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Electrophoretic Analysis of Proteins from Chicken after Irradiation and during Cold Storage

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ABSTRACT

The SDS/polyacrylamide gel electrophoresis method was tested to determine whether it was suitable for the detection of the pre-irradiation of chicken. The electrophoretic pattern of unirradiated Pectoralis major *muscle contained 26 proteins in the range 372 391 to 15 026 daltons. The radiosensitivities of various proteins in the untreated samples were investigated. Irradiation with 6 kGy caused the complete disappearance of five proteins but induced the appearance of six new proteins as well as considerably altering the molecular weights (MW) of many proteins. Samples irradiated with 10 and 20 kGy were also distinguishable from either unirradiated or the 6-kGy irradiated samples. At least 19 and 25 new protein fractions were induced upon irradiation with 10 and 20 kGy, respectively. Changes in unirradiated and irradiated muscle proteins were followed during cold storage at 4 ± 1°C. Slower rates of protein breakdown characterized the higher irradiation doses. At the end of shelf-life of different samples, i.e. 12 (0 kGy), 32 (6 kGy), 55 (10 kGy) and 75 (20 kGy) days, the numbers of protein bands were dramatically increased to 48, 46, 48 and 61, respectively. All of these bands were characterized by a partially reduced MW.*

INTRODUCTION

The joint FAO/IAEA/WHO (1980) Expert Committee on the wholesomeness of irradiated food (WHO, 1981) recommended the acceptability of any

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foodstuff irradiated up to an overall average dose of 10 kGy. However, studies have been in progress in many laboratories on the chemical changes induced by irradiation with the objective of establishing control techniques for identifying irradiated foods. Many electrophoresis techniques have been suggested to find a recommended method for the identification of pre-irradiation treatments. Most previous investigations have been carried out on protein components, e.g. fibrillar and sarcoplasmic, with different buffer solutions used for extraction in conjunction with simple techniques for the resolution, such as vertical starch gel electrophoresis, agar and agarose gel electrophoresis, immunoelectrophoresis and isoelectric focusing pattern (Radola, 1969; Bruaux *et al.*, 1970; Bugyaki & Van der Steichelen-Rogier, 1973; Halliday *et al.*, 1976; Hassan & Emam, 1988). However, most of the previous researchers in this field did not clearly demonstrate the application of their electrophoretic techniques to the detection of irradiation treatments.

The present investigation was undertaken with the dual objectives of elucidating the nature of reactions that occur on irradiation of chicken muscle proteins and establishing control techniques for the recognition of pre-irradiation treatments.

MATERIALS AND METHODS

Materials

Eight hundred washed, eviscerated and slaughtered carcasses of 8-week-old chickens weighing approximately 1000 g were obtained from the General Poultry Company, Cairo, Egypt. The carcasses were sealed in 30 × 20 cm bags made of 0·135-mm-thick polyethylene. The carcasses were kept for 6 h at 2°C and then subjected to the irradiation process. The carcasses were divided into four treatment groups of 200 carcasses each. After treatments and/or storage randomly selected carcasses were subjected to microbiological (Hegazy, 1988), chemical/biochemical (Hassan *et al.*, 1988*a*,*b*; Hegazy, 1988) and sensory (Hegazy, 1988) assessments as well as the electrophoretic analysis which is the subject of the present investigation.

Methods

Irradiation process

The polyethylene-packed chicken carcasses were irradiated at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo. The irradiation facility used was an Egypt Industrial Mega Gamma 1, model 'AECL JS 6500', irradiator provided with automatic conveyors.

Cartons (48·2 × 58·4 × 91·4 cm), designed as irradiation containers, were filled with the packed carcasses. The Cobalt-60 irradiation source produced an average dose rate of 1·14 kGy/h as measured by red Perspex dosimeter type 3034 (United Kingdom Atomic Energy Authority, Harwell, Berkshire). Irradiation doses applied in the present study were 0, 6, 10 and 20 kGy. The temperature of the irradiation chamber ranged from 10–15°C. After completion of the irradiation treatments, the temperatures of the 0, 6, 10 and 20 kGy chicken carcasses were found to be 7·5, 9·7, 11·3 and 12·7°C, respectively.

