Food

S. Cabo Verde, M. Luísa Botelho, R. Tenreiro [1], M. João Trigo[2]

Objectives

With the purpose to apply the food irradiation as a food safety tool, the Radiation Technology group at the ITN is developing two projects:

1 - "Sanitation of chicken eggs by ionizing radiation". The aims of this project is to study the impact of radicidation in egg or egg-products ; and the *Salmonella* and *Campylobacter* detection by PCR technique to be applied as a monitoring program to the eggs production line.

2 - "Application of Ionising Radiation to Vegetables" – The aim of this project is to evaluate the impact of irradiation on microbial, and functional parameters in vegetables.

Results

1 - "Sanitation of chicken eggs by ionizing radiation"- In previous published studies [1, 2] we proposed a radicidation dose of 1.5 kGy, in order to guarantee organoleptic acceptable and safe egg and egg-products, as a preventive tool to eliminate to non-detectable levels the potential pathogenic microorganisms present in/on eggs.

In order to replace the hard laborious and time consuming standard methods (ISO 6579:2002 and ISO 70272:1995(E)), two Polymerase Chain Reaction (PCR) techniques were developed. The first was a conventional PCR with *Salmonella invA* and *Campylobacter* 16S-specific primers to detect these pathogens directly from eggs and eggs-products. The validation was performed by artificial contamination of egg and egg-products, with *Salmonella* and *Campylobacter* reference strains. The DNA extraction was carried out by boiling from 1ml of crude 18h-enriched spiked cultures. Positive results were obtained for all spiked samples, after agarose gel electrophoresis of the PCR products it were visualized a DNA band corresponding to the amplification of a 284 bp fragment for *Salmonella* spp and a 287 bp fragment for *Campylobacter* spp.. The second method evaluated was a Real-time PCR with *Salmonella invA* and *Campylobacter 16S* specific primers in order to detect these pathogens in shell eggs. The SYBR Green I dye was used for the non-specific detection of dsDNA. DNA-chloroform extraction was used to obtain purified DNA. After optimization, the real-time SYBR Green PCRs were applied to detect *S. typhimurium* CCUG 31969 and *C. jejuni* CCUG 11284 in artificially contaminated enrichment cultures of shell eggs (Fig. 1). The specificity of the reactions was determined by the melting temperature (T_m), which was consistently specific for the amplicons obtained, also confirmed by gel analysis. The mean peak T_m obtained with for *S. typhimurium* was 86.84 ± 0.04ºC and 87.56 ± 0.07ºC for *C. jejuni*. The optimized protocol successfully detected *S. typhimurium* and *C. jejuni* in artificially contaminated shell eggs down to the level of 1 to 10 CFU/ml.

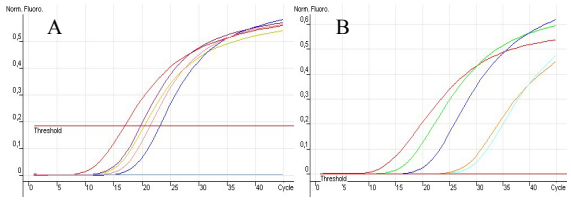

Fig. 1. Fluorescent curves ampflification for spiked egg samples with (A) *S. typhimurium* and (B) *C. jejuni*.

The implementation of the real-time PCR with the enrichment step reduces the *Salmonella* and *Campylobacter* detection time to 23h, instead of the 5 to 7 days required by the standard culture methods.

2 - "Application of Ionising Radiation to Vegetables" – Several samples of minimal vegetables were irradiated in the gamma facility. Results showed that irradiation doses of 0.5 and 1.0 kGy decreases the microbial count extends the shelf-life of lettuce leaves during 12 days. (Internal Report IAEA).

