A C T A A L I M E N T A R I A P O L O N I C A
Vol. XII (XXXVI), No. 3—4 1986

WALDEMAR UCHMAN
JAN ZABIELSKI
PIOTR KONIECZNY
WŁODZIMIERZ FISZER

RADIATION PASTEURIZATION OF BOVINE BLOOD PLASMA. I. EFFECT OF DOSE ON THE TOTAL NUMBER OF AEROBIC BACTERIA OF LIQUID AND SPRAY-DRIED PREPARATION

Institute of Food Technology Animal of Origin, Agricultural University, Poznań

Key words: food irradiation, slaughter by-products, bovine blood plasma preparation.

The quantitative effect of gamma radiation on natural microbial contamination of liquid bovine blood plasma and its spray-dried preparation was studied. An exponential decrease of natural contamination of the materials as the result of radiation treatment with the doses up to 8 kGy was found. The parameters of the bacteria elimination were calculated. The results indicate also that the best pasteurizing effect of bovine blood plasma could be achieved through the combined treatment of spray-drying and irradiation.

Blood and its fractions represent an important source of high nutritional quality proteins and may be utilized as a colouring, binding and filling agent [2]. Also the transformation of blood plasma into meat analogue is possible [9, 13].

To achieve high quality and bacteriologically safe product, the hygienization of blood components should be performed prior to the use or during processing. The total bacteria count of fresh liquid plasma could be sometimes as high as 10^5-10^6, depending on the collection system. The species of *Staphylococaceae* and *Enterobacteriaceae* are usually predominant in the contamination [8].

Radiation technology offers certain possibilities for the reduction of microbial load, because since 1977 radiation treatment is considered as a safe physical process comparable to the heating or freezing [10].

The sterilizing effect of gamma radiation on blood constituents was studied with the promissing results even earlier [1, 4] with respect to

medical applications. Radiation pasteurized and dehydrated animal blood and blood proteins are expected to obtain in 1985 clearances for the use in food processing [5, 6]. There is, however, a lack of literature data on the microbial effect of radiation treatment of these slaughter by-products.

The present studies were undertaken to determine the quantitative effect of the radiation dose on the total number of aerobic bacteria in naturally contaminated liquid and spraydried preparation of bovine blood plasma. The presence of yeasts and moulds in powdered material was also determined.

MATERIALS AND METHODS

Bovine blood was collected during commercial slaughter of animals and immediately centrifuged to separate the plasma protein solution. The blood plasma proteins in powdered form were prepared from the liquid one through the spray-drying 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 powder was 71.2%. The liquid material contained 7.6% of the crude protein.

Both the liquid plasma and powdered blood plasma proteins were taken for irradiation.

10 g of the powder or 10 cm^3 of the liquid were transferred into sterile glass vessels and irradiated in a laboratory type radiation source PXM-Gamma 20 ^{60}Co. The absorbed dose of radiation was determined with Fricke's dosimeter according to the standard procedure recomended by the IAEA [11]. The dose rate was 0.038 kGy min^{-1}.

The samples of liquid blood plasma were irradiated with the doses of 0.3; 0.5; 1.0; 3.0; 4.0; 8.0 kGy. The doses of 0.1; 1.0; 2.0; 4.0; 6.0 kGy were applied for the powdered preparation. Separate portions of the material were collected for each of the dose levels applied. The doses of 4.0 kGy and 2.0 kGy were chosen for the additional determination of bacteria regrowth during storage of irradiated samples. The temperature inside the device was 30 ± 2°C during treatment.

For microbiological analyses the conventional dilution procedure was used. The samples of material before and after irradiation were transferred into peptone dilution fluid (0.1% peptone, 0.85% NaCl). The Plate Count Agar (Difco) was used for determination of the total number of aerobic bacteria. Appropriate dilutions were plated on two parallel Petrie dishes. Incubation was carried out at 30°C for 72 h.

The same procedure was applied for the determination of bacteria regrowth during postradiation storage. To compare the dynamics of these processes in liquid and powdered material, storage of the samples was performed at 30°C, that was under the conditions favourable to bacteria

regrowth. The examinations of a liquid material were carried out 12, 24 and 72 h after the treatment, while those of powdered plasma — after 3, 6 and 10 weeks of storage.

