X-RAY ANALYSIS OF BEEF SAUSAGE AND CHICKEN PATTIES MANUFACTURED WITH OR WITHOUT PROTEASE INHIBITOR.

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SUMMARY:

Protease inhibitor that isolated from potato tubers (alpha-variety) was used in its crude form in the processing of beef sausage and chicken patties. The processed samples that contained 0, 1, 2, 3% of protease inhibitor were stored at 4 C for two weeks through which X-ray analysis was considered. Experimental analysis indicated that the investigated samples containing the protease inhibitors showed a better configuration and higher retention of the responsed elements (31 elements) which correlated with the structure of both beef sausage and chicken patties.

INTRODUCTION:

Today, many areas of X-rays analysis have been developed such as X-ray absorption, diffraction, fluorescence, emission, absorption edge, low angle scattering, K-capture,soft X-rays, and most recently, the electron probe. In direct quantitative analysis, X-ray fluorescence is now the most important technique with X-ray absorption which is quite valuable in many cases, (Pomeranz and Meloan 1971). On the other hand, the X-ray powder photograph of a substance is also a valuable characteristic property which can be used for identification, particularly for solids which give no useful vibrational spectra.

Potatoes are among several major crops which contain higher concentrations of protease inhibitors; Santarius and Belitz (1978). These are proteins that inhibitor prevent the activities of the pancreatic digestive protease including trypsin, chymotrypsin, elastase and carboxypeptidase A and B (Ryan & Hass, 1981). Potatoes are almost unique in that they also contain carboxypeptidase inhibitor; previously isolated only in tomatoes (Hass et al., 1979); in addition they are a rich source of plasma kallikrein inhibitors(Richardson, 1977). Enteroxkinase-inhibiting activity was also found in tubers of two potato cultivars (Lau et al., 1980).

It was aimed through such research to use the crude protease inhibitor (1,2 and 3%) in processing of beef sausage and chicken patties. Such trend was considered to see to what extent the protease inhibitor that isolated from potato could control the activity of protease enzymes during storage of the aforementioned products for two weeks at 4°C. In such case, X-ray analysis of the aformentioned samples was compared.

MATERIALS AND METHODS:

A. Materials:

* Beef meat, spices and chicken samples:

\- Beef meat and spices were obtained from "RAMADA" refrigerator of the"GERCO"Company; related to the Ministry of supplies, Egypt. The spices mixture are composed of the following ingredients.

Ingredients	Weight/gm	Ingredients	Weight/gm
Black pepper	20	Red pepper	10
Vitamin C	20	Nut meg	10
Cardamom	10	Cubeb (powder)	10
Coriander	20	TOTAL	100

\- "EL SHARK EL AWSAT" Company that located in "KALYOBIAH" governorate (EGYPT) is the source of frozen chicken samples; with an individual weight that ranged between 850 up to 1100 gm.

** Potato tubers (alpha variety):

The Horticulture Department, Agricultural Research Centre, Ministry of Agriculture is the source of potato tubers (alpha variety) that used as a source of extraction of protease inhibitor.

B. Technological Methods:

* Extraction of protease inhibitor was performed as described by Belitz et al., (1971).

** Manufacturing of beef sausage and chicken patties:

The formula of beef asausage and chicken patties were based on using the previous meat sources which were grinded by an electrical meat mincer and remixed with spices and starch in preparing of the control sample (Free of protease inhibitor; P.I.).On the other hand,other samples were manufactured in the presence of the crude P.I. to reach the level of 1, 2 and 3% of the recipe formula as follows:

Ingredients	Control (Free of P.I)	Levels of protease inhibitor (P.I) in the tested samples		
		1%	2%	3%
Meat (Beef or chicken)	90	90	90	90
Spices	1	1	1	1
Protease inhibitor	0	1	2	3
Starch	3	2	1	0
Water	6	6	6	6

With respect to the beef sausage samples, they were prepared by stuffing the responsed mixture in a natural casings (small intestine) after which they were kept in a refrigerator at 4°C for 15 days. The chicken patties were prepared by using the previous formula after shapping in patties forms and the produced samples were wrapped in a parchement paper and kept at 4°C for 15 days. Before analysis, both of the beef sausage and chicken patties samples were dried at 60°C overnight.