Preparation of sample

Samples for electrophoresis were prepared according to the procedure of Stegman *et al.* (1983). A 10-g subsample prepared from six minced *Pectoralis major* muscles was defatted three times with cold acetone and then dried under vacuum at 20°C. The dried muscle tissue was milled (Fritsch Pulueristte mill type 14072, Ser. No. 504) with liquid nitrogen to prevent any temperature rise. Dried muscle tissue was transferred to Pyrex test tubes by being picked up with a finely drawn glass rod. The tubes were stoppered, frozen in liquid N_2 and stored at −70°C until the end of the shelf-life of the 20-kGy samples, 75 days, when all the samples were subjected to electrophoresis.

SDS/polyacrylamide gel electrophoresis

Slab gels were prepared according to the methods of Laemelli (1970) using a Pharmacia vertical gel electrophoresis apparatus (GE 2/4 LS) with a power supply unit (EPS 500/400). Gel sample buffer (0·01M Tris-HCl, 0·001M EDTA, 1% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, pH 8·0) was added to each tube of muscle fibre. The tubes were flame-sealed and then incubated at 110°C for 15 min. The tubes were opened and the samples stirred with a microsyringe needle before an aliquot containing an estimated 20 μg of protein was transferred to the gel. The stacking and resolution gels contained, respectively, 4% and 12·6% polyacrylamide (both 2·7% cross-linked methylene-bis-acrylamide). Separating gel buffer consisted of 0·375M Tris-HCl, 0·1% (w/v) SDS and pH 8·8. Electrophoresis buffer contained 0·05M Tris-HCl, 0·384M glycine, 0·1% (w/v) SDS and a final pH of 8·3. Gel preparation, electrophoresis conditions, fixing, staining and destaining were performed as described by Stegman *et al.* (1983).

Scanning was carried out using a Chromoscan 200 densitometer (Joyce–Loebl) fitted with an interference filter with a maximum transmission at 660 nm to trace each zone in the destained gels. Protein bands were characterized in the electrophoretic pattern by estimating the molecular weight (MW) from the relationship between the mobility of muscle and

marker proteins, and also by the methods described by Etlinger *et al.* (1976), Andrews (1981) and Young and Davey (1981).

Cold storage

Packed chicken carcasses were kept in a refrigerator at $4 \pm 1°C$ from immediately after irradiation until the end of shelf-life of each treatment. The shelf-lives of 0, 6, 10 and 20 kGy irradiated samples were 12, 32, 55 and 75 days (at $4 \pm 1°C$), respectively, as estimated by sensory and chemical analysis (Hassan *et al.*, 1988*a,b*; Hegazy, 1988).

RESULTS AND DISCUSSION

Identification of proteins from *P. major* muscle of unirradiated chicken

As shown in Fig. 1 and Table 1, 26 protein bands with molecular weights (MW) from 372 391 to 15 026 daltons were resolved from *P. major* muscle of unirradiated fresh chicken.

TABLE 1

Effect of Irradiation Treatments on the *Pectoralis major* Muscle Proteins of Chicken resolved by SDS/Polyacrylamide Gel Electrophoresis