The Oxytetracycline Glucose Yeast Extract Agar was used for the determination of presence or absence of moulds and yeasts in powdered blood plasma. After the incubation at 25°C for 3 to 5 days, the positive plates were examined microscopically to confirm the presence of yeasts.

RESULTS AND DISCUSSION

The initial contamination of liquid blood plasma varied significantly in particular determinations (Table 1), while the contamination of spray-dried material was relatively uniform. As it follows from these data, the process of spray-drying itself reduced the level of contamination from 10^5-10^6 in the liquid material to the value of 10^3 per 1 g in the powdered preparation.

Table 1. The effect of radiation dose on the number of aerobic bacteria of liquid and spray-dried bovine blood plasma

Material	Radiation dose kGy	Number of aerobic bacteria		Surviving fraction N_D/N_0 per cent
		prior to irradiation N_0	after the treatment N_D	
Liquid plasma (a)	0.3	1.7×10^5	8.5×10^1	0.050
	0.5	1.5×10^5	7.4×10^1	0.050
	1.0	1.7×10^5	5.1×10^1	0.030
	3.0	1.9×10^6	5.0×10^1	0.030
	4.0	9.0×10^5	2.0×10^1	0.002
	8.0	3.2×10^6	0.0	0.0
Spray-dried preparation (b)	0.1	3.5×10^3	2.0×10^3	60.4
	1.0	2.2×10^3	2.3×10^2	10.6
	2.0	3.3×10^3	8.0×10^1	2.6
	4.0	3.6×10^3	2.0×10^2	5.6
	6.0	4.3×10^3	0.0	0.0

Note: (a) — counts per 1 cm^3; (b) — counts per 1 g. Mean values of 4 repl cat ons

The dynamics of microflora reduction in irradiated materials was different for liquid plasma and spray-dried preparation and this can be calculated from the surviving curves. Assuming, that the relationship between the dose D (kGy) and surviving fraction of bacteria N_D/N_0 (per cent) could have an exponential nature, than according to the formula:

$$(N_D/N_0) = a \times D^{-b}$$

were:
b — rate of the reduction,
a — constant value,
this is possible to calculate the values of both "b" and "a".

Under the experimental conditions the calculated values were a = 0.015 and b = −1.3596 for the liquid plasma while for spray-dried preparation 9.420 and −0.7642 respectively.

These exponential curves are plotted in semi-log scale and this is shown on Fig. 1.

Taking logs, one may calculate that the most pronounced effect of irradiation of the liquid material was obtained for the doses up to 1 kGy, because the initial cantamination was reduced by over 3 log cycles (from 100% to 0.015%). Treatment with the higher doses was less effective.