Published, accepted or in press work

1. S. Cabo Verde, R. Tenreiro, M. L. Botelho, 2004. Sanitation of chicken eggs by ionising radiation: HACCP and inactivation studies. *Radiat. Phys. Chem.* 71: 29-33.
2. P. Pinto, R. Ribeiro, L. Sousa, S. Cabo Verde, M.G. Lima, M. Dinis, A. Santana, M. L. Botelho. 2004. Sanitation of chicken eggs by ionising radiation: functional and nutritional assessment. *Radiat. Phys. Chem.* 71: 35-38.
3. S. Cabo Verde, Tenreiro, R., Botelho, M. L., 2004. Foodborne Agents and Bioterrorism Prevention – A Portuguese Case Study on Ionizing Irradiation, Radiation Inactivation of Bioterrorism Agents, NATO Advanced Research Workshop.
4. S. Cabo Verde, Hjelm, E., Herrmann, B., Tenreiro, R., Botelho, M. L., 2004. Detection of *Salmonella* spp. and *Campylobacter* spp. in eggs by Real-Time SYBR Green PCR. Manuscript to be submitted.

[1] Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Portugal.
[2] Instituto Nacional de Investigação Agrária, Oeiras, Portugal.

Environmental Control

M. L. Botelho, S. Cabo Verde, P. Matos, Sílvia Xisto[1], Pet Mazarello[1]

Objectives

"Environmental control of surgical rooms at the army Hospitals" - This project, is focusing on the development and improvement of alternative techniques to control the environment in surgical rooms leading to the detection and identification of nosocomial microrganisms, in order to construct a database that could demonstrate the relation between the improvement of the air born conditions and the infection hospital agents. In a far future we pretend to use the database for early detection and prevention of nosocomial infections.

Results

Air samples were collected twice, spaced by a six month period at the army hospital surgery room. Sampling was planned previously taking into account critical points, by means of air sampler biocollector and sedimentation plates.. As seen on Fig.: 1 the cfu/m^3 for air collection and cfu/plate in each phase, before (BS), during (DS) and after surgery (AS), were calculated taking in account the medium value of all sampling collected in each local. The air bioburden average values (P= 95%) obtained in the first sampling with the biocollector MAS100 were 1900 ± 1000 cfu/m^3, 1100 ± 24 cfu/m^3 and 2300 ± 420 cfu/m^3, for BS, DS and AS, respectively. The sedimentation plates results were 25 ± 8 cfu/plate BF, 22 ± 4 cfu/plate DS and 32 ± 5 cfu/plate AS. For the second sampling period, the average bioburden values were 687 ± 172 cfu/m^3; 480 ± 139 cfu/m^3 and 1270 ± 140 cfu/m^3, BS, DS and AS, respectively. The sedimentation plates results were BS 38 ± 26, DS 35 ± 11 and AS 16 ± 4 cfu/plate. A better visualization of these results is presented in Figures 1.

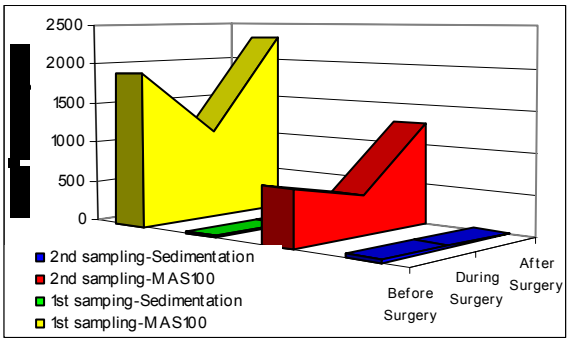

Fig. 1. Average values of cfu/m^3 of air bioburden for the two performed samplings, before, during and after an orthopedic surgery in the Army Hospital.

Comparing bioburden values between the two used techniques the efficiency of microorganism's collection is 100 fold higher for MAS 100 than for sedimentation. This result indicates that the air monitoring method applied in the surgery rooms by the hospital (sedimentation) is not the most appropriate to obtain a significant sampling of all natural airborne microorganisms. Bioburden collected by the MAS100, decreases during surgery and increases after surgery. This fact could be due to lesser movement during surgery by the surgical team. In other to evaluate the resistance/sensibility of the recovered strains, antibiograms were performed using four antibiotics administrated to surgery patients. 90% of all strains were susceptible to all antibiotics. One of the strains isolated during surgery by the biocolector, identified as *Pseudomonas fluorescens*, showed resistance to Ceriax® and Cefoxitin® with halos of 20 mm, but susceptibility to the other two antibiotics.