C. Analytical method:

X-ray fluorescence of the samples was figured using the X-ray apparatus Model 84200/10 produced by the Applied Research Laboratory, Switzerland. Experiments were carried out at the chemical department of the X-ray fluorescence laboratory of the Egyption Organization for Standardization and Quality Control, Ministry of Industry. Samples were held in the usual apparatus holder and the source of the X-ray bean was "Cu-K" with a wave length of 1.5405 °A. However, angles were measured from zero to 90 using lithium 2000 crystal and from 45 to 150 using FOPET crystal. The responsed elements of the periodical table was computed in "K count/Second" as well as their relative intensities within the aforementioned angles. The method however had been mentioned in details by Nishiuchi and Takahashi (1966).

RESULTS AND DISCUSSION:

The X-ray intensities of the investigated samples given in Tables (1 and 2)and the relative distribution area % of the 31 elements were used to compare between the beef and chicken products that manufactured with and without using the crude form of protease inhibitor. X-ray analysis of both the stored and the unstored beef sausage samples indicated a very distinctive sharp distribution area of the specific 31 elements which forming a nomen-clature fingerprints as seen in Figs. (1 and 2). These figures are characterized by neighbour areas namely "A" to "G" which constructed the group of the identified 31 elements as given in Table (3).

As a result of adding the "P.I" in the beef sausage samples, the characterizing elements (neighbouring groups) in the unstored samples seem to be in symmetric forms as those of the samples which prepared without the inhibitor.The only differences lie in the intensities of elements No., 13 (Ca), 14 (K), 15 (Cl) and 17 (S). On contrary, during storage of the control sample at 4 C several changes took place in the intensity of the investigated elements, a trend which may be related to the disturbance occuring in mass distribution of the elements within the investigated samples. This simply means that the activity of protease enzymes as well as the autolysis of the tested samples may lead to a type of breakdown of the protein molecules. Such previous trend was out of detection in the beef sausage containing the P.I. as seen in Table (3).

Regarding the pattern of changes in the X-ray diagram in the form of elements configuration of the investigated chicken patties, the data of Table (4) that resumed from Figs. (3 and 4) inidicated the main following trends.

** The characterized area in cm^2 of group "C" (17.8 C_1 + 9.2 C_2)that represents 27.0% of the total distributed areas reached to 38.5% (11.3 C_1 + 27.2 C_2) and to 29.8 (11.6 C_1 + 18.2 C_2) for the samples stored for one and two weeks at 4 C respectively.

** Elements of group "F" and "G" indicate a reversible pattern of changes. The former one having a relative distribution area of 6.3, 11.7 and 5.60% for 0, 1 and 2 weeks of storage at 4°C, while the latter one showed a value of 13.6, 8.3 and 16.8% at a corresponding similar conditions of storage.

** As a result of adding the protease inhibitor, the rupturing of element distribution was minimized greatly. For instance, the main "C" group showed appproximatly a constant area of distribution whatever the added % of the inhibitor or whatever the storage period that extended to two weeks at 4°C.

The aforementioned results indicated the presence of a degree of unbalance in the mass channels of the distribution of the 31 elements (that identified by X-ray) during storage of the investigated samples prepared without protease inhibitor. Since protein is the major constituents in the beef sausage and chicken patties, so any deterioration in protein as a result of protease enzymes, will reflect on the distribution pattern of these elements. This is held true since it could be imaged that within any given food item, elements acting as a structural configuration of specific span within which the other chemical constituents are distributed. Subsequently, the previous results proved the capability of protease inhibitor that added during processing of beef sausage and chicken patties in minimizing and/or controlling the disorders of the distribution pattern of the investigated elements that occurred during storage of the control sampled at 4°C; i.e. free of P.I. as a result of the activity of protease enzymes.

CONCLUSION:

As a result of adding protease inhibitor "P.I." in the beef sausage samples or in chicken patties the characterized elements (neighbouring groups) that were identified by X-ray analysis in the unstored samples seem to be in symmetic forms as that of the samples manufactured without P.I. The only differences lie in the intensities of elements No, 13 (Ca), 14(K), 15(Cl) and 17(S). On contrary, during storage, several changes took place in the intensities of the tested elements, a trend which may be related to the changes occur in mass equilibrium distribution of the elements within the investigated samples. This simply means that the activity of protease enzymes as well as the autolysis of the tested samples may lead to a type of breakdown of the protein molecules. This is held true since it could be imaged that within any given food items, elements acting as a structural configuration of specific span within which the other chemical constituents are distributed. However, results indicated the capability of protease enzymes in minimizing the disorders of the distribution pattern of elements that occurred in the control samples.