In contrast to it, the dose of 1 kGy reduced the initial contamination

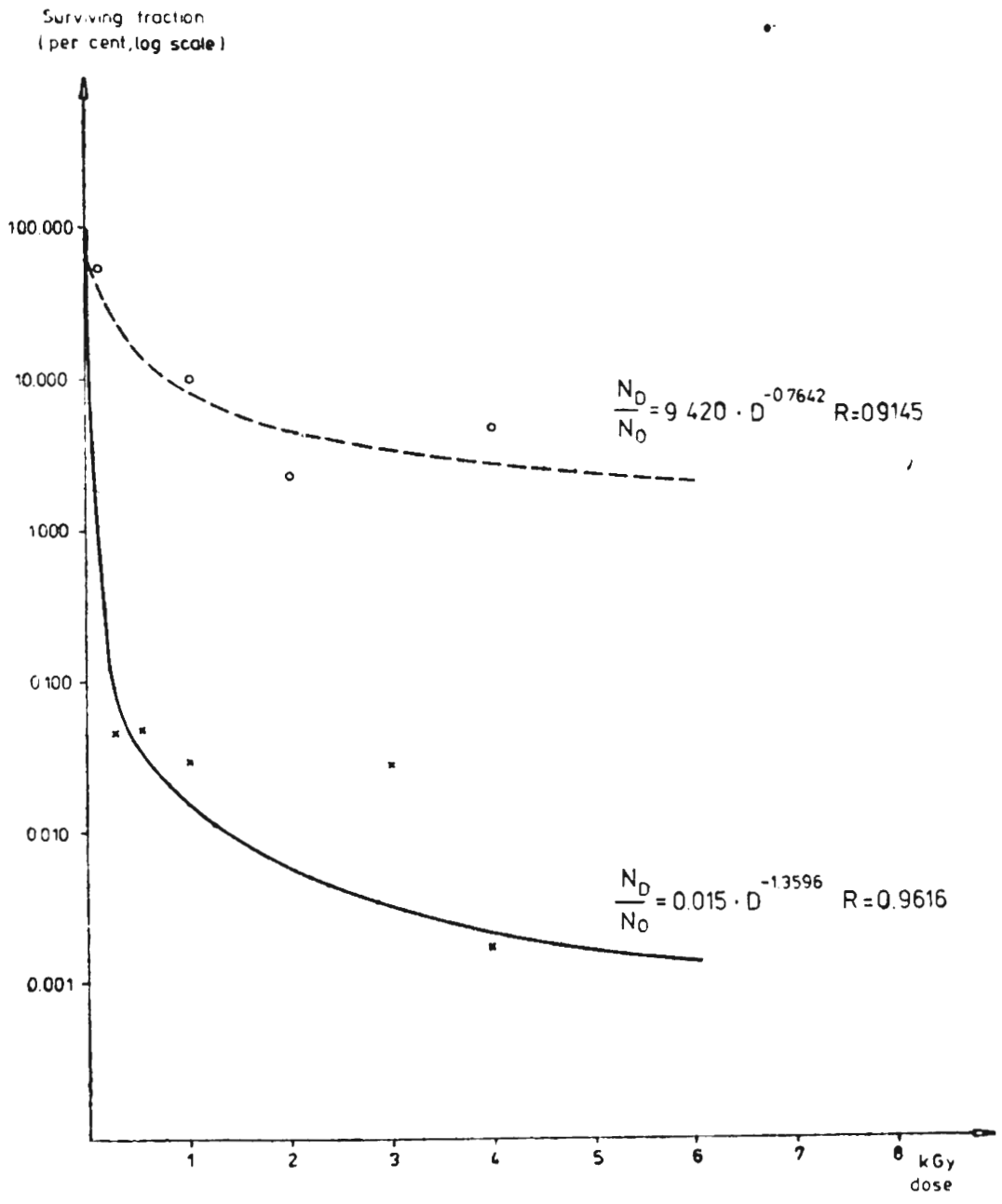

Fig. 1. Effect of radiation dose on surviving fraction of aerobic bacteria in liquid (x ——— x) and spray-dried preparation (o - - - - o) of bovine blood plasma

of spray-dried preparation from 100% to 9.42%, that was by 1 log cycle. Such a difference confirms the general experience that vegetative forms of microorganisms are much more sensitive in high moisture environment in comparison with the dehydrated medium [3]. Also the relatively radiation resistant bacterial spores were probably predominant in the contamination of spray-dried preparation, since the heat treatment during drying had reduced the number of vegetative forms.

Elimination of bacteria from the liquid plasma was very effective, but from the practical point of viev, the preservation of that material by irradiation only would be aimless. As this can be calculated from an exponential formula, the contamination of liquid plasma after treatment with the dose of 4 kGy was lowered to the level of 10^1 per cm^3. During 48 h of post-treatment storage under the conditions favourable for bacterial regrowth (30°C), the development of aerobic bacteria was very rapid and reached the level comparable with untreated samples (Fig. 2). Certain

