Isoelectric Focusing And Carnosine (β- Alanyl Histidine Dipeptide) Content As Tools For Identification Of Ostrich, Beef, Chicken And binary Mixtures Of Raw Meat

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ABSTRACT

Twenty meat samples from the thigh of ostrich, beef, chicken, in addition a binary mixtures of ostrich and beef meat (3:1) and ostrich and chicken meat (3:1) were used in this study. Isoelectric points (PI) (using an immobilized pH gradient), protein banding, amount percentage, peaks and molecular weights of each band were determined in raw meat extracts using polyacrylamide isoelectric focusing method (IEF) and one dimension SDS polyacrylamide gel electrophoresis.

The results showed variations in the number, amount percentage, molecular weight of each band for each species as well as binary mixtures.

High performance liquid chromatography (HPLC) was used to determine the amount of carnosine (β- Alanyl histidine dipeptide in meat samples. Results revealed that a significant difference between ostrich, beef, chicken meats.

Isoelectric focusing, SDS gel electrophoresis and the HPLC, methods can be achieved to be tools for identification of ostrich, beef, chicken or meat as well as in that of mixture.

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INTRODUCTION

Identification of various species of meat is becoming very important.Variations in the price pattern of different meat varieties have further created the problem of adulteration and substitution of low priced meat with high priced ones. The substitution of expensive meat with the cheapest meat is unethical and objectionable for health, religious and economic reasons. Consumption of products containing undeclared flesh can cause allergies in sensitized individuals *(1).*

From the beginning of the 1995 ratites (ostriches, emus, rheas) have received an increasing attention as meat producing animals. Most of commercially high quality usable ostrich meat is taken from the legs *(2).*

Isoelectric focusing (IEF) is a modern analytical tool employed by biochemists to analyse and characterestize several biological materials *(3)*, also it can be used for identification of meat species based on the banding pattern of muscle protein and the specific characteristic peaks *(4)*.Sheep and goat meat and horse and donkey meat could not be differentiated by this technique *(5).* Aqueous urea solution extracts of heated ostrich muscle was used as an antigen for the production of precipitating rabbit antiostrich sera, it was possible to specifically identify raw, heated (70-95°C) and air dried-salted ostrich meat by means of gel immunodiffusion tests. The sera of ostrich did not react with chicken, turkey, horse meat or with beef in any form *(6).*

Carnosine (β -alanyl histidine) is a dipeptide composed of the amino acids, histidine and beta-alanine. Carnosine is found in relatively high concentration in several tissues, most notably in skeletal muscle, heart muscle and brain *(7).* Carnosine and anserine are the major dipeptides in skeletal muscle tissue of most vertebrates *(8)*. These dipeptides play important roles in physiological

functions as such as a potent intracellular pH-buffer inhibition of oxidative reactions *(9,10).* Moreover histidine dipeptides were used as a tool for identification of skeletal muscle of different species *(11)* .

Ratite meat shows some characteristics of poultry muscle (Low fat, rapid PH decline) as well as beef muscle (intense red color) *(12).* Ostrich meat characterized by tenderness, low fat and cholesterol content *(13).*

Scientific knowledge about ostrich meat characterization is incomplete and very fragmented. No available literatures were found delaing with isoelectric focusing (IEF) or carnosine content of ostrich meat. So the aim of this study was to determine, the isoelecteric point (PI) and carnosine content of ostrich meat compared with chicken and beef meat also binary mixtures of ostrich and beef and ostrich and chicken were also analyzed to be used as tools for identification.

MATERIAL AND METHODS

Meat samples and sources

Twenty-meat samples (40 g each) were used during our study. Twelve samples were obtained from the thigh of ostrich, beef (beef) and chicken meat (4 samples from each species). The remaining eight samples were used for preparation of binary mixtures four samples for ostrich & beef (3:1) and four samples for ostrich & chicken (3:1) mixture *(14).*

Ostrich meat was purchased from an ostrich farm, beef meats from butchers ,and chicken meat from the market .

