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RADIATION PASTEURIZATION OF BOVINE BLOOD PLASMA. II. EFFECT OF DOSE ON FUNCTIONAL PROPERTIES OF SPRAY DRIED PREPARATION

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The effect of gamma radiation on the functional properties of spray dried bovine blood plasma preparation was studied for a dose range of up to 50 kGy. Solubility of the preparation, its gellation capacity and viscosity of dispersions remained unaffected after treatment with doses up to 5 kGy. The results indicate that pasteurizing doses of a radiation up to 5 kGy can be considered safe from the technological point of view.

The use of gamma rediation appeared to be very effective for an improvement of the bacteriological quality of spray — dried preparation of bovine blood plasma [7, 8]. In quantitative terms, the efficiency of treatment is strictly related to the radiation dose absorbed by the material. On the other hand, the probability of a post radiation damage of molecules of the preparation is also related to the dose and therefore the problem arises of a technologically reasonable dose [5].

There are relatively little data on the behaviour of blood protein preparaitons after radiation treatment. According to Urbain [9], who investigated the effect of radiation on bovine serum albumin, the interaction of radiation induced free radicals leads not only to intramolecular reactions but also to intermolecular ones, in spite of the restricted migration of sensitive sites in solid state [5]. The post radiation changes of protein at the molecular level resulted in a decrease of albumin solubility in water and salt solutions. Since the sedimentation constant increased without any simultaneous changes of the molecular weight of albumin molecule, these effects were due to the change of shape of the

molecules. The primary structure of albumin was not altered after low dose irradiation.

It becomes obvious that the above changes may be reflected in the functional properties of radiation pasteurized bovine blood plasma preparations; the present studies were undertaken to determine the radiation dose that may be applied safely to the material, without worsening its functional properties.

MATERIALS AND METHODS

Bovine blood was collected during commercial shaughter of animals and immediately centrifuged to separate the plasma protein solution. The blood plasma protein preparation was obtained by the spray — drying of the solution in an Anhydro type of dryer (7500 rpm, 200°C at the top and 75°C at the bottom of dryer).

The content of crude protein (N × 6.25) in the preparation was 71.2 per cent and albumin was predominant [7].

Irradiation of the preparation was performed in a laboratory type device PXM-Gamma 20 ^{60}Co in conditions described previously [8]. The doses applied were 1.0; 2.0; 3.0; 4.0; 5.0; 10.0; 16.7; 20.0 and 50.0 kGy.

The following parameters were taken to check the most important functional properties: solubility of the preparation, gellation ability, emulsifying capacity and viscosity. The analyses were performed with the preparation dispersed in water and in a 2% NaCl solution.

To determine the solubility of the preparation, dispersions of the material in distilled water and a 2% NaCl solution were stirred for 30 min at room temperature.

The liquid part of the dispersions was then separated by centrifuging at 8000 × G for 20 min. Protein solubility was expressed as the ratio of its concentration in the supernatant to that in the dispersion.

A modification of the Leas Concentration Endpoint method [6] was used to check the gel forming ability of the irradiated preparation. To demonstrate the effect of temperature on LCE, incubation of test tubes was carried out in temperatures varying from 60°C to 90°C with 5°C intervals. The LCE value was expressed in per cent of the preparation forming gel self-sustaining in test tubes while positioned upside down.

A method based on the principles described by Webb N.B. et al. [10] was used for the determination of emulsifying capacity. The results were expressed in cm^3 of oil emulsified by 100 mg of protein.

Measurements of the viscosity of 10% preparation dispersions in water and 2% NaCl solution were performed using a Hoeppler Rheo-Viscosimeter, following the mode of operation. The principle of this method is based on measurements of the time of glass bead movement through

a cylinder filled with the dispersion. The dynamic viscosity of the sample was expressed in cP.

To calculate the effect of radiation dose and the kind of solvent on investigated parameters, the variance analysis was applied. Also the interactions among the above factors and the lowest significant differences for mean values were computed.

RESULTS AND DISCUSSION

Table 1 summarizes the results of statistical treatment of experimental data. It is evident that the main effects e.g. radiation dose and solvent, were influencing all the functional parameters determined the experiment.