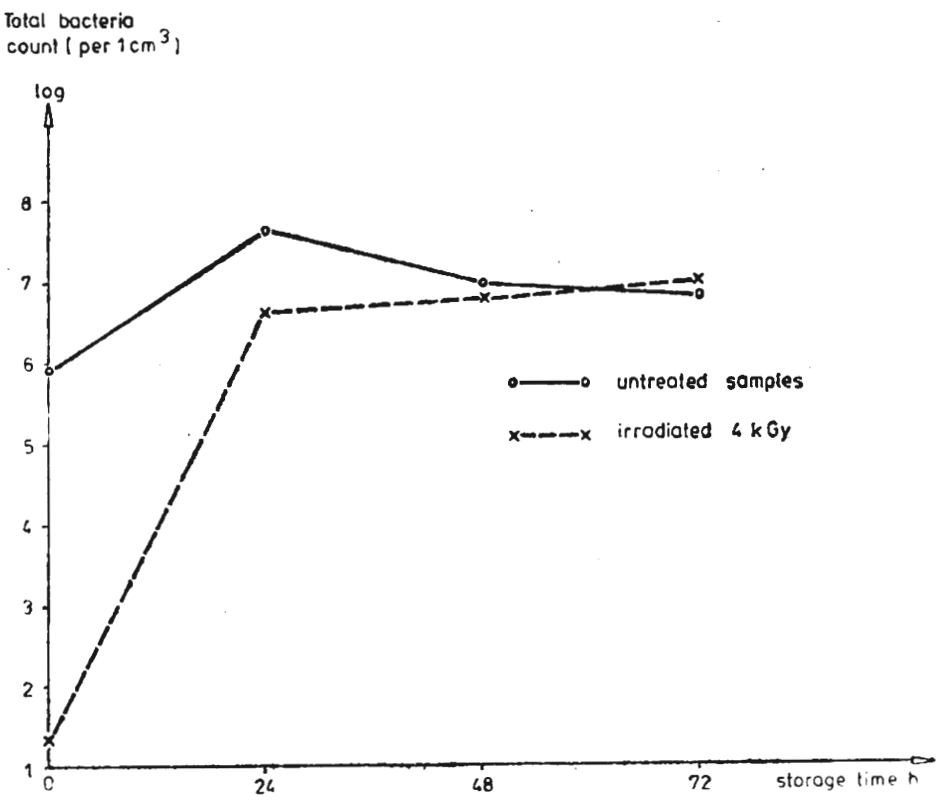

Fig. 2. Changes of the number of aerobic bacteria of liquid bovine blood plasma during storage in 30°C

increase of the total number of aerobic bacteria was also observed in spray-dried preparation treated with 2 kGy, but after 6 weeks of storage the level of contamination was remaining stable and lower in comparison with untreated samples (Fig. 3). There are two reasons that may be offered for an explanation of the increase of bacteria count in the beginning of storage period: the cells were able to repair damages caused by radiation [3], and, changes of water activity [7]. In the latter case, the changes of water bind-