The air was collected in the surgery room, before and after a cleaning procedure. Bioburden results (before: 838 ± 218 cfu/m^3; after: 1350 ± 575 cfu/m^3) demonstrate the inadequacy of the hospital cleaning protocols to eliminate potential nosocomial microorganisms.

The hospital data obtained so far point to the usefulness of the implemented HACCP study to assess infection risk and nosocomial antibiotic and disinfectant resistant strains. Nevertheless, more data should be gathered before we can implement new actions that can effectively reduce the risk of infection. Once the detection of an outbreak can easily be detected, corrective actions can be implemented.

Published, accepted or in press work

1. M.L. Botelho, S. Cabo Verde, P. Matos, P. Mazarelo, R. Tenreiro, 2004. Detection and prevention of bioterrorism agents – Portuguese case studies. Radiation Inactivation of Bioterrorism Agents, NATO Advanced Research Workshop, in press.

[1] Laboratório Militar, Lisboa, Portugal.

Effluents

M. L. Botelho, Rita Melo, Joaquim Branco, Vanda Farinha, Luisa Alves

Objectives

To implement wastewater (WW) treatment by ionizing radiation in Portugal, R&D work is being done. A study case using gamma radiation on slaughterhouse wastewater samples was carried out. The impact of radiation was evaluated by chemical and microbiological parameters. Taking into account these technical parameters, economical feasibility studies were also undertaken in order to plan and foreseen the best design for a future implementation of this technology.

Results

The results obtained at a low dose rate (0.9 kGy.h^{-1}) show a decrease in Chemical Oxygen Demand (COD), Biologycal Oxigen Demand (BOD) and colour pointing out an improvement in WW quality. After irradiation at 25-35 kGy, COD show a reduction of 35 % compared to non-irradiated samples. At 7 kGy COD value increase which could be explained by the gamma radiation's scissor effect (number molecular low weight increasing). See Fig.1. Therefore, results showed that high gamma radiation doses are adequate to lower the organic matter weight on the effluent, achieving an important goal for the slaughterhouse.

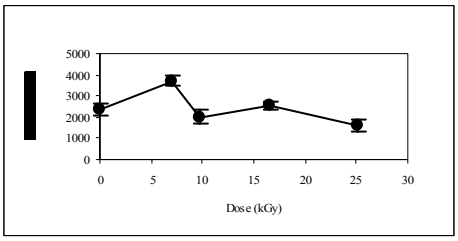

Fig. 1. COD vs. absorbed radiation dose.(n=3; α=0.05)

As shown in Fig. 2, BOD decreases to at least 50% at 25 kGy. An exception occurs at 7 kGy where the value increases by 20%. With the exception of the lowest dose, gamma radiation effect can be associated to the decrease of BOD, leading to a better water quality.

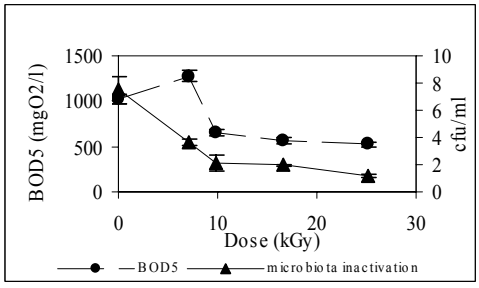

Fig. 2. BOD and inactivation curve of microbiota vs. absorbed dose.

The similar platforms at 17 kGy and at 25 kGy demonstrate the relationship between microbiota inactivation and BOD. Studies carried out for the higher dose rate (3 kGy h^{-1}), show an inactivation of 5 log of c.f.u. at 7 kGy, corresponding to an efficiency of 99,999 %. For the 0.9 kGy.h^{-1} dose rate, at the same absorbed dose, an inactivation of 4 log c.f.u, was observed, showing a lesser efficiency (99,986 %). Therefore, the high dose rate was shown to be more efficient for the cleanness process of the tertiary treatment. Survivor strains at higher doses (up to 30 kGy) are being studied by means of kinetics growth and molecular biology, techniques in order to understand their bioremediation capacity and the mutation effects probability. Besides, an attempt to find out the survivor mechanisms is under our scope. These studies stress the advantages of the use of gamma radiation for slaughterhouse WW chemical and biological remediation.

