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The Destruction of *Salmonella* Bacteria in Refrigerated Liquid Whole Egg with Gamma Radiation

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Thermal pasteurization being a borderline process, it was attempted to use ionizing radiation for rendering whole egg, intended for preservation by other methods than freezing, free from *Salmonelleae*. Initial densities of about 10^7/ml of *S. typhimurium* and *S. senftenberg* were used. Irradiation was carried out with a ca. 1500 c Co^{60}-source, the inoculated whole egg being packed in lacquered tins and kept at ca. 1°C during this treatment.

Six decimal reductions of the *Salmonella*-species tested were obtained at dosages of 2×10^5 rad, nine reductions at slightly over 5×10^5 rad. 'No effect of the use of specific recovery media or pre-treatments on the apparent dose value required for obtaining these reductions could be established.

The odour of the whole egg and its functional properties appeared to have been impaired at dosages of 10^4 rad, while the colour suffered from ca. 2×10^5 rad. Therefore, the radiation pasteurization of liquid whole egg at ca. 1°c cannot be considered a promising process.

LA DESTRUCTION DE LA BACTÉRIE *SALMONELLE* DANS L'OEUF ENTIER LIQUIDE RÉFRIGÉRÉ AU MOYEN DU RAYONNEMENT GAMMA

Vu que la pasteurization thermique est un procédé marginal, on essaya l'emploi du rayonnement ionizant pour rendre libre de *Salmonellae* l'oeuf entier destiné à la conservation par autres moyens que le gel. On utilisa des densités initiales d'environ 10^7/ml. de *S. typhimurium* et de *S. senftenberg*. Le rayonnement se fit avec une source de Co^{60} d'environ 1500 c, l'oeuf entier inoculé étant enfermé dans des boîtes métalliques laquées, et maintenu à environ 1°C durant ce traitement.

Six diminutions décimales des espèces de *Salmonella* mises à l'essai furent obtenues pour des doses de 2×10^5 rad, et neuf diminutions pour un peu plus de 5×10^5 rad. On ne put établir aucun effet sur la valeur de dose apparente nécessaire pour donner ces diminutions, que pourrait rendre l'emploi des agents spécifiques de rétablissement ou des traitements préalables.

Il y avait une détérioration apparente de l'odeur de l'oeuf entier et de ses qualités fonctionnelles à des doses de 10^4 rad, et un changement de couleur à partir d'environ 2×10^5 rad. Ainsi on ne peut guère considérer comme une méthode de promesse la pasteurization au rayonnement de l'oeuf entier liquide à environ 1°C.

УНИЧТОЖЕНИЕ *Salmonella* БАКТЕРИИ В ОХЛАЖДЕННОМ ЦЕЛОМ ЖИДКОМ ЯЙЦЕ ПРИ ПОМОЩИ ГАММА-ОБЛУЧЕНИЯ

Для уничтожения *Salmonella* в целых яйцах, хранение которых производится не в холодильниках, были сделаны попытки применить ионизирующее излучение, поскольку термическая пастеризация является неизбирательным процессом. Исходные концентрации *S. typhimurium* и *S. senftenberg* были 10^7/мл. Облучение производилось источником Co^{60} мощностью ~1500 кюри, причем зараженное яйцо помещали в жестяную запаянную банку и выдерживали при ~1°C в течение всего опыта.

Шесть десятикратно разбавленных образцов, зараженных *Salmonella* были подвергнуты облучению дозами в 2×10^5 рад, а девять образцов–дозами немного выше 5×10^5 рад. Применяемая питательная среда и предварительная обработка не влияли на величину дозы, требующейся для указанных разбавлений.

Дозы в 10^4 рад вызывали появление запаха и меняли функциональные свойства целого яйца, а дозы в 2×10^5 рад приводили к изменению окраски. Следовательно, применение радиационной пастеризации целого жидкого яйца при ~1°C следует признать бесперспективным.

DIE VERNICHTUNG VON SALMONELLA BAKTERIEN IN GEKÜHLTER EIFLÜSSIGKEIT MIT HILFE VON γ-STRAHLUNG

Da thermale Pasteurisierung hier nicht angewendet werden kann, wurde versucht, Eier, für die andere Konservierungsmethoden als Tiefkühlung vorgesehen waren, durch ionisierende Strahlung von Salmonella zu befreien. Als Ausgangskonzentration wurde etwa 10^7/ml von S. Typhimurium und S. Senftenberg verwendet. Die Bestrahlung wurde mit Hilfe einer etwa 1500 C Co-^{60}-Quelle durchgeführt. Die infizierten Eier wurden in gelackte Dosen verpackt und während der Behandlung auf ca. 1°C gehalten.

