

EFFECT OF E-BEAM IRRADIATION ON THE MICROBIAL QUALITY OF MINIMALLY PROCESSED PRODUCTS: A CASE OF A COMMERCIALIZED READY TO EAT SALAD.

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Abstract

Fresh vegetables and commercialized ready to eat salad could be contaminated by pathogenic foodborne microorganisms and may constitute a potential health-risk product. Hence, the use of safe disinfecting treatment like E-beam irradiation could be a useful tool to ensure pathogens inactivation and shelf-life extension. In this study, freshly packaged ready-to-eat salads were collected from supermarket and analyzed for naturally occurring microorganisms, including aerobic plate count, *Staphylococcus* spp., yeasts, molds and *Clostridium perfringens*. Then, in a second step, ready to eat salads were processed at an E-Beam accelerator. Doses were ranging from 2kGy to 4kGy. Irradiated salads were analyzed for total aerobic plate count, *Staphylococcus* spp., yeasts and molds and *Clostridium perfringens* during 15 days of storage period at 4°C. The validity of processing treatment at 2kGy to 4kGy was challenged by artificial contamination of sterilized salad using *Staphylococcus aureus* strain (ATCC 25823). Results showed that E-beam irradiation at a dose of 4 kGy reduced concentrations of total aerobic plate count and yeast by 5 and 3 Log 10 CFU/g respectively, and inactivated *Staphylococcus* spp and molds. Hence, irradiation at 4 kGy dose contribute to maintain a satisfactory limit for naturally occurring microorganisms and extended the shelf life of commercialized ready to eat salads during more than 10 days of storage period at refrigeration temperature. Concerning resistant sporulating bacteria, E-beam irradiation at 4 kGy dose reduced the concentration of *Clostridium perfringens* by 1,5 Log10 CFU/g, initial mean concentration was estimated at (2,6 Log10 CFU/g). Results corroborate the use of E-Beam irradiation for food preservation as an efficient physical treatment for packaged ready to eat salads.

1. INTRODUCTION

Socio-economic development during last decades in Tunisia has led to tremendous changes in eating habits such as the consumption of ready to eat products, mainly fresh vegetables and commercialized ready to eat salad

[1]. Consumption of ready to eat salad provides many benefits for human health related to its nutritional properties and it is considered as a time-saving product for consumers. Fresh vegetables do not undergo bacterial heat treatment before consumption. Therefore, spoilage microorganisms could contaminate fresh salads and cause several food-borne diseases as well as industrial economic losses [2]. Microorganisms, including aerobic plate count, yeasts, molds and pathogenic foodborne microorganisms such as *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* spp and *Listeria monocytogenes* are detected in ready to eat salad [3,4,5, 6]. Commercialized ready to eat salads could be contaminated during processing steps (trimming, washing, peeling, cutting, slicing, and shredding) and mainly after packaging step [7]. To meet consumers demand in providing nutritious, safe, and sustainable supply of food, industrials are investing in non-thermal processing for pathogens elimination and shelf-life extension. Hence, the use of safe disinfecting treatment like food irradiation seems to be a good alternative to ensure good quality and safe salads [8,9]. Food irradiation is considered as commercial use for sanitary applications based on control spoilage and food borne pathogenic microorganisms as well as prevent the spread of invasive insects pests [10]. Among irradiation tools, E beam and gamma irradiation is widely used for food irradiation, mainly for fresh produce irradiation. They can maintain food quality and address food safety without significantly affecting a food's sensory or nutritional attributes [11, 12].

This study, aimed at evaluating the effect of E-beam irradiation combined with cold temperature storage technology on naturally occurring and artificially contaminating selected microorganisms in order to extend the shelf life of commercialized ready to eat salad.

2. MATERIAL AND METHODS

2.1. Samples Collection

A Total of 15 ready to eat salads were collected from end-point commercialized products (supermarket) located in north of Tunisia. Commercialized ready to eat salads were composed of lettuce, cherry tomato, red cabbage, rocket leaves and corn, are packaged in plastic boxes, the shelf life indicated in salads is 4 to 6 days. Samples were immediately kept at 4 ± 1 °C and analysed within 24h.