Identification	*0 kGy*		*6 kGy*		*10 kGy*		*20 kGy*	
	Number of band	*MW*	*Number of band*	*MW*	*Number of band*	*MW*	*Number of band*	*MW*
α-Macroglobulin	1	372 391	—	—	—	—	—	—
Myosin heavy chain (MHC)	2	219 615	1	214 615	1	244 714	1	267 656
α-Actin (α-AC)	3	109 807	3	96 235	3	76 473	6	82 355
Lactoperoxidase	4	94 120	4	85 650	—	—	7	73 836
Plasminogen	5	87 397	—	—	—	—	—	—
Transferrin	6	77 162	5	69 072	7	52 870	—	—
Phosphorylase	7	70 204	6	59 068	8	48 942	11	49 796
Pyruvate kinase	8	55 616	7	53 198	—	—	—	—
Heavy chain of immunoglobulin G (IgG)	9	50 087	8	45 317	10	40 400	13	40 785
Actin (AC)	10	40 400	9	40 212	11	35 246	14	32 816
Pepsin	11	33 720	10	33 197	12	29 636	15	30 372
Carboxypeptidase A	12	32 199	11	32 566	13	28 081	16	29 033
β-Tropomyosin (β-TM)	13	30 052	12	30 589	14	27 364	17	27 808
α-Tropomyosin (α-TM)	14	28 550	14	27 190	16	24 126	19	24 126
Carbonic anhydrase	15	27 018	15	25 876	17	22 840	20	23 274
Myosin light chain 1, fast	16	22 901	18	21 683	22	19 204	24	20 392
Tropomyosin 1, slow	17	21 412	20	20 344	24	18 419	26	18 700
Tropomyosin C, fast	18	20 588	21	19 247	25	17 918	27	17 843
β-Lactoglobulin	19	19 290	22	18 184	27	16 728	28	16 960
Myosin light chain 2, fast	20	17 515	23	16 960	29	15 601	31	15 658
Myoglobin	21	17 164	—	—	30	15 159	32	15 240
Avidin	22	16 663	—	—	—	—	33	14 947
Myosin light chain 3, fast	23	16 099	24	15 715	31	14 443	35	14 370
Haemoglobin	24	15 601	26	14 741	—	—	—	—
α-Lactalbumin	25	15 267	27	14 346	—	—	—	—
Lysozyme	26	15 026	—	—	—	—	—	—

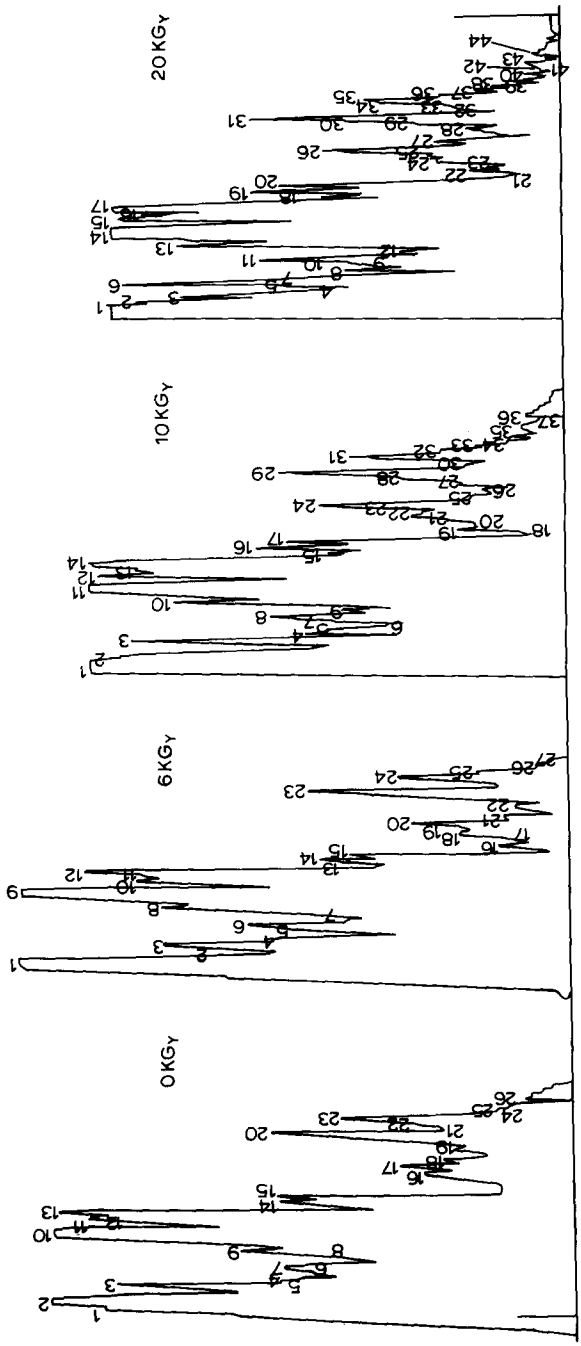

Fig. 1. Effect of irradiation treatments in non-frozen state on the proteins of *Pectoralis major* muscle of chicken as identified by SDS polyacrylamide gel electrophoresis.