Eine Verminderung um 6 Grössenordnungen der Zahl der untersuchten Salmonella-Spezies wurde durch eine Dosis von 2×10^5 rad erreicht, während eine Verminderung von 9 Grössenordnungen bei etwas über 5×10^5 rad eintrat. Die Verwendung von speziellen Wiederbelebungsmitteln und Vorbehandlungen ergaben keine Veränderungen des Dosiswertes, der zur Erzielung der erwähnten Verminderung nötige war. Der Geruch und die funktionellen Eigenschaften der Eier wurden bei Dosen von 10^4 rad beeinträchtigt, während Farbenveränderungen bei ca. 2×10^5 rad auftraten. Die Pasteurisierung von Eiflüssigkeit durch Bestrahlung bei 1°C kann daher nicht als eine günstige Methode betrachtet werden.

It seems to be well established now, that the future use of ionizing radiation in the processing of fresh perishable foods, if any, will be limited to low dosage treatments, especially pasteurization and disinfestation.[8]

Because the thermal pasteurization of liquid whole egg, being a typical borderline process, continues to present occasional problems, it was considered worthwhile to carry out a few tentative tests on the possibilities of using ionizing radiation for this purpose. The irradiation of frozen whole egg has been rather well studied;[2,13] therefore in the present study it was specifically attempted to irradiate liquid egg under refrigeration, as it seems hardly practicable to pre-freeze liquid eggs for pasteurization purposes prior to preservation methods other than freezing.

At the same time it was investigated whether Alper and Gillies's observation that a strain of *Escherichia coli*, carrying radiolesions, was better recovered in slightly unfavourable media than in commonly used optimal ones[7] was also valid for *Salmonelleae* irradiated in foods.

EXPERIMENTAL DETAILS

Raw material

Fresh hens' eggs were washed with 0·5% chloramine solution, thereupon broken out and the yolks and whites obtained mixed under aseptic conditions. The product so obtained was immediately cooled to about 2°C. It had an aerobic and anaerobic plate count of less than 10^2 organisms/1 ml.

Adequate portions of this material were inoculated with *Salmonella typhimurium* and *S. senftenberg* in initial densities of about 10^7/ml. The first strain was chosen because it is one of the most important causes of human salmonellosis in The Netherlands,[4] the latter because rather heat-resistant varieties of this serotype have been encountered in the United States.[1] The rather high initial densities were considered necessary to allow a sufficient safety margin for the opportunities which may exist in egg-breaking plants for a few *Salmonella*-infected hen's eggs to contaminate rather large amounts of whole egg and for the subsequent considerable increase in numbers of *Salmonelleae* in such lots.[2,3]

The refrigerated inoculated material was

canned in lacquered tins, measuring ca. 7 × 1·5 cm and kept at about 2°C throughout subsequent procedures.

Irradiation

Irradiation was carried out in the Department of Radiology, Municipal Hospitals, The Hague, under the direction of Mr. H. R. Marcuse, Health Physicist. The source used consisted of about 1500 c Co^{60}-fuel rods.

In order to be able to irradiate at a continuous range of dosages, cans containing inoculated egg were stacked to a height of about 50 cm and transferred to an aluminium container, maintained at 0–1°C. Cans irradiated at a level of ca. 10^4, 3×10^4, 1×10^5, 2×10^5 and 5×10^5 rad were used for further investigation. Radiation at this level required about 10 hr exposure to the γ-source.

During the experiments the temperature in the whole egg never exceeded 2°C.

Bacteriological examination of irradiated samples

Irradiated samples were examined both by enrichment in liquid media and by direct counts in solid media using optimal as well as selective media.

