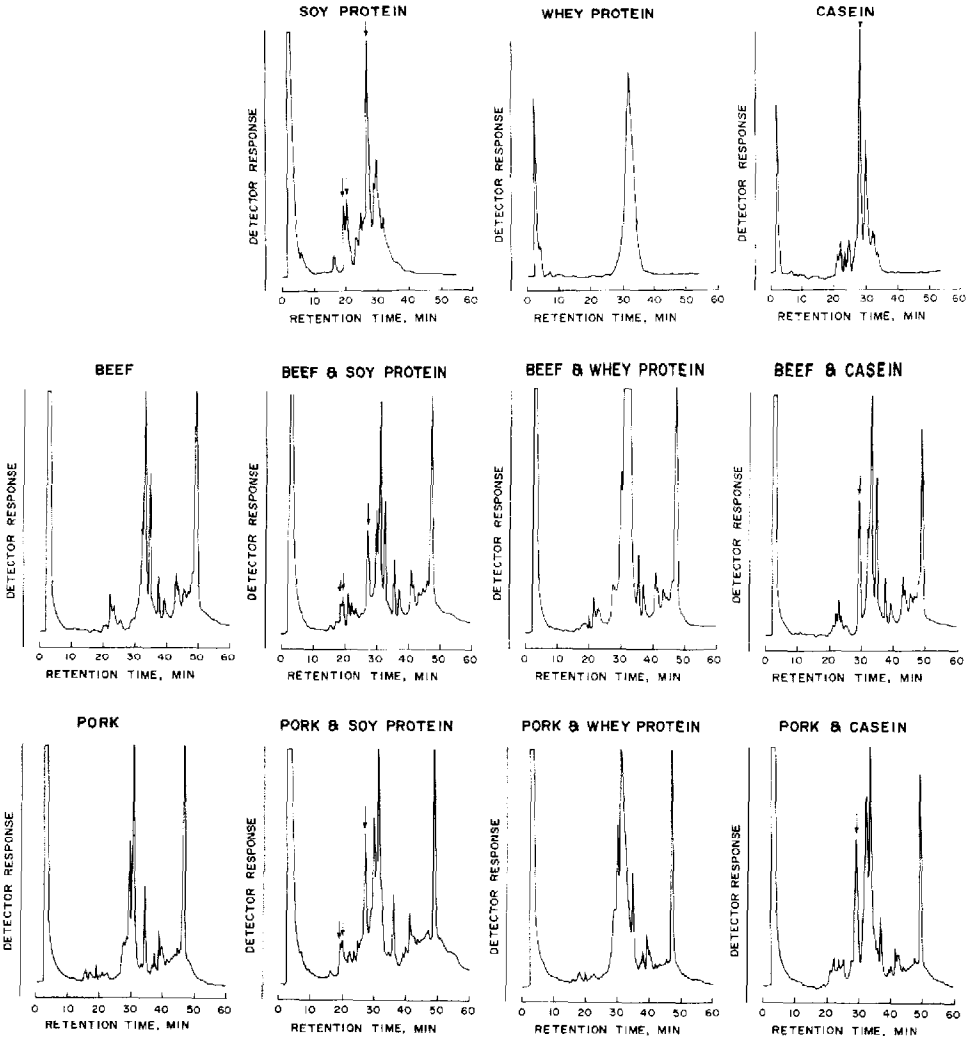

Fig. 1. Chromatograms of non-meat protein standards, beef and pork samples before and after spiking. Arrows indicate characteristic peaks of non-meat proteins in standards and spiked meat samples.

Protein content of non-meat protein standards was 90.3%, 50.4% and 88.4% for isolated soy protein, whey protein concentrate and caseinate, respectively. Results obtained from HPLC analysis of non-meat protein standards are shown in Table I and Figs. 1 and 2. Isolated soy protein and caseinate yielded several peaks with a major peak having more than a third of total area, whereas whey protein concentrate had only one peak (Table I). When area of major peak of each non-meat protein was plotted against amount of protein injected, a linear response was obtained over protein concentration range injected (50–750 μg). Soy protein had the lowest detector response factor, and caseinate had the highest.