2.2. Samples irradiation

Irradiation was carried out using electron-beam accelerator (CIRCE 3, SGN, France) with an energy of 10 MeV located at the ionizing radiation facility in the National Center for Nuclear Sciences and technologies (CNSTN). Fresh ready to eat salads were irradiated in their package (250g; one box per dose) at room temperature at doses ranging from 2 to 4 kGy with an average dose rate of 40kGy/min. Non irradiated samples (0 kGy) were used as control.

2.3. Microbial analysis

Samples were analysed for naturally occurring microorganisms including total aerobic plate count, *Staphylococcus* spp., yeasts, molds and *Clostridium perfringens* during 15 days of storage period at 4°C. 25g of each sample was diluted with 225 ml of Peptone Water (Biokar diagnostics, France) and homogenized by stomacher (AES, 400ml) for 2 min. Then, serial dilution was performed for inoculation in triplicate on appropriate media following the analysis method shown in table 1. Artificially contamination of sterilized ready to eat salad (exposed to 4kGy dose) was performed using 10^7 CFU/ml of *Staphylococcus aureus* (ATCC 25823).

TABLE 1. METHOD OF MICROBIAL ANALYSIS

Microorganism	Method
Total aerobic plate count	ISO 4833-2:2013
<i>Staphylococcus aureus</i>	ISO 6888-1:2004
<i>Clostridium perfringens</i>	ISO 15213:2003
Yeast and molds	ISO 08-059:2001

3. RESULTS AND DISCUSSION

3.1. Effect of E. beam irradiation on total aerobic plate count

After irradiation at 2, 3 and 4kGy, concentrations of aerobic plate count were determined during 15 days storage period (Fig 1).

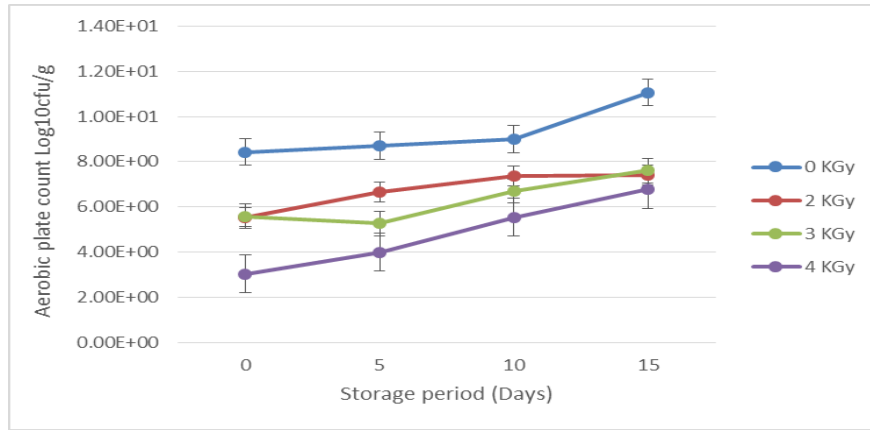


FIG.1. Concentration of aerobic plate count in commercialized ready to eat salads irradiated at 2, 3 and 4kGy and stored at 4°C for 15 days.

Initial mean concentration of aerobic plate count in commercialized ready to eat salads was 8,43 Log₁₀ CFU/g. The E beam reduction Log scale at 2, 3, and 4kGy doses was 2.88, 2.85 and 5.41 Log₁₀ CFU/g respectively. The E-beam treatment allowed to comply with recommended criteria for fresh fruits and vegetables regarding aerobic plate counts and reached satisfactory limit (3 log CFU/g) < 5 log CFU/g. [6]. During 15 days storage period, aerobic plate count increased in both irradiated and non-irradiated samples, which is similar to gamma irradiation effect [7]. For irradiated samples at 4kGy, the mean concentration of aerobic plate counts at 15 days was lower by 1,66 Log₁₀ CFU/g than initial counts of non-irradiated samples at (T₀).