1-Determination of isoelectric focusing (IEF)

The isoelectric focusing was determined according to the method described previously by Ò Farrel *(15).* The method based on separationof protein on polyacrylamide gel (PAGE) using pH gradients.

Extracts from meat samples were obtained by mincing and homogenizing 2 g. of meat with 10 ml 0.09% saline solution. The supernatant was separately collected in labeled sterilized screw capped bottles and kept at – 20°C till used. The running procedure for IEF using carrier Ampholine 2% w/v pH 3.5-10 (LKB, Bromma, Sweden) carrier ampholyte was carried out. On completion of IEF the gel was stained by Coomassie Brillent Blue-R-250 *(16).* Densitometry (Densitometer, G 700 biorad, USA) and calculation of peak areas were carried out.

2-Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried on meat samples (200 mg/ml) after homogenization in 10% SDS and centrifuged. The supernatants were used for SDS-PAGE as described proviously by Laemmli *(17),* method. The technique is basd by on the separation of proteins according to size and determination the relative molecular mass of protein. Detection of protein after gel electrophoresis was carried out after staining the polyacrylamide gel with silver stain *(16).*

3-HPLC method for determination of β alanyl histidine

Extraction and determination of carnosine were carried out *(11).*

a. Tissue extraction

Thirty grams of raw lean muscle sample was added to 30 ml 0.9 % saline and 120 ml of 5-sulphosalicylic acid (BHD-POOLE, Great Britian) then homogenized for 1 min. The homogenate was centrifuged at 5°C for 1h. The supernatant fractions were filtered through Millipore pre- filter (type AW) and refiltered by (type GS., 0.22 µm diameter pores). The samples were centrifuged at 8000 r.p.m. for 4.5 min.

b. Determination by HPLC

The dipeptide in 5 ml of extract was separated on a hypersiel column.The column was operated at 30 °C. Carnosine was detected with a fluorescent detector GBC supplied with quaternary pump linked to a winchrom 3.1.6 software with IBM computer. The concentration of carnosine was expressed as

µmol/ g of meat and retention time was 10 min by using HPLC type GBC Australia

Statistical analysis of the data was carried out *(18).*

RESULTS AND DISCUSSION

Isoelectric focusing (IEF)

This method is ideal for separation of amphoteric substances such as protein and it is based on the separation of molecules according to their different PI the method has a high resolution and separate protein differ in their PI as little as 0.01 of a pH unit *(19).*

The study revealed that the raw meats of the species under investigation have a definte number of visible protein bands (Fig. 1) which are 11, 11 , 9 , 11 13 for ostrich, beef, chicken, binary mixtures of ostrich & beef and ostrich & chicken respectively. Each band has a definite amount percentage which are variable (Table 1). Also, variations in isoelectric point for each species was ranged from 8.4279-5.0617 for beef 8.4279-5.0011 for chicken, 8.2766-5.2438 for ostrich & beef and 8.5940 -4.7909 for ostrich & chicken. All these variations help in meat species identification.

The previous study *(20)* found that equal number of bands in raw camel and sheep [9]. The difference in the PI and amount percentage of each band and also thickness of bands help in differentiation. Similarly cattle and sheep had the same number of bands [10] while poultry and pigs had [15] bands but the protein banding patterns were more discernible in the PH range which helping in determining the species–specific patterns *(4)*. Our results showed that ostrich, beef and mixture of ostrich and beef had the same number of bands [11]. The difference was definite in the IEF, amount percentage and the molecular weight of each band which is helpful in ostrich meat identification.

SDS-PAGE polyacrylamide gel electrophoresis :

SDS-PAGE polyacrylamide gel electrophoresis revealed that the number of bands were (ostrich, beef, chicken, ostrich beef mixture and ostrich chicken mixture 8, 7, 7 8 and 10 respectively. Each band has a specific molecular weight and specific amount percentage which distinguish it from other bands expressed in Kilo Dalton (KD) as shown Table (2) and Fig. (2). Molecular weight variations aids in identification.