Data obtained from HPLC analysis of unspiked and spiked meat samples are

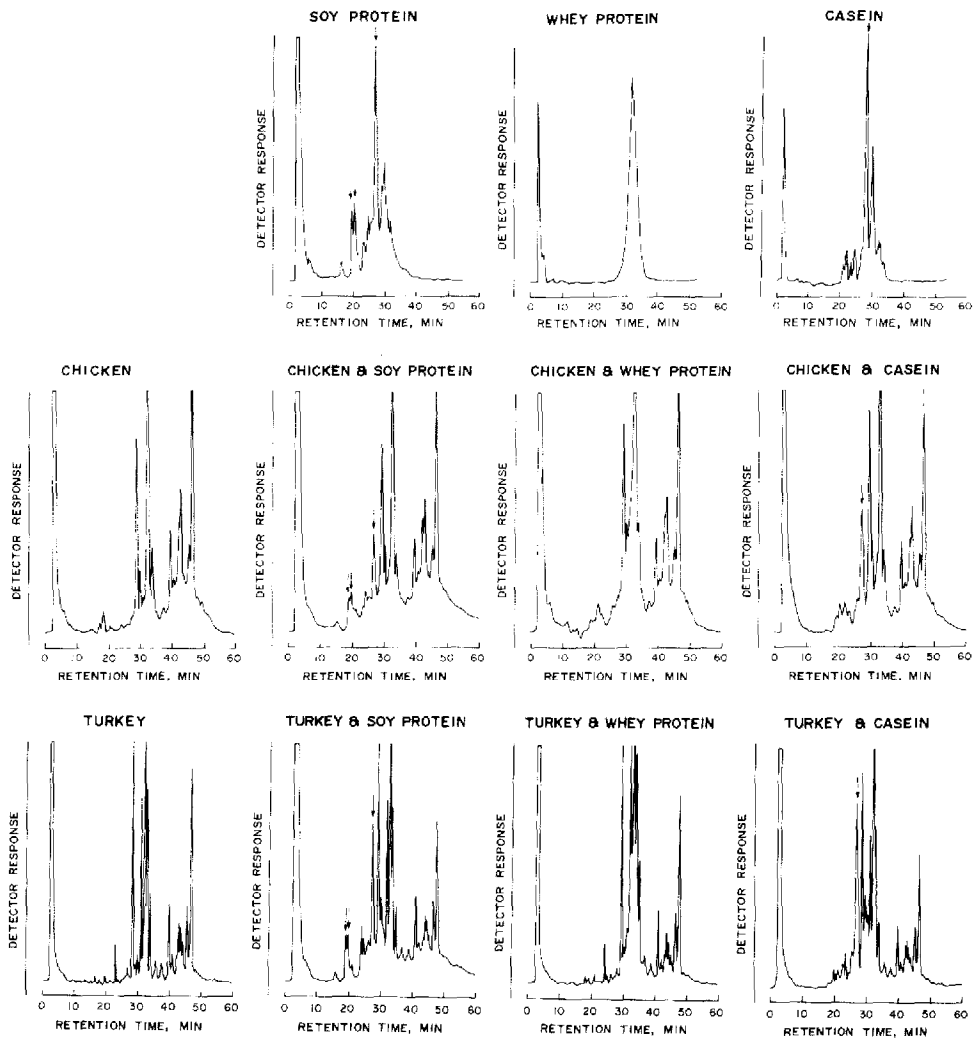

Fig. 2. Chromatograms of non-meat protein standards, chicken and turkey samples before and after spiking. Arrows indicate characteristic peaks of non-meat proteins in standards and spiked poultry samples.

TABLE II

ANALYSIS OF BEEF SAMPLES BY HPLC

Additional peaks to meat sample are italicized. – = Peak is absent or with area less than 2%.

*Relative retention time***	*Percent of total area**			
	Beef	*Beef + soy protein*	*Beef + whey protein*	*Beef + caseinate*
0.76	–	*2.0*	–	–
0.78	–	*3.1*	–	–
0.82	2.4	2.2	2.6	2.1
0.86	2.0	2.0	2.5	3.1
1.07	–	*11.7*	2.2	*16.2*
1.18	9.2	8.8	8.8	11.8
1.21	22.6	28.2	32.3	30.3
1.27	12.4	12.5	58.0	8.7
1.38	2.5	5.2	3.2	2.4
1.44	2.0	2.8	2.0	2.0
1.58	4.2	3.0	2.0	2.5
1.60	3.4	–	–	
1.67	3.5	–	–	2.0
1.78	4.3	2.0	2.0	3.8
1.85	28.3	30.0	24.0	19.7

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

TABLE III

ANALYSIS OF PORK SAMPLES BY HPLC

Additional peaks to meat sample are italicized. – = Peak is absent or with area less than 2%.