The heaviest band, which was identified only in the unirradiated samples, was characterized as α-macroglobulin. The heavy chains of myosin (MHC—219 651) and α-actin (α-AC—109 807) were easily identified, followed by some glycolytic enzymes, lactoperoxidase (94 120), plasminogen (87 397), transferrin (77 162), phosphorylase (PE—70 204) and pyruvate kinase (55 616). The tropomyosin bands were identified as β-tropomyosin (β-TM—30 052) and α-tropomyosin (α-TM—28 550). Two forms of α-tropomyosin could be differentiated, the slower migrating form exemplified by the slow fibre ($T1_s$—21 412) and the other faster form (TC_f—20 588). Other myosins were resolved: the fast myosin chains, myosin light chain 1, fast ($LC1_f$—22 901), myosin light chain 2, fast ($LC2_f$—17 515), and myosin light chain 3, fast ($LC3_f$—16 099). The protein bands, which could also be characterized from the electrophoretic pattern, were arranged according to their mobility (Table 1) as follows: β-lactoglobulin (19 290), myoglobin (17 164), avidin (16 663), haemoglobin (15 601), α-lactalbumin (15 267) and lysozyme (15 026).

Effect of irradiation

It is very difficult to trace all the changes which occurred in the 26 proteins liberated from the tested muscles. It is convenient, however, to make a general survey about the differences between the irradiated and the untreated samples. Major differences can be detected (Fig. 1) in the patterns of irradiated and fresh muscle, e.g. disappearance of some lines, weakening of others and alterations in MW. A number of diverse mechanisms were operative in bringing about the changes in proteins following irradiation.

Radiosensitive polypeptide chains

The first band (Fig. 1), which has been characterized as α-macroglobulin (372 391), was completely absent in all irradiated samples. A cleavage, or a scission of its polypeptide chain into smaller molecular weight fragments, may have occurred. Both the processes of aggregation and fragmentation are predictable (Clark & Richards, 1971). Similar radiosensitivity was also exhibited by plasminogen (87 397—5th band) and pyruvate kinase (55 616—8th band), i.e. the two polypeptide chains were completely absent from the electrophoretic patterns for all radiation doses. Recombination of the peptide fragments to reconstitute the identical polypeptide chain was not observed (Table 1).

The transferrin (77 162—6th band) polypeptide chain was found to be less radiosensitive. Degradation of transferrin was directly related to the radiation dose, with the molecule being eliminated at 20 kGy.

Other polypeptide chains underwent chain scission into smaller molecular

weight fragments at specific radiation energies. Recombination of these fragments may, however, occur at higher radiation energies. Among these polypeptide chains, lactoperoxidase (94 120—4th band), myoglobin (17 164—21st band) and avidin (16 663—22nd band) were detected (Table 1). Lactoperoxidase was initially identified in the untreated samples by its MW of 94 120. At 6 kGy irradiation its chain showed signs of disintegration to give an 85 650-MW compound (band 4) and at 10 kGy it was completely eliminated. Lactoperoxidase reappeared with a MW of 73 836 after irradiation at an irradiation dose of 20 kGy. Similar trends were evident for both myoglobin and avidin (Table 1 and Fig. 1).