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Table (1): X-ray intensities as k counts/sec. of the main elements scattered within the beef sausage. (unstored samples).

Element No.	Angels	Elements and their symbols		X-Ray intensities (k counts/sec) of the: Sample without P.I.	Samples containing P.I. 1 %	2 %	3 %
1.	54.98	Sodium	Na	0.27785	0.34892	0.40692	0.434880
2.	45.08	Mangnesium	Mg	0.27005	0.35992	0.38904	0.439010
3.	45.03	Copper	Cu	0.52204	0.65108	0.65515	0.679100
4.	41.8	Zirconium	Zr	1.01427	1.08630	1.05133	1.100300
5.	15.31	Cadmium	Cd	0.57807	0.67015	0.62803	0.686150
6.	48.67	Nickel	Ni	0.47386	0.54699	0.53896	0.585960
7.	14.04	Tin	Sn	0.37700	0.45906	0.43303	0.492040
8.	52.8	Cobalt	Co	0.41196	0.48784	0.46285	0.525900
9.	87.17	Barium	Ba	0.11901	0.18497	0.19997	0.258980
10.	28.26	Lead	Pb	2.69115	2.84036	2.77728	2.862410
11	30.45	Arsenic	As	0.87718	0.97225	0.94622	1.002250
12.	57.52	Iron	F	0.69508	0.84501	0.83103	0.929140
13.	113.09	Calcium	Ca	1.35922	1.45206	1.56829	1.746190
14.	136.69	Potassium	K	6.31305	5.81535	6.59365	6.354210
15.	65.49	Chlorine	Cl	49.45490	37.63510	40.25770	35.051700
16.	75.85	Sulfur	S	10.07320	9.21396	10.27260	10.345800
17.	89.56	Phosphorus	P	4.14917	3.21521	3.30920	3.361470
18.	41.35	Mercury	Hg	3.08384	3.26415	3.21210	3.239120
19.	22.55	Zirconium	Zr	1.01427	1.08630	1.05133	1.100300
20.	18.42	Ruthenium	Ru	2.52088	2.60403	2.38270	2.430780
21.	31.89	Selenium	Se	0.85622	0.92626	0.91424	0.982285
22.	26.62	Rubidium	Rb	1.01924	1.11540	1.08729	1.120370
23.	62.97	Manganese	Mn	0.37299	0.44405	0.41884	0.499990
24.	38.06	Platinum	Pt	3.08384	3.26415	3.20210	3.239120
25.	109.21	Silicon	Si	0.40004	0.57702	0.67697	0.672160
26.	20.33	Molybdenum	Mo	1.00831	1.08530	1.05530	1.109340
27.	145.12	Aluminum	Al	0.24484	0.35190	0.40692	0.429840
28.	14.61	Indium	In	0.29298	0.28496	0.29092	1.824360
29.	21.4	Neodymium	Nb	1.00732	1.08729	1.03027	1.077350
30.	69.36	Chromium	Cr	0.33402	0.39683	0.38904	0.455980
31.	69.41	Promethium	Pm	0.29298	0.28496	0.29092	1.824360

P.I. = Protease inhibitor.

Table (2): X-Ray intensities as k counts/sec. of the main elements scattered within the chicken patties. (Unstored samples).