Table 1. The effect of variability factors on functional parameters of bovine blood plasma preparation

Parameter	Level of significance of calculated F values for:		
	dose A	kind of solvent B	interaction A×B
Solubility of the preparation	0.01	0.01	0.29
Gellation — total effect	0.01	0.01	0.01
Gellation — temperature	0.01	0.01	
Emulsifying capacity	0.01	0.01	0.02
Viscosity of 10% preparate solution	0.01	0.01	0.01

Changes of functional parameters as influenced by the main effect — radiation dose are presented in Table 2. The solubility of the preparation decreased with the increase of the radiation dose. In contrast to this, the emulsifying capacity of irradiated preparation was improved. Both effects were particularly pronounced for doses higher than 5 kGy, or 10 kGy, when is a significant decrease of viscosity.

Gellation capacity seems to be the most sensitive to radiation treatment, because a dose as low as 1 kGy caused a significant decrease of the amount of preparation required to form self-sustaining gel. After treatment of the preparation with a dose of 50 kGy that value was almost twice as high as for control, unirradiated samples. It is interesting that changes of the parameters as listed in Table 2, are mutually interrelated (Table 3).

These calculations suggest that the reason of changes appearing in dehydrated blood protein preparation after radiation treatment may be of similar nature for all the tested parameters.

A probable explanation of this interrelation is provided by changes of viscosity which indicate that the biophysical properties of protein were

Table 2. The effect of radiation dose on functional parameters of irradiated bovine blood plasma preparation (mean values of two solvents)

Radiation dose kGy	Changes of functional parameters			
	solubility (a)	gellation (b)	emulsifying capacity (c)	viscosity (d)
0.00	97.767	8.222	51.667	1.548
1.00	97.650	8.833	47.750	1.981
2.00	95.250	9.278	58.112	1.719
3.00	94.767	9.444	51.398	1.643
4.00	96.367	9.500	54.178	1.659
5.00	95.167	9.667	59.537	1.693
10.00	89.650	10.000	61.395	1.416
16.70	77.567	14.167	84.692	1.175
20.00	65.483	14.667	94.943	1.266
50.00	54.800	15.944	124.328	1.063
Lowest significant difference at $P < 0.05$	3.889	0.310	5.460	0.032

Note: (a) per cent of total N; (b) per cent of protein forming self sustaining gell; (c) cm^3 of oil per 100 mg of total protein; (d) 10% solution of preparation, cP.

Table 3. Correlations between changes of functional parameters vs radiation dose

	Solubility	Gellation	Emulsifying capacity	Viscosity
Solubility		−0.9718	−0.9852	0.8645
Gellation	−0.9718		0.9605	−0.8728
Emulsifying capacity	−0.9852	0.9605		−0.8764
Viscosity	0.8645	−0.8728	−0.8764	

Note: the critical r value is 0.7646 at $P < 0.01$.

affected at the colecular level as a result of treatment [1]. On the basis of the experimental data for solubility changes from Table 2, one may calculate the dose — effect relation (see Fig.).

The experimental data fit into the following formula:

$$\frac{N_D}{N_0} = e^{-0.0126D}$$

with an accuracy of 0.9564 as measured by the correlation between the results and the above formula, where:

N_0 — solubility of control samples,

N_D — solubility of samples theated with does D, kGy.