Polypeptide chains exhibiting gradual degradation by increasing the dose level

With this category of protein chains some fragmentations have occurred, leading to a reduction in MW. Recombination processes also occur simultaneously on increasing the dose levels. For example, the heavy chain of immunoglobulin G was characterized in the untreated muscle by its MW of 50 087 (9th band). The application of 6, 10 and 20 kGy produced MW alterations to 45 317 (8th band), 40 400 (10th band) and 40 785 (13th band), respectively. Similar trends were also observed for actin (10th band), β-tropomyosin (13th band), α-tropomyosin (14th band), tropomyosin 1, slow (17th band), and β-lactoglobulin (19th band). A continuous decrease in MW as a function of raising the dose level was observed (Table 1) in tropomyosin C, fast (18th band), and myosin light chain 3, fast (23rd band).

Polypeptide chains exhibiting initial degradation followed by further polymerization

Irradiation of this group of proteins produced a considerable reduction in the MW at certain irradiation doses that were followed by progressive polymerization as the dose level increased (Table 1). This group of proteins included myosin heavy chain, α-actin, phosphorylase, pepsin, carboxypeptidase A, carbonic anhydrase, myosin light chain 1, fast ($LC1_f$), and myosin light chain 2, fast ($LC2_f$).

Myosin heavy chain (MHC) was characterized in the untreated muscle with a MW of 219 615 (2nd band). Its MW was transformed to 214 615 (1st band), 244 714 (1st band) and 267 656 (1st band) by applications of 6, 10 and 20 kGy, respectively. It is evident that a 6-kGy irradiation dose produced fragmentation, indicated by a loss in MW, but at increased dose levels gradual repolymerization was induced, causing the MW to increase.

Similar effects are seen with α-actin (α-AC), phosphorylase, pepsin,

carboxypeptidase A, carbonic anhydrase, and myosin light chains 1 and 2, fast (Table 1 and Fig. 1).

The last three bands in the electrophoretic pattern of the untreated muscle (haemoglobin, 24th band; α-lactalbumin, 25th band; and lysozyme, the last band) were not easily traced after irradiation treatments (Table 1 and Fig. 1). This may be attributed to their presence in minor quantities, their low but almost similar MWs and changes induced in these fractions.

Effect of cold storage

Hassan *et al.* (1988*a*,*b*) and Hegazy (1988) found that the shelf-life of unirradiated chilled chicken ($4 \pm 1°C$) was mainly limited by the deterioration of sensory attributes. These correlated well with the microbial load and protein breakdown products. The shelf-lives of samples irradiated with 6, 10 and 20 kGy were also mainly limited by sensory attributes which showed a correlation with lipid oxidation products rather than protein breakdown.

The protein patterns of both the untreated and irradiated samples during cold storage are confused by protein fragmentation occurring as a result of residual proteolytic activity in the muscle. The application of radiation did not accomplish a complete microbiological or enzymatic inactivation. Proteolytic activity of microbial and muscle enzyme origins causes the breakdown of different muscle proteins. The electrophoretic pattern results obtained during cold storage ($4 \pm 1°C$) of both untreated or irradiated samples are shown in Table 3 and Fig. 2. The electrophoretic pattern of untreated samples originally contained 26 bands with a corresponding MW ranging between 372 391 and 15 026. At the end of the shelf-life of these samples (12 days) the number of protein bands increased to 48, accompanied by a reduction in the MW range to 244 714 and 11 813. It is evident that most of the original bands were subjected to proteolysis, causing the breakdown of protein chains. The 1st and 2nd bands in the fresh muscles, for example, which were characterized as α-macroglobulin (MW = 372 391) and myosin heavy chain (MW = 219 615), underwent a considerable reduction in their MW after 12 days of cold storage, being reduced to 244 714 and 171 300, respectively.

In the samples irradiated with 6 kGy, electrophoretic patterns initially contained 27 protein bands ranging in MW from 214 615 to 14 346. After 12 days of cold storage, 32 protein bands could be distinguished in the gel with molecular weight range between 199 186 and 12 046. A relatively slow rate of protein breakdown was observed during storage compared to that observed in unirradiated samples. During further cold storage up to 32 days (the end of shelf-life of the 6-kGy lot), a progressive increase in the number of protein bands occurred to about 46 bands of lower MW ranging between174 795

TABLE 2
Radiation-Induced New Protein Fractions from *Pectoralis major* Muscle of Chicken