Element No.	Angels	Elements and their symbols		X-Ray intensities (k counts/sec) of the: Sample without P.I.	Samples containing P.I. 1 %	2 %	3 %
1.	54.98	Sodium	Na	0.09285	0.20403	0.19692	0.17904
2.	45.08	Mangnesium	Mg	0.09904	0.21893	0.21985	0.20793
3.	45.03	Copper	Cu	0.48101	0.68009	0.72112	0.67015
4.	41.8	Zirconium	Zr	0.88622	0.96122	0.98228	0.92427
5.	15.31	Cadmium	Cd	0.45101	0.54111	0.53505	0.51906
6.	48.67	Nickel	Ni	0.24002	0.49999	0.49701	0.46790
7.	14.04	Tin	Sn	0.24994	0.36399	0.35206	0.33001
8.	52.8	Cobalt	Co	0.17400	0.42984	0.42801	0.42296
9.	87.17	Barium	Ba	0.01301	0.09795	0.09795	0.10898
10.	28.26	Lead	Pb	2.99464	2.91049	3.01272	3.09686
11.	30.45	Arsenic	As	0.82513	0.88423	0.92427	0.88324
12.	57.52	Iron	F	0.69806	1.05599	0.93900	0.83401
13.	113.09	Calcium	Ca	1.78149	2.17053	1.88030	1.52528
14.	136.69	Potassium	K	11.07030	10.99450	10.99960	11.22540
15.	65.49	Chlorine	Cl	7.77176	7.20174	6.41435	6.26599
16.	75.85	Sulfur	S	20.27040	21.42860	23.33770	21.11500
17.	89.56	Phosphorus	P	7.77795	6.60181	6.12186	5.65602
18.	41.35	Mercury	Hg	3.27120	3.26514	3.35137	3.23514
19.	22.55	Zirconium	Zr	0.88622	0.96122	0.98228	0.92427
20.	18.42	Ruthenium	Ru	2.19146	2.27451	2.31455	2.13732
21.	31.89	Selenium	Se	0.79413	0.86427	0.88920	0.84519
22.	26.62	Rubidium	Rb	1.00732	1.03931	1.06335	1.00027
23.	62.97	Manganese	Mn	0.11990	0.39385	0.37390	0.35190
24.	38.06	Platinum	Pt	3.27120	3.26514	3.35137	3.23514
25.	109.21	Silicon	Si	0.32805	0.52085	0.43488	0.49793
26.	20.33	Molybdenum	Mo	0.87221	0.93718	0.97324	0.93321
27.	145.12	Aluminum	Al	0.07405	0.26387	0.20999	0.20793
28.	14.61	Indium	In	0.17904	0.24186	0.26501	0.24988
29.	21.4	Neodymium	Nb	0.87023	0.94026	0.95020	0.89824
30.	69.36	Chromium	Cr	0.07496	0.32095	0.33287	0.31292
31.	69.41	Promethium	Pm	0.17904	0.24186	0.26501	0.24988

P.I. = Protease inhibitor.

Table (3): Element groups and their relative distribution areas in beef sausage samples processed with or without protease inhibitor.

Storage period (weeks)	Neighbours area in character	Beef sausage free of inhibitor				Beef sausage manufactured with 1%inhibitor				2%inhibitor				3% inhibitor			
		N.D.E From	N.D.E To	Area in cm^2	R.D.A %	N.D.E From	N.D.E To	Area in cm^2	R.D.A %	N.D.E From	N.D.E To	Area in cm^2	R.D.A %	N.D.E From	N.D.E To	Area in cm^2	R.D.A %
Zero	A	1	8	1.40	11.10	1	6	1.20	11.30	1	7	1.10	10.20	1	7	1.00	9.50
	B	9	11	1.80	14.20	9	11	1.13	10.70	9	11	1.10	10.20	9	11	0.95	9.00
	C	12	19	6.50	51.40	12	19	5.60	52.90	12	19	6.00	55.50	12	19	5.70	54.10
	D	19	21	0.50	4.00	19	25	0.45	4.3	19	21	0.45	4.10	19	21	0.43	4.10
	E	23	25	1.10	8.70	23	25	1.00	9.40	23	25	1.00	9.20	23	25	0.88	8.30
	F	25	27	0.60	4.70	25	27	0.33	3.10	25	27	0.37	3.40	25	27	0.38	3.60
	G	28	31	0.75	5.90	28	31	0.88	8.30	28	31	0.80	7.40	27	30	1.20	11.40
One	A	2	8	2.20	15.40	2	7	2.10	16.20	2	7	1.60	12.30	2	8	1.40	11.40
	B	9	11	1.10	7.70	9	11	1.10	8.50	9	11	1.10	8.40	9	11	0.95	7.70
	C	12	18	7.30	50.90	12	18	6.50	50.10	12	18	7.10	54.40	12	18	6.80	55.20
	D	18	21	1.40	9.70	18	21	1.30	10.00	18	21	1.30	9.90	18	21	1.27	10.30
	E	23	25	Zero	Zero	23	25	Zero	Zero	23	25	Zero	Zero	23	25	Zero	Zero
	F	25	27	0.83	5.80	25	27	0.77	5.90	25	27	0.66	5.10	25	27	0.59	4.80
	G	28	31	1.50	10.50	28	31	1.20	9.30	28	31	1.30	9.90	28	31	1.30	10.60
Two	A_1	1	3	0.20	2.50	2	7	1.10	9.50	2	7	1.10	9.70	2	7	1.10	10.40
	A_2	3	5	0.20	2.50												
	B	9	11	0.60	7.60	9	11	0.93	8.00	9	11	0.87	7.70	9	11	0.86	8.10
	C	12	19	5.20	65.90	12	19	6.00	51.60	12	19	6.40	56.70	12	19	5.70	53.90
	D	19	21	0.32	4.10	19	21	0.50	4.30	19	21	0.45	4.80	19	21	0.45	4.30
	E	23	25	0.60	7.60	23	25	1.40	12.00	23	25	0.88	7.80	23	25	0.93	8.80
	F	25	27	Zero	Zero	25	27	0.40	3.40	25	27	0.30	2.70	25	27	0.34	3.20
	G	27	30	0.77	9.80	27	30	1.30	11.20	27	30	1.20	10.60	27	30	1.20	11.30