3.2. Effect of E. beam irradiation on *Staphylococcus* spp.

After irradiation at 2, 3 and 4kGy, concentrations of *Staphylococcus* spp. were determined during 15 days storage period (Fig. 2.).

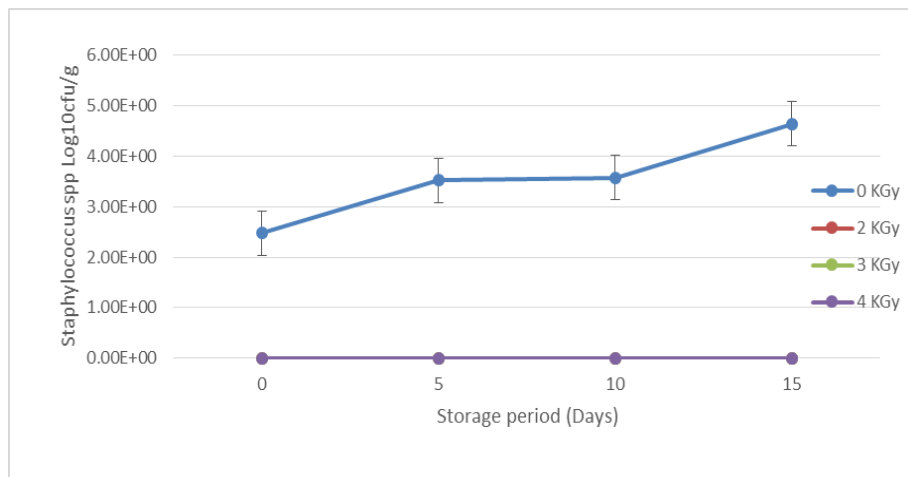


FIG.2. Concentration of *Staphylococcus* spp in commercialized ready to eat salads irradiated at 2, 3 and 4kGy and stored at 4°C for 15 days.

Initial mean concentration of *Staphylococcus* spp. in commercialized ready to eat salads was 2,5 Log₁₀ CFU/g. The E beam reduction Log scale at 2, 3, and 4kGy doses was 2.5, 2.5 and 2,5 Log₁₀ CFU/g respectively, leading to a total inactivation of *Staphylococcus* spp. In this case, E beam ensure pathogens control of ready to eat salad by maintaining satisfactory limit < 20 of *Staphylococcus* spp. [6]. During 15 days storage period, *Staphylococcus* spp was not detected in irradiated salads and still increasing for non-irradiated. These results were similar to those observed for the effect of gamma irradiation on *Staphylococcus* spp [7].

Regarding artificial contamination by 7 Log CFU/g of *Staphylococcus aureus* strain (ATCC 25823), E beam reduction Log scale at 2, 3, and 4kGy doses was 1.3, 1.5 and 3 Log₁₀ CFU/g respectively (Fig. 3.)

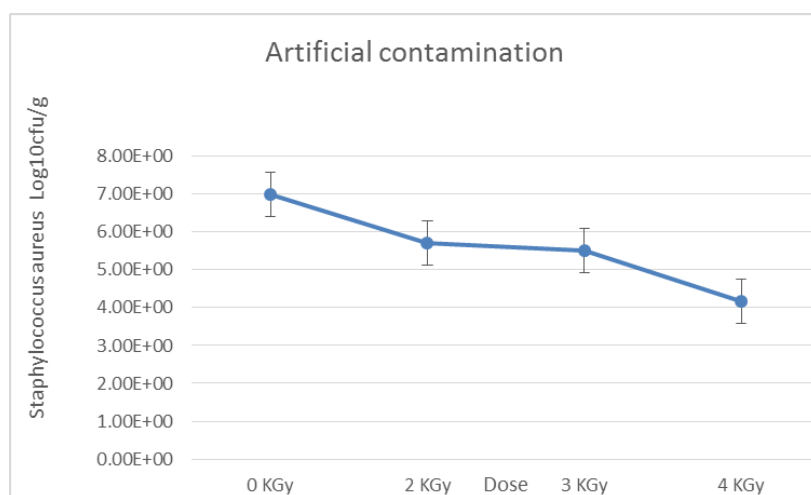


FIG. 3. Concentration of *Staphylococcus aureus* (ATCC 25823) in inoculated ready to eat salad irradiated at 2, 3 and 4kGy