Our study revealed that the number of bands in each sample after IEF gel was increased than that after SDS gel electrophoresis. This phenomenon was explained Wilson and Walker *(19)* who stated that a protein may show a single band on a SDS –added gel but may show three bands on an IEF gel this occurs when a protein exists in mono – di- and triphosphorylated forms and the difference of a couple of phosphate groups has no significant effect on the overall relative molecular mass of the protein.

Table (1): Isoelectric point and amount percentage of protein bands for ostrich , beef , chicken, binary mixtures of ostrich & beef (3:1) and ostrich & chicken (3:1)

No. of band	IEP of marker	Amount %	IEP of Ostrich	Amount %	IEP of Beef	Amount %	IEP of Ostrich &Beef	Amount %	IEP of chicken	Amount %	IEP of Ostrich & chicken	Amount %
1	8.80	7.63	8.4279	8.87	8.8863	1.69	8.2766	9.32	8.4279	16.9	8.5940	4.98
2	7.20	12.2	7.9710	7.37	8.4869	5.50	7.7844	4.04	7.8170	12.1	8.2306	8.55
3	6.60	25.2	7.6234	5.18	8.2192	6.45	7.5916	3.36	7.1798	6.05	7.5704	6.38
4	5.90	11.6	7.0253	13.7	7.7411	2.39	7.2402	5.46	6.8983	12.6	7.1899	3.94
5	5.10	26.1	6.8838	6.13	7.4969	4.18	7.0947	2.96	6.6698	9.18	7.0698	3.03
6	4.60	8.28	6.6652	7.10	7.3413	4.27	6.9371	6.51	6.1866	13	6.9226	5.77
7			6.0416	13	6.9762	10.1	6.8309	3.34	5.5499	8.43	6.6511	11.2
8			5.6433	10	6.7688	8.28	6.6511	9.27	5.2966	8.50	6.1335	13.8
9			5.4520	6.11	6.6605	7.61	6.0677	23.9	5.0011	13.2	5.6621	8.31
10			5.2438	5.77	6.0677	19.7	5.4641	5.62			5.4097	4.52
11			5.0617	16.4	5.5933	29.8	5.2438	26.1			5.1057	9.59
12											4.9189	6.12
13											4.7909	12.08
Sum		100		99.7		100		99.9		100		99.4

Table (2) Electrophoretic pattern of meat protein of ostrich, beef, binary mixtures of ostrich & beef (3:1) , chicken and mixture of ostrich & chicken (3:1) by one dimension SDS gel electrophoresis.

No. of band	Marker (molecular weight)	Amount %	Ostrich (molecular weight)	Amount %	beef (molecular weight)	Amount %	Mixture of Ostrich & beef (molecular weight)	Amount %	Chicken (molecular weight)	Amount %	Mixture of Ostrich & chicken (molecular weight)	Amount %
1	205	10.2	227.36	8.88	145.17	21.3	212.20	10.3	142.69	19.8	205	10.3
2	116	30.2	147.70	14.2	113.55	8.77	140.25	12.5	113.55	5.91	142.69	11.8
3	97.40	25.9	115.02	7.78	107.89	23.1	113.55	6.83	78.327	22	111.63	6.82
4	66	8.72	108.82	20.1	63.520	14.7	107.89	12.2	61.721	18.4	106.52	6.60
5	45	9.55	84.667	5.95	56.625	8.77	84.010	8.61	57.720	10	92.235	7.33
6	29	15.1	64.748	14.8	45	5.92	62.315	20.6	34.464	11.1	78.939	10.3
7			57.170	8.93	26.394	17.1	40.957	12.7	25.181	12.3	60.551	13.8
8			26.812	19.1			25.983	15.8			52.955	9.42
9											33.927	10.5
10											24.403	12.6
Sum		99.6		99.7		99.6		99.5		99.6		99.5