6 kGy		*10 kGy*		*20 kGy*	
Number of band	*MW*	*Number of band*	*MW*	*Number of band*	*MW*
2	135 952	2	124 130	2	167 941
13	27 718	4	66 914	3	125 955
16	23 465	5	61 178	4	94 120
17	22 658	6	57 100	5	87 397
19	20 688	9	46 048	8	63 444
25	15 267	15	25 340	9	57 483
		18	22 189	10	51 596
		19	21 359	12	46 048
		20	20 941	18	25 117
		21	19 599	21	22 539
		23	18 991	22	21 520
		26	17 303	23	20 992
		28	16 039	25	19 510
		32	14 087	29	16 221
		33	13 904	30	15 920
		34	13 638	34	14 641
		35	13 279	36	14 110
		36	12 841	37	13 814
		37	12 449	38	13 595
				39	13 382
				40	12 996
				41	12 783
				42	12 632
				43	12 449
				44	12 200

and 12 467 (Fig. 2). The obvious retardation of the rate of protein breakdown could be attributed to the effect of irradiation that drastically reduced the microbial load (Idziak & Incze, 1968; Kiss & Farkas, 1972; Josephson *et al.*, 1974; Hassan, 1977, 1980).

The electrophoretic patterns of both 10 and 20 kGy irradiated muscles contained 37 and 44 bands (at 0 time) with corresponding MW ranges of 244 714–12 449 and 267 656–12 200, respectively. At this relatively high irradiation level two types of reactions might have taken place during storage: polymerization and cleavage (radiation after-effects). The numbers of protein bands distinguished after 12 days (4 ± 1°C) in the electrophoretic patterns of the two samples were 34 (208 902–12 097 MW range) and 36 (244 714–12 063 MW range). This means that a reduction in the protein

TABLE 3
Effect of Cold Storage on the *Pectoralis major* Muscle Proteins of Irradiated Chicken resolved by SDS/Polyacrylamide Gel Electrophoresis