The enrichment media were brain heart infusion broth, MULLER's tetrathionate broth[12] and OSBORNE and STOKES's selenite bile brilliantgreen sulphapyridin medium.[14] About 30 g of the irradiated material was both directly seeded into these media and after 1 hr incubation in nutrient broth at 37°C, followed by 6 hr further incubation in nutrient broth, to which 5 μg/ml of chloramphenicol had been added.[7] Enrichment cultures so obtained were further examined in the usual way.[10]

The solid media used were crystal violet neutral red bile lactose mannitol agar,[9] nutrient agar and TDYM-agar.[11] These media were again seeded both with the freshly irradiated samples of whole egg and with material having been pre-incubated in nutrient broth plus chloramphenicol at 37°C. Adequate proportions of colonies obtained in plates were confirmed as *Salmonelleae* in the usual way.[10]

General examination of irradiated samples

The freshly irradiated samples were also tested for taste, odour, colour and for significant colloidal properties. The latter were assessed by carrying out a dispersion test, in which 5 ml of whole egg is gently shaken with 45 ml of water and thereupon allowed to settle. In this assay, untreated whole egg forms a copious sediment, while irradiated samples of liquid egg show very much less sediment and more complete dispersion, the latter increasing with the dose of radiation to which the samples have been subjected.

RESULTS

Judged by the results of the method of direct plate counts, "total inactivation", which implies reduction from ca. 10^7 to <10/ml, of all *Salmonelleae* was obtained at dosages of 2×10^5 rad. No significant influence of media or pre-treatments on the recovery of irradiated cells could be detected in these experiments.

Based on the outcome of enrichment tests, reduction from ca. 10^7 to $<10^{-2}$ viable *Salmonelleae* per 1 ml of whole egg, required dosages of at least 5×10^5 rad. Because this dosage was the highest one tested, complete inactivation of *Salmonelleae* was not attained in a few of the tests. Here again, no consistent effect of the use of specific

TABLE 1. Relative radiation resistance of the strains of *S. typhimurium* and *S. senftenberg* tested

Dose of γ-radiation (rad)	Numbers of surviving cells of	
	S. typhimurium	*S. senftenberg*
0	$0{\cdot}6 \times 10^7$	$0{\cdot}6 \times 10^7$
10^4	$0{\cdot}8 \times 10^7$	$0{\cdot}9 \times 10^5$
3×10^4	$0{\cdot}1 \times 10^7$	$0{\cdot}2 \times 10^4$
1×10^5	$0{\cdot}7 \times 10^3$	$0{\cdot}05 \times 10^2$
2×10^5	<10	<10
5×10^5	<10	<10

media or pre-treatments could be established. The strain of *S. senftenberg* tested appeared to be less radiation resistant than the strain of *S. typhimurium*, as the figures in Table 1 show. These differences did not, however, materially influence the doses of radiation required in practice for inactivation.

General examination of the irradiated samples showed that the odour of all materials had been impaired already at dosages of 10^4 rad. Colloidal changes also became obvious at this dosage, while colour impairment occurred invariably at 2×10^5 rad, and in some tests even at lower dosages.

DISCUSSION OF RESULTS

The bactericidal effects of γ-radiation on *Salmonelleae* observed in the present investigation are in good agreement with those obtained by NICKERSON *et al.*[13]. The fact that BROOKS and colleagues in Britain found lower inactivation doses for *Salmonella* in eggs[2] seems quite obvious in view of the very much lower initial density (ca. 10/ml vs. 10^7/ml in the American and our assays) used by that group.

We have been unable to find any influence of the post-irradiation medium on the recovery of irradiated *Salmonelleae* analogous to that described by ALPER and GILLIES for *E. coli* strain B. Our findings are thus in agreement with the general conclusions of DAVYDOFF[5] and FREEMAN and BRIDGES[6] who investigated other species.

The denaturation effects which were encountered in our experiments were more serious than those observed by both NICKERSON *et al.* and BROOKS *et al.* This is most probably due to the fact that we deliberately carried out irradiation at +1°C not ca. −20°C and also used high doses. The off-flavours noticed will most probably have little effect on the taste of baked goods, although they may exert a detrimental influence on the flavour of pastry fillings; while the international egg products trade, where judgement of lots is based on the flavour of the *raw* merchandise, would have to be duly informed if radiation pasteurization might become a reality. The observed loss of colloidal ("functional") properties of irradiated whole egg will most probably affect the quality of baked goods.

Our conclusion must therefore be that radiation pasteurization of liquid whole egg, refrigerated but not frozen, cannot be considered a promising process.

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