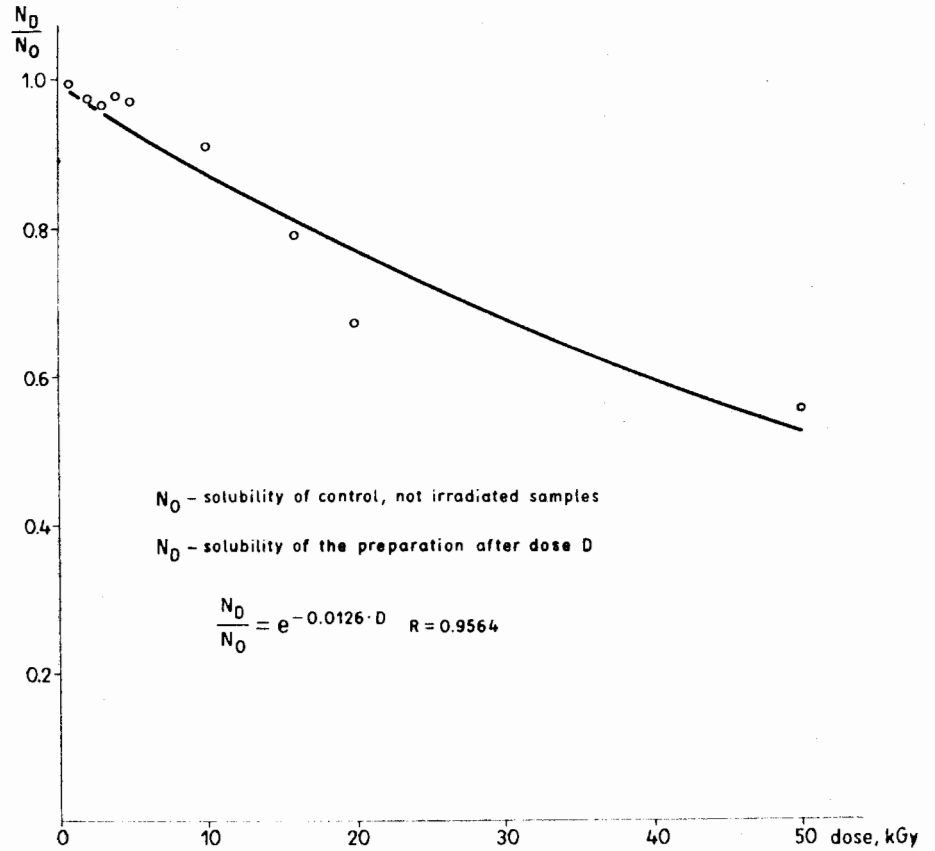

Fig. Changes of relative solubility of irradiated blood protein preparation as a function of dose

The N_D/N_O ratio stands for the amount of protein molecules remaining undamaged after irradiation, an d it is therefore possible to use the target theory for a calculation of the molecular weight of the radiation sensitive component of the preparation, as demonstrated by Leyko [4].

In this case:

$$\frac{N_D}{N_0} = e^{-0.0126\,D} = e^{-\frac{1}{D_{37}}D}$$

D_{37} — radiation dose that reduces the amount of undamaged molecules to the value of 0.37 N_O, kGy.

Since the dose of radiation indicates in general the amount of energy dissipated per unit of mass, according to the Poissons distribution law [3], for D_{37} all radiation sensitive macromolecules (targets) of a given mass should be damaged by the quanta of energy.

Taking 79.365 kGy = D_{37} for the transformations described in detail by Leyko [4], it is possible to calculate that the molecular weight of the target would be 72854 daltons. This value differs by 5.6 per cent only

from the molecular mass of albumin (69000 daltons). The content of albumin in spray dried bovine blood plasma was predominant. Radiolytic changes of albumin at the molecular level are therefore responsible for alterations of the functional properties of the preparations. These effects however, are particularly pronounced at higher doeses, even in the case of a parameter as sensitive as gellation (Table 4).

Table 4. The effect of temperature and dose range on the concentration of preparation forming the self sustaining gell (per cent)

Radiation dose kGy	Temperature of gellation°C					
	60	65	70	75	80	90
0.00	11.5 (a)	9.5 (a)	7.5 (a)	6.0 (a)	5.5 (a)	4.0 (a)
1.00	13.5 (b)	10.0 (a)	7.5 (a)	6.0 (a)	4.5 (b)	4.0 (a)
2.00	14.5 (c)	11.0 (b)	7.0 (a)	5.5 (a)	5.0 (ab)	4.5 (a)
3.00	13.5 (b)	11.0 (b)	8.0 (a)	6.0 (a)	5.0 (ab)	4.5 (a)
4.00	13.5 (b)	11.5 (b)	8.0 (a)	6.0 (a)	5.9 (a)	4.0 (a)
5.00	14.0 (bc)	11.5 (b)	7.5 (a)	6.5 (a)	6.0 (a)	4.5 (a)
10.00	14.5 (c)	11.5 (b)	8.5 (b)	6.0 (a)	6.0 (a)	4.5 (a)
16.70	18.0 (d)	16.0 (c)	14.0 (c)	12.0 (b)	10.5 (d)	7.5 (b)
20.00	17.0 (e)	16.5 (c)	15.5 (d)	13.0 (b)	11.5 (e)	8.5 (c)
50.00	18.0 (d)	17.5 (d)	16.5 (e)	15.5 (c)	13.5 (f)	10.5 (d)

Note: identical letters within the columns — lack of significant difference at $P < 0.05$.

