CHANGES IN THE SOLUBLE MUSCLE PROTEINS AND IZOENZYMES OF LACTATDEHYDROGENASE IN IRRADIATED BEEF MEAT

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Introduction.

The application of ionizing irradiation for preservation of food products, continuously attracts the attention and interest of research workers, because of the big possibilities which are offered by this technology. Underlined is the following advantage - effective elimination of microorganisms causing deterioration of the products and their storage under plus temperatures.

Results from rcent investigations show, that a number of cell enzymes are radioresistant (3,5,12,14) and exercise autolytical processes, which in turn contribute for the decrease of storage life of irradiated food products.

In this aspect considerable interest present the changes in the protein and enzyme molecules of irradiated meat, which is the scope of the present investigations.

Material and Mehtodics.

Object of investigation.

Used was m.longissimus dorsi of two year old beef with a maximum standardisation of the material according to age,origin, and rearing conditions. Immediately after slaughter of the animals, were taken samples from the muscle and were hermetically closed in envelopes.

Irradiation.

The irradiation treatment of the samples was made with Co^{60} gamma irradiation under room temperature with doses of 1, 2,5, and 5 M rads, with a regime of the irradiation of 1,2 M rads per hour.

Treatment of the material.

10 g of each sample was homogenized with quartz sand in 0,15 M sodium chlorid media in 1 : 1 relation. The received homogenate was centrifuged on 10.000 r/m for 30 minutes. To the supernatant was added double quantity chilled chlorophorm and after mixing, was centrifuged on 8.000 r/m for 20 minutes. After repetition of the pro-

cedure, the delapidated supernatant, containing the soluble muscle proteins, were then electrophoretically and biochemically studied. All manipulations were effected under low temperatures (0° to +2°C).

Electrophoretic quantitative studies on proteins and LDH.

The soluble muscle priteins were fractionated electrophoretically in 1% agar gel under veronal-meidnal buffer, having pH 8,6 and ionic strength of 0,55. The dry proteinograms were stained with Amidoblack-10B and the fractions were determined with densitometer ERI-10 "zeiss".

The electrophoretic deviding of the isoenzymes of LDH was effected under the method of Wime (16,17).

The values of this protein total were measured by the photometric method of Kingsley (7). For the determination of the total LDH activity we used the method of Sevela,Tovarek (15) based on the reaction :

$$\text{sodium lactat} + \text{NAD} \xrightarrow{\text{LDH}} \text{pyruvat} + \text{NADH}_2$$

followed by colorimetric measurement of the resulted pyruvat after the reaction with 2,4 dinitrophenylhidrasin.

Results.

Characteristic of the radiation action.

In the electrophoretic deviding of the soluble muscle proteins upon agar gel were received eight fractions, which we mark from the anode to the cathode consecutively by roman figures (fig 1)

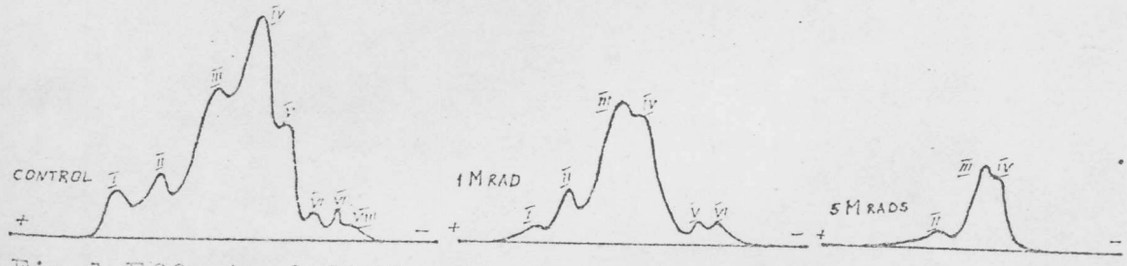

Fig.1.Effect of different doses of irradiation on the spectrum of soluble muscle proteins.