0 kGy/12 days		*6 kGy/12 days*		*10 kGy/12 days*		*20 kGy/12 days*		*10 kGy/32 days*		*20 kGy/32 days*		*20 kGy/55 days*	
Number of band	*MW*	*Number of band*	*MW*	*Number of band*	*MW*	*Number of band*	*MW*	*Number of band*	*MW*	*Number of band*	*MW*	*Number of band*	*MW*
1	244 714	1	199 186	1	208 902	1	244 714	1	199 186	1	219 615	1	190 333
2	171 300	2	167 941	2	178 437	2	155 727	2	147 672	2	171 300	2	171 300
3	124 130	3	140 409	3	101 964	3	118 958	3	138 145	3	97 329	3	155 727
4	100 764	4	117 328	4	81 571	4	101 964	4	125 955	4	81 571	4	138 145
5	89 218	5	91 117	5	66 914	5	85 650	5	114 200	5	76 473	5	80 801
6	72 584	6	74 478	6	59 895	6	76 473	6	85 650	6	69 072	6	63 444
7	67 440	7	65 884	7	53 531	7	62 977	7	71 375	7	61 618	7	58 664
8	62 518	8	59 068	8	48 117	8	59 895	8	59 479	8	50 087	8	54 503
9	57 483	9	45 558	9	40 785	9	54 903	9	54 554	9	47 583	9	49 796
10	53 531	10	37 077	10	37 898	10	48 117	10	51 909	10	45 078	10	44 842
11	50 382	11	33 588	11	33 457	11	41 780	11	45 558	11	42 825	11	40 211
12	45 078	12	28 935	12	30 158	12	34 123	12	39 837	12	39 837	12	35 539
13	41 577	13	27 808	13	27 451	13	31 145	13	35 687	13	38 408	13	32 320
14	39 109	14	26 599	14	25 265	14	29 033	14	32 320	14	37 077	14	29 843
15	36 602	15	23 595	15	24 263	15	27 718	15	30 589	15	35 836	15	28 741
16	34 959	16	22 074	16	22 598	16	25 191	16	28 935	16	34 536	16	27 104
17	33 457	17	20 344	17	21 305	17	23 791	17	27 899	17	33 588	17	26 353
18	29 534	18	19 554	18	20 588	18	21 683	18	26 765	18	30 052	18	24 126
19	28 081	19	18 824	19	19 918	19	20 638	19	25 567	19	26 849	19	22 840
20	26 765	20	17 769	20	19 421	20	19 735	20	24 471	20	24 194	20	21 628
21	24 826	21	16 994	21	18 824	21	19 118	21	23 086	21	23 337	21	20 992
22	24 126	22	16 471	22	18 031	22	18 301	22	21 863	22	21 683	22	20 296
23	23 148	23	16 129	23	17 303	23	17 659	23	20 738	23	20 152	23	19 247
24	22 539	24	15 890	24	16 794	24	17 268	24	20 011	24	18 824	24	17 881
25	21 628	25	15 322	25	16 376	25	16 794	25	19 247	25	17 843	25	17 268
26	20 941	26	14 157	26	15 744	26	16 009	26	18 419	26	17 303	26	16 695
27	20 296	27	13 770	27	14 844	27	15 715	27	17 843	27	16 761	27	16 283
28	19 334	28	13 424	28	14 394	28	14 973	28	17 303	28	15 861	28	15 686
29	18 824	29	13 116	29	13 488	29	14 370	29	17 027	29	15 240	29	15 079
30	18 223	30	12 899	30	13 176	30	13 859	30	16 728	30	14 666	30	14 741
31	17 843	31	12 235	31	12 938	31	13 488	31	16 314	31	13 949	31	14 180
32	17 303	32	12 046	32	12 577	32	13 096	32	15 460	32	13 361	32	13 747
33	16 994			33	12 306	33	12 821	33	15 026	33	13 096	33	13 403
34	16 314			34	12 097	34	12 503	34	14 349			34	13 076
35	15 715					35	12 323	35	13 836			35	12 577
36	15 159					36	12 063	36	13 279			36	12 306
37	14 869							37	12 918				
38	14 641												
39	14 227												
40	13 926												
41	13 660												
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46	12 323												
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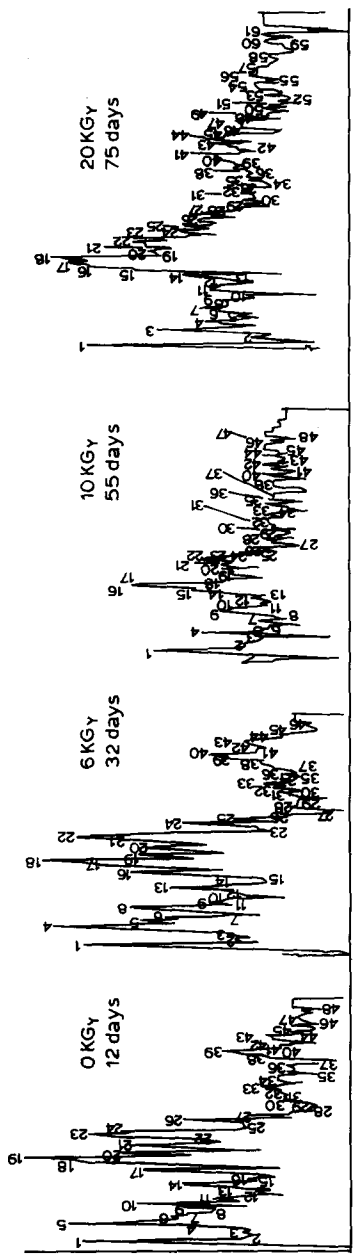

Fig. 2. Effect of irradiation treatments and cold storage ($4 \pm 1°C$) up to the end of shelf-life on the electrophoretic pattern of *Pectoralis major* muscle proteins of chicken.