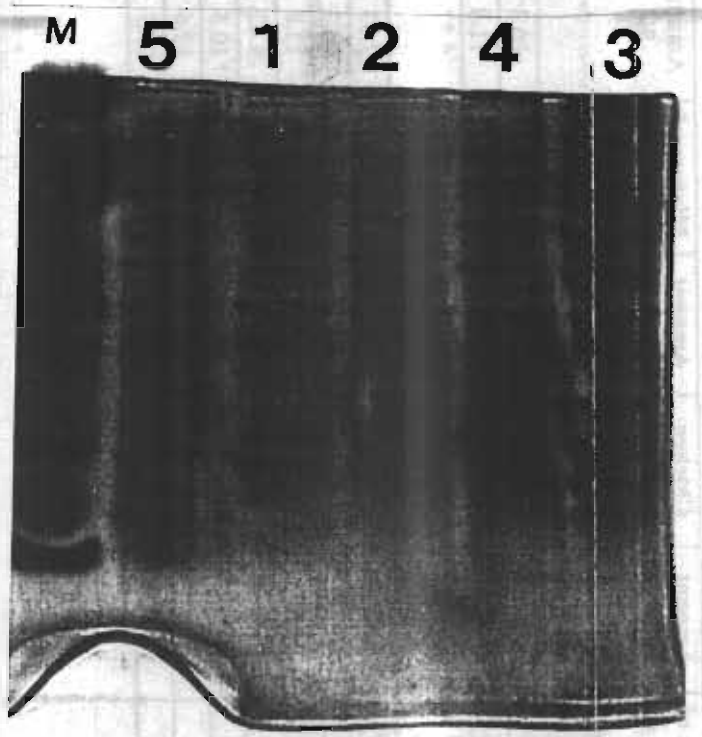


Fig. (1) :Showing isoelectric focusing on polyacrylamide gel for marker {M} and soluble proteins from extracts of meat from ostrich {1}, beef {2}, chicken {3}, ostrich & beef {4} and ostrich & chicken {5}(Comassie Brillent Blue R250).

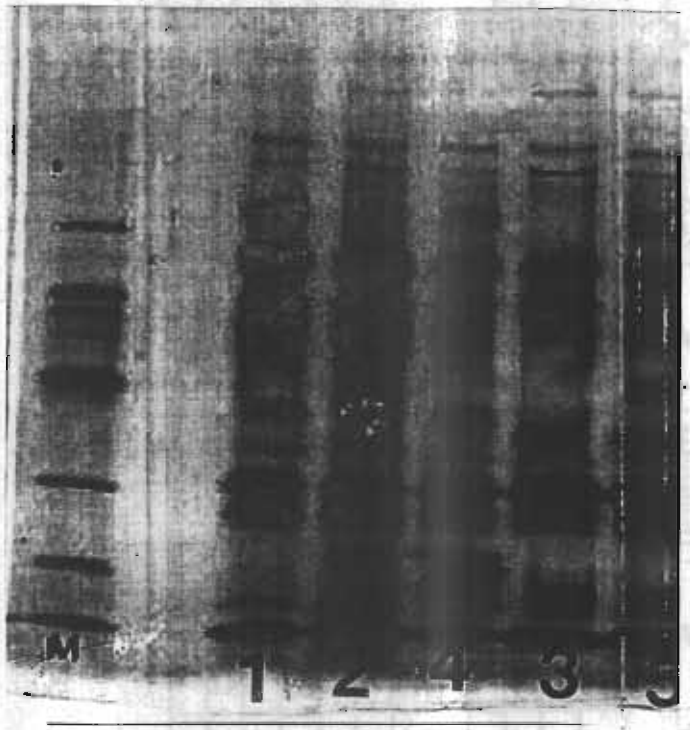


Fig. (2): Showing electrophoretic pattern of marker {M} and soluble proteins from meat extracts of from ostrich {1}, beef {2}, chicken {3}, ostrich & beef {4} and ostrich & chicken {5} by one dimension SDS polyacrylamid gel which differ in their molecular weight (KD) (Silver stain).