The results shown in table 1, indicate expressive changes in the relative contents of protein in the different fractions of the soluble muscle proteins, after irradiation. The proteinograms are most sensitively changed in the region of I,VII and VIII fractions. The observed changes are in relation to the doses of irradiation (fig 1) With values of 1 M rad, considerably decrease I,IV, and V fractions. Relatively lightly decrease fraction VI. Most stable to irradiation were fractions II and III, which in regard to relative content are as those of the controls.

With irradiation of 2,5 M rads, this tendency is still more underlined. All fractions exibit lower values in comparison with those

TABLE 1. EFFECT OF IONIZING RADIATION ON SOLUBLE PROTEINS

Dose /Mrads/	Fractions of soluble muscle proteins								Total soluble protein, %
	I	II	III	IV	V	VI	VII	VIII	
0,0	10,62	13,73	24,72	29,62	12,56	7,86	5,89	3,46	100,00
1,0	6,89	12,53	24,24	20,17	4,76	5,39	-	-	70,98
2,5	4,08	10,51	17,53	14,09	0,0	5,02	-	-	49,77
5,0	-	2,87	17,39	14,00	traces	traces	-	-	39,73

TABLE 2. EFFECT OF IONIZING RADIATION ON THE TOTAL ACTIVITY AND ISOENZYMES OF LDH

Dose (Mrads)	LDH Isoenzymes								Total LDH activity %	
	LDH-2		LDH-3		LDH-4		LDH-5			
	1 day	7 days	1 day	7 days	1 day	7 days	1 day	7 days	1 day	7 days
0,0	14,31	9,95	17,61	14,80	20,30	20,01	48,38	48,00	100,00	100,00
1,0	8,37	3,80	18,23	7,48	20,70	20,02	49,27	46,06	96,00	81,41
2,5	0,00	0,00	4,38	3,80	14,36	14,30	38,19	34,00	70,88	70,00
5,0	-	-	3,24	3,15	18,79	4,73	43,79	38,00	64,88	55,29

of the control and those irradiated with 1 M rad.

When the meat is irradiated with 5 M rads, totally falls off, fraction I from the soluble protein, and strongly decrease in protein content fractions II,III, and IV, while V and VI are only present in traces (table 1).

Total soluble protein from irradiated muscles decrease in percent content proportionally to the strength of the irradiation. Similar tendency is exibited in the total activity of LDH in the soluble protein (fig.3)

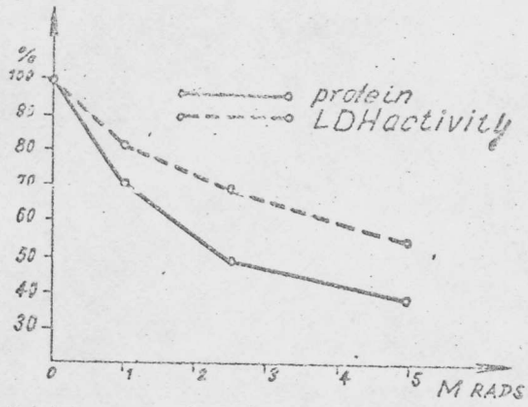

Fig.3. Relations of percent content of soluble proteins and total LDH activity

On table 2 are shown the changes wich occur with the isoenzymes and the total LDH activity in muscles studied immediately and 7 days after irradiation and kept under 2°C. It is seen that in doses of 1 M rad, LDH-2 activity decreases, while LDH-3, LDH-4, and LDH-5 are stable with this strength of irradiation. In the studies od samples irradiated with 1 M rad and a 7 days storage period under 2°C, was observed a strong decrease in the LDH-2 and LDH-3 activity in comparison with the control and the samples studied immediately after irradiation with L M rad. In the same time LDH-4 and LDH-5 do not change sensitively in activity.