N.D.E = Number of the distributed elements.
R.D.A% = Relative distribution area %.

Table (4): Element groups and their relative distribution areas in chicken patties samples processed with and without protease inhibitor.

Storage period weeks	Neighbours area in character	Chicken patties free of inhibitor				Chicken patties 1% inhibitor				Chicken patties 2% inhibitor				Chicken patties 3% inhibitor			
		N.D.E From	N.D.E To	Area in cm^2	R.D.A %	N.D.E From	N.D.E To	Area in cm^2	R.D.A %	N.D.E From	N.D.E To	Area in cm^2	R.D.A %	N.D.E From	N.D.E To	Area in cm^2	R.D.A %
Zero	A	2	8	2.30	24.00	2	7	1.40	23.30	2	7	1.40	24.80	2	7	1.00	14.10
	B	9	11	1.70	17.80	9	11	1.00	16.60	9	11	0.90	15.90	9	11	1.10	15.60
	C_1	12	15	0.88	9.20	11	15	0.58	9.60	12	15	0.66	11.70	12	15	1.00	14.10
	C_2	15	19	1.20	12.60	15	19	1.20	19.90	15	19	0.66	11.70	15	19	1.40	19.80
	D	19	21	0.38	4.00	19	21	0.36	6.00	19	21	0.35	6.20	19	21	0.42	5.90
	E	23	25	1.20	12.60	23	25	0.70	11.60	23	25	0.75	13.30	23	25	1.10	15.60
	F	25	27	0.60	6.30	25	27	0.30	5.00	25	27	0.48	8.50	25	27	0.46	6.50
	G	27	30	1.30	13.60	28	30	0.48	8.00	28	30	0.45	7.90	28	30	0.59	8.30
One	A	2	8	1.20	15.60	2	7	1.20	16.50	2	7	1.40	16.50	2	8	1.70	19.20
	B	9	11	0.90	11.70	9	11	0.96	13.20	9	11	0.84	9.90	9	11	0.78	8.80
	C_1	12	15	0.87	11.30	12	15	0.85	11.70	12	15	0.85	10.00	12	15	0.76	8.60
	C_2	15	18	2.10	27.20	15	18	1.00	13.70	15	18	2.00	23.60	15	18	2.20	24.20
	D	18	21	1.10	14.30	18	21	0.95	13.00	18	21	1.00	11.80	18	21	0.88	9.90
	E	22	24	Zero	Zero	22	24	Zero	Zero	22	24	Zero	Zero	22	24	Zero	Zero
	F	24	27	0.90	11.70	25	27	0.52	7.20	25	27	0.60	7.10	25	27	0.73	8.20
	G	28	30	0.64	8.30	27	30	1.80	24.70	27	30	1.80	21.20	27	30	1.80	20.30
Two	A	2	7	1.10	15.40	2	7	1.10	15.00	2	7	0.20	3.20	2	7	1.10	14.80
	B	9	11	0.95	13.30	9	11	1.20	16.40	9	11	0.80	12.70	9	11	0.93	12.50
	C_1	12	15	0.83	11.60	12	15	0.70	9.60	12	15	0.80	12.70	12	15	0.78	10.50
	C_2	15	19	1.30	18.20	15	19	1.50	20.50	15	19	1.70	26.90	15	19	1.50	20.20
	D	19	21	0.40	5.60	19	21	0.40	5.50	19	21	0.50	7.90	19	21	0.44	6.00
	E	23	25	0.98	13.70	23	25	0.88	12.00	23	25	0.90	14.20	23	25	1.00	13.50
	F	25	27	0.40	5.60	25	27	0.34	4.60	25	27	0.32	5.10	25	27	0.37	5.00
	G	27	30	1.20	16.80	27	30	1.20	16.40	27	30	1.10	17.40	27	30	1.30	17.50