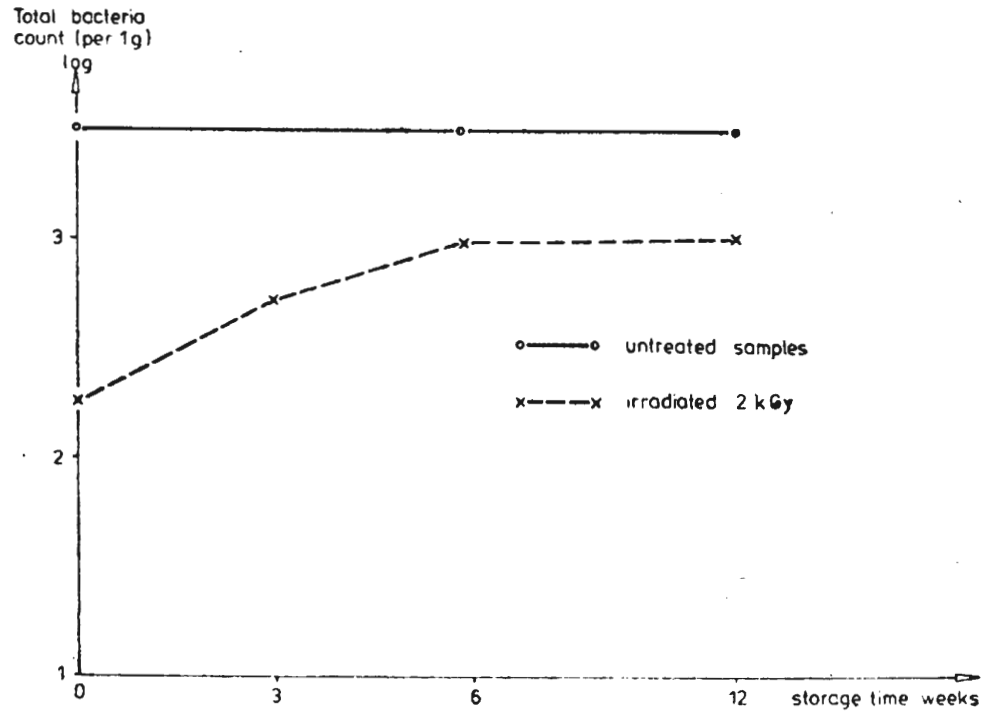

Fig. 3. Changes of the number of aerobic bacteria in spray-dried bovine blood plasma preparation during storage in 30°C

ing capacity in irradiated protein preparation should result in the release of certain amount of water and thus leading to the increase of water activity in the sealed glass vessels used for irradiation and storage of the samples. This hypothesis, however, should require more accurate investigations.

The elimination of yeasts and moulds from the plasma preparation was also effective (Table 2). For the doses higher than 1 kGy, the number of these microorganisms would be lower than 10^2 per 1g, similarly to the number of bacteria after the same treatment. This remains in agreement with the information of Farkas [3] indicating that radiation resistance of yeasts and moulds is generally of the same order as for vegetative bacteria.

Table 2. The presence of yeasts and moulds in spray-dried bovine blood plasma preparation after radiation treatment

Radiation dose kGy	Dilutions					
	10^{-2}		10^{-3}		10^{-4}	
	yeasts	moulds	yeasts	moulds	yeasts	moulds
0.0	+	+	+	+	+	+
1.0	+	−	+	−	+	−
2.0	−	−	−	−	−	−
4.0	−	−	−	−	−	−

Note: + positive in at least one of 4 replicates,
− negative in all replicates

The radiation dose of 2 kGy was obviously too small to achieve the pasteurizing effect in spray-dried preparation. For practical purposes the dose of 10 kGy, accepted by Joint FAO/IAEA/WHO Commision [10] as a wholesome and safe, could be applied. The calculated effect of microbial decontamination would be than 1.62% for surviving fraction or approximately 10^{-2} when discussed in the terms of probability. Taking into account the reduction of microflora in liquid plasma through the spray-drying process (10^{-2}-10^{-3}), under the experimental conditions the multiplied effect of the mild heat treatment and irradiation with 10 kGy would be as high as 10^{-4}-10^{-5}. The more precise technological governing of the pasteurizing effect would be possible when the radiation resistance of bacteria species predominating in contamination are known.

The radiation treatment of spray-dried bovine blood plasma preparation requires, however, the further investigations on the marginal conditions of the process with respect to probable changes of technological and functional properties of the material [12].

CONCLUSIONS

1. Radiation treatment of bovine blood plasma and its spray-dried preparation caused an exponential decrease of the number of aerobic bacteria in these materials.
2. The most pronounced effect of bacteria reduction in liquid blood plasma and spray-dried preparation was found for the radiation doses up to 1 kGy. The dynamics of these changes was significantly higher in the liquid material in comparison with spray-dried preparation.
3. For storage purposes the combined treatment of spray-drying and medium dose radiation provides the highest pasteurizing effect and thus offers a meat protein substitute of high microbial quality.
4. The additional investigations are needed before the potential technological application of that technique and particular attention should be paid on probable changes in functional properties of spray-dried preparation pasteurized with the doses up to 10 kGy.

This work was partially supported by the Maria Skłodowska-Curie Fund (Project PL-ARS-108).

LITERATURE

1. Antoni F., Pangil W., Schwarz O., Simonich W., Kozinets G., Schulman W., Arky J.: Radiosterilization of Medical Products. IAEA, Vienna 1967, 107.
2. Bengtsson O., Holmqvist O.: Fleischwirtschaft 1984 (3), 64, 334.