As a result of irradiation of the samples with 2,5 M rads all isoenzymes of LDH, immediately after irradiation as well as after & days, decrease in acitivity in comparison to the control and the samples irradiated with 1 M rad, while LDH-2 is only present in traces. In LDH-4 and LDH-5 a relative stability is observed after 7 days storage.

After irradiation of the samples with 5 M rads is observed a very different behaviour of LDH-isoenzymes (fig 2). LDH-2 completely looses activity, LDH-3 decreases highly, while LDH-4 and LDH-5 keep values close to those of the control. Noted changes exist only with LDH-4, after 7 days of storage, which decreases sharply in activity.

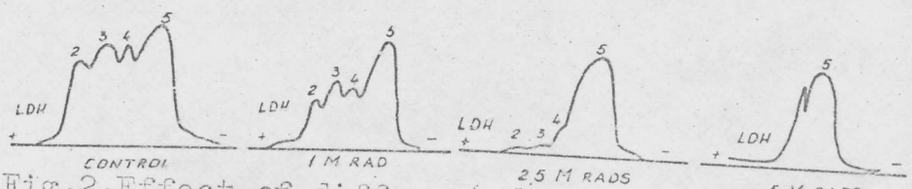

Fig.2.Effect of different doses of irradiation on the isoenzymes spectrum of muscle LDH

Total activity measured after the method of Sevela,Tovarek, reflects in a general way the decreased value of lactatdehydrogenasae activity paralel to the irradiation values and storage prolongation of the samples.

Discussion.

From the obse4ved changes in the soluble proteins and isoenzymes of LDH in muscles, could be assumed, that under the influence of gamma rays, denaturation processes occur, unevenly acting upon the individual soluble proteins and isoenzyme molecules. This could be assumed from the enrichment of the clear homogenate after a short period of rest, with sediment of flocules, and decrease in soluble proteins and lactatdehydrogenasae activity. It was een that the rapid mobile fractions of soluble proteins towards the anode and cathode and LDH-2, LDH-3, and to a certain extend LDH-4, are more unstable to irradiation treatment. Possibly, in these structures, changes in the covalent bonds are easier for the polypeptide chains, or the hydrogen and ionogene bonds between the side chains (2,4) which lead to a modification of the secondary, tertiary (and with isoenzymes and to the fourth structure) of molecules, so as to loose their specific physico-chemical properties, exibited in the case with the change of their behaviour in the electric field. This explanation is partially based on the investigations of Alexander (1), which show, that ionic irradiations lead to a desorganisation of parts of molecules by destroying the secondary bonds under the action of additional charges, as well as the conception of Joly (6) for the denaturation processes.

It is possible that the lost of the said protein fractions is due to desintegration or radiolyses of some molecules, or aggregation of others (6,11), which in turn changes the properties of solubility and electromobility observed in our experiments.

It is interesting to be noted that a very well expressed "post irradiation effect" exists with a large consecutive aggregation which envolves a big percent (above 50% with irradiation of 5 M rads) of the proteins and their separation from the solution. The remaining in the solution proteins are stable to irradiation action and are characterized with smallest mobility in the electric field.

Most interesting is the fact, that the decrease of total LDH activity under the action of irradiation is not as a result of equivalent decrease of activity for the separate isoenzymes. The resluts from the investigations show, that gamma rays affect the activity of isoenzymes which predominately are formed of B subunits. Reversively, LDH-5 built up by the A/AAAA/ subunits rest radioresistent. This data permit us to accept, that possibly the decrease of the LDH activity wit with irradiation is in relation to the degree of the part taken by the B subunits in the given isoenzymes. This discovery, gives the possibility for a new characteristic for the isoenzymic forms of LDH radioresistency. This moment is of a big theoretical value, as to now, only the total activity of enzymes under irradiation is studied (8,12,13). The behaviour of the separate molecules forms has not been object of investigations by other authors. It is of course understood, that this needs a detailed further study and experimental confirmation, so as to be used for proving irradiation treatment of meat. Similar investigations are being under way in our laboratory, to prove the different action of irradiation upon isoenzymes systems.

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