*Relative retention time***	*Percent of total area**			
	Pork	*Pork + soy protein*	*Pork + whey protein*	*Pork + caseinate*
0.76	–	*2.4*	–	–
0.78	–	*3.2*	–	–
1.07	–	*17.1*	–	*20.0*
1.12	2.8	–	9.6	–
1.17	13.3	23.4	16.7	24.4
1.21	28.3	35.7	61.1	30.0
1.37	6.1	5.4	10.1	3.1
1.48	2.0	–	4.8	–
1.54	2.8	–	4.0	–
1.58	4.5	2.0	6.6	2.8
1.85	27.4	18.7	23.3	17.1

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

TABLE IV

ANALYSIS OF CHICKEN SAMPLES BY HPLC

Additional peaks to meat sample are italicized. – = Peak is absent or with area less than 2%.

*Relative retention time***	*Percent of total area**			
	Chicken	*Chicken + soy protein*	*Chicken + whey protein*	*Chicken + caseinate*
0.76	–	*2.0*	–	–
0.78	–	*2.0*	–	–
1.02	–	–	–	*2.5*
1.07	–	*5.7*	–	*13.3*
1.15	12.3	12.5	14.4	17.3
1.19	3.2	3.2	4.6	5.4
1.27	32.3	28.6	56.7	34.0
1.33	5.1	4.4	5.7	5.2
1.61	2.0	2.0	2.1	2.4
1.66	4.9	6.1	7.6	6.0
1.68	6.1	9.2	9.2	9.8
1.78	3.1	4.3	5.1	4.8
1.85	20.3	16.2	20.5	17.4

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

TABLE V

ANALYSIS OF TURKEY SAMPLES BY HPLC

Additional peaks to meat sample are italicized. – = Peak is absent or with area less than 2%.

*Relative retention time***	*Percent of total area**			
	Turkey	*Turkey + soy protein*	*Turkey + whey protein*	*Turkey + caseinate*
0.76	–	*3.0*	–	–
0.78	–	*4.3*	–	–
0.91	2.0	3.1	2.0	–
0.96	–	*2.3*	–	–
1.02	–	*4.5*	–	*2.2*
1.07	–	*14.5*	–	*18.4*
1.12	12.4	16.5	12.3	11.8
1.16	–	7.3	2.0	7.3
1.18	–	–	3.2	4.5
1.22	8.1	8.5	15.1	7.8
1.27	24.9	23.5	37.6	23.5
1.29	11.0	9.2	15.4	9.5
1.35	3.0	2.6	6.0	2.8
1.57	3.8	4.1	3.7	3.2
1.69	3.6	2.8	2.0	3.0
1.71	2.7	2.6	2.0	2.0
1.78	5.3	4.6	4.7	4.3
1.85	13.0	10.1	11.1	8.5

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

presented in Tables II-V and Figs. 1 and 2. Characteristic peaks of soy protein with relative retention times of 0.76, 0.78 and 1.07, and that of caseinate with relative retention time of 1.07 were present in all meat samples spiked with these non-meat proteins. These characteristic peaks were well separated with no interference from meat samples. The amount of added soy protein or caseinate was calculated from area of the peak with relative retention time of 1.07. The area of this peak was measured reliably at a spiking level of 1% and increased proportionally as the level of spiking with these two non-meat proteins increased to 5%. Meat samples spiked with soy protein can be distinguished from those spiked with caseinate by the presence of the two peaks with relative retention times of 0.76 and 0.78. These two peaks were always present in meat samples spiked with soy protein at the three levels used. In the case of whey protein, however, the major peak overlapped with peaks of meat samples. In meat samples with whey protein at the three levels used, areas of peaks with relative retention time of 1.21 and 1.27 increased significantly. The increase in area was detected reliably at a spiking level of 1% and was proportional to the level of spiking.

Ten unknowns of unspiked and spiked meat samples with the three non-meat proteins were prepared and tested by the HPLC method. The identity of non-meat proteins was determined correctly in all spiked meat samples. Non-meat proteins were quantified accurately in only meat samples spiked with soy protein or caseinate. It is therefore recommended that the HPLC method is used for qualitative detection of all three non-meat proteins, and for quantitative determination of soy protein and caseinate in raw meat products extended with the non-meat proteins. The applicability of the HPLC method to sterilized extended meat products was not tested in this study. This will be the subject of a future project.

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