N.D.E. = Number of the distributed elements.
R.D.A % = Relative distribution area %.

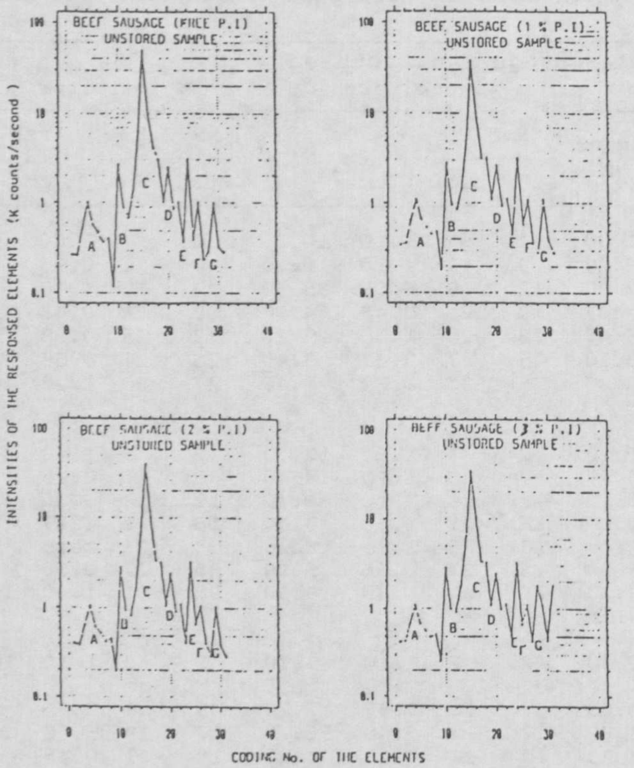
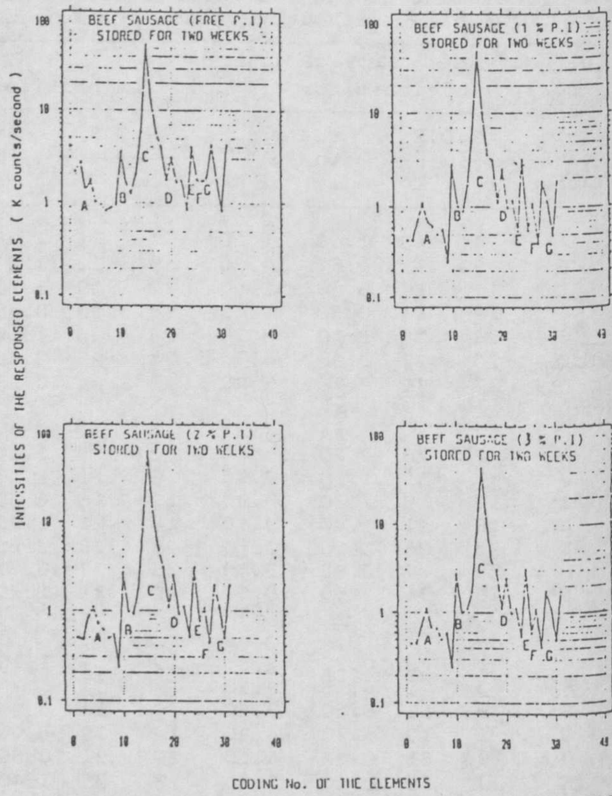