From the technological point of view, the most interesting temperature gellation ranges are 70-75°C. For these temperatures the concentrations of the preparation required to form self-sustaining gell did not differ significantly from control, unirradiated samples up to the dose of 5 kGy. While performing gellation in lower temperatures, the effect of a 1 kGy dose becomes evident and is reflected in the results of the variance analysis.

Except for the emulsifying capacity, the 2% NaCl solution enhanced the effect of radiation treatment on the functional properties of bovine

Table 5. Effect of the solvent on functional properties of irradiated bovine blood plasma preparation (Total effect as calculated from analysis, of variance, mean values)

Parameter	Solvent		Lowest significant difference at $P < 0.05$
	water	2% NaCl	
Solubility of the preparation (a)	89.997	82.897	1.739
Gellation (b)	11.367	10.578	0.139
Emulsifying capacity (c)	67.630	69.570	2.442
Viscosity of 10% preparate solution (d)	1.642	1.391	0.014

Note: descriptions of a, b, c, d — identical as in Table 2.

blood plasma preparation (Table 5). The presence of salt is a factor promoting intermolecular reactions due to a reduction of the diffuse part of the electric double layer [2], but it would be rather difficult to give an explanation of this phenomenon on the basis of the experimental results. Since the effect of salt was also taken into account while calculating the data in Table 2, the dose of 5 kGy should be considered safe from the technological point of view. The application of doses higher than 5 kGy for radiation pasteurization of bovine blood plasma preparation may lead to a significant worsening of its functional properties. Under the conditions of the experiment, radiation treatment of the preparation with 5 kGy reduced the number of bacteria to 2.7 per cent of the initial contamination [7] and thus improved its microbial quality.

CONCLUSIONS

1. The application of gamma radiation for the pasteurization of spray dried bovine blood plasma preparation with a dose range up to 5 kGy is safe from the technological point of view.
2. A significant worsening of solubility, gellation capacity and viscosity of the material may be expected after treatment with doses higher than 5 kGy. Undesirable changes of functional properties are due to radiolytic effects appearing in albumin at molecular level.
3. Technologically undesirable changes of solubility, gellation and viscosity after high dose treatment were enhanced in 2% NaCl solution.
4. In the light of the experimental results obtained, it is recommended to make additional investigations on the quality of meat products manufactured with the use of a radiation pasteurized preparation.

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RADIACYJNA PASTERYZACJA PLAZMY KRWI BYDLĘCEJ
II. WPŁYW DAWKI NA WŁAŚCIWOŚCI FUNKCJONALNE PREPARATU SUSZONEGO ROZPYŁOWO

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Streszczenie

W przeprowadzonych badaniach dokonano oceny zakresu zmienności niektórych wyróżników funkcjonalnych suszonego rozpyłowo preparatu plazmy krwi bydlęcej, poddanego działaniu promieniowania gamma ^{60}Co w celu obniżenia zakażenia mikrobiologicznego. Wielkość dawek promieniowania jonizującego wynosiła od 1 kGy do 50 kGy. Ocenę zmian właściwości funkcjonalnych przeprowadzono na podstawie badania rozpuszczalności preparatu, jego zdolności żelowania (LCE) i emulgowania tłuszczu, a także lepkości 10% roztworu preparatu. Oznaczenia przeprowadzane były z użyciem roztworów preparatu w wodzie destylowanej i 2% NaCl.

Wyniki przeprowadzonych analiz wykazały istotność wpływu zabiegu napromieniowania oraz rodzaju rozpuszczalnika na wszystkie badane parametry (tabela 1). Dla dawek promieniowania jonizującego wyższych od 5 kGy zaobserwowano statystycznie istotne pogorszenie rozpuszczalności preparatu, jego zdolności żelowania oraz lepkości (tabela 2), co spowodowane było radiolitycznymi zmianami albumin na poziomie cząsteczkowym. W przeciwieństwie do tego, zdolność emulgowania tłuszczu napromienionego preparatu wzrastała wraz z dawką promieniowania. Technologicznie niepożądane zmiany rozpuszczalności, zdolności żelowania i lepkości preparatu po napromienieniu były większe, jeśli oznaczenia tych wyróżników wykonywano w 2% roztworze NaCl (tabela 5).

Z przeprowadzonych badań wynika, że możliwa jest radiacyjna pasteryzacja suszonego rozpyłowo preparatu plazmy krwi bydlęcej przy użyciu dawek do 5 kGy, natomiast zastosowanie dawek wyższych prowadzić może do istotnego pogorszenia jego przydatności technologicznej.