Determination of carnosine by HPLC

Typical separation of the histidine dipeptide carnosine in raw skeletal muscles from ostrich, beef and chickens as well as of mixture are shown Table (3) and Fig. (5). Variation in the carnosine content in different species is of medicolegal importance in identification of studied meat. Ostrich had the highest concentration compared with the other species.

The carnosine content showed slight difference than that previously was obtained by *(11)*, in thigh of beef meat, this may attributed to variation of histidine dipetides in different muscles of the individual species as concentration of histidine dipeptides in the shoulder of pig was lesser than in leg *(11)*. On the same side breast meat (white muscle) of chicken, duck and turkey contained higher concentrations of carnosine and ansesine than that of in the thigh *(8,10,21)*. The previous study by *(10)* found that carnosine and anserine are affected by diet and their concentrations vary widely with species and muscle type.

Table (3):Showing carnosine content (µmol/g) of meat in ostrich, beef, chicken, ostrich + beef and ostrich + chicken (mean ± SD).

Meat type	Ostrich	Beef	Ostrich & beef	Chicken	Ostrich & chicken
Carnosine content	20±1.2	12.35±0.6	17.5±0.5	9.3±0.6	15.6±0.3

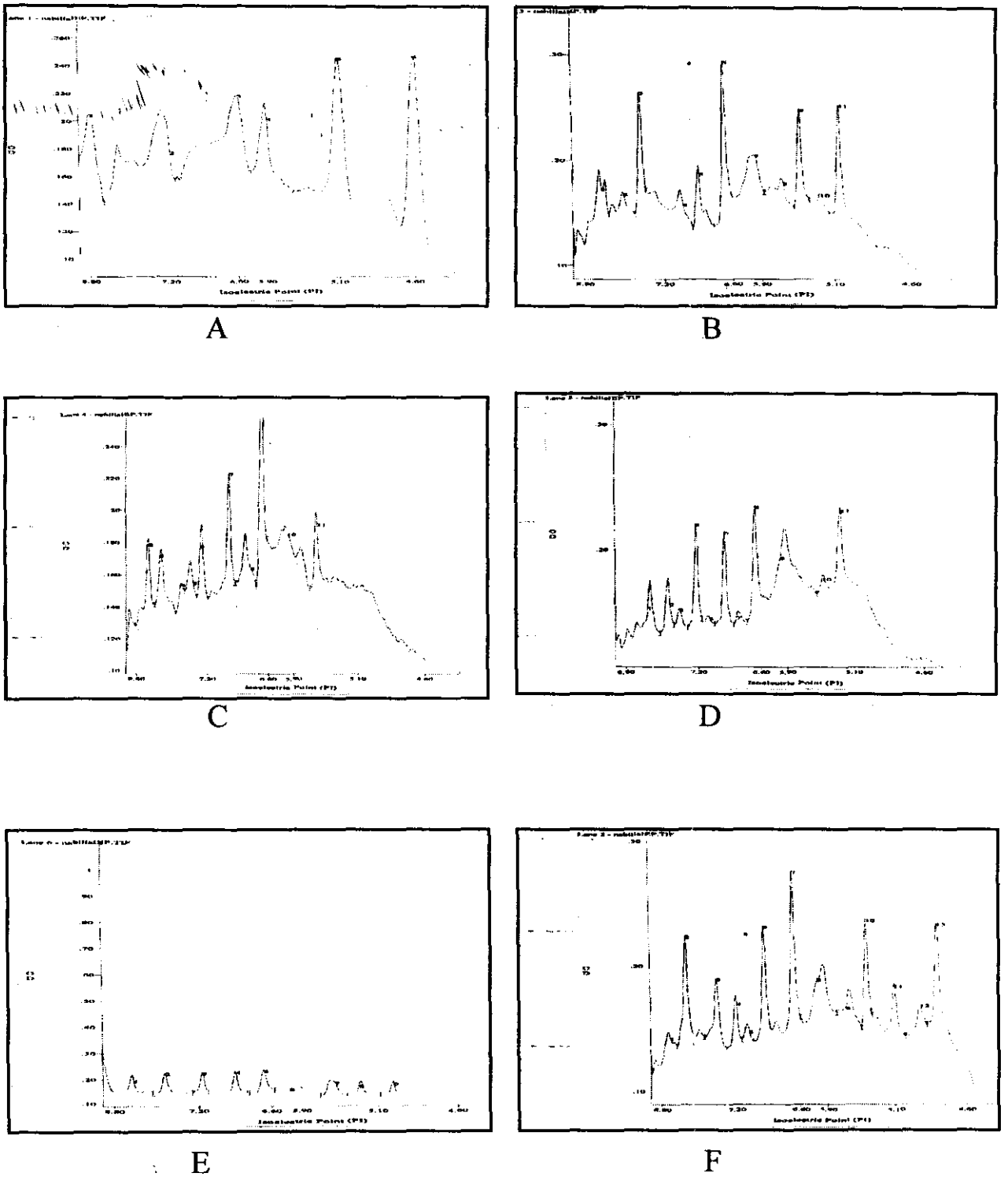
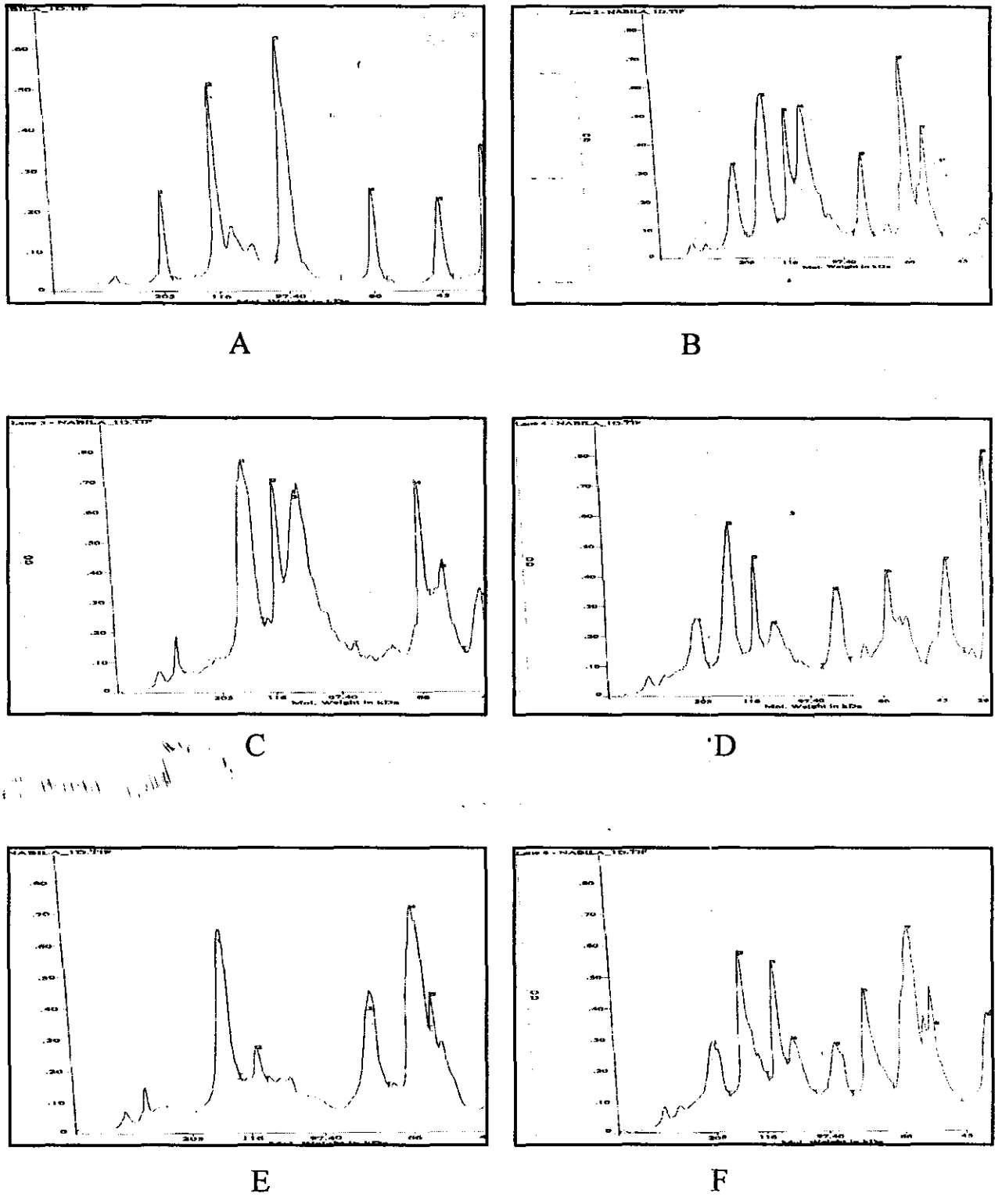