FIG (1) X-RAY DIAGRAM OF 31 ELEMENTS WHICH FORMING THE "A" TO "G" GROUPS OF THE UNSTORED BEEF SAUSAGE SAMPLES



FIC (2) X-RAY DIAGRAM OF 31 ELEMENTS WHICH FORMIN THE "A" TO "G" GROUPS OF THE BEEF SAUSAGE SAMPLES STORED FOR TWO WEEKS

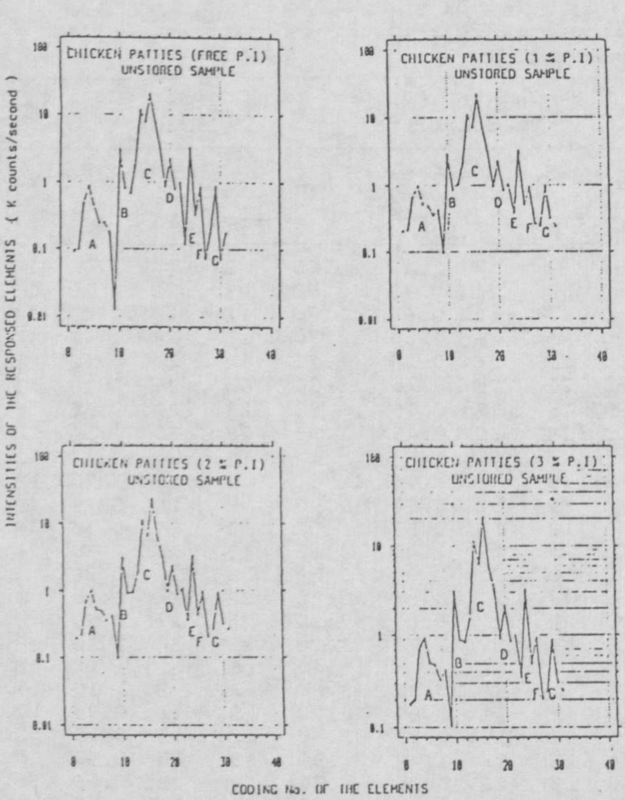


FIG (3) X-RAY DIAGRAM OF 31 ELEMENTS WHICH FORMING THE "A" TO "G" GROUPS OF THE UNSTORED CHICKEN PATTIES SAMPLES

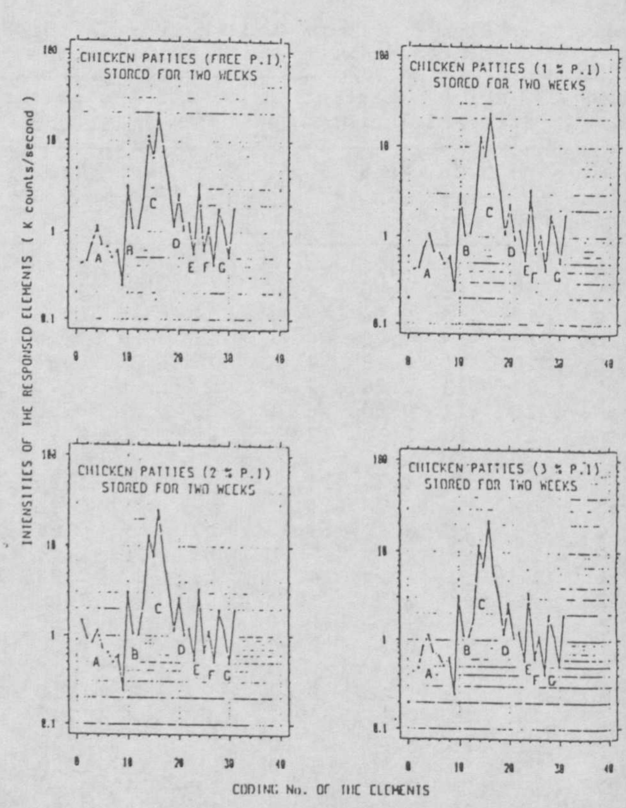


FIG (4) X-RAY DIAGRAM OF 31 ELEMENTS WHICH FORMING THE "A" TO "G" GROUPS OF THE CHICKEN PATTIES SAMPLES STORED FOR TWO WEEKS