3. Farkas J.: Microbiological Implications of Food Irradiation. IFFIT Technical Report No 18, Wageningen, the Netherlands 1981, 4.
4. Frischauf H.: Report of a Panel on Radiation Sterilization of Biological Tissues for Transpl. IAEA, Vienna 1970, 47.
5. Henon Y.: Food Irradiation Newsletter, IAEA, Vienna 1984 (8), 1, 41.
6. Van Kooij J. G.: Food Irradiation Newsletter, IAEA, Vienna 1983 (7), 3, 3.
7. Langerak D. Is.: Food Irradiation Now. Nijhoff — Junk Publishers, the Hague (Boston) London 1981, 40.
8. Otto R. G.: Bakteriologische Stufenkontrollen in Blutplasmagewinnungsanlagen zur Erkennung von Hygienerisiken. Doctoral thesis. Der Tierarztlichen Hochschule Hannover 1983, 92.
9. Swingler G. R., Lawrie R. A.: Meat Science 1977 (1), 3, 161.
10. Technical Report 659: Wholesomeness of Irradiated Food. WHO, Genewa 1981, 31.
11. Technical Report 178: Manual of Food Irradiation Dosimetry. IAEA, Vienna 1977, 89.
12. Uchman W., Fiszer W., Konieczny P., Mastyński J., Wierzbicki S., Wojciechowski J., Zabielski J.: Investigations of the Influence of Various Concentration of Bovine Blood Plasma Preparations on Some Meat Products Properties. Fourth Annual Report of the Project PL-ARS-108, 1983, 32.
13. Young R. H., Lawrie R. A.: J. Fd Technology 1974 (9), 171.

Manuscript received: June, 1985
Authors address: 60-623 Poznań, Mazowiecka 48

W. Uchman, J. Zabielski, P. Konieczny, W. Fiszer

RADIOWA PASTERYZACJA PLAZMY KRWI BYDLĘCEJ. I. WPŁYW DAWKI NA OGÓLNĄ LICZBĘ BAKTERII TLENOWYCH PLAZMY PŁYNNEJ I PREPARATU SUSZONEGO ROZPYŁOWO

Instytut Technologii Produktów Zwierzęcych, Akademia Rolnicza, Poznań

Streszczenie

W przeprowadzonych badaniach oceniono wpływ dawek promieniowania gamma ^{60}Co na zmiany ogólnej liczby bakterii tlenowych plazmy płynnej i preparatu suszonego rozpyłowo.

Stwierdzono, że w zakresie dawek od 0,1 kGy do 8,0 kGy następuje wykładnicze zmniejszenie ogólnej liczby bakterii w napromienionym materiale. Ilościowo najwyższe efekty napromieniania występowały przy dawkach niskich — do 1 kGy. Z przeprowadzonych obliczeń wynika także, że dynamika zmian liczby bakterii tlenowych była wielokrotnie wyższa w plazmie płynnej niż w preparacie suszonym rozpyłowo. Pomimo wysokiej skuteczności redukcji liczby bakterii w plazmie płynnej, celowość zastosowania radiacji, jako jedynego czynnika utrwalającego jest dyskusyjna ze względu na intensywny rozwój mikroflory resztkowej. Po 48 h przechowywania próbek plazmy płynnej napromienionej dawką 4 kGy w temp. 30°C, poziom skażenia był zbliżony do wartości próbek kontrolnych, nienapromienionych.

Ogólna liczba bakterii tlenowych w suszonym rozpyłowo preparacie napromienionym dawką 2 kGy wzrastała nieznacznie do 6 tygodnia przechowywania w 30°C, po czym następowała stabilizacja poziomu skażenia mikrobiologicznego. Dawka promieniowania 2 kGy spowodowała również skuteczne obniżenie liczby drożdży i pleśni w suszonym rozpyłowo preparacie. Z przeprowadzonych obliczeń wynika, że w warunkach doświadczalnych utrwalanie plazmy krwi bydlęcej metodą kombinowaną, to jest suszenia rozpyłowego, a następnie napromieniania preparatu dawką 10 kGy prowadzić będzie do zmniejszenia liczby bakterii tlenowych w produkcie o 10^{-4}-10^{-5} raza w porównaniu ze skażeniem początkowym plazmy płynnej.