One of the major problems of the slaughterhouse wastewater it is its red colour, due to blood, oil and greases. Fig.3 shows that the colour intensity after irradiation at 30 kGy is similar to the one obtained *in situ* after the anaerobic process. This fact stresses the advantages of the use of gamma radiation for slaughterhouse WW treatment.

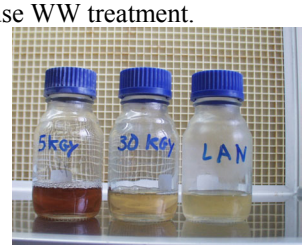

Fig. 3. Slaughterhouse wastewater after filtration at 5 kGy, 30 kGy and after anaerobic lagoon (LAN).

Cost-effectiveness analysis for the implementation of WW treatment by gamma radiation shows that an integrated gamma radiation treatment is economically favorable and urgent taking in account the sector legislation.

Published, accepted or in press work

1. Melo R., Branco, J., Botelho, M.L. 2004. Impact of Ionizing Radiation on Slaughterhouse Wastewater - chemical parameters - *Water Research*.

2. Botelho M.L., Microbiological Aspects in Wastewater, *Int. Symp. on "Advanced Oxidation Technologies for Wastewater Treatment*", Warsaw, Poland, 18 June 2004.

3. Melo, R., Branco, J., Botelho, M.L, Slaughterhouse, Sewage Treatment using Gamma Radiation – Economical Feasibility Study, IAEA Report, Sept. 2004, pp 1-17.

4. Botelho M L.,. Progress report of the Research Project IAEA 12017/R0/RBF "Impact of ε-beam and γ radiation on the wastewater and drinking water: Comparative studies" May 2003 – 2004.

Modification of Polymeric Molecular Structures by Gamma Irradiation

L.M. Ferreira, A.N. Falcão, M.H. Gil[1]

Objectives

Production of new grafted copolymeric LDPE based materials suitable for bioapplications, using the technique of graft copolymerisation induced by gamma radiation.

Results

The studies performed made possible the selection of experimental protocols adequate for the production of new materials with high grafting yields and homogeneous distribution. These were used in the preparation of new LDPE films with enhanced hydrophilic properties. All irradiations experiments were carried out at the UTR ^{60}Co facility.

The hydration capacity of the new materials were obtained by the following expression:

$$Hydration\ (\%) = [(W_w - W_d)/ W_d] \times 100$$

where W_d and W_w represent the weights of dry and wet grafted films, respectively.

Figures 1 and 2 show the isothermal water absorption and dehydration curves as a function of time for two PE-g-HEMA films grafted at 14.0 and 268.0 %, prepared by gamma irradiation in the absence of air at a dose rate of 0.3 kGy.h^{-1}, with a $[HEMA]_i$= 15 % (V/V), during 10 and 30 hours respectively.

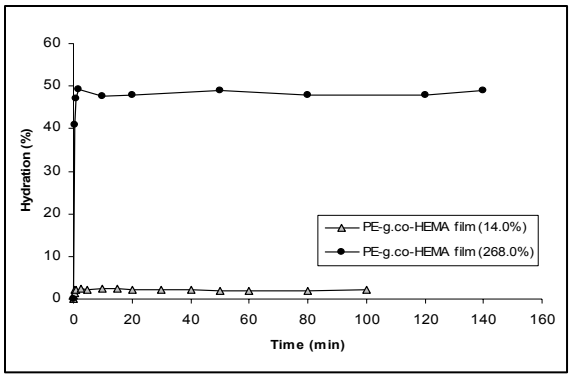

Fig.1. Hydration capacity of LDPE grafted films at 37 ºC in physiological serum as function of time.