Fig. (3): Densitometric scans of isolectric focusing of protein marker {A}, meat of ostrich {B}, beef {C}, ostrich & beef {D} , chicken {E} and ostrich & chicken {F}



Fig,. (4): Electrophoretic pattern of soluble proteins from extracts of meats from protein marker {A} ostrich {B}, beef {C}, chicken {D}, ostrich & beef {E} and ostrich & chicken {F} by one dimension SDS polyacrylamide gel

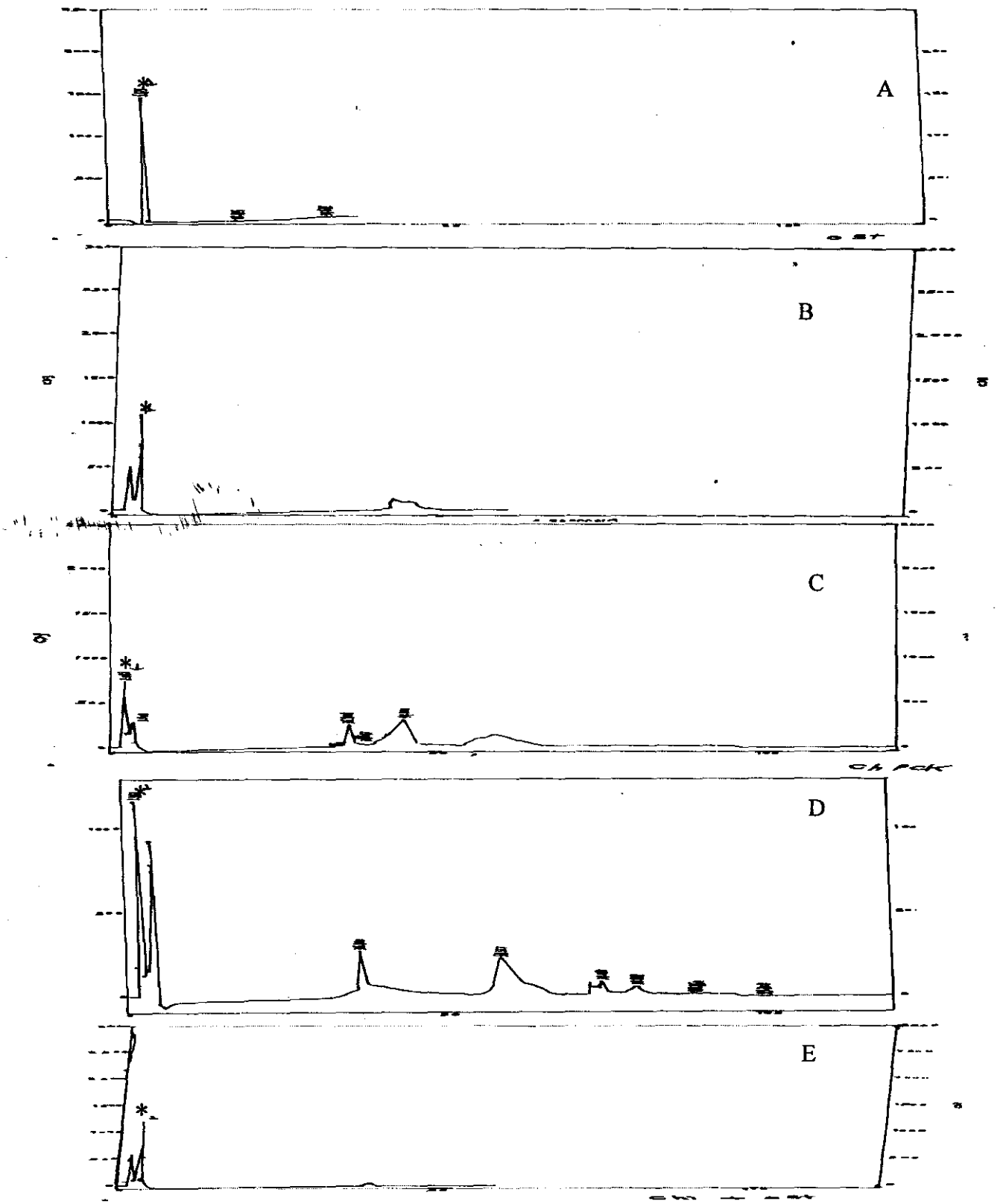


Fig. (5) Showing chromatogram of carnosine contents in , different samples using HPLC : ostrich meat {A}, beef meat {B}, chicken meat,{C} mixture of ostrich & beef meat {D} and binary mixture of ostrich & chicken meat {E} . * Carnosine

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الملخص العربى

تعيين نقطة تساوى الجهد الكهربى وكمية الكارنوزين للاستعراف على لحوم النعام والأبقار والدواجن