D₁₀ values were determined, as the irradiating dose needed to reduce microorganisms by 90% for irradiated samples. D₁₀ value of *Staphylococcus aureus* (ATCC 25823) in inoculated ready to eat salad and irradiated by E beam was 1.6 kGy and it was higher than D₁₀ value of *Staphylococcus aureus* irradiated by gamma irradiation [7].

3.3. Effect of E. beam irradiation on yeast load

After irradiation at 2, 3 and 4kGy, the concentrations of yeast were determined during 15 days storage period (Fig. 4).

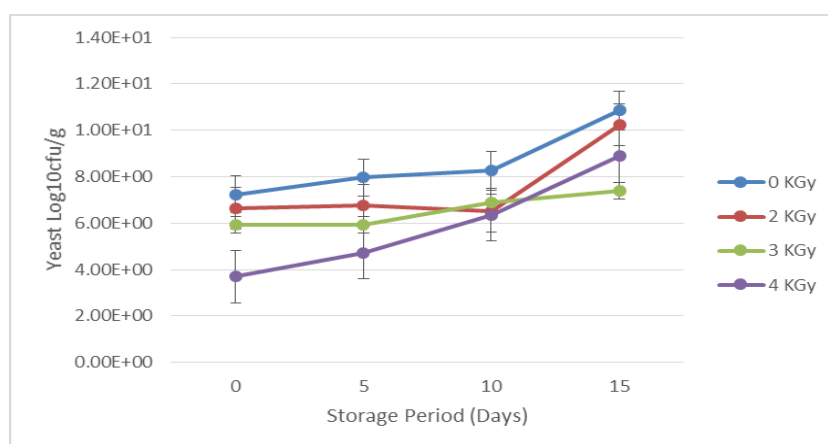


FIG.4. Concentration of yeasts in salad irradiated at 2, 3, and 4kGy and stored at 4°C for 15 days.

Initial mean concentration of yeasts in commercialized ready to eat salads was 7,25 Log₁₀ CFU/g. The E beam reduction Log scale at 2, 3, and 4kGy doses was 0,6, 1,4 and 3,6 Log₁₀ CFU/g respectively, leading to maintain satisfactory limit < 4 Log CFU/g [6]. During 15 days storage period, mean concentration of yeast increased for irradiated and non-irradiated salads. The concentration of yeast for irradiated during this period was always lower than control (0kGy). Previous study reported the same results of yeast load after gamma irradiation treatment [7].

3.4. Effect of E beam irradiation on molds load

After irradiation at 2, 3 and 4kGy, concentrations of molds were determined during 15 days storage period as presented in Fig. 5.

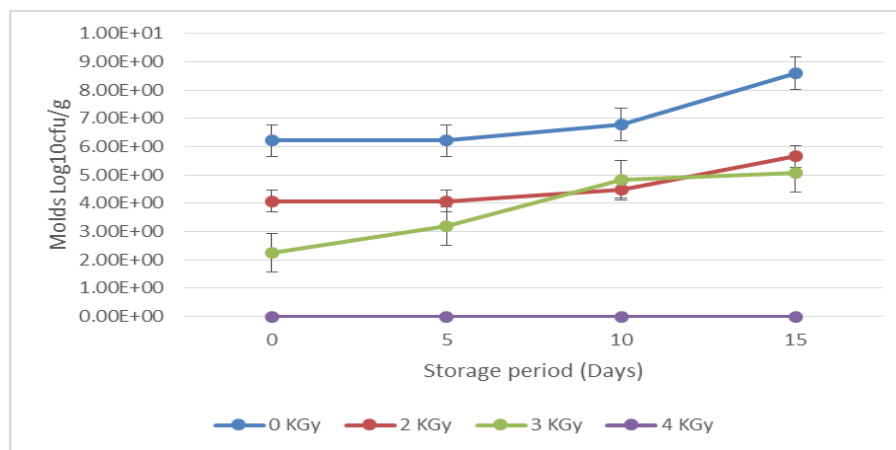


FIG.5. Concentration of molds in salad irradiated at 2, 3 and 4kGy and stored at 4°C for 15 days.