Independently of the grafting degree both films studied attained their maximum hydration capacity (≅ 4 % for PE-g-HEMA film with 14.0 % of graft and ≅ 45.0 % for PE-g-HEMA film with 268.0 % of graft) in less than 2 minutes.

Isothermal dehydration data shows that, for the 268.0 % grafted film, the dehydration kinetic is almost 7 times slower that of the hydration kinetic. For the poor grafted film the kinetics ratio is almost 1:1.

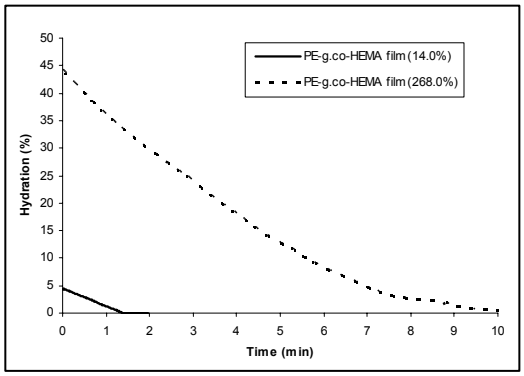

Fig. 2. Dehydration behavior of LDPE grafted films at 37 ºC as function of time.

TGA curves of these new copolymeric films (Figure 3) shows that, with increasing grafting yield, the thermal behavior of the copolymers increasingly approaches that of polyHEMA, and departures from that of pure LDPE.

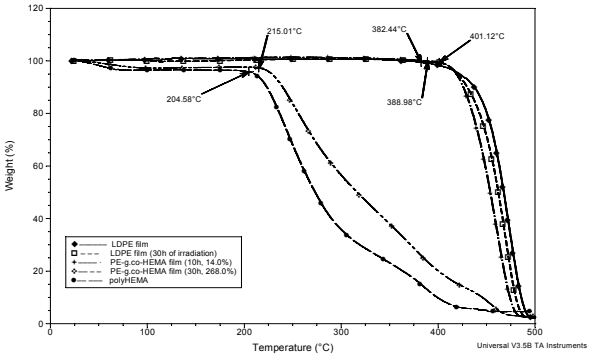

Fig.3. TGA thermograms of LDPE film, LDPE film irradiated in MeOH, polyHEMA and two PE-g-HEMA films grafted at 14.0% and 268.0%, obtained by gamma irradiation in absence of air (DR= 0.3 kGy.h^{-1}, $[HEMA]_i$= 15 % (V/V)).

This behavior suggests that with the increase of grafting yield, the grafted HEMA branches begin to show the typical reticulated structure of polyHEMA. This was confirmed by FTIR analysis.

Above 425 ºC the thermal behavior of the high grafted film slightly approaches that of the LDPE backbone. This is an evidence that the new copolymeric film keeps part of the desirable LDPE structural identity, even when the grafting yield is high.

Published, accepted or in press work

L.M. Ferreira, A.N. Falcão, M.H. Gil, Modification of LDPE Molecular Structure by Gamma Irradiation for Bioapplications, *Proc. 6th Int. Symp. on Ionizing Radiation and Polymers*, Houffalize, Belgium, 25-30 September 2004, *Nucl. Inst. and Meth.B.*

[1],Dept. of Chemical Eng., Faculty of Science and Technology, Coimbra University, Portugal

Characterisation of chitosan based copolymers for biomedical applications

M.H. Casimiro, J.P. Leal, M.H. Gil[1]

Objectives

Our attention has been focused on the characterisation of grafted copolymers of chitosan/HEMA (particles and films), prepared by chemical and γ-radiation induced polymerisation in order to obtain a biocompatible vehicle for sustained released of drugs.

Results

Previous data have show that graft copolymerisation induced by γ-radiation leads to higher grafting yields with the maintenance of the thermal stability of the backbone structure - chitosan. The grafting percentage is obtained by the following expression:

$$\text{Grafting (\%)} = \frac{\text{wt of monomer grafted}}{\text{wt of chitosan}} \text{x } 100$$

To find out if obtained copolymers (particles) maintain the chitosan natural antimicrobial activity, qualitative microbiological assays were done by immersing an amount of copolymer in Tryptic Soy Agar (TSA) medium and put to incubate at 30 ºC. Results (see table1) show microbial growth after 24h of incubation for samples obtained by chemical polymerisation, while copolymeric samples obtained by γ irradiation begin to present growth only after 48h. This evidences that γ irradiation method leads to chitosan based copolymers with antimicrobial properties.

Table 1: Results of the daily observation of chitosan and polymeric samples in TSA medium at 303 K.

Sample	Incubation time (h)			
	24	48	72	120
Chitosan	N.G.	N.G	N.G	N.G
17.6% - 1h chem. polym.	**G.**	**G.**	**G.**	**G.**
17.8% - 0.4h γ polym.	N.G.	**G.**	**G.**	**G.**
48.2% - 0.6h γ polym.	N.G.	N.G.	**R.G.**	**R.G.**
138.8 % - 6h γ polym.	N.G.	N.G.	N.G.	N.G.

Legend: **G.** ↔ Microbiological growth
N. G. ↔ No growth
R. G. ↔ Reduced growth

Concerning the evaluation of various synthesis conditions on physical, chemical and microbiological properties of chitosan/pHEMA membranes, the obtained results suggest that:

1. Hydration capacity of non-irradiated membranes increases with the increasing in its chitosan and/or HEMA content (Figure 1).
2. γ irradiation of chitosan/pHEMA membranes conduces to a more crosslinked network structure, and this to a decrease in its hydration capacity and to an improvement in its thermal decomposition temperature (Figures 1 and 2).
3. Chitosan/pHEMA membranes present good barrier properties against microbes, since no bacteria was found on the TSA medium under the membranes pads.

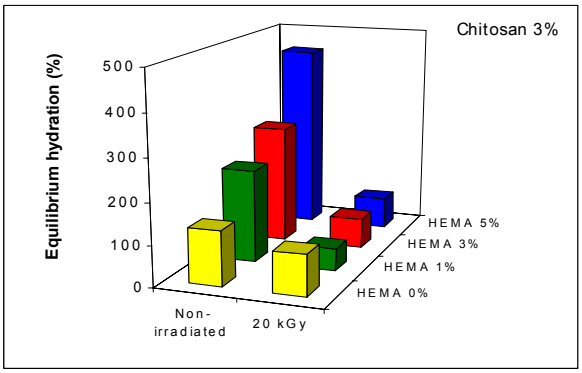

Fig.1. Hydration capacity of non-irradiated and irradiated membranes at 37ºC in physiological solution.

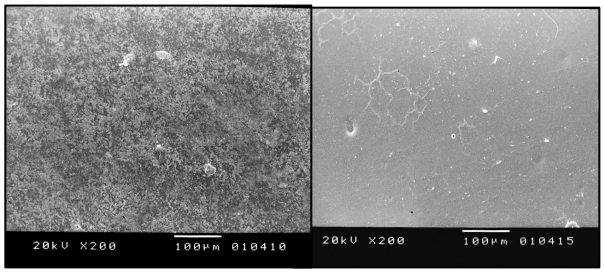

Fig.2. SEM micrographs of chitosan (3%)/HEMA (1%) surface.

Published, accepted or in press work

1. M.H. Casimiro, M.L. Botelho, J.P. Leal, M.H. Gil, Study on chemical, UV and gamma radiation induced grafting of 2-hydroxyethyl methacrylate onto chitosan, *Rad. Phys. Chem*. **76** (2005) 731-735 (in press).
2. M.H. Casimiro, J.P. Leal, M.H. Gil, Characterisation of gamma irradiated chitosan/pHEMA membranes for biomedical purposes, *Proc. 6th Int.l Symp. on Ionizing Radiation and Polymers*, Houffalize, Belgium, 25-30 September 2004, *Nucl. Inst. And Meth. B* (in press).

[1]Dept. of Chemical Eng., Faculty of Science and Technology, Coimbra University, Portugal