bands by three (in the case of 10 kGy) and eight (in the case of 20 kGy) took place during 12 days of storage. It seems likely that some combinations between radical pairs involved in the initial bond scission would re-form the original compounds. With the progression of cold storage ($4 \pm 1°C$) up to 32 days, 37 (199 186–12 918 MW range) and 33 (219 615–13 096 MW range) protein bands (Fig. 2 and Table 3) could be resolved in those samples previously irradiated with 10 and 20 kGy, respectively. The rate of hydrolysis of protein molecules was slow during the first 32 days of cold storage of these samples followed by an extensive breakdown of protein chains, particularly by the end of shelf-life of each irradiated lot, i.e. 55 days (10 kGy) and 75 days (20 kGy). At the end of the shelf-life of the 10-kGy irradiated lot, for example, the electrophoretic pattern was heavily loaded with 48 protein bands with an average MW ranging between 186 195 and 13 488. It is apparent that a continuous fragmentation of molecules occurred with a consequent MW decrease. The first band (MHC) in the electrophoretic pattern of 10-kGy irradiated samples (Tables 1 and 3) showed a reduction in MW all over the storage period: 244 714 (0 days), 208 902 (12 days), 199 186 (32 days) and finally 186 195 (Fig. 2) at the end of shelf-life (55 days). The 20-kGy irradiated lot showed similar trends (Table 3 and Fig. 2). At the end of their shelf-life the electrophoretic pattern contained 61 bands (Fig. 2) with very small quantities of MW ranging between 167 941 and 9063. In fact all these protein chains degenerated into many small entities that could not be considered as identifiable native proteins. The mechanism of protein breakdown or degradation cannot be expected to be similar in differently treated samples. This might be attributed to alterations in the chemical characteristics of the medium, enzyme activity and the microbial profile of the tested samples induced by different irradiation doses. Immediately after irradiation, the psychrophilic bacterial counts of the carcasses receiving 0, 6, 10 and 20 kGy were $2{\cdot}3 \times 10^5$, $4{\cdot}0 \times 10^2$, $8{\cdot}5 \times 10^1$ and $1{\cdot}3 \times 10^1$ cell/g, respectively. During subsequent cold storage, progressive increases in the psychrophilic bacterial counts of unirradiated 6, 10 and 20 kGy irradiated samples reached $2{\cdot}0 \times 10^8$, $8{\cdot}9 \times 10^8$, $7{\cdot}9 \times 10^7$ and $2{\cdot}9 \times 10^7$ cell/g after 12, 32, 55 and 75 days of cold storage ($4 \pm 1°C$), respectively (Hegazy, 1988).

Radiation-induced protein fractions

The absorption of radiation energy by polypeptide chains causes different types of reactions depending upon the dose absorbed, temperature and the presence or absence of oxygen. Among these reactions are the breaking of peptide bonds, primary deamination, secondary deamination with main scission, oxidation of sulphydryl groups, and combination with and addition to polypeptide chains (Delincee, 1982). The results shown in Table 2

reveal that irradiation treatments with 6, 10 and 20 kGy induced 6, 19 and 25 protein fractions with estimated MWs between 135 952 and 15 267, 124 130 and 12 449, and 167 941 and 12 200, respectively. It should also be noted that a proportional relationship exists between the irradiation dose and the number of small fragments detected. The extent to which these changes took place and the time period over which the phenomena occurred depended ultimately on the applied irradiation dose.

In conclusion, the electrophoretic technique used in the present study could be used to detect prior irradiation treatments of chicken meat. The disappearance of α-macroglobulin, plasminogen, myoglobin, avidin and lysozyme as well as the appearance of six new protein fractions appear characteristic of chicken flesh that has received a 6-kGy irradiation dose. Higher irradiation doses of 10 and 20 kGy are characterized by the complete elimination of 8 and 7 bands, respectively, as well as the disappearance of pyruvate kinase, haemoglobin and α-lactalbumin. The absence of transferrin, alterations in MW and the radiation induction of 25 new protein fractions are typically associated with 20-kGy treatments. These differences clearly demonstrate the potential of this electrophoretic technique in the identification of irradiated chicken.

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