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أجريت هذه الدراسة على ٢٠ عينة لحوم أخذت من الفخذ لكل من النعام والأبقار والدواجن مع خليط لكل من لحوم النعام والابقار (١:٣) وكذلك خليط من لحوم النعام والدواجن (١:٣) (اربع عينات لكل منهم).

تم فصل مكونات بروتين العضلات فى كل من النعام والأبقار والدواجن باستخدام نقطة تساوى الجهد الكهربى وكذلك تحديد وزنه الجزيئى.

ولقد أوضحت النتائج اختلاف فى عدد وكمية بروتين العضلات وكذلك وزنه الجزيئى فى العينات محل الدراسة مما يساعد فى الاستعراف.

وتعتبر هذه الدراسة التى تم فيها تحديد نقطة تساوى الجهد الكهربى وكذلك الكمية والوزن الجزيئى لبروتين لحوم النعام من الدراسات الهامه التى يحتاج اليها الطب الشرعى.

كذلك دلت النتائج على وجود اختلاف فى كمية الكارنوزين فى جميع العينات محل الدراسة حيث وجد أن لحوم النعام تحتوى على أعلى كمية من الكارنوزين بمقارنتها بلحوم البقر والدواجن.

ولقد خلصت هذه الدراسة الى انه يمكن استخدام طريقة نقطة تساوى الجهد الكهربى لبروتين العضلات الى جانب قياس كمية الكارنوزين فى الاستعراف على لحوم هذة الحيوانات وإستبيان حالات الغش التجارى