Initial mean concentration of molds in ready to eat salads was 6,22 Log₁₀ CFU/g. Irradiation at 4 kGy ensured total inactivation of molds, contributing to maintain satisfactory limit < 4 Log CFU/g [6]. During 15 days storage period, total inactivation of molds still persist for irradiated salads at 4kGy comparatively to control (0kGy). This finding highlighted the use of E-beam treatment at 4kGy for food preservation after packaging process, as it extends its shelf-life with a reduced processing time comparatively to Gamma irradiation.

3.5. Effect of E beam on *Clostridium perfringens* load

The effect of E beam irradiation on *Clostridium perfringens* load is shown in Fig. 6.

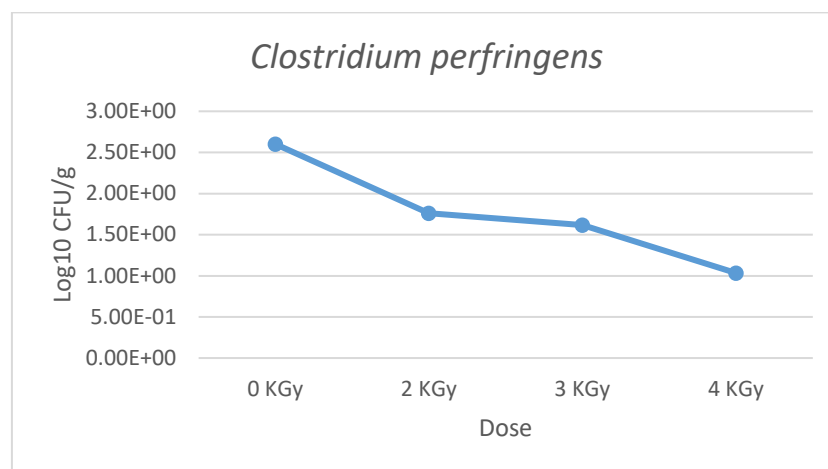


FIG.6. Concentration of *Clostridium perfringens* in salad irradiated at 2, 3 and 4kGy

Initial mean concentration of *Clostridium perfringens* in ready to eat salads was 2,6 Log₁₀ CFU/g. The E beam reduction Log scale at 2, 3, and 4kGy doses was 0,84; 0,98 and 1,57 Log₁₀ CFU/g respectively. The dose of 4 kGy is efficient against *Clostridium perfringens* especially that D₁₀ value of *Clostridium perfringens* is estimated at 3kGy. Spores are more resistant to ionizing irradiation treatment than bacteria and viruses that highlighted usefulness of *Clostridium perfringens* as indicators of irradiation treatment efficiency for food preservation.

Appearance of salads before and after E beam treatment (at 4kGy dose) during 15 days of storage period showed that irradiated samples preserved a sensory properties comparatively to control (Fig.7).



FIG.7. Appearance of salads before and after E beam treatment (at 4kGy dose);
a: Control; b: Irradiated salad after 10 days; c: Irradiated salad after 15 days.

4. CONCLUSION

In conclusion, results corroborate the use of E-Beam irradiation as a treatment for ready to eat salads at a dose of 4kGy after packaging process and prior to commercialization. Its effectiveness depends on initial mean concentration of naturally occurring microorganisms. E-Beam irradiation seems to be more adequate for ready to eat food treatment to avoid contamination occurring during packaging process and to extend its shelf-life as an environmentally friendly process with a